

Brazilian Health Regulatory Agency

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Volume II – Monographs

Pharmaceutical Ingredients and Specialties

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PHARMACEUTICAL INGREDIENTS AND SPECIALTIES

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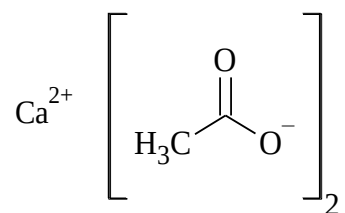
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POLYSORBATE 20	IF292-00
POLYSORBATE 40	IF293-00
POLYSORBATE 60	IF294-00
POLYSORBATE 80	IF295-00
PRAZIQUANTEL	IF296-00
PRAZIQUANTEL TABLETS	EF203-00
PREDNISONE	IF297-00
PREDNISONE TABLETS	EF204-00

PROGESTERONE	IF298-00
PROPYLPARABEN	IF299-00
PROPYLTHIOURACIL	IF300-01
TESTOSTERONE PROPIONATE	IF301-00
RABEPRAZOLE SODIUM	IF302-00
RABEPRAZOLE SODIUM TABLETS	EF205-00
RIFAMPICIN	IF303-00
RIFAMPICIN CAPSULES	EF206-00
RIFAMPICIN ORAL SUSPENSION	EF207-00
RITONAVIR CAPSULES	EF208-00
SUCROSE	IF304-00
ORAL REHYDRATION SALTS	EF209-00
METHYL SALICYLATE	IF305-00
SIMVASTATIN	IF306-00
SIMVASTATIN CAPSULES	EF210-00
SIMVASTATIN TABLETS	EF211-00
ORGAN PRESERVATION SOLUTIONS	EF212-00
SOLUTIONS FOR PERITONEAL DIALYSIS	EF213-00
SOLUTIONS FOR HEMOFILTRATION AND HEMODIAFILTRATION	EF214-00
SOLUTIONS FOR IRRIGATION	EF215-00
SULFADIAZINE	IF307-00
SULFADIAZINE TABLETS	EF216-00
SULFAMETHOXAZOLE	IF308-01
SULFAMETHOXAZOLE AND TRIMETHOPRIM TABLETS	EF217-00
SULFAMETHOXAZOLE AND TRIMETHOPRIM ORAL SUSPENSION	EF218-00
SULFAMETHOXYPYRIDAZINE	IF309-00
SULFANILAMIDE	IF310-01
ATROPINE SULFATE	IF311-00
ATROPINE SULFATE MONOHYDRATE INJECTION SOLUTION	EF219-00
BARIUM SULFATE	IF312-00
CALCIUM SULFATE	IF313-00
CEFPIROME SULFATE	IF314-00
CEFPIROME SULFATE POWDER FOR INJECTION SOLUTION	EF220-00
EPHEDRINE SULFATE	IF315-00
EPHEDRINE SULFATE INJECTION SOLUTION	EF221-00
STREPTOMYCIN SULFATE POWDER FOR INJECTION SOLUTION	EF222-00
INDINAVIR SULFATE	IF317-00
MAGNESIUM SULFATE	IF318-00
MORPHINE SULFATE	IF319-01
MORPHINE SULFATE TABLETS	EF223-00
MORPHINE SULFATE INJECTION SOLUTION	EF224-00
NEOMYCIN SULFATE	IF320-00
POTASSIUM SULFATE	IF321-00
PSEUDOEPHEDRINE SULFATE	IF322-01
SALBUTAMOL SULFATE	IF323-00
SALBUTAMOL SULFATE TABLETS	EF225-00

SALBUTAMOL SULFATE ORAL SOLUTION	EF226-00
SODIUM SULFATE	IF324-00
ZINC SULFATE	IF325-00
FERROUS SULFATE	IF326-00
FERROUS SULFATE TABLETS	EF227-00
FERROUS SULFATE HEPTAHYDRATE	IF316-00
FERROUS SULFATE ORAL SOLUTION	EF228-00
SELENIUM SULFIDE	IF327-00
SODIUM SULFITE	IF328-00
SULPIRIDE	IF329-01
ANTIMONY POTASSIUM TARTRATE	IF330-00
ANTIMONY SODIUM TARTRATE	IF331-00
METOPROLOL TARTRATE TABLETS	EF229-00
POTASSIUM SODIUM TARTRATE	IF332-00
THEOPHYLLINE	IF333-01
TERCONAZOLE	IF334-01
TERCONAZOLE CREAM	EF230-00
THIABENDAZOLE	IF335-00
THIABENDAZOLE TABLETS	EF231-00
THIABENDAZOLE POMADE	EF232-00
THIABENDAZOLE ORAL SUSPENSION	EF233-00
THIAMAZOLE	IF336-00
TOLMETIN SODIUM	IF337-00
TRETINOIN	IF338-00
TRETINOIN CREAM	EF234-00
TRETINOIN GEL	EF235-00
TRIMETHOPRIM	IF339-01
UREA	IF340-00
WARFARIN SODIUM	IF341-00
WARFARIN SODIUM TABLETS	EF236-00
AMARANTH RED	IF342-00
AMARANTH RED ALUMINUM LAKE	IF343-00
PONCEAU RED	IF344-00
PONCEAU RED ALUMINUM LAKE	IF345-00
ZIDOVUDINE	IF346-01
ZIDOVUDINE CAPSULES	EF237-00
ZIDOVUDINE INJECTION SOLUTION	EF238-00
ZIDOVUDINE ORAL SOLUTION	EF239-00
ZIDOVUDINE LAMIVUDINE TABLETS	EF240-00

CALCIUM ACETATE*Calcii acetat*C₄H₆CaO₄; 158.17

calcium acetate; 09629

Acetic acid calcium salt (1:2)

[62-54-4]

Contains no less than 99.0% and no more than 100.5% of C₄H₆CaO₄ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, odorless, hygroscopic powder.

Solubility. Freely soluble in water, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. Complies with calcium ion reactions **(5.3.1.1)**.

B. Complies with acetate ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 7.2 to 8.2. Determine in aqueous solution at 5% (w/v).

Barium. Proceed as described in *Atomic emission spectrometry (5.2.13.2)*. Use a spectrophotometer with a barium hollow cathode lamp and select the emission line at 455.4 nm.

Sample solution: weigh, accurately, about 5 g of the sample and transfer to a 100 mL volumetric flask. Complete the volume with water and homogenize.

Standard solution: prepare the reference solution using barium standard solution (0.1% Ba), diluted with water.

Procedure: calculate the content of barium in the sample from the readings obtained. No more than 0.005% (50 ppm).

Strontium. Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*, use *Method II*. Use a spectrophotometer with a flame fed with a mixture of air-acetylene, magnesium hollow cathode lamp, and select the emission line at 460.7 nm.

Sample solution: weigh, accurately, about 2 g of the sample and transfer to a 100 mL volumetric flask. Complete the volume with water and homogenize.

Procedure: calculate the content of strontium in the sample from the readings obtained. No more than 0.05% (500 ppm).

Fluoride. Determine the concentration of fluoride ion potentiometrically. Use a selective electrode for fluoride ion. Prepare and store all solutions in plastic containers.

Buffer solution: transfer to a 250 mL volumetric flask 73.5 g of sodium citrate dihydrate. Top off the volume with water. Homogenize.

Sample solution: transfer 2 g of the sample to a beaker, add 20 mL of water and 2 mL of hydrochloric acid, maintain under agitation until complete dissolution of the sample. Add 50 mL of the *Buffer solution* and top off with water to 100 mL.

Standard solution: dissolve in water an amount, accurately weighed, of sodium fluoride CRS to obtain a solution with 1.1052 mg/mL. Transfer 20 mL of this solution to a 100 mL volumetric flask with 50 mL of the *Buffer solution*. Complete volume with water and homogenize, obtaining a solution at 100 µg/mL of fluoride ion. From this solution, transfer to successive 100 mL volumetric flasks that have, each one, 50 mL of *Buffer solution* and 2 mL of hydrochloric acid, 100 µL, 200 µL, 300 µL and 500 µL of *Standard solution*. Complete the volume with water, transfer each solution to different beakers, and conduct the potentiometric readings to build the analytical curve.

Procedure: conduct the potentiometric readings and calculate the amount of fluoride from the readings obtained. No more than 0.005% (50 ppm).

Magnesium. Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*, use *Method II*. Use a spectrophotometer with a flame fed with a mixture of air-acetylene, magnesium hollow cathode lamp, and select the emission line at 285.2 nm.

Sample solution: prepare a solution with 200 mg of the sample in 100 mL of water.

Procedure: calculate the content of magnesium in the sample from the readings obtained. No more than 0.05% (500 ppm).

Nitrate. Dissolve 1 g of the sample in 10 mL of water and add 5 mg of sodium chloride, 0.05 mL of indigo carmine RS and, under agitation, 10 mL of nitrogen-free sulfuric acid. An intense blue color persists for, at least, 10 minutes.

Potassium. Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*, use *Method II*. Use a spectrophotometer with a flame fed with a mixture of air-acetylene, magnesium hollow cathode lamp, and select the emission line at 766.7 nm.

Sample solution: weigh, accurately, about 1.25 g of the sample and transfer to a 100 mL volumetric flask. Complete the volume with water and homogenize.

Procedure: calculate the content of potassium in the sample from the readings obtained. No more than 0.05% (500 ppm).

Sodium. Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*, use *Method II*. Use a spectrophotometer with a flame fed with a mixture of air-acetylene, magnesium hollow cathode lamp, and select the emission line at 589 nm.

Sample solution: weigh, accurately, about 1 g of the sample and transfer to a 100 mL volumetric flask. Complete the volume with water and homogenize.

Procedure: calculate the content of sodium in the sample from the readings obtained. No more than 0.05% (500 ppm).

Aluminum (5.3.2.10). Dissolve 4 g of the sample in 100 mL of water and proceed as described in *Limit test for aluminum*. No more than 0.0001% (1 ppm).

Arsenic (5.3.2.5). Use *Method I*. Weigh 1.5 g of the sample and proceed according to *Limit test for arsenic*. No more than 0.0002% (2 ppm).

Lead (5.3.2.12). No more than 0.001% (10 ppm).

Chlorides (5.3.2.1). Dissolve 1 g of the sample in 40 mL of water and proceed as described in *Limit test for chlorides*. No more than 0.0354% (354 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve 1 g of the sample in 10 mL of water, add 6 mL of 3 M hydrochloric acid and complete volume to 25 mL using water. Proceed as described in *Limit test for heavy metals*. No more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Dissolve 2 g of the sample in 40 mL of water and proceed as described in *Limit test for sulfates*. No more than 0.06% (600 ppm).

Water (5.2.20.1). No more than 7.0%

Easily oxidizable substances. Dissolve 2 g of the sample in 10 mL of water in ebullition. Add a few glass beads, 6 mL of 5 M sulfuric acid and 0.3 mL of potassium permanganate RS. Mix, heat the ebullition for five minutes. Allow to stand until all the precipitate has decanted. A pink color remains in the supernatant solution.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 300 mg of the sample and dissolve in 150 mL of water, with 2 mL of 3 M hydrochloric acid. Maintain under agitation and add approximately 30 mL of 0.05 M disodium edetate VS from a 50 mL burette. Add 15 mL of M sodium hydroxide and 300 mg of hydroxynaphthol blue. Continue the titration until the endpoint, of blue color. Each mL of 0.05 M disodium edetate VS is equivalent to 7.909 mg of C₄H₆CaO₄.

PACKAGING AND STORAGE

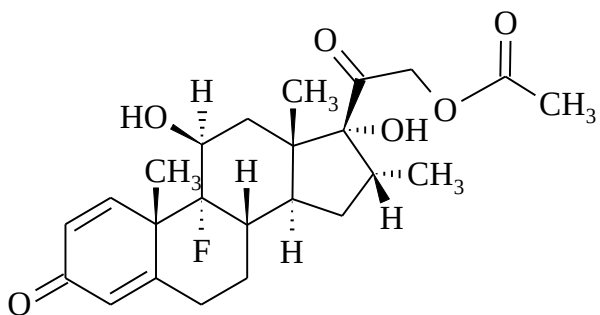
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Pharmaceutical adjuvant used in solutions for dialysis.

DEXAMETHASONE ACETATE*Dexamethasoni acetas* $C_{24}H_{31}FO_6$; 434.50

dexamethasone acetate; 02819

(11 β , 16 α)-9-fluoro-11,17-dihydroxy-16-methyl-3,20-dioxopregna-1,4-diene-21-yl acetate
[1177-87-3]

Contains no less than 97.0% and no more than 102.0% of $C_{24}H_{31}FO_6$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Practically insoluble in water, freely soluble in dioxane, ethyl alcohol and in methyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +82 to +88, in relation to the desiccated substance. Determine in solution at 1% (w/v) in dioxane.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of dexamethasone acetate CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range from 200 nm to 400 nm, of sample solution at 15 $\mu\text{g/mL}$ in methyl alcohol, there are maximum and minimum absorption points only on the same wavelengths as the ones observed in the spectrum of the similar dexamethasone acetate CRS solution. The absorptivities, calculated in the maximum absorbance wavelength of approximately 239 nm, do not differ more than 3% for the sample and standard solutions.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph with an ultraviolet detector at 254 nm; column with 250 mm of length and

4.6 mm of internal diameter, packed with silica chemically bonded to a phenyl group (4 μm); *Mobile phase* flow of 1.0 mL/minute.

Formate buffer pH 3.6: transfer 1.32 g of ammonium formate to a 1000 mL volumetric flask, add 900 mL of water and homogenize. Adjust pH to 3.6 with formic acid and complete the volume with water the same solvent.

Mobile phase: mixture of *Formate buffer pH 3.6* and acetonitrile (3:2). Make adjustments if necessary.

Test solution: transfer, quantitatively, approximately 200 mg of dexamethasone acetate to a 100 mL volumetric flask, dissolve in acetonitrile, complete the volume with the same solvent and homogenize. Transfer 40 mL of the solution to a 100 mL volumetric flask, dilute with *Formate buffer pH 3.6* to 100 mL and homogenize.

Procedure: inject 10 μL of the *Test solution*, record the chromatograms and measure the areas under the peaks obtained. The column efficiency must be no less than 5400 theoretical plates. The sum of the areas under the secondary peaks, except the main peak area, is of no more than 2.0% of the total area under the peaks obtained, including the main peak area. No individual impurity obtained with the *Test solution* can be superior to 1.0%, compared to the total area under the peaks obtained.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven, under reduced pressure, at 105 °C, for three hours. No more than 0.4%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph with an ultraviolet detector at 254 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (10 μm); *Mobile phase* flow of 2.0 mL/minute.

Buffer solution pH 6.0: transfer 3 mL of *M* sodium hydroxide solution, 138 mL of 0.5 *M* potassium chloride and 50 mL of 0.5 *M* monobasic potassium phosphate to a 1000 mL volumetric flask. Dilute with water to 1000 mL and homogenize.

Mobile phase: mixture of water and acetonitrile (55:45). Make adjustments if necessary.

Diluent: mixture of acetonitrile and *Buffer solution pH 6.0* (1:1).

Sample solution: weigh, accurately, about 25 mg of the sample and transfer to a 250 mL volumetric flask. Add 100 mL of *Diluent* and leave in ultrasonic bath until obtaining a clear solution. Complete volume with *Diluent* and homogenize.

Standard solution: weigh, accurately, about 25 mg of dexamethasone acetate CRS, transfer to a 250 mL volumetric flask and dissolve with the *Diluent*. Complete volume with *Diluent* and homogenize.

Inject 20 µL replicates of the *Standard solution*. The column efficiency must be no less than 1500 theoretical plates. The retention factor must be lower than 2.0. The tailing factor is no more than 2.0. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{24}H_{31}FO_6$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light. Store between 15 °C and 30 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-inflammatory.

DEXAMETHASONE ACETATE CREAM

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{24}H_{31}FO_6$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay* method, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 240 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 μ m), maintained at temperature of 40 °C; *Mobile phase* flow of 1.2 mL/minute.

Mobile phase: mixture of methyl alcohol and water (65:35).

Sample solution: transfer an amount of sample, accurately weighed, equivalent to 2 mg of dexamethasone acetate. Add 40 mL of methyl alcohol and leave in ultrasonic bath, stirring with a glass rod until it dissolves. Transfer quantitatively to a 100 mL volumetric flask, complete the volume with the same solvent and homogenize.

Standard solution: weigh, accurately, about 20 mg of dexamethasone acetate CRS and transfer to a 100 mL volumetric flask. Add 50 mL of methyl alcohol and leave in ultrasonic bath to dissolve. Complete the volume with methyl alcohol and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Inject 20 μ L replicates of the *Standard solution*. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2%.

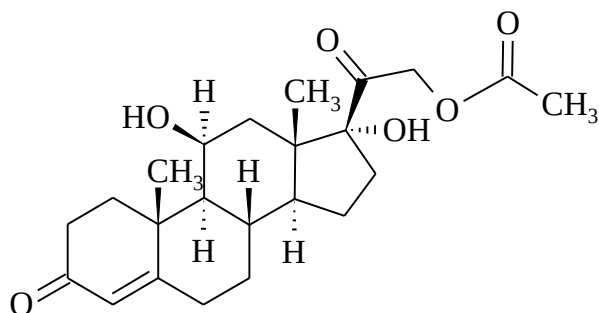
Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{24}H_{31}FO_6$ in the cream, from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers and protected from excessive heat.

LABELING

Comply with current legislation.

HYDROCORTISONE ACETATE*Hydrocortisoni acetat* $C_{23}H_{32}O_6$; 404.50

hydrocortisone acetate; 04666

11 β ,17-dihydroxy-3,20-dioxopregn-4-ene-21-yl acetate

[50-03-3]

Contains no less than 97.0% and no more than 103.0% of $C_{23}H_{32}O_6$, in relation to the desiccated substance.

DESCRIPTION

Physical-chemical characteristics. White crystalline powder. *Melting point (5.2.2):* around 220 °C, with decomposition.

Solubility. Practically insoluble in water, slightly soluble in absolute ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +158 to +167, in relation to the desiccated substance. Determine in solution at 10 mg/mL in dioxane.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample dispersed in potassium bromide there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of hydrocortisone acetate CRS, prepared identically.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of methylene chloride, ether, methyl alcohol and water (73:15:10:2) as mobile phase. Apply to the plate, separately, 5 μ L of each of the freshly prepared solutions described below.

Test solution: dissolve 10 mg of the sample in methyl alcohol:methylene chloride (1:9), complete the volume to 10 mL with the same solvent and homogenize.

Solution (1): dissolve 20 mg of hydrocortisone acetate CRS in methyl alcohol:methylene chloride (1:9), complete the volume to 20 mL with the same solvent and homogenize.

Solution (2): dissolve 10 mg of hydrocortisone acetate CRS in *Solution (1)*, complete the volume to 10 mL with the same solvent and homogenize.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with the *Test solution* corresponds in position, color and intensity to the one obtained with *Solution (1)*. Nebulize the plate with alcoholic solution of sulfuric acid RS. Heat at 120 °C for 10 minutes or until stains appear and allow to cool. The main stain obtained with the *Test solution* corresponds in position, color and dimensions to the main stain obtained with *Solution (1)* when examined at daylight and under ultraviolet light (365 nm). The *Solution (2)* chromatogram demonstrates two clearly separated stains.

C. Add approximately 2 mg of sample in 2 mL of sulfuric acid and shake until full dissolution. Wait five minutes. An intense red brown color will develop, with green fluorescence, which is especially intense when viewed at ultraviolet light (365 nm). Add to this solution 10 mL of water and homogenize. The color fades and the fluorescence under ultraviolet light remains.

D. Complies with acetyl group reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of acetonitrile and water (40:60).

Sample solution: weigh, accurately, about 25 mg of the sample and transfer to a 10 mL volumetric flask. Dissolve, complete the volume with methyl alcohol and homogenize.

Standard solution: weigh, accurately, about 2 mg of hydrocortisone acetate CRS and 2 mg of cortisone acetate. Transfer to a 100 mL volumetric flask, dissolve, complete the volume with *Mobile phase* and homogenize. Pipette 1 mL of this solution, transfer to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Inject 20 µL of the *Standard solution*. The resolution between the peaks of hydrocortisone acetate and of cortisone acetate is no less than 4.2.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of each impurity in the sample. For any individual impurity, no more than 1.0% of the area under the main peak obtained with the *Standard solution* and a peak with an area not bigger than 0.5% of the area under the main peak obtained in the *Standard solution*. For the total of impurities found, no more than 1.5% of the area under the main peak obtained with the *Standard solution*. Ignore peaks with area smaller than 0.05% of the area under the main peak obtained in the *Standard solution*.

Loss on drying (5.2.9.1). Determine in 0.5 g of sample. Dry in oven at 105 °C for three hours. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 0.1 g of the sample and dissolve in ethyl alcohol. Complete the volume to 100 mL with the same solvent and homogenize. Dilute, successively, in ethyl alcohol until concentration of 0.002% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 241 nm, using ethyl alcohol for zero adjustment. Calculate the content of $C_{23}H_{32}O_6$ in the sample from the readings obtained.

PACKAGING AND STORAGE

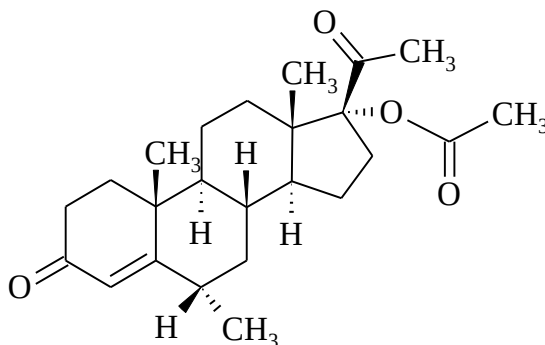
In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Corticosteroid.

MEDROXYPROGESTERONE ACETATE*Medroxyprogesteroni acetat* $C_{24}H_{34}O_4$; 386.53

medroxyprogesterone acetate; 05563

6-methyl-3,20-dioxopregn-4-ene-17-yl acetate

[71-58-9]

Contains no less than 97.0% and no more than 103.0% of $C_{24}H_{34}O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to nearly white crystalline powder.

Solubility. Practically insoluble in water, soluble in dioxane and moderately soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +45 to +51. Determine in solution at 1% (w/v) in dioxane.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of medroxyprogesterone acetate CRS, prepared identically. It is not necessary to Dry the sample.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of the 0.001% (w/v) sample solution in ethyl alcohol, there is a maximum point at 241 nm, identical to the one observed in the spectrum of a similar medroxyprogesterone acetate CRS solution.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of acetonitrile and water (3:2). Make adjustments if necessary.

Sample solution: weigh, accurately, about 62.5 g of medroxyprogesterone acetate and transfer to a 25 mL volumetric flask. Dissolve, complete the volume with the *Mobile phase* and homogenize.

Standard solution: dissolve an amount, accurately weighed, of medroxyprogesterone acetate CRS in the *Mobile phase* and dilute adequately, to obtain a solution at 50 µg/mL.

Resolution solution: dissolve adequately an amount of megestrol acetate and medroxyprogesterone acetate CRS in *Mobile phase* to obtain a solution at 40 µg/mL for both.

Inject 20 µL replicates of the *Resolution solution*. The resolution between the peaks of megestrol acetate and of medroxyprogesterone acetate is no less than 1.5. Inject replicates of the *Standard solution*. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 3.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of each impurity present in the sample. No more than 1.0% of any individual impurity. No more than 1.5% of the total impurities found. Ignore peaks with area smaller than 0.05% of the main peak obtained with the *Standard solution*.

Limit of related medroxyprogesterone acetate compound A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G, as support, and a mixture of hexane, *tert*-butyl methyl ether, tetrahydrofuran (45:45:10) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.2 g of the sample in methylene chloride, complete the volume to 10 mL with the same solvent and homogenize.

Solution (2): dissolve 0.2 g of medroxyprogesterone acetate CRS and 1 mg of related medroxyprogesterone acetate CRS compound A in methylene chloride and complete the volume to 10 mL with the same solvent.

Develop the chromatogram. Remove the plate, allow it to air dry and develop the chromatogram again. Remove the plate, let it dry in oven at 120 °C for 10 minutes. Nebulize the plate with an alcoholic *p*-toluene sulfonic acid solution 0.02 g/mL. Heat the plate at 120 °C for 10 minutes. Examine under ultraviolet light (365 nm). Any blue fluorescent stain with *R_f* value higher than the main medroxyprogesterone acetate stain obtained from *Solution (1)* has maximum intensity equal to the stain obtained with the corresponding *R_f* value obtained on *Solution (2)* (0.5%).

Loss on drying (5.2.9.1). Determine in 1.0 g of sample. Dry in oven at 105 °C for three hours. No more than 1.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group, kept at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of acetonitrile and water (60:40).

Sample solution: weigh, accurately, about 25 mg of the sample and transfer to a 50 mL volumetric flask. Add 30 mL of *Mobile phase* and leave in ultrasonic bath for 20 minutes. Complete the volume with the same diluent and homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: dissolve an amount, accurately weighed, of medroxyprogesterone acetate CRS in the *Mobile phase* and dilute adequately to obtain a solution at 100 µg/mL.

Inject 20 µL replicates of the *Standard solution*. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%. The tailing factor is no more than 2.0.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₄H₃₄O₄ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

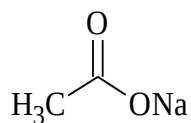
In hermetically closed, opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Progestogen.

SODIUM ACETATE***Natrii acetat*** $C_2H_3NaO_2$; 82.03

sodium acetate; 00087

Acetic acid sodium salt (1:1)

[127-09-3]

 $C_2H_3NaO_2 \cdot 3H_2O$; 136.08

sodium acetate trihydrate; 00088

Acetic acid sodium salt hydrate (1:1:3)

[6131-90-4]

Contains no less than 99.0% and no more than 101.0% of $C_2H_3NaO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Colorless, clear crystals, or white, granular crystalline powder, or white flakes. Effloresces to hot dry air.

Solubility. Very soluble in water, soluble in ethyl alcohol.

IDENTIFICATION

A. Complies with acetate ion reactions (5.3.1.1).

B. Complies with sodium ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. The 10% (w/v) aqueous preparation is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). Prepare a solution that has 5% (w/v) of $C_2H_3NaO_2$ and proceed as described in *Determination of pH*. Between 7.5 and 9.2.

Insoluble matter. Dissolve the equivalent to 20 g of sodium acetate anhydrous in 150 mL of water. Prepare this solution in a beaker and heat until ebullition. Cover the beaker with a watch glass and leave it in water bath for one hour. Filter in a previously weighed filter, wash and dry at 105 °C until constant weight. No more than 0.05% (500 ppm).

Calcium and magnesium. Weigh the equivalent to 0.2 g of sodium acetate anhydrous and dissolve in 20 mL of water. Add 2 mL of each of the following reagents: 6 M ammonium hydroxide,

ammonium oxalate RS and 12% (w/v) sodium phosphate dibasic. No turbidity is developed for five minutes.

Potassium. Weigh the equivalent to 3 g of sodium acetate anhydrous and dissolve in 5 mL of water. Acidify the solution with a few drops of *M* acetic acid and add five drops of sodium cobaltinitrite RS. No precipitate is formed.

Arsenic (5.3.2.5). Dissolve the equivalent to 1 g of sodium acetate anhydrous in 35 mL of water and proceed according to *Limit test for arsenic*. No more than 0.0003% (3 ppm).

Chlorides (5.3.2.1). The equivalent to 1 g of sodium acetate anhydrous does not present more chlorides than the equivalent to 1.0 mL of 0.01 *M* hydrochloric acid VS. No more than 0.035% (350 ppm).

Iron (5.3.2.4). Proceed as described on *Method I* using 20 mL of the preparation obtained in *Aspect of the preparation*. Use 0.2 mL of *Standard iron solution (100 ppm)*. No more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve the equivalent to 2 g of sodium acetate anhydrous in a 25 mL volumetric flask using water as solvent and proceed as described in *Limit test for heavy metals*, using 2 mL of *Standard lead solution (10 ppm Pb)*. No more than 0.001% (10 ppm).

Sulfates (5.3.2.2). The equivalent to 10 g of sodium acetate anhydrous does not present more sulfates than the equivalent to 1 mL of standard sulfuric acid (H_2SO_4 0.005 *M* VS). No more than 0.005% (50 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C, until constant weight. The hydrate form loses 38% to 41% of its weight; the anhydrous form loses no more than 1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Weigh an amount equivalent to 0.2 g of sodium acetate previously desiccated, dissolve in 25 mL of glacial acetic acid and heat if necessary for full dissolution. Add two drops of 1-naphtholbenzein TS. Titrate with 0.1 *M* perchloric acid VS. Carry out the blank test and make the necessary corrections. Each mL of 0.1 *M* perchloric acid VS is equivalent to 8.203 mg of $\text{C}_2\text{H}_3\text{NaO}_2$.

PACKAGING AND STORAGE

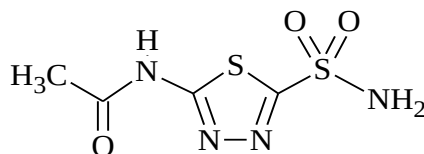
In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Pharmaceutical adjuvant used in solutions for dialysis.

ACETAZOLAMIDE***Acetazolamidum***

$C_4H_6N_4O_3S_2$; 222.25

acetazolamide; 00063

N-[5-(Aminosulfonyl)-1,3,4-thiadiazol-2-yl] acetamide

[59-66-5]

Contains no less than 98.5% and no more than 101.0% of $C_4H_6N_4O_3S_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder. Presents polymorphism.

Solubility. Very slightly soluble in water, slightly soluble in ethyl alcohol. Soluble in diluted alkali hydroxide solutions.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of acetazolamide acetate CRS, prepared identically. If the spectrum of the sample is not identical to that of the standard, dissolve the sample and the standard separately in ethyl alcohol, evaporate to dryness and repeat the test with the residues.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 230 nm to 260 nm, of 0.003% (w/v) solution in 0.01 *M* sodium hydroxide, there is a maximum point at 240 nm and the absorbance is of 0.49 to 0.52. In the ultraviolet absorption spectrum, in the range of 260 nm to 350 nm, of 0.00075% (w/v) solution in 0.01 *M* sodium hydroxide, there is a maximum point at 292 nm and the absorbance is of 0.43 to 0.46.

PURITY TESTS

Aspect of the preparation. Dissolve 1 g of the sample in 10 mL of *M* sodium hydroxide. The preparation obtained is not more opalescent than the *Reference suspension II* (**5.2.25**) and is not more intensely stained than the *Color reference solution* (**5.2.12**), prepared as described next.

Color reference solution: mix 4.8 mL of *Ferric chloride base solution*, 1.2 mL of *Cobalt II chloride base solution* and 14 mL of 1% (v/v) hydrochloric acid. Dilute 12.5 mL of this solution with 87.5 mL of 1% (v/v) hydrochloric acid.

Related substances. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ as support, and a mixture of ammonia, ethyl acetate and isopropyl alcohol (20:30:50) as

mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): 5 mg/mL sample solution in a mixture of ethyl alcohol and ethyl acetate (1:1).

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL, with a mixture of ethyl alcohol and ethyl acetate (1:1).

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). Any secondary stain from the chromatogram obtained with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (1.0%).

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Dissolve 0.96 g of the sample in 20 mL of water, heat to ebullition until full dissolution. Cool down by shaking and filter. Proceed as described in *Limit test for sulfates*, using 1 mL of standard sulfuric acid (H_2SO_4 0.005 M VS). No more than 0.05% (500 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample, in oven, between 100 °C and 105 °C. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.2 g of the sample in 25 mL of dimethylformamide. Titrate with 0.1 M ethanolic sodium hydroxide VS, determining the endpoint potentiometrically. Each mL of 0.1 M ethanolic sodium hydroxide VS is equivalent to 22.225 mg of $\text{C}_4\text{H}_6\text{N}_4\text{O}_3\text{S}_2$.

PACKAGING AND STORAGE

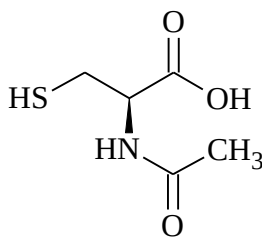
In hermetic containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Diuretic.

ACETYLCYSTEINE
Acetylcysteinum

C₅H₉NO₃S; 163.19
acetylcysteine; 00067
N-Acetyl-L-cysteine
[616-91-1]

Contains no less than 98.0% and no more than 102.0% of C₅H₉NO₃S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly colorless crystalline powder.

Solubility. Freely soluble in water and in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 104 °C to 110 °C.

Specific optical rotation (5.2.8): +21 to +27, in relation to the desiccated substance. Add to a 25 mL volumetric flask 1.25 g of the sample, 1 mL of 1% (w/v) disodium edetate, 3.75 mL of sodium hydroxide RS and homogenize. Complete the volume with phosphate buffer pH 7.0.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of acetylcysteine CRS, prepared identically.

B. Dissolve approximately 1 g of the sample in 20 mL of water and add 0.05 mL of 5% (w/v) sodium nitroprusside and 0.05 mL of ammonium hydroxide. A dark violet color develops.

PURITY TESTS

Aspect of the preparation. The 5% (w/v) sample preparation is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). 2.0 to 2.8. Determine in 1% (w/v) solution in carbon dioxide-free water.

Heavy metals (5.3.2.3). Humidify 2 g of the sample, carefully, drop by drop, with 2 mL of nitric acid and proceed as described on *Method III*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample, in an oven at 70 °C, under reduced pressure, until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 2 g of the sample. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh, accurately, about 0.14 g of the sample, dilute in 60 mL of water and add 10 mL of 2 *M* hydrochloric acid. Cool down in an ice bath, add 10 mL of potassium iodide RS and titrate with 0.05 *M* iodine VS, determining the endpoint potentiometrically or using 1 mL of starch TS as indicator. Each mL of 0.05 *M* iodine VS is equivalent to 16.319 mg of C₅H₉NO₃S.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph with an ultraviolet detector at 214 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: dissolve 6.8 g of monobasic sodium phosphate in 1000 mL of water. Filter and adjust pH to 3.0 with phosphoric acid.

Internal standard solution: transfer approximately 1 g of DL-phenylalanine to a 200 mL volumetric flask and complete the volume with freshly prepared 0.05% (w/v) sodium metabisulfite. Homogenize.

Sample solution: weigh, accurately, about 1 g of the sample and transfer to a 100 mL volumetric flask. Complete the volume with 0.05% (w/v) sodium metabisulfite and homogenize. Transfer 5 mL of this solution and 5 mL of the *Internal standard solution* to a 100 mL volumetric flask and complete the volume with 0.05% (w/v) sodium metabisulfite.

Standard solution: transfer, quantitatively, about 0.1 g of acetylcysteine CRS to a 10 mL volumetric flask and complete the volume with 0.05% (w/v) sodium metabisulfite. Transfer 5 mL of this solution and 5 mL of the *Internal standard solution* to a 100 mL volumetric flask and complete the volume with 0.05% (w/v) sodium metabisulfite, obtaining a solution at 0.5 mg/mL of acetylcysteine CRS and 0.25 mg/mL of DL-phenylalanine.

Inject 5 µL replicates of the *Standard solution*. The resolution between the peaks corresponding to acetylcysteine and to DL-phenylalanine is no less than 6. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 5 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to acetylcysteine and to DL-phenylalanine. Calculate the content of C₅H₉NO₃S in the sample from the responses obtained for the acetylcysteine/DL-phenylalanine ratio, with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

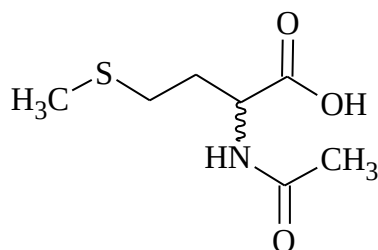
LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Mucolytic.

N-ACETYL-L-METHIONINE
Acetylmethioninum



$C_7H_{13}NO_3S$; 191.25

n-acetyl-l-methionine; 11029

N-Acetyl-DL-methionine

[1115-47-5]

Contains no less than 98.0% of $C_7H_{13}NO_3S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder.

Solubility. Soluble in water and in ethyl alcohol in ebullition.

Physicochemical constants.

Melting range (5.2.2): 114 °C to 116 °C.

Specific optical rotation (5.2.8): -0.05 to +0.05. Dissolve 1.00 g of the sample in 25% (w/v) hydrochloric acid and complete the volume to 50 mL.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of n-acetyl-dl-methionine CRS, prepared identically.

B. Dissolve 10 mg of the sample in 1 mL of distilled water and add, successively, under agitation, 1 mL of 5 M sodium hydroxide, 1 mL of glycerol and 0.3 mL of 5% (w/v) sodium nitroprusside. Heat between 35 °C and 40 °C, for 10 minutes, and cool down in an ice bath for two minutes. Add 1.5 mL of hydrochloric acid RS and shake. A purple red color develops.

PURITY TESTS

Aspect of the preparation. Dissolve 0.2 g of the sample in 2 mL of distilled water. The preparation obtained is clear (5.2.25). Add 38 mL of distilled water and reserve this solution for other tests.

Iron (5.3.2.4). Use *Method I*. Determine in 10 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.005% (50 ppm).

Chlorides (5.3.2.1). Determine in 10 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.015% (150 ppm).

Heavy metals (5.3.2.3). Determine in 10 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C, for four hours. No more than 2.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.3 g of the sample and transfer to an Erlenmeyer flask with cap. Add 100 mL of water, 5 g of dibasic potassium phosphate, 2 g of monobasic potassium phosphate, and 2 g of potassium iodide. Shake until complete dissolution. Add 50 mL of 0.05 M iodine VS, shake and allow to stand for 30 minutes. Titrate the excess iodine with 0.1 M sodium thiosulfate VS, add 3 mL of starch TS near the endpoint, and continue with the titration until the blue color disappears. Carry out the blank test and make the necessary corrections. Each mL of 0.05 M iodine VS is equivalent to 9.562 mg of $C_7H_{13}NO_3S$.

PACKAGING AND STORAGE

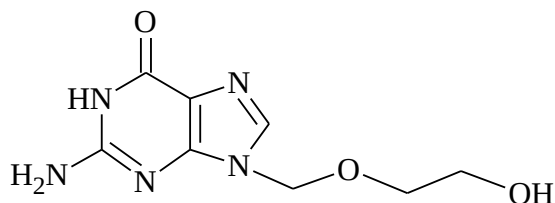
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Lipotropic.

ACYCLOVIR***Aciclovirum***

$C_8H_{11}N_5O_3$; 225.21

acyclovir; 00082

2-Amino-1,9-di-hydro-9-[(2-hydroxyethoxy)methyl]-6*H*-purin-6-one
[59277-89-3]

Contains no less than 98.0% and no more than 101.0% of $C_8H_{11}N_5O_3$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water, freely soluble in dimethyl sulfoxide and very slightly soluble in ethyl alcohol. Soluble in diluted solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are conducted.

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of acyclovir CRS, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 350 nm, of 0.0015% (w/v) solution in 0.1 *M* hydrochloric acid, there is maximum absorption at 255 nm and a sloped shoulder around 274 nm, identical to the ones observed in the similar acyclovir CRS solution spectrum.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ as support, and a mixture of chloroform, methyl alcohol and ammonium hydroxide (80:20:2) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): transfer 0.1 g of the sample to a 10 mL volumetric flask, dissolve in dimethyl sulfoxide and complete the volume with the same solvent, to obtain a 10 mg/mL solution.

Solution (2): 0.2 mg/mL acyclovir CRS solution in dimethyl sulfoxide.

Solution (3): 0.1 mg/mL acyclovir CRS solution in dimethyl sulfoxide.

Solution (4): 0.05 mg/mL acyclovir CRS solution in dimethyl sulfoxide.

Solution (5): 0.01 mg/mL acyclovir CRS solution in dimethyl sulfoxide.

Procedure: develop the chromatogram. Remove the plate, dry the stains with dry air stream. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than the ones obtained with *Solution (2)*, *Solution (3)*, *Solution (4)* and *Solution (5)*. The sum of the impurities observed must be no more than 2.0%.

Limit of guanine. Proceed as described on method **B**. from *Assay*. Calculate the content of guanine in the sample, from the responses obtained with the peak related to guanine in the *Standard solution* and *Sample solution*. No more than 0.7%.

Water (5.2.20.1). No more than 6.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 0.15 g of the sample in 60 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 22.52 mg of $C_8H_{11}N_5O_3$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m to 10 μ m); *Mobile phase* flow of 3.0 mL/minute.

Mobile phase: mixture of water and glacial acetic acid (100:0.1).

Guanine solution: weigh, accurately, about 8.75 mg of guanine, transfer to a 500 mL volumetric flask and dissolve in 50 mL of 0.1 M sodium hydroxide. Complete the volume with water and homogenize.

Sample solution: weigh, accurately, about 0.1 g of the sample and transfer to a 200 mL volumetric flask using 20 mL of 0.1 M sodium hydroxide. Add 80 mL of water, leave in ultrasonic bath for 15

minutes and shake mechanically for 15 minutes. Complete the volume with water and homogenize. Transfer 10 mL to a 50 mL volumetric flask, complete the volume with 0.01 M sodium hydroxide and homogenize.

Standard solution: transfer, quantitatively, approximately 25 mg of acyclovir CRS to a 50 mL volumetric flask, dissolve in 5 mL of 0.1 M sodium hydroxide, complete the volume with water and homogenize. Transfer 10 mL of this solution to a 50 mL volumetric flask, add 2 mL of *Guanine solution*, complete the volume with 0.01 M sodium hydroxide and homogenize, to obtain a concentration of 0.1 mg/mL of acyclovir CRS and 0.7 µg/mL of guanine.

Inject 20 µL replicates of the *Standard solution*. The relative retention times are approximately 0.2 for guanine and 1 for acyclovir. The tailing factor for the peaks analyzed is no more than 2.0. The resolution between acyclovir and guanine is no less than 2.0. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₈H₁₁N₅O₃ in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In hermetic containers, at temperature below 25 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiviral.

ACYCLOVIR TABLETS

Contains no less than 95.0% and no more than 105.0% the declared amount of $C_8H_{11}N_5O_3$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), at the range of 230 nm to 350 nm, of the sample solution obtained in *Assay*, there is maximum absorption at 255 nm and a sloped shoulder around 274 nm.

B. Proceed as described in *Limit of guanine*. The main stain obtained with the *Solution (2)* corresponds in position, color and intensity to the stain obtained with *Solution (3)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddle, 50 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 255 nm (**5.2.14**) using the same solvent for zero adjustment. Calculate the amount of $C_8H_{11}N_5O_3$ dissolved in the medium, comparing the readings obtained with the acyclovir CRS solution at the concentration 0.001% (w/v). Alternatively, make calculations considering $A(1\%, 1\text{ cm}) = 560$, at 255 nm, in 0.1 M hydrochloric acid.

Tolerance: no less than 80% (Q) of the declared quantity of $C_8H_{11}N_5O_3$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of 13.5 M ammonium hydroxide, methyl alcohol and methylene chloride (2:20:80) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Shake, for 15 minutes, an amount of powder equivalent to 0.25 g of acyclovir with 10 mL of dimethyl sulfoxide. Filter.

Solution (2): dilute 0.7 volumes of *Solution (1)* to 100 volumes with dimethyl sulfoxide.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.7%).

Limit of guanine. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using cellulose F₂₅₄ as support, and a mixture of *n*-propyl alcohol, 13.5 M ammonium hydroxide and 5% (w/v) ammonium sulfate (10:30:60) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Transfer an amount of powder equivalent to 0.25 g of acyclovir to a 50 mL volumetric flask. Add 25 mL of 0.1 M sodium hydroxide, shake for 10 minutes and complete the volume with 0.1 M sodium hydroxide. Let the undissolved material decant before the application on the plate.

Solution (2): transfer 1 mL of *Solution (1)* to a 10 mL volumetric flask and complete the volume with 0.1 M sodium hydroxide.

Solution (3): dissolve 5 mg of acyclovir CRS in 10 mL of 0.1 M sodium hydroxide.

Solution (4): dissolve 5 mg of guanine in 100 mL of 0.1 M sodium hydroxide.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). Any secondary stain, corresponding to guanine, obtained in the chromatogram with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (4)* (1.0%). Discard the stains present in the solvent application point.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 0.1 g of acyclovir to a 100 mL volumetric flask, add 60 mL of 0.1 M sodium hydroxide, leave in an ultrasonic bath for 15 minutes or more, if necessary, and complete the volume with 0.1 M sodium hydroxide. Homogenize and filter. Transfer 15 mL of filtrate to a 100 mL volumetric flask, add 50 mL of water, 5.8 mL of 2 M hydrochloric acid and complete the volume with water. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with 0.1 M hydrochloric acid and homogenize, obtaining a solution at 0.0015% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 255 nm (**5.2.14**), using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of C₈H₁₁N₅O₃ in the tablets from the readings obtained. Alternatively, make calculations considering A (1%, 1 cm) = 560, at 255 nm, in 0.1 M hydrochloric acid.

PACKAGING AND STORAGE

In hermetic containers, at temperature below 25 °C.

LABELING

Comply with current legislation.

ACYCLOVIR CREAM

Contains not less than 90.0% and not more than 110.0% the declared amount of $C_8H_{11}N_5O_3$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 230 nm to 350 nm, of the sample solution obtained in *Assay*, there is maximum absorption at 255 nm and a sloped shoulder around 274 nm, identical to the ones observed in the similar acyclovir CRS solution spectrum.

B. The main stain of the chromatogram from *Solution (2)*, obtained in *Limit of guanine*, corresponds in position and intensity to the one obtained with *Solution (3)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

PURITY TESTS

Limit of guanine. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using cellulose F₂₅₄ as support. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): weigh an amount of cream equivalent to 30 mg of acyclovir, transfer to a graded centrifuge tube, add 3 mL of 0.1 M sodium hydroxide and shake to obtain the cream dispersion. Add 5 mL of a mixture of chloroform and *n*-propyl alcohol (1:2), shake, centrifuge and dilute the upper aqueous layer to 5 mL with 0.1 M sodium hydroxide. Homogenize.

Solution (2): transfer 1 mL of *Solution (1)* to a 10 mL volumetric flask and complete the volume with 0.1 M sodium hydroxide.

Solution (3): dissolve 6 mg of acyclovir CRS in 10 mL of 0.1 M sodium hydroxide.

Solution (4): dissolve 6 mg of guanine in 100 mL of 0.1 M sodium hydroxide.

Develop the chromatogram, initially using ethyl acetate as mobile phase, and let it run along the plate. Remove the plate and allow to air dry. Develop the chromatogram again using, as mobile phase, a mixture of *n*-propyl alcohol, 13.5 M ammonium hydroxide and 5% (w/v) ammonium sulfate (10:30:60). Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain, corresponding to guanine, obtained in the chromatogram with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (4)* (1%). Discard the stains present in the solvent application point.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

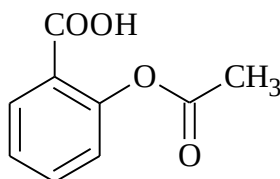
Transfer an amount of sample equivalent to 7.5 mg of acyclovir to a separator funnel using 50 mL of 0.5 M sulfuric acid and shake. Add 50 mL of ethyl acetate, shake, wait until the separation of phases and collect the lower aqueous phase. Wash the organic phase with 20 mL of 0.5 M sulfuric acid, collect the aqueous phase and join to the previous combination. Transfer the aqueous combinations to a 100 mL volumetric flask and complete the volume with 0.5 M sulfuric acid. Homogenize and filter, discarding the first milliliters of filtrate. Transfer 10 mL of the filtrate to a 50 mL volumetric flask and complete the volume with water. Prepare an acyclovir CRS solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 255 nm **(5.2.14)**, using 0.1 M sulfuric acid for zero adjustment. Calculate the amount of $C_8H_{11}N_5O_3$ in the cream, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, in a dry place and at temperature between 15 °C and 25 °C.

LABELING

Comply with current legislation.

ACETYLSALICYLIC ACID***Acidum acetylsalicylicum***

$C_9H_8O_4$; 180.16

acetylsalicylic acid; 00089

2-(acetyloxy)benzoic acid

[50-78-2]

Contains no less than 99.5% and no more than 101.0% of $C_9H_8O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical-chemical characteristics. White crystalline powder or colorless crystals. Melting point (5.2.2): around 143 °C.

| **Solubility.** Slightly soluble in water, easily soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of acetylsalicylic acid CRS, prepared identically.

B. Mix a small amount of sample with water, heat for a couple of minutes. Cool. Add one or two drops of ferric chloride RS. A violet red color develops.

PURITY TESTS

Aspect of the preparation. Dissolve 1 g of the sample in 9 mL of ethyl alcohol. The preparation is clear (5.2.25) and colorless (5.2.12).

Related substances. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 237 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow of 1.0 mL/minute. Prepare the solutions described below immediately before use.

Mobile phase: mixture of phosphoric acid, acetonitrile and water (2:400:600).

Sample solution: dissolve 0.10 g of the sample in acetonitrile and complete the volume to 10 mL with the same solvent.

Standard solution 1: dissolve 50.0 mg of salicylic acid to a volume of 50 mL with *Mobile phase*. Dilute 1 mL of this solution with *Mobile phase* to 100 mL.

Standard solution 2: dissolve 10.0 mg of salicylic acid to a volume of 10 mL with *Mobile phase*. Dilute 1 mL of this solution with 0.2 mL of the *Sample solution* to 100 mL with *Mobile phase*.

Inject 10 µL replicates of the *Standard solution 2*. The resolution between acetylsalicylic acid and salicylic acid is no less than 6.0. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution 1* and the *Sample solution*, record the chromatograms for seven times the salicylic acid retention time, and measure the areas under the peaks. Disregard peaks with areas below 0.25 times the area under the main peak obtained with the *Standard solution 1*.

Any secondary peak obtained with the *Sample solution* must be smaller than the main peak obtained with the *Standard solution 1* (0.1%). The sum of the areas under the secondary peaks obtained with the *Sample solution* must be lower than 2.5 times the area under the main peak obtained with the *Standard solution 1* (0.25%).

Heavy metals (5.3.2.3). Dissolve 2 g of the sample in acetone, complete the volume to 25 mL with the same solvent and add 2 mL of water. Add 1.2 mL of thioacetamide RS and 2 mL of acetate buffer pH 3.5. Homogenize and allow to stand for five minutes. Any color developed is not darker than the standard, prepared with 25 mL of acetone, 2 mL of *Standard lead solution (10 ppm Pb)*, 1.2 mL of thioacetamide RS and 2 mL of acetate buffer pH 3.5. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample, in a desiccator with silica gel, at room temperature, under reduced pressure, until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 1 g of sample, transfer to a 250 mL Erlenmeyer flask with cap, and dissolve in 10 mL of ethyl alcohol. Add 50 mL of 0.5 M sodium hydroxide VS. Allow to stand for one hour. Add 0.2 mL of phenolphthalein TS as indicator and titrate with 0.5 M hydrochloric acid VS. Carry out the blank test and make the necessary corrections. Each mL of 0.5 M sodium hydroxide VS is equivalent to 45.040 mg of C₉H₈O₄.

PACKAGING AND STORAGE

In perfectly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Analgesic; anti-pyretic; non-steroid anti-inflammatory; platelet aggregation inhibitor; used also for relief of migraine and in ischemic cardiopathy.

ACETYLSALICYLIC ACID TABLETS

Contains no less than 95.0% and no more than 105.0% the declared amount of $C_9H_8O_4$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 0.5 g of acetylsalicylic acid to a centrifuge tube and shake with 10 mL of ethyl alcohol for a couple of minutes. Centrifuge. Remove the clear supernatant and evaporate to dryness in water bath at 60 °C, for one hour. Dry the residue in vacuum oven at 60 °C, for one hour. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of acetylsalicylic acid CRS, prepared identically.

B. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 0.5 g of acetylsalicylic acid and dissolve in 10 mL of 5 M sodium hydroxide. Boil for two or three minutes. Cool down. Add an excess of M of sulfuric acid. A crystalline precipitate with characteristic odor of acetic acid forms. Add ferric chloride RS to the solution. An intense violet color develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). No more than five minutes.

Uniformity of unit doses (5.1.6). Complies with the test. For 100 mg tablets, proceed as described in *Assay*, employing volumetric solutions at 0.1 M.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.05 M acetate buffer pH 4.5, 500 mL.

Apparatus: baskets, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and, if necessary, dilute in 0.05 M acetate buffer pH 4.5 until adequate concentration. Immediately measure the absorbances of the solutions at 265 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the amount of $C_9H_8O_4$ dissolved in the medium, comparing the readings obtained with the acetylsalicylic acid CRS solution at the concentration 0.008% (w/v), prepared at the moment of use. Ethyl alcohol can be used to dissolve the standard before the dilution in 0.05 M acetate buffer pH 4.5. The volume of ethyl alcohol cannot exceed 1% of the total volume of the standard solution.

Tolerance: no less than 80% (Q) of the declared quantity of $C_9H_8O_4$ dissolve in 30 minutes.

PURITY TESTS

Salicylic acid. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 0.2 g of acetylsalicylic acid to a 100 mL volumetric flask. Add 4 mL of ethyl alcohol and shake. Complete the volume to 100 mL with cooled water, keeping the temperature below 10 °C. Filter immediately and transfer 50 mL of filtrate to a Nessler tube. Prepare the salicylic acid CRS solution at 0.01% (w/v). Transfer 3 mL of this solution to a Nessler tube, add 2 mL of ethyl alcohol and water in sufficient amount for 50 mL. Add 1 mL of ferric ammonium sulfate RS2 to the standard and sample solutions. The violet color produced with the sample solution must not be more intense than the one obtained with the standard solution.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

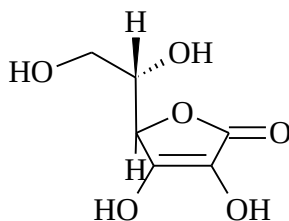
Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 0.5 g of acetylsalicylic acid to a 250 mL Erlenmeyer flask and add 30 mL of 0.5 M sodium hydroxide. Boil, carefully, for 10 minutes and titrate the excess 0.5 M sodium hydroxide VS with 0.5 M hydrochloric acid VS, using phenol red TS as indicator. Conduct the blank test and make the necessary corrections. Each mL of 0.5 M sodium hydroxide VS is equivalent to 45.040 mg of $C_9H_8O_4$.

PACKAGING AND STORAGE

In perfectly closed containers and protected from light.

LABELING

Comply with current legislation.

ASCORBIC ACID***Acidum ascorbicum***

C₆H₈O₆; 176.12
 ascorbic acid; 00104
 L-ascorbic acid
 [50-81-7]

Contains no less than 99.0% and no more than 100.5% of C₆H₈O₆, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, or slightly yellow, fine crystalline powder. In solid state it is stable to air, but in solution it oxidizes quickly. Its aqueous preparation is clear.

Solubility. Freely soluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 189 °C to 192 °C, with decomposition.

Specific optical rotation (5.2.8): +20.5 to +21.5, determined in 10% (w/v) solution, in carbon dioxide-free water.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of ascorbic acid CRS, prepared identically.

B. Add to an aliquot of 2% (w/v) solution alkaline cupric tartrate RS and allow to stand at room temperature. A change of color is observed due to the slow reduction of cupric tartrate. Under heating, the reduction is faster.

PURITY TESTS

pH (5.2.19). 2.2 to 2.5. Determine in aqueous solution at 5% (w/v).

Heavy metals (5.3.2.3). Dissolve 1 g in 25 mL of water. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample, in a vacuum desiccator, under sulfuric acid atmosphere, for 24 hours. No more than 0.4%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.2 g of the sample and dissolve in a mixture of 100 mL of water and 25 mL of *M* sulfuric acid. Add 3 mL of starch TS and titrate immediately with 0.05 *M* iodine VS. Each mL of 0.05 *M* iodine VS is equivalent to 8.806 mg of C₆H₈O₆.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vitamin.

ASCORBIC ACID TABLETS

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_6H_8O_6$.

IDENTIFICATION

Weigh and pulverize the tablets. From the powder, prepare a 2% (w/v) solution of ascorbic acid in ethyl alcohol, homogenize and filter. Proceed as described on tests **A.** and **B.** for *Identification* in the *Ascorbic acid* monograph, using 2 mL of the solution obtained.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute, if necessary, with water to obtain a 0.5 mg/mL ascorbic acid solution. Transfer a volume equivalent to approximately 2 mg of ascorbic acid to a 50 mL Erlenmeyer flask, add 5 mL of metaphosphoric acetic acid RS and titrate with standard dichlorophenol-indophenol solution, until achieving a pink color, persistent for 5 seconds. Conduct a blank test with the mixture of 5.5 mL metaphosphoric acetic acid RS and 15 mL of water. Calculate the amount of $C_6H_8O_6$ dissolved, from the titer of the standard dichlorophenol-indophenol solution.

Tolerance: no less than 75% (Q) of the declared quantity of $C_6H_8O_6$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh and pulverize 20 tablets. Use an amount of powder equivalent to 0.2 g of ascorbic acid. Proceed as described in *Assay* in the *Ascorbic acid* monograph.

B. Weigh and pulverize 20 tablets. Use an amount of powder equivalent to 0.15 g of ascorbic acid. Dissolve in a mixture of 30 mL of water and 20 mL of *M* sulfuric acid. Titrate with 0.1 *M* ceric ammonium sulfate VS, using ferroin TS as indicator. Each mL of 0.1 *M* ceric ammonium sulfate VS is equivalent to 8.806 mg of $C_6H_8O_6$.

PACKAGING AND STORAGE

In hermetic, opaque containers.

LABELING

Comply with current legislation.

ASCORBIC ACID INJECTION SOLUTION

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_6H_8O_6$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of ethyl alcohol and water (120:20) as mobile phase. Apply to the plate, separately, 2 μ L of each of the freshly prepared solutions described below.

Solution (1): dilute the injection solution in water, to obtain a 5 mg/mL ascorbic acid solution.

Solution (2): 5 mg/mL aqueous solution of ascorbic acid CRS.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. Dilute the injection solution in ethyl alcohol, until a concentration of 2% (w/v), and filter. Proceed as described in test **B.** of *Identification* from the *Ascorbic acid* monograph.

C. Transfer a volume of injection solution equivalent to 50 mg of ascorbic acid to a test tube. Add 0.2 mL of 2 M nitric acid and 0.2 mL of 0.1 M silver nitrate. A grey precipitate is produced.

D. Complies with sodium ion reactions (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 6.1 to 7.1.

PURITY TESTS

Limit of oxalate. Dilute a volume of injection solution equivalent to 50 mg of ascorbic acid with water to 5 mL. Add 0.2 mL of glacial acetic acid and 0.5 mL of calcium chloride RS. No turbidity is produced in an interval of one minute.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 1.2 EU/mg of ascorbic acid.

ASSAY

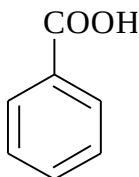
Transfer a volume of injection solution equivalent to approximately 0.2 g of ascorbic acid to an Erlenmeyer flask. Add 100 mL of carbon dioxide-free water and proceed as described in *Assay* on the *Ascorbic acid* monograph as of "and 25 mL of M sulfuric acid...". Each mL of 0.05 M iodine VS is equivalent to 8.806 mg of $C_6H_8O_6$.

PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELING

Comply with current legislation.

BENZOIC ACID*Acidum benzoicum*

C₇H₆O₂; 122.12
benzoic acid; 00115
Benzoic acid
[65-85-0]

Contains no less than 99.5% and no more than 100.5% of C₇H₆O₂ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, crystalline powder or colorless crystals.

Solubility. Moderately soluble in water, soluble in water in ebullition, freely soluble in ethyl alcohol and fatty acids.

Physicochemical constants.

Melting range (5.2.2): 121 °C to 124 °C.

IDENTIFICATION

A. Prepare a saturated solution of benzoic acid in water and filter twice. Add to a portion of the filtrate ferric chloride solution RS. The formation of orange precipitate occurs. Add 1 mL of 3 M sulfuric acid to another portion of 10 mL of the filtrate and cool the mixture. There is the formation of a white precipitate, soluble in ethyl ether, in approximately 10 minutes.

B. Dissolve 5 g of sample in 100 mL of ethyl alcohol. Complies with benzoate ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Dissolve 5 g of sample in 100 mL of ethyl alcohol. The preparation obtained is clear **(5.2.25)**.

Oxidizable substances. Dissolve 2 g of sample in 10 mL of water in ebullition, cool and filter. Add to the filtrate 1 mL of 5% (v/v) sulfuric acid and 0.2 mL of 0.02 M potassium permanganate. A persistent pink color forms for, at least, five minutes.

Carbonizable substances. Dissolve 0.5 g of sample in 5 mL of sulfuric acid RS. After five minutes, the solution is not more intensely stained than the one prepared by diluting 12.5 mL of the *Color solution H (5.2.12)* to 100 mL with hydrochloric acid RS.

Halogenated compounds and halides. Prepare the solutions as described below.

Note: all glassware used must be free from chlorides. A way to achieve that is by filling the glassware with a 50% (w/v) nitric acid solution and allow it to stand for at least 12 hours or in ultrasonic bath as long as necessary. On the following day, wash the glassware with water and store it filled with water. It is recommended to have glassware reserved specifically for executing this test.

Solution (1): dissolve 6.7 g of sample in a mixture of 40 mL of 0.1 M sodium hydroxide and 50 mL of ethyl alcohol and complete the volume to 100 mL with water. Add to 10 mL of this solution 7.5 mL of sodium hydroxide solution RS, 0.125 g of nickel-aluminum alloy, and heat in water bath for 10 minutes. Let it cool down to room temperature, filter and wash with three portions, of 3 mL each, of ethyl alcohol. Wash with 25 mL of water.

Solution (2): prepare this solution similarly to *Solution (1)*, but without using the sample.

Solution (3): chloride standard solution (8 ppm Cl).

Add to four 25 mL volumetric flasks, separately, 10 mL of *Solution (1)*, 10 mL of *Solution (2)*, 10 mL of *Solution (3)* and 10 mL of water. Add to each flask 5 mL of ferric ammonium sulfate RS1, 2 mL of nitric acid RS and 5 mL of mercury thiocyanate RS. Complete the volume of each flask to 25 mL with water. Allow to stand in water bath at 20 °C for 15 minutes. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Measure the absorbance of *Solution (1)* at 460 nm, using *Solution (2)* for zero adjustment. Measure the absorbance of *Solution (3)* at 460 nm, using water for zero adjustment. The absorbance of *Solution (1)* is not higher than the absorbance of *Solution (3)* (300 ppm).

Heavy metals (5.3.2.3). Use *Method II*. Weigh 2 g of sample, dissolve in ethyl alcohol and complete the volume to 25 mL. Prepare the standard solution using ethyl alcohol as solvent. No more than 0.001% (10 ppm).

Water (5.2.20.1). Dissolve the sample in a mixture of methyl alcohol and pyridine (1:2). No more than 0.7%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.05%.

ASSAY

Accurately weigh about 200 mg of the sample and dissolve in 20 mL of ethyl alcohol. Titrate with 0.1 M sodium hydroxide VS, using phenol red TS until formation of a violet color, corresponding to the titration endpoint. Each mL of 0.1 M sodium hydroxide VS is equivalent to 12.212 mg of C₇H₆O₂.

PACKAGING AND STORAGE

In tightly closed and opaque containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS/CATEGORY

Antimicrobial; preservative

BORIC ACID***Acidum boricum***

H₃BO₃; 61.83
boric acid; 00116
Boric acid
[10043-35-3]

Contains no less than 99.5% and no more than 100.5% of H₃BO₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder, unctuous to touch, or colorless brilliant crystals.

Solubility. Soluble in water, freely soluble in water in ebullition and 85% (v/v) glycerol, soluble in ethyl alcohol.

IDENTIFICATION

Dissolve, by mildly heating, 0.1 g of the sample in 5 mL of methyl alcohol. Add 0.1 mL of sulfuric acid and lead the solution to ignition. A flame with green edges is observed.

PURITY TESTS

Aspect of the preparation. Dissolve 3.3 g of the sample in 80 mL of water in ebullition. Cool down and dilute to 100 mL with carbon dioxide-free water. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). 3.8 to 4.8. Determine in the preparation obtained in *Aspect of the preparation*.

Solubility in ethyl alcohol. Dissolve 1 g of the sample in 10 mL of ethyl alcohol in ebullition. The preparation is colorless **(5.2.12)** and no more opalescent than the *Reference suspension II (5.2.25)*.

Organic impurities. Heat the sample progressively at red heat. It does not get dark.

Heavy metals (5.3.2.3). Use *Method I*. Dissolve 1 g of the sample in 23 mL of water, add 2 mL of acetic acid *M* and proceed as described in *Limit test for heavy metals*. No more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Determine in 2.7 g of the sample. No more than 0.045% (450 ppm).

Loss on drying (5.2.9.1). Dry over silica gel for five hours. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 1 g of the sample and dissolve in 100 mL of water with 15 mL of mannitol, by heating. Titrate with *M* sodium hydroxide VS, using 0.5 mL of phenolphthalein TS as indicator, until turning pink. Each mL of *M* sodium hydroxide VS is equivalent to 61.832 mg of H_3BO_3 .

PACKAGING AND STORAGE

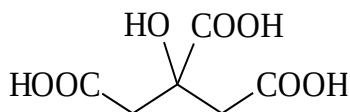
In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Antiseptic and pharmaceutical adjuvant.

CITRIC ACID*Acidum citricum* $C_6H_8O_7$; 192.12

citric acid; 00134

2-hydroxy-1,2,3-propanetricarboxylic acid

[77-92-9]

 $C_6H_8O_7 \cdot H_2O$; 210.14

monohydrate citric acid; 09852

Hydrated 2-hydroxy-1,2,3-propanetricarboxylic acid (1:1)

[5949-29-1]

Contains no less than 99.5% and no more than 100.5% of $C_6H_8O_7$ in relation to the anhydrous substance.

DESCRIPTION

Physical-chemical characteristics. Colorless, translucent crystals or white crystalline powder. Efflorescent on hot and dry air. The hydrated form is slightly deliquescent in humid air. Melting point **(5.2.2)**: approximately 153 °C, with decomposition.

Solubility. Very soluble in water, freely soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated at 105 °C for two hours, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of citric acid CRS, prepared identically.

B. Dissolve 1 g of the substance in 10 mL of water. The solution is strongly acidic.

C. Dissolve 0.5 g of the substance in 5 mL of water and neutralize with *M* sodium hydroxide. Add 10 mL of calcium chloride RS and heat until ebullition. A white precipitate is formed.

D. Complies with citrate ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Dissolve 2 g of the sample in water and complete the volume to 10 mL with the same solvent. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

Easily carbonizable substances. Transfer, quantitatively, about 0.5 g of the powdered sample to a test tube washed in advance with sulfuric acid, containing 5 mL of sulfuric acid. Heat for one hour at 90 °C. The solution must be only yellow, not brown.

Oxalic acid. Weigh the equivalent to 0.8 g of citric acid and dissolve in 4 mL of water. Add 3 mL of hydrochloric acid and 1 mL of granulated zinc. Boil for one minute and cool for two minutes. Transfer the liquid supernatant to a test tube with 0.25 mL of 1% (w/v) phenylhydrazine hydrochloride solution and heat until ebullition. Cool down quickly, transfer to a graduated tube and add an equal volume of hydrochloric acid and 0.25 mL of potassium ferricyanide RS. Shake and allow to stand for 30 minutes. The pink color developed in the solution must not be more intense than the one developed by the oxalic acid standard, prepared in the same way, using 4 mL of a 0.01% (w/v) oxalic acid solution.

Aluminum (5.3.2.10). Weigh, accurately, about 20 g of the sample and proceed as described in *Limit test for aluminum*, using 40 mL of the *Standard aluminum solution (2 ppm Al)*. No more than 0.2 ppm (0.00002%), when citric acid is used in solutions for dialysis.

Sulfates (5.3.2.2). Dissolve 3.2 g of the sample in 40 mL of water and proceed as described in *Limit test for sulfates*, using 1 mL of the 0.005 M standard sulfuric acid solution. No more than 0.015% (150 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Accurately weigh about 2 g of the sample and dissolve in sodium hydroxide RS. Dilute to 25 mL with water and proceed as described in *Limit test for heavy metals*. After adding the reagent thioacetamide and diluting with water, homogenize and heat at 80 °C, then allow it to stand for two minutes. No more than 0.001% (10 ppm).

Water (5.2.20.1). For the anhydrous form, determine in 2 g of the sample. No more than 1%. For the hydrated form, determine in 0.5 g of the sample. Between 7.5 and 9.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Citric acid targeted at production of parenteral preparation complies with the following tests.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.5 EU/mg of citric acid anhydrous, if the finished product is not submitted to a later procedure for removing bacterial endotoxins.

ASSAY

Weigh, accurately, about 0.5 g of the sample and dissolve in 50 mL of water, heating at low heat, if necessary, until full dissolution. Titrate with M sodium hydroxide VS, using phenolphthalein TS as indicator. Each mL of M sodium hydroxide VS is equivalent to 64.040 mg of C₆H₈O₇.

PACKAGING AND STORAGE

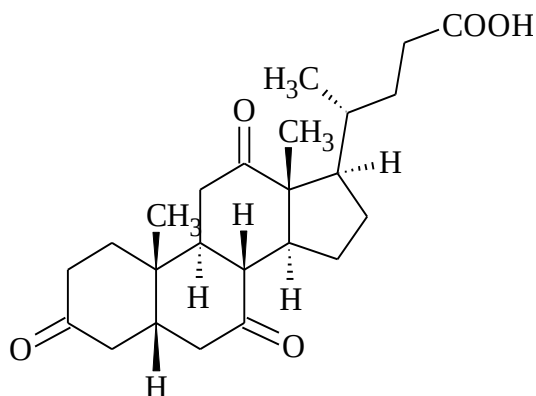
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Acidulant.

DEHYDROCHOLIC ACID*Acidum dehydrocholicum*C₂₄H₃₄O₅; 402.53

dehydrocholic acid; 00157

(5 β)-3,7,12-trioxocholan-24-oic acid

[81-23-2]

Contains no less than 98.5% and no more than 101.0% of C₂₄H₃₄O₅, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder.

Solubility. Very slightly soluble in water, slightly soluble in glacial acetic acid and ethyl alcohol. Soluble in alkali hydroxides and carbonates.

Physicochemical constants.

Melting range (5.2.2): 231 °C to 240 °C. The interval between the beginning and the end of the melting does not exceed 3 °C.

Specific optical rotation (5.2.8): +29.0 to +32.5. Determine in solution at 2% (w/v) in dioxane.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of dehydrocholic acid CRS, prepared identically.

PURITY TESTS

Barium. Dissolve 2 g of the sample in 100 mL of water and boil for two minutes. Add 2 mL of hydrochloric acid RS, boil for another two minutes, cool and filter. Wash the filter with water and complete the volume to 100 mL with the same solvent. Add 1 mL of *M* sulfuric acid to 10 mL of the filtrate. The solution obtained must not get turbid or precipitate.

Heavy metals (5.3.2.3). Use *Method III*. Use 1 g of the sample, heating the solution at 80 °C before adding thioacetamide RS. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample, in an oven. Dry in oven at 105 °C, for two hours. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.3%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.25 g of the sample previously desiccated and dissolve in 30 mL of ethyl alcohol. Shake until full dissolution, heating in water bath, if necessary, and add two drops of phenolphthalein TS and 30 mL of water. Titrate with 0.1 *M* sodium hydroxide VS. Each mL of 0.1 *M* sodium hydroxide VS is equivalent to 40.252 mg of C₂₄H₃₄O₅.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Cholagogue.

STEARIC ACID***Acidum stearicum***

stearic acid; 00182
Octadecanoic acid
[57-11-4]

Mixture of stearic acid ($C_{18}H_{36}O_2$, 284.48) and palmitic acid ($C_{16}H_{32}O_2$, 256.43). May contain antioxidant.

DESCRIPTION

Physical characteristics. White to yellow-white powder, or white, flaky and waxy crystals, or white to faintly yellow solid masses. Slight odor, similar to non-rancid sebum.

Solubility. Practically insoluble in water, freely soluble in chloroform and ethyl ether, soluble in ethyl alcohol and in petroleum ether.

Physicochemical constants.

Congearing temperature (5.2.4): no less than 54 °C.

IDENTIFICATION

It complies with the requirements from the *Acidity value* in *Purity Tests*.

PURITY TESTS

Acidity. Shake, for two minutes, 5 g of the sample melted with an equal volume of hot water, cool and filter. Add one drop of methyl orange TS to the filtrate. No red color develops.

Acidity value (5.2.29.7). 194 to 212.

Iodine value (5.2.29.10). No more than 4.0.

Paraffin and other non-saponifiable substances. Boil approximately 1 g of the sample with 30 mL of water and 0.5 g of sodium carbonate anhydrous. The resulting preparation, while hot, is clear or, at most, slightly opalescent.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.001% (10 ppm).

Residue by incineration (5.2.10). Determine in 4 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study and identification of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

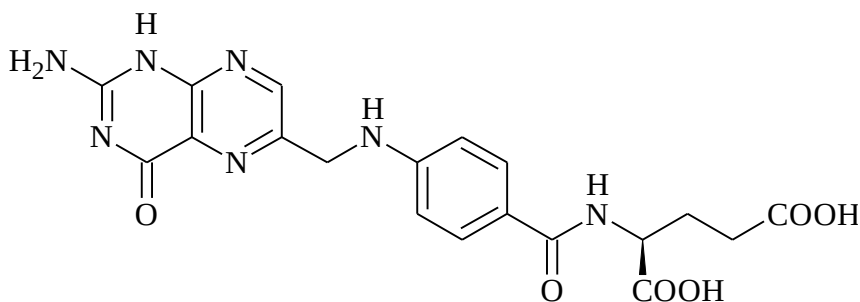
Comply with current legislation.

CATEGORY

Raw material for preparation of sodium, magnesium, zinc stearates and other pharmacotechnical adjuvants.

FOLIC ACID

Acidum folicum



$C_{19}H_{19}N_7O_6$; 441.40

folic acid; 00194

N-[4-[[[(2-amino-3,4-di-hydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid
[59-30-3]

Contains no less than 97.0% and no more than 102.0% of $C_{19}H_{19}N_7O_6$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Orange-yellow crystalline powder.

Solubility. Practically insoluble in water, insoluble in ethyl alcohol. Soluble in alkali hydroxide solutions. Soluble in hydrochloric acid, producing pale yellow solutions.

Physicochemical constants.

Specific optical rotation (5.2.8): +18 to +22, in relation to the anhydrous substance. Determine in 1.0% (w/v) solution in 0.1 M sodium hydroxide.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Chromatographic purity. Proceed as described in *Assay*. Use the *Concentrated sample solution* as *Test solution*.

Procedure: inject 10 μ L of the *Test solution*. Record the chromatogram for, at least, two times the folic acid retention time and measure the areas under the peaks. The sum of the areas under all peaks, except the one corresponding to folic acid, is not higher than 2.0% of the sum of the areas under all peaks recorded, including the one corresponding to folic acid. Do not consider peaks related to the solvent.

Water (5.2.20.1). No more than 8.5%.

Residue by incineration (5.2.10). Determine 1 g of the sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 280 nm; column with 250 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (3 µm to 10 µm), kept at room temperature; *Mobile phase* flow of 1.2 mL/minute.

Mobile phase: transfer 2 g of monobasic potassium phosphate to a 1000 mL volumetric flask. Add 650 mL of water, 15 mL of 0.5 M tetrabutylammonium hydroxide in methyl alcohol, 7 mL of M phosphoric acid, 270 mL of methyl alcohol and homogenize. Adjust pH to 5.0 with M phosphoric acid or 6 M ammonium hydroxide. Complete the volume with water, homogenize and filter.

Note: protect the solutions described below from direct light.

Internal standard solution: dissolve 50 mg of methylparaben in 1 mL of methyl alcohol, dilute to 25 mL with *Mobile phase* and homogenize.

Concentrated sample solution: weigh, accurately, about 0.1 g of the sample and transfer to a 100 mL volumetric flask. Add 40 mL of *Mobile phase* and 1 mL of 10% (v/v) ammonium hydroxide. Complete the volume with the *Mobile phase* and homogenize.

Sample solution: transfer 4 mL of the *Concentrated sample solution* and 4 mL of the *Internal standard solution* to a 50 mL volumetric flask. Complete the volume with the *Mobile phase* and homogenize.

Standard stock solution: prepare a folic acid CRS solution at 1 mg/mL in *Mobile phase*, using 1 mL of 10% (v/v) ammonium hydroxide for each 100 mL of solution.

Standard solution: transfer 4 mL of the *Standard stock solution* and 4 mL of the *Internal standard solution* to a 50 mL volumetric flask. Complete the volume with the *Mobile phase* and homogenize.

Inject 10 µL replicates of the *Standard solution*. The resolution between methylparaben and folic acid is, at minimum, 2.0. The relative standard deviation of the replicate areas of the ratio between the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₉H₁₉N₇O₆ in the sample from the responses obtained for the folic acid/methylparaben ratio with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Hematopoietic.

FOLIC ACID TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{19}H_{19}N_7O_6$.

IDENTIFICATION

Weigh and pulverize the tablets. Shake an amount of powder equivalent to 50 mg of folic acid with 100 mL of 0.1 M sodium hydroxide heated between 40 °C and 50 °C. Allow to cool and filter. Adjust the filtrate pH to 3.0 with hydrochloric acid. Cool the solution down to 5 °C, filter and wash the precipitate with cold water, until the washing waters do not respond to the reaction from chlorides **(5.3.1.1)**. Wash the precipitate with acetone and dry at 80 °C, for one hour. Transfer 10 mg of the residue to a 100 mL volumetric flask, dissolve in 0.1 M sodium hydroxide and complete the volume with the same solvent. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with 0.1 M sodium hydroxide, obtaining a 0.001% (w/v) solution. In the ultraviolet absorption spectrum **(5.2.14)**, at the range of 230 nm to 386 nm, there are maximum peaks of the solution obtained at 256 nm, 283 nm and 365 nm. The ratio between the absorbance values measured at 256 nm and 365 nm is comprised between 2.80 and 3.00.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 500 mL.

Apparatus: paddles, 50 rpm. Use amber tanks or perform the test in specific lighting conditions.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium and proceed as described in Assay.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{19}H_{19}N_7O_6$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 283 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octylsilane group (5 µm), kept at room temperature; *Mobile phase* flow of 1 mL/minute.

Mobile phase: mixture of 0.05 M monobasic potassium phosphate and acetonitrile (93:7). Adjust the mixture pH to 6.0 with 5 M sodium hydroxide.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 20 mg of folic acid to a 100 mL volumetric flask, add 50 mL of 0.1 M sodium hydroxide and complete the volume with the same solvent. Homogenize. Centrifuge, transfer 5 mL of the supernatant to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Standard solution: accurately weigh about 20 mg of folic acid CRS and transfer to a 100 mL volumetric flask. Complete the volume with the 0.1 M sodium hydroxide and homogenize. Transfer 5 mL of this solution to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₉H₁₉N₇O₆ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers protected from light.

LABELING

Comply with the current legislation.

PHOSPHORIC ACID***Acidum phosphoricum***

H₃PO₄; 97.99
phosphoric acid; 00199
Phosphoric acid
[7664-38-2]

Contains no less than 85.0% and no more than 88.0% of H₃PO₄.

DESCRIPTION

Physical characteristics. Colorless, clear, corrosive, syrupy liquid.

Solubility. Miscible in water and in ethyl alcohol.

IDENTIFICATION

When carefully neutralized with *M* sodium hydroxide, using phenolphthalein TS as indicator, complies with the phosphate ion reactions **(5.3.1.1)**.

PURITY TESTS

Phosphorous and hypophosphorous acid. Dilute 6 mL of the sample in 14 mL of water. Heat 5 mL of the dilution in water bath and add 2 mL of silver nitrate RS. The mixture must not get dark or form a precipitate.

Alkali phosphates. Transfer 1 mL of the sample to a Nessler tube and add 6 mL of ethyl ether and 2 mL of ethyl alcohol. No turbidity is produced.

Substances precipitable by ammonia. For preparing the *Sample solution*, dissolve 10 g of the sample and dilute to 100 mL with water. Pipette 6.7 mL of the *Sample solution* and complete the volume to 10 mL with water. Add 8 mL of ammonia RS. The preparation obtained is not more opalescent than the mixture of 6.7 mL of *Sample solution* in 18 mL of water.

Sulfates. Dilute 6 mL of the sample in 90 mL of water and add 1 mL of barium chloride RS. No precipitate is formed immediately.

Arsenic (5.3.2.5). Use 15 mL of the *Sample solution* obtained in *Substances precipitable by ammonia*. No more than 0.0002% (2 ppm).

Chlorides (5.3.2.1). Add to 15 mL of the *Sample solution* obtained in *Substances precipitable by ammonia* 1 mL of silver nitrate RS. Prepare the standard concomitantly mixing 1 mL of nitric acid RS, 0.21 mL of 0.01 *M* hydrochloric acid VS, 1 mL of silver nitrate RS and 13.79 mL of water. After five minutes protected from light, observe the tubes in the longitudinal axis from top to bottom, and over a dark background. If the sample preparation presents opalescence, it must be less intense than that of the standard preparation. No more than 0.005% (50 ppm).

Iron (5.3.2.4). Use 2 mL of the *Sample solution* obtained in *Substances precipitable by ammonia*. Use 1 mL of *Standard iron solution (10 ppm Fe)* as standard. No more than 0.005% (50 ppm).

Heavy metals (5.3.2.3). No more than 0.001% (10 ppm).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 1 g of the sample, transfer to an Erlenmeyer flask and add 10 g of sodium chloride and 30 mL of water. Titrate with *M* sodium hydroxide VS using phenolphthalein TS as indicator. Carry out the blank test and make the necessary corrections. Each mL of *M* sodium hydroxide VS is equivalent to 49.000 mg of H₃PO₄.

PACKAGING AND STORAGE

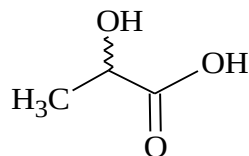
In tightly closed glass containers.

LABELING

Comply with current legislation.

CATEGORY

Preparation of diluted phosphoric acid.

LACTIC ACID
Acidum lacticum

C₃H₆O₃; 90.08

lactic acid; 00274

2-hydroxypropanoic acid.

[50-21-5]

Mixture of 2-hydroxypropanoic acid and its condensation products, such as lactoyllactic acid and polylactic acid and water. The equilibration between lactic acid and polylactic acids depends on concentration and temperature. Lactic acid is usually a racemate ((*RS*)-lactic acid), but the isomer *S* (+) may prevail. Contains no less than 88.0% and no more than 92.0% of C₃H₆O₃.

DESCRIPTION

Physical characteristics. Colorless or slightly yellow viscous liquid.

Solubility. Miscible with water and ethyl alcohol.

Physicochemical constants.

Optical rotation (5.2.8): -0.05° to +0.05°, for racemic lactic acid.

IDENTIFICATION

A. Dissolve 1 g of the sample in water. The solution is strongly acidic (pH lower than 4).

B. Complies with lactate ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. Dissolve 5 g of the sample in 42 mL of *M* sodium hydroxide and dilute to 50 mL with water. The preparation obtained is not more stained than the *Standard color solution SC F* (5.2.12).

Sugars and other reducer substances. Add to 10 mL of hot alkaline cupric tartrate RS five drops of the sample. No red precipitate is produced.

Easily carbonizable substances. Wash a test tube with sulfuric acid and allow to drain for 10 minutes. Add to the test tube 5 mL of sulfuric acid and, carefully, add 5 mL of the sample, in such a way that the liquids are not mixed. Keep the tube at temperature of 15 °C. After 15 minutes, no dark color develops in the interface between the two acids.

Ether-insoluble substances. Dissolve 1 g of the sample in 25 mL of ethyl ether. The solution is not more opalescent than the solvent used for the test.

Oxalic, citric and phosphoric acids. Add to 5 mL of the preparation obtained in *Aspect of the preparation* ammonia RS until a slightly alkaline pH (between 8 and 10). Add 1 mL of calcium chloride solution RS. Heat in a water bath for five minutes. Any opalescence in the preparation, before or after heating, is not more intense than that of a mixture of 1 mL of water and 5 mL of the preparation obtained in *Aspect of the preparation*.

Calcium (5.3.2.7). Dilute 5 mL of the preparation obtained in *Aspect of the preparation* to 15 mL with water and proceed as described in *Limit test for calcium*. No more than 0.02% (200 ppm).

Chlorides (5.3.2.1). Add to 10 mL of 1% (w/v) sample solution, acidified with nitric acid, a few drops of 0.1 M silver nitrate. No opalescence is produced immediately.

Sulfates (5.3.2.2). Add to 10 mL of 1% (w/v) sample solution two drops of hydrochloric acid and 1 mL of barium chloride RS. No turbidity is produced.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.001% (10 ppm).

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Transfer, quantitatively, about 1 g of the sample to a vial with lid, add 10 mL of water and 20 mL of *M* sodium hydroxide. Close the vial and allow to stand for 30 minutes. Add 0.5 mL of phenolphthalein TS and titrate with *M* hydrochloric acid VS until the pink color disappears. Each mL of *M* sodium hydroxide is equivalent to 90.080 mg of $C_3H_6O_3$.

PACKAGING AND STORAGE

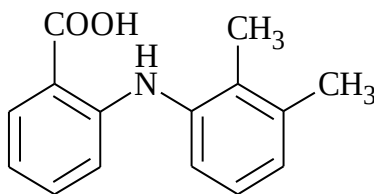
In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Buffering agent.

MEFENAMIC ACID*Acidum mefenamicum*C₁₅H₁₅NO₂; 241.29

mefenamic acid; 00286

2-[(2,3-dimethylphenyl)amino]benzoic acid

[61-68-7]

Contains no less than 98.0% and no more than 102.0% of C₁₅H₁₅NO₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white micro-crystalline powder. Presents polymorphism.

Solubility. Practically insoluble in water, moderately soluble in ethyl alcohol and methyl alcohol. Soluble in alkali hydroxide solutions.

IDENTIFICATION

The identification tests B. and C. can be omitted if test A. is conducted. The identification test A. can be omitted if tests B. and C. are conducted.

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of mefenamic acid CRS, prepared identically. If the spectrum of the sample is not identical to that of the standard, dissolve the sample and the standard separately in ethyl alcohol, evaporate to dryness and repeat the test with the residues.

B. In the ultraviolet absorption spectrum (**5.2.14**), at the range of 250 nm to 380 nm, of 0.002% (w/v) solution in mixture of *M* hydrochloric acid and methyl alcohol (1:99), there are maximum peaks at 279 nm and 350 nm. The ratio between the absorbance values measured at 279 nm and 350 nm is comprised between 1.1 and 1.3.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described in *Assay*. Prepare the *Standard solution* and the *Test solution* as described below.

Test solution: transfer, quantitatively, approximately 100 mg of the sample to a 100 mL volumetric flask, dissolve in *Mobile phase*, complete the volume with the same solvent and homogenize.

Standard solution: dissolve an accurately weighed amount of mefenamic acid CRS in *Mobile phase* to obtain a 10 µg/mL solution.

Procedure: inject, separately, 10 µL of the *Test solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks obtained. No individual impurity obtained with the *Test solution* is higher than 0.1%, nor the sum of all impurities is higher than 0.5% when compared to the main peak obtained with the *Standard solution*.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for four hours. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group; *Mobile phase* flow of 1.0 mL/minute.

Phosphate buffer pH 5.0: 50 mM monobasic ammonium phosphate, pH 5.0, adjusted with 3 M ammonium hydroxide.

Mobile phase: mixture of acetonitrile, *Phosphate buffer pH 5.0* and tetrahydrofuran (46:40:14). Degas and filter.

Sample solution: weigh, accurately, about 0.1 g of the sample and transfer to a 500 mL volumetric flask. Complete the volume with the *Mobile phase* and homogenize.

Standard solution: dissolve an accurately weighed amount of mefenamic acid CRS in *Mobile phase* to obtain a 0.2 µg/mL solution.

Inject 10 µL replicates of the *Standard solution*. The column efficiency is not lower than 8200 theoretical plates. The tailing factor is no more than 1.6. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₅H₁₅NO₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

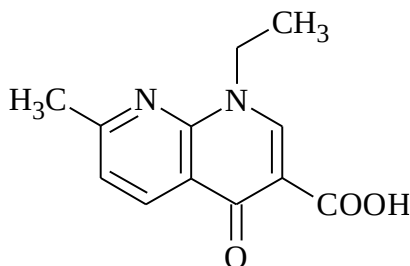
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Analgesic.

NALIDIXIC ACID***Acidum nalidixicum***C₁₂H₁₂N₂O₃; 232.24

nalidixic acid; 00294

1-ethyl-1,4-di-hydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid

[389-08-2]

Contains no less than 99.0% and no more than 101.0% of C₁₂H₁₂N₂O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to nearly white or pale yellow crystalline powder.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol. Soluble in diluted alkali hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 225 °C to 231 °C.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are conducted.

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of nalidixic acid CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 230 nm to 350 nm, of 0.0005% (w/v) sample solution in 0.1 M sodium hydroxide, there are maximum points at 258 nm and 334 nm. The ratio between the absorbance values measured at 258 nm and 334 nm is comprised between 2.2 and 2.4.

C. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

PURITY TESTS

Light absorption. Dissolve 1.5 g of the sample in a 50 mL volumetric flask with methylene chloride and complete the volume with the same solvent. Homogenize. The solution absorbance **(5.2.14)** measured at 420 nm is no more than 0.10.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel F₂₅₄ as support, and a mixture of ammonia RS, methylene chloride and ethyl alcohol (10:20:70) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.2 g of the sample in methylene chloride and complete the volume to 10 mL with the same solvent. Homogenize.

Solution (2): transfer 1 mL of *Solution (1)* to a 20 mL volumetric flask and complete the volume with methylene chloride.

Solution (3): dissolve 10 mg of nalidixic acid CRS in methylene chloride and complete the volume to 10 mL with the same solvent. Homogenize.

Solution (4): transfer 2 mL of *Solution (2)* to a 10 mL volumetric flask and complete the volume with methylene chloride.

Solution (5): transfer 1 mL of *Solution (4)* to a 10 mL volumetric flask and complete the volume with methylene chloride.

Solution (6): transfer 1 mL of *Solution (4)* to a 25 mL volumetric flask and complete the volume with methylene chloride.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (5)* (0.1%), and no more than one stain is more intense than the stain obtained with *Solution (6)* (0.04%).

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven between 100 °C and 105 °C for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

ASSAY

Weigh, accurately, about 0.25 g of the sample and dissolve in 30 mL of dimethylformamide, neutralized in advance, using thymolphthalein TS as indicator. Titrate with 0.1 M lithium methoxide VS, using thymolphthalein TS as indicator. Use a magnetic stirrer and take precautions to avoid absorption of atmospheric carbon dioxide. Each mL of 0.1 M lithium methoxide VS is equivalent to 23.224 mg of C₁₂H₁₂N₂O₃.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

NALIDIXIC ACID TABLETS

Contains no less than 95.0% and no more than 105.0% of the declared amount of $C_{12}H_{12}N_2O_3$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Use an amount of powder equivalent to 1 g of nalidixic acid, add 50 mL of chloroform, shake for 15 minutes, filter and evaporate the filtrate to dryness. The residue complies with the test **A.** in *Identification of the Nalidixic acid* monograph.

B. Dry the residue obtained in test **A.** of *Identification* at 105 °C for two hours. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 230 nm to 350 nm, of 0.0008% (w/v) residue solution in 0.1 M sodium hydroxide, there are maximum points at 258 nm and 334 nm.

C. Dry the residue obtained in test **A.** of *Identification* at 105 °C for two hours. Melting temperature (**5.2.2**): around 228 °C.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit dose (5.1.6). Complies with the test.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of 5 M ammonia, methylene chloride and ethyl alcohol (20:30:50) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Dissolve an amount of powder equivalent to 0.1 g nalidixic acid in 50 mL of methylene chloride, shake for 15 minutes, filter and evaporate the filtrate to dryness. Dissolve the residue in 5 mL of methylene chloride.

Solution (2): transfer 1 mL of *Solution (1)* to a 200 mL volumetric flask and complete the volume with methylene chloride. Pipette 5 mL, transfer to a 10 mL volumetric flask, complete the volume with methylene chloride and homogenize.

Solution (3): Pipette 4 mL of *Solution (2)*, transfer to a 10 mL volumetric flask, complete the volume with methylene chloride and homogenize.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). No stain obtained in the chromatogram with *Solution (1)*, in addition to the main stain,

must be more intense than the stain obtained with *Solution (2)* (0.25%), and no more than one stain is more intense than the stain obtained with *Solution (3)* (0.1%).

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 8.6, 900 mL.

Apparatus: paddle, 60 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in 0.01 M sodium hydroxide until adequate concentration. Measure the absorbances of the solutions at 334 nm (5.2.14), using a mixture of 0.01 M sodium hydroxide and dissolution medium, in the same proportion of the test solution, for zero adjustment. Calculate the amount of nalidixic acid dissolved in the medium, comparing the readings obtained with the nalidixic acid CRS solution at the concentration 0.00055% (w/v) in 0.01 M sodium hydroxide.

Tolerance: no less than 80% (Q) of the declared quantity of nalidixic acid dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 0.1 g of nalidixic acid to a 200 mL volumetric flask, add 150 mL of M sodium hydroxide, shake for three minutes and complete the volume with the same solvent. Allow the solution to stand for 15 minutes. Transfer 2 mL to a 200 mL volumetric flask and complete the volume with water. Prepare a standard solution in the same conditions. Measure the absorbance of the resulting solution at 334 nm, using 0.01 M sodium hydroxide for zero adjustment. Calculate the amount of $C_{12}H_{12}N_2O_3$ in the tablets from the readings obtained. Alternatively, make calculations considering $A(1\%, 1\text{ cm}) = 494$, at 334 nm, in 0.01 M sodium hydroxide.

PACKAGING AND STORAGE

In hermetic containers and protected from light.

LABELING

Comply with current legislation.

NALIDIXIC ACID ORAL SUSPENSION

Contains no less than 92.5% and no more than 107.5% of the declared amount of $C_{12}H_{12}N_2O_3$.

IDENTIFICATION

Transfer 5 mL of the sample to a separation funnel, add 30 mL of water and 20 mL of 10% (w/v) decahydrate sodium carbonate. Homogenize. Extract with two portions of 30 mL of chloroform. Acidify the aqueous solution with 5 M hydrochloric acid, add 40 mL of chloroform and shake. Collect the chloroform layer and transfer to a separation funnel, wash with 10 mL of water, and add 0.5 mL of 5 M hydrochloric acid. Filter the chloroform layer in cotton and evaporate the filtrate to dryness. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 230 nm to 350 nm, of 0.0008% (w/v) residue solution in 0.1 M sodium hydroxide, there are two maximum points, at 258 nm and 334 nm.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

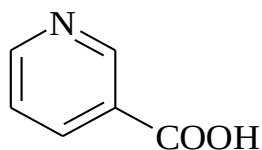
Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer a volume or mass of oral suspension, equivalent to 0.12 g of nalidixic acid, to a 100 mL volumetric flask. Add 0.01 M sodium hydroxide, shake and complete the volume with the same solvent. Transfer 2 mL of this solution to a 250 mL volumetric flask and complete the volume with 0.01 M sodium hydroxide. Filter, if necessary. Measure the absorbance of the resulting solution at 334 nm, using 0.01 M sodium hydroxide for zero adjustment. Calculate the amount of $C_{12}H_{12}N_2O_3$ in the oral suspension, considering $A(1\%, 1\text{ cm}) = 494$, at 334 nm, in 0.01 M sodium hydroxide.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

NICOTINIC ACID***Acidum nicotinicum*** $C_6H_5NO_2$; 123.11

nicotinic acid; 00296

3-pyridinecarboxylic acid

[59-67-6]

Contains no less than 99.0% and no more than 101.0% of $C_6H_5NO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical-chemical characteristics. White crystalline powder or white crystals. *Melting point (5.2.2):* around 235 °C.

Solubility. Moderately soluble in water, freely soluble in water in ebullition and in ethyl alcohol in ebullition. Freely soluble in alkali hydroxide and carbonate solutions.

IDENTIFICATION

The identification tests B. and C. can be omitted if test A. is conducted. The identification test A. can be omitted if tests B. and C. are conducted.

A. In the infrared absorption spectrum **(5.2.14)** of the sample, previously desiccated, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of nicotinic acid CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 300 nm of the *Sample solution* obtained in the method from *Assay*, there are maximum and minimum points only at the same wavelengths of similar nicotinic acid CRS solution. The ratio between the absorbance values measured at 237 nm and 262 nm is comprised between 0.46 and 0.50.

C. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the main stain obtained in the chromatogram of *Solution (3)*.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 250 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), maintained at temperature of 15 °C; *Mobile phase* flow of 1.0 mL/minute.

Eluent A: mix 2 mL of glacial acetic acid in 950 mL of water pH 5.6 adjusted in advance with diluted ammonia, dilute with water to 1000 mL and homogenize.

Eluent B: mixture of acetonitrile and methyl alcohol (50:50).

Mobile Phase gradient: adopt the gradient system described on the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 - 10	100	0	isocratic
10 - 30	100 → 20	0 → 80	linear gradient
30 - 35	20	80	isocratic
35 - 40	20 → 100	80 → 0	linear gradient
40 - 48	100	0	isocratic

Solution (1): dissolve 0.12 g of the sample, accurately weighed, in 200 µL of diluted ammonia, and dilute to 10 mL with *Eluent A*.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with *Eluent A*. Dilute 1 mL of this solution to 10 mL with *Eluent A* and homogenize.

Solution (3): prepare a solution with 12 µg/mL of impurity A (6-methylnicotinic acid) and impurity B (6,6'-dinicotinic acid) in *Eluent A* or dissolve the content from the vial with mixture of impurities from nicotinic acid CRS (with impurities A and B) in 1 mL of *Eluent A*.

Inject 10 µL replicates of the *Solution (3)*. The resolution between the peaks of impurities A and B is no less than 1.5. In relation to nicotinic acid (retention time around six minutes), the relative retention time of impurity A is around 2.7; of impurity B is around 2.8.

Procedure: inject, separately, 10 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of each impurity present in *Solution (1)*. Any individual impurity presents no more than 0.5 times the area under the main peak of the chromatogram obtained with *Solution (2)* (0.05%). The total of impurities found is no more than 0.5 times the area under the main peak of the chromatogram obtained with *Solution (2)* for total impurities (0.05%). Disregard peaks with areas below 0.3 times the area under the main peak obtained in the chromatogram of *Solution (2)* (0.03%).

Chlorides (5.3.2.1). Accurately weigh about 0.5 g of the sample and dissolve in 40 mL of heated water. Proceed as described in *Limit test for chlorides*. Use 0.30 mL of 0.01 M hydrochloric acid VS. No more than 0.02% (200 ppm).

Sulfates (5.3.2.2). Accurately weigh about 0.5 g of the sample and dissolve in 40 mL of heated water. Proceed as described in *Limit test for sulfates*. Use 0.20 mL of 0.005 M sulfuric acid VS. No more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Mix 1 g of the sample with 4 mL of M acetic acid, dilute in water to 25 mL, heat carefully the preparation until full dissolution and cool. Proceed with the pH adjustment and dilution with water, as described on *Method I*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for one hour. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 200 mg of the sample and dissolve in *Phosphate buffer pH 7.0*. Complete the volume to 500 mL with the same solvent. Transfer 5 mL of this solution to a 100 mL volumetric flask, complete with *Phosphate buffer pH 7.0* and homogenize. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbance of the resulting solutions at 262 nm, using *Phosphate buffer pH 7.0* for zero adjustment. Calculate the content of $C_6H_5NO_2$ in the sample from the readings obtained.

Phosphate buffer pH 7.0: dissolve 6.8 g of monobasic sodium phosphate in 1000 mL of water. Adjust to pH 7.0 with 50% (w/v) sodium hydroxide solution.

PACKAGING AND STORAGE

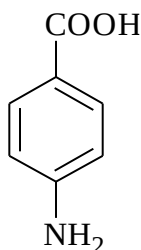
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vitamin.

PARA-AMINOBENZOIC ACID*Acidum 4-aminobenzoicum* $C_7H_7NO_2$; 137.14

para-aminobenzoic acid; 00098

4-aminobenzoic acid

[150-13-0]

Contains no less than 98.5% and no more than 101.5% of $C_7H_7NO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder, or white or yellow-white needles. Darkens when exposed to air and light.

Solubility. Slightly soluble in water, freely soluble in ethyl alcohol. Freely soluble in alkali hydroxide and carbonate solutions.

Physicochemical constants.

Melting range (5.2.2): 186.0 °C to 189.0 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of para-aminobenzoic acid CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 1% (w/v) solution in isopropyl alcohol, there is a maximum point at 288 nm. The absorbance at 288 nm is of approximately 1.370.

PURITY TESTS

Related substances 1. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 270 nm; column with 150 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to an octylsilane group (5 µm), kept at room temperature; *Mobile phase* flow of 1 mL/minute.

Mobile phase: mixture of acetonitrile, methyl alcohol and aqueous solution with 1.5 g/L of dibasic potassium phosphate and 2.5 g/L of sodium 1-octanesulfonate adjusted in advance to pH 2.2 with phosphoric acid (70:80:600).

Sample solution: dissolve, accurately, about 25 mg of the sample in *Mobile phase* and dilute to 100 mL with the same solvent.

Standard solution: dissolve, accurately, about 25 mg of 4-nitrobenzoic acid and 25 mg of benzocaine in methyl alcohol and dilute to 100 mL with the same solvent. Dilute 1 mL to 50 mL with *Mobile phase*. Dilute 1 mL of the resulting solution to 10 mL with *Mobile phase*.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms for, at least, 11 times the para-aminobenzoic acid retention time, and measure the area under the peaks. The area under the 4-nitrobenzoic acid peak in the *Sample solution* is not greater than the corresponding peak at the *Standard solution* (0.2%). The area under the benzocaine peak in the *Sample solution* is not greater than the corresponding peak at the *Standard solution* (0.2%). No other impurity must have a peak with area greater than 0.5 times the area under the peak obtained with 4-nitrobenzoic acid in the *Standard solution*. Do not consider peaks with area 0.1 times inferior to the area under the 4-nitrobenzoic acid peak obtained with the *Standard solution* (0.02%).

Related substances 2. Proceed as described in *Gas chromatography (5.2.17.5)*. Use a chromatograph equipped with a flame ionization detector; capillary column with 30 m of length and 0.32 mm of internal diameter, in melted silica, filled with poly(methyl(95)phenyl(5))siloxane, with 0.5 µm of film thickness. The injector temperature must be adjusted to 280 °C, the detector temperature to 300 °C, and the column temperature must be programmed to start at 130 °C for four minutes, with increase from 130 °C to 180 °C at 20 °C per minute and maintain at 200 °C for five minutes (total: 11.5 minutes). Use purified helium as carrier gas, flow at 1 mL/minute.

Internal standard solution: dissolve 20.0 mg of lauric acid in methylene chloride and dilute to 100 mL with the same solvent.

Solution (1): dissolve 1.0 g of the sample in 10 mL of a 84 g/L sodium hydroxide solution and extract with two portions of 10 mL of methylene chloride. Combine the liquids and wash with 5 mL of water, filter with sodium sulfate anhydrous. Wash the filter with methylene chloride. Evaporate in water bath at 50-60 °C to obtain a volume between 1 mL and 5 mL. Add 1.0 mL of the *Internal standard solution* and dilute to 10 mL with methylene chloride.

Solution (2): dissolve 20 mg of aniline in methylene chloride and dilute to 100 mL with the same solvent.

Solution (3): dissolve 20 mg of p-toluidine in methylene chloride and dilute to 100 mL with the same solvent.

Solution (4): dilute 0.50 mL of *Solution (2)*, 0.50 mL of *Solution (3)* and 10.0 mL of the *Internal standard solution* to 100 mL with methylene chloride.

Procedure: inject the volume of 2 µL of the *Solution (1)* and *Solution (4)* into the gas chromatograph, using a 1:10 flow division. Calculate the ratio (R1) between the area under the peak corresponding to aniline and the area under the peak corresponding to the internal standard in the chromatogram

obtained with *Solution (4)*; calculate the ratio (R2) between the area under the peak corresponding to aniline and the area under the peak corresponding to the internal standard in the chromatogram obtained with *Solution (1)*. R2 is not higher than R1 (10 ppm). Calculate the ratio (R3) between the area under the peak corresponding to p-toluidine and the area under the peak corresponding to the internal standard in the chromatogram obtained with *Solution (4)*; calculate the ratio (R4) between the area under the peak corresponding to p-toluidine and the area under the peak corresponding to the internal standard in the chromatogram obtained with *Solution (1)*. R4 is not higher than R3 (10 ppm).

Heavy metals (5.3.2.3). Suspend 1 g of the sample in 15 mL of distilled water and add an amount of 6 M ammonium hydroxide until dissolution. Add acetic acid RS until the mixture gets slightly acidic to litmus paper and add 2 mL of the same acid. Proceed as described in *Method I*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). No more than 0.2%.

Residue by incineration (5.2.10). No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 100 mg of sample, transfer to a 250 mL Erlenmeyer flask and dissolve in 50 mL of water with heating. Titrate with 0.1 M sodium hydroxide VS, determining the endpoint potentiometrically. Each mL of 0.1 M sodium hydroxide VS is equivalent to 13.714 mg of $C_7H_7NO_2$.

PACKAGING AND STORAGE

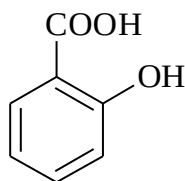
In tightly closed and opaque containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Topical protector.

SALICYLIC ACID***Acidum salicylicum***C₇H₆O₃; 138.12

salicylic acid; 00340

2-hydroxybenzoic acid

[69-72-7]

Contains no less than 99.5% and no more than 101.0% of C₇H₆O₃, calculated in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or white or colorless crystals in the shape of fine needles. The synthetic product is white. When obtained from methyl salicylate of natural origin, it may have a yellow or pink color.

Solubility. Slightly soluble in water, freely soluble in ethyl alcohol, moderately soluble in fatty oils.

Physicochemical constants.

Melting range (5.2.2): 158 °C to 161 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of salicylic acid CRS, prepared identically.

B. The retention time of the main peak from the *Solution (1)* chromatogram, obtained in *Related substances*, corresponds to the one from the main peak of the *Solution (2)*.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 270 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow of 0.5 mL/minute.

Mobile phase: mixture of glacial acetic acid, methyl alcohol and water (1:40:60). Make the necessary adjustments with glacial acetic acid.

Solution (1): weigh, accurately, about 0.5 g of sample, transfer to a 100 mL volumetric flask, dissolve and complete the volume with *Mobile phase*.

Solution (2): prepare a 5 mg/mL salicylic acid solution CRS in *Mobile phase*.

Solution (3): prepare a 0.1 mg/mL phenol solution in *Mobile phase*.

Solution (4): prepare a 0.25 mg/mL 4-hydroxyisophthalic acid solution in *Mobile phase*.

Solution (5): prepare a 0.5 mg/mL 4-hydroxybenzoic acid solution in *Mobile phase*.

Solution (6): dilute 1 mL of *Solution (3)* to 10 mL with *Mobile phase*.

Solution (7): dilute 1 mL of *Solutions (3), (4) and (5)* to 10 mL with *Mobile phase*.

Solution (8): dilute 1 mL of *Solution (7)* to 10 mL with *Mobile phase*.

Inject, separately, 10 µL of *Solutions (6) and (7)* and record the chromatogram. The relative retention times in relation to phenol are approximately 0.70 for 4-hydroxybenzoic acid and 0.90 for 4-hydroxyisophthalic acid. The resolution between 4-hydroxyisophthalic acid and phenol is not inferior to 1.0. The third peak obtained with *Solution (7)* corresponds to the main peak of phenol in the chromatogram obtained with *Solution (6)*.

Inject 10 µL of *solution (8)*. Adjust the detector sensitivity so that the main peak height is, at least, 70% of the total of the full scale.

Procedure: inject, separately, 10 µL of the *Solution (1)* and of *Solution (8)*, record the chromatograms and measure the areas of the peaks. The area under the peak related to 4-hydroxybenzoic acid, obtained with *Solution (1)*, is not greater than the area under the peak obtained with *Solution (8)* (0.1%). The area under the peak related to 4-hydroxyisophthalic acid, obtained with *Solution (1)*, is not greater than the area under the peak obtained with *Solution (8)* (0.05%). The area under the peak related to phenol, obtained with *Solution (1)*, is not greater than the area under the peak obtained with *Solution (8)* (0.02%). Any other impurity recorded in the chromatogram of *Solution (1)* does not have a greater area than the area obtained with the peak of 4-hydroxyisophthalic acid from *Solution (8)* (0.05%). The sum of the areas under all peaks obtained with the *Solution (1)*, except that under the solvent peak, is not more than two times the area under the 4-hydroxybenzoic acid peak, obtained with *Solution (8)* (0.2%). Do not consider peaks with an area less than 0.01 times that presented by the main peak in the chromatogram obtained with *Solution (8)*.

Chlorides (5.3.2.1). Dissolve, by heating, 1.0 g of the sample in 50 mL of distilled water. Allow it to cool, filter to a 50 mL volumetric flask, wash the filter until completing 50 mL in the flask. A volume of 25 mL of filtrate does not have more chloride than the one corresponding to 0.20 mL of 0.01 M hydrochloric acid. No more than 0.014% (140 ppm).

Sulfates (5.3.2.2). Add to 25 mL of the filtrate, obtained in *Chlorides*, two drops of hydrochloric acid and 3 mL of barium chloride RS. The preparation obtained is not more opalescent than 0.2 mL of 0.005 M sulfuric acid in 25 mL of the solution with two drops of hydrochloric acid RS and 3 mL of barium chloride. No more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Dissolve 1.0 g in 15 mL of ethyl alcohol and add 15 mL of water. Proceed as described in *Limit test of heavy metals, Method II*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.05%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.5 g of the sample and dissolve in 25 mL of ethyl alcohol, neutralized in advance with 0.1 M sodium hydroxide. Use phenolphthalein TS as indicator and titrate with 0.1 M sodium hydroxide VS, until a pink color appears. Each mL of 0.1 M sodium hydroxide VS is equivalent to 13.812 mg of $C_7H_6O_3$.

PACKAGING AND STORAGE

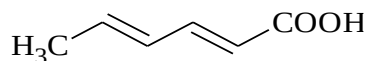
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Keratolytic.

SORBIC ACID*Acidum sorbicum* $\text{C}_6\text{H}_8\text{O}_2$; 112.13

sorbic acid; 00346

(2*E*,4*E*)-2,4-hexadienoic acid

[110-44-1]

Contains no less than 99.0% and no more than 101.0% of $\text{C}_6\text{H}_8\text{O}_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 132 °C to 136 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of sorbic acid CRS, prepared identically.

B. Dissolve 50 mg of the sample in water and complete volume to 250 mL. Dilute 2 mL of this solution to 200 mL with 0.1 *M* hydrochloric acid. In the ultraviolet absorption spectrum **(5.2.14)**, at the range of 230 nm to 350 nm of the solution obtained, there is a maximum peak at 264 nm (± 2 nm). The absorbance at 264 nm is of 0.43 to 0.51.

PURITY TESTS

Aspect of the preparation. The 5% (w/v) sample preparation in ethyl alcohol is clear **(5.2.25)** and colorless **(5.2.12)**.

Limit of aldehydes. Dissolve 1 g of the sample in a mixture with 50 mL of isopropyl alcohol and 30 mL of water. Adjust the pH of the solution to 4.0 with 0.1 *M* hydrochloric acid or 0.1 *M* sodium hydroxide and complete the volume to 100 mL with water. Add to 10 mL of the solution 1 mL of bleached fuchsine RS and allow to stand for 30 minutes. The color produced must not be more intense than the one obtained in solution prepared by the addition of 1 mL of bleached fuchsine RS in a mixture of 1.5 mL of standard acetaldehyde solution (100 ppm $\text{C}_2\text{H}_4\text{O}$), 4 mL of isopropyl alcohol and 4.5 mL of water. (0.15%, calculated as $\text{C}_2\text{H}_4\text{O}$).

Heavy metals (5.3.2.3). Use *Method II*. No more than 0.001% (10 ppm).

Water (5.2.20.1). Determine in 2 g of substance. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of substance. No more than 0.2%.

ASSAY

Accurately weigh 0.1 g of the sample and dissolve in 20 mL of ethyl alcohol. Titrate with 0.1 *M* sodium hydroxide VS, using 0.2 mL of phenolphthalein TS as indicator, until turning rosy. Each mL of 0.1 *M* sodium hydroxide VS is equivalent to 11.213 mg of C₆H₈O₂.

PACKAGING AND STORAGE

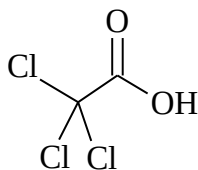
In tightly closed containers, protected from light and excessive heat.

LABELING

Comply with current legislation

CATEGORY

Antimicrobial preservative, especially against fungi and yeasts.

TRICHLOROACETIC ACID*Acidum trichloraceticum* $C_2HCl_3O_2$; 163.39

trichloroacetic acid; 00366

2,2,2-trichloroacetic acid

[76-03-9]

Contains no less than 98.0% and no more than 100.5% of $C_2HCl_3O_2$.

DESCRIPTION

Physical characteristics. White, crystalline mass or colorless very deliquescent crystals.

Solubility. Very soluble in water and in ethyl alcohol.

IDENTIFICATION

A. Add to 0.5 mL of the solution obtained in *Limit test for chlorides* 2 mL of pyridine and 5 mL of concentrated sodium hydroxide RS solution. Shake vigorously and heat in water bath at 60 °C to 70 °C for five minutes. The superior phase presents an intense red color.

B. The solution obtained in *Limit test for chlorides* is strongly acidic.

PURITY TESTS

Chlorides (5.3.2.1). Dissolve 2.5 g of the sample in water and complete to 25 mL with the same solvent. Pipette 5 mL of this solution and complete the volume to 15 mL with water. The solution complies with the *Limit test for chlorides*. No more than 0.01% (100 ppm).

Residue by incineration (5.2.10). No more than 0.1%, determined in 1.0 g of the sample.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.150 g of the sample in 20 mL of water. Titrate with 0.1 M sodium hydroxide VS using phenolphthalein TS as indicator. Each mL of 0.1 M sodium hydroxide VS corresponds to 16.339 mg of $C_2HCl_3O_2$.

PACKAGING AND STORAGE

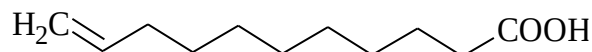
In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

It has caustic action.

UNDECYLENIC ACID*Acidum undecylenicum*

C₁₁H₂₀O₂; 184.28
undecylenic acid; 00367
10-undecenoic acid
[112-38-9]

Contains no less than 97.0% and no more than 102.0% of C₁₁H₂₀O₂.

DESCRIPTION

Physical characteristics. Colorless or pale yellow liquid, or white or pale yellow crystalline mass, depending on the temperature.

Solubility. Practically insoluble in water, freely soluble in ethyl alcohol, fatty oils and essential oils.

Physicochemical constants.

Relative density (5.2.5): 0.910 to 0.913.

Congearing temperature (5.2.4): between 21 °C and 24 °C.

Refractive index (5.2.6): between 1.447 and 1.450, determined at (25.0 ± 0.5) °C.

IDENTIFICATION

Dissolve 0.1 g of the sample in a mixture of 2 mL of *M* sulfuric acid and 5 mL of glacial acetic acid. Add, drop by drop, 0.25 mL of potassium permanganate RS. The potassium permanganate solution gets discolored.

PURITY TESTS

Water-soluble acids. Add 5 mL of water to 5 mL of the sample, homogenize and filter the aqueous layer in filter paper moistened with water. Add one drop of methyl orange TS and titrate with 0.01 *M* sodium hydroxide VS. No more than 1 mL of 0.01 *M* sodium hydroxide VS is necessary to obtain a color identical to the one produced by one drop of methyl orange TS in 5 mL of water.

Iodine value (5.2.29.10). 131 to 138.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.001% (10 ppm).

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.15%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.75 g of the sample and dissolve in 50 mL of ethyl alcohol. Titrate with 0.1 M sodium hydroxide VS, using 0.2 mL of phenolphthalein TS as indicator, until turning rosy persistently for no less than 30 seconds. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium hydroxide VS is equivalent to 18.428 mg of $C_{11}H_{20}O_2$.

PACKAGING AND STORAGE

In tightly closed, non-metallic containers, protected from light.

LABELING

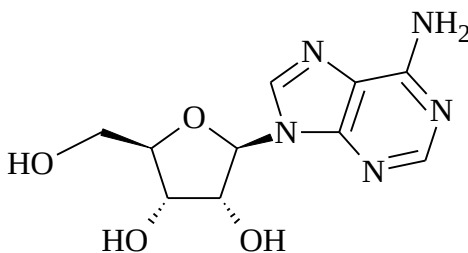
Comply with current legislation.

THERAPEUTIC CLASS

Topical antifungal.

ADENOSINE

Adenosinum



$C_{10}H_{13}N_5O_4$; 267.25

adenosine; 00419

9-β-D-Ribofuranosyl-9H-purine-6-amine
[58-61-7]

Contains no less than 99.0% and no more than 101.0% of $C_{10}H_{13}N_5O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Moderately soluble in water, soluble in heated water, practically insoluble in ethyl alcohol. Dissolves in diluted mineral acids.

Physicochemical constants.

Melting range (5.2.2): 233 °C to 238 °C.

Specific optical rotation (5.2.8): -45 to -49, in relation to the desiccated substance. Determine in solution at 2.5% (w/v) in *M* hydrochloric acid. Examine within 10 minutes after the preparation.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of adenosine CRS, prepared identically.

PURITY TESTS

Acidity and alkalinity. Disperse 2.5 g of the sample in 50 mL of water, heat until ebullition, wait until cool and filter at vacuum. Dilute to 100 mL with water. Add to 10 mL of the filtrate 0.1 mL of bromocresol purple TS and 0.3 mL of 0.01 *M* hydrochloric acid VS. The solution turns yellow. Add 0.4 mL of 0.01 *M* sodium hydroxide VS. The solution turns blue-violet.

Chromatographic purity. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 μm), kept at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Sulfate buffer: dissolve 6.8 g of potassium sulfate monobasic and 3.4 g of tetrabutylammonium sulfate in 800 mL of water, adjust to pH 6.5 with 2 M potassium hydroxide and complete the volume to 1000 mL with water. Homogenize.

Mobile phase: mixture of *Sulfate buffer* and 0.0001% (w/v) sodium azide solution (60:40). Filter and degas.

Solution (1): weigh, accurately, about 20 mg of adenosine CRS and 20 mg of inosine, transfer to a volumetric flask and complete the volume to 100 mL with *Mobile phase*.

Solution (2): prepare a sample solution in the concentration of 1 mg/mL in mobile phase.

Procedure: inject, separately, 20 µL of each solution and record the chromatograms for no less than twice the retention time of the main peak. The retention times related to adenosine, which retention time is of approximately one minute, are about 0.29 for uridine, 0.34 for adenine, 0.42 for inosine and 0.42 for guanosine. Determine the percentage of each impurity found in *Solution (2)*, in which the individual content of guanosine, inosine and uridine are no more than 0.1% in relation to adenosine. The content of adenine is no more than 0.2% in relation to adenosine. The sum of all areas under all peaks obtained, except the main peak, is inferior to 0.5%. The test will only be valid if the resolution between the peaks obtained with *Solution (1)* is no less than 1.5 and the tailing factor is no more than 2.5. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2%.

Ammonia. Suspend 0.5 g of the sample in 10 mL of water, shake for 30 seconds and filter. Dilute the filtrate to 15 mL with water, homogenize and use this solution as sample. Dilute 1 mL of 0.0314% (w/v) ammonia chloride solution in 100 mL of water. As standard solution, use 2 mL of the previous solution and add 13 mL of water. Add 0.3 mL of alkaline potassium tetraiodomercurate(II) solution, in both solutions, homogenize and allow to stand for five minutes. The yellow color produced in the sample must be, at maximum, equal to that of the standard. No more than 0.0004% (4 ppm).

Chlorides (5.3.2.1). Suspend 0.2 g of the sample in 10 mL of water, shake for 30 seconds and filter. Dilute the filtrate to 15 mL with water. The solution complies with the *Limit test for chlorides*. No more than 0.007% (70 ppm).

Sulfates. Suspend 0.75 g of the sample in 15 mL of water, shake for 30 seconds and filter. Use the filtrate as sample solution. Prepare standard solution with 0.30 mL of 0.005 M sulfuric acid in 15 mL of water. Add to the standard and sample solutions 2 mL of barium chloride RS, 1 mL of 3 M hydrochloric acid, 30 mL of water, and homogenize. The turbidity of sample solution must not be greater than that of the standard. No more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Use *Method II*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of sample. Dry in oven at 105 °C for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.4.5)*. Dissolve 0.1 g of the previously desiccated sample in 20 mL of glacial acetic acid and add 30 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 26.724 mg of $C_{10}H_{13}N_5O_4$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-arrhythmic.

AGAR-AGAR

Agar

agar-agar; 09547

Agar

[9002-18-0]

Dry, colloidal, hydrophilic substance extracted from *Gelidium cartilagineum* L algae. (Gaillon) – GELIDIACEAE, *Gracilaria confervoides* L. (Greville) – SPHAEROCOCCACEAE and related red algae (Class RHODOPHYCEAE).

MACROSCOPIC DESCRIPTION

Presented in bundles, consisting of membranous strips agglutinated or in the form of granules or flakes. May present orange-yellow, yellow-gray, slightly yellow color or be colorless. It is resistant when humid and brittle when dry.

MICROSCOPIC DESCRIPTION

In assembly on water, agar presents itself as granular and somewhat filamentous; fragments of spike from sponges and some frustules from diatoms may be present. Eventually, according to the origin, there may be frustules from *Arachnoidiscus ehrenbergii* Baillon, which are characterized by their disc shape with 100 µm to 300 µm of diameter.

MICROSCOPIC DESCRIPTION OF POWDER

Pulverized agar presents a white, yellow-white or slightly yellow color. In assembly in hydrated chloral, its fragments are clear, more or less granular, striated and angular, and may occasionally have frustules from diatoms.

IDENTIFICATION

A. Dissolve by heating 0.1 g of the sample in 50 mL of water. Cool down. Carefully add to 1 mL of mucilage 3 mL of water, to form two different layers. Add 0.1 mL of 0.05 M iodine solution. A violet-brown color develops at the interface. Shake the mixture. The liquid acquires a pale yellow color.

B. Add 0.5 mL of hydrochloric acid to 5 mL of the mucilage obtained in the test **A.** from *Identification*. Heat for 30 minutes in water bath. Add 1 mL of 0.67% (w/v) barium chloride solution. White turbidity develops after 30 minutes.

C. Heat 0.5 g with 50 mL of water in water bath until dissolution. Only a few fragments remain insoluble. When cooling, the solution gels between 35 °C and 30 °C. Heat the gel obtained in water bath. There is no liquefaction at temperature below 85 °C.

PURITY TESTS

Intumescence index (5.4.1.11). Determine over sample powdered to a semi-fine powder (5.2.11). The intumescence index is no less than 10 and differs from no more than 10% of the value declared on the label.

Insoluble foreign materials. Weigh 5 g of the sample powdered to a semi-fine powder (**5.2.11**), add 100 mL of water and 14 mL of hydrochloric acid RS. Carefully boil for 15 minutes, shaking frequently. Filter the hot liquid in a sintered glass funnel tared in advance, wash the filter with hot water, and dry at temperature between 100 °C and 105 °C. No more than 1.0%.

Gelatin. Weigh 1 g of the sample, add 100 mL of water and heat in water bath until dissolution. Allow to cool down to 50 °C. Add to 5 mL of the previous solution 5 mL of 10% (w/v) picric acid. No turbidity is developed after 10 minutes.

Determination of water (5.4.1.4). Determine in 1 g of the sample powdered to a semi-fine powder (**5.2.11**), in oven at temperature between 100 °C and 105 °C, until constant weight. No more than 20.0%.

Total ash (5.4.1.5.1). Not greater than 5.0% in relation to the desiccated substance.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

In tightly closed containers protected from humidity.

LABELING

Comply with current legislation.

CATEGORY

Adjuvant (thickener).

STERILE WATER FOR IRRIGATION

Sterilis aqua irrigandi

Sterile water for irrigation is prepared with sterile *Water for injection* adequately packaged. It contains no antimicrobial substances or addition of other substances.

PURITY TESTS

Oxidizable substances. Boil 100 mL of the sample with 10 mL of 2 M sulfuric acid. For sterile water for irrigation, in vials with volume lower than 50 mL, add 0.4 mL of 0.02 M potassium permanganate and boil for five minutes; when the volume in the vial is of 50 mL or higher, add 0.2 mL of 0.02 M potassium permanganate and boil for five minutes. If a precipitate forms, cool in ice bath down to room temperature and filter in sintered filter. The pink color does not disappear completely.

Water conductivity (5.2.24). Complies with the test.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.25 EU/mL of sample.

PACKAGING AND STORAGE

Preserve in glass or plastic containers for single dose. Glass containers are preferably of type I or type II glass (6.1). The containers must store volumes of more than 1000 mL and be designed to allow quick emptying.

LABELING

Comply with current legislation. The label must indicate that there was no addition of antimicrobial agents or other substances. The designations "Only for irrigation" and "Not for injection" appear highlighted on the label.

Note: for microbiological recommendations, please refer to *Water for pharmaceutical use* (5.5.3.6).

WATER FOR INJECTION

Aqua ad injectabile

H₂O; 18.02

water for injection; 09320

Water

[7732-18-5]

Water for injection is the ingredient used in the preparation of drugs for parenteral administration, as a vehicle, in the dissolution or dilution of substances or preparations. Other examples of pharmaceutical applications are: manufacturing of active ingredients for parenteral use, for final washing of equipment, tubes and containers used in parenteral preparations, and in cleaning certain equipment items.

Water for injection is obtained by distillation of adequately treated water, in equipment which parts in contact with water are of neutral glass, quartz or another appropriate material. It can also be obtained through a process equivalent or superior to distillation, in the removal of chemical contaminants, microorganisms and bacterial endotoxins. The process for obtaining must be validated.

To ensure that the water meets the quality requirements demanded, its production must be monitored, through validated procedures, for parameters of electric conductivity, total organic carbon, endotoxins, and microbial count.

Sterilized water for injection. Sterilized water for injection is the *water for injection* that, after sterilization, was stored in inert containers, such as in polished 316L stainless steel, kept closed, at temperature of 80 °C to 85 °C and under recirculation, for a maximum period of 24 hours, in such conditions that ensure compliance with the bacterial endotoxins test. Sterilized water is free from addition of any substance.

DESCRIPTION

Physical characteristics. Clear, colorless, tasteless and odorless liquid.

PURITY TESTS

Complies with the tests described in *Purity tests* on the *Purified water* monograph.

Sterilized water for injection complies with the tests described in *Purity tests* on the *Purified water* monograph and with the additional test presented next.

Contamination by particles: subvisible particles (5.1.7.1). Complies with test 1 or 2, depending on the volume of the containers.

BIOLOGICAL SAFETY TESTS

Count of total number of heterotrophic bacteria (5.5.3.6.1). Complies with the test. No more than 10 CFU/100 mL.

Research on total and fecal coliforms (5.5.3.6.2). Complies with the test.

Research on *Pseudomonas aeruginosa* (5.5.3.6.3). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.25 EU/mL.

Sterilized water for injection also complies with the Sterility test (5.5.3.2.1).

PACKAGING AND STORAGE

Stored and distributed in adequate conditions to ensure the physical-chemical and microbiological properties required are maintained.

LABELING

Comply with current legislation.

PURIFIED WATER

Aqua purificata

H₂O; 18.02

purified water; 09879

Water

[7732-18-5]

Purified water is drinking water that has undergone some type of treatment to remove possible contaminants and meet the purity requirements established in this monograph. It is prepared by distillation, ion exchange, reverse osmosis or another adequate process. It must be free from addition of any dissolved substances. It is generally used in the preparation of drugs that do not require sterile or pyrogen-free water, targeted at non-parenteral use.

DESCRIPTION

Physical characteristics. Clear, colorless, tasteless and odorless liquid.

PURITY TESTS

Acidity or alkalinity. Add to 20 mL of the sample 0.05 mL of phenol red TS. If the solution is yellow, it turns red, with the addition of 0.1 mL of 0.01 M sodium hydroxide; if it is red, it turns yellow, with the addition of 0.15 mL of 0.01 M hydrochloric acid.

Total organic carbon (5.2.30). Alternatively, replaces the test for oxidizable substances. No more than 0.50 mg/L.

Oxidizable substances. Boil 100 mL of the sample with 10 mL of 1 M sulfuric acid. Add 0.2 mL of 0.02 M potassium permanganate VS and leave in ebullition for five minutes. The remaining solution is faintly rosy.

Water conductivity (5.2.24). No more than 1.3 µS/cm at 25.0 °C. The user must define the adequate maximum limit for the specific application, as described in *Water for pharmaceutical use (chapter 8.5, volume 1)*. Alternatively, it replaces the tests for *Ammonium, Calcium and Magnesium, Chlorides, Nitrates and Sulfates*.

Ammonium. Add 1 mL of alkaline mercuric potassium iodide RS1 to 20 mL of the sample. After five minutes, examine the solution in the vertical axis of the tube. The solution is not more intensely stained than the standard by the addition of 1 mL of alkaline mercuric potassium iodide RS1 to a mixture of 4 mL of standard ammonium solution (1 ppm NH₄) and 16 mL of ammonia-free water. No more than 0.00002% (0.2 ppm).

Calcium and magnesium. Add 2 mL of ammonium chloride buffer pH 10.0, 0.5 mL of eriochrome black T and 5 µL of 0.05 M sodium edetate to 100 mL of the sample. A clear blue color is produced. No more than 1 ppm.

Chlorides. Add 1 mL of nitric acid RS and 0.2 mL of 0.1 M silver nitrate to 10 mL of the sample. The solution does not present changes in the appearance for at least 15 minutes.

Nitrates. Transfer 5 mL of sample to a test tube immersed in icy water, add 0.4 mL of 10% (w/v) potassium chloride and 0.1 mL of 0.1% (w/v) diphenylamine solution. Drip, under agitation, 5 mL of nitrogen-free sulfuric acid. Transfer the tube to a water bath at 50 °C. After 15 minutes, any blue color developed in the solution is not more intense than that of the standard solution, prepared concomitantly and in the same manner, using a mixture of 4.5 mL of nitrate-free water and 1 mL of standard nitrate solution 2 ppm in NO₃, recently prepared. No more than 0.00002% (0.2 ppm).

Sulfates. Add 0.1 mL of 2 M hydrochloric acid and 0.1 mL of 6.1% (w/v) aqueous barium chloride solution to 10 mL of the sample. The solution does not present changes in the appearance for at least one hour.

BIOLOGICAL SAFETY TESTS

Count of total number of heterotrophic bacteria (5.5.3.6.1). Complies with the test. No more than 100 CFU/mL.

Research on total and fecal coliforms (5.5.3.6.2). Complies with the test.

Research on *Pseudomonas aeruginosa* (5.5.3.6.3). Complies with the test.

The modality of sterile purified water, used in the preparation of eyedrops and other processes that cannot go through final sterilization by heat or filtration, must also comply with the sterility test (5.5.3.2.1).

PACKAGING AND STORAGE

In inert containers, such as in glass or polished 316L stainless steel, adequately identified, which ensure the physical-chemical and microbiological properties required. If it is necessary to stock, the purified water must be stored and distributed in adequate conditions to prevent microbial growth and avoid any other contamination.

LABELING

Comply with current legislation.

ULTRAPURE WATER

Aqua ultra purificata

H₂O; 18.02

ultrapure water; 09880

Water

[7732-18-5]

Ultrapure water is purified water that has undergone additional treatment to remove potential contaminants and meet the purity requirements established in this monograph. It is prepared by the complementation of a group of processes, such as distillation, ion exchange, reverse osmosis, among others. It has no dissolved substance. In general, it is used in the preparation of drugs targeted at non-parenteral use, but that require water with high purity, or in most laboratory test procedures that require readings at low concentrations or where the water purity may affect the sensitivity, reproducibility or robustness of the analytical method.

DESCRIPTION

Physical characteristics. Clear, colorless, tasteless and odorless liquid.

PURITY TESTS

Water conductivity (5.2.24). No more than 0.055 µS/cm at 25.0 °C.

Total organic carbon (5.2.30). No more than 0.50 mg/L. **Note:** *this test is optional. It must be employed if the specific application requires such control.*

BIOLOGICAL SAFETY TESTS

Count of total number of heterotrophic bacteria (5.5.3.6.1). Complies with the test. No more than 10 CFU/100 mL.

Research on total and fecal coliforms (5.5.3.6.2). Complies with the test.

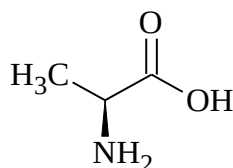
Research on *Pseudomonas aeruginosa* (5.5.3.6.3). Complies with the test.

PACKAGING AND STORAGE

In polymer or glass containers, according to the application, that ensure the physical-chemical and microbiological properties required. If it is necessary to stock, the ultrapure water must be stored and distributed within 24 hours, at maximum, and in adequate conditions to prevent microbial growth and avoid any other contamination.

LABELING

Identify correctly the container targeted at this type of water.

ALANINE***Alaninum***C₃H₇NO₂; 89.09

alanine; 00451

L-Alanine

[56-41-7]

Contains no less than 98.5% and no more than 101.5% of C₃H₇NO₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder or colorless crystals.

Solubility. Freely soluble in water, very slightly soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +13.7 to +15.1, in relation to the desiccated substance. Determine in 10% (w/v) solution in 6 M hydrochloric acid.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of alanine CRS, prepared identically.

B. The main stain of the chromatogram from *Solution (2)*, obtained in *Substances detectable by ninhydrin*, corresponds in position, color and intensity to the one obtained with *Solution (4)*.

PURITY TESTS

Aspect of the preparation. Dissolve 2.5 g of the sample in water and complete the volume to 50 mL with the same solvent. Dilute 10 mL of this solution to 20 mL with water. The preparation obtained is clear (5.2.25) and no more intensely stained than the *Color reference solution (5.2.12)*, prepared as described next.

Color reference solution: mix 2.4 mL of ferric chloride base solution, 1 mL of cobalt II chloride base solution, 0.4 mL of cupric sulfate base solution and 6.2 mL of 1% (v/v) hydrochloric acid solution. Mix 5 mL of the solution obtained with 95 mL of 1% (v/v) hydrochloric acid.

pH (5.2.19). 5.5 to 7.0. Determine in solution at 5% (w/v).

Substances detectable by ninhydrin. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of water, glacial acetic acid and butyl alcohol (20:20:60) as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in water.

Solution (2): dilute 1 mL of *Solution (1)* to 50 mL with water.

Solution (3): dilute 5 mL of *Solution (2)* to 20 mL with water.

Solution (4): 0.2 mg/mL alanine solution CRS in water.

Solution (5): dissolve 10 mg of alanine CRS and 10 mg of glycine CRS in water and dilute to 25 mL with the same solvent.

Develop the chromatogram. Remove the plate and allow to air dry. Nebulize with ninhydrin RS. Heat the plate at 105 °C for 15 minutes and examine immediately. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (3)* (0.5%). The test is only valid if the chromatogram obtained with *Solution (5)* presents two clearly separated main stains.

Ammonium. Prepare a small chamber using two watch glasses with 60 mm of diameter, placed edge to edge. Adhere to the inner wall of the upper watch glass, with a few drops of water, a strip of red litmus paper with 5 mm × 5 mm. In the lower glass, suspend 50 mg of the sample, finely powdered, in 0.5 mL of water. Add 0.3 g of magnesium oxide, mix quickly with a glass rod, and close the chamber joining the two watch glasses. Heat at 40 °C for 15 minutes. The litmus paper must not acquire a more intense blue color than a strip of red litmus paper from a preparation conducted simultaneously, and in the same conditions, with 0.1 mL of 0.0296% (w/v) ammonium chloride, 0.5 mL of water and 0.3 g of magnesium oxide solution. No more than 0.02% (200 ppm).

Chlorides (5.3.2.1). No more than 0.05% (500 ppm).

Iron (5.3.2.4). Use *Method III*. No more than 0.003% (30 ppm).

Heavy metals (5.3.2.3). Use *Method I*. No more than 0.0015% (15 ppm).

Sulfates (5.3.2.2). No more than 0.03% (300 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at temperature between 100 °C and 105 °C for three hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium* (**5.3.3.5**). Accurately weigh about 80 mg of the sample and dissolve in 3 mL of anhydrous formic acid. Add 30 mL of glacial acetic acid and titrate with 0.1 M perchloric acid VS. Determine the endpoint potentiometrically or use 0.1 mL of 1-naphtholbenzein TS until the color changes to green. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 8.909 mg of C₃H₇NO₂.

PACKAGING AND STORAGE

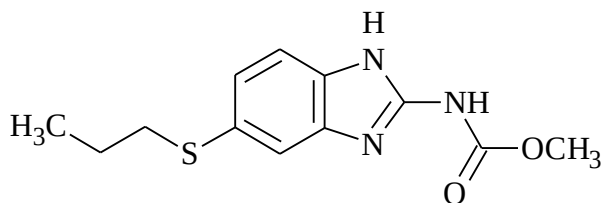
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Amino acid.

ALBENDAZOLE***Albendazolum***

$C_{12}H_{15}N_3O_2S$; 265.33

albendazole; 00458

Methyl ester of [6-(propylthio)-1*H*-benzimidazol-2-yl]carbamic acid

[54965-21-8]

Contains no less than 98.0% and no more than 102.0% of $C_{12}H_{15}N_3O_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder, unctuous to tact.

Solubility. Practically insoluble in water and in ethyl alcohol, freely soluble in anhydrous formic acid, soluble in glacial acetic acid, very slightly soluble in isopropyl alcohol. Very slightly soluble in 0.1 *M* hydrochloric acid and insoluble in 0.1 *M* sodium hydroxide.

Physicochemical constants.

Melting range (5.2.2): 208 °C to 210 °C.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are conducted.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of albendazole CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14) in the range of 200 nm to 400 nm of the sample solution obtained in method B. from Assay, there are maximum absorption points identical to those observed in the spectrum of the standard solution.

C. The main stain of the chromatogram from *Solution (4)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (1)*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of chloroform, ethyl ether and glacial acetic acid (60:10:10) as mobile

phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 50 mg of albendazole CRS in glacial acetic acid and complete the volume to 10 mL with the same solvent.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with glacial acetic acid.

Solution (3): dissolve 100 mg of the sample in glacial acetic acid and complete the volume to 10 mL with the same solvent.

Solution (4): dilute 5 mL of *Solution (3)* to 10 mL with glacial acetic acid.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (3)*, different from the main stain, is not more intense than the main stain obtained with *Solution (2)* (0.5%).

Loss on drying (5.2.9.1). Determine in 2 g of sample. Dry in oven at 105 °C, for four hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrimetric determination in non-aqueous medium (5.3.3.5)*. Accurately weigh about 0.4 g of the sample previously desiccated and dissolve in 30 mL of glacial acetic acid. Heat if necessary. Cool and add five drops of methylrosanilinium chloride TS. Titrate with 0.1 M perchloric acid VS until turning to an emerald green color. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 26.533 mg of C₁₂H₁₅N₃O₂S.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh, accurately, about 25 mg of sample and dissolve in 25 mL of 2% (w/v) hydrochloric acid in methyl alcohol. Complete the volume to 50 mL with water. Transfer 5 mL to a 50 mL volumetric flask and complete the volume with 0.1 M hydrochloric acid. Dilute, successively, in 0.1 M sodium hydroxide, until concentration of 0.0005% (w/v). Prepare a standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 309 nm, using 0.1 M sodium hydroxide for zero adjustment. Calculate the content of C₁₂H₁₅N₃O₂S in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anthelmintic.

ALBENDAZOLE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{12}H_{15}N_3O_2S$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 10 mg of albendazole to a 50 mL volumetric flask and add 25 mL of 2% (v/v) hydrochloric acid in methyl alcohol. Shake for 10 minutes, complete the volume with the same solvent and filter. Dilute the filtrate to a concentration of 0.001% (w/v) with the same solvent. Prepare a standard solution using albendazole CRS in the same concentration. In the ultraviolet absorption spectrum (**5.2.14**) of the sample solution, in the range of 200 nm to 400 nm, there are maximum and minimum points only at the same wavelengths of the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). No more than 15 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter, transfer 10 mL to a 250 mL volumetric flask and complete the volume with 0.1 M sodium hydroxide. Transfer 90 mg of albendazole CRS to a 250 mL volumetric flask, add 10 mL of 2% (v/v) hydrochloric acid in methyl alcohol and homogenize. Complete the volume with 0.1 M hydrochloric acid. Transfer 5 mL of this solution to a 200 mL volumetric flask and complete the volume with 0.1 M sodium hydroxide. Measure the absorbances at 308 nm and 350 nm, using the same solvent for zero adjustment. Calculate the amount of $C_{12}H_{15}N_3O_2S$ dissolved in the medium, by the formula: $22.5C(Aa/AP)$, where C is the concentration, in $\mu\text{g/mL}$, of albendazole in the standard solution, and Aa and Ap are the differences between the absorbances at 308 nm and 350 nm, obtained for the sample solution and for the standard solution, respectively.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{12}H_{15}N_3O_2S$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 10 mg of albendazole to a 50 mL volumetric flask and add 25 mL of 2% (v/v) hydrochloric acid in methyl alcohol. Shake for 10 minutes, complete the volume with distilled water and filter. Dilute successively to a concentration of 0.0008% (w/v) using 0.1 M sodium hydroxide as solvent. Prepare a standard solution in the same concentration, using the same solvents. Measure the absorbances of the solutions at 308 nm, using 0.1 M sodium hydroxide for zero adjustment. Calculate the amount of $C_{12}H_{15}N_3O_2S$ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: solution of 0.5 g ammonium phosphate monobasic in 1000 mL of mixture of water and methyl alcohol (4:6).

Internal standard solution: weigh, accurately, about 150 mg of parbendazole CRS. Transfer to a 50 mL volumetric flask, add 5 mL of 1% (v/v) sulfuric acid in methyl alcohol and complete the volume with methyl alcohol.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 100 mg of albendazole to a 50 mL volumetric flask, add 5 mL of 1% (v/v) sulfuric acid in methyl alcohol and 20 mL of methyl alcohol. Shake for 15 minutes, complete the volume with methyl alcohol and filter. Transfer 5 mL of the filtrate and 5 mL of the *Internal standard solution* to a 50 mL volumetric flask and complete the volume with methyl alcohol.

Standard solution: weigh, accurately, about 100 mg of albendazole CRS and transfer to a 50 mL volumetric flask, add 5 mL of 1% (v/v) sulfuric acid in methyl alcohol, and complete the volume with methyl alcohol. Transfer 5 mL of this solution and 5 mL of the *Internal standard solution* to a 50 mL volumetric flask and complete the volume with methyl alcohol.

The column efficiency is no less than 4000 theoretical plates/meter. The resolution between albendazole and parbendazole is no less than 2.0. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{12}H_{15}N_3O_2S$ in the sample solution from the responses obtained with the *Standard solution* and the *Sample solution* in relation to the *Internal standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

ALBENDAZOLE ORAL SUSPENSION

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{12}H_{15}N_3O_2S$. Albendazole oral suspension is a mixture of albendazole with one or more coloring, flavoring, buffering, sweetening and preservative agents, in aqueous vehicle.

IDENTIFICATION

Dilute an adequate volume of the suspension in a mixture of methyl alcohol and hydrochloric acid (99:1) to obtain a concentration of 1 mg/mL. Filter, if necessary, transfer 1 mL of the filtrate to a 100 mL volumetric flask, complete the volume with 0.1 M sodium hydroxide and homogenize. In the ultraviolet absorption spectrum **(5.2.14)** of the resulting solution, in the range of 200 nm to 400 nm, there are maximum and minimum points only at the same wavelengths of similar albendazole CRS solution.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.5 to 5.5.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*, using a chromatograph equipped with an ultraviolet detector at 308 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm); *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: dissolve 11 g of monobasic sodium phosphate in 800 mL of water and add 1200 mL of methyl alcohol.

Sample solution: transfer a volume of suspension corresponding to 0.1 g of albendazole to a 100 mL volumetric flask and complete the volume with a mixture of methyl alcohol and hydrochloric acid (99:1). Dilute, successively, to a concentration of 100 µg/mL, using *Mobile phase* as solvent.

Standard solution: weigh, accurately, about 50 mg of albendazole CRS. Transfer to a 50 mL volumetric flask and complete the volume with the mixture of methyl alcohol and hydrochloric acid (99:1). Dilute, successively, until a concentration of 100 µg/mL, using *Mobile phase* as solvent.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is no less than 8000 theoretical plates/meter. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2%.

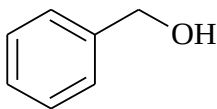
Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{12}H_{15}N_3O_2S$ in the oral suspension, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, at room temperature.

LABELING

Comply with current legislation.

BENZYL ALCOHOL*Alcohol benzylicus*

C₇H₈O; 108,14

benzyl alcohol; 00471

Benzenemethanol

[100-51-6]

Contains no less than 98.0% and no more than 100.5% of C₇H₈O.

DESCRIPTION

Physical characteristics. Oily, clear and colorless liquid.

Solubility. Soluble in water, miscible with ethyl alcohol.

Physicochemical constants.

Relative density (5.2.5): 1.043 to 1.049 g/mL.

Refractive index (5.2.6): 1.538 to 1.541. Determine at 20 °C.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed among sodium chloride or potassium bromide plates, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of benzyl alcohol CRS, prepared identically.

PURITY TESTS**Clarity of the solution.**

Hydrazine solution: transfer 1 g of hydrazine sulfate to a 100 mL volumetric flask, dissolve and complete the volume with water. Homogenize. Allow to stand for four to six hours.

Methenamine solution: transfer 2.5 g of methenamine to a 100 mL volumetric flask, add 25 mL of water and shake until dissolving.

Primary opalescent suspension: transfer 25 mL of the *Hydrazine solution* to the 100 mL volumetric flask with the *Methenamine solution*, complete the volume with water and homogenize. Allow to stand for 24 hours (this suspension is stable for two months, if maintained in a closed glass vial with no defects. The suspended particles may adhere to the glass and must be re-dispersed by shaking before use.)

Opalescence standard: transfer 15 mL of the *Primary opalescent suspension* to a 1000 mL volumetric flask, complete the volume with water and homogenize (this solution must not be used after 24 hours of preparation.)

Reference suspensions: transfer 5 mL of the *Opalescence standard* to a 100 mL volumetric flask, complete the volume with water and homogenize, to obtain the *Reference suspension A*. Transfer 10 mL of the *Opalescence standard* to another 100 mL volumetric flask, top off with water and homogenize, to obtain the *Reference suspension B*.

Sample solution: dissolve 2 g of the sample in 60 mL of water.

Procedure: transfer, separately, the same amount of *Sample solution*, *Reference suspension A*, *Reference suspension B* and water to colorless clear glass tubes, with internal diameter of 15 mm to 25 mm, to obtain, approximately, 40 mm of depth. Compare the solutions, employing a dark background and incident light. The light diffusion must be such that the *Reference suspension A* can be easily distinguished from water and that the *Reference suspension B* can be easily distinguished from the *Reference suspension A*. The *Sample solution* is as clear as water or is no more opalescent than the *Reference suspension A*.

Color of the solution. Transfer, separately, the same amount of *Sample solution*, obtained in *Clarity of the solution*, and water to colorless clear glass tubes, with internal diameter of 15 mm to 25 mm, to obtain, approximately, 40 mm of depth. The *Sample solution* has the same color as water.

Related substances. Proceed as described in *Gas chromatography (5.2.17.5)*. Use a chromatograph equipped with a flame ionization detector; capillary column with 30 m of length and 0.32 mm of internal diameter, in melted silica, filled with macrogol 20000, with 0.5 µm of film thickness. The injector temperature must be adjusted to 200 °C, the detector temperature to 310 °C, and the column temperature must be programmed to start at 50 °C going to 220 °C for 34 minutes, with increases of 5 °C per minute and maintain at 220 °C for 35 minutes (total: 69 minutes). Use purified helium as carrier gas, with linear speed of 25 cm/second.

When benzyl alcohol is not targeted at use in parenteral solutions, prepare the *Solutions (1), (2) and (3)*.

When benzyl alcohol is targeted at use in parenteral solutions, prepare the *Solutions (1), (2) and (4)*.

Sample solution: substance to be assessed.

Solution (1): dissolve 0.10 g of ethylbenzene in the *Sample solution* and dilute to 10 mL with the same solution. Dilute 2.0 mL of this solution to 20 mL with the *Sample solution*.

Solution (2): dissolve 2.0 g of dicyclohexyl in the *Sample solution* and dilute to 10 mL with the same solution. Dilute 2 mL of this solution to 20 mL with the *Sample solution*.

Solution (3): dissolve 0.75 g of benzaldehyde and 0.50 g of cyclohexylmethanol in the *Sample solution* and dilute to 25 mL with the same solution. Add 1 mL of this solution to a mixture of 2 mL of *Solution (1)* and 3 mL of *Solution (2)*, and dilute to 20 mL with the *Sample solution*.

Solution (4): dissolve 0.25 g of benzaldehyde and 0.50 g of cyclohexylmethanol in the *Sample solution* and dilute to 25 mL with the same solution. Add 1 mL of this solution to a mixture of 2 mL of *Solution (1)* and 2 mL of *Solution (2)*, and dilute to 20 mL with the *Sample solution*.

Inject, without the air plug, 0.1 µL replicates of *Solution (3)* or *Solution (4)*. The relative retention times are approximately 0.28 for ethylbenzene, 0.59 para dicyclohexyl, 0.68 for benzaldehyde, and 0.71 for cyclohexylmethanol, in relation to benzyl alcohol (retention time of approximately 26 minutes). The resolution between benzaldehyde and cyclohexylmethanol peaks obtained with the chromatogram of *Solution (3)* or of *Solution (4)* is no less than 3.

Procedure: inject, separately, without the air plug, 0.1 µL of the *Sample solution* and of *Solution (3)* or *Solution (4)*, record the chromatograms and measure the areas under the peaks. If any peak recorded in the chromatogram obtained with the *Sample solution* presents the same retention time of the peaks related to ethylbenzene or dicyclohexyl, make the necessary corrections, subtracting the areas obtained with *Solutions (3)* or *(4)* from the ones obtained with the *Sample solution*. Any peaks recorded in the chromatogram obtained with the *Sample solution* must be included in the assessments for the sum of other peaks. For calculating the impurities, subtract the areas under the peaks obtained with the *Solution (3)* or *(4)* from the corresponding ones obtained with the *Sample solution*.

For benzyl alcohol not targeted at parenteral use, the area under the benzaldehyde peak obtained with the *Sample solution* is not greater than the difference of area obtained with *Solution (3)* (0.15%). The area under the cyclohexylmethanol peak obtained with the *Sample solution* is not greater than the difference of area obtained with *Solution (3)* (0.10%). The sum of the other peaks with relative retention times lower than the benzyl alcohol time obtained with the *Sample solution* is no more than four times the area corrected under the ethylbenzene peak obtained with *Solution (3)* (0.04%). The sum of the other peaks with relative retention times higher than the benzyl alcohol time in the *Sample solution* is no more than the area corrected under the dicyclohexyl peak obtained with *Solution (3)* (0.3%). Disregard peaks with area below 0.01 times the area corrected under the ethylbenzene peak in the chromatogram obtained with *Solution (3)* (0.0001%).

For benzyl alcohol targeted at parenteral use, the area under the benzaldehyde peak obtained with the *Sample solution* is not greater than the difference of area obtained with *Solution (4)* (0.05%). The area under the cyclohexylmethanol peak obtained with the *Sample solution* is not greater than the difference of area obtained with *Solution (4)* (0.10%). The sum of the other peaks with relative retention times lower than the benzyl alcohol time obtained with the *Sample solution* is no more than four times the area corrected under the ethylbenzene peak obtained with *Solution (4)* (0.02%). The sum of the other peaks with relative retention times higher than the benzyl alcohol time in the *Sample solution* is no more than the area corrected under the dicyclohexyl peak obtained with *Solution (4)* (0.2%). Disregard peaks with area below 0.01 times the corrected area of the ethylbenzene peak in the chromatogram obtained with *Solution (4)* (0.0001%).

Acidity. Add 1 mL of phenolphthalein TS to 50 mL of ethyl alcohol and neutralize with 0.1 M sodium hydroxide. Dissolve 10 mL of sample in 10 mL of neutralized ethyl alcohol and titrate with 0.1 M sodium hydroxide, until the rosy color remains for, at least, 30 seconds. No more than 1 mL is consumed.

Peroxide index. Accurately weigh about 5 g of sample and transfer to a 250 mL Erlenmeyer flask. Add 30 mL of a mixture of glacial acetic acid and chloroform (3:2), shake and add 0.5 mL of saturated

potassium iodide solution. Shake for one minute and add 30 mL of water. Slowly titrate with 0.01 *M* sodium thiosulfate VS, under constant agitation, until the yellow color fades. Add 5 mL of starch TS and continue the titration, under vigorous agitation, until the blue color disappears. Carry out a blank test (the volume spent on blank must not exceed 0.1 mL). The value of peroxides is equal to the difference between the volumes (mL) of sodium thiosulfate spent in the sample and in blank, multiplied by 10 and divided by the sample mass (g). No more than 5.

Limit of non-volatile residues. Evaporate 10 g of the sample, in water bath, and dry the residue at 105 °C for one hour. Cool in desiccator and weigh. The residue weighs no more than 5 mg. No more than 0.05% of non-volatile residues are found.

ASSAY

Weigh, accurately, about 0.9 g of the sample, add 15 mL of a recently prepared mixture of pyridine and acetic anhydride (7:1) and boil in reflux for 30 minutes. Cool, add 25 mL of water and 0.25 mL of phenolphthalein TS and titrate with *M* sodium hydroxide VS. Carry out a blank test. Calculate the percentage of C₇H₈O, according to the formula:

$$\frac{10,814(Vb - Va)}{Ma}$$

where

Va = volume (mL) of titrant spent for the sample;

Vb = is the volume (mL) of titrant spent for the blank;

Ma = mass (g) of benzyl alcohol that was titrated.

PACKAGING AND STORAGE

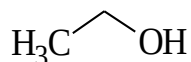
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Local anesthetic; antimicrobial.

ETHYL ALCOHOL*Alcohol ethylicus*C₂H₆O; 46.07

ethyl alcohol; 00475

Ethanol

[64-17-5]

Contains no less than 95.1% (v/v), corresponding to 92.55% (w/w), and no more than 96.9% (v/v), corresponding to 95.16% (w/w), of C₂H₆O at 20 °C, calculated from the relative density, employing the alcoholometric table **(5.2.26)**. For absolute ethyl alcohol, contains no less than 99.5% (v/v), corresponding to 99.18% (w/w) of C₂H₆O at 20 °C, calculated from the relative density, employing the alcoholometric table **(5.2.26)**.

DESCRIPTION

Physical characteristics. Colorless, clear, volatile, inflammable and hygroscopic liquid.

Solubility. Miscible with water and with methylene chloride.

Physicochemical constants.

Relative density (5.2.5): 0.805 to 0.812, determined at 20 °C. For absolute ethyl alcohol, no more than 0.793, determined at 20 °C.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of ethyl alcohol CRS.

PURITY TESTS**Clarity of the solution (5.2.25).**

Hydrazine solution: transfer 1 g of hydrazine sulfate to a 100 mL volumetric flask, dissolve, complete the volume with water and shake. Allow to stand for four to six hours.

Methenamine solution: transfer 2.5 g of methenamine to a 100 mL volumetric flask, add 25 mL of water and shake until dissolving.

Primary opalescent suspension: transfer 25 mL of the *Hydrazine solution* to the 100 mL volumetric flask with the *Methenamine solution*. Shake and allow to stand for 24 hours (this suspension is stable for two months, if maintained in a closed glass vial with no defects. The suspended particles may adhere to the glass and must be re-dispersed by shaking before use.)

Opalescence standard: transfer 15 mL of the *Primary opalescent suspension* to a 1000 mL volumetric flask, complete the volume with water and shake (this solution must not be used after 24 hours of preparation).

Reference suspensions: transfer 5 mL of the *Opalescence standard* to a 100 mL volumetric flask, complete the volume with water and shake to obtain the *Reference suspension A*. Transfer 10 mL of the *Opalescence standard* to another 100 mL flask, top off with water and shake to obtain the *Reference suspension B*.

Sample solution A: sample to be examined.

Sample solution B: dilute 1 mL of *Sample solution A* to 20 mL of water and allow to stand for five minutes before use.

Procedure: transfer a portion of *Sample solution A* and *Sample solution B* to colorless clear glass tubes, with internal diameter of 15 mm to 25 mm, to obtain approximately 40 mm of depth. Similarly, transfer portions of *Reference suspension A*, *Reference suspension B* and water to different tubes. Compare the *Sample solution A*, *Sample solution B*, *Reference suspension A*, *Reference suspension B* and water, employing dark background and light. The analyst must be capable of distinguishing the opalescence obtained with *Reference suspensions A* and *B*. The *Sample solution A* and the *Sample solution B* have the same clarity as water or do not present more opalescence than the *Reference suspension A*.

Color of the solution (5.2.12).

Standard stock solution: combine 3 mL of *Ferric chloride base solution*, 3 mL of *Cobalt chloride base solution*, 2.4 mL of *Cupric sulfate base solution* and 1.6 mL of diluted hydrochloric acid (10 mg/mL).

Standard solution: transfer 1 mL of *Standard stock solution* to a 100 mL volumetric flask, complete the volume with diluted hydrochloric acid (10 mg/mL) and shake. Use this solution soon after the preparation.

Procedure: transfer a portion of *Standard solution* to a colorless clear glass tube, with internal diameter of 15 mm to 25 mm, to obtain approximately 40 mm of depth. Transfer to a similar tube the same volume of sample and to another tube the same amount of water. The *Sample solution* has the appearance of water or has a color not more intense than that of the *Standard solution*.

Acidity or alkalinity. Add 20 mL of carbon dioxide free-water to 20 mL of the sample and add 0.1 mL of phenolphthalein TS. The solution must be colorless. Add 1.0 mL of 0.01 M sodium hydroxide. The solution turns pink (30 ppm, expressed as acetic acid).

Light absorption. Record the ultraviolet absorption spectrum of the sample between 200 nm and 400 nm, employing a cuvette with 1 cm of optical path, using water as blank. The sample presents maximum absorbance of 0.08 at 240 nm, 0.06 between 250 and 260 nm, and 0.02 between 270 and 340 nm.

Limit of non-volatile residues. Evaporate 100 mL of the sample, in water bath and dry the residue at 105 °C for one hour. Cool in desiccator and weigh. The residue weighs no more than 2.5 mg. No more than 0.025%.

Volatile organic impurities. Proceed as described in *Gas chromatography (5.2.17.5)*. Use a chromatograph with flame ionization detector; capillary column with 30 m of length and 0.53 mm of internal diameter, filled with stationary phase bonded to cyanopropylphenyl (6%) and dimethylpolysiloxane (94%), with 1.8 µm of thickness; column temperature of 40 °C to 240 °C (40 °C maintained for 12 minutes after the injection, increased to 240 °C for 12 to 32 minutes, and maintained at 240 °C for the period of 32 to 42 minutes), injector temperature of 200 °C and detector temperature of 280 °C; use helium at 35 cm/s as carrier gas and split ratio of 1:20; carrier gas flow of 1 mL/minute.

Sample solution A: sample of ethyl alcohol to be tested.

Sample solution B: transfer 150 µL of 4-methyl-2-pentanol to a 100 mL volumetric flask and complete the volume with the sample. Homogenize. Transfer 10 mL of this solution to a 50 mL volumetric flask and complete the volume with the sample. Homogenize.

Standard solution A: transfer 100 µL of methyl alcohol to a 50 mL volumetric flask and complete the volume with the sample. Homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with the sample. Homogenize.

Standard solution B: transfer 50 µL of methyl alcohol and 50 µL of acetaldehyde to a 50 mL volumetric flask and complete the volume with the sample. Homogenize. Transfer 100 µL of this solution to a 10 mL volumetric flask and complete the volume with the sample. Homogenize.

Standard solution C: transfer 150 µL of acetal to a 50 mL volumetric flask and complete the volume with the sample. Homogenize. Transfer 100 µL of this solution to a 10 mL volumetric flask and complete the volume with the sample. Homogenize.

Standard solution D: transfer 100 µL of benzene to a 100 mL volumetric flask and complete the volume with the sample. Homogenize. Transfer 100 µL of this solution to a 50 mL volumetric flask and complete the volume with the sample. Homogenize.

Procedure: inject, separately, 1 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. Calculate the sum of all amounts of acetaldehyde and acetal, expressed as acetaldehyde, according to the formula:

$$\text{Acetaldehyde (ppm)} = [(10 \times \text{AE})/(\text{AT} - \text{AE})] + [(30 \times \text{CE})/(\text{CT} - \text{CE})]$$

where

AE = area under the acetaldehyde peak obtained in the chromatogram of *Sample solution A*;

AT = area under the acetaldehyde peak obtained in the chromatogram of *Standard solution B*;

CE = area under the acetal peak obtained in the chromatogram of *Sample solution A*;

CT = area under the acetal peak obtained in the chromatogram of *Standard solution C*;

Calculate the amount of benzene according to the formula:

$$\text{Benzene (ppm)} = (2\text{BE})/(\text{BT} - \text{BE})$$

where

BE = area under the benzene peak obtained in the chromatogram of *Sample solution A*;

BT = area under the benzene peak obtained in the chromatogram of *Standard solution D*;

Disregard any peaks with area inferior to 0.03 times the area under the peak corresponding to the 4-methyl-2-pentanol obtained in the chromatogram of *Sample solution B* (9 ppm). The area under the peak corresponding to methyl alcohol obtained in the chromatogram of *Sample solution A* cannot be greater than half the area under the corresponding peak obtained in the chromatogram of *Standard solution A*. The amount of acetaldehyde found in the *Sample solution A* must be no more than 10 ppm. The amount of benzene found in the *Sample solution A* must be no more than 2 ppm. The total impurities obtained in the chromatogram of *Sample solution B* cannot be higher than the area corresponding to the peak of 4-methyl-2-pentanol, obtained in the same chromatogram.

ASSAY

Determine the amount of C_2H_6O at 20 °C, from the relative density, employing the alcoholometry table **(5.2.26)**.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

ALLOPURINOL TABLETS

Contains no less than 93.0% and no more than 107.0% of the declared amount of $C_5H_4N_4O$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 350 nm, of the sample solution obtained in *Assay*, there are maximum and minimum points only at the same wavelengths of the standard solution.

B. Weigh and pulverize the tablets. Mix in a mortar an amount of powder equivalent to 50 mg of allopurinol with 10 mL of 0.1 M sodium hydroxide. Filter, acidify the filtrate with M acetic acid and allow to stand for 10 to 15 minutes. Filter, wash the precipitate with portions of 3 mL of absolute ethyl alcohol and, then, with 4 mL of anhydrous ethyl ether. Dry under air stream for 15 minutes and Dry in oven at 105 °C for three hours. In the infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of allopurinol CRS, prepared identically.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in 0.01 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 250 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_5H_4N_4O$ dissolved in the medium, comparing the readings obtained with that of the allopurinol CRS solution at the concentration of 0.001% (w/v), prepared as described next. Transfer 20 mg of allopurinol CRS to a 100 mL volumetric flask, using 5 mL of 0.1 M sodium hydroxide. Leave in ultrasonic bath for 10 minutes, shake mechanically for another 10 minutes and complete the volume with the *Dissolution medium*. Dilute successively with the same medium to an adequate concentration.

Tolerance: no less than 75% (Q) of the declared quantity of $C_5H_4N_4O$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 230 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Eluent A: mixture of one volume of methyl alcohol and nine volumes of 0.125% (w/v) monobasic potassium phosphate.

Eluent B: mixture of three volumes of methyl alcohol and seven volumes of 0.125% (w/v) monobasic potassium phosphate.

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 - 30	100 → 0	0 → 100	linear gradient

Solution (1): shake, using an ultrasonic bath, an amount of powder from tablets equivalent to 0.1 g of allopurinol with 10 mL of 0.1 M sodium hydroxide for one minute. Then, dilute to a 200 mL volumetric flask with *Eluent A* and filter.

Solution (2): dilute one volume of *Solution (1)* to 100 volumes with *Eluent A*. Dilute 1 mL of this solution to 10 mL with *Eluent A*.

Solution (3): dissolve, in *Eluent A*, 10 mg of 5-amino-1H-pyrazole-4-carboxamide (*Allopurinol impurity A*) CRS, 5 mg of 5-formylamino-1H-pyrazole-4-carboxamide (*Allopurinol impurity B*) CRS, 5 mg of 5-(4H-1,2,4-triazole-4-yl)-1H-pyrazole-4-carboxamide (*Allopurinol impurity C*) CRS, 5 mg of 5-amino-1H-pyrazole-4-carboxylate ethyl (*Allopurinol impurity D*) CRS and 5 mg of 5-(formylamino)-1H-pyrazole-4-carboxylate ethyl (*Allopurinol impurity E*) CRS. Add 20 mL of *Solution (1)* and dilute, immediately, to 100 mL with *Eluent A*. Dilute 1 mL of the resulting solution to 100 mL using the same solvent.

Inject 20 µL of *Solution (3)*. In the conditions described for the test, the relative retention times are approximately 0.55 for *Allopurinol impurity A*, 0.79 for *Allopurinol impurities B* and *C*, 1.0 for allopurinol, 3.39 for *Allopurinol impurity D*, and 3.61 for *Allopurinol impurity E*. The test is only valid if the resolution between peaks corresponding to *Allopurinol impurity A* and to allopurinol is no less than 3.0.

Procedure: inject, separately, 20 µL of *Solutions (1)*, *(2)* and *(3)*. Record the chromatograms for no less than five times the retention time of allopurinol. In the chromatogram obtained with *Solution (1)*, the area under any peak corresponding to *Allopurinol impurity A* is not greater than the area under the corresponding peak obtained in the chromatogram of *Solution (3)* (0.2%); the area under any unresolved double peak corresponding to the *Allopurinol impurities B* and *C* is not greater than the area under the corresponding double peak obtained in the chromatogram of *Solution (3)* (0.2%); the area under any of the peaks corresponding to *Allopurinol impurity D* or to *Allopurinol impurity E* is not greater than the area under the corresponding peaks obtained in the chromatogram of *Solution (3)* (0.1%); the area under any other secondary peak is not greater than the area under the peak related to allopurinol in the chromatogram obtained with *Solution (2)* (0.1%); the sum of the areas under any secondary peak is not greater than three times the area under the peak related to

allopurinol in the chromatogram obtained with *Solution (2)* (0.3%). Do not consider any peak with area inferior to 0.2 times the area under the peak related to allopurinol in the chromatogram obtained with *Solution (2)*.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 0.1 g of allopurinol to a 100 mL volumetric flask. Add 20 mL of 0.1 M sodium hydroxide, leave in ultrasonic bath or shake mechanically for 15 minutes. Complete the volume with water and homogenize. Filter. Carry out successive dilutions to a concentration of 0.001% (w/v), using 0.1 M hydrochloric acid as solvent. Prepare a standard solution in the same conditions. Measure the absorbances of the resulting solutions at 250 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_5H_4N_4O$ in the tablets from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 563$, at 250 nm.

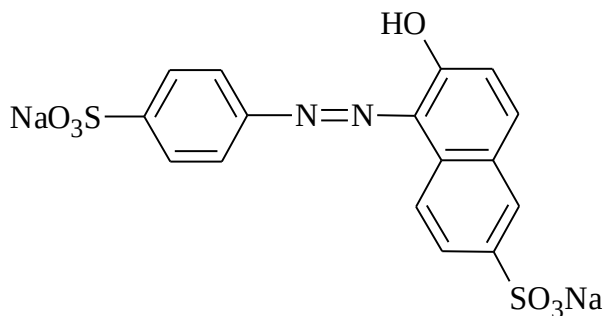
PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

SUNSET YELLOW



$C_{16}H_{10}N_2Na_2O_7S_2$; 452.36

sunset yellow; 11240

Sodium salt of the 6-hydroxy-5-[2-(4-sulfophenyl)diazenyl]-2-naphthalenesulfonic acid (2:1)
[2783-94-0]

Contains no less than 85% of $C_{16}H_{10}N_2Na_2O_7S_2$.

DESCRIPTION

Physical characteristics. Reddish-orange fine, hygroscopic powder. Orange-yellow aqueous solution.

Solubility. Soluble in water, ethyl alcohol, methyl alcohol and glycerol, insoluble in ethyl ether, acetone and mineral oil. Poorly stable in the presence of reducing agents.

IDENTIFICATION

In the visible, ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 700 nm, of 0.001% (w/v) solution in 0.02 M ammonium acetate (pH 5.6), there are maximum points at 481, 312, 234 and 211 nm and minimum points at 348, 286 and 218 nm, identical to those observed in the spectrum of similar sunset yellow CRS solution.

PURITY TESTS

Subsidiary coloring agents. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support, and a mixture of butyl alcohol, ethyl alcohol, water and ammonium hydroxide (50:25:25:10) as mobile phase. Apply to the plate, separately, 2 µL of each one of the freshly prepared solutions described below.

Solution (1): 0.25 g of the sample in 10 mL of 0.5 M sodium hydroxide.

Solution (2): 0.05 g of sunset yellow CRS in 10 mL of 0.5 M sodium hydroxide.

Solution (3): dilute *Solution (2)* in order to obtain a solution at 0.25 mg/mL, with the same diluent.

Solution (4): dilute *Solution (1)* in order to obtain a solution at 1.25 mg/mL, with the same diluent.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ambient light and ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. The secondary stains obtained with *Solution (1)* must not be more intense than those obtained with *Solution (3)* (1%) and *Solution (4)* (5%).

Alternatively, a mixture of butyl alcohol, water, glacial acetic acid (20:12:5) can be employed as mobile phase. Chromatographic paper can be used instead of silica gel G, employing the conditions described previously and observing the stains also for transparency.

Lead, copper, tin, zinc. Proceed as described in *Atomic absorption spectrometry (5.2.13)*. Weigh 2 g of the sample, use a silica crucible and burn, slowly, over an asbestos mesh ($\pm 350\text{ }^{\circ}\text{C}$); take to the muffle for 12 hours, without exceeding the temperature of $450\text{ }^{\circ}\text{C}$. Remove the crucible and cool. Mix the residue with approximately 2 mL of water and add two drops of 50% (w/v) magnesium nitrate. Dry over an electric plate and return to the muffle for three to four hours or until the residue is white or yellowish. Then, drip 1 to 2 mL of nitric acid, 1 mL of water and heat over an electric plate until it nearly dries. Dissolve the metallic nitrates with 5 mL of water. If necessary, centrifuge. Take to the atomic absorption spectrometer, calibrated in advance, and read the concentration of each one of the metals. No more than 0.001% (10 ppm) of lead, 0.002% (20 ppm) of copper, 0.025% (250 ppm) of tin and 0.005% (50 ppm) of zinc.

Chlorides and sulfates. For determination of chlorides, weigh 0.5 g of the sample, dissolve in 200 mL of water, acidify with 8 mL of 25% (v/v) nitric acid and titrate with 0.1 M silver nitrate VS, using potentiometer with combined silver electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 5.85 mg of NaCl.

For determination of sulfates, weigh 0.5 g of the sample and dissolve in 100 mL of water in water bath. Add 35 g of sulfate-free sodium chloride and shake. Transfer to a 200 mL volumetric flask and complete the volume with saturated sodium chloride solution. Homogenize. After one hour, filter in filter paper and transfer an aliquot of 100 mL of filtrate to a 600 mL beaker, dilute to 300 mL with water and acidify with hydrochloric acid RS, adding a slight excess. Heat until boiling and drip, with agitation, 25 mL of 12% (w/v) barium chloride or until there no longer is precipitation. Allow to stand for four hours. Separate the barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to a dry crucible, weighed in advance, and calcinate in muffle at $500\text{ }^{\circ}\text{C}$ for one hour. Cool in desiccator and weigh. Calculate the content of sulfates according to the formula:

$$\% \text{ sulfates} = \frac{N \times 0,6085 \times 100}{p}$$

where

N = mass in grams of barium sulfate;

p = mass in grams of the sample used in the precipitation.

No more than 5% of chlorides and sulfates.

Water-insoluble substances. Dissolve 5 g of the sample in 200 mL of hot water ($80\text{ }^{\circ}\text{C}$ to $90\text{ }^{\circ}\text{C}$) with agitation. Cool down to room temperature. Filter in filtering plate, dried and weighed in advance. Wash with cold water until the washing waters become colorless. Dry the filter with residue on oven at $120\text{ }^{\circ}\text{C}$ for four hours and weigh. No more than 0.5%.

Arsenic (5.3.2.5). Use *Method I*. Determine in 1 g of the sample. No more than 0.0001% (1 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Determine in 0.5 g of the sample. No more than 0.004% (40 ppm).

ASSAY

Prepare sample solution as described in *Identification* and determine the absorbance on the maximum absorption, at approximately 481 nm **(5.2.14)**. Calculate the content of the sample according to the formula:

$$\frac{A \times 100}{564 \times p} = \% \text{ de amarelo crepúsculo na amostra}$$

where

A = absorbance measured;

p = mass of sample in grams.

Alternatively, it is possible to consider A (1%, 1 cm) = 564 at 481 nm.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

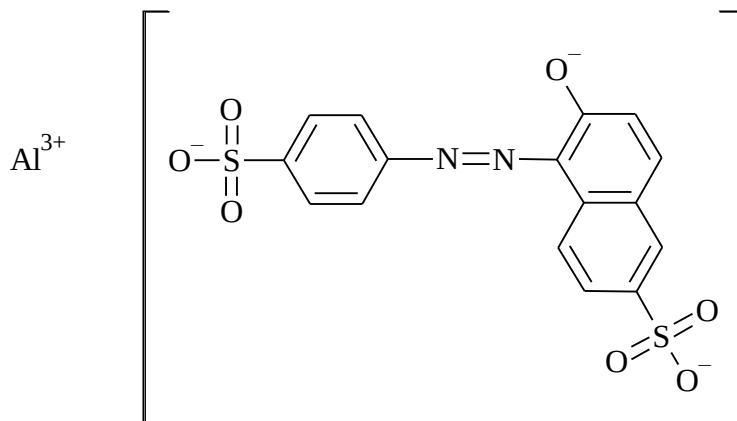
LABELING

Comply with current legislation.

CATEGORY

Dye.

SUNSET YELLOW ALUMINUM LAKE



$C_{16}H_9AlN_2O_7S_2$, 432.36

sunset yellow aluminum lake; 11424

[15790-07-5]

Contains no less than 95% and no more than 105% of the content of $C_{16}H_9AlN_2O_7S_2$. Dye comprised mainly of the sodium salt of the 6-hydroxy-5-[2-(4-sulfophenyl)diazenyl]-2-naphthalenesulfonic acid (2:1) - sunset yellow - over alumina substrate.

DESCRIPTION

Physical characteristics. Orange-yellow fine powder. It is hygroscopic.

Solubility. Practically insoluble in water and in ethyl alcohol. Soluble in *M* sodium hydroxide, but the dye decomposes slowly in alkaline pH.

IDENTIFICATION

A. In the visible, ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 700 nm, of 0.001% (w/v) sample solution in 0.02 *M* ammonium acetate (pH 5.6), dissolved in advance in *M* sodium hydroxide, there are maximum points at approximately 481 nm, 312 nm, 234 nm and 211 nm, and minimum points at 348 nm, 286 nm and 218 nm, identical to those observed in the spectrum of similar sunset yellow CRS solution.

B. Transfer 0.15 g of the sample to a 60 mL beaker and dissolve with about 20 mL of hot 30% (w/v) acetic acid, until it turns only opalescent. Cool and divide the solution into two test tubes. Add to one of them 2 mL of morin solution at 3 mg/mL in recently prepared ethyl alcohol. Observe the green fluorescence that develops under the ultraviolet light (254 nm), comparing with the tube with no reactive.

PURITY TESTS

Subsidiary coloring agents. Proceed as described in *Thin layer chromatography* (**5.2.17.1**) using silica gel G as support, and a mixture of butyl alcohol, ethyl alcohol, water and ammonium hydroxide (50:25:25:10) as mobile phase. Apply to the plate, separately, 2 μ L of each of the freshly prepared solutions described below:

Solution (1): 0.25 g of the sample in 10 mL of 0.5 M sodium hydroxide.

Solution (2): 0.05 g of sunset yellow CRS in 10 mL of 0.5 M sodium hydroxide.

Solution (3): dilute *Solution (2)* in order to obtain a solution at 0.25 mg/mL, with the same diluent.

Solution (4): dilute *Solution (1)* in order to obtain a solution at 1.25 mg/mL, with the same diluent.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ambient light and ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. The secondary stains obtained with *Solution (1)* must not be more intense than those obtained with *Solution (3)* (1%) and *Solution (4)* (5%).

Alternatively, a mixture of butyl alcohol, water and glacial acetic acid (20:12:5) can be employed as mobile phase. Chromatographic paper can be used instead of silica gel G, employing the conditions described previously and observing the stains also for transparency.

Chlorides and sulfates. For determination of chlorides, weigh 10 g of the sample, shake with 250 mL of water and leave in contact for 30 minutes. Filter. Measure 50 mL of the filtrate, equivalent to 2 g of the sample, dilute to 200 mL with water, acidify with 8 mL of 25% (v/v) nitric acid and titrate with 0.1 M silver nitrate VS, using potentiometer with combined silver electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 5.85 mg of NaCl.

For determination of sulfates, measure another 50 mL of the filtrate, dilute to 300 mL with water, acidify with hydrochloric acid RS and another 1 mL of excess. Heat until boiling and drip, with agitation, 25 mL of 12% (w/v) barium chloride. Allow to stand for four hours. Separate the barium sulfate by filtration, wash with hot water, dry the paper with residue, transfer to a dry crucible, weighed in advance, and calcinate in muffle at 500 °C for one hour. Cool in desiccator and weigh. Calculate the content of sulfates, according to the formula:

$$\% \text{ sulfates} = \frac{N \times 0,6086 \times 100}{p}$$

where

N = mass in grams of barium sulfate;

p = mass in grams of the sample used in the precipitation.

No more than 2% of chlorides and sulfates.

Loss on drying (5.2.9.1). Weigh approximately 0.5 g. Dry in oven at 120 °C for four hours or at 135 °C for three hours. No more than 20%.

Residue by incineration (5.2.10). Weigh about 0.1 g of the sample in a crucible dried and weighed in advance and incinerate at 800 °C for two hours. It must contain between 40% and 55%.

ASSAY

Prepare sample solution as described in method **A.** in *Identification* and determine the absorbance on the maximum absorption, in approximately 481 nm (**5.2.14**). Calculate the content of the sample, according to the formula:

$$\frac{A \times 100}{564 \times p} = \% \text{ of sunset yellow in the sample at 481 nm where}$$

A = absorbance measured;

p = mass of sample in grams.

PACKAGING AND STORAGE

In perfectly closed containers, protected from light.

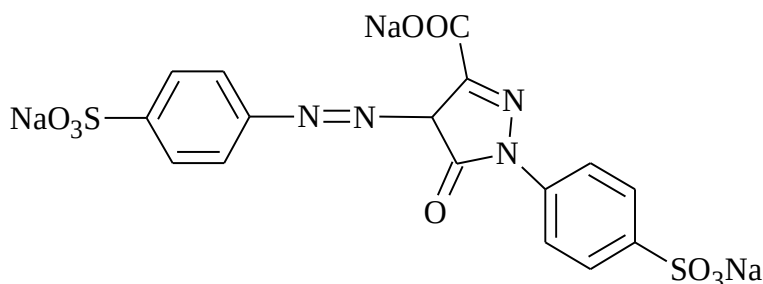
LABELING

Comply with current legislation.

CATEGORY

Dye.

TARTRAZINE YELLOW



$C_{16}H_9N_4Na_3O_9S_2$; 534.36

tartrazine yellow; 11341

Sodium salt of 4,5-di-hydro-5-oxo-1-(4-sulfophenyl)-4-[2-(4-sulfophenyl)diazenyl]-1*H*-pyrazole-3-carboxylic acid (3:1)

[1934-21-0]

Contains no less than 85% of $C_{16}H_9N_4Na_3O_9S_2$.

DESCRIPTION

Physical characteristics. Orange fine, brilliant and hygroscopic powder. Yellowish aqueous solution.

Solubility. Soluble in water, methyl alcohol and glycerol. Slightly soluble in ethyl alcohol. Insoluble in ethyl ether, acetone, mineral oil and fats.

IDENTIFICATION

In the visible, ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 700 nm, of 0.001% (w/v) solution in 0.02 *M* ammonium acetate (pH 5.6), there are maximum points at 426 nm, 257 nm and 203 nm and minimum points at 311 and 221 nm, identical to those observed in the spectrum of similar tartrazine yellow CRS solution.

PURITY TESTS

Subsidiary coloring agents. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support, and a mixture of butyl alcohol, ethyl alcohol, water and ammonium hydroxide (50:25:25:10) as mobile phase. Apply to the plate separately 2 μ L of each of the freshly prepared solutions described below.

Solution (1): 0.25 g of the sample in 10 mL of 0.5 *M* sodium hydroxide.

Solution (2): 0.05 g of tartrazine yellow CRS in 10 mL of 0.5 *M* sodium hydroxide.

Solution (3): dilute *Solution (2)* in order to obtain a solution at 0.05 mg/mL, with the same diluent.

Solution (4): dilute *Solution (1)* in order to obtain a solution at 0.25 mg/mL, with the same diluent.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ambient light and ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position,

color and intensity to the one obtained with *Solution (2)*. The secondary stains obtained with *Solution (1)* must not be more intense than those obtained with *Solution (3)* (0.2%) and *Solution (4)* (1%).

Alternatively, a mixture of butyl alcohol, water and glacial acetic acid (20:12:5) can be employed as mobile phase. Chromatographic paper can be used instead of silica gel G, employing the conditions described previously, and observing the stains also for transparency.

Lead, copper, tin, zinc. Proceed as described in *Atomic absorption spectrometry (5.2.13)*. Weigh 2 g of the sample, use a silica crucible and burn, slowly, over an asbestos mesh ($\pm 350\text{ }^{\circ}\text{C}$); take it to the muffle for 12 hours, without exceeding the temperature of $450\text{ }^{\circ}\text{C}$. Remove the crucible and cool. Mix the residue with approximately 2 mL of water and add two drops of 50% (w/v) magnesium nitrate. Dry over an electric plate and return to the muffle for three to four hours or until the residue is white or yellowish. Then, drip 1 to 2 mL of nitric acid, 1 mL of water and heat over an electric plate until it nearly dries. Dissolve the metallic nitrates with 5 mL of water. If necessary, centrifuge. Take to the atomic absorption spectrometer calibrated in advance and read the concentration of each one of the metals. No more than 0.001% (10 ppm) of lead, 0.002% (20 ppm) of copper, 0.025% (250 ppm) of tin and 0.005% (50 ppm) of zinc.

Chlorides and sulfates. For determination of chlorides, weigh 0.5 g of the sample, dissolve in 200 mL of water, acidify with 8 mL of 25% (v/v) nitric acid and titrate with 0.1 M silver nitrate VS using potentiometer with combined silver electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 5.85 mg of NaCl.

For determination of sulfates, weigh 0.5 g of the sample and dissolve with 100 mL of water in water bath. Add 35 g of sulfate-free sodium chloride and shake. Transfer to a 200 mL volumetric flask and complete the volume with saturated sodium chloride solution. Homogenize. After one hour, filter in filter paper and transfer an aliquot of 100 mL of filtrate to a 600 mL beaker, dilute to 300 mL with water and acidify with hydrochloric acid RS, adding a slight excess. Heat until boiling and drip, with agitation, 25 mL of 12% (w/v) barium chloride, or until there no longer is precipitation. Allow to stand for four hours. Separate the barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to a dry crucible, weighed in advance, and calcinate in muffle at $500\text{ }^{\circ}\text{C}$ for one hour. Cool in desiccator and weigh. Calculate the content of sulfates, according to the formula:

$$\% \text{ sulfates} = \frac{N \times 0,6085 \times 100}{p}$$

where

N = mass in grams of barium sulfate;

p = mass in grams of the sample used in the precipitation.

No more than 6% of chlorides and sulfates.

Water-insoluble substances. Dissolve 5 g of the sample in 200 mL of hot water ($80\text{ }^{\circ}\text{C}$ to $90\text{ }^{\circ}\text{C}$), with agitation. Cool down to room temperature. Filter in filtering plate, dried and weighed in advance. Wash with cold water, until the washing waters become colorless. Dry the filter with residue on oven at $120\text{ }^{\circ}\text{C}$ for four hours and weigh. No more than 0.5%.

Arsenic (5.3.2.5). Use *Method I*. Determine in 3 g of the sample. No more than 0.0001% (1 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Determine in 0.5 g of the sample. No more than 0.004% (40 ppm).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Prepare sample solution as described in *Identification* and determine the absorbance of the maximum absorption, at approximately 426 nm **(5.2.14)**. Calculate the content of the sample, according to the formula:

$$\frac{A \times 100}{536,6 \times p} = \% \text{ de amarelo de tartrazina na amostra}$$

where

A = absorbance measured;

p = mass of sample in grams.

Alternatively, it is possible to consider A (1%, 1 cm) = 536.6 at 426 nm.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Dye.

STARCH

Amylum

starch; 00657

Starch

[9005-25-8]

Starch is obtained from fruits, roots and other parts of different vegetables. Maize starch (*Zea mays* L., Poaceae), rice starch (*Oryza sativa* L., Poaceae), wheat starch (*Triticum aestivum* L., Poaceae), tapioca starch (*Manihot utilissima* Pohl, Euphorbiaceae) and potato starch (*Solanum tuberosum* L., Solanaceae) are considered officinal. Starches obtained from different botanical origins may not have identical properties when used for pharmaceutical purposes. Chemically, starch is a mixture of polymers that corresponds to the formula $(C_6H_{10}O_5)_n$. Maize starch has approximately 27% of amylose and 73% of amylopectin.

DESCRIPTION

Physical characteristics. White, odorless and insipid powder. When examined in thin layer, must not present visible impurities or dirt.

Solubility. Practically insoluble in cold water, ethyl alcohol and organic solvents.

MICROSCOPIC DESCRIPTION

Rice starch (Figure 1). Very small, polyhedral grains, with sharp angles and straight edges, commonly gathered into groups, with diameter from 2 μm to 10 μm (4 μm to 6 μm , in average). Rounded grains are rare and the hilum is frequently absent or appears as a tiny dot.

Potato starch (Figure 2). Simple, irregularly ovoid or sub-spherical grains, rarely grouped in pairs or trios, characteristic. Ovoid grains are unevenly elongated or triangular, with 30 μm to 100 μm of diameter. Sub-spherical grains measure 10 μm to 35 μm . The hilum is round, eccentrically arranged in the narrowest part of the grain, with very clear and concentric striations.

Tapioca starch (Figure 3). The grains vary from 25 μm to 35 μm of diameter, irregularly rounded, in the shape of a thimble, with a trunked sphere in one or several sides, pointy, linear or star-shaped, central and very clear hilum.

Maize starch (Figure 4). A mix of grains with two shapes. When coming from the periphery of the albumen, they are polyhedral, tightly compressed, showing a rounded, split or star-shaped hilum, and measure in average 14 μm to 20 μm of diameter. When coming from the most central part of the albumen, they display a poorly angular contour, irregularly rounded, and are elongated, ovoid or pyriform, with a greater hilum; and measure in average 10 μm to 35 μm . The smaller grains are, at times, grouped, being similar to compound grains.

Wheat starch (Figure 5). Two forms of grains, clearly differentiated and with almost no intermediate forms: large, lenticular, round, oval and sub-reniform grains, sometimes split on the margins; present concentric layers with no clear distinction, as well as hilum in the form of a central dot or a simple line; measure, in average, 28 μm to 35 μm of diameter. Seen in profile, they are elliptic, elongated, nearly fusiform, with a very wide groove at times. The smaller grains are rounded,

faceted by mutual compression, measuring 2 µm to 9 µm (5 µm to 7 µm, in average) of diameter. They also present in some groups from two to four grains.

IDENTIFICATION

A. Mix 1 g of the sample with 2 mL of cold water. Pour over 15 mL of water in ebullition. Boil, in low heat, for two minutes, under agitation. Cool. A gelatinous, light-colored and translucent product is formed.

B. Add to the gelatinous mixture obtained in test **A.** from *Identification* one drop of iodine RS. A blue color develops, disappears with boiling and returns with cooling.

PURITY TESTS

pH (5.2.19). 4.5 to 7.0 for maize starch and 5.0 to 8.0 for potato starch. Determine in 20 g of the sample. Transfer the sample to a non-metallic vial and add 100 mL of water. A paste is formed. Shake, continuously, for five minutes, at moderate speed.

Oxidizing substances. Transfer 4 g of the sample to a 125 mL Erlenmeyer flask. Add 50 mL of water. Cover and shake for five minutes. Transfer to a centrifuge tube with capacity of 50 mL and centrifuge. Transfer 30 mL of the clear supernatant to a 125 mL Erlenmeyer flask with a ground stopper. Add 1 mL of glacial acetic acid and 1 g of potassium iodide. Put a stopper, shake and allow to stand for 30 minutes, protected from direct light. Add 1 mL of starch TS and titrate with 0.001 M sodium thiosulfate VS until the blue color disappears. Carry out the blank test and make the necessary corrections. Each mL of 0.001 M sodium thiosulfate VS is equivalent to 17 µg of oxidizing agent, calculated as hydrogen peroxide. No more than 2.8 mL of 0.001 M sodium thiosulfate VS are consumed (0.002%, calculated as H₂O₂).

Sulfur dioxide. Mix 20 g of the sample with 200 mL of water until obtaining a homogeneous suspension. Filter. Add to 100 mL of the clear filtrate 3 mL of starch TS and titrate with 0.02 M iodine VS until a permanent blue color. No more than 5.4 mL of 0.02 M VS iodine are consumed (0.008%).

Iron (5.3.2.4). Dissolve the residue obtained in the test of *Residue by incineration* in 8 mL of hydrochloric acid, under soft heating. Dilute to 100 mL with water and homogenize. Transfer 25 mL to a Nessler tube, add 12 mL of water and proceed as described in *Method I*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C, until constant weight. No more than 15.0%.

Residue by incineration (5.2.10). Determine in 2 g of the sample. No more than 0.6%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

In tightly closed containers, protected from humidity. The label must indicate the botanical origin.

LABELING

Comply with current legislation.

CATEGORY

Pharmaceutical adjuvant.

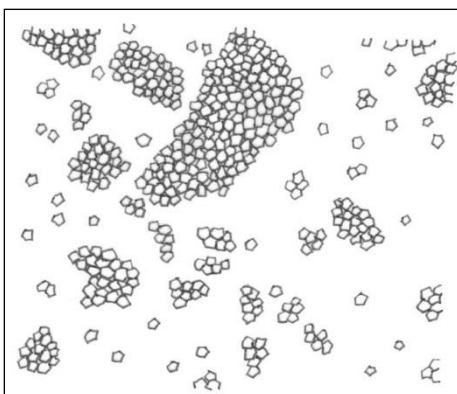


Figure 1 – Rice starch.

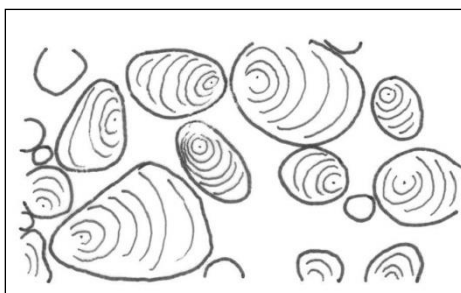


Figure 2 – Potato starch.

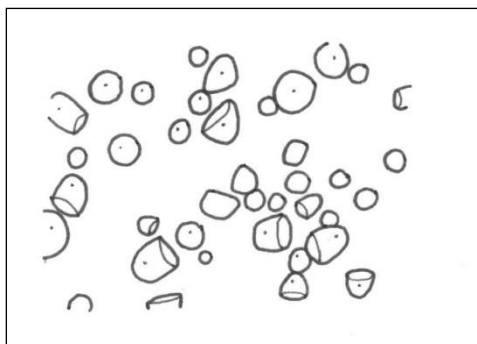


Figure 3 – Tapioca starch.

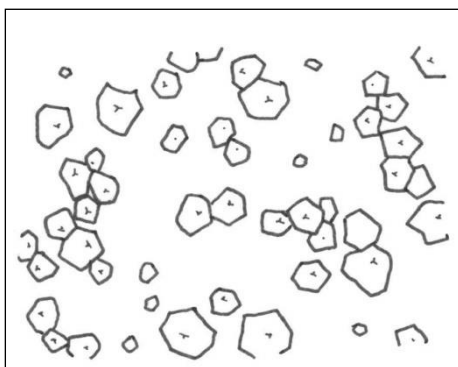


Figure 4 – Corn starch.

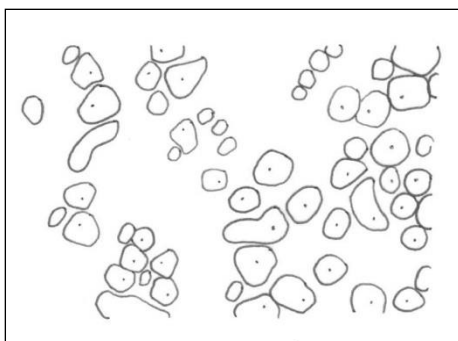
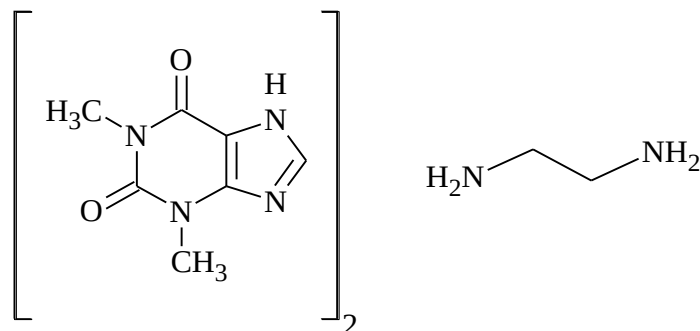


Figure 5 – Wheat starch.

AMINOPHYLLINE***Aminophyllinum*** $(C_7H_8N_4O_2)_2 \cdot C_2H_8N_2$; 420.43 $C_7H_8N_4O_2$; 180.17 $C_2H_8N_2$; 60.10

aminophylline; 00685

3,9-Di-hydro-1,3-dimethyl-1*H*-purine-2,6-dione with 1,2-ethanediamine (2:1)

[317-34-0]

Aminophylline is a combination of theophylline with ethylenediamine, which contains no less than 84.0% and no more than 87.4% of the declared amount of theophylline ($C_7H_8N_4O_2$), and no less than 13.5% and no more than 15.0% of ethylenediamine ($C_2H_8N_2$), in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or slightly yellow powder or granules.

Solubility. Soluble in carbon dioxide-free water, practically insoluble in absolute ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the precipitate obtained on test **B.** in *Identification*, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of theophylline CRS, prepared identically.

B. Dissolve 0.5 g of the sample in 20 mL of water, add 1 mL of 3 *M* hydrochloric acid, with constant agitation. Filter and wash the precipitate with small portions of cold water. Dry at 105 °C for one hour. The precipitate obtained melts between 270 °C and 274 °C.

C. Transfer 10 mg of the desiccated precipitate, obtained on test **B.** of *Identification*, to a porcelain capsule, add 1 mL of hydrochloric acid and 0.1 g of potassium chlorate. Evaporate in water bath to dryness. Invert the capsule over a container with a few drops of 6 *M* ammonium hydroxide. The residue acquires a purple color, which disappears with the addition of alkaline hydroxide solution.

D. The precipitate obtained on test **B.** of *Identification* complies with the xanthine reactions **(5.3.1.1).**

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel HF₂₅₄ as support, and a mixture of concentrated solution of ammonia, acetone, chloroform and butyl alcohol (10:30:30:40) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.2 g of the powdered sample in 2 mL of carbon dioxide-free water and dilute to 10 mL with methyl alcohol.

Solution (2): transfer 0.5 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with methyl alcohol.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any stain obtained in the chromatogram of *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram of *Solution (2)* (0.5%).

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Water (5.2.20.1). Determine in 2 g of the sample. No more than 1.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.15%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Ethylenediamine

Dissolve 0.25 g of the sample in 30 mL of water. Titrate with 0.1 M hydrochloric acid VS using 0.1 mL of bromocresol green TS as indicator, until turning green. Each mL of 0.1 M hydrochloric acid VS is equivalent to 3.005 mg of ethylenediamine (C₂H₈N₂).

Theophylline

Use one of the methods described below.

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 150 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mix 200 mL of methyl alcohol, 0.96 g of sodium 1-pentanesulfonate monohydrate and complete the volume to 1000 mL with water. Adjust the pH to 2.9 ± 0.1 with glacial acetic acid.

Diluent: mixture of water and methyl alcohol (4:1).

Sample solution: weigh, accurately, about 24 mg of the sample, transfer to a 250 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Standard solution: dissolve an accurately weighed amount of theophylline CRS in *Diluent* and dilute adequately to obtain a 80 µg/mL solution.

Resolution solution: prepare a theobromine CRS solution at 80 µg/mL using *Standard solution* as diluent. Transfer 20 mL to a 25 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Inject 10 µL replicates of the *Resolution solution*. The relative retention times are of 0.65 for theobromine and 1.0 for theophylline. The resolution between the theophylline and theobromine peaks is no less than 3.0. The tailing factor for the theophylline peak is no more than 2.0. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of theophylline (C₇H₈N₄O₂) in the sample from the responses obtained for theophylline with the *Standard solution* and *Sample solution*.

B. Dry the sample in oven at 135 °C until constant weight. Accurately weigh about 0.2 g of the sample, dissolve in 100 mL of water and heat, if necessary. Cool. Add 20 mL of 0.1 M silver nitrate and shake. Titrate with 0.1 M sodium hydroxide VS using 1 mL of bromothymol blue TS as indicator. Each mL of 0.1 M sodium hydroxide VS is equivalent to 18.016 mg of theophylline (C₇H₈N₄O₂).

PACKAGING AND STORAGE

In closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Bronchodilator.

AMINOPHYLLINE TABLETS

Contains no less than 80.6% and no more than 90.8% of theophylline ($C_7H_8N_4O_2$), and no less than 10.9% of ethylenediamine ($C_2H_8N_2$), of the declared amount of aminophylline.

IDENTIFICATION

A. Weigh and pulverize the tablets. Shake an amount of powder equivalent to 0.5 g of aminophylline with 20 mL of water and filter. Add to the filtrate, under constant agitation, 1 mL of 2 M hydrochloric acid, allow to stand for a few minutes and filter. Reserve the filtrate for the test **C.** of *Identification*. Wash the residue with small amounts of cold water, recrystallize in hot water and dry in oven at 105 °C until constant weight. In the infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of aminophylline CRS, prepared identically.

B. The residue obtained in the test **A.** of *Identification* melts around 271 °C.

C. Add to the filtrate reserved on test **A.** of *Identification* 0.2 mL of benzyl chloride, alkalize with 5 M ammonium hydroxide and shake vigorously. Filter and wash the residue with cold water, recrystallize in a mixture of water and ethyl alcohol (10:30) and dry in oven at 100 °C until constant weight. The crystals obtained melt around 250 °C.

D. Dissolve 10 mg of the residue obtained on test **A.** of *Identification* in 1 mL of hydrochloric acid. Add 0.1 g of potassium chloride and evaporate to dryness. A reddish residue is obtained, and it turns purple under exposure to ammonia vapor.

E. Weigh and pulverize the tablets. Mix an amount of powder equivalent to 0.25 g of aminophylline with 5 mL of water and filter. Add to 2 mL of filtrate 2 mL of 1% (w/v) of cupric sulfate and homogenize. A dark blue color develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in water until adequate concentration. Measure the absorbances of the solutions at 269 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of theophylline ($C_7H_8N_4O_2$) dissolved in the medium, comparing the readings obtained with that of the theophylline CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of theophylline ($C_7H_8N_4O_2$) dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Ethylenediamine

Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 0.3 g of aminophylline to a 150 mL Erlenmeyer flask, dissolve in 20 mL of water and heat at 50 °C for 30 minutes, shaking occasionally. Titrate with 0.05 M sulfuric acid VS, using bromocresol green TS solution as indicator, until the color changes to green-blue. Each mL of 0.05 M sulfuric acid VS is equivalent to 3.005 mg of ethylenediamine ($C_2H_8N_2$).

Theophylline

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize, to a fine powder, 20 tablets. Transfer an amount of powder equivalent to 80 mg of aminophylline [$(C_7H_8N_4O_2)_2 \cdot C_2H_8N_2$] to a 200 mL volumetric flask. Add 20 mL of 0.1 M sodium hydroxide and 60 mL of water and shake mechanically for 10 minutes. Complete the volume with water, homogenize and filter. Transfer 5 mL of the filtrate to a 200 mL volumetric flask and complete the volume with 0.01 M sodium hydroxide, obtaining a 0.001% concentration (w/v). Measure the absorbance of the resulting solution at 275 nm, using 0.01 M sodium hydroxide VS for zero adjustment. Calculate the amount of theophylline ($C_7H_8N_4O_2$) in the tablets considering $A(1\% \text{ } 1 \text{ cm}) = 650$, at 250 nm, in 0.01 M sodium hydroxide VS.

B. Weigh and pulverize, to a fine powder, 20 tablets. Transfer an amount of powder equivalent to 2 g of aminophylline [$(C_7H_8N_4O_2)_2 \cdot C_2H_8N_2$] to a 200 mL volumetric flask, using a mixture of 50 mL of water and 15 mL of 6 M ammonium hydroxide, and leave with occasional agitation for 30 minutes, heating at 50 °C if necessary, to dissolve the aminophylline. Cool the mixture down to room temperature, if it was heated. Complete the volume with water and homogenize. Centrifuge about 50 mL of mixture, pipette the clear portion of the supernatant, equivalent to 250 mg of aminophylline, dilute with water, if necessary, to reach approximately 40 mL and transfer to a 250 mL Erlenmeyer flask. Add 8 mL of 6 M ammonium hydroxide and 20 mL of 0.1 M silver nitrate VS. Heat to ebullition for 15 minutes. Cool between 5 °C and 10 °C for 20 minutes and filter, preferably in crucible under reduced pressure. Wash the precipitate with three portions of 10 mL of water.

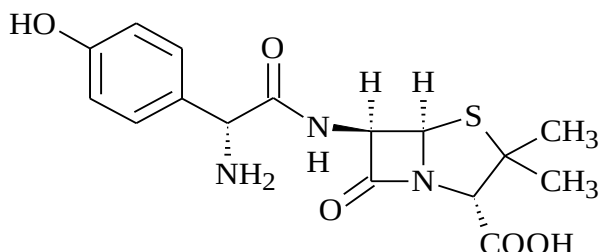
Acidify the combined filtrate and the washes with nitric acid and add 3 mL of acid. Cool, add 2 mL of ferric ammonium sulfate RS and titrate the excess silver nitrate with 0.1 M ammonium thiocyanate VS. Each mL of 0.1 M silver nitrate VS is equivalent to 18.016 mg of theophylline ($C_7H_8N_4O_2$).

PACKAGING

In tightly closed containers.

LABELING

Comply with current legislation.

AMOXICILLIN***Amoxicillinum***

$C_{16}H_{19}N_3O_5S$; 365.40

amoxicillin; 00734

(2*S*,5*R*,6*R*)-6-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
[26787-78-0]

$C_{16}H_{19}N_3O_5S \cdot 3H_2O$; 419.45

amoxicillin trihydrate; 00736

(2*S*,5*R*,6*R*)-6-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid hydrate (1:3)
[61336-70-7]

The potency is no less than 900 µg and no more than 1050 µg of amoxicillin ($C_{16}H_{19}N_3O_5S$) per milligram, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Slightly soluble in water, ethyl alcohol and methyl alcohol. Insoluble in acetonitrile. Soluble in alkali hydroxide solutions.

Physicochemical constants.

Specific optical rotation (5.2.8): +290 to +315, in relation to the anhydrous substance. Determine in 0.2% (w/v) solution in carbon dioxide-free water.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of amoxicillin hydrate CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 to 400 nm, of the 0.002% (w/v) solution in ethyl alcohol, there are maximum points at 230 nm and at 274 nm, identical to the ones observed in a similar amoxicillin trihydrate CRS solution.

C. The retention time of the main peak from the *Solution (1)* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Solution (2)*.

PURITY TESTS

Related substances. Proceed as described on method **B.** of Assay. Prepare solutions as described below.

Test solution: transfer, quantitatively, approximately 30 mg of the sample to a 20 mL volumetric flask, complete the volume with *Eluent A* and homogenize. Prepare immediately before use.

Reference solution: use the *Solution (3)* described on method **B.** of Assay.

Blank solution: *Eluent A*.

Procedure: inject 50 µL of the *Test solution*, *Reference solution* and *Blank solution*, record the chromatograms and measure the areas under all peaks obtained. No secondary peak on the chromatogram obtained with the *Test solution* has an area greater than the area under the main peak obtained with the *Reference solution* (1.0%). Disregard any peak obtained in the *Blank solution* chromatogram.

Crystallinity. Suspend a few particles of the sample in mineral oil, transfer to a glass slide and examine on a microscope equipped with polarized light. The particles display birefringence, which is extinguished when moving the sample, through micrometric adjustment.

pH (5.2.19). 3.5 to 6.0. Determine in aqueous solution at 0.2% (w/v).

Limit of *N,N*-dimethylaniline (5.3.2.13). No more than 20 ppm.

Water (5.2.20.1). 11.5% to 14.5%. Determine in 0.3 g of sample.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 1%.

BIOLOGICAL SAFETY TESTS

Amoxicillin targeted at parenteral preparation and, when indicated on the label that the substance is sterile, the sample complies with the following tests.

Sterility (5.5.3.2.1). Complies with the test. Dissolve 6 g of the sample in 800 mL of *Fluid II* with polysorbate 80 (5 mg/mL) and sufficient amount of sterile penicillinase, to inactivate the amoxicillin. Shake to full dissolution and employ the *Direct inoculation method*.

Bacterial endotoxins (5.5.2.2). No more than 0.25 EU/mg of amoxicillin.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Note: the dilutions of Standard and sample solutions must be prepared simultaneously.

Sample solution: dissolve an accurately weighed amount of the sample in sterile water, to obtain a 0.1 mg/mL amoxicillin solution. Dilute successively with *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)* to obtain solutions in the concentration range suitable to the standard curve.

Standard solution: dissolve an accurately weighed amount of amoxicillin trihydrate CRS in sterile water, to obtain a 0.1 mg/mL amoxicillin solution. Dilute, successively, with *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)* to obtain solutions in the concentration range suitable to the standard curve.

Procedure: proceed as described in *Microbiological assay by agar diffusion (5.5.3.3.1)*, using cylinders. Calculate the sample potency, in µg of amoxicillin per milligram, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Buffer solution pH 5.0: dissolve 6.805 g of monobasic potassium phosphate in 250 mL of water, adjust pH to 5.0 with sodium hydroxide RS and complete the volume to 1000 mL with water.

Eluent A: mixture of acetonitrile and *Buffer solution pH 5.0* (1:99).

Eluent B: mixture of acetonitrile and *Buffer solution pH 5.0* (20:80).

Mobile phase gradient: adopt the gradient system described on the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 – t	92	8	isocratic
t – (t + 25)	92 → 0	8 → 100	linear gradient
(t + 25) – (t + 40)	0	100	isocratic
(t + 40) – (t + 55)	92	8	isocratic

t = retention time of amoxicillin determined with *Solution (3)*.

Solution (1): weigh, accurately, about 30 mg of the sample, transfer to a 50 mL volumetric flask, complete the volume with *Eluent A* and homogenize.

Solution (2): weigh, accurately, about 30 mg of amoxicillin CRS, transfer to a 50 mL volumetric flask, complete the volume with *Eluent A* and homogenize.

Solution (3): dilute 2 mL of *Solution (2)* to 20 mL with *Eluent A*. Transfer 5 mL to a 20 mL volumetric flask, complete the volume with the same solvent and homogenize.

Solution (4): dissolve 4 mg of *cefadroxil CRS* in *Eluent A* and complete the volume to 50 mL with the same solvent. Add to 5 mL of this solution 5 mL of *Solution (2)* and complete the volume to 100 mL with the same solvent.

Solution (5): dilute 1 mL of *Solution (2)* to 20 mL with *Eluent A*. Transfer 1 mL to a 50 mL volumetric flask, complete the volume with the same solvent and homogenize.

Inject 50 µL replicates of *Solution (3)* in isocratic elution to determine the retention time (t) of amoxicillin.

Inject 50 µL replicates of *Solution (4)* using the gradient system. The resolution between amoxicillin and cefadroxil is no less than 2. If necessary, adjust the proportion of *Eluent A* and *Eluent B* from the *Mobile phase*.

Inject 50 µL replicates of *Solution (5)* using the gradient system. The signal/noise ratio is higher than 3. Make adjustments, if necessary.

If it is necessary to make adjustments in the composition of the *Mobile phase* to obtain the necessary resolution, the adjusted composition must be applied since the starting time in the gradient to conduct the assay

Procedure: inject 50 µL of *Solution (1)* and *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the content in µg of amoxicillin (C₁₆H₁₉N₃O₅S) per milligram in the sample from the content of the standard and the responses obtained with *Solution (1)* and *Solution (2)*. The relative standard deviation of the replicate areas under the peaks recorded is no more than 1%.

C. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Prepare the standard solution in the same conditions than the sample solution.

PACKAGING AND STORAGE

In tightly closed containers, at temperature between 15 °C and 25 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

AMOXICILLIN AND POTASSIUM CLAVULANATE TABLETS

Contains no less than 90.0% and no more than 120.0% of the declared amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and potassium clavulanate ($C_8H_8KNO_5$).

IDENTIFICATION

The retention times of the main peaks of the *Sample solution* chromatogram, obtained in the *Assay*, correspond to the ones from the main peaks of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Disintegration test (5.1.4.1). No more than 45 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute, if necessary, in water until adequate concentration. Proceed as described in *Assay*. Calculate the amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and potassium clavulanate ($C_8H_8KNO_5$) dissolved in the medium, comparing the responses obtained with those from amoxicillin solution CRS and lithium clavulanate CRS at the concentrations of 0.05% (w/v) and 0.02% (w/v), respectively, prepared in the same solvent.

Tolerance: no less than 85% (Q) of the declared quantity of amoxicillin ($C_{16}H_{19}N_3O_5S$) and no less than 80% (Q) of the declared quantity of potassium clavulanate ($C_8H_8KNO_5$) dissolve in 30 minutes.

PURITY TESTS

Water (5.2.20.1). No more than 7.5%, if the labeled amount of amoxicillin is up to 250 mg. No more than 10.0%, if the labeled amount of amoxicillin is higher than 250 mg and lower than 500 mg. No more than 11.0%, if the labeled amount of amoxicillin is higher than 500 mg.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph with an ultraviolet detector at 220 nm; column with 300 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 µm to 10 µm); *Mobile phase* flow of 2.0 mL/minute.

Buffer pH 4.4: dissolve 7.8 g of monobasic sodium phosphate in 900 mL of water. Adjust pH to 4.4 ± 0.1 with phosphoric acid or sodium hydroxide. Complete the volume to 1000 mL with water and homogenize.

Mobile phase: mixture of *Buffer pH 4.4* and methyl alcohol (95:5).

Sample solution: dissolve at least 10 tablets in water, in a volumetric flask that results in a concentration not superior, in amoxicillin, to 3 mg/mL, with agitation, for 30 minutes. Filter or centrifuge and dilute aliquot of the resulting clear solution until obtaining a concentration of amoxicillin at 0.5 mg/mL. Use this solution within one hour.

Standard solution: dissolve amounts of amoxicillin CRS and lithium clavulanate CRS, accurately weighed, in water, in order to obtain a solution with 0.5 mg/mL and 0.2 mg/mL, respectively.

Inject 20 µL replicates of the *Standard solution*. The column efficiency, determined for each analyte, is no less than 550 theoretical plates. The relative retention times are approximately 0.5 for clavulanic acid and 1.0 for amoxicillin. The tailing factor for the peak of each analyte is no more than 1.5. The resolution between the amoxicillin and clavulanic acid peaks is no less than 3.5. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amounts of amoxicillin and potassium clavulanate in the tablets, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

AMOXICILLIN AND POTASSIUM CLAVULANATE POWDER FOR INJECTION SOLUTION

Contains no less than 90.0% and no more than 120.0% of the declared amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and potassium clavulanate ($C_8H_8KNO_5$).

IDENTIFICATION

The retention times of the main peaks of the *Sample solution* chromatogram, obtained in the *Assay*, correspond to the ones from the main peaks of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Determination of volume (5.1.2). Complies with the test. Determine in the injection solution, reconstituted as indicated on the label.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

pH (5.2.19). 8.0% to 10.0%. Determine in the reconstituted solution containing the equivalent to 10% (w/v) of amoxicillin.

Water (5.2.20.1). Determine in 0.5 g. No more than 3.5%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Dissolve the content of the ampoule vial in water to a LAL reagent, in order to obtain a solution at 10 mg/mL of amoxicillin. No more than 2.5 EU/mL of this solution.

ASSAY

Proceed as described in *Assay* from the monograph *Amoxicillin and potassium clavulanate tablets*. Prepare the *Sample solution* as described below.

Sample solution: mix the contents of 10 units. Transfer an amount of powder equivalent to 0.1 g of amoxicillin to a 200 mL volumetric flask, add water until dissolution and complete the volume with the same solvent. Homogenize. Use this solution within one hour.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amounts of amoxicillin and potassium clavulanate in the injection solution, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

AMOXICILLIN AND POTASSIUM CLAVULANATE POWDER FOR ORAL SUSPENSION

Contains no less than 90.0% and no more than 120.0% of the declared amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and potassium clavulanate ($C_8H_8KNO_5$).

IDENTIFICATION

The retention times of the main peaks of the *Sample solution* chromatogram, obtained in the *Assay*, correspond to the ones from the main peaks of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test. Determine in the oral suspension, reconstituted as indicated on the label.

Determination of weight (5.1.1). Complies with the test.

PURITY TESTS

Water (5.2.20.1). No more than 7.5%, if the labeled amount of amoxicillin after reconstitution is up to 40 mg/mL. No more than 10.0%, if the labeled amount of amoxicillin after reconstitution is higher than 40 mg/mL and lower than 50 mg/mL. No more than 11.0%, if the labeled amount of amoxicillin after reconstitution is higher than 50 mg/mL and lower than 80 mg/mL. No more than 12.0%, if the labeled amount of amoxicillin after reconstitution is higher than 80 mg/mL.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Assay* from the monograph *Amoxicillin and potassium clavulanate tablets*. Prepare the *Sample solution* as described below.

Sample solution: reconstitute the powder for oral suspension as indicated on the label. Dilute quantitatively a volume of suspension in water, in order to obtain a solution with 0.5 mg/mL of amoxicillin. Shake mechanically for 10 minutes and filter. Use this solution within one hour.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amounts of amoxicillin and potassium clavulanate in the oral suspension, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

AMOXICILLIN TRIHYDRATE CAPSULES

Contains no less than 90.0% and no more than 120.0% of the declared amount of $C_{16}H_{19}N_3O_5S$. Amoxicillin trihydrate capsules are comprised of amoxicillin trihydrate, with or without one or more suitable lubricant, diluent and drying agents, included in gelatin capsules.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.02% (w/v) solution in ethyl alcohol, there are maximum points at 230 nm and at 274 nm, identical to the ones observed in the spectrum of a similar amoxicillin trihydrate CRS solution.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G 60 as support, and a mixture of methyl alcohol, chloroform, water and acetone (9:8:3:1) as mobile phase. Apply, separately, to the plate 5 μ L of each of the solutions described next, which must be used within 10 minutes after their preparation.

Solution (1): sample solution with 0.4% (w/v) of amoxicillin trihydrate in 0.1 M hydrochloric acid.

Solution (2): solution at 0.4% (w/v) of amoxicillin trihydrate CRS in 0.1 M hydrochloric acid.

Develop the chromatogram. Remove the plate, allow to air dry and nebulize with ninhydrin RS. Heat in oven at 110 °C for 15 minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 90 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in water until adequate concentration. Measure the absorbances of the solutions at 272 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the amount of $C_{16}H_{19}N_3O_5S$ dissolved in the medium, comparing the readings obtained with that of the amoxicillin trihydrate solution CRS at the concentration of 0.01% (w/v), prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{16}H_{19}N_3O_5S$ dissolve in 90 minutes.

PURITY TESTS

Water (5.2.20.1). Determine in 0.3 g of the sample. No more than 14.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: Kocuria rhizophila ATCC 9341.

Culture media: culture medium number 1, for microorganism maintenance, sterile saline solution for inoculum standardization; culture medium number 11, for base layer and inoculum layer on the plate.

Sample solution: remove the content of the capsules and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 0.1 g of amoxicillin to a 100 mL volumetric flask, complete the volume with water and shake for about 30 minutes. Transfer 1 mL of this solution to a 100 mL volumetric flask, complete the volume with *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* and homogenize. Dilute, successively, to obtain concentrations of 0.05 µg/mL, 0.10 µg/mL and 0.20 µg/mL, using *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Standard solution: weigh an amount of amoxicillin trihydrate CRS equivalent to 25 mg of amoxicillin, transfer to a 50 mL volumetric flask and complete the volume with water. Transfer 1 mL of the solution obtained to a 100 mL volumetric flask, complete the volume with *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* and homogenize. Dilute, successively, to obtain concentrations of 0.05 µg/mL, 0.10 µg/mL and 0.20 µg/mL, using *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Procedure: add 21 mL of culture medium number 11 to each plate, wait until it solidifies, add 4 mL of 0.5% inoculum and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Add to the cylinders 0.2 mL of the freshly prepared solutions. Calculate the sample potency, in mg of amoxicillin per capsule, from the potency of standard and responses obtained with the *Standard solutions* and with the *Sample solutions*.

B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Remove the content of the capsules and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder, accurately weighed, to a volumetric flask, add water, shake for three to five minutes and complete the volume with the same solvent, to obtain an amoxicillin solution at 2.00 mg/mL. Prepare a standard solution in the same conditions.

PACKAGING AND STORAGE

In tightly closed containers, at temperature between 15 °C and 25 °C.

LABELING

Comply with current legislation.

AMOXICILLIN TRIHYDRATE POWDER FOR ORAL SUSPENSION

Contains no less than 90.0% and no more than 120.0% of the declared amount of $C_{16}H_{19}N_3O_5S$. Amoxicillin trihydrate used in the production meets the specifications described on the monograph *Amoxicillin*. Amoxicillin trihydrate powder for oral suspension is a mixture of one or more agents suitable for suspension, containing or not coloring, flavoring, preserving agents, buffers, sweeteners and stabilizers.

IDENTIFICATION

Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G 60 as support and a mixture of methyl alcohol, chloroform, water and acetone (9:8:3:1) as mobile phase. Apply separately to the plate 5 μ L of each of the solutions described next, which must be used within 10 minutes after their preparation.

Solution (1): sample solution with 0.4% (w/v) of amoxicillin trihydrate in 0.1 M hydrochloric acid.

Solution (2): solution at 0.4% (w/v) of amoxicillin trihydrate CRS in 0.1 M hydrochloric acid.

Develop the chromatogram. Remove the plate, allow to air dry and nebulize with ninhydrin RS. Heat in oven at 110 °C for 15 minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test for *Liquid products in multi-dose containers*. Determine in the reconstituted suspension, as indicated on the label.

pH (5.2.19). 5.0 to 7.5. Determine in the reconstituted suspension, as indicated on the label.

Determination of weight (5.1.1). Complies with the test.

PURITY TESTS

Water (5.2.20.1). No more than 3.0%. Determine in 0.3 g of the sample.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: Kocuria rhizophila ATCC 9341.

Culture media: medium number 1, for microorganism maintenance, sterile saline solution for inoculum standardization, and medium number 11, for base layer and inoculum layer on the plate.

Sample solution: transfer the equivalent to 250 mg of amoxicillin to a 250 mL volumetric flask. Complete the volume with water and shake for about 30 minutes. Transfer 1 mL of this solution to a 100 mL volumetric flask, complete the volume with *Solution 2 (Potassium phosphate buffer, sterile, pH 8.0)* and homogenize. Dilute, successively, to obtain concentrations of 0.05 µg/mL, 0.10 µg/mL and 0.20 µg/mL, using *Solution 2 (Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Standard solution: weigh an amount of amoxicillin trihydrate CRS equivalent to 25 mg of amoxicillin and transfer to a 50 mL volumetric flask. Complete the volume with water and homogenize. Transfer 1 mL of this solution to a 100 mL volumetric flask, complete the volume with *Solution 2 (Potassium phosphate buffer, sterile, pH 8.0)* and homogenize. Dilute, successively, to obtain concentrations of 0.05 µg/mL, 0.10 µg/mL and 0.20 µg/mL, using *Solution 2 (Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Procedure: add 21 mL of culture medium number 11 to each plate, wait until it solidifies, add 4 mL of 0.5% inoculum and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Add to the cylinders 0.2 mL of the freshly prepared solutions. Calculate the sample potency, in mg of amoxicillin per milliliter, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

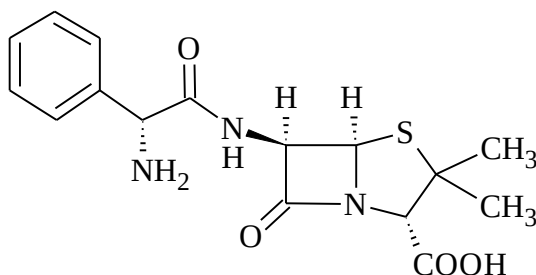
B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Reconstitute the content of 10 units, as indicated on the label. Mix and homogenize the content of the vials. Transfer an amount, accurately measured, of reconstituted oral suspension to a volumetric flask, add water, shake for three to five minutes and complete the volume with the same solvent, to obtain an amoxicillin solution at 2.00 mg/mL. Prepare a standard solution in the same conditions.

PACKAGING AND STORAGE

In tightly closed containers, at temperature between 15 °C and 25 °C.

LABELING

Comply with current legislation.

AMPICILLIN**Ampicillinum**C₁₆H₁₉N₃O₄S; 349.41

ampicillin; 00738

(2*S*,5*R*,6*R*)-6-[[*(2R)*-2-amino-2-phenylacetyl]amino]- 3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2- carboxylic acid
[69-53-4]

C₁₆H₁₉N₃O₄S.3H₂O; 403.45

ampicillin trihydrate; 00742

(2*S*,5*R*,6*R*)-6-[[*(2R)*-2-amino-2-phenylacetyl]amino]- 3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2- carboxylic acid hydrate (1:3)
[7177-48-2]

The potency is no less than 960 µg and no more than 1005 µg of ampicillin (C₁₆H₁₉N₃O₄S) per milligram, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White to slightly yellowish crystalline powder. Presents polymorphism.

Solubility. Slightly soluble in water and methyl alcohol, practically insoluble in absolute ethyl alcohol. Soluble in diluted acidic and alkaline solutions.

Physicochemical constants.

Melting range (5.2.2): 199 °C to 202 °C, with decomposition.

Specific optical rotation (5.2.8): +280 to +305, in relation to the anhydrous substance. Determine in solution at 0.25% (w/v).

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of ampicillin CRS or ampicillin trihydrate CRS, prepared identically.

B. The retention time of the main peak from the *Solution (1)* chromatogram, obtained in method **C** of Assay, corresponds to the one from the main peak of the *Solution (3)*.

PURITY TESTS

Related substances. Proceed as described on method **C.** of Assay. Prepare the solutions described below.

Test solution: dissolve an amount of sample equivalent to 27.0 mg of ampicillin in *Eluent A* and complete the volume to 10 mL with the same solvent. Prepare immediately before use.

Reference solution: dilute 1.0 mL of *Solution (2)*, described on method **C.** of Assay, to 20 mL with *Eluent A*.

Blank solution: *Eluent A*.

Procedure: inject 50 µL of the *Test solution*, *Reference solution* and *Blank solution*, record the chromatograms and measure the areas of all peaks obtained. No secondary peak on the chromatogram obtained with the *Test solution* has an area greater than the area of the main peak obtained with the *Reference solution* (1.0%). Disregard any peak obtained in the blank chromatogram.

pH (5.2.19). 3.5 to 6.0. Determine in aqueous solution at 1% (w/v).

Crystallinity. Suspend a few particles of sample in mineral oil. Transfer to a glass slide and examine in microscope equipped with polarized light. The particles display birefringence, which is extinguished when moving the sample, through micrometric adjustment.

Limit of *N,N*-dimethylaniline (5.3.2.13). Use *Method II*. No more than 0.002% (20 ppm).

Water (5.2.20.1). No more than 2.0% for the anhydrous form and no more than 12% to 15% for the hydrated form.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Ampicillin targeted at production of parenteral preparations complies with the following tests.

Sterility (5.5.3.2.1). Complies with the test. Dissolve 6 g of the sample in 800 mL of *Fluid II* with a sufficient amount of β -lactamase to inactivate ampicillin, shake to full dissolution and proceed as described in *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). No more than 0.15 EU/mg of ampicillin.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Note: the dilutions of Standard and sample solutions for the standard curve must be prepared simultaneously.

Sample solution: dissolve an accurately weighed amount of the sample in sterile water, to obtain a 0.1 mg/mL solution. Dilute successively with *Solution 2* (0.1 M potassium phosphate buffer, sterile, pH 8.0) to obtain solutions in the concentration range suitable to the standard curve.

Standard solution: dissolve an accurately weighed amount of ampicillin CRS in sterile water, to obtain a 0.1 mg/mL solution. Dilute, successively, with *Solution 2* (0.1 M potassium phosphate buffer, sterile, pH 8.0) to obtain solutions in the concentration range suitable to the standard curve.

Procedure: proceed as described in *Microbiological assay by agar diffusion* (5.5.3.3.1). Calculate the potency of the sample, in µg of C₁₆H₁₉N₃O₄S per milligram, from the potency of standard and responses obtained with the *Standard solutions* and with the *Sample solutions*.

B. Proceed as described in *Iodometric test of antibiotics* (5.3.3.10). Prepare the standard solution in the same conditions than the sample solution.

C. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Eluent A: mixture of diluted acetic acid, 0.2 M monobasic potassium phosphate, acetonitrile and water (0.5:50:50:899.5).

Eluent B: mixture of diluted acetic acid, 0.2 M monobasic potassium phosphate, acetonitrile and water (0.5:50:400:549.5).

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – t	85	15	isocratic
t – (t + 30)	85 → 0	15 → 100	linear gradient
(t + 30) – (t + 45)	0	100	isocratic
(t + 45) – (t + 60)	85	15	isocratic

t = retention time of ampicillin determined with *Solution (2)*.

Solution (1): dissolve an amount of sample equivalent to 27.0 mg of ampicillin in *Eluent A* and complete the volume to 50 mL with the same solvent.

Solution (2): dissolve 27.0 mg of ampicillin anhydrous CRS in *Eluent A* and complete the volume to 50 mL with the same solvent.

Solution (3): dissolve 2.0 mg of cefradine CRS in *Eluent A* and complete the volume to 50 mL with the same solvent. Add to 5.0 mL of this solution 5.0 mL of *Solution (2)*.

Inject 50 µL replicates of *Solution (2)* in isocratic elution to determine the retention time (t) of ampicillin.

Inject 50 µL replicates of *Solution (3)* using the gradient system. The resolution between ampicillin and cefradine is no less than 3. If necessary, adjust the proportion of *Eluent A* and of *Eluent B* from the *Mobile phase*.

If it is necessary to make adjustments in the composition of the *Mobile phase* to obtain the necessary resolution, the adjusted composition must be applied since the starting time in the gradient to conduct the assay.

Procedure: inject 50 µL of *Solution (1)* and *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the content in µg of ampicillin (C₁₆H₁₉N₃O₄S) per milligram in the sample from the content of the standard and the responses obtained with *Solution (1)* and *Solution (2)*. The relative standard deviation of replicate areas under the peaks recorded is no more than 1%.

PACKAGING AND STORAGE

In hermetically closed containers, at temperature below 30 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

AMPICILLIN CAPSULES

Contains no less than 90.0% and no more than 120.0% of the declared amount of $C_{16}H_{19}N_3O_4S$.

IDENTIFICATION

Proceed as described in test **B.** of *Identification* from the *Ampicillin* monograph. Prepare *Solution (1)* as described next.

Solution (1): weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Shake an amount of powder equivalent to 0.125 g of ampicillin with 4.2% (w/v) sodium bicarbonate and dilute to 50 mL with the same solvent. Filter.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in cupric sulfate buffer to adequate concentration. Transfer 10 mL to a test tube with cap, heat in water bath at 75 °C for 30 minutes and cool quickly. Measure the absorbances of the solutions at 320 nm **(5.2.14)**, using aliquot of the dissolution medium diluted in cupric sulfate buffer, without heating, for zero adjustment. Calculate the amount of $C_{16}H_{19}N_3O_4S$ dissolved in the medium, comparing the readings obtained with that of the ampicillin solution CRS at the concentration of 0.0022% (w/v), prepared in the same conditions.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{16}H_{19}N_3O_4S$ dissolve in 45 minutes.

PURITY TESTS

Water (5.2.20.1). No more than 4%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics by agar diffusion (5.5.3.3)* by the agar diffusion method.

Note: *the dilutions of Standard solution and Sample solution must be prepared simultaneously.*

Sample solution: weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder, accurately weighed, to a volumetric flask and dilute with sterile water, to obtain an ampicillin solution at 0.1 mg/mL. Shake for three to five minutes. Dilute successively with *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)* to obtain solutions in the concentration range suitable to the standard curve.

Standard solution: dissolve an accurately weighed amount of ampicillin CRS in sterile water, to obtain a 0.1 mg/mL solution. Dilute successively with *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)* to obtain solutions in the concentration range suitable to the standard curve.

Procedure: proceed as described in *Microbiological assay by agar diffusion (5.5.3.3.1)*. Calculate the amount in mg of $C_{16}H_{19}N_3O_4S$ in the capsules, from the potency of standard and responses obtained with the *Standard solutions* and with the *Sample solutions*.

B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder, accurately weighed, to a volumetric flask, add water, shake for three to five minutes and complete the volume with the same solvent, to obtain an ampicillin solution at 2.50 mg/mL. Prepare a standard solution in the same conditions.

PACKAGING AND STORAGE

In hermetically closed containers, between 15 °C and 25 °C.

LABELING

Comply with current legislation.

AMPICILLIN TABLETS

Contains no less than 90.0% and no more than 120.0% of the declared amount of $C_{16}H_{19}N_3O_4S$.

IDENTIFICATION

Proceed as described in test **B.** of *Identification* from the *Ampicillin* monograph. Prepare *Solution (1)* as described next.

Solution (1): weigh and pulverize the tablets. Shake an amount of powder equivalent to 0.125 g of ampicillin with 4.2% (w/v) sodium bicarbonate and dilute to 50 mL with the same solvent. Filter.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in cupric sulfate buffer, to adequate concentration. Transfer 10 mL to a test tube with cap, heat in water bath at 75 °C for 30 minutes and cool quickly. Measure the absorbances of the solutions at 320 nm **(5.2.14)**, using aliquot of the dissolution medium diluted in cupric sulfate buffer, without heating, for zero adjustment. Calculate the amount of $C_{16}H_{19}N_3O_4S$ dissolved in the medium, comparing the readings obtained with that of the ampicillin solution CRS at the concentration of 0.0022% (w/v), prepared in the same conditions.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{16}H_{19}N_3O_4S$ dissolve in 45 minutes.

PURITY TESTS

Water (5.2.20.1). No more than 4.0%

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)* by the agar diffusion method.

Note: the dilutions of Standard and sample solutions for the standard curve must be prepared simultaneously.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder, accurately weighed, to a volumetric flask and dilute with sterile water to obtain an ampicillin solution at 0.1 mg/mL. Shake for three to five minutes. Dilute successively with *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)*, to obtain solutions in the concentration range suitable to the standard curve.

Standard solution: dissolve an accurately weighed amount of ampicillin CRS in sterile water, to obtain a 0.1 mg/mL solution. Dilute successively with *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)*, to obtain solutions in the concentration range suitable to the standard curve.

Procedure: proceed as described in *Microbiological assay by agar diffusion (5.5.3.3.1)*. Calculate the amount in mg of $C_{16}H_{19}N_3O_4S$ in the tablets, from the potency of standard and responses obtained with the *Standard solutions* and with the *Sample solutions*.

B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Weigh and pulverize 20 tablets. Transfer an amount of powder accurately weighed to a volumetric flask, add water, shake for three to five minutes and complete the volume with the same solvent, to obtain an ampicillin solution at 2.50 mg/mL. Prepare a standard solution in the same conditions.

PACKAGING AND STORAGE

In hermetically closed containers, protected from humidity, at temperature below 30 °C.

LABELING

Comply with current legislation.

AMPICILLIN POWDER FOR ORAL SUSPENSION

Contains no less than 90.0% and no more than 120.0% of the declared amount of ampicillin ($C_{16}H_{19}N_3O_4S$). Ampicillin powder for oral suspension is the mixture of ampicillin with one or more coloring, flavoring, buffering, sweetening and preservative agents.

IDENTIFICATION

Proceed as described in test **B.** of *Identification* from the *Ampicillin* monograph. Prepare *Solution (1)* as described next.

Solution (1): reconstitute the oral suspension as indicated on the label. Shake an amount of oral suspension, equivalent to 0.125 g of ampicillin, in 4.2% (w/v) sodium bicarbonate solution and dilute to 50 mL with the same solvent. Filter.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test for *Liquid products in multi-dose containers*. Determine in the reconstituted suspension as indicated on the label.

pH (5.2.19). 5.0 to 7.5. Determine in the reconstituted suspension as indicated on the label.

Determination of weight (5.1.1). Complies with the test. Determine in the non-reconstituted powder.

Uniformity of unit doses (5.1.6). Complies with the test for solids stored in single-dose containers.

PURITY TESTS

Water (5.2.20.1). No more than 2.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)* by the agar diffusion method.

Note: the dilutions of *Standard solution* and *Sample solution* must be prepared simultaneously.

Sample solution: reconstitute the suspension as indicated on the label. Transfer an amount of suspension, accurately measured, to a volumetric flask and dilute with sterile water to obtain an ampicillin solution at 0.1 mg/mL. Shake for three to five minutes. Dilute successively with *Solution*

2 (0.1 M potassium phosphate buffer, sterile, pH 8.0) to obtain solutions in the concentration range suitable to the analytical curve.

Standard solution: dissolve an accurately weighed amount of ampicillin CRS in sterile water, to obtain a 0.1 mg/mL solution. Dilute successively with *Solution 2* (0.1 M potassium phosphate buffer, sterile, pH 8.0) to obtain solutions in the concentration range suitable to the analytical curve.

Procedure: proceed as described in *Microbiological assay by agar diffusion (5.5.3.3.1)*. Calculate the amount in mg of $C_{16}H_{19}N_3O_4S$ in the oral suspension, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Reconstitute the content of 10 units, as indicated on the label. Mix and homogenize the content of the vials. Transfer an amount of reconstituted oral suspension, measured accurately, to a volumetric flask, add water, shake for three to five minutes and complete the volume with the same solvent, to obtain an ampicillin solution at 2.50 mg/mL. Prepare a standard solution in the same conditions.

PACKAGING AND STORAGE

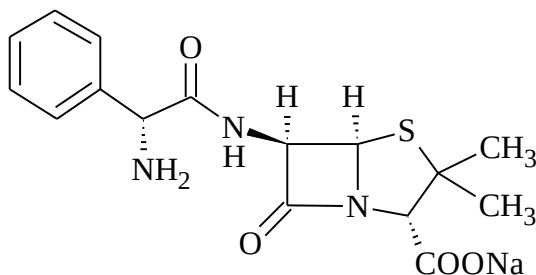
In tightly closed containers, protected from humidity, at temperature below 25 °C.

LABELING

Comply with current legislation.

AMPICILLIN SODIUM

Ampicillinum natricum



$C_{16}H_{18}N_3NaO_4S$; 371.39

ampicillin sodium; 00741

Sodium salt of (2*S*,5*R*,6*R*)-6-[[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (1:1)
[69-52-3]

The potency is no less than 856 µg and no more than 960 µg of ampicillin ($C_{16}H_{19}N_3O_4S$) per milligram, calculated in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder, hygroscopic. Presents polymorphism.

Solubility. Very soluble in water.

Physicochemical constants.

Specific optical rotation (5.2.8): +258 to +287, in relation to the anhydrous substance. Determine in 0.25% (w/v) solution having 0.4% (w/v) potassium biphthalate solution as solvent.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of ampicillin sodium CRS, prepared identically.

B. The retention time of the main peak from the *Solution (1)* chromatogram, obtained in method **C.** of Assay, corresponds to the one from the main peak of the *Solution (3)*.

C. Complies with sodium ion reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described on method **C.** from Assay. Prepare the solutions described below.

Test solution: dissolve 31.0 mg of the sample in *Eluent A* and complete the volume to 10 mL with the same solvent. Prepare immediately before use.

Reference solution: dilute 1.0 mL of *Solution (2)*, described on method **C.** of Assay to 20 mL with *Eluent A*.

Blank solution: *Eluent A*.

Procedure: inject 50 µL of the *Test solution*, *Reference solution* and *Blank solution*, record the chromatograms and measure the areas under all peaks obtained. No secondary peak on the chromatogram obtained with the *Test solution* has an area greater than the area under the main peak obtained with the *Reference solution* (1.0%). Disregard any peak obtained in the blank chromatogram.

pH (5.2.19). 8.0% to 10.0%. Determine in aqueous solution at 1% (w/v).

Water (5.2.20.1). No more than 2.0%.

Crystallinity. Suspend a few particles of the sample in mineral oil, transfer to a glass slide and examine, on a microscope equipped with polarized light. The particles display birefringence, which is extinguished when moving the sample, through micrometric adjustment.

Limit of *N,N*-dimethylaniline (5.3.2.13). Use *Method II*. No more than 0.002% (20 ppm).

Methylene chloride. Proceed as described in *Gas chromatography (5.2.17.5)*. Use a chromatograph equipped with flame ionization detector; glass column with 1.5 m of length and 4 mm of internal diameter, packaged with silanized diatomaceous support (particles of up to 120 µm), washed with acid, coated with macrogol 1000 at 10% (w/w), column temperature of 60 °C; injector temperature of 100 °C; detector temperature of 150 °C; nitrogen as carrier gas; flow of 40 mL/minute.

Internal standard solution: 0.2% (v/v) aqueous ethylene chloride solution.

Standard solution: transfer 1 mL of 0.2% (v/v) aqueous methylene chloride solution to a 10 mL volumetric flask. Add 1 mL of the *Internal standard solution*, complete the volume with water and homogenize.

Sample solution: dissolve 1 g of the sample in water and transfer to a 10 mL volumetric flask. Add 1.0 mL of the *Internal standard solution*, complete the volume with water and homogenize.

Procedure: inject, separately, 1 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and calculate the percentage (w/w) of methylene chloride, considering as 1.325 g/mL the density value at 20 °C. No more than 0.2% (w/w).

BIOLOGICAL SAFETY TESTS

Ampicillin sodium targeted at parenteral preparation and, when indicated on the label that the substance is sterile, the sample complies with the following tests.

Sterility (5.5.3.2.1). Complies with the test. Employ the *Membrane filtration method*. Dissolve 6 g of the sample in 800 mL of *Fluid II* with a sufficient amount of sterile penicillinase, to inactivate ampicillin, shake to full dissolution and proceed as described.

Bacterial endotoxins (5.5.2.2). No more than 0.15 EU/mg of ampicillin.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method. Dissolve, separately, ampicillin sodium CRS and sample in sterile water, to obtain a solution at the concentration of 0.1 mg/mL each. Dilute the solutions obtained in Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0), to obtain the concentrations used in the standard curve. Calculate the sample potency, in µg of ampicillin per milligram, from the potency of standard and responses obtained with the standard and sample solutions.

B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Prepare the standard solution in the same conditions than the sample solution.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Eluent A: mixture of diluted acetic acid, 0.2 M monobasic potassium phosphate, acetonitrile and water (0.5:50:50:899.5).

Eluent B: mixture of diluted acetic acid, 0.2 M monobasic potassium phosphate, acetonitrile and water (0.5:50:400:549.5).

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – t	85	15	isocratic
t – (t + 30)	85 → 0	15 → 100	linear gradient
(t + 30) – (t + 45)	0	100	isocratic
(t + 45) – (t + 60)	85	15	isocratic

t = retention time of ampicillin determined with *Solution (2)*.

Solution (1): dissolve 31.0 mg of the sample in *Eluent A* and complete the volume to 50 mL with the same solvent.

Solution (2): dissolve 31.0 mg of ampicillin sodium CRS in *Eluent A* and complete the volume to 50 mL with the same solvent.

Solution (3): dissolve 2.0 mg of cefradine CRS in *Eluent A* and complete the volume to 50 mL with the same solvent. Add to 5.0 mL of this solution 5.0 mL of *Solution (2)*.

Inject 50 µL replicates of *Solution (2)* in isocratic elution to determine the retention time (t) of ampicillin.

Inject 50 µL replicates of *Solution (3)* using the gradient system. The resolution between ampicillin and cefradine is no less than 3. If necessary, adjust the proportion of *Eluent A* and of *Eluent B* from the *Mobile phase*.

If it is necessary to make adjustments in the composition of the *Mobile phase* to obtain the necessary resolution, the adjusted composition must be applied since the starting time in the gradient to conduct the assay

Procedure: inject 50 µL of *Solution (1)* and *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the content in µg of ampicillin ($C_{16}H_{19}N_3O_4S$) per milligram in the sample from the content of the standard, the responses obtained with *Solution (1)* and *Solution (2)* and divide the result by 1.063. The relative standard deviation of replicate areas under the peaks recorded is no more than 1%.

PACKAGING AND STORAGE

In hermetically closed containers, at temperature below 30 °C. If targeted at production of injectable pharmaceutical dosage forms, it must be packaged in sterile containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

AMPICILLIN SODIUM POWDER FOR INJECTION SOLUTION

Contains no less than 90.0% and no more than 115.0% of the declared amount of $C_{16}H_{19}N_3O_4S$.

IDENTIFICATION

Proceed as described in test **B.** of *Identification* from the *Ampicillin sodium* monograph.

CHARACTERISTICS

pH (5.2.19). 8.0% to 10.0%. Determine in aqueous solution at 1% (w/v).

Determination of weight (5.1.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Water (5.2.20.1). No more than 2.0%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Employ the *Membrane filtration method*. Dissolve 6 g of the sample in 800 mL of *Fluid II*, with a sufficient amount of sterile penicillinase to inactivate ampicillin, shake to full dissolution and proceed as described.

Bacterial endotoxins (5.5.2.2). No more than 0.15 EU/mg of ampicillin.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method. Reconstitute the content of 10 vials as indicated on the label. Dissolve, separately, standard and sample in sterile water, to obtain a solution at the concentration of 0.1 mg/mL. Dilute, separately, the standard and sample solution, in *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)*, to the concentrations used in obtaining the standard curve. Calculate the sample potency, in µg of ampicillin per milligram, from the potency of standard and responses obtained with the standard and sample solutions.

B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Reconstitute the content of 10 vials as indicated on the label. Mix and homogenize the content of the vials. Transfer an amount, accurately measured, of reconstituted suspension to a volumetric flask, add water and complete the volume with the same solvent, to obtain an ampicillin solution at 2.50 mg/mL. Prepare a standard solution in the same conditions.

PACKAGING AND STORAGE

In hermetically closed containers, at temperature below 25 °C.

LABELING

Comply with current legislation.

AMPICILLIN TRIHYDRATE CAPSULES

Contains ampicillin trihydrate equivalent to no less than 90.0% and no more than 120.0% of the declared amount of ampicillin ($C_{16}H_{19}N_3O_4S$).

IDENTIFICATION

A. Proceed as described in test **B.** of *Identification* from the *Ampicillin* monograph. Prepare *Solution (1)* as described next.

Solution (1): weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Shake an amount of powder in 4.2% (w/v) sodium bicarbonate solution and dilute with the same solvent, to obtain an ampicillin ($C_{16}H_{19}N_3O_4S$) solution at 2.5 mg/mL. Filter.

B. Weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 10 mg of ampicillin to a beaker, add 1 mL of water and 2 mL of mixture of alkaline cupric tartrate RS and water (2:6). A violet color immediately develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in cupric sulfate buffer to adequate concentration. Transfer an aliquot of 10 mL to a test tube with cap, submit to heating in water bath at 75 °C for 30 minutes and cool quickly. Measure the absorbances of the solutions at 320 nm (**5.2.14**), using dissolution medium (water) in cupric sulfate buffer in the same proportion employed in the preparation of sample solutions and standard solution, without heating, for zero adjustment. Calculate the amount of $C_{16}H_{19}N_3O_4S$ dissolved in the medium comparing the readings obtained with that of the ampicillin solution CRS at the concentration of 0.0022% (w/v), prepared in the same conditions.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{16}H_{19}N_3O_4S$ dissolve in 45 minutes.

PURITY TESTS

Water (5.2.20.1). 10.0 to 15.0.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics by agar diffusion (5.5.3.3.1)* for ampicillin.

Sample solution: weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder, accurately weighed, to a volumetric flask, add *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)*, shake for three to five minutes and complete the volume with the same solvent, to obtain an ampicillin ($C_{16}H_{19}N_3O_4S$) solution at 0.1 mg/mL. Dilute successively, with the same solvent, to the concentration range of the standard curve.

Standard solution: dissolve an accurately weighed amount of ampicillin CRS in sterile water and dilute with the same solvent, to obtain a 0.1 mg/mL solution. Dilute successively in *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)* to the concentration range of the standard curve.

Procedure: proceed as described in *Microbiological assay by agar diffusion (5.5.3.3.1)*. Calculate the amount in mg of ampicillin ($C_{16}H_{19}N_3O_4S$) in the capsules, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder, accurately weighed, to a volumetric flask, add water, shake for three to five minutes and complete the volume with the same solvent, to obtain an ampicillin solution at 2.50 mg/mL. Prepare a standard solution in the same conditions.

PACKAGING AND STORAGE

In perfectly closed containers, at temperature below 30 °C.

LABELING

Comply with current legislation.

AMPICILLIN TRIHYDRATE TABLETS

Contains ampicillin trihydrate equivalent to no less than 90.0% and no more than 120.0% of the declared amount of ampicillin ($C_{16}H_{19}N_3O_4S$).

IDENTIFICATION

A. Proceed as described in test **B.** of *Identification* from the *Ampicillin* monograph. Prepare *Solution (1)* as described next.

Solution (1): weigh and pulverize the tablets. Shake an amount of powder in 4.2% (w/v) sodium bicarbonate solution and dilute with the same solvent to obtain an ampicillin ($C_{16}H_{19}N_3O_4S$) solution at 2.5 mg/mL. Filter.

B. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 10 mg of ampicillin to a beaker and proceed as described on test **B.** of *Identification* from the monograph *Ampicillin trihydrate capsules*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). No more than 15 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute with cupric sulfate buffer to adequate concentration. Proceed as described in *Dissolution test* on the monograph *Ampicillin trihydrate capsules*.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{16}H_{19}N_3O_4S$ dissolve in 45 minutes.

PURITY TESTS

Water (5.2.20.1). 9.5% to 12%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)* for Ampicillin, by the agar diffusion method.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder, accurately weighed, to a volumetric flask, add *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)*, shake for three to five minutes and complete the volume with the same solvent, to obtain an ampicillin ($C_{16}H_{19}N_3O_4S$) solution at 0.1 mg/mL. Dilute successively, with the same solvent, to the concentrations of the standard curve.

Standard solution: dissolve an accurately weighed amount of ampicillin CRS in sterile water and dilute with the same solvent, to obtain a 0.1 mg/mL solution. Dilute, successively, in *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)* to the concentration range of the standard curve.

Procedure: proceed as described in *Microbiological assay by agar diffusion (5.5.3.3.1)*. Calculate the amount in mg of ampicillin ($C_{16}H_{19}N_3O_4S$) in the tablets, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Weigh and pulverize 20 tablets. Transfer an amount of powder, accurately weighed, to a volumetric flask, add water, shake for three to five minutes and complete the volume with the same solvent, to obtain an ampicillin solution at 2.50 mg/mL. Prepare a standard solution in the same conditions.

PACKAGING AND STORAGE

In perfectly closed containers, protected from humidity, at temperature below 30 °C.

LABELING

Comply with current legislation.

AMPICILLIN TRIHYDRATE POWDER FOR ORAL SUSPENSION

Contains no less than 90.0% and no more than 120.0% of the declared amount of $C_{16}H_{19}N_3O_4S$. The powder for oral suspension contains one or more coloring, flavoring, buffering, sweetening and preservative agents.

IDENTIFICATION

Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica G as support, and a mixture of acetone, water, toluene and glacial acetic acid (650:100:100:25) as mobile phase. Apply to the plate, separately, 2 μ L of each of the freshly prepared solutions described below.

Solution (1): sample solution with 5 mg/mL of ampicillin, in a mixture of acetone and 0.1 M hydrochloric acid (4:1).

Solution (2): solution at 5 mg/mL of ampicillin CRS, in a mixture of acetone and 0.1 M hydrochloric acid (4:1).

Develop the chromatogram. Remove the plate and allow to air dry. Nebulize the plate with 0.3% (w/v) ninhydrin in ethyl alcohol. Dry in oven at 90 °C for 15 minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test. Determine in the reconstituted oral suspension, as indicated on the label.

pH (5.2.19). 5.0 to 7.5. Determine in the reconstituted oral suspension, as indicated on the label.

Determination of weight (5.1.1). Complies with the test.

PURITY TESTS

Water (5.2.20.2). No more than 2.5% in sample with 50 mg/mL of ampicillin after reconstitution or no more than 5.0% in sample with 100 mg/mL of ampicillin.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Reconstitute the content of 10 units, as indicated on the label. Mix and homogenize the content of the vials. Transfer an amount, accurately measured, of reconstituted oral suspension to a volumetric flask, add water, shake for three to five minutes and complete the volume with the same

solvent, to obtain an ampicillin ($C_{16}H_{19}N_3O_4S$) solution at 2.50 mg/mL. Transfer 2 mL of this solution to a 250 mL Erlenmeyer flask with lid. Prepare a standard solution in the same conditions.

Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: Kocuria rhizophila ATCC 9341.

Culture media: culture medium number 1, for microorganism maintenance; culture medium number 11, for base layer and inoculum preparation.

Sample solution: reconstitute the content as indicated on the label. Transfer a volume of oral suspension to a volumetric flask and dilute with *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)*, to obtain a 0.1 ampicillin solution. Dilute to obtain concentrations of 0.05 µg/mL, 0.1 µg/mL and 0.2 µg/mL, using *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Standard solution: accurately weigh approximately 25 mg of ampicillin CRS, transfer to a 250 mL volumetric flask and complete the volume with sterile water. Dilute to obtain concentrations of 0.05 µg/mL, 0.1 µg/mL and 0.2 µg/mL, using *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Procedure: add 20 mL of culture medium number 11 to each plate, wait until it solidifies, add 5 mL of 0.5% inoculum and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Add to the cylinders 0.2 mL of the freshly prepared solutions. Calculate the sample potency, in µg of ampicillin per milliliter of the reconstituted suspension, from the potency of standard and responses obtained with the *Standard solutions* and with the *Sample solutions*.

B. Proceed as described in *Iodometric test of antibiotics (5.3.3.10)*. Reconstitute the content of 10 units, as indicated on the label. Mix and homogenize the content of the vials. Transfer an amount, accurately measured, of reconstituted oral suspension to a volumetric flask, add water, shake for three to five minutes and complete the volume with the same solvent, to obtain an ampicillin solution at 2.50 mg/mL. Prepare a standard solution in the same conditions.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph with ultraviolet detector at 254 nm; pre-column with 50 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); stored at room temperature; *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: mixture of water, acetonitrile, *M* monobasic potassium phosphate and *M* acetic acid (909:80:10:1).

Diluent: mix 10 mL of *M* monobasic potassium phosphate and 1 mL of *M* acetic acid. Dilute with water to 1000 mL.

Sample solution: reconstitute the suspension as described on the product label. Transfer a volume of oral suspension equivalent to 0.1 g of ampicillin to a 100 mL volumetric flask, add 75 mL of *Diluent* and homogenize. If necessary, leave in ultrasonic bath. Complete the volume with the same solvent.

Standard solution: dissolve an accurately weighed amount of ampicillin CRS in *Diluent* and dilute with the same solvent to obtain a 1 mg/mL solution.

Resolution solution: dissolve an accurately weighed amount of caffeine in *Standard solution* and dilute with the same solvent to obtain a 0.12 mg/mL solution.

Inject 20 µL replicates of the *Resolution solution*. The resolution between caffeine and ampicillin is no less than 2.0. Inject 20 µL replicates of the *Standard solution*. The tailing factor is no more than 1.4. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₆H₁₉N₃O₄S in the powder for oral suspension from the responses obtained with the *Standard solution* and the *Sample solution*.

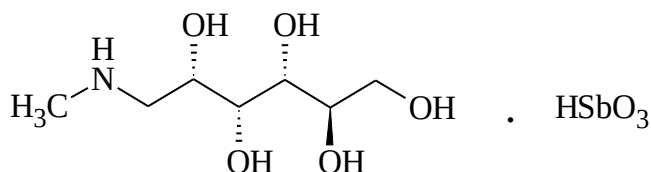
PACKAGING AND STORAGE

In tightly closed containers, protected from humidity and at temperature below 25 °C.

LABELING

Comply with current legislation.

MEGLUMINE ANTIMONIATE



$\text{C}_7\text{H}_{17}\text{NO}_5 \cdot \text{HSbO}_3$; 365.98

meglumine antimoniate; 05587

D-glucitol-1-deoxy-1-(methylamino) trioxoantimonate(1-)

[133-51-7]

Meglumine antimoniate is constituted of the pentavalent *N*-methylglucamine antimony salt. Contains no less than 26% and no more than 28% of pentavalent antimony (Sb^{5+}), in relation to meglumine antimoniate.

DESCRIPTION

Physical characteristics. White, slightly yellow powder.

Solubility. Soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. Dissolve 6 g of the sample in 20 mL of water. Acidify 2 mL of this solution with hydrochloric acid RS and add thioacetamide RS, prepared at the moment of use. Orange precipitate is produced.

B. Dissolve 6 g of the sample in 20 mL of water. Dilute 1 mL of this solution with 9 mL of water. Acidify with 5 mL of 0.3% (v/v) sulfuric acid and add 4 mL of alkaline mercuric potassium iodide RS. A yellow color develops after a few seconds.

PURITY TESTS

pH (5.2.19). 5.5 to 7.5. Determine in 30% (w/v) solution in carbon dioxide-free water.

Trivalent antimony. Proceed as described in *Atomic absorption spectrometry with hydride generation (5.2.13.1.2)*, system in batch, atomization in quartz cell, wavelength of 217.6 nm and monochromator resolution of (0.20 ± 0.10) nm.

Sample solution: prepare a 0.3% (w/v) sample solution in water and dilute this solution by a factor at 500 times, using the same solvent.

Standard solution: prepare a 0.1% (w/v) trivalent antimony solution, by dilution of antimony potassium tartrate ($\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$) in water.

Reducer solution: prepare, immediately, the 1% (w/v) sodium borohydride solution in 0.1% (w/v) solution.

Citric acid solution: prepare a 4% (w/v) citric acid solution in water.

Procedure: adapt the reaction vial in the hydride generator system, wait 30 seconds for the system purge, and proceed to the determination, according to recommendations from the manufacturer, specific to the equipment used. The maximum interval for mixing the diluted *Sample solution* or the *Standard solution* with the *Citric acid solution* must be five seconds before the introduction in the equipment. Build the analytical curve with aliquots of 0.1 mL of *Standard solution* of antimony in the following concentrations: 0.1 mg/L; 0.2 mg/L; 0.3 mg/L; 0.4 mg/L and 0.5 mg/L, prepared every day by sequential dilution in water. Put between 0.20 mL and 0.80 mL of the diluted *Sample solution* or the *Standard solution* of antimony in the reaction vial and add 10 mL of *Citric acid solution*. No more than 0.04 mg of trivalent antimony per milliliter of the 0.3% (w/v) meglumine antimoniate solution corresponds to 1.33% of trivalent antimony from the substance analyzed.

Heavy metals. The determinations must be made by *Atomic absorption spectrometry (5.2.13.1)*, with *graphite furnace (5.2.13.1.4)* or *hydride generation (5.2.13.1.2)*, by *Optical emission spectrometry with inductively coupled plasma (5.2.13.2.2)* or by *Mass spectrometry with inductively coupled plasma (5.2.13.3)*. No more than 9 mg/L, in the 30% (w/v) meglumine antimoniate solution, corresponding to 0.003% (30 ppm) of heavy metals in the substance analyzed, for the sum of concentration of the following elements: aluminum, arsenic, bismuth, cadmium, lead, copper, chromium, manganese, mercury, nickel and zinc.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*, use *Method I*. Employ the following conditions: air plus acetylene flame, wavelength 217.6 nm, monochromator resolution of (0.20 ± 0.10) nm.

Sample solution: prepare a 30% (w/v) meglumine antimoniate solution in water and, then, dilute by a factor of 2500 times, with 6 M hydrochloric acid.

Standard solution: prepare a 0.1% (w/v) trivalent antimony solution, in water, using antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$).

Procedure: build the analytical curve with the *Standard solution* of antimony in the following concentrations: 10 mg/L; 20 mg/L; 30 mg/L; 40 mg/L and 50 mg/L, by sequential dilution in 6 M hydrochloric acid. From the concentration of Sb determined, calculate the content of Sb in meglumine antimoniate.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiprotozoal.

MEGLUMINE ANTIMONIATE INJECTION SOLUTION

Contains no less than 92.0% and no more than 108.0% of pentavalent antimony (Sb^{5+}) in relation to the declared amount of Sb^{5+} . Each 1.5 g of meglumine antimoniate contains 405 mg of Sb^{5+} .

IDENTIFICATION

A. Acidify 2 mL of the injection solution with hydrochloric acid RS and add thioacetamide RS, prepared at the moment of use. Orange precipitate is developed.

B. Dilute 1 mL of the injection solution with 9 mL of water. Acidify this solution with 5 mL of 0.3% (v/v) sulfuric acid and add 4 mL of alkaline mercuric potassium iodide RS. A yellow color develops after a few seconds.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.1.19). 5.5 to 7.5.

PURITY TESTS

Trivalent antimony. Dilute the injection solution with water by a factor of 50,000 times and proceed as described in *Trivalent antimony*, in the *Meglumine antimoniate* monograph.

Heavy metals. Proceed as described in *Heavy metals* in the *Meglumine antimoniate* monograph. No more than 0.0009% (9 mg/L) of injection solution.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.5 EU/mg of meglumine antimoniate.

Toxicity (5.5.2.3). Complies with the test. Inject, intravenously, the equivalent to 1 mg/g of weight of the animal.

ASSAY

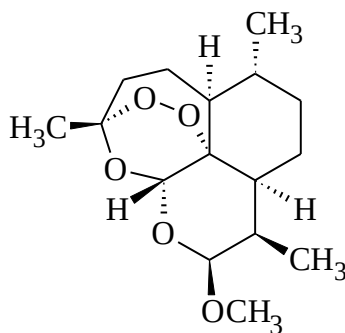
Dilute the injection solution with water by a factor of 2500 times with 6 M hydrochloric acid and proceed as described in *Assay* on the *Meglumine antimoniate* monograph.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

ARTEMETHER***Artemetherum***

$C_{16}H_{26}O_5$; 298.38

artemether; 00885

(3*R*,5*aS*,6*R*,8*aS*,9*R*,10*S*,12*R*,12*aR*)-Decahydro-10-pyrano[4,3-*j*]-1,2-benzodioxepin
[71963-77-4]

methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-

Contains no less than 98.0% and no more than 102.0% of $C_{16}H_{26}O_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, fine, crystalline powder.

Solubility. Practically insoluble in water, freely soluble in absolute ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 86 °C to 90 °C.

Specific optical rotation (5.2.8): +166 to +173. Determine in 1% (w/v) solution in absolute ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of artemether CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described in *Assay*. Prepare *Solution (1)* and *Solution (2)* as described next.

Solution (1): 10 mg/mL sample solution in *Mobile phase*.

Solution (2): 0.05 mg/mL sample solution in *Mobile phase*.

Procedure: inject separately 20 µL of *Solution (1)* and of *Solution (2)*. Record the chromatograms and measure the area under the peaks. The area of any secondary peak obtained with *Solution (1)* is not greater than the area from the main peak, obtained with *Solution (2)* (0.5%). At maximum a secondary peak obtained with *Solution (1)* presents an area greater than half the area under the main peak obtained with *Solution (2)* (0.25%). The sum of the areas under all the secondary peaks obtained with *Solution (1)* is not greater than twice the area under the main peak obtained with *Solution (2)* (1.0%). Do not consider peaks with an area less than 0.1 times that presented by the main peak in the chromatogram obtained with *Solution (2)*.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in phosphorus pentoxide atmosphere, in oven at 60 °C, under reduced pressure, for four hours. No more than 0.5%.

Residue by incineration (5.2.10). No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 216 nm; chromatographic column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), maintained at temperature of 30 °C; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: mixture of acetonitrile and water (62:38).

Sample solution: dissolve an accurately weighed amount of the sample in *Mobile phase* to obtain a 4 mg/mL solution.

Standard solution: dissolve an accurately weighed amount of artemether CRS in *Mobile phase* to obtain a 4 mg/mL solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₆H₂₆O₅ in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

THERAPEUTIC CLASS

Antimalarial.

ARTEMETHER INJECTION SOLUTION

Contains no less than 95.0% and no more than 105.0% of the declared amount of $C_{16}H_{26}O_5$.

IDENTIFICATION

A. Transfer a volume of injection solution equivalent to 50 mg of artemether to a beaker, add 25 mL of acetone, shake and filter. Evaporate the filtrate at temperature of 40 °C and dry the residue in desiccator for 24 hours. Proceed as described in test **A.** of *Identification* from the *Artemether* monograph.

B. Add 6 mL of absolute ethyl alcohol to a volume of injection solution equivalent to 30 mg of artemether. Transfer five drops to a porcelain capsule and add one drop of vanillin RS. A pink color develops.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

PURITY TESTS

Related substances. Proceed as described in *Related substances* in the *Artemether* monograph. Prepare solutions as described below.

Solution (1): dilute a volume of the injection solution in *Mobile phase* to obtain a 10 mg/mL solution.

Solution (2): dilute the *Solution (1)* in *Mobile phase* to obtain a 0.05 mg/mL solution.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Pyrogens (5.5.2.1). Complies with the test.

ASSAY

Proceed as described in *Assay* in the *Artemether* monograph. Prepare the *Sample solution* as described next.

Diluent: mixture of isopropanol and acetonitrile (75:25).

Sample solution: dilute a volume of the injection solution in *Diluent* to obtain a 4 mg/mL solution.

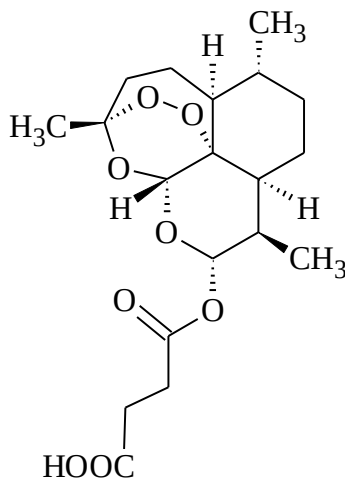
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{16}H_{26}O_5$ in the injection solution from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In type I glass containers, protected from light, at a temperature below 25 °C.

LABELING

Comply with current legislation.

ARTESUNATE***Artesunatum***

$C_{19}H_{28}O_8$; 384.43

artesunate; 09673

1-[(3*R*,5*aS*,6*R*,8*aS*,9*R*,10*S*,12*R*,12*aR*)-decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-ylidene] ester of butanedioic acid
[88495-63-0]

Contains no less than 98.0% and no more than 102.0% of $C_{19}H_{28}O_8$ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, fine and crystalline powder.

Solubility. Very slightly soluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 132 °C to 135 °C.

Specific optical rotation (5.2.8): +4.5 to +6.5. Determine in solution at 1% (w/v) in methylene chloride.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of artesunate CRS, prepared identically.

B. The retention time of the main peak from the chromatogram of the *Sample solution*, obtained in *Assay*, corresponds to that of the main peak of the *Standard Solution*.

PURITY TESTS

pH (5.2.19). 3.5 to 4.5. Determine in 1% (w/v) suspension in carbon dioxide-free water.

Related substances. Proceed as described in *Assay*. Prepare solutions as described below.

Solution (1): 4 mg/mL sample solution in acetonitrile.

Solution (2): 0.04 mg/mL sample solution in acetonitrile.

Procedure: inject separately 20 µL of each solution. Record the chromatograms and measure the areas under the peaks. The area of any secondary peak obtained with *Solution (1)* is not greater than the area from the main peak obtained with *Solution (2)* (1.0%). At maximum a peak obtained with *Solution (1)* presents an area greater than half the area under the main peak obtained with *Solution (2)* (0.5%). The sum of the areas under all the secondary peaks obtained with *Solution (1)* is not greater than twice the area under the main peak obtained with *Solution (2)* (2.0%). Disregard peaks with areas below 0.1 times the area under the main peak obtained with *Solution (2)*.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Water (5.2.20.1). Determine in 2 g of the sample. No more than 0.5%.

Residue by incineration (5.2.10). No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 216 nm; column with 125 mm of length and 3 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), maintained at temperature of 30 °C; *Mobile phase* flow of 0.6 mL/minute.

Buffer pH 3.0: dissolve 1.36 g of monobasic sodium phosphate in 900 mL of water. Adjust pH to 3.0 with phosphoric acid, complete the volume to 1000 mL with water and homogenize.

Mobile phase: mixture of *Buffer pH 3.0* and acetonitrile (50:50).

Sample solution: dissolve an accurately weighed amount of acetonitrile, to obtain a 2 mg/mL solution.

Standard solution: dissolve an accurately weighed amount of artesunate CRS in acetonitrile, to obtain a 2 mg/mL solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₉H₂₈O₈ in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimalarial.

ARTESUNATE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{19}H_{28}O_8$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 50 mg of artesunate to a beaker, add 25 mL of acetone, shake and filter. Evaporate the filtrate in water bath and leave the residue in a desiccator, under silica gel, for 24 hours. The residue obtained complies with the test **A.** in *Identification* of the *Artesunate* monograph.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described on the method from *Assay*.

PURITY TESTS

Related substances. Proceed as described in *Related substances* in the *Artesunate* monograph. Prepare solutions as described below.

Solution (1): weigh and pulverize the tablets. Transfer an amount of powder equivalent to 0.4 g of artesunate to a beaker and add 20 mL of ethyl alcohol. Shake vigorously and filter. Transfer 5 mL of the filtrate to a 25 mL volumetric flask and complete the volume with *Mobile phase*.

Solution (2): transfer 1 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Assay* in the *Artesunate* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Transfer, quantitatively, an amount of powder equivalent to 0.25 g of artesunate to a 25 mL volumetric flask, add 20 mL of ethyl alcohol and shake mechanically for 10 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of the filtrate to a 25 mL volumetric flask and complete the volume with *Mobile phase*.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₉H₂₈O₈ in the tablets from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

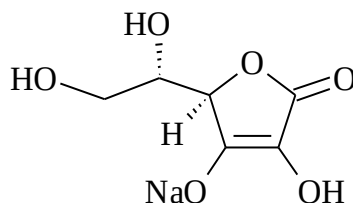
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

SODIUM ASCORBATE

Natrii ascorbas



$C_6H_7NaO_6$; 198.11

sodium ascorbate; 00107

Sodium salt of L-ascorbic acid (1:1)

[134-03-2]

Contains no less than 99.0% and no more than 101.0% of $C_6H_7NaO_6$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish crystalline powder.

Solubility. Freely soluble in water, moderately soluble in ethyl alcohol and practically insoluble in methylene chloride.

Physicochemical constants.

Specific optical rotation (5.2.8): +103 to +108 Determine in 100 mg/mL solution in water.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of sodium ascorbate CRS, prepared identically.

B. Weigh 1 g of the sample and dissolve in 50 mL of water. Add to 4 mL of this solution 1 mL of 0.1 M hydrochloric acid. The resulting solution reduces the alkaline cupric tartrate RS slowly, at room temperature, and more quickly under heating.

C. Complies with sodium ion reactions (5.3.1.1).

D. Prepare a 10% (w/v) solution of the sample. Add to 1 mL of this solution 0.2 mL of nitric acid RS and 0.2 mL of 1.7% (w/v) silver nitrate. There is formation of gray precipitate.

PURITY TESTS

Aspect of the preparation. Dissolve 5 g of the sample in water and complete the volume to 50 mL with the same solvent. This preparation is not more intensely stained than the standard solution, prepared by diluting 5 mL of the *Color reference solution* described next, in 95 mL of 1% (w/v) hydrochloric acid. Proceed as described in *Color of liquids* (5.2.12).

Color reference solution: mix 1.5 parts of the ferric chloride base solution, 1.2 parts of cupric sulfate base solution, 1.5 parts of the cobalt II chloride base solution and 0.8 parts of 1% (w/v) hydrochloric acid.

pH (5.2.19). 7.0 to 8.0. Determine in solution at 10% (w/v).

Volatile organic impurities. Proceed as described in *Gas chromatography (5.2.17.5)*. Use a chromatograph equipped with a flame ionization detector, using a mixture of nitrogen, synthetic air and hydrogen (1:1:10) as auxiliary gases to the detector flame; capillary column with 30 m of length and 0.53 mm of internal diameter, packed with stationary phase bonded to phenyl and methylpolysiloxane (5:95), with film thickness of 5 µm; column temperature from 35 °C to 260 °C (35 °C maintained for five minutes, increase to 175 °C at 8 °C per minute, increased to 260 °C at 35 °C and maintained at this temperature for no less than 16 minutes), injector temperature of 70 °C and detector temperature at 260 °C; use helium as carrier gas; carrier gas flow of 1 mL/minute.

Sample solution: accurately weigh approximately 1 g of the sample and dissolve in 50 mL of organic compounds-free water.

Standard solution: prepare a solution, in organic compounds-free water, containing in each mL 10 µg of methylene chloride, 1 µg of chloroform, 2 µg of benzene, 2 µg of dioxane and 2 µg of trichlorethylene.

Inject, separately, 1 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the area under the peaks. Identify, based on the retention time, any peaks present in the sample solution chromatogram. The presence and identification of the peaks in the chromatogram must be established by comparing the chromatograms of the *Sample Solution* and *Standard Solution*. The sample must have no more than 2 ppm of benzene, 50 ppm of chloroform, 100 ppm of dioxane, 500 ppm of methylene chloride and 100 ppm of trichlorethylene.

Oxalic acid. Allow the following preparations to stand for one hour. The opalescence of the *Sample preparation* is not more intense than that of the *Standard preparation*.

Sample preparation: dissolve 0.25 g of sample in 5 mL of water. Add 1 mL of diluted acetic acid and 0.5 mL of calcium chloride RS. No more than 0.3% (3000 ppm).

Standard preparation: dissolve 70 mg of oxalic acid in water and complete to the volume of 500 mL with the same solvent. Add to 5 mL of this solution 1 mL of diluted acetic acid and 0.5 mL of calcium chloride RS.

Sulfates (5.3.2.2). Dissolve 1.6 g of the sample in 40 mL of water and proceed as described in *Limit test for sulfates*, using 0.5 mL of 0.005 M standard sulfuric acid solution. No more than 0.015% (150 ppm).

Copper. Proceed as described in *Flame atomic absorption spectrometry (5.2.13.1.1)*. Use a spectrophotometer equipped with a flame fed by a mixture of air-acetylene, copper hollow cathode lamp, and select the emission line at 324.8 nm.

Sample solution: transfer 2 g of the sample to a 25 mL volumetric flask and complete the volume with nitric acid RS. No more than 5 ppm of copper.

Iron. Proceed as described in *Flame atomic absorption spectrometry (5.2.13.1.1)*. Use a spectrophotometer equipped with a flame fed by a mixture of air-acetylene, iron hollow cathode lamp, and select the emission line at 248.3 nm.

Sample solution: transfer 5 g of the sample to a 25 mL volumetric flask and complete the volume with nitric acid RS. No more than 2 ppm of iron.

Nickel. Proceed as described in *Flame atomic absorption spectrometry (5.2.13.1.1)*. Use a spectrophotometer equipped with a flame fed by a mixture of air-acetylene, nickel hollow cathode lamp, and select the emission line at 232.0 nm.

Sample solution: transfer 10 g of the sample to a 25 mL volumetric flask and complete the volume with nitric acid RS. No more than 1 ppm of nickel.

Heavy metals (5.3.2.3). Dissolve 2 g of the sample in water and proceed as described in *Method I*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample, in oven at 105 °C, until constant weight. Not more than 0.25%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.4 g of the sample and dissolve in a mixture of 100 mL of water and 25 mL of 9.8% (w/v) sulfuric acid. Titrate immediately with 0.1 mL *M* iodine VS and add 3 mL of starch I, near the endpoint. Each mL of 0.1 *M* iodine VS is equivalent to 19.81 mg of C₆H₇NaO₆.

PACKAGING AND STORAGE

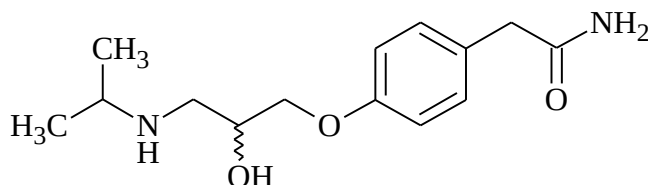
In non-metallic containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Excipient.

ATENOLOL***Atenololum***

$C_{14}H_{22}N_2O_3$; 266.34

atenolol; 00911

4-[2-Hydroxy-3-[(1-methylethyl)amino]propoxy]benzeneacetamide

[29122-68-7]

Contains no less than 98.0% and no more than 102.0% of $C_{14}H_{22}N_2O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Very slightly soluble in water, freely soluble in methyl alcohol, soluble in glacial acetic acid and ethyl alcohol, practically insoluble in acetonitrile.

Physicochemical constants.

Melting range (5.2.2): 152 °C to 155 °C.

Optical rotation (5.2.8): +0.10° to -0.10°. Determine in solution at 1% (w/v) in water.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of atenolol CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 230 nm to 350 nm, of 0.01% (w/v) solution in methyl alcohol, there are maximum and minimum points only at the same wavelengths of similar atenolol CRS solution. The ratio between the absorbance values measured at 275 nm and 282 nm is comprised between 1.15 and 1.20.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of ammonium hydroxide and methyl alcohol (3:97) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): 10 µg/mL sample solution in methyl alcohol.

Solution (2): 10 µg/mL atenolol CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

PURITY TESTS

Chlorides. Dissolve 1 g of the sample in 100 mL of 0.15 M nitric acid, add 1 mL of silver nitrate RS and homogenize. Any turbidity developed is not more intense than that of the mixture of 2.8 mL of 0.01 M hydrochloric acid, 98.6 mL of 0.15 M nitric acid and 1 mL of silver nitrate RS. No more than 0.1% (1000 ppm).

Chromatographic purity. Proceed as described on method **B.** from Assay. Prepare *Solution (1)* and *Solution (2)* as described next.

Solution (1): dissolve an accurately weighed amount of the sample in *Mobile phase* to obtain a 0.1 mg/mL solution.

Solution (2): transfer 0.5 mL of *Solution (1)* to a 100 mL volumetric flask and dilute with *Mobile phase* to obtain a 0.5 µg/mL solution.

Procedure: inject, separately, 50 µL of *Solution (1)* and of *Solution (2)*, record the chromatograms for a period of time corresponding to no less than six times the atenolol peak retention time and measure the areas under the peaks. No secondary peak obtained with *Solution (1)* must present an area greater than half the area under the main peak obtained with *Solution (2)* (0.25%). The sum of the areas under the secondary peaks obtained with *Solution (1)* must not be greater than the area under the main peak obtained with *Solution (2)* (0.5%).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C, until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 25 mg of the sample and dissolve in methyl alcohol. Complete the volume to 50 mL with the same solvent. Dilute, successively, in methyl alcohol until concentration of 0.01% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 275 nm, using methyl alcohol for zero adjustment. Calculate the content of C₁₄H₂₂N₂O₃ in the sample, from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 226 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow of 0.6 mL/minute.

Mobile phase: dissolve 1.1 g of sodium heptanesulfonate and 0.71 g of sodium phosphate dibasic anhydrous in 700 mL of water. If necessary, adjust pH to 3.0 with phosphoric acid RS. Add 300 mL of methyl alcohol and 2 mL of dibutylamine and homogenize.

Sample solution: dissolve an accurately weighed amount of the sample in *Mobile phase* to obtain a 10 µg/mL solution.

Standard solution: dissolve an accurately weighed amount of atenolol CRS in *Mobile phase* to obtain a 10 µg/mL solution.

Inject 10 µL replicates of *Standard solution*. The column efficiency is no less than 5000 theoretical plates. The tailing factor is no more than 2.0. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₄H₂₂N₂O₃ in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS.

Anti-hypertensive.

ATENOLOL TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{14}H_{22}N_2O_3$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 230 nm to 350 nm of the sample solution obtained in method **A.** from *Assay*, displays maximum absorption points at 275 nm and 282 nm, identical to those observed in the spectrum of the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 25 mL volumetric flask containing 15 mL of methyl alcohol and leave in ultrasonic bath until the tablet disintegration. Proceed as described in method **A.** of *Assay* from "Heat the resulting suspension...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, dilute with 0.1% (v/v) phosphoric acid to concentration of 10 µg/mL and proceed as described on method **B.** of *Assay* from the *Atenolol* monograph. Calculate the amount of $C_{14}H_{22}N_2O_3$ dissolved in the medium, comparing the readings under the peaks obtained with the atenolol CRS solution at the concentration of 10 mg/mL, prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{14}H_{22}N_2O_3$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets, transfer an amount of powder equivalent to 0.25 g of atenolol to a 250 mL volumetric flask and add 150 mL of methyl alcohol. Heat the resulting suspension at 60 °C for 10 minutes, shaking occasionally. Shake mechanically for 15 minutes, cool and complete the volume with methyl alcohol. Homogenize and filter. Dilute, successively, in methyl alcohol until concentration of 0.01% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 275 nm, using methyl alcohol for zero adjustment. Calculate the amount of $C_{14}H_{22}N_2O_3$ in the tablets, from the readings obtained.

B. Proceed as described in method **B.** of *Assay* in the *Atenolol* monograph. Prepare the *Sample solution* as described next.

Sample solution: transfer 10 tablets to a 1000 mL volumetric flask, add 500 mL of *Mobile phase* and leave in ultrasonic bath for 15 minutes, or until full disintegration of the tablets. Complete the volume with the same solvent, homogenize and centrifuge. Dilute successively, in the same solvent, to a concentration of 10 µg/mL.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{14}H_{22}N_2O_3$ in the tablets from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

ATENOLOL AND CHLORTALIDONE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of atenolol ($C_{14}H_{22}N_2O_3$) and chlortalidone ($C_{14}H_{11}ClN_2O_4S$).

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of *M* ammonium hydroxide and butyl alcohol (1:5) as mobile phase. Apply to the plate, separately, 10 μ L of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Transfer an amount of powder equivalent to 10 mg of chlortalidone to a 10 mL volumetric flask and add 8 mL of methyl alcohol. Shake mechanically for 15 minutes and complete the volume with the same solvent. Homogenize and filter.

Solution (2): prepare 1 mg/mL chlortalidone CRS solution in methyl alcohol.

Solution (3): prepare 4 mg/mL atenolol CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The stains related to atenolol and chlortalidone, obtained with *Solution (1)*, correspond in position, color and intensity to the ones obtained with *Solution (2)* and with *Solution (3)*.

B. The retention times of the main peaks of the *Sample solution* chromatogram, obtained in the *Assay*, correspond to the ones from the main peaks of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a volumetric flask, obtaining a chlortalidone solution with concentration of 0.025% (w/v). Add a mixture of water and acetonitrile (1:1) equivalent to 50% of the flask volume. Shake mechanically for 20 minutes until full tablet disintegration and complete the volume with the same solvent. Proceed as described in method of *Assay* from the preparation of the *Standard solution*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium and proceed as described in *Assay*. Prepare the *Sample solution*, the *Standard solution* and the *Diluent* as described next.

Diluent: mixture of acetonitrile and 1.8 M sulfuric acid (1000:32).

Sample solution: mixture of 10 mL of dissolution medium after the test and 3 mL of *Diluent*.

Standard solution: prepare a atenolol CRS solution in a mixture of water and *Diluent* (750:225), obtaining a solution at 0.00085 L mg of atenolol and 0.00085 L' mg of chlortalidone, per milliliter, where L and L' are the declared amounts, in milligrams, in the tablets, of atenolol and chlortalidone, respectively.

Tolerance: no less than 80% (Q) of the declared quantity of atenolol ($C_{14}H_{22}N_2O_3$) and 70% (Q) of the declared quantity of chlortalidone ($C_{14}H_{11}ClN_2O_4S$) dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 275 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow of 1.7 mL/minute.

Mobile phase: mixture of water, acetonitrile and 1.8 M sulfuric acid (740:250:8) and 930 mg of sodium octyl sulfate per liter of mixture.

Sample solution: weigh and pulverize 10 tablets. Transfer an amount of powder equivalent to 25 mg of chlortalidone to a 50 mL volumetric flask, add 40 mL of the mixture of water and acetonitrile (1:1) and shake mechanically for 20 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 25 mL of the filtrate to a 50 mL volumetric flask and complete the volume with water, obtaining a 0.25 mg/mL solution.

Standard solution: dissolve an amount of atenolol CRS and chlortalidone CRS, accurately weighed, in a mixture of water and acetonitrile (3:1) to obtain a solution at 1 mg/mL and 0.25 mg/mL, respectively.

Inject 10 μ L replicates of the *Standard solution*. The relative retention times are approximately 0.8 for atenolol and 1.0 for chlortalidone. The resolution between chlortalidone and atenolol is no less than 3.0. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

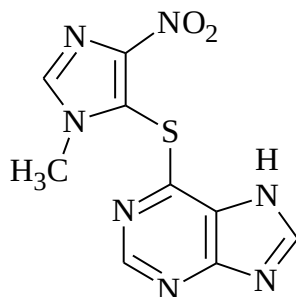
Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of atenolol ($C_{14}H_{22}N_2O_3$) and chlortalidone ($C_{14}H_{11}ClN_2O_4S$) in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

AZATHIOPRINE***Azathioprinum***

$C_9H_7N_7O_2S$; 277.26

azathioprine; 00984

6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9*H*-purine
[446-86-6]

Contains no less than 98.0% and no more than 101.5% of $C_9H_7N_7O_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Pale yellow powder.

Solubility. Practically insoluble in water and ethyl alcohol. Soluble in diluted solutions of alkali hydroxides and slightly soluble in diluted mineral acid solutions.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of azathioprine CRS, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 230 nm to 350 nm, of the 0.00075% (w/v) solution prepared in 0.1 *M* hydrochloric acid, there is a maximum absorption at 280 nm, identical to that observed in the spectrum of similar solution of azathioprine CRS.

C. Heat 20 mg of the sample with 100 mL of water and filter. Add to 5 mL of the filtrate 1 mL of hydrochloric acid and 10 mg of powdered zinc and allow to stand for 5 minutes. A yellow color develops. Filter. Add 0.1 mL of sodium nitrite RS and 0.1 g of sulfamic acid. Shake until the bubbles disappear. Add 1 mL of 2-naphthol RS. A pink precipitate is produced.

PURITY TESTS

Acidity or alkalinity. Weigh, accurately, about 2 g of the sample, transfer to a 100 mL volumetric flask and shake for 15 minutes. Filter. No more than 0.1 mL of 0.02 *M* sodium hydroxide is spent to neutralize 20 mL of the filtrate, using methyl red TS as an indicator. No more than 0.2 mL of 0.01 *M* hydrochloric acid is spent to neutralize 20 mL of the filtrate, using methyl red TS as an indicator.

Limit of mercaptopurine. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of butyl alcohol, ethyl alcohol and water (4:1:1) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 20 mg/mL sample solution in ammonia RS.

Solution (2): 0.2 mg/mL mercaptopurine solution in ammonia RS.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Then, submit to iodine vapors. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram with *Solution (2)* (1.0%).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven, under reduced pressure, at 105 °C, for five hours. No more than 1.0%.

Residue by incineration (5.2.10). No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh about 0.25 g of the sample and dissolve in 25 mL of dimethylformamide. Titrate with 0.1 M tetrabutylammonium VS, determining the endpoint potentiometrically. Each mL of 0.1 M tetrabutylammonium hydroxide VS is equivalent to 27.726 mg of C₉H₇N₇O₂S.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer, quantitatively, about 0.1 g of azathioprine to a 500 mL volumetric flask and add 300 mL of 0.1 M hydrochloric acid. Leave it in water bath for 30 minutes. Cool and complete the volume with the same solvent. Homogenize. Carry out the dilution to a concentration of 0.001% (w/v), using the same solvent. Prepare a standard solution in the same conditions. Measure the absorbance of the solutions at 280 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the content of C₉H₇N₇O₂S in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Immunosuppressant.

AZATHIOPRINE TABLETS

Contains no less than 93.0% and no more than 107.0% of the declared amount of $C_9H_7N_7O_2S$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using microcrystalline cellulose as support, and a mixture of butyl alcohol saturated with 6 M sodium hydroxide as mobile phase. Apply to the plate, separately, 5 μ L of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Transfer an amount of powder equivalent to 0.2 g of azathioprine to a small vial and add 10 mL of 6 M sodium hydroxide. Homogenize and filter.

Solution (2): prepare a 20 mg/mL solution of azathioprine CRS in 6 M sodium hydroxide.

Develop the chromatogram. Remove the plate, allow to air dry and examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. Weigh and pulverize the tablets. Use an amount of powder equivalent to 20 mg of azathioprine and proceed as described on test **C.** of *Identification* from the *Azathioprine* monograph.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in water until adequate concentration. Measure the absorbances of the solutions at 280 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_9H_7N_7O_2S$ dissolved in the medium, comparing the readings obtained with that of the azathioprine CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_9H_7N_7O_2S$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 0.15 g of azathioprine to a 500 mL volumetric flask. Dissolve in 20 mL of dimethylsulfoxide and complete the volume with 0.1 M hydrochloric acid. Filter. Dilute, successively, in 0.1 M hydrochloric acid to a concentration of 0.00075% (w/v). Prepare the standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 280 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_9H_7N_7O_2S$ in the tablets from the readings obtained. Alternatively, make calculations considering $A(1\%, 1\text{ cm}) = 628$, at 280 nm, in 0.1 M hydrochloric acid.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 300 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (10 μm), kept at room temperature; *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: dissolve 1 g of sodium 1-heptanesulfonate in 700 mL of water, add 300 mL of methyl alcohol and homogenize. Adjust the solution pH to 3.5 with M hydrochloric acid.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 50 mg of azathioprine to a 100 mL volumetric flask. Add 25 mL of methyl alcohol and 1 mL of ammonium hydroxide to the flask and take to an ultrasonic bath for two minutes. Complete the volume with methyl alcohol and homogenize. Transfer 10 mL of the solution to a 50 mL volumetric flask, complete the volume with water and homogenize.

Standard solution: weigh, accurately, about 25 mg of azathioprine CRS and transfer to a 50 mL volumetric flask. Add 15 mL of methyl alcohol and 0.5 mL of ammonium hydroxide and leave in ultrasonic bath for two minutes. Complete the volume with methyl alcohol and homogenize. Transfer 10 mL of the solution to a 50 mL volumetric flask, complete with water and homogenize.

Inject 10 μL replicates of the *Standard solution*. The column efficiency is no less than 800 theoretical plates. The tailing factor is no more than 1.5. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

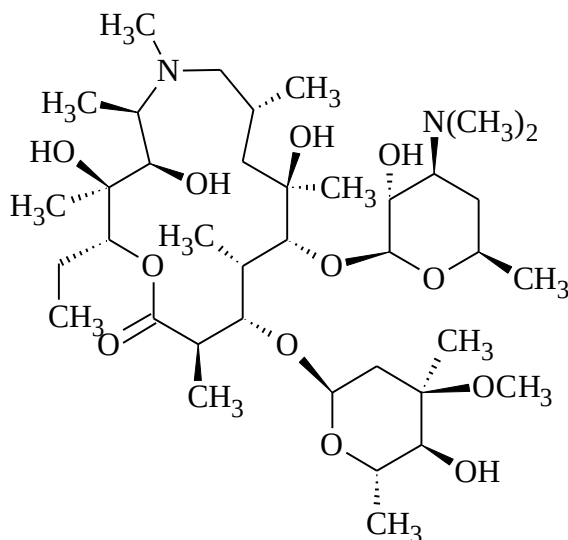
Procedure: inject, separately, 10 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the area under the peaks. Calculate the amount of $C_9H_7N_7O_2S$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

AZITHROMYCIN***Azithromycinum***

$C_{38}H_{72}N_2O_{12}$; 749.00

azithromycin; 00997

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribohexopyranosyl)oxy]-2-ethyl-3,4,10-tri-hydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xilo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one [83905-01-5]

$C_{38}H_{72}N_2O_{12} \cdot H_2O$; 767.01

azithromycin monohydrate; 10657

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribohexopyranosyl)oxy]-2-ethyl-3,4,10-tri-hydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xilo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one hydrate [121470-24-4]

$C_{38}H_{72}N_2O_{12} \cdot 2H_2O$; 785.03

azithromycin di-hydrate; 00998

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribohexopyranosyl)oxy]-2-ethyl-3,4,10-tri-hydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- β -*D*-xilo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one di-hydrate [117772-70-0]

The potency is no less than 945 μ g and no more than 1030 μ g of azithromycin ($C_{38}H_{72}N_2O_{12}$) per milligram, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, crystalline powder.

Solubility. Very slightly soluble in water, freely soluble in ethyl alcohol and methyl alcohol. Very slightly soluble in alkali hydroxide solutions. Moderately soluble in acidic solutions.

Physicochemical constants.

Specific optical rotation (5.2.8): -45 to -49, in relation to the anhydrous substance. Determine in 2% (w/v) solution in ethyl alcohol, at 20 °C.

IDENTIFICATION

In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of azithromycin CRS, prepared identically.

PURITY TESTS

pH (5.2.19). 9.0 to 11.0. Determine in solution at 0.2% (w/v) in a mixture of water and methyl alcohol (1:1).

Heavy metals (5.3.2.3). Use *Method IV*. No more than 0.0025% (25 ppm).

Water (5.2.20.1). No more than 5.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Moisten the sample with 2 mL of nitric acid and five drops of sulfuric acid. No more than 0.3%.

Crystallinity. Suspend a few particles of the sample in mineral oil, transfer to a glass slide and examine on a microscope equipped with polarized light. The particles display birefringence, which is extinguished when moving the sample, through micrometric adjustment.

ASSAY

Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: Kocuria rhizophila ATCC 9341.

Culture media: culture medium number 1, for microorganism maintenance; culture medium number 3, for inoculum standardization; and culture medium number 11, for base layer and inoculum preparation.

Sample solution: weigh, accurately, about 25 mg of the sample, transfer to a 25 mL volumetric flask using 10 mL of methyl alcohol. Shake mechanically for 15 minutes and complete the volume with methyl alcohol. Filter. Dilute, successively, the resulting solution in *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)*, to obtain solutions at 0.1 µg/mL, 0.2 µg/mL and 0.4 µg/mL.

Standard solution: accurately weigh approximately 25 mg of azithromycin CRS, transfer to a 25 mL volumetric flask and complete the volume with methyl alcohol. Dilute, successively, the resulting solution in *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)*, to obtain solutions at 0.1 µg/mL, 0.2 µg/mL and 0.4 µg/mL.

Procedure: add 20 mL of culture medium number 11 to each plate, wait until it solidifies, add 5 mL of 1% inoculum and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Add to the cylinders 0.2 mL of the freshly prepared solutions. Calculate the potency

of the sample, in μg of azithromycin per milligram, from the potency of standard and responses obtained with the *Standard solutions* and with the *Sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

AZITHROMYCIN CAPSULES

Contains no less than 90.0% and no more than 110.0% of the declared amount of azithromycin ($C_{38}H_{72}N_2O_{12}$).

IDENTIFICATION

Weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 0.25 g of azithromycin to a 50 mL volumetric flask, dissolve in methyl alcohol and complete the volume with the same solvent. Filter. Evaporate the filtrate in water bath and weigh 1.5 mg of the residue. Proceed as described in *Identification* in the *Azithromycin* monograph.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Water (5.2.20.1). No more than 5.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Assay* in the *Azithromycin* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 25 mg of azithromycin to a 25 mL volumetric flask and complete the volume with methyl alcohol. Shake for 15 minutes and filter. Dilute, successively, the resulting solution in *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)*, to obtain solutions at concentrations between 0.1 µg/mL and 0.4 µg/mL.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

AZITHROMYCIN POWDER FOR ORAL SUSPENSION

Contains no less than 90.0% and no more than 110.0% of the declared amount of azithromycin ($C_{38}H_{72}N_2O_{12}$). Azithromycin powder for oral suspension is the mixture of azithromycin with one or more flavoring, buffering, sweetening and suspending agents.

IDENTIFICATION

Transfer an amount of powder equivalent to 0.2 mg of azithromycin to a 50 mL volumetric flask, complete the volume with methyl alcohol. Homogenize and filter. Evaporate the filtrate in water bath until obtaining the residue. Proceed as described in *Identification* in the *Azithromycin* monograph.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test. Determine in the diluent vial.

pH (5.2.19). 8.5 to 11.0. Reconstitute the suspension as described on the product label.

Determination of weight (5.1.1). Complies with the test. Determine in the non-reconstituted powder.

Uniformity of unit doses (5.1.6). Applied when the powder is packaged in single dose. Complies with the test. Reconstitute the suspension as described on the product label.

PURITY TESTS

Water (5.2.20.1). No more than 1.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Assay* in the *Azithromycin* monograph. Prepare the *Sample solution* as described next.

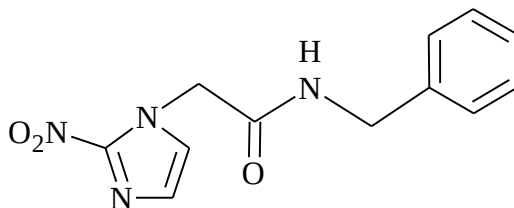
Sample solution: reconstitute the suspension as described on the product label. Transfer a volume of oral suspension, recently homogenized and free from bubbles, containing the equivalent to 0.2 g of azithromycin to a 20 mL volumetric flask using 10 mL of methyl alcohol. Shake and complete the volume with the same solvent. Filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask and complete the volume with *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)*. Dilute until concentrations of 0.1 µg/mL, 0.2 µg/mL and 0.4 µg/mL, using *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

BENZNIDAZOLE***Benznidazolium***

$C_{12}H_{12}N_4O_3$; 260.25

benznidazole; 01153

2-Nitro-*N*-(phenylmethyl)-1*H*-imidazole-1-acetamide

[22994-85-0]

Contains no less than 99.0% and no more than 101.0% of $C_{12}H_{12}N_4O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Slightly yellow crystalline powder, stable on air.

Solubility. Practically insoluble in water, very soluble in dimethylsulfoxide, slightly soluble in methyl alcohol, very slightly soluble in ethyl alcohol, isopropyl alcohol and glycerol. Very slightly soluble in 0.1 *M* sodium hydroxide and 0.1 *M* hydrochloric acid.

Physicochemical constants.

Melting range (5.2.2): 188 °C to 190 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of benznidazole CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the sample solution obtained in *Assay*, there is maximum absorption at 316 nm, identical to that observed in the spectrum of the standard solution. The absorbance at 316 nm is of approximately 0.352.

C. The main stain of the chromatogram from *Solution (1)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of chloroform, ethyl acetate, methyl alcohol and glacial acetic acid (40:40:15:5) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): 25 mg/mL sample solution in acetone.

Solution (2): 25 mg/mL of benznidazole CRS solution in acetone.

Solution (3): dilute the *Solution (2)* in acetone to obtain a 125 µg/mL solution.

Develop the chromatogram. Remove the plate and allow to air dry. Heat at 110 °C for 10 minutes. Let it cool and examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram with *Solution (3)* (0.5%).

Chlorides. Dissolve 30 mg of the sample in 3 mL of methyl alcohol using a test tube and add 5 mL of 12% (v/v) nitric acid and 5 mL of silver nitrate RS. Turbidity is not formed.

Loss on drying (5.2.9.1). Determine in oven at 105 °C for four hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer, quantitatively, about 0.12 g of the sample to a 200 mL volumetric flask and add 150 mL of methyl alcohol. Shake, mechanically, until complete dissolution. Complete the volume with the same solvent and homogenize. Dilute, successively, in 0.1 M hydrochloric acid, to a concentration of 0.0012% (w/v). Prepare a standard solution in the same concentration and using the same solvents. Determine the absorbances of the solutions at 316 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the content of C₁₂H₁₂N₄O₃ in the sample from the readings obtained.

PACKAGING AND STORAGE

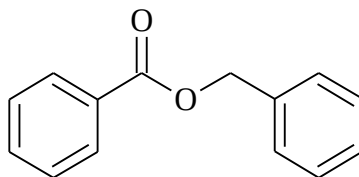
In hermetically closed containers and protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-Chagas.

BENZYL BENZOATE*Benzylis benzoas* $C_{14}H_{12}O_2$; 212.25

benzyl benzoate; 01155

Benzoic acid phenyl methyl ester

[120-51-4]

Contains no less than 99.0% and no more than 100.5% of $C_{14}H_{12}O_2$.**DESCRIPTION**

Physical-chemical characteristics. Colorless, clear, oily liquid, with faintly aromatic odor. By cooling, forms colorless crystals. Its freezing point is around 17 °C.

Solubility. Practically insoluble in water. Miscible in ethyl alcohol, ethyl ether, chloroform and fixed oils. Practically insoluble in glycerol.

Physicochemical constants.

Relative density (5.2.5): 1.116 to 1.120.

Refractive index (5.2.6): 1.568 to 1.570.

IDENTIFICATION

*The identification tests **B.** and **C.** can be omitted if test **A.** is conducted.*

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of benzyl benzoate CRS, prepared identically.

B. Dissolve 2 g of the sample in 25 mL of 0.5 M potassium hydroxide in ethyl alcohol, boil for 20 minutes and evaporate the ethyl alcohol in water bath. Cool and add 20 mL of water. Extract with two portions of 15 mL of ethyl ether and reserve the aqueous layer. Evaporate the ethereal layer in water bath. The colorless, oily residue, comprised of benzyl alcohol, presents ebullition point between 203 °C and 208 °C. Heat one drop of the residue with 5 mL of sodium carbonate RS and 1 mL of potassium permanganate RS. An odor of benzoic aldehyde is produced.

C. Add 10 mL of 10% (w/v) sulfuric acid to the aqueous layer obtained on test **B.** of *Identification*. A white, crystalline precipitate of benzoic acid is produced. Wash with water and dry in oven, under reduced pressure at 70 °C, for three to four hours. The melting point of the precipitate is, approximately, 121 °C.

PURITY TESTS

Acidity. Dissolve 1 g of the sample in 10 mL of previously neutralized ethyl alcohol. Add 0.2 mL of phenolphthalein TS and 0.2 mL of 0.1 M sodium hydroxide. A rosy color develops.

Aldehyde. Transfer 10 g of the sample to an Erlenmeyer flask with 50 mL of ethyl alcohol and 5 mL of 3.5% (w/v) hydroxylamine hydrochloride. Homogenize and allow to stand for 10 minutes. Add 1 mL of bromophenol blue TS and titrate with 0.1 M sodium hydroxide VS until a slightly green color. Carry out the blank test and make the necessary corrections. The volume of 0.1 M sodium hydroxide VS consumed in the sample titration must be no more than 0.5 mL (0.05% benzaldehyde).

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.05%.

ASSAY

Accurately weigh about 2 g of the sample, transfer to an Erlenmeyer flask and add 50 mL of 0.5 M ethanolic potassium hydroxide VS. Adapt to the flask a reflux condenser and boil for one hour. Cool and titrate the excess potassium hydroxide with 0.5 M hydrochloric acid VS, adding two drops of phenolphthalein TS. Carry out the blank test and make the necessary corrections. Each mL of 0.5 M potassium hydroxide VS is equivalent to 106.120 mg of $C_{14}H_{12}O_2$.

PACKAGING AND STORAGE

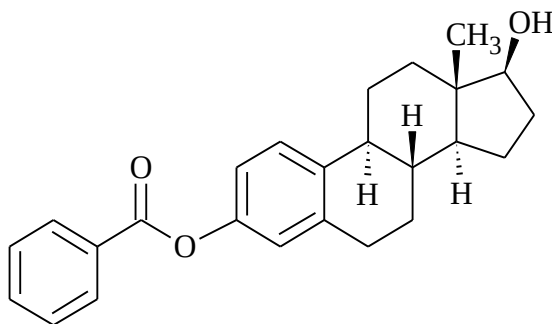
In tightly closed containers, protected from light and excessive heat.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Scabicide.

ESTRADIOL BENZOATE*Estradioli benzoas*C₂₅H₂₈O₃; 376.50

estradiol benzoate; 03597

(17 β)-Estra-1,3,5(10)-triene-3,17-diol 3-benzoate

[50-50-0]

Contains no less than 97.0% and no more than 103.0% of C₂₅H₂₈O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellow-white crystalline powder, stable on air.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol and in vegetable oils.

Physicochemical constants.

Melting range (5.2.2): 191 °C to 196 °C.

Specific optical rotation (5.2.8): +57 to +63, in relation to the desiccated substance. Determine in sample solution at 1.0% (w/v) in dioxane.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of estradiol benzoate CRS, prepared identically.

B. Dissolve 2 mg of sample in 2 mL of sulfuric acid. The solution becomes green-yellow with blue fluorescence. Add 2 mL of distilled water; the color becomes orange.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 230 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μ m), kept at temperature of 30 °C; *Mobile phase* flow of 1.0 mL/minute.

Eluent A: mixture of water and acetonitrile (40:60).

Eluent (B): acetonitrile.

Mobile phase: adopt the gradient system described in the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 - 20	100	0	isocratic
20 - 21	100 → 10	0 → 90	linear gradient
21 - 31	10	90	isocratic

Solution (1): weigh, accurately, 20 mg of the sample, dissolve in acetonitrile and dilute to 10 mL with the same solvent.

Solution (2): dissolve 5 mg of estradiol benzoate for CRS system suitability (containing impurities A (estradiol), B (17 β -hydroxy-4-methyl-estra-1,3,5(10)-triene-3-yl benzoate), C (estra-1,3,5(10)-triene-3,17 β -di-yl dibenzoate), E (17 α -hydroxy-estra-1,3,5(10)-triene-3-yl benzoate) and G (estrone benzoate)) in acetonitrile and dilute to 2.5 mL with the same solvent.

Solution (3): dilute 0.5 mL of *Solution (1)* to 100 mL with acetonitrile.

Use the chromatogram provided with the estradiol benzoate for CRS system suitability and the chromatogram obtained with *Solution (2)* to identify the peaks related to impurities A, B, C, E and G. The relative retention times in relation to estradiol benzoate (retention time of approximately 19 minutes) are about 0.3 for impurity A; about 1.1 for impurity E; about 1.2 for impurity B; about 1.3 for impurity G; and about 1.5 for impurity C.

Procedure: inject, separately, 10 μ L of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Multiply the areas under the peaks related to impurities A and C by the following correction factors: impurity A – 3.3; impurity C – 0.7. Calculate the percentage of each impurity found. The area obtained for impurity C is not greater than the area under the main peak obtained in the chromatogram of *Solution (3)* (0.5%). The areas under each of the peaks related to impurities B, E and G are not greater than 0.6 times the area under the main peak obtained in the chromatogram of *Solution (3)* (0.3%). The area obtained for impurity A is not greater than 0.4 times the area under the main peak obtained in the chromatogram of *Solution (3)* (0.2%). For the other impurities, the areas under each of the peaks are not greater than 0.2 times the area under the main peak obtained in the chromatogram of *Solution (3)* (0.10%). The sum of the areas of all impurities found in the chromatogram of *Solution (1)* is not greater than double the area under the main peak obtained in the chromatogram of *Solution (3)* (1.0%). Disregard peaks with areas below 0.1 times the area under the main peak obtained with *Solution (3)* (0.05%).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105 °C for three hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 0.5 g of the sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 25 mg of the sample and dissolve in ethyl alcohol. Dilute to 250 mL with the same solvent. Transfer 10 mL of this solution to a 100 mL volumetric flask, dilute and complete the volume with ethyl alcohol. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 231 nm, using ethyl alcohol for zero adjustment. Calculate the content of $C_{25}H_{28}O_3$ in the sample from the readings obtained.

PACKAGING AND STORAGE

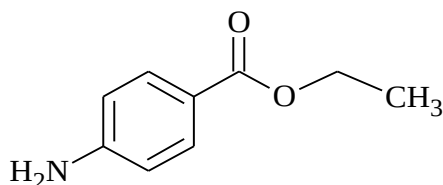
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Estrogen.

BENZOCAINE***Benzocainum***

$C_9H_{11}NO_2$; 165.19

benzocaine; 01159

4-aminobenzoic acid ethylic ester

[94-09-7]

Contains no less than 98.0% and no more than 102.0% of $C_9H_{11}NO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Very slightly soluble in water, freely soluble in ethyl alcohol. Soluble in diluted acid solutions.

Physicochemical constants.

Melting range (5.2.2): 88 °C to 92 °C. The interval between the beginning and the end of the melting is no more than 2 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated in phosphorous pentoxide anhydrous atmosphere for three hours, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of benzocaine CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of the 0.0005% (w/v) solution in chloroform, there is maximum absorption at 278 nm, identical to that observed in the spectrum of similar benzocaine CRS solution. The respective absorptivities, calculated in relation to the desiccated base and at the maximum absorbance wavelength, of approximately 278 nm, do not differ more than 3%.

C. Dissolve 20 mg of the sample with 10 mL of water in the presence of a few drops of 3 M hydrochloric acid. Add five drops of sodium nitrite RS solution and 2 mL of a solution prepared by dissolving 100 mg of 2-naphthol in 5 mL of M sodium hydroxide. Orange red precipitate is developed.

D. The 4% (w/v) sample solution in ethyl alcohol complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. The 5% (w/v) ethylic preparation is clear (5.2.25) and colorless (5.2.12).

Acidity or alkalinity. Dissolve 0.5 g of the sample in 10 mL of ethyl alcohol previously neutralized with 0.05 mL of phenolphthalein TS. Add 10 mL of carbon dioxide-free water. The solution remains colorless. No more than 0.5 mL of 0.01 M sodium hydroxide VS is spent to promote the indicator turning.

Related substances. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel GF₂₅₄ as support, and a mixture of chloroform with 0.75% anhydrous ethyl alcohol as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): transfer 100.0 mg of the sample, accurately weighed, to a 10 mL volumetric flask and dilute with anhydrous ethyl alcohol.

Solution (2): dissolve an accurately weighed amount of benzocaine CRS in anhydrous ethyl alcohol to obtain a 0.10 mg/mL solution.

Develop the chromatogram and allow the mobile phase to run three-quarters of the plate length. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm and 365 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, has maximum intensity equal to that obtained with *Solution (2)* (1.0%).

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry under phosphorus pentoxide for three hours. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph with an ultraviolet detector at 285 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to a phenyl group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Aqueous solution: mixture of 980 mL of water, 20 mL of acetic acid and 1 mL of triethylamine. Adjust pH to values between 2.95 and 3.00, if necessary. Homogenize.

Mobile phase: mixture of *Aqueous solution* and methyl alcohol (40:60).

Sample solution: weigh, accurately, about 24 mg of the sample, transfer to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 10 mL of this solution to a 100 mL volumetric flask and complete the volume with the same solvent. Homogenize.

Standard solution: dissolve an accurately weighed amount of benzocaine CRS in *Mobile phase* and dilute adequately to obtain a solution at 0.024 mg/mL.

Inject 10 µL replicates of the *Standard solution*. The tailing factor for the benzocaine peak is no more than 2.0. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₉H₁₁NO₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

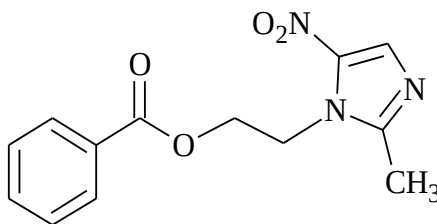
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anesthetic.

METRONIDAZOLE BENZOATE***Metronidazoli benzoas***C₁₃H₁₃N₃O₄; 275.26

metronidazole benzoate; 01166

2-Methyl-5-nitro-1*H*-imidazole-1-ethanol 1-benzoate

[13182-89-3]

Contains no less than 98.5% and no more than 101.0% of C₁₃H₁₃N₃O₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to yellowish-white crystalline powder or flakes.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 99 °C to 102 °C.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of metronidazole benzoate CRS, prepared identically.

PURITY TESTS

Acidity. Dissolve 2 g of the sample in 40 mL of mixture of dimethylformamide and water (1:1), neutralized in advance with 0.02 *M* hydrochloric acid or 0.02 *M* sodium hydroxide, using 0.2 mL of methyl red TS as indicator. No more than 0.25 mL of 0.02 *M* sodium hydroxide VS is necessary to change the color of the indicator.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 235 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an di-isobutyl octadecylsilane group (5 µm) with specific surface area of 180 m²/g, pore size of 8 nm and carbon load of 10%; *Mobile phase* flow of 1.0 mL/minute.

Eluent A: prepare a 0.15% (w/v) monobasic potassium phosphate solution in water and adjust the pH to 3.2 with phosphoric acid.

Eluent (B): acetonitrile.

Mobile phase: adopt the gradient system described in the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 5	80	20	isocratic
5 – 15	80 → 55	20 → 45	linear gradient
15 – 40	55	45	isocratic

Diluent: mixture of *Eluent A* and *Eluent B* (55:45).

Solution (1): weigh, accurately, about 0.1 g of sample, transfer to a 10 mL volumetric flask, dissolve and complete the volume with *Diluent*.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with *Diluent*. Transfer 1 mL of this solution to a 10 mL volumetric flask and complete the volume with *Diluent*.

Solution (3): transfer, quantitatively, about 5.0 mg of impurity A (metronidazole), 5.0 mg of impurity B (2-methyl-5-nitroimidazole) and 5.0 mg of impurity C (benzoic acid) to a 50 mL volumetric flask, dissolve and complete the volume with *Diluent*. Transfer 1 mL of the solution to a 10 mL volumetric flask and complete the volume with *Diluent*.

Inject, separately, 10 µL of *Solution (3)* and record the chromatogram. The relative retention times in relation to metronidazole benzoate (retention time of about 20 minutes) are around 0.17 for impurity B, 0.20 for impurity A and 0.70 for impurity C. The resolution between impurity A and impurity B is no less than 2.0.

Procedure: inject, separately, 10 µL of the *Solution (1)*, *Solution (2)* and *Solution (3)*, record the chromatograms and measure the areas under the peaks. The areas under the peaks related to impurities A, B and C, obtained with *Solution (1)*, are not greater than the areas under the respective peaks obtained with *Solution (3)* (0.1% for each impurity). Any other impurity recorded in the chromatogram of *Solution (1)* does not have a greater area than the area obtained under the main peak (metronidazole benzoate) from *Solution (2)* (0.1%). The sum of the areas under all peaks obtained with the *Solution (1)*, except that under the solvent peak and that under the main peak, is not greater than two times the area under the main peak (metronidazole benzoate) obtained with *Solution (2)* (0.2%). Do not consider peaks with an area less than 0.1 times that presented by the main peak in the chromatogram obtained with *Solution (2)* (0.01%).

Heavy metals (5.3.2.3). Use *Method IV*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 80°C for 3 hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 0.25 g of the sample in 50 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically or using methylrosanilinium chloride TS until the color changes to blue-green. Each mL of 0.1 M perchloric acid VS is equivalent to 27.526 mg of $C_{13}H_{13}N_3O_4$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-bacterial; antiprotozoal.

METRONIDAZOLE BENZOATE ORAL SUSPENSION

Contains no less than 95.0% and no more than 105.0% of the declared amount of $C_{13}H_{13}N_3O_4$.

IDENTIFICATION

Use one of the methods described below.

A. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 250 nm to 400 nm of the sample solution obtained in method **A.** of Assay, there is maximum absorption at 308 nm, identical to that observed in the spectrum of the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.5 to 6.5. Determine in the reconstituted oral suspension as indicated on the label.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer a volume of oral suspension equivalent to 0.4 g of metronidazole benzoate to a 100 mL volumetric flask, add 10 mL of dimethylformamide and 60 mL of ethyl alcohol. Leave in ultrasonic bath for 15 minutes, shake mechanically for 15 minutes, complete the volume with ethyl alcohol, homogenize and filter. Dilute, successively, in ethyl alcohol until concentration of 0.002% (w/v). Prepare a standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 308 nm, using ethyl alcohol for zero adjustment. Calculate the amount of $C_{13}H_{13}N_3O_4$ in the oral suspension from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 150 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of methyl alcohol and water (50:50).

Sample solution: transfer a volume of oral suspension equivalent to 0.2 g of metronidazole benzoate to a 50 mL volumetric flask, add 35 mL of methyl alcohol and leave in ultrasonic bath for 15 minutes. Cool down to room temperature, complete the volume with the same solvent, homogenize and

filter. Transfer 5 mL of the filtrate to a 100 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 0.2 mg/mL solution.

Standard solution: accurately weigh about 50 mg of metronidazole benzoate CRS, transfer to a 25 mL volumetric flask, add 20 mL of methyl alcohol and leave in ultrasonic bath for 15 minutes. Cool down to room temperature, complete the volume with the same solvent and homogenize. Transfer 5 mL to a 50 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 0.2 mg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{13}H_{13}N_3O_4$ in the oral suspension from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

POTASSIUM BICARBONATE
Kalii hydrogenocarbonas

KHCO₃; 100.11
potassium bicarbonate; 01248
Carbonic acid potassium salt (1:1)
[298-14-6]

Contains no less than 99.0% and no more than 101.0% of KHCO₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder or colorless crystals. When heated, the dry substance or in solution gradually converts into potassium carbonate.

Solubility. Freely soluble in water and practically insoluble in ethyl alcohol.

IDENTIFICATION

A. Add to 5 mL of the solution in *Aspect of the preparation* 0.1 mL of phenolphthalein TS. The solution acquires a pale pink color. Heat. The gas evaporates and the color becomes red.

B. Complies with carbonate ion reactions (5.3.1.1).

C. Complies with bicarbonate ion reactions (5.3.1.1).

D. The solution obtained in *Aspect of the preparation* complies with the potassium ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. Dissolve 5 g of the sample in carbon dioxide-free water and complete the volume to 100 mL with the same solvent. The preparation obtained is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). No more than 8.6. Determine in the preparation obtained in *Aspect of the preparation*.

Sodium. Proceed as described in *Flame atomic absorption spectrometry (5.2.13.1.1)*, use *Method I*. Use a spectrometer with air-acetylene flame, with light emitter source at 589.6 nm. Prepare solutions as described below.

Solution (1): dissolve 1 g of the sample in water and complete the volume to 100 mL with the same solvent.

Solution (2): prepare a 0.1% (w/v) sodium chloride solution in water. Prepare the solutions of the analytical curve by sequential dilution in water.

Add to *Solution (1)* and to *Solution (2)* an amount equivalent to 0.5% (v/v) of a 1% (w/v) cesium chloride solution. No more than 0.5% of sodium (5000 ppm).

Ammonia (5.3.2.6). Use 10 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.002% (20 ppm).

Calcium (5.3.2.7). Use 10 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.001% (10 ppm).

Chlorides (5.3.2.1). Determine in 2.4 g of the sample. No more than 0.015% (150 ppm).

Iron (5.3.2.4). Determine in 2.5 g of the sample. No more than 0.002% (20 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve 2 g of the sample in 25 mL of water and proceed as described in *Limit test for heavy metals*, without the need to adjust pH. No more than 0.001% (10 ppm).

Sulfates (5.3.2.2). Determine in 8 g of the sample. No more than 0.015% (150 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in silica gel for four hours. No more than 0.3%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.8 g of the sample in 50 mL of carbon dioxide-free water. Add 0.1 mL of methyl orange TS. Titrate with *M* hydrochloric acid VS until the yellow color starts changing to yellow-pink. Heat carefully and boil for two minutes. The solution turns yellow. Cool and titrate until obtaining a yellow-pink color. Each mL of *M* hydrochloric acid VS is equivalent to 100.100 mg of KHCO_3 .

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Pharmacotechnical adjuvant.

SODIUM BICARBONATE

Natrii hydrogenocarbonas

NaHCO₃; 84.01
sodium bicarbonate; 01249
Sodium salt of carbonic acid (1:1)
[144-55-8]

Contains no less than 99.0% and no more than 101.0% of NaHCO₃.

DESCRIPTION

Physical characteristics. White, crystalline powder. When heated, dry or in solution, converts gradually into sodium carbonate.

Solubility. Soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. Prepare a 5% (w/v) sodium bicarbonate solution in carbon dioxide-free water. Add to 5 mL of this solution 0.1 mL of phenolphthalein TS solution. A rosy color develops. Under heating, gas is released and the solution color changes to red.

B. Complies with reactions from carbonate and bicarbonate ions (5.3.1.1).

C. Complies with sodium ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. The sample preparation at 5% (w/v) in carbon dioxide-free water is clear (5.2.25) and colorless (5.2.12).

Ammonia (5.3.2.6). Dilute 10 mL of the preparation described in *Aspect of the preparation* to 15 mL with water. Proceed as described in *Limit test for ammonia*. No more than 0.002% (20 ppm).

Arsenic (5.3.2.5). Use *Method I*. Add to 1.5 g of the sample 3.5 M sulfuric acid until the effervescence stops. Proceed as described in *Limit test for arsenic*. No more than 0.0002% (2 ppm).

Carbonates. The pH (5.2.19) of the preparation described in *Aspect of the preparation*, freshly prepared, is no more than 8.6.

Calcium (5.3.2.7). Neutralize the suspension of 1 g of the sample in 10 mL of water with hydrochloric acid and dilute to 15 mL with water. Proceed as described in *Limit test for calcium*. No more than 0.01% (100 ppm).

Chlorides (5.3.2.1). Add to 7 mL of the preparation described in *Aspect of the preparation* 2 mL of nitric acid and dilute to 15 mL with water. Proceed as described in *Limit test for chlorides*. No more than 0.015% (150 ppm).

Iron (5.3.2.4). Use *Method III*. Dissolve 1.0 g of the sample in 5 mL of hydrochloric acid. Proceed as described in *Limit test for iron*. No more than 0.002% (20 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve 2 g of the sample in the mixture of 2 mL of hydrochloric acid and 18 mL of water. Proceed as described in *Limit test for heavy metals*. No more than 0.001% (10 ppm).

Sulfates (5.3.2.2). Suspend 1 g of the sample in 10 mL of water and add hydrochloric acid until neutralizing. Proceed as described in *Limit test for sulfates*. No more than 0.015% (150 ppm).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 1.5 g of the sample in 50 mL of carbon dioxide-free water. Titrate with *M* hydrochloric acid VS using 0.2 mL of methyl orange TS as indicator. Each mL of *M* hydrochloric acid VS corresponds to 84.010 mg of NaHCO₃.

PACKAGING AND STORAGE

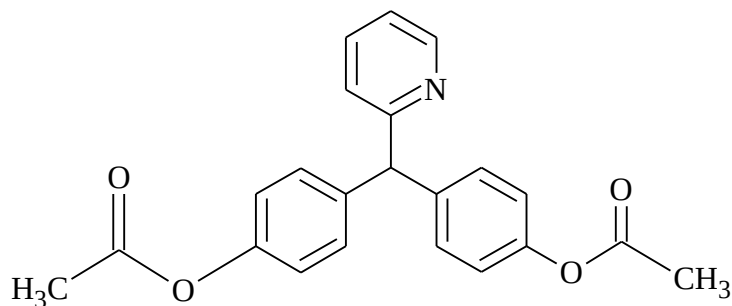
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antacid.

BISACODYL***Bisacodylum***

$C_{22}H_{19}NO_4$; 361.40

bisacodyl; 01287

4,4'-(2-pyridinylmethylene)bis-phenol 1,1'-diacetate

[603-50-9]

Contains no less than 98.0% and no more than 101.0% of $C_{22}H_{19}NO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol. Soluble in diluted mineral acids.

Physicochemical constants.

Melting range (5.2.2): 131 °C to 135 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample desiccated at 105 °C, until constant weight, and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of bisacodyl CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 350 nm, of 0.001% (w/v) sample solution in 0.6% (w/v) methanolic potassium hydroxide, there is a maximum point at 248 nm and a shoulder at 290 nm. The absorbance at 248 nm is of approximately 0.632 to 0.672.

C. Nebulize the chromatographic plate obtained in *Related substances* with the mixture of 0.05 M iodine solution and M sulfuric acid (50:50). The main stain obtained with *Solution (2)* chromatogram corresponds in position, color and intensity to that obtained with *Solution (3)*.

PURITY TESTS

Acidity or alkalinity. Shake 1.0 g of the sample with 20 mL of carbon dioxide-free water. Heat until boiling, cool and filter. No more than 0.2 mL of 0.01 M sodium hydroxide is spent to neutralize the

filtrate, using methyl red TS as an indicator. No more than 0.4 mL of 0.01 M hydrochloric acid is spent to neutralize the filtrate, using the same indicator.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of xylene and methyl-ethyl-ketone (50:50) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.2 g of the sample in acetone and complete the volume to 10 mL with the same solvent.

Solution (2): dilute 1.0 mL of *Solution (1)* to 10 mL with acetone.

Solution (3): dissolve 20 mg of bisacodyl CRS in acetone and complete the volume to 10 mL with the same solvent.

Solution (4): dilute 1.0 mL of *Solution (1)* to 100 mL with acetone.

Solution (5): dilute 5.0 mL of *Solution (4)* to 10 mL with acetone.

Develop the chromatogram. Remove the plate and allow to air dry. If necessary, heat the plate at 105 °C for 10 minutes. Examine under ultraviolet light (254 nm). Any secondary stain obtained with *Solution (1)*, different from the main stain, must not be more intense than the stain obtained with *Solution (4)* (1.0%), and no other stain must be more intense than the stain obtained in the chromatogram with *Solution (5)* (0.5%).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105 °C, until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Dissolve 0.250 g of the sample in 70 mL of glacial acetic acid, add two drops of 1-naphtholbenzein TS and titrate with 0.1 M perchloric acid VS. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 36.139 mg of C₂₂H₁₉NO₄.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Cathartic.

BISACODYL TABLETS

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{22}H_{19}NO_4$. The tablets must be coated.

IDENTIFICATION

A. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

B. Weigh and pulverize the tablets. Extract an amount of powder equivalent to 50 mg of bisacodyl with chloroform, filter, evaporate the filtrate to dryness and dissolve the residue with 10 mL of 0.5% (v/v) sulfuric acid solution. Add to 2 mL of the solution obtained 50 μ L of potassium mercury iodide RS. A white precipitate is formed.

C. Add sulfuric acid to 2 mL of the solution obtained on test **B.** of *Identification*. A violet color develops.

D. Boil 2 mL of the solution obtained on test **B.** of *Identification* with a little bit of nitric acid. A yellow color develops. Cool and add 5 M sodium hydroxide. A yellowish brown color develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test. Carry out the acid step in 0.1 M hydrochloric acid for 120 minutes. The second step must be carried out with 1.5% (w/v) sodium bicarbonate solution for 60 minutes.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described in *Assay*. Prepare solution with final concentration of 0.5 mg/mL.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of xylene and methylethylketone (50:50) as mobile phase. Apply to the plate, separately, 10 μ L of each one of the freshly prepared solutions described below.

Solution (1): shake an amount of powder equivalent to 20 mg of bisacodyl with 2 mL of acetone for 10 minutes, centrifuge and use the liquid supernatant.

Solution (2): dilute 3 mL of *Solution (1)* to 100 mL with acetone.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, not being related to excipients, is not more intense than that obtained with *Solution (2)* (3%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 265 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica gel chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 2.0 mL/minute.

0.074 M sodium acetate buffer: weigh 10.06 g of sodium acetate trihydrate, dissolve and complete the volume to 1000 mL with water. Adjust pH to 7.4 with 2.5% (v/v) acetic acid.

Mobile phase: mixture of *0.074 M sodium acetate buffer* and acetonitrile (50:50).

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 50 mg of bisacodyl to a 100 mL volumetric flask and add 12 mL of water. Shake mechanically for 15 minutes and submit to ultrasonic bath, at room temperature, for 15 minutes. Add 50 mL of water, shake mechanically and leave in ultrasonic bath for 15 minutes. Complete the volume with acetonitrile, homogenize and centrifuge for 15 minutes. Filter the supernatant and use the filtrate in the determinations.

Standard solution: dissolve an accurately weighed amount of bisacodyl CRS in acetonitrile and dilute adequately to obtain a 0.5 mg/mL solution.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₂₂H₁₉NO₄ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

BISACODYL SUPPOSITORIES

Contains not less than 90.0% and not more than 110.0% the declared amount of $C_{22}H_{19}NO_4$.

IDENTIFICATION

A. Proceed as described in *Related substances*. Apply to the chromatographic plate 2 μ L of each one of the solutions and use 1% (w/v) bisacodyl CRS in acetone, as *Solution (2)*. The main stain of the *Solution (1)* chromatogram corresponds to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. Dissolve an amount of suppositories containing the equivalent to 0.15 g of bisacodyl in 150 mL of petroleum ether. Filter, wash the residue with petroleum ether until it is free from oily material and dry at 100 °C. Dissolve the residue in a minimum amount of chloroform, slightly heated, and dissolve in 10 mL of 0.5% (v/v) sulfuric acid. Add to 2 mL of the solution obtained 50 μ L of potassium mercury iodide RS. A white precipitate is formed.

D. Add sulfuric acid to 2 mL of the solution obtained on test **C.** of *Identification*. A violet color develops.

E. Boil 2 mL of the solution obtained on test **C.** of *Identification* with a little bit of nitric acid. A yellow color develops. Cool and add 5 M sodium hydroxide. A yellowish brown color develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described on method **B.** from *Assay*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of xylene and methylethylketone (50:50) as mobile phase. Apply to the plate, separately, 10 μ L of each one of the freshly prepared solutions described below.

Solution (1): shake an amount of suppositories containing the equivalent to 20 mg of bisacodyl with 20 mL of petroleum ether and filter. Wash the residue with petroleum ether until it is free from oily material and dissolve in 2 mL of acetone.

Solution (2): dilute 3 mL of *Solution (1)* to 100 mL with acetone.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (3%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve an amount of suppositories containing the equivalent to 0.1 g of bisacodyl in 80 mL of glacial acetic acid, neutralized previously with 0.02 M perchloric acid VS, using 1-naphtholbenzein TS to verify the neutralization. Titrate with 0.02 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.02 M perchloric acid VS is equivalent to 7.228 mg of $C_{22}H_{19}NO_4$.

B. Proceed as described in Assay in the *Bisacodyl tablets* monograph. Prepare the *Sample solution* as described next.

Sample solution: transfer an amount of suppositories containing the equivalent to 0.1 g of bisacodyl to a 500 mL separation funnel and add 150 mL of *n*-hexane. Shake mechanically until the suppositories are dissolved. Add 50 mL of acetonitrile, shake for 1 minute and wait for the separation of phases. Transfer the lower phase to a 200 mL volumetric flask. Extract the remaining content in the separation funnel with two portions of 50 mL of acetonitrile, gather the lower layers in the 200 mL volumetric flask and complete the volume with acetonitrile. Shake and filter.

Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{22}H_{19}NO_4$ in the suppositories from the responses obtained with the *Standard solution* and the *Sample solution*.

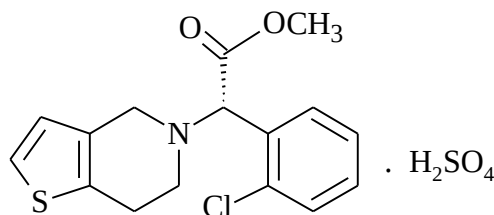
PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

CLOPIDOGREL BISULFATE
Clopidogreli hydrogenosulfas



$C_{16}H_{16}ClNO_2S \cdot H_2SO_4$; 419.89

clopidogrel bisulfate; 02319

(2*S*)-(2-chlorophenyl) [6,7-dihydrothiene[3,2-*c*]pyridine-5(4*H*)-yl] methyl acetate, sulfate (1:1)
 [120202-66-6]

Contains no less than 99.0% and no more than 101.0% of $C_{16}H_{16}ClNO_2S \cdot H_2SO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white powder. Presents polymorphism.

Solubility. Freely soluble in water and methyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +54.0 to +58.0, in relation to the desiccated substance. Determine in solution at 1% (w/v) in methyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of clopidogrel bisulfate CRS, prepared identically.

B. Complies with sulfate ion reactions (5.3.1.1).

PURITY TESTS

Enantiomeric purity. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica gel OJ for chiral separation (10 μ m), maintained at room temperature; *Mobile phase* flow of 0.8 mL/minute.

Mobile phase: mixture of heptane and ethyl alcohol anhydrous (85:15).

Sample solution: dissolve 0.1 g of the sample in 25 mL of ethyl alcohol anhydrous and dilute to 50 mL with heptane.

Standard solution: dissolve 10 mg of *clopidogrel for assessment of the CRS system suitability* (containing impurities B and C) in 2.5 mL of ethyl alcohol anhydrous and dilute to 5 mL with heptane.

Inject 10 µL replicates of the *Standard solution*. The relative retention times are about 0.6 for impurity C and 0.7 for impurity B. The resolution between the peaks of impurities C and B is no less than 2.0. The signal-noise ratio is no less than 20 for impurity C.

Procedure: inject, separately, 10 µL of the *Sample solution* and the *Standard solution*, record the chromatograms for no less than 1.25 times the retention time of clopidogrel. Calculate the content of impurity C. No more than 0.5%.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph with ultraviolet detector at 220 nm; column with 150 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane groups (5 µm), with deactivated base, maintained at 30 °C; *Mobile phase* flow of 1.0 mL/minute.

Diluent: mixture of acetonitrile and *Eluent A* (60:40).

Eluent A: mixture of sodium pentanesulfonate monohydrate 0.96 g/L, with pH adjusted to 2.5 with phosphoric acid, and methyl alcohol (95:5).

Eluent B: mixture of acetonitrile and methyl alcohol (95:5).

Mobile Phase gradient: adopt the gradient system described in the following table.

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 - 3	89.5	10.5	isocratic
3 - 48	89.5 → 31.5	10.5 → 68.5	linear gradient
48 - 68	31.5	68.5	isocratic

Solution (1): dissolve 65 mg of the sample, accurately weighed, in the *Diluent*, dilute to 10 mL with the same solvent and homogenize.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL using the *Diluent* and homogenize. Dilute 1 mL of this solution to 10 mL using the *Diluent* and homogenize.

Solution (3): dissolve 5 mg of *clopidogrel impurity A CRS* in the *Diluent*, dilute to 25 mL with the same solvent and homogenize.

Resolution solution: dissolve 32 mg of *clopidogrel for assessment of the CRS system suitability* (containing impurities B and C) in the *Diluent*, add 0.5 mL of *Solution (3)*, dilute to 5 mL with the same solvent and homogenize.

Inject 10 µL replicates of the *Resolution solution*. The relative retention time of impurity A is of approximately 0.4, and of impurity B, of 1.1, in relation to the main peak.

Procedure: inject, separately, 10 µL of the *Solution (1)* and *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of each impurity reported. No more than two times the area under the main peak of the chromatogram obtained with *Solution (2)* for impurity A (0.2%). No more than three times the area under the main peak of the chromatogram

obtained with *Solution (2)* for impurity B (0.3%). No more than the area under the main peak of the chromatogram obtained with *Solution (2)* for other impurities (0.1%). No more than five times the area under the main peak of the chromatogram obtained with *Solution (2)* for total impurities (0.5%). Disregard peaks with areas below 0.5 times the area under the main peak obtained with *Solution (2)* (0.05%).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105 °C for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.16 g of the sample in a mixture with 10 mL of acetone, 10 mL of methyl alcohol and 30 mL of water. Titrate with 0.1 M sodium hydroxide VS, determining the endpoint potentiometrically. Precipitate may form during the titration. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium hydroxide VS is equivalent to 20.99 mg of $C_{16}H_{16}ClNO_2S \cdot H_2SO_4$.

PACKAGING AND STORAGE

Store protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Platelet aggregation inhibitor.

CLOPIDOGREL BISULFATE TABLETS

Clopidogrel bisulfate tablets have the equivalent to no less than 90.0% and no more than 110.0% of the declared amount of $C_{16}H_{16}ClNO_2S$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 250 nm to 300 nm, of the sample solution obtained in *Procedure for content uniformity*, there are maximum absorption points only at the same wavelengths as those observed in the spectrum obtained with the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 50 mL volumetric flask. Add 30 mL of 0.1 M hydrochloric acid, leave in ultrasonic bath for five minutes, complete the volume with the same solvent and filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask and complete the volume with the same solvent. Filter through a 0.45 µm porosity filter. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 270 nm **(5.2.14)**, using 0.1 M hydrochloric acid for zero adjustment.

DISSOLUTION TEST (5.1.5)

Dissolution medium: hydrochloric acid buffer pH 2.0, 1000 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot from the dissolution medium, filter immediately and dilute in hydrochloric acid buffer pH 2.0 until adequate concentration. Measure the absorbances of the solutions at 240 nm **(5.2.14)**, using hydrochloric acid buffer pH 2.0 for zero adjustment. Calculate the amount of $C_{16}H_{16}ClNO_2S$ dissolved in the medium, comparing the readings obtained with that of the clopidogrel bisulfate solution CRS containing the equivalent to 0.003% (w/v) of clopidogrel, prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{16}H_{16}ClNO_2S$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 220 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to ovomucoid protein, maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of 0.1 M monobasic potassium phosphate and acetonitrile (75:25).

Sample solution: weigh and pulverize the tablets. Transfer an amount of powder equivalent to 50 mg of clopidogrel to a 50 mL volumetric flask. Add about 30 mL of methyl alcohol. Leave in ultrasonic bath for five minutes. Shake mechanically for 30 minutes and complete the volume with methyl alcohol. Homogenize and filter. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with methyl alcohol.

Standard solution: weigh, accurately, the equivalent to 25 mg of clopidogrel and transfer to a 25 mL volumetric flask. Dissolve in 5 mL of methyl alcohol, using ultrasonic bath, if necessary. Complete the volume with methyl alcohol and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with methyl alcohol.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{16}H_{16}ClNO_2S$ in the tablets, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers at room temperature.

LABELING

Comply with current legislation.

SODIUM BORATE***Natrii boras***

$\text{Na}_2\text{B}_4\text{O}_7$; 201.22
sodium borate; 00117
Boron sodium oxide
[1330-43-4]

$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$; 381.37
sodium sulfate decahydrate; 10051
Borax
[1303-96-4]

Contains no less than 99.0% and no more than 105.0% of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$.

DESCRIPTION

Physical characteristics. White crystalline powder or colorless crystals.

Solubility. Soluble in water, very soluble in water in ebullition, freely soluble in glycerol, insoluble in ethyl alcohol.

IDENTIFICATION

A. Dissolve 0.2 g of the sample in carbon dioxide-free water and complete to 5 mL with the same solvent. Add 0.1 mL of phenolphthalein TS. A red color develops. Add 5 mL of 85% (v/v) glycerol. The color disappears.

B. The solution prepared identically to the solution from test **A.** of *Identification* complies with the borate ion reactions **(5.3.1.1)**.

C. The solution prepared identically to the solution from test **A.** of *Identification* complies with the sodium ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Dissolve 8 g of the sample in carbon dioxide-free water and complete the volume to 100 mL with the same solvent. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). 9.0 to 9.6. Determine in the preparation obtained in *Aspect of the preparation*.

Carbonate and bicarbonate. In a test tube, add 5 mL of 5% (w/v) aqueous sample solution and 1 mL of 3 M hydrochloric acid. There is no effervescence.

Sulfates (5.3.2.2). Use 25 mL of the preparation obtained in *Aspect of the preparation* and proceed as described in *Limit test for sulfates*. Prepare the standard solution using 3 mL of standard sulfate solution and 12 mL of water. No more than 0.005% (50 ppm).

Heavy metals (5.3.2.3). Use 10 mL of the preparation obtained in *Aspect of the preparation* and proceed as described in *Method I*. Prepare standard solution using *Standard lead solution (1 ppm)*. No more than 0.0025% (25 ppm).

Arsenic (5.3.2.5). Use *Method I*. Use 12.5 mL of the preparation obtained in *Aspect of the preparation* and proceed as described in *Limit test for arsenic*. No more than 0.0005% (5 ppm).

Ammonia (5.3.2.6). Dilute 3 mL of the preparation obtained in *Aspect of the preparation* to 14 mL with water and proceed as described in *Limit test for ammonia*. Prepare the standard solution using a mixture of 2.5 mL of *Standard ammonia solution (1 ppm)* and 7.5 mL of water. No more than 0.001% (10 ppm).

Calcium (5.3.2.7). Use 7.5 mL of the preparation obtained in *Aspect of the preparation* and proceed as described in *Limit test for calcium*. Prepare the standard solution using a mixture of 6 mL of *Standard calcium solution (10 ppm)* and 9 mL of water. No more than 0.01% (100 ppm).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.3 g of the sample and dissolve in 50 mL of water. Add a few drops of methyl red TS and titrate with 0.1 M hydrochloric acid VS. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M hydrochloric acid VS is equivalent to 19.069 mg of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$.

PACKAGING AND STORAGE

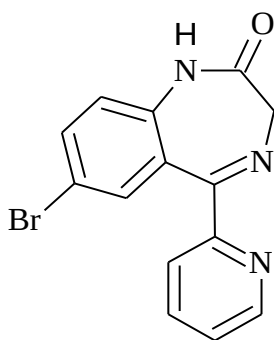
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiseptic agent, detergent, astringent for mucosa membranes.

BROMAZEPAM***Bromazepamum***

$C_{14}H_{10}BrN_3O$; 316.15

bromazepam; 01366

7-bromo-1,3-di-hydro-5-(2-pyridyl)-2H-1,4-benzodiazepin- 2-one

[1812-30-2]

Contains no less than 99.0% and no more than 101.0% of $C_{14}H_{10}BrN_3O$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, or slightly yellowish, crystalline powder.

Solubility. Insoluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 237 °C to 238.5 °C, with decomposition.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, previously desiccated until constant weight, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of bromazepam CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 220 nm to 350 nm, of 0.0005% (w/v) solution in methyl alcohol, there are maximum and minimum points only at the same wavelengths of bromazepam CRS, prepared identically.

C. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel GF₂₅₄ as support, and a mixture of diethylamine and ethyl ether (30:70) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 1 mg/mL sample solution in a mixture of methyl alcohol and methylene chloride (1:9).

Solution (2): 1 mg/mL sample solution of bromazepam CRS in a mixture of methyl alcohol and methylene chloride (1:9).

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of ethyl alcohol, triethylamine, methylene chloride and petroleum ether (5:5:20:70) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below. The assay must be conducted protected from light.

Solution (1): 10 mg/mL sample solution in a mixture of methyl alcohol and methylene chloride (1:9).

Solution (2): dilute *Solution (1)* in a mixture of methyl alcohol and methylene chloride (1:9), to obtain a 20 µg/mL sample solution.

Develop the chromatogram. Remove the plate and allow it to dry on air stream for 20 minutes. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.2%).

Loss on drying (5.2.9.1). Determine in 1 g of the sample, in oven at 80 °C, under reduced pressure, for four hours. No more than 0.2%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 0.25 g of sample, dissolve in 20 mL of glacial acetic acid and add 50 mL of acetic anhydride. Titrate with 0.1 M perchloric acid solution VS and determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 31.615 mg of C₁₄H₁₀BrN₃O.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anxiolytic.

BROMAZEPAM TABLETS

Contains no less than 93.0% and no more than 110.0% of the declared amount of $C_{14}H_{10}BrN_3O$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the sample solution obtained in *Assay*, there are maximum and minimum points identical to those observed in the spectrum of the standard solution.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ as support, and a mixture of ethyl acetate and 25% (v/v) ammonium hydroxide (100:1) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Use an amount of powder equivalent to 25 mg of bromazepam and add 10 mL of methyl alcohol. Homogenize and filter.

Solution (2): 2.5 mg/mL bromazepam CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. *Procedure for content uniformity. Prepare the solutions protected from light.* Weigh individually and transfer each tablet to a 100 mL volumetric flask. Proceed as described in *Assay*, as of "Add 70 mL of 0.1 M methanolic sulfuric acid...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: simulated gastric fluid (with enzyme), 900 mL.

Apparatus: paddles, 50 rpm.

Time: 20 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter, cool at 20 °C and dilute, if necessary, in simulated gastric fluid (without enzyme) until adequate concentration. Measure the absorbances of the solutions at 239 nm (**5.2.14**), using simulated gastric fluid (without enzyme) for zero adjustment. Calculate the amount of $C_{14}H_{10}BrN_3O$ dissolved in the medium, comparing the readings obtained with that of the bromazepam CRS solution at the concentration of 0.00033% (w/v), prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{14}H_{10}BrN_3O$ dissolve in 20 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Note: *prepare the solutions protected from light.*

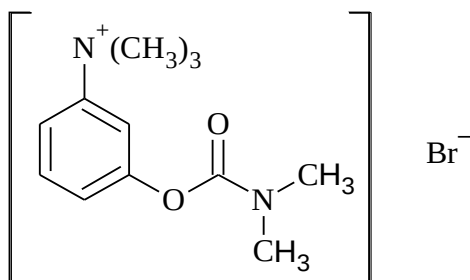
Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer an amount of powder, accurately weighed, equivalent to 60 mg of bromazepam to a 100 mL volumetric flask. Add 70 mL of 0.1 M methanolic sulfuric acid and leave in ultrasonic bath for 20 minutes. Complete the volume with the same solvent, centrifuge and filter, if necessary. Carry out successive dilutions to a concentration of 0.0006% (w/v), using the same solvent. Prepare a standard solution in the same conditions. Measure the absorbances of the resulting solutions at 239 nm, using 0.1 M methanolic sulfuric acid for zero adjustment. Calculate the amount of $C_{14}H_{10}BrN_3O$ in the tablets from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

NEOSTIGMINE BROMIDE*Neostigmini bromidum*C₁₂H₁₉BrN₂O₂; 303.20

neostigmine bromide; 06287

3-[[[(dimethylamino)carbonyl]oxy]-N,N,N-trimethylbenzenaminium bromide

[114-80-7]

Contains no less than 98.0% and no more than 102.0% of C₁₂H₁₉BrN₂O₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, or nearly white, crystalline powder or colorless crystals. It is hygroscopic. Its solutions are neutral to litmus paper.

Solubility. Very soluble in water, soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 171 °C to 176 °C, with decomposition.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated at 105 °C for three hours, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of neostigmine bromide CRS, prepared identically.

B. The aqueous solution in the ratio 1:50 complies with the bromide reactions **(5.3.1.1)**.

PURITY TESTS

Sulfate. Dissolve 0.25 g of the sample in 10 mL of water, add 1 mL of hydrochloric acid and 1 mL of barium chloride. It does not produce turbidity immediately.

Loss on drying (5.2.9.1). Dry the sample at 105 °C for three hours. No more than 2.0%.

Residue by incineration (5.2.10). No more than 0.15%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve, quantitatively, about 0.75 g of the sample in a mixture of 70 mL of glacial acetic acid and 20 mL of mercury acetate RS. Add four drops of methylrosanilinium chloride TS and titrate with 0.1 M perchloric acid VS until the color turns greenish-blue. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 30.32 mg of $C_{12}H_{19}BrN_2O_2$.

PACKAGING AND STORAGE

In hermetic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Cholinergic.

SODIUM BROMIDE

Natrii bromidum

NaBr; 102.89
sodium bromide; 01445
Sodium bromide
[7647-15-6]

Contains no less than 98.0% and no more than 100.5% of NaBr, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder or colorless or opaque crystals, sparingly hygroscopic.

Solubility. Freely soluble in water and soluble in ethyl alcohol.

IDENTIFICATION

A. Complies with bromide ion reactions (5.3.1.1).

B. The 10% (w/v) solution complies with sodium ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. Transfer 10 g of the sample to a 100 mL volumetric flask, dissolve in carbon dioxide-free water and complete the volume with the same solvent. The preparation is clear (5.2.25) and colorless (5.2.12).

Acidity or alkalinity. Add to 10 mL of the preparation obtained in *Aspect of the preparation* 0.1 mL of bromothymol blue TS. No more than 0.5 mL of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is necessary to promote the indicator turning.

Bromides. Add to 10 mL of the preparation obtained in *Aspect of the preparation* 1 mL of starch solution TS, 0.1 mL of a 10% (w/v) potassium iodide solution and 0.25 mL of 0.5 M sulfuric acid. Protect from light for five minutes. A blue or violet color must not develop.

Chlorides. Transfer 1 g of the sample to an Erlenmeyer flask and dissolve in 20 mL of 20% (w/v) nitric acid. Add 5 mL of concentrated hydrogen peroxide and heat in water bath until the solution becomes completely colorless. Wash the flask walls with a little bit of water and heat in water bath for 15 minutes. Cool, dilute to 50 mL with water, add 5 mL of 0.1 M silver nitrate VS and 1 mL of dibutyl phthalate. Homogenize and titrate with 0.1 M ammonium thiocyanate solution VS using 5 mL of ammonium ferric sulfate RS as indicator. No more than 1.7 mL of 0.1 M silver nitrate solution VS are necessary to promote the indicator turning (0.6%). Record the volume of 0.1 M silver nitrate VS used.

Iodides. Add to 5 mL of the preparation obtained in *Aspect of the preparation* 0.15 mL of ferric chloride RS and 2 mL of chloroform. Shake and observe the phases. The chloroform phase is colorless.

Sulfates (5.3.2.2). Use 15 mL of the preparation obtained in *Aspect of the preparation* and proceed as described in *Limit test for sulfates*. No more than 0.01% (100 ppm).

Barium. Add to 5 mL of the preparation obtained in *Aspect of the preparation* 5 mL of distilled water and 1 mL of diluted sulfuric acid RS. After 15 minutes, any opalescence observed is not more intense than that of a mixture of 5 mL of the preparation obtained in *Aspect of the preparation* and 6 mL of water.

Heavy metals (5.3.2.3). Use *Method III*. Use 20 mL of the preparation obtained in *Aspect of the preparation* and proceed as described in *Limit test for heavy metals*. Prepare a reference solution using *lead solution (10 ppm Pb)*. No more than 0.001% (10 ppm).

Iron (5.3.2.4). Use *Method I*. Dilute 5 mL of the preparation obtained in *Aspect of the preparation* to 10 mL with water and proceed as described in *Limit test for iron*. No more than 0.002% (20 ppm).

Magnesium and alkaline earth metals (5.3.2.9). Use 10 g of sample and proceed as described in *Limit test for magnesium and alkaline earth metals*. The volume of 0.01 M disodium edetate VS used is no more than 5 mL. No more than 0.02% (200 ppm), calculated as calcium.

Loss on drying (5.2.9.1). Determine in 1 g of sample, in oven between 100 °C and 105 °C, for three hours. No more than 3.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Transfer quantitatively about 2 g of the sample to a 100 mL volumetric flask, dissolve in water and complete the volume with the same solvent. Add to 10 mL of this solution 50 mL of water, 5 mL of 20% (w/v) nitric acid, 25 mL of 0.1 M silver nitrate VS, 2 mL of dibutyl phthalate and homogenize. Titrate with 0.1 M ammonium thiocyanate VS using 2 mL of ammonium ferric sulfate RS as indicator, shaking vigorously until the indicator turns. Correct the volume, subtracting the volume of 0.1 M silver nitrate VS spent on the test for *Chlorides* in *Purity tests*. Each mL of 0.1 M silver nitrate VS is equivalent to 10.289 mg of NaBr.

PACKAGING AND STORAGE

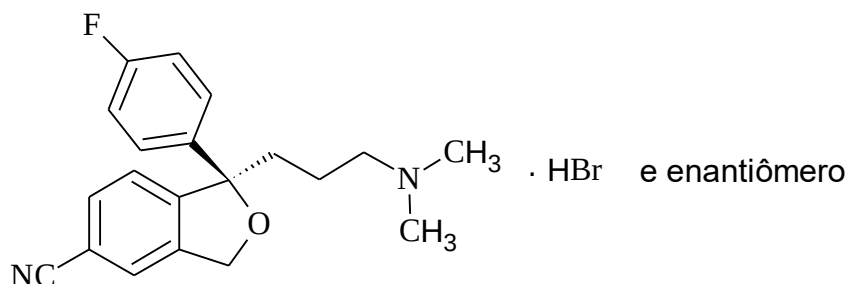
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Sedative, hypnotic, anticonvulsant.

CITALOPRAM HYDROBROMIDE*Citaloprami hydrobromidum*C₂₀H₂₁FN₂O·HBr; 405.30

citalopram hydrobromide; 02162

1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofuran-carbonitrile hydrobromide (1:1)

[59729-32-7]

Contains no less than 98.0% and no more than 101.0% of C₂₀H₂₁FN₂O·HBr, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Moderately soluble in water, soluble in methyl alcohol and ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 182 °C to 189 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of citalopram hydrobromide CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.001% (w/v) solution in 0.1 M hydrochloric acid, there is maximum absorption at 239 nm, identical to that observed in the spectrum of citalopram hydrobromide CRS, prepared identically.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of water, butyl alcohol and acetic acid (15:12:3) as mobile phase. Prepare the mobile phase 24 hours beforehand and discard the organic layer. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 1 mg/mL sample solution in water.

Solution (2): 1 mg/mL solution of citalopram hydrobromide CRS in water.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

E. Complies with bromide ion reactions **(5.3.1.1)**.

PURITY TESTS

Water (5.2.20.1). Determine in 0.25 g of the sample. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer, quantitatively, the equivalent to 10 mg of the sample to a 100 mL volumetric flask, dissolve in 0.1 M hydrochloric acid and complete the volume with the same solvent. Dilute successively, with the same solvent, to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 239 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the content of $C_{20}H_{21}FN_2O.HBr$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 239 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of 0.3% (v/v) triethylamine, with pH adjusted to 6.6 with phosphoric acid, and acetonitrile (55:45).

Sample solution: transfer the equivalent to 10 ng of the sample to a 50 mL volumetric flask and complete the volume with water. Transfer 5 mL to a 25 mL volumetric flask and complete the volume with the same solvent, obtaining a solution at 40 μ g/mL.

Standard solution: transfer the equivalent to 10 mg of citalopram hydrobromide CRS, accurately weighed, to a 50 mL volumetric flask and complete the volume with water. Transfer 5 mL to a 25 mL volumetric flask and complete the volume with the same solvent, obtaining a solution at 40 μ g/mL.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{20}H_{21}FN_2O \cdot HBr$ in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

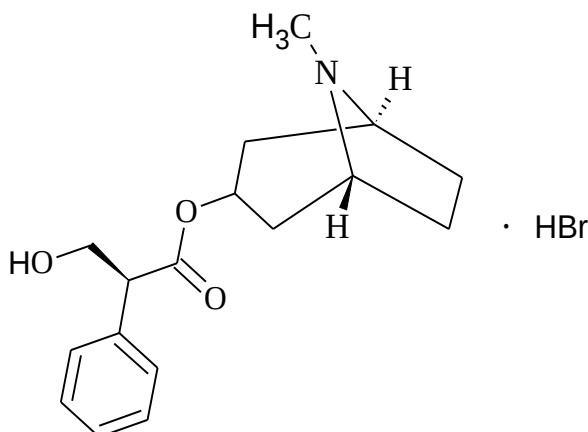
In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antidepressant.

HYOSCYAMINE HYDROBROMIDE*Hyoscyamini hydrobromidum*C₁₇H₂₃NO₃.HBr; 370.29

hyoscyamine hydrobromide; 04727

(α S)-(3-endo)-8-methyl-8-azabicyclo[3.2.1]octa-3-yl ester hydrobromide of α -(hydroxymethyl) benzene acetic ester
[306-03-6]

Contains no less than 98.5% and no more than 100.5% of C₁₇H₂₃NO₃.HBr, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder. Deliquescent to air and sensitive to light.

Solubility. Very soluble in water and in ethyl alcohol.

IDENTIFICATION

A. Put 10 mg of the sample in a porcelain capsule, add five drops of nitric acid and heat in water bath until complete evaporation. After cooling, add to the residue a few drops of 0.5 M ethanolic potassium hydroxide. A violet color is produced.

B. Add to 1 mL of 5% (w/v) aqueous sample solution gold chloride RS drop by drop, until formation of precipitate. Add a small amount of diluted hydrochloric acid and heat until the precipitate dissolves. After cooling, small lustrous, reddish brown slides must be formed, and they can be accompanied of needles with the same color (distinction between atropine and scopolamine).

C. Add silver nitrate RS to a 5% (w/v) aqueous sample solution. A yellowish-white precipitate, insoluble in nitric acid, is formed.

PURITY TESTS

Other alkaloids. Dissolve 250 mg of the sample in 1 mL of 0.1 M hydrochloric acid, dilute to 15 mL with water and separate in two portions. Add to a portion of 5 mL of solution a few drops of platinic chloride RS; a precipitate must not form immediately. Add to the other portion of 5 mL of solution

2 mL of ammonia RS; the mixture may develop a slight opalescence, but must not present turbidity or immediate precipitation.

Loss on drying (5.2.9.1). Dry in oven at 105 °C, for two hours. No more than 1.0%.

Residue by incineration (5.2.10). No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve about 700 mg of the sample, accurately weighed, in a mixture of 50 mL of glacial acetic acid and 10 mL of mercury acetate RS. Add one drop of methylrosanilinium chloride TS and titrate with 0.1 M perchloric acid VS until a greenish-blue color appears. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 37.028 mg of $C_{17}H_{23}NO_3 \cdot HBr$.

PACKAGING AND STORAGE

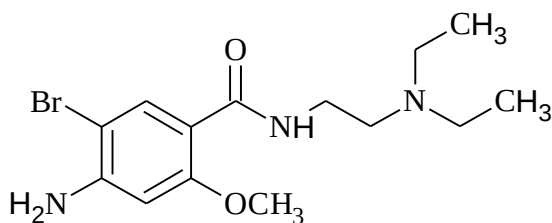
In hermetic, opaque containers.

LABELING

Comply with current legislation.

CATEGORY

Anticholinergic.

BROMOPRIDE***Bromopridum***C₁₄H₂₂BrN₃O₂; 344.25

bromopride; 01471

4-Amino-5-bromo-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide

[4093-35-0]

Contains no less than 99.0% and no more than 102.0% of C₁₄H₂₂BrN₃O₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to ivory white crystalline powder.

Solubility. Practically insoluble in water. Moderately soluble in acetonitrile. Slightly soluble in ethyl alcohol. Soluble in diluted mineral acid solutions.

Physicochemical constants.

Melting range (5.2.2): 151 °C to 155 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of bromopride CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 230 nm to 350 nm of the sample solution obtained in method **B.** of *Assay*, there is maximum absorption at 274 nm, identical to that observed in the spectrum of bromopride CRS, prepared identically.

PURITY TESTS

Aspect of the preparation. Dissolve 1 g of the sample in 10 mL of 0.5 *M* hydrochloric acid. The preparation obtained is clear **(5.2.25)**.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.003% (30 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample, in oven at 105 °C, for 4 hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Weigh, accurately, about 0.17 g of sample, transfer to a 150 mL Erlenmeyer flask and dissolve in 80 mL of glacial acetic acid. Add 2 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 34.425 mg of $C_{14}H_{22}BrN_3O_2$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh, accurately, about 0.1 g of the sample to a 100 mL volumetric flask. Add 50 mL of 0.1 M hydrochloric acid, leave in ultrasonic bath for 10 minutes and complete the volume with the same solvent. Dilute, successively, with 0.1 M hydrochloric acid, to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 274 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the content of $C_{14}H_{22}BrN_3O_2$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiemetic.

BROMOPRIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{14}H_{22}BrN_3O_2$.

IDENTIFICATION

In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the sample solution obtained in *Assay*, there is maximum absorption at 274 nm, identical to that observed in the spectrum of the standard solution.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity: grind each tablet to a fine powder, transfer quantitatively to a 100 mL volumetric flask, add 50 mL of 0.1 M hydrochloric acid, and leave in ultrasonic bath for 15 minutes. Dilute, successively, in 0.1 M hydrochloric acid, to a concentration of 0.001% (w/v) and proceed as described in *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 500 mL.

Apparatus: baskets, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and filter. Measure the absorbances of the solutions at 274 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the amount of $C_{14}H_{22}BrN_3O_2$ dissolved in the medium, comparing the readings obtained with that of the bromopride CRS solution at the concentration of 0.002% (w/v), prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{14}H_{22}BrN_3O_2$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry* (**5.2.14**). Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 10 mg of bromopride to a 100 mL volumetric flask, add 50 mL of 0.1 M hydrochloric acid and leave in ultrasonic bath for 10 minutes. Complete the volume with 0.1 M hydrochloric acid, homogenize and filter. Dilute, successively, in 0.1 M hydrochloric acid, to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 274 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{14}H_{22}BrN_3O_2$ in the tablets, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

BROMOPRIDE ORAL SOLUTION

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{14}H_{22}BrN_3O_2$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 2.8 to 3.7.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph with an ultraviolet detector at 310 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to a phenyl group (5 mm); *Mobile phase* flow of 1.0 mL/minute.

Buffer pH 7.0: dissolve 1.361 g of monobasic potassium phosphate in 900 mL of water, add 2 mL of triethylamine, adjust pH to 7.0 ± 0.05 with phosphoric acid and dilute to 1000 mL with water.

Mobile phase: mixture of *Buffer pH 7.0* and acetonitrile (60:40).

Diluent: mixture of water and acetonitrile (3:2).

Sample solution: transfer a volume of sample equivalent to 8 mg of bromopride to a 100 mL volumetric flask, complete the volume with *Diluent* and homogenize, obtaining a 80 µg/mL solution.

Standard solution: Transfer 40 mg of bromopride CRS to a 50 mL volumetric flask, dissolve in acetonitrile and complete the volume with the same solvent. Transfer 1 mL to a 10 mL volumetric flask and complete the volume with *Diluent*, obtaining a 80 mg/mL solution.

Inject 10 µL replicates of the *Standard solution*. The column efficiency is no less than 3500 theoretical plates/meter. The relative standard deviation of the areas under the bromopride peak is no more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{14}H_{22}BrN_3O_2$ in the oral solution, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

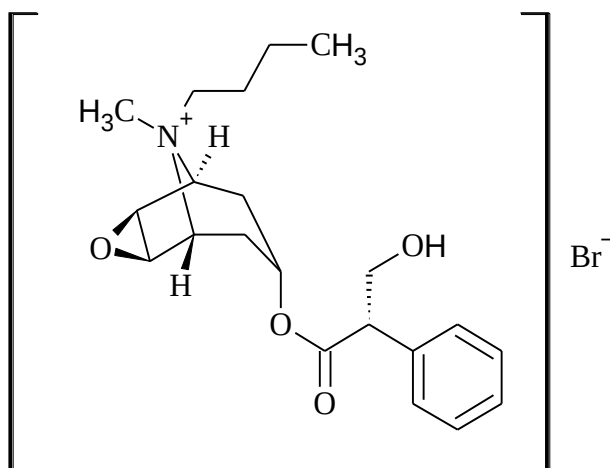
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

HYOSCINE BUTYLBROMIDE

Scopolamini butylbromidum



$C_{21}H_{30}BrNO_4$; 440.38

hyoscine butylbromide; 03517

(1 α ,2 β ,4 β ,5 α ,7 β)-9-butyl-7-[(2S)-3-hydroxy-1-oxo-2-phenylpropoxy]-9-methyl-3-oxa-9-azoniatricyclo[3.3.1.0^{2,4}] nonane bromide
[149-64-4]

Contains no less than 98.0% and no more than 101.0% of $C_{21}H_{30}BrNO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Freely soluble in water, slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 139 °C to 141 °C.

Specific optical rotation (5.2.8): -18 to -20, in relation to the desiccated substance. Determine in solution at 10% (w/v) in water.

IDENTIFICATION

The test **A.** can be omitted if the tests **B.** and **C.** are carried out. The test **B.** can be omitted if the tests **A.** and **C.** are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of hyoscine butylbromide CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

C. Complies with bromide ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5.5 to 6.5. Determine in 10% (w/v) solution in carbon dioxide-free water.

Related substances. Proceed as described on method **B.** from Assay. Prepare solutions as described below:

Solution (1): weigh, accurately, about 0.1 g of the sample and transfer to a 10 mL volumetric flask using the *Mobile phase*. Complete the volume with the same solvent and homogenize.

Solution (2): weigh, accurately, about 10 mg of hyoscine hydrobromide CRS, transfer to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 10 mL to a 50 mL volumetric flask, complete the volume with the *Mobile phase* and homogenize.

Solution (3): transfer 5 mL of *Solution (2)* to a 10 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Resolution solution: add to 10 mL of *Solution (2)* 10 µL of *Solution (1)*.

Inject 20 µL of the *Resolution solution*. The resolution between scopolamine and butylscopolamine is no less than 5. The relative standard deviation of the areas under the scopolamine peak is no more than 2.0%.

Procedure: inject, separately, 20 µL of *Solution (1)*, *Solution (2)* and *Solution (3)*, record the chromatograms for no less than double the retention time of the main peak and measure the areas under the peaks. The area under the peak corresponding to scopolamine eventually present in the chromatogram obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (3)* (0.1%). The area of any other secondary peak obtained with *Solution (1)*, except for the peak corresponding to scopolamine, is not greater than the area under the main peak obtained with *Solution (2)* (0.2%). Disregard the peaks related to the solvent and to the bromide ion, which appear early in the chromatogram.

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105 °C, until constant weight. No more than 2.5%.

Residue by incineration (5.2.10). Determine in 0.5 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh about 0.4 g of the sample and dissolve in 50 mL of water. Titrate with 0.1 M silver nitrate VS and determine the endpoint potentiometrically. Use silver indicator electrode and silver/silver chloride reference electrode. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M silver nitrate VS corresponds to 44.037 mg of $C_{21}H_{30}BrNO_4$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 210 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane (5 μ m to 10 μ m), maintained at room temperature; *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: 2 g of sodium lauryl sulfate in a mixture of 0.001 M hydrochloric acid and methyl alcohol (370:680).

Sample solution: dissolve an accurately weighed amount of the sample in 0.001 M hydrochloric acid, to obtain a 0.4 mg/mL solution.

Sample solution: dissolve an accurately weighed amount of hyoscine butylbromide CRS in 0.001 M hydrochloric acid, to obtain a 0.4 mg/mL solution.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{21}H_{30}BrNO_4$ in the sample from the responses obtained with the *Standard solution* and with the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with the current legislation.

THERAPEUTIC CLASS

Antispasmodic.

HYOSCINE BUTYLBROMIDE TABLETS

Contains no less than 92.5% and no more than 107.5% the declared amount of $C_{21}H_{30}BrNO_4$. The tablets must be coated.

IDENTIFICATION

A. Weigh and pulverize the tablets. Shake an amount of powder equivalent to 50 mg of hyoscine butylbromide with 20 mL of chloroform. Filter, evaporate to dryness and resuspend the residue with 5 mL of acetonitrile. Evaporate to dryness at 50 °C, under reduced pressure, for 1 hour. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of hyoscine butylbromide CRS.

B. Weigh and pulverize the tablets. Shake an amount of powder equivalent to 50 mg of hyoscine butylbromide with 20 mL of chloroform. Filter, evaporate to dryness, resuspend the residue with 50 mL of water and filter. In the ultraviolet absorption spectrum **(5.2.14)** of the filtrate, at the range of 230 nm to 350 nm, there are maximum absorption points at 252 nm, 257 nm and 264 nm.

C. Use 1 mg of the residue obtained on method **A.** of *Identification* in this monograph and proceed as described on method **B.** of *Identification* from the *Hyoscine butylbromide* monograph.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 25 mL volumetric flask and add 15 mL of 0.001 M hydrochloric acid. Shake mechanically for 15 minutes to disintegrate the tablet. Leave in ultrasonic bath for 15 minutes, complete the volume with the same solvent and centrifuge for 15 minutes. If necessary, filter the supernatant. Proceed as described on the method from *Assay*.

PURITY TESTS

Limit of scopolamine. Proceed as described in method **B.** of *Assay* in the *Hyoscine butylbromide* monograph. Prepare solutions as described below.

Solution (1): weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to approximately 0.1 g of hyoscine butylbromide, accurately weighed, to a 10 mL volumetric flask, add a volume of 0.001 M hydrochloric acid sufficient to dissolve the drug and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent and homogenize. If necessary, filter the supernatant.

Solution (2): weigh, accurately, about 10 mg of hyoscine hydrobromide CRS, transfer to a 100 mL volumetric flask, complete the volume with 0.001 M hydrochloric acid and homogenize. Transfer 5 mL to a 50 mL volumetric flask, complete the volume with the same solvent and homogenize.

Resolution solution: transfer 10 µL of *Solution (1)* to a 10 mL volumetric flask, complete the volume with *Solution (2)* and homogenize.

Inject 20 µL replicates of the *Resolution solution*. The resolution between the scopolamine and butylscopolamine peaks is no less than 5. The relative standard deviation of the areas under the peaks recorded between the replicates is no more than 2.0%.

Procedure: inject, separately, 20 µL of each solution, record the chromatograms and measure the areas under the peaks. The area under the peak corresponding to scopolamine, obtained with *Solution (1)*, must not be greater than the area under the main peak obtained with *Solution (2)* (0.1%).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel 60 GF₂₅₄ as support, and a mixture of formic acid, water, ethyl alcohol and methylene chloride (0.5:1.5:9:9) as mobile phase. Apply, separately, 2 µL of each one of the solutions, freshly prepared, described next, and allow the mobile phase to migrate around 4 cm above the point of application in the chromatographic plate.

Solution (1): weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to approximately 20 mg of hyoscine butylbromide to a 5 mL volumetric flask. Add a volume of 0.01 M hydrochloric acid sufficient to dissolve the drug and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent and homogenize. Centrifuge for 15 minutes. If necessary, filter the supernatant.

Solution (2): dilute 3 mL of *Solution (1)* to 100 mL with 0.01 M hydrochloric acid.

Solution (3): dilute 1 mL of *Solution (1)* to 50 mL with 0.01 M hydrochloric acid.

Solution (4): dilute 1 mL of *Solution (1)* to 400 mL with 0.01 M hydrochloric acid.

Develop the chromatogram. Remove the plate, dry in oven at 60 °C for 15 minutes and nebulize with potassium iodide and bismuth subnitrate RS. Allow the plate to dry, nebulize with 5% (w/v) sodium nitrite and examine immediately. The main stain obtained in the chromatogram of *Solution (1)* presents R_f of approximately 0.45. Any secondary stain obtained in the chromatogram of *Solution (1)*, with R_f smaller than that of the main stain, is not more intense than the stain obtained with *Solution (2)* (3%); and no more than two stains are more intense than the stain obtained with *Solution (4)* (0.25%). Any secondary stain, with R_f smaller than that of the main stain, is not more intense than the stain obtained with *Solution (3)* (2%); and no more than one stain is more intense than the stain obtained with *Solution (4)* (0.25%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in method **B.** of Assay in the *Hyoscine butylbromide* monograph. Prepare the sample solution as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 40 mg of hyoscine butylbromide to a 100 mL volumetric flask, add 60 mL of 0.001 M hydrochloric acid, leave in ultrasonic bath for 15 minutes, complete the volume with the same solvent and centrifuge for 15 minutes. If necessary, filter the supernatant.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{21}H_{30}BrNO_4$ in the tablets, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

HYOSCINE BUTYLBROMIDE INJECTION SOLUTION

Contains no less than 92.5% and no more than 107.5% the declared amount of $C_{21}H_{30}BrNO_4$. It can be prepared in water for injection or in another adequate solvent.

IDENTIFICATION

A. Use a volume of injection solution equivalent to 0.1 g of hyoscine butylbromide. Evaporate to dryness and resuspend the residue with chloroform. Evaporate to dryness and resuspend the residue with 5 mL of acetonitrile. Evaporate to dryness, at 50 °C, under reduced pressure, for 1 hour. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of hyoscine butylbromide CRS.

B. In the ultraviolet absorption spectrum **(5.2.14)**, at the range of 230 nm to 350 nm, of the *Sample solution* obtained in *Assay*, there are maximum absorption points at 252 nm, 257 nm and 264 nm.

C. Use 1 mg of the residue obtained on method **A.** of *Identification* in this monograph and proceed as described on method **B.** of *Identification* from the *Hyoscine butylbromide* monograph.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.7 to 5.5.

PURITY TESTS

Limit of scopolamine. Proceed as described in method **B.** of *Assay* in the *Hyoscine butylbromide* monograph. Prepare solutions as described below.

Solution (1): dilute, if necessary, a volume of injection solution in 0.001 M hydrochloric acid to prepare a 10 mg/mL solution.

Solution (2): weigh, accurately, 10 mg of hyoscine hydrobromide CRS, transfer to a 100 mL volumetric flask, complete the volume with 0.001 M hydrochloric acid and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with the same solvent and homogenize.

Resolution solution: transfer 10 µL of *Solution (1)* to a 10 mL volumetric flask, complete the volume with *Solution (2)* and homogenize.

Inject 20 µL replicates of the *Resolution solution*. The resolution between the scopolamine and butylscopolamine peaks is no less than 5. The relative standard deviation of the areas under the peaks recorded between the replicates is no more than 2.0%.

Procedure: inject, separately, 20 µL of each solution, record the chromatograms and measure the areas under the peaks. The area under the peak corresponding to scopolamine obtained with *Solution (1)* must not be greater than the area under the main peak obtained with *Solution (2)* (0.1%).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel 60 GF₂₅₄ as support, and a mixture of formic acid, water, ethyl alcohol and methylene chloride (0.5:1.5:9:9) as mobile phase. Apply, separately, 2 µL of each one of the freshly prepared solutions, described next, and allow the mobile phase to migrate around 4 cm above the point of application in the chromatographic plate.

Solution (1): dilute, if necessary, a volume of sample to prepare a 20 mg/mL hyoscine butylbromide solution in 0.01 M hydrochloric acid.

Solution (2): dilute 3 mL of *Solution (1)* to 100 mL with 0.01 M hydrochloric acid.

Solution (3): dilute 1 mL of *Solution (1)* to 50 mL with 0.01 M hydrochloric acid.

Solution (4): dilute 1 mL of *Solution (1)* to 400 mL with 0.01 M hydrochloric acid.

Develop the chromatogram. Remove the plate, dry at 60 °C for 15 minutes and nebulize with potassium iodide and bismuth subnitrate RS. Allow the plate to dry, nebulize with 5% (w/v) sodium nitrite, and examine immediately. The main stain obtained in the chromatogram of *Solution (1)* presents R_f of approximately 0.45. Any secondary stain obtained in the chromatogram of *Solution (1)*, with R_f smaller than that of the main stain, is not more intense than the stain obtained with *Solution (2)* (3%), and no more than two stains are more intense than the stain obtained with *Solution (4)* (0.25%). Any secondary stain, with R_f smaller than that of the main stain, is not more intense than the stain obtained with *Solution (3)* (2%), and no more than one stain is more intense than the stain obtained with *Solution (4)* (0.25%).

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 555 EU/mg of hyoscine butylbromide.

ASSAY

Proceed as described in method **B.** of Assay in the *Hyoscine butylbromide* monograph. Prepare the *Sample solution* as described next.

Sample solution: transfer a volume of injection solution equivalent to 40 mg of hyoscine butylbromide to a 100 mL volumetric flask, complete the volume with 0.001 M hydrochloric acid and homogenize.

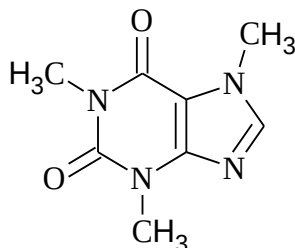
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₂₁H₃₀BrNO₄ in the injection solution, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELING

Comply with the current legislation.

CAFFEINE
Coffeinum

$C_8H_{10}N_4O_2$; 194.19

caffeine; 01642

3,7-di-hydro-1,3,7-trimethyl-1*H*-purine-2,6-dione

[58-08-2]

$C_8H_{10}N_4O_2 \cdot xH_2O$;

caffeine hydrate; 11382

3,7-di-hydro-1,3,7-trimethyl-1*H*-purine-2,6-dione, hydrate (1:1)

[75639-14-4]

Contains no less than 98.5% and no more than 101.0% of $C_8H_{10}N_4O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder or white, brilliant acicular crystals. Sublimes easily under heat action. The hydrate form is efflorescent to air.

Solubility. Moderately soluble in water and ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 235 °C to 239 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of caffeine CRS, prepared identically.

B. Dissolve about 5 mg of sample in 1 mL of hydrochloric acid in a watch glass or porcelain capsule, add 50 mg of potassium chlorate and evaporate in water bath to dryness. Invert the watch glass over another watch glass with a small amount of 6 *M* ammonium hydroxide. The residue acquires a purple color, which disappears with the addition of *M* sodium hydroxide.

C. Add to 2 mL of a saturated aqueous preparation of sample 0.1 mL of iodine RS. The preparation is clear. Add 0.1 mL of diluted hydrochloric acid. A brown precipitate is formed, and dissolves after neutralization with diluted sodium hydroxide solution.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G₂₅₄ as support, and a mixture of ammonia, acetone, chloroform and butyl alcohol (10:30:30:40) as mobile phase. Apply, separately, to the plate 10 µL of each solution described below.

Solution (1): dissolve 0.2 g of the sample in a mixture of methyl alcohol and chloroform (4:6) and complete the volume to a 10 mL volumetric flask.

Solution (2): transfer 0.5 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with a mixture of methyl alcohol and chloroform (4:6).

Develop the chromatogram over a course of 15 cm. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). If other stains, in addition to the main stain, appear in the chromatogram obtained with *Solution (1)*, none is more intense than the stain of the chromatogram obtained with *Solution (2)* (0.5%).

Other alkaloids. Add drops of potassium mercury iodide RS to 5 mL of a 0.02% (w/v) solution. It must not precipitate.

Arsenic (5.3.2.5). Use *Method 1*. No more than 0.0003% (3 ppm).

Lead (5.3.2.12). No more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Mix 2 g of the sample with 5 mL of 0.1 M hydrochloric acid and 10 mL of water and heat to dissolution. After cooling, complete to 50 mL and use 25 mL of this solution for the heavy metals assay. Proceed as described in *Method I*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 115 °C until constant weight. No more than 0.5% for caffeine anhydrous. No more than 8.5% for caffeine hydrate.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Dissolve 0.4 g of sample, accurately weighed, with heating, in 40 mL of acetic anhydride. Cool and add 80 mL of toluene. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 19.47 mg of C₈H₁₀N₄O₂.

PACKAGING AND STORAGE

In hermetic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Central stimulant.

CALAMINE

ZnO; 81.41
calamine; 01646
Calamine
[8011-96-9]

Calamine is zinc oxide, with a small proportion of ferric oxide (Fe_2O_3 , 159.69), which provides, after ignition, no less than 98.0% and no more than 100.5% of zinc oxide (ZnO).

DESCRIPTION

Physical characteristics. Rosy or reddish brown amorphous, non-palpable powder, depending on the color of the variety and amount of ferric oxide present, and also on the process through which it is incorporated.

Solubility. Practically insoluble in water. Dissolves, with effervescence, in hydrochloric acid.

IDENTIFICATION

A. Dissolve 1 g of the sample in 10 mL of 3 M hydrochloric acid and filter. The filtrate complies with zinc ion reactions (**5.3.1.1**).

B. Dissolve 1 g of the sample in 10 mL of 3 M hydrochloric acid, heat to boiling and filter. There is a reddish color after the addition of ammonium thiocyanate RS.

PURITY TESTS

Calcium. Digest 1 g of the sample in 25 mL of 3 M hydrochloric acid for 30 minutes. Filter, to remove insoluble ferric oxide, add 6 M sodium hydroxide to the filtrate, until the first precipitate formed is dissolved again, and then add another 5 mL of 6 M sodium hydroxide. Add to 10 mL of this solution 2 mL of 3.5% (w/v) ammonium oxalate. No more than a slight turbidity is produced.

Calcium or Magnesium. Add to another portion of 10 mL of the solution prepared for the *Calcium* test 2 mL of 12% (w/v) of sodium phosphate dibasic heptahydrate. No more than a slight turbidity is produced.

Lead. Add to 1 g of the sample 15 mL of water, shake, then add 3 mL of glacial acetic acid and heat in water bath until it dissolves. Filter and add five drops of potassium chromate RS. No turbidity is observed.

Acid-insoluble substances. Weigh 2 g and add 50 mL of 3 M hydrochloric acid. If an insoluble residue remains, collect in a tared filter, wash with water and dry at 105 °C for 1 hour, cool and weigh. The residue weight does not exceed 40 mg (2.0%).

Alkaline substances. Digest 1 g with 20 mL of water in water bath for 15 minutes, filter and add two drops of phenolphthalein TS. If a red color is produced, no more than 0.2 mL of 0.05 M sulfuric acid is required to remove it.

Arsenic (5.3.2.5). Use *Method 1*. Use a solution of 3.5 M sulfuric acid and a 40% (w/v) tin chloride solution in hydrochloric acid. The limit is 0.0008% (8 ppm).

Loss on ignition (5.2.9.2). Weigh about 2 g of the sample, calcinate at 500 °C until constant weight. No more than 2.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Calcinate, quantitatively, about 1.5 g of calamine. Make the digestion with 50 mL of 0.5 M sulfuric acid VS to this recently calcinated sample, applying low heat, until there no longer is dissolution. Filter the mixture and wash the residue in the filter, with hot water, until the final wash is neutral to litmus paper. Add to the filtrate combined with the washes 2.5 g of ammonium chloride, cool, add methyl orange TS and titrate with M sodium hydroxide VS. Each mL of 0.5 M sulfuric acid VS is equivalent to 40.69 mg of zinc oxide.

PACKAGING AND STORAGE

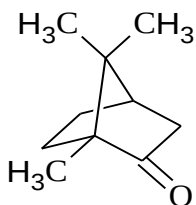
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Astringent; antipruritic.

CAMPHOR***Camphora***

$C_{10}H_{16}O$; 152.23

camphor; 01677

1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one

[76-22-2]

DESCRIPTION

Physical characteristics. White or colorless crystals, crystalline masses or granules. Volatilizes slowly at room temperature.

Solubility. Slightly soluble in water, very soluble in ethyl alcohol, freely soluble in volatile and fixed oils.

Physicochemical constants.

Melting range (5.2.2): 174 °C to 179 °C.

Specific optical rotation (5.2.8): +41 to +43 for natural camphor. Synthetic camphor is the racemic, optically inactive form. Determine in solution at 10% (w/v) in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of camphor CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.1% (w/v) sample solution in ethyl alcohol, there is maximum absorption at (289 ± 1) nm.

C. Add to powdered camphor (obtained by treatment with a small amount of ethyl alcohol) one drop of 1.0% (w/v) vanillin and one drop of sulfuric acid; a yellow color will appear, and gradually change to purple, violet and blue. This test is only positive for natural camphor.

D. Heating camphor powder in a container covered with watch glass, a sublimate composed of isotropic pyriform crystals gathered in radial groups is obtained.

PURITY TESTS

Aspect of the preparation. The 10% (w/v) preparation in hexane is clear **(5.2.25)**.

Residue by evaporation. Heat, in water bath, 2.0 g of the sample in a tared capsule, until full sublimation. Dry the residue at 120 °C for three hours, cool and weigh. The residue weight must not exceed 0.05%.

Halogens. Mix 0.1 g of camphor finally divided with 0.2 g of sodium peroxide, in a dry porcelain crucible. Heat slowly until full incineration. Dissolve the residue in 25 mL of tepid water, acidify with nitric acid, filter the solution and transfer to a comparison tube. Wash the crucible and the filter with 10 mL of hot water (twice) and filter, adding the washing waters to the filtered solution. Add to the filtrate 0.5 mL of 0.1 M silver nitrate, dilute with water to 50 mL and homogenize. The turbidity must not exceed the one produced on a blank test, with the same amounts of the same reagents and 0.05 mL of 0.02 M hydrochloric acid (0.035%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

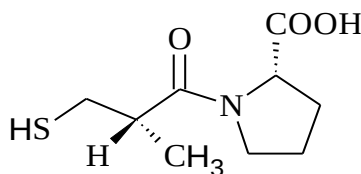
In hermetic containers. Avoid excessive heat.

LABELING

Comply with the current legislation. The label must indicate the origin, whether natural or synthetic.

THERAPEUTIC CLASS

Topical antipruritic.

CAPTOPRIL***Captoprilum***C₉H₁₅NO₃S; 217.29

captopril; 01699

1-[(2S)-3-Mercapto-2-methyl-1-oxopropyl]-L-proline

[62571-86-2]

Contains no less than 97.5% and no more than 102.0% of C₉H₁₅NO₃S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Soluble in water, freely soluble in methyl alcohol. Soluble in diluted alkali hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 105 °C to 108 °C.

Specific optical rotation (5.2.8): -156 to -161, in relation to the desiccated substance. Determine in 2% (w/v) solution in carbon dioxide-free water.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of captopril CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

pH (5.2.19). 2.0 to 2.6. Determine in 2% (w/v) sample solution in carbon dioxide-free water.

Related substances. Proceed as described on method **B.** from *Assay*. Prepare the *Test solutions* as described next.

Solution (1): transfer 50 mg of the sample to a 100 mL volumetric flask, dissolve with *Mobile phase*, complete the volume with the same solvent and homogenize.

Solution (2): transfer 1 mL of *Solution (1)* to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Solution (3): dissolve 10 mg of the sample in 20 mL of *Mobile phase*, add 0.25 mL of 0.05 M iodine and complete the volume to 100 mL with the same solvent. Homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, homogenize and complete the volume with the same solvent.

Procedure: inject, separately, 20 µL of each solution, record the chromatograms for no less than three times the retention time of captopril and measure the areas under the peaks. The test is only valid if the chromatogram obtained with *Solution (3)* presents three peaks and the resolution between the two peaks with the highest retention times is no less than 2.0. The three peaks correspond, respectively, to excess iodine, captopril, and captopril disulfide formed. The area of any secondary peak obtained in the chromatogram with *Solution (1)* is no more than half the area under the main peak obtained in the chromatogram with *Solution (2)* (1.0%). The sum of the areas of all peaks obtained with *Solution (1)*, except that of the main peak, is no more than the area under the main peak obtained with *Solution (2)* (2.0%). Disregard peaks related to the solvent or with area below 10% the area under the main peak, obtained in the chromatogram with *Solution (2)* (0.2%).

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for 3 hours. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Transfer, quantitatively, about 0.15 g of sample to a 125 mL Erlenmeyer flask and dissolve in 50 mL of water. Titrate with 0.05 M iodine VS, determining the endpoint potentiometrically or using 1 mL of starch TS. Each mL of 0.05 M iodine VS is equivalent to 21.729 mg of C₉H₁₅NO₃S.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of 0.11% (v/v) phosphoric acid and methyl alcohol (45:55).

Note: protect the solutions described next from exposure to air and use them within 8 hours.

Sample solution: dissolve an accurately weighed amount of the sample in *Mobile phase* to obtain a 0.5 mg/mL solution.

Standard solution: dissolve an accurately weighed amount of captopril CRS in *Mobile phase* to obtain a 0.5 mg/mL solution.

Inject 20 µL of the *Solution (3)* obtained in *Related substances*. The test is only valid if the chromatogram obtained presents three peaks and the resolution between the two peaks with the highest retention times is no less than 2.0. Inject 20 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas of the recorded peaks is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₉H₁₅NO₃S in the sample from the responses obtained with the *Standard* and *sample solutions*.

PACKAGING AND STORAGE

In hermetic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-hypertensive.

CAPTOPRIL TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_9H_{15}NO_3S$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of toluene, glacial acetic acid and methyl alcohol (75:25:1) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Transfer the equivalent to 0.1 g of captopril to a 25 mL volumetric flask, add 15 mL of methyl alcohol, leave in ultrasonic bath for 30 minutes, shaking occasionally. Complete the volume with the same solvent, homogenize and filter.

Solution (2): prepare a 4 mg/mL captopril CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow to air dry. Nebulize with mercuric diphenylcarbazone RS. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method B. from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a volumetric flask of adequate capacity, containing 5 mL of water, and wait for the tablet to completely disintegrate. Add a volume of mixture of ethyl alcohol and water (1:1), corresponding to half the capacity of the flask. Leave in an ultrasonic bath for 15 minutes. Shake mechanically for 15 minutes, complete the volume with the same solvent, homogenize and filter. Dilute successively, with the same solvent, to a concentration of 0.002% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 212 nm **(5.2.14)**, using a mixture of ethyl alcohol and water (1:1) for zero adjustment. Calculate the amount of $C_9H_{15}NO_3S$ in each tablet, from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 50 rpm.

Time: 20 minutes.

Procedure: immediately after the test, take an aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid, until adequate concentration. Measure the absorbances at 212 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_9H_{15}NO_3S$ dissolved in the medium, comparing the readings obtained with that of the captopril CRS solution at the concentration of 0.0025% (w/v), prepared in 0.1 M hydrochloric acid.

Tolerance: no less than 80% (Q) of the declared quantity of $C_9H_{15}NO_3S$ dissolve in 20 minutes.

PURITY TESTS

Limit of captopril disulfide. Proceed as described on method **B.** from Assay. Inject, separately, 20 μ L of the *Test solution* and the *Sample solution*. The area under the peak related to captopril disulfide, obtained in the *Sample solution*, must not be greater than the area under the peak related to captopril disulfide obtained in the *Test solution*. No more than 3.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh and pulverize 20 tablets. Use an amount of powder equivalent to approximately 0.15 g of captopril, transfer to a 125 mL Erlenmeyer flask and add 50 mL of water. Leave in ultrasonic bath for 15 minutes and shake mechanically for 15 minutes. Proceed as described on method A. from Assay in the *Captopril* monograph, as of "Titrate with 0.05 M iodine VS...".

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of 0.11% (v/v) phosphoric acid and methyl alcohol (45:55).

Captopril disulfide solution: prepare a 1 mg/mL solution of captopril disulfide CRS in *Mobile phase*.

Test solution: transfer 3 mL of the *Captopril disulfide solution* to a 100 mL flask, complete with *Mobile phase* and homogenize.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 50 mg of captopril to a 50 mL volumetric flask, add 30 mL of *Mobile phase*, leave in ultrasonic bath for 15 minutes and shake mechanically for 15 minutes. Complete the volume with the same solvent, homogenize and filter.

Standard solution: transfer 0.1 g of captopril CRS to a 100 mL volumetric flask, add 3 mL of the *Captopril disulfide solution* and complete the volume with *Mobile phase*.

Inject 20 µL replicates of the *Standard solution*. The relative retention times are approximately 0.5 for captopril and 1.0 for captopril disulfide. The resolution between the peaks of captopril and captopril disulfide must be of no less than 2. The relative standard deviation of the replicate areas from the recorded peaks must be of no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_9H_{15}NO_3S$ in the tablets, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

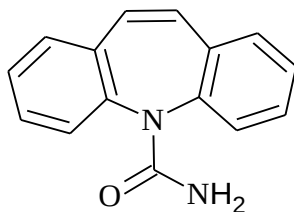
In tightly closed containers.

LABELING

Comply with current legislation.

CARBAMAZEPINE

Carbamazepinum



C₁₅H₁₂N₂O; 236.27

carbamazepine; 01710

5*H*-Dibenz[*b,f*]azepine-5-carboxamide

[298-46-4]

Contains no less than 98.0% and no more than 102.0% of C₁₅H₁₂N₂O, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish-white crystalline powder. Presents polymorphism.

Solubility. Practically insoluble in water, soluble in methyl alcohol, moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 189 °C to 193 °C.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of carbamazepine CRS, prepared identically.

PURITY TESTS

Acidity or alkalinity. Weigh 2.5 g of the sample and transfer to a 50 mL volumetric flask. Add 20 mL of water. Shake, complete the volume with water and homogenize. Filter. Add to an aliquot of 20 mL one drop of phenolphthalein TS. Titrate with 0.01 *M* sodium hydroxide. Carry out in parallel a blank test. No more than 1 mL is required for each 1 g of sample. Add to the other aliquot of 20 mL one drop of methyl red TS. Titrate with 0.01 *M* hydrochloric acid. Carry out in parallel a blank test. No more than 1 mL is required for each 1 g of sample.

Related substances.

Use one of the methods described below.

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of toluene and methyl alcohol (70:30) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described next, in a mixture of chloroform and ethyl alcohol (1:1).

Solution (1): 50 mg/mL sample solution.

Solution (2): 0.05 mg/mL sample solution.

Solution (3): 50 mg/mL carbamazepine CRS solution.

Solution (4): 0.05 mg/mL iminodibenzyl solution.

Solution (5): 0.05 mg/mL carbamazepine related substance B CRS (iminostilbene) solution.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm and 365 nm). Nebulize with 0.5% (w/v) potassium dichromate in *M* sulfuric acid. Any secondary stain obtained in the chromatogram with *Solution (1)* is not more intense than the stains obtained with *Solutions (4)* and *(5)* (0.1%). Heat at 140 °C for 15 minutes and observe under ultraviolet light (254 nm and 365 nm). Any stain obtained in the chromatogram with *Solution (1)*, with *R_f* value lower than the main stain, is not more intense than that obtained with *Solution (2)* (0.1%).

B. Proceed as described on method **B.** from *Assay*. Prepare the following solutions.

Sample solution: accurately weigh about 100 mg of sample. Transfer to a 50 mL volumetric flask, dissolve, complete the volume with methyl alcohol and homogenize. Transfer 25 mL of this solution to a 50 mL volumetric flask, complete with *Mobile phase* and homogenize.

Standard solution: dissolve an amount, accurately weighed, of carbamazepine CRS, carbamazepine related substance A CRS (10,11-di-hydrocarbamazepine) and carbamazepine related substance B CRS (iminostilbene) in methyl alcohol, to obtain a concentration of 0.02 mg/mL from each substance. Transfer 5 mL of this solution to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize, obtaining a solution at 1 µg/mL.

Resolution solution: dissolve an amount, accurately weighed, of carbamazepine CRS and carbamazepine related substance A CRS (10,11-di-hydrocarbamazepine) in methyl alcohol, to obtain a solution at 100 µg/mL and 500 µg/mL, respectively. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*.

Inject 20 µL replicates of the *Resolution solution*. The resolution between the peaks of carbamazepine related substance A and carbamazepine is no less than 1.70. The relative standard deviation of the replicate areas of the recorded peaks is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*. Record the chromatograms and measure the areas under the peaks. Calculate the amount in mg of any impurity found on the *Sample solution*, from the responses obtained. No more than 0.2% of individual impurities and 0.5% of total impurities.

Chlorides (5.3.2.1). Add 20 mL of water to 0.5 g of the sample and let it boil for 10 minutes. Cool and filter, quantitatively, to a comparison tube. No more than 0.014% (140 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Heat, to ebullition, 1 g of the sample in 20 mL of water for 10 minutes, cool and filter, quantitatively, to a comparison tube. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 2 g of the sample. Dry in oven at 100 °C to 105 °C for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

X-ray diffraction (5.2.31). The sample's X-ray diffraction pattern, without prior treatment, present maximum diffraction points only on the same angles as those observed in the diffraction spectrum of carbamazepine CRS, prepared identically.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 50 mg of the sample. Transfer to a 50 mL volumetric flask, add 25 mL of methyl alcohol and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent and homogenize. Dilute, successively, in methyl alcohol until concentration of 0.001% (w/v). Prepare a standard solution, in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 285 nm, using methyl alcohol for zero adjustment. Calculate the content of $C_{15}H_{12}N_2O$ in the sample from the readings obtained. Alternatively, calculate using $A(1\%, 1\text{ cm}) = 490$, at 285 nm, in methyl alcohol.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 230 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: methyl alcohol and water (70:30).

Sample solution: dissolve an accurately weighed amount of the sample in methyl alcohol to obtain a 2 mg/mL solution. Leave in an ultrasonic bath for 15 minutes. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with mobile phase and homogenize, obtaining a solution at 0.2 mg/mL.

Standard solution: dissolve an accurately weighed amount of carbamazepine CRS in methyl alcohol to obtain a 1 mg/mL solution. Leave in ultrasonic bath for 15 minutes, complete the volume with the same solvent and homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with mobile phase and homogenize, obtaining a solution at 0.2 mg/mL.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₅H₁₂N₂O in the sample from the responses obtained with the *Standard* and *sample solutions*.

PACKAGING AND STORAGE

In hermetic containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anticonvulsant.

CARBAMAZEPINE TABLETS

Contains no less than 92.0% and no more than 108.0% of the declared amount of $C_{15}H_{12}N_2O$.

IDENTIFICATION

Weigh and pulverize the tablets. Heat in water bath an amount of powder equivalent to 0.2 g of carbamazepine with 15 mL of acetone. Filter. Wash with two portions of 5 mL of hot acetone. Evaporate the filtrate to approximately 5 mL and cool in ice bath to crystallization. Filter the crystals and wash the filter with 3 mL of cold acetone. Dry in oven at 70 °C, under reduced pressure, for 30 minutes. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of carbamazepine CRS, prepared identically.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). No more than five minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 1% (w/v) sodium lauryl sulfate in water; 900 mL.

Apparatus: paddles, 75 rpm.

Time: 60 minutes, with collecting times at 15 and 60 minutes.

Procedure: take aliquots of the dissolution mediums at the times determined and filter. Measure the absorbances at 285 nm **(5.2.14)**, using the *Dissolution medium* for zero adjustment. Calculate the amount of $C_{15}H_{12}N_2O$ dissolved in the medium, comparing the readings obtained with that of the carbamazepine CRS solution at the concentration of 0.002% (w/v), prepared in 1% (w/v) sodium lauryl sulfate, with prior addition of methyl alcohol to ensure the dissolution. The concentration of methyl alcohol in the standard solution cannot exceed 1% (v/v).

Tolerance: no less than 45% (Q) dissolve in 15 minutes; no less than 75% (Q) of the declared quantity of $C_{15}H_{12}N_2O$ dissolve in 60 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 50 mg of carbamazepine to a 100 mL volumetric flask, add 70 mL of methyl alcohol and leave in ultrasonic bath for 30 minutes. Complete the volume with the same solvent, homogenize and filter. Carry out successive dilutions, to a concentration of 0.001% (w/v), using methyl alcohol as solvent. Prepare a standard solution, in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 285 nm, using methyl alcohol for zero adjustment. Calculate the amount of $C_{15}H_{12}N_2O$ in the tablets, from the readings obtained. Alternatively, calculate using $A(1\%, 1\text{ cm}) = 490$ at 285 nm, in methyl alcohol.

B. Proceed as described in method **B.** of *Assay* in the *Carbamazepine* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 100 mg of carbamazepine to a 50 mL volumetric flask, add 25 mL of methyl alcohol and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize, obtaining a solution at 0.2 mg/mL.

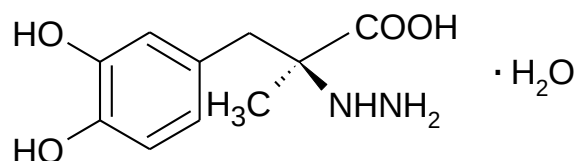
Procedure: inject, separately, 20 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{15}H_{12}N_2O$ in the tablets from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In hermetic containers, protected from light.

LABELING

Comply with current legislation.

CARBIDOPA*Carbidopum*C₁₀H₁₄N₂O₄·H₂O; 244.25

carbidopa monohydrate; 11134

(2S)-3-(3,4-di-hydroxyphenyl)-2-hydrazinyl-2-methylpropanoic acid monohydrate

[38821-49-7]

C₁₀H₁₄N₂O₄; 226.23

carbidopa; 01731

(2S)-3-(3,4-di-hydroxyphenyl)-2-hydrazinyl-2-methylpropanoic acid

[28860-95-9]

Contains no less than 98.0% and no more than 102.0% of C₁₀H₁₄N₂O₄·H₂O.

DESCRIPTION

Physical-chemical characteristics. White or yellowish-white powder. Melting point **(5.2.2)**: around 197 °C, with decomposition.

Solubility. Slightly soluble in water and very slightly soluble in ethyl alcohol. Soluble in diluted mineral acid solutions.

Physicochemical constants.

Specific optical rotation (5.2.8): -21 to -23.5, in relation to the monohydrate sample. Prepare a sample solution at 10 mg/mL, in a 70% (w/v) aluminum chloride solution (use the hexahydrate form of aluminum salt) filtered in advance and with pH adjusted to 1.5 using 0.25 M sodium hydroxide.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of carbidopa CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 240 nm to 300 nm, of a carbidopa solution at 40 µg/mL in a mixture of hydrochloric acid and methyl alcohol (9:100), there are maximum absorption points only at the same wavelengths and with the same relative intensities as those observed in the carbidopa CRS spectrum, prepared identically.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Organic impurities. Limit of methyldopa and carbidopa related compound A. Proceed as described in Assay. Prepare the solutions described below.

Standard impurities solution: dissolve an amount, accurately weighed, of methyldopa CRS and carbidopa related substance A CRS in *Mobile phase*, to obtain a solution with concentration of 2.5 µg/mL from each of these impurities.

The relative retention times of methyldopa, carbidopa and carbidopa related substance A are approximately 0.8, 1.0 and 1.8, respectively.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard impurities solution*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of methyldopa and carbidopa related substance A according to the formula:

$$\text{Porcentagem de impureza} = \left(\frac{r_U}{r_S} \right) \times \left(\frac{C_S}{C_U} \right) \times 100$$

where

r_U = area under the peak of methyldopa or carbidopa related substance A from the *Sample solution*;
 r_S = area under the peak of methyldopa or carbidopa related substance A from the *Standard impurities solution*;

C_S = concentration of methyldopa CRS or carbidopa related substance A CRS from the *Standard impurities solution* (µg/mL);

C_U = concentration of the *Sample solution* (µg/mL).

The sample must present no more than 0.5% of methyldopa and no more than 0.5% of carbidopa related substance A.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 100 °C, under reduced pressure of no more than 5 mmHg, until constant weight. Cool and filter. Loses 6.9% to 7.9% of its weight.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 280 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of ethyl alcohol and 0.05 M monobasic sodium phosphate with pH adjusted to 2.7 with phosphoric acid (5:95).

Sample solution: dissolve an accurately weighed amount of the sample in *Mobile phase* to obtain a solution with concentration of 0.5 mg/mL of carbidopa.

Standard solution: dissolve an accurately weighed amount of carbidopa CRS in the *Mobile phase* to obtain a 0.5 mg/mL solution. Use low heating and ultrasonic bath, if necessary, to dissolve.

System suitability solution: prepare a solution at 0.1 mg/mL of carbidopa CRS and 0.1 mg/mL of methyldopa CRS using *Mobile phase* as diluent.

Inject 20 µL replicates of the *System suitability solution* and the *Standard solution*. The resolution between methyldopa and carbidopa must be no less than 0.9 in the chromatograms with the *System suitability solution*. The relative standard deviation of the areas under the peak of carbidopa, obtained with the *Standard solution*, must be no more than 1.5%.

Note: the relative retention time of methyldopa and carbidopa is around 0.8 and 1.0, respectively.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of $C_{10}H_{14}N_2O_4 \cdot H_2O$ in the sample from the responses obtained with the *Standard solution* and with the *Sample solution*.

PACKAGING AND STORAGE

In hermetically closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Dopaminergic agent.

BISMUTH SUBCARBONATE***Bismuthi subcarbonas***

(BiO)₂CO₃; 509.97

bismuth subcarbonate; 01747

Bismuth carbonate oxide

[5892-10-4]

Contains no less than 97.6% and no more than 100.7% of (BiO)₂CO₃, in relation to the desiccated substance, equivalent to no less than 80.0% and no more than 82.5% of bismuth.

DESCRIPTION

Physical characteristics. White powder.

Solubility. Practically insoluble in water and ethyl alcohol. Dissolves, with effervescence, in diluted mineral acids and glacial acetic acid.

IDENTIFICATION

A. Complies with bismuth ion reactions **(5.3.1.1)**.

B. Complies with carbonate ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Shake 5 g of the sample with 10 mL of water. Add 20 mL of nitric acid and heat until dissolution. Cool and dilute to 100 mL with water. The preparation obtained is colorless **(5.2.12)** and less opalescent than the *Reference suspension II* **(5.2.25)**.

Copper. Add to 5 mL of the preparation obtained in *Aspect of the preparation* 2 mL of ammonia, dilute to 50 mL with water and filter. Add to 10 mL of the filtrate 1 mL of 0.1% (w/v) sodium diethyldithiocarbamate solution. The color of the preparation is not more intense than that of a reference solution prepared in parallel, in the same conditions, using a mixture of 0.25 mL of *Standard copper solution (10 ppm Cu)* and sufficient water for 10 mL, instead of filtrate. No more than 0.005% (50 ppm).

Alkaline metals and earth metals. Add to 1 g of the sample 10 mL of water and 10 mL of acetic acid RS. Heat to ebullition for two minutes, cool and filter. Wash the residue with 20 mL of distilled water. Add to the filtrate 2 mL of hydrochloric acid RS and 20 mL of water. Heat to ebullition and pass hydrogen sulfide in the solution, until the entire bismuth is precipitated. Filter, wash the residue with water, evaporate in water bath to dryness and add 0.5 mL of sulfuric acid. Incinerate carefully and let it breathe. The residue mass is no more than 10 mg (1.0%).

Nitrates. Transfer 0.25 g of the sample to a 125 mL Erlenmeyer flask, add 20 mL of distilled water, 0.05 mL of indigo carmine VS and, carefully, 30 mL of sulfuric acid. Titrate immediately with indigo carmine VS, until turning into a stable blue color. The volume of indigo carmine VS spent is no more than the volume equivalent to 1 mg of NO₃ (0.4%).

Silver. Add to 2 g of the sample 1 mL of water and 4 mL of nitric acid. Heat, carefully, until dissolution, cool and dilute to 11 mL with water. Add 2 mL of *M* hydrochloric acid, homogenize and allow to stand for five minutes protected from light. Any opalescence developed is not more intense than that of a standard, prepared by mixing 10 mL of standard silver solution (5 ppm Ag), 1 mL of nitric acid and 2 mL of *M* hydrochloric acid. No more than 0.0025% (25 ppm).

Arsenic (5.3.2.5). Transfer 0.6 g of the sample to a distillation flask. Add 5 mL of water, 7 mL of sulfuric acid and cool. Add 5 g of reducer mixture and 10 mL of hydrochloric acid. Heat, gradually, to ebullition, for 15 to 30 minutes, and continue heating, so that the distillation is carried out regularly until the flask volume reduces to half or until the condenser fills with vapor for five minutes. The distillation must be interrupted before the formation of sulfur trioxide. Collect the distillate in a tube with 15 mL of water chilled in an ice bath. Wash the condenser with water, dilute the distillate to 25 mL with the same solvent and proceed as described in *Visual method*. Prepare the reference solution using a mixture of 3 mL of *Standard arsenic solution* (1 ppm As) and 22 mL of water. No more than 0.0005% (5 ppm).

Lead. Proceed as described in *Atomic absorption spectrometry* (5.2.13.1), use *Method II*. Dissolve 12.5 g of the sample in 75 mL of a mixture of equal volumes of water and lead-free nitric acid. Heat to ebullition for one minute, cool and dilute to 100 mL with water. For the preparation of reference lead solutions, use appropriate amounts of standard lead solution and 37% (v/v) lead-free nitric acid. Measure the absorbances of the solutions at 283.3 nm, using a hollow cathode lamp as source of radiation and air-acetylene flame. No more than 0.002% (20 ppm).

Chlorides (5.3.2.1). Determine in 0.7 g of the sample, dissolved with 8 mL of nitric acid. Transfer to a 100 mL volumetric flask, complete with water and homogenize. No more than 0.05% (500 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample, in oven at 105 °C. No more than 1.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.25 g of the sample in 2 mL of nitric acid and dilute to 100 mL with water. Proceed as described in *Complexometric titrations* (5.3.3.4) for *Bismuth*. Each mL of 0.05 *M* disodium edetate VS is equivalent to 12.749 mg of (BiO)₂CO₃, corresponding to 10.449 mg of bismuth.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antacid.

CALCIUM CARBONATE***Calcii carbonas***

CaCO₃; 100.09

calcium carbonate; 01748

Carbonic acid calcium salt (1:1)

[471-34-1]

Contains no less than 98.5% and no more than 100.5% of CaCO₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, fine, microcrystalline powder.

Solubility. Practically insoluble in water. Slightly soluble in water, when in presence of ammoniacal salts or carbon dioxide. Practically insoluble in ethyl alcohol. Dissolves with effervescence in *M* acetic acid, 3 *M* hydrochloric acid and 2 *M* nitric acid.

IDENTIFICATION

A. Introduce, in a test tube, about 0.1 g of the sample and suspend with 2 mL of water. Then, add 3 mL of 2 *M* acetic acid and close the tube immediately with a stopper connected in advance to a U-shaped glass tube. The mixture effervesces. Connect to the other end of the U-shaped tube a second test tube, with 0.1 *M* barium hydroxide. Heat slowly the tube with the sample. A precipitate forms in the second tube and dissolves in 6 *M* hydrochloric acid.

B. Complies with calcium ion reactions (5.3.1.1).

PURITY TESTS

Acid-insoluble substances. Weigh 5 g of sample and drip hydrochloric acid, with agitation, until the effervescence stops. Then, transfer to a 200 mL flask and complete the volume with water. Filter on adequate filter paper. Wash the residue until the final wash does not present reaction for chloride (5.3.1.1). Incinerate and cool in desiccator. The residue weight is not more than 10 mg (0.2%).

Magnesium and alkaline metals. Mix 1 g of the sample with 35 mL of distilled water. Add, carefully, 3 mL of hydrochloric acid and boil the solution for one minute. Quickly add 40 mL of oxalic acid RS. Shake vigorously until there is precipitation. Heat, immediately add two drops of methyl red TS and add 6 *M* ammonium hydroxide until the mixture is alkaline. Cool at room temperature and transfer to a 100 mL volumetric flask. Complete the volume with water and allow to stand for four hours. Filter on adequate filter paper. Put 50 mL of the filtrate in a porcelain capsule, add 0.5 mL of sulfuric acid and reduce the volume in water bath to a small volume. Heat on an electric plate to decomposition and volatilization of ammonium salts. Incinerate the residue at 600 °C until constant weight. The residue weight is no more than 5 mg (1%).

Arsenic (5.3.2.5). Use *Method I*. Dissolve 1 g of the sample in 80 mL of diluted acetic acid. After the effervescence stops, boil for two minutes, cool and complete the volume to 100 mL with diluted acetic acid. Filter, if necessary, in sintered glass filter. No more than 0.0004% (4 ppm).

Chlorides (5.3.2.1). Weigh 1 g of the sample, add 10 mL of distilled water and, carefully, add 10 mL of 2 M nitric acid, shaking until dissolution. No more than 0.035% (350 ppm).

Barium. Weigh, accurately, about 2.5 g of the sample, transfer quantitatively to a tall form beaker, add 3 M hydrochloric acid until the effervescence stops and heat until ebullition, to eliminate the dissolved carbon dioxide. Transfer quantitatively to a 25 mL volumetric flask and complete the volume with water. Transfer to a test tube 5 mL of the sample solution and add 5 mL of calcium sulfate RS. After 15 minutes, the preparation is not more opalescent than 5 mL of the sample solution with 5 mL of water.

Iron. Proceed as described in *Atomic absorption spectrometry (5.2.13.1), Method I*. No more than 0.02% (200 ppm).

Sample solution: weigh, accurately, about 0.5 g of the sample and transfer to a tall form beaker. Add 5 mL of 3 M hydrochloric acid and heat until ebullition to eliminate carbon dioxide. Transfer, quantitatively, to a 25 mL volumetric flask, complete the volume with water and homogenize.

Sulfate (5.3.2.2). Use 5 mL of the sample solution obtained in the barium test. No more than 0.25% (2500 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Use 10 mL of the sample solution obtained in the barium test. No more than 0.002% (20 ppm).

Loss on drying. (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 200 °C for four hours. No more than 2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.1 g of the sample, previously desiccated, and transfer to a 250 mL Erlenmeyer flask. Add 50 mL of water and 2 mL of diluted hydrochloric acid, cover with watch glass and shake until dissolution of calcium carbonate. Calculate the theoretical equivalence point and titrate with 0.05 M disodium edetate VS, until approximately 2 mL before this volume. Add 8 mL of sodium hydroxide RS and 150 mg of hydroxynaphthol blue indicator. Continue titrating with 0.05 M disodium edetate VS until a blue color. Each mL of 0.05 M disodium edetate VS is equivalent to 5.004 mg of CaCO₃.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antacid, nutritional supplement, phosphorus chelator.

LITHIUM CARBONATE***Lithium carbonas***

Li_2CO_3 ; 73.89
lithium carbonate, 01749
[554-13-2]

Contains no less than 98.5% and no more than 100.5% of Li_2CO_3 .

DESCRIPTION

Physical characteristics. White or nearly white powder.

Solubility. Slightly soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. When moistened with hydrochloric acid, gives a red color to the non-luminous flame.

B. Dissolve 0.2 g in 1 mL of hydrochloric acid. Evaporate in water bath to dryness. The residue dissolves in 3 mL of ethyl alcohol.

C. Complies with carbonate ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Suspend 10 g of the sample in 30 mL of water and dissolve by adding 22 mL of nitric acid. Neutralize with sodium hydroxide solution RS and dilute with water to 100 mL. The preparation is clear **(5.2.25)** and colorless **(5.2.12)**.

Chloride (5.3.2.1). Proceed as described in *Limit test for chlorides*, using 5.0 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.02% (200 ppm).

Sulfate (5.3.2.2). Disperse 1.25 g in 5 mL of water and dissolve by adding 70% (w/v) hydrochloric acid. Boil for two minutes. Cool and add sodium hydroxide RS solution until neutralization. Dilute to 25 mL with water. Proceed as described in *Limit test for sulfates*. No more than 0.02% (200 ppm).

Arsenic (5.3.2.5). Add to 1.5 g of sample 3.5 M sulfuric acid until the effervescence stops and proceed as described in *Limit test for arsenic*. No more than 0.0002% (2 ppm).

Calcium (5.3.2.7). Use 5 mL of the preparation obtained in *Aspect of the preparation*. Proceed as described in *Limit test for calcium*. No more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Use 10 mL of the preparation obtained in *Aspect of the preparation* and proceed as described in *Method II*. No more than 0.002% (20 ppm).

Iron (5.3.2.4). Use 5 mL of the solution obtained in *Aspect of the preparation* and proceed as described in *Limit test for iron*. No more than 0.002% (20 ppm).

Magnesium (5.3.2.8). Dilute 1 mL of the preparation obtained in *Aspect of the preparation* to 10 mL with water. Use 6.7 mL of this preparation and proceed as described in *Limit test for magnesium*. No more than 0.015% (150 ppm).

Potassium. Dissolve 1 g of the sample in 10 mL of 70% (w/v) hydrochloric acid and dilute to 50 mL with water. Use a potassium chloride solution with 0.5 mg of potassium per mL as reference. Proceed as described in *Atomic emission spectrometry (5.2.13.2)*. Measure the intensity of emission at 766.5 nm. No more than 0.03% (300 ppm).

Sodium. Dissolve 1 g of the sample in 10 mL of 25% (w/v) hydrochloric acid and dilute to 50 mL with water. Use a sodium chloride solution with 0.3 mg of sodium per mL as reference. Proceed as described in *Atomic emission spectrometry (5.2.13.2)*. Measure the intensity of emission at 589 nm. No more than 0.03% (300 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 200 °C for four hours. No more than 1.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.5 g of the sample in 25 mL of *M* hydrochloric acid VS. Titrate with *M* sodium hydroxide solution VS using methyl orange TS as indicator. Carry out the blank test and make the necessary corrections. Each mL of *M* hydrochloric acid VS is equivalent to 36.945 mg Li_2CO_3 .

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antidepressant.

MAGNESIUM CARBONATE***Magnesii carbonas***

MgCO₃; 84.31

magnesium carbonate; 01750

Carbonic acid magnesium salt (1:1)

[546-93-0]

MgCO₃.xH₂O

magnesium carbonate hydrate; 11383

Carbonic acid magnesium salt hydrate

[23389-33-5]

Magnesium carbonate is a mixture of magnesium carbonate hydrate and basic magnesium carbonate hydrate. It must contain no less than 40.0% and no more than 43.5% of MgO.

DESCRIPTION

Physical characteristics. Presents under two varieties: light and heavy. Light magnesium carbonate: white, light, friable mass or white, very fine, light powder. Heavy magnesium carbonate: white, granular powder. Both, when shaken with water, make it sparingly alkaline to litmus paper.

Solubility. Practically insoluble in water and ethyl alcohol; dissolves in cold, in appreciable amount, in water saturated with carbon dioxide. Dissolves with effervescence in diluted acids.

IDENTIFICATION

A. When treated with diluted mineral acids, produces effervescence,

B. The solution obtained in test **A.** of *Identification* complies with the magnesium ion reactions **(5.3.1.1)**.

PURITY TESTS

Arsenic (5.3.2.5). Add to 1 g of sample 10 mL of water and 5 mL of brominated hydrochloric acid RS, eliminate the excess bromine with a few drops of tin(II) chloride RS, and proceed as described in *Limit test for arsenic*. Use *Method I*. No more than 0.0005% (5 ppm).

Calcium (5.3.2.7). Accurately weigh about 1 g of the sample and carefully add 22 mL of water and 3 mL of sulfuric acid. Add 50 mL of ethyl alcohol and allow the mixture to stand for no less than 12 hours. If there is separation of magnesium sulfate crystals, heat the mixture around 50 °C to dissolve them. Filter in an asbestos-coated Gooch crucible washed in advance with *M* sulfuric acid, water and ethyl alcohol, calcinated and tared. Wash the Gooch crucible several times with a mixture of two volumes of ethyl alcohol and one volume of *M* sulfuric acid. Dry it with bright red, cool and weigh quickly. The weight of the calcium sulfate thus obtained, multiplied by 0.4119, results in the weight of CaO in the sample. The sample must contain no more than the equivalent to 0.7% of CaO.

Iron (5.3.2.4). Use Method III. Weigh 2.5 g of sample and dissolve in 19 mL of 3 *M* hydrochloric acid. When the effervescence stops, complete the volume to 25 mL with water. Use 5 mL on the *Limit test for iron*. No more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Use Method I Use 8 mL of the solution prepared in the *Limit test for iron* and neutralize with concentrated ammonia solution. Add 2 mL of acetic acid RS (Pb) and carry on as described in the *Limit test for heavy metals*. No more than 0.0025% (25 ppm).

Sulfates (5.3.2.2). Use 10 mL of the solution prepared in the *Limit test for iron*. No more than 0.12% (1200 ppm).

Chlorides (5.3.2.1). Accurately weigh about 2.0 g of sample, add 15 mL of 2 M nitric acid, 25 mL of water and 1 mL of 0.25 M silver nitrate. Complete the volume to 50 mL with water. If it produces opalescence, it must not be more intense than that produced by 0.1 mg of chloride in equal volume of liquid, using the same reagents. No more than 0.02% (200 ppm).

Hydrochloric acid-insoluble substances. Mix 5 g of the sample with 75 mL of water and add, under agitation, hydrochloric acid in small portions, until full dissolution. Boil for five minutes. Collect the insoluble residue in a filter and wash until the washing waters no longer comply with the chloride reaction. Incinerate, let it cool and filter. The residue must weigh no more than 0.0025 g (0.05%).

Water-soluble substances. Add to 50 mL of recently boiled water 1 g of sample and take to ebullition for five minutes. Filter, evaporate the filtrate to dryness and dry the residue at 110 °C, for one hour. The residue must weigh no more than 0.01 g (1%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 1 g of sample, add 50 mL of 0.5 M sulfuric acid VS, 0.5 mL of methyl orange TS and dose the excess acid with M sodium hydroxide VS. Carry out the blank test and make the necessary corrections. Subtract from the volume of 0.5 M acid VS consumed the corresponding to CaO determined in *Purity tests*. The difference will be the volume of 0.5 M sulfuric acid VS that is equivalent to magnesium carbonate. Each mL of 0.5 M sulfuric acid VS is equivalent to 0.02015 mg of MgO and to 0.02804 g of CaO.

PACKAGING AND STORAGE

In closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antacid and laxative.

POTASSIUM CARBONATE***Kalii carbonas*** K_2CO_3 ; 138.21

potassium carbonate; 01751

Carbonic acid potassium salt (2:1)

[584-08-7]

Contains no less than 99.5% and no more than 100.5% of K_2CO_3 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, granular and hygroscopic powder.

Solubility. Freely soluble in water and practically insoluble in ethyl alcohol.

IDENTIFICATION

A. The 0.1% (w/v) sample solution complies with carbonate ion reactions **(5.3.1.1)**.

B. The 0.1% (w/v) sample solution complies with potassium ion reactions **(5.3.1.1)**.

PURITY TESTS

Insoluble matter. Dissolve 10 g of the sample in 100 mL of water using a beaker. Heat the covered beaker to ebullition, in water bath, for one hour. Filter the solution in a tared funnel with medium porosity (10 μ m to 15 μ m). Wash with hot water. Dry at 105 °C, cool in desiccator and weigh. No more than 0.01% (100 ppm).

Calcium and magnesium. Add hydrochloric acid to 20 mL of 10% (w/v) sample solution until acidic reaction to litmus paper, add 5 mL of ammonium oxalate RS, 2 mL of sodium phosphate dibasic heptahydrate RS and 10 mL of ammonium hydroxide. Allow to stand, in a cool place, for 24 hours. If there is formation of precipitate, filter and wash with 2% (v/v) ammonium hydroxide. Dry and calcinate until constant weight. The residue mass is no more than 0.4 mg. No more than 0.02%.

Cyanide. Add to 15 mL of 10% (w/v) sample solution 0.5 mL of ferrous sulfate RS and 0.5 mL of ferric chloride RS. Add hydrochloric acid RS until a strongly acidic reaction. A blue color is not developed.

Chlorides (5.3.2.1). Acidify 30 mL of the 10% (w/v) sample solution with nitric acid SR until an acidic reaction to litmus paper. Add 1 mL of silver nitrate RS, complete the volume to 50 mL with water and homogenize. If opalescence is produced, it must not be more intense than that produced by 0.35 mg of chloride ion treated in the same conditions. No more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Acidify 20 mL of the 10% (w/v) sample solution with hydrochloric acid SR until an acidic reaction to litmus paper. Add 1 mL of barium chloride RS, complete the volume to 50 mL with water and heat in water bath for 15 minutes. If opalescence is produced, it must not be more intense than that produced by 0.2 mg of sulfate ion treated in the same conditions. No more than 0.01% (100 ppm).

Heavy metals (5.3.2.3). Dissolve 4 g of the sample in 10 mL of water, add 15 mL of hydrochloric acid RS and heat until ebullition. Add one drop of phenolphthalein TS and neutralize with *M* sodium hydroxide until a slightly pink color. Cool and dilute with water to 25 mL. Proceed as described in *Method I*. No more than 0.0005% (5 ppm).

Iron (5.3.2.4). Dissolve 10 g of the sample in 25 mL of water and slowly add 14 mL of hydrochloric acid. When the effervescence stops, heat to ebullition for a couple of minutes. Cool and dilute to 50 mL with water. Proceed as described in *Method I* using 5 mL of the solution obtained. Use 0.1 mL of *Standard iron solution (100 ppm Fe)*. No more than 0.001% (10 ppm).

Arsenic (5.3.2.5). Use 10 mL of 10% (w/v) sample solution and add 5 mL of hydrochloric acid RS. Prepare the standard with *Standard arsenic solution (1 ppm As)* and proceed as described in *Method I*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 0.3 g of the sample. Dry in oven at 180 °C for four hours. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Transfer, quantitatively, about 1.5 g of the sample previously desiccated to an Erlenmeyer flask. Add 150 mL of water and four drops of methyl orange TS. Titrate with *M* hydrochloric acid VS. Each mL of *M* hydrochloric acid VS is equivalent to 69.105 mg of K₂CO₃.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Alkalinizing agent and diuretic.

SODIUM CARBONATE***Natrii carbonas***

Na_2CO_3 ; 105.99

sodium carbonate; 01752

Sodium salt of carbonic acid (2:1)

[497-19-8]

$\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$; 124.00

sodium carbonate monohydrate; 11399

Sodium salt of carbonic acid hydrate (2:1:1)

[5968-11-6]

Contains no less than 99.5% and no more than 100.5% of Na_2CO_3 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Colorless crystals or white powder. On humid air and in a cool place, absorbs water; at 100 °C, becomes anhydrous.

Solubility. Freely soluble in water and in water in ebullition. Insoluble in ethyl alcohol.

IDENTIFICATION

A. Complies with sodium ion reactions **(5.3.1.1)**.

B. Complies with carbonate ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. The 10% (w/v) aqueous preparation is clear **(5.2.25)** and colorless **(5.2.12)**.

Alkalinity. The aqueous sample solution is strongly alkaline to litmus paper.

Calcium and Magnesium. Determine in 20 mL of the preparation obtained in *Aspect of the preparation*. Add hydrochloric acid until acidic reaction to litmus paper. Add 5 mL of 0.25 M ammonium oxalate, 2 mL of 0.3 M sodium phosphate dibasic heptahydrate and 10 mL of ammonia. Allow to stand, in a cool place, for 24 hours. If there is precipitation, filter in an adequate paper filter, wash with 2% (w/v) ammonia solution, dry and calcinate to constant weight. The residue must weigh no more than 0.4 mg (0.02%).

Cyanide. Determine in 15 mL of the preparation obtained in *Aspect of the preparation*. Add 0.5 mL of 0.5 M ferrous sulfate and 0.5 mL of 0.3 M ferric chloride. Add 3 M hydrochloric acid until a strongly acidic reaction. The liquid must not acquire a blue color.

Arsenic (5.3.2.5). Use *Method I*. Determine in 10 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.001% (10 ppm).

Chlorides (5.3.2.1). Determine in 10 mL of the preparation obtained in *Aspect of the preparation*. Add *M* nitric acid until acidic reaction to litmus paper. Add 1 mL of 0.25 *M* silver nitrate, complete the volume to 50 mL with water and homogenize. If opalescence is produced, it must not be more intense than that produced by 0.1 mg of chloride ion in equal volume of liquid, using the same reagents. No more than 0.01% (100 ppm).

Iron (5.3.2.4). Use *Method I*. Determine in 20 mL of the preparation obtained in *Aspect of the preparation*. Add acetic acid until acidic reaction to litmus paper. If a pink or red color is produced, it must not be more intense than that obtained with 0.02 mg of ferric ion in equal volume of liquid, using the same reagents. No more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Determine in 10 mL of the preparation obtained in *Aspect of the preparation*. Add acetic acid until acidic reaction to litmus paper. No more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Determine in 20 mL of the preparation obtained in *Aspect of the preparation*. Add 3 *M* hydrochloric acid until acidic reaction to litmus paper. Add 1 mL of 0.5 *M* barium chloride, complete the volume with water to 50 mL and homogenize. Heat in water bath for 10 minutes. If opalescence is produced, it must not be more intense than that produced by 0.8 mg of sulfate ion in equal volume of liquid, using the same reagents. No more than 0.04% (400 ppm).

Loss on drying (5.2.9.1). Determine in oven at 105 °C for four hours. No more than 0.5% for the anhydrous substance and between 12% and 15% for the hydrate substance.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 1 g of the sample in 25 mL of water. Titrate with *M* hydrochloric acid VS using 0.2 mL of methyl orange TS. Each mL of *M* hydrochloric acid VS is equivalent to 52.99 mg of Na₂CO₃ or to 62.0 mg of Na₂CO₃·H₂O.

PACKAGING AND STORAGE

In hermetic containers.

LABELING

Comply with current legislation.

CATEGORY

Pharmacotechnical adjuvant (alkalinizing agent).

CARBOPLATIN INJECTION SOLUTION

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_6H_{12}N_2O_4Pt$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support and a mixture of acetone and water (80:20) as mobile phase. Apply, separately, to the plate 10 μ L of each solution described next.

Sample solution: injection solution diluted in water, if necessary, to obtain a 10 mg/mL carboplatin solution.

Standard solution: 10 mg/mL carboplatin CRS solution in water.

Note: use the *Sample solution* and the *Standard solution* within two hours.

Develop the chromatogram. Remove the plate and allow to air dry for two hours. Nebulize with a mixture of 5.6 g of tin(II) chloride in 10 mL of hydrochloric acid (the dissolution may not be complete; if necessary, filter) and 90 mL of water with 1 g of potassium iodide, prepared immediately before use. Heat the plate at 100 °C for 10 minutes. The stain obtained in the chromatogram with the *Sample solution* corresponds in size, color and position to that obtained with the *Standard solution*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak obtained with the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.0 to 7.0.

PURITY TESTS

Limit of cyclobutane-1,1-dicarboxylic acid. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (10 μ m), maintained at room temperature and *Mobile phase* flow of 2.0 mL/minute.

Solution (1): dissolve 8.5 g of tetrabutylammonium sulfate in 80 mL of water, add 3.4 mL of phosphoric acid and adjust pH to 7.55 with sodium hydroxide.

Mobile phase: mixture of water, acetonitrile and *Solution (1)* (88:10:2). Degas and filter.

Sample solution: dilute the injection solution in water to obtain a 1 mg/mL carboplatin solution. Use this solution within two hours.

Standard solution: 0.01 mg/mL cyclobutane-1,1- dicarboxylic acid solution in water.

Resolution solution: mixture of *Sample solution* and *Standard solution* (1:1).

The resolution between the carboplatin and the cyclobutane-1,1-dicarboxylic acid peaks is no less than 2.5. The relative standard deviation of the areas under the cyclobutane-1,1-dicarboxylic acid peak between the replicates is no more than 2.0%.

Procedure: inject, separately, 100 µL of the *Sample solution*, the *Standard solution* and the *Resolution solution*. Record the chromatograms for no less than two and a half times the retention time of the peak corresponding to carboplatin. The area under the peak corresponding to the cyclobutane-1,1-dicarboxylic acid peak, obtained in the *Sample solution* chromatogram, is not greater than the area under the peak obtained in the *Standard solution* chromatogram (1.0%).

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.54 EU/mg of carboplatin.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 230 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to aminopropylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: prepare a mixture of acetonitrile and water (87:13). Degas and filter.

Sample solution: injection solution diluted in water to obtain a 1 mg/mL carboplatin solution.

Standard solution: 1 mg/mL carboplatin CRS solution in water.

Note: use the *Sample solution* and the *Standard solution* within two hours.

The retention factor is no less than 4.0 for the main peak, the number of theoretical plates is no less than 5000 and the tailing factor is no more than 2.0. The relative standard deviation of the areas under the carboplatin peak between the replicates is no more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₆H₁₂N₂O₄Pt in the injection solution from the responses obtained for the *Standard* and *sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and free from contact with metals.

LABELING

Comply with current legislation.

CARRAGEENAN

carrageenan; 01798

Carrageenan

[9000-07-1]

Carrageenan is the hydrophile colloid obtained from the extraction with water or with alkaline aqueous solution of some members of the *Rhodophyceae* class (red algae), used as gelling, emulsifying, stabilizer, suspensor and viscosity increase agent. It is a mixture of sulfated polysaccharides, usually comprised of potassium, sodium, calcium, magnesium and ammonium sulfate esters, and galactose and 3,6-anhydrogalactose copolymers. The structural families are identified by the position of the sulfate group and the presence or absence of anhydrogalactose. Such hexoses are alternated in the α -1,3 and β -1,4 bonds in the polymer. The prevalent copolymers in the colloid are designated as *kappa*, *iota* and *lambda* type carrageenan. The *kappa* family consists of *kappa* and *iota*. *Kappa*-carrageenan is usually D-galactose-4-sulfate and 3,6-anhydro-D-galactose alternated, and *iota*-carrageenan is similar, except that 3,6-anhydrogalactose is sulfated on carbon 2. There are different intermediate compositions between *kappa*-carrageenan and *iota*-carrageenan, depending on the degree of sulfation on carbon 2. Due to the helicoidal tertiary structure, which allows gelling, the *kappa* family is the most important commercially. On *lambda*-carrageenan, the monomeric units are usually D-galactose-2-sulfate (bond 1,3) and D-galactose-2,6-disulfate (bond 1,4). This carrageenan is not gelling. The content of sulfate ester in carrageenan is 18% to 40%.

DESCRIPTION

Physical characteristics. White or yellowish, almost odorless powder.

Solubility. Soluble in hot water (80 °C). Disperses more easily if moistened first in ethyl alcohol, glycerol and syrup.

Physicochemical constants.

Viscosity (5.2.7): no less than 5 cP at 75 °C. Transfer 7.5 g of the sample to a 600 mL beaker weighed in advance, add 450 mL of water and disperse under agitation for 15 minutes. Add water to 500 g of weight and heat in water bath, with continuous agitation, until the temperature of 80 °C is reached. Add water to adjust the loss by evaporation, cooling to a range of 76 °C to 77 °C and maintain in bath, at constant temperature of 75 °C. Use an adequate rotational viscometer and adapt a rotating body with 1.88 cm of diameter and 6.51 cm of height, with depth immersion of 8.10 cm. Let the rotating body spin in the sample at 30 rpm for six rotations and carry out the reading on the scale. Convert the reading to centipoises, multiplying by the constant of the rotating body and the speed employed.

IDENTIFICATION

A. Prepare an uniform dispersion at 2% (w/v) of the sample in water and heat in water bath at 80 °C (*Preparation A*). After cooling, the dispersion becomes more viscous and may form a gel.

B. Add to 10 mL of *Preparation A*, obtained in test **A.** of *Identification*, still hot, four drops of 10% (w/v) potassium chloride, homogenize and cool. A frail texture of gel indicates predominance of

kappa carrageenan; an elastic gel indicates predominance of *iota* carrageenan. If the solution does not form gel, the predominant carrageenan is of the *lambda* type.

C. Dilute one portion of *Preparation A*, obtained in test **A.** of *Identification*, in four parts of water and add two to three drops of 0.05% (w/v) methylthionine chloride in ethyl alcohol. A fibrous blue precipitate is formed.

D. Obtain the infrared absorption spectrum (**5.2.14**) of the gelling and non-gelling fractions by the procedure described next. Disperse 2 g of the sample in 200 mL of 2.5% (w/v) potassium chloride and shake for one hour. Allow to stand for 18 hours, shake again for one hour and transfer to a centrifuge tube. If it is not possible to transfer because the dispersion is very viscous, dilute with 200 mL of 2.5% (w/v) potassium chloride. Centrifuge at approximately 1000 *g* for 15 minutes. Remove the clear supernatant liquid, resuspending the residue in 200 mL of 2.5% (w/v) potassium chloride and centrifuge again. Coagulate the combined supernatants, by adding two volumes of 90% (v/v) ethyl alcohol. Retrieve the clump and the sediment. Wash the clump with 250 mL of 90% (v/v) ethyl alcohol. Remove excess liquid from the clump by pressure and dry at 60 °C for two hours. The material thus obtained is the non-gelling fraction, *lambda* carrageenan. Disperse the sediment in 250 mL of cold water, heat at 90 °C for 10 minutes and cool down to 60 °C. Coagulate the mixture, with two volumes of 90% (v/v) ethyl alcohol. Retrieve, wash and dry the clump as described previously. The material thus obtained is the gelling fraction, *kappa* and *iota* carrageenan. Prepare, for each fraction, films with 5 mm of thickness (when dry) on an even non-adhering surface and obtain the infrared absorption spectra from each film. Carrageenan presents a wide range of absorption, typical of polysaccharides, within 1000 cm⁻¹ and 1100 cm⁻¹. The maximum absorption points are 1065 cm⁻¹ and 1020 cm⁻¹ for the gelling and the non-gelling fractions, respectively. Other characteristic absorption bands and their intensities related to the absorbance at 1050 cm⁻¹ are displayed on the following table.

Wavelength (cm ⁻¹)	Molecular fraction	Absorbances related to 1050 cm ⁻¹		
		<i>Kappa</i>	<i>Iota</i>	<i>Lambda</i>
1220 to 1260	Sulfate ester	0.7 to 1.2	1.2 to 1.6	1.4 to 2.0
928 to 933	3,6-Anhydrogalactose	0.3 to 0.6	0.2 to 0.4	0 to 0.2
840 to 850	Galactose-4-sulfate	0.3 to 0.5	0.2 to 0.4	---
825 to 830	Galactose-2-sulfate	---	---	0.2 to 0.4
810 to 820	Galactose-6-sulfate	---	---	0.1 to 0.3
800 to 805	3,6-Anhydrogalactose-2-sulfate	0 to 0.2	0.2 to 0.4	---

PURITY TESTS

Insoluble acidic material.

Transfer 2 g of the sample, accurately weighed, to a 250 mL beaker with 150 mL of water and 1.5 mL of sulfuric acid. Cover with a watch glass and heat in vapor bath for six hours. Frequently rub the beaker walls with a glass rod with rubber at the top, replenishing water lost by evaporation. Add 500 mg, accurately weighed, of an auxiliary filtration agent. Filter the preparation in a funnel with filtering plate with a 2.4 cm fiberglass layer, desiccated and weighed in advance. Wash the residue several times with hot water. Dry at 105 °C for three hours, cool in desiccator and weigh. The difference between the final weight and the sum of weights of the funnel, the fiberglass and the auxiliary filtration agent is the weight of the insoluble acidic material. No more than 2%.

Arsenic (5.3.2.5). No more than 0.0003% (3 ppm).

Lead (5.3.2.12). No more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.004% (40 ppm).

Loss on drying (5.2.9.1). Dry under pressure of no more than 10 mmHg at 70 °C, for 18 hours. No more than 12.5%.

Total ash (5.4.1.5.1). No more than 35%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

In hermetic containers, preferably in a cool place.

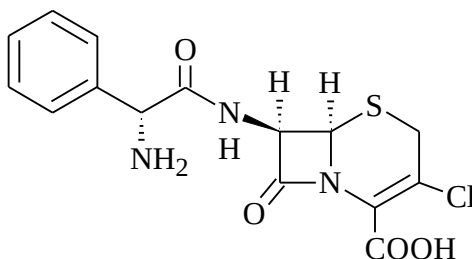
LABELING

Comply with current legislation.

CATEGORY

Excipient.

CEFACLOR
Cefaclorum



$C_{15}H_{14}ClN_3O_4S$; 367.80

cefaclor; 01824

(6*R*,7*R*)-7-[[[(2*R*)-2-amino-2-phenylacetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2- carboxylic acid
[53994-73-3]

$C_{15}H_{14}ClN_3O_4S \cdot H_2O$; 385.82

cefaclor monohydrate; 09368

(6*R*,7*R*)-7-[[[(2*R*)-2-amino-2-phenylacetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2- carboxylic acid hydrate (1:1)
[70356-03-5]

Contains no less than 950 µg and no more than 1020 µg of cefaclor ($C_{15}H_{14}ClN_3O_4S$) per milligram, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water, practically insoluble in methyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +101 to +111, in relation to the anhydrous substance. Determine in 1% (w/v) sample solution in 1% (w/v) hydrochloric acid.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cefaclor CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak obtained with the *Standard solution*.

PURITY TESTS

pH (5.2.19). 3.0 to 4.5. Determine in aqueous suspension at 2.5% (w/v).

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm or 10 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Diluent: dissolve 2.4 g of monobasic sodium phosphate in 1000 mL of water and adjust the pH to 2.5 with phosphoric acid.

Eluent A: dissolve 6.9 g of monobasic sodium phosphate in 1000 mL of water and adjust the pH to 4.0 with phosphoric acid.

Eluent B: mixture of *Eluent A* and acetonitrile (55:45).

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 30	95 → 75	5 → 25	linear gradient
30 – 45	75 → 0	25 → 100	linear gradient
45 – 55	0	100	isocratic
55 – 60	0 → 95	100 → 5	linear gradient
60 – 70	95	5	isocratic

Sample solution: transfer, quantitatively, about 50 mg of the sample to a 10 mL volumetric flask, add a volume of *Diluent* sufficient to dissolve and leave in ultrasonic bath, if necessary. Complete the volume with *Diluent*, homogenize and filter. Use this solution within three hours, if stored at room temperature, or within 20 hours, if stored under refrigeration.

Standard solution: dissolve an accurately weighed amount of cefaclor CRS in *Diluent*, to obtain a 50 µg/mL solution.

Resolution solution: dissolve an amount of delta-3-cefaclor CRS in *Standard solution* to obtain a 50 µg/mL solution.

Inject 20 µL replicates of the *Resolution solution*. The retention time for the cefaclor peak must be between 23 minutes and 29 minutes. The tailing factor for the cefaclor peak is no more than 1.2. The resolution between delta-3-cefaclor and cefaclor is no less than 2.0. Inject the *Diluent*. Disregard any peak related to the *Diluent*.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of each related substance according to the formula:

$$0,01 \times C \times P (r_i/r_p)$$

where

C = concentration, in mg/mL, of cefaclor in the *Standard solution*;

P = potency, in µg/mg, of cefaclor CRS;

r_i = area under the peak of each related substance in the chromatogram obtained with the *Sample solution*;

r_p = area under the peak related to cefaclor in the chromatogram obtained with the *Standard solution*.

No more than 1.0% for any related substance and no more than 3.0% for the sum of all related substances. Disregard any peak with result below 0.1%.

Water (5.2.20.1). 3.0% to 6.5%.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 265 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m); *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. Adjust pH to 2.5 ± 0.1 with phosphoric acid. Add 220 mL of methyl alcohol and shake.

Sample solution: transfer, quantitatively, about 15 mg of the sample to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Use this solution within eight hours, if stored at room temperature, or within 20 hours, if stored under refrigeration.

Standard solution: transfer, quantitatively, about 15 mg of cefaclor CRS to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Use this solution within 8 hours, if stored at room temperature, or within 20 hours, if stored under refrigeration.

Resolution solution: prepare a solution, in *Mobile phase*, with about 0.3 mg/mL of cefaclor CRS and 0.3 mg/mL of delta-3-cefaclor CRS.

Inject 20 μ L replicates of the *Resolution solution*. The tailing factor for the cefaclor peak is no more than 1.5. The resolution between cefaclor and delta-3-cefaclor is no less than 2.5. The relative standard deviation of the areas under the cefaclor peak between the replicates is no more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content in μ g of cefaclor ($C_{15}H_{14}ClN_3O_4S$) per milligram of the sample, from the content of the standard and the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

CEFACLOX CAPSULES

Contains cefaclor monohydrate equivalent to no less than 90.0% and no more than 120.0% of the declared amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$).

IDENTIFICATION

The retention time of the main peak from the chromatogram obtained with the *Sample solution*, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in water until adequate concentration. Measure the absorbances of the solutions at 264 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_{15}H_{14}ClN_3O_4S$ dissolved in the medium, comparing the readings obtained with that of the cefaclor CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{15}H_{14}ClN_3O_4S$ dissolve in 30 minutes.

PURITY TESTS

Related substances. Proceed as described in *Related substances* in the *Cefaclor* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh 20 capsules, remove the content and weigh again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 50 mg of cefaclor to a 10 mL volumetric flask. Complete the volume with *Diluent*, homogenize and filter. Use this solution within three hours, if stored at room temperature, or within 20 hours, if stored under refrigeration.

Inject 20 μ L replicates of the *Resolution solution*. The retention time for the cefaclor peak must be between 23 minutes and 29 minutes. The tailing factor for the cefaclor peak is no more than 1.2. The resolution between the peaks of delta-3-cefaclor and cefaclor is no less than 2.0. Inject the *Diluent*. Disregard any peak related to the *Diluent*.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of each related substance using the following formula:

$$0,01 \times C \times P (r_i/r_p)$$

where

C = concentration, in mg/mL, of cefaclor in the *Standard solution*;

P = potency, in µg/mg, of cefaclor CRS;

r_i = area under the peak of each related substance in the chromatogram obtained with the *Test solution*;

r_p = area under the peak related to cefaclor in the chromatogram obtained with the *Standard solution*.

No more than 1.0% for any related substance and no more than 3.0% for the sum of all related substances. Disregard any peak with result below 0.1%.

Water (5.2.20.1). No more than 8.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 265 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine and adjust pH to 2.5 ± 0.1 with phosphoric acid. Add 220 mL of methyl alcohol and mix.

Sample solution: weigh 20 capsules, remove the content and weigh again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 30 mg of cefaclor to a 100 mL volumetric flask, add 70 mL of *Mobile phase* and leave in ultrasonic bath until dissolution. Complete the volume with the same solvent, homogenize and filter.

Standard solution: dissolve an accurately weighed amount of cefaclor CRS in *Mobile phase* to obtain a final concentration of 0.3 mg/mL.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of cefaclor (C₁₅H₁₄ClN₃O₄S) in the capsules, from the responses obtained with the *Standard solution* and with the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

CEFACLOR ORAL SUSPENSION

Contains no less than 80% and no more than 120% the declared amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$). The oral suspension may have coloring agents, suspending agents, flavoring, buffering, sweetening and preservative agents in aqueous vehicle.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 190 nm to 310 nm, of a 30 µg/mL sample solution, diluted in water and filtered, there is maximum absorption at 264 nm.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Diluent: dissolve 2.4 g of monobasic sodium phosphate in 1000 mL of water and adjust the pH to 2.5 with phosphoric acid.

Eluent A: dissolve 6.9 g of monobasic sodium phosphate in 1000 mL of water and adjust the pH to 4.0 with phosphoric acid.

Eluent B: prepare mixture of *Eluent A* and acetonitrile (55:45).

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 30	95 → 75	5 → 25	linear gradient
30 – 45	75 → 0	25 → 100	linear gradient
45 – 55	0	100	isocratic
55 – 60	0 → 95	100 → 5	linear gradient
60 – 70	95	5	isocratic

Sample solution: dilute an amount of sample in the *Diluent* to obtain a solution with concentration of 5 mg/mL. Shake and leave in ultrasonic bath, if necessary. Filter.

Standard solution: dissolve an accurately weighed amount of cefaclor CRS in *Diluent* to obtain a solution with concentration of 0.05 mg/mL.

Resolution solution: dissolve an accurately weighed amount of delta-3-cefaclor CRS in the *Standard solution* to obtain a solution with concentration of 0.05 mg/mL.

Inject 20 µL of the *Resolution solution*. The resolution between the peaks related to delta-3-cefaclor and cefaclor is no less than 2.0.

Procedure: inject, separately, 20 µL of *Standard solution* and *Sample solution*, record the chromatograms for no less than twice the retention time of the main peak and measure the areas under the peaks.

The maximum percentage tolerated is 1.0% for each individual impurity and no more than 3.0% for the sum of all impurities present. Disregard peaks related to the solvent or with a result below 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 265 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water with 10 mL of triethylamine and adjust pH to 2.5 ± 0.1 with phosphoric acid. Add 220 mL of methyl alcohol and mix.

Sample solution: weigh an amount of suspension, equivalent to 75 mg of cefaclor, and transfer to a 250 mL volumetric flask. Shake for 30 minutes, complete the volume with *Mobile phase* and homogenize, obtaining a 0.3 mg/mL solution. Filter in quantitative filter.

Standard solution: dissolve an accurately weighed amount of cefaclor CRS in *Mobile phase* to obtain a solution with final concentration of 0.3 mg/mL.

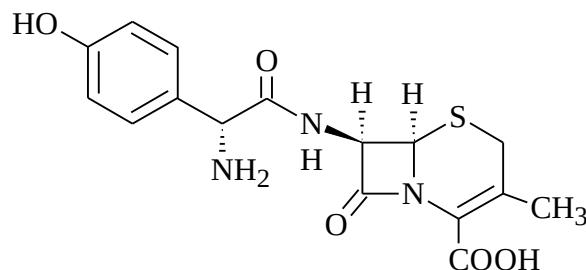
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$) in the sample, from the responses obtained with the *Standard solution* and with the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, at room temperature and protected from light.

LABELING

Comply with current legislation.

CEFADROXIL*Cefadroxilum* $C_{16}H_{17}N_3O_5S$; 363.39

cefadroxil; 01825

(6*R*,7*R*)-7-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
[50370-12-2]

 $C_{16}H_{17}N_3O_5S \cdot H_2O$; 381.40

cefadroxil monohydrate; 11384

(6*R*,7*R*)-7-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hydrate (1:1)
[66592-87-8]

The potency is no less than 950 µg and no more than 1050 µg of cefadroxil ($C_{16}H_{17}N_3O_5S$) per milligram, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or nearly white powder.

Solubility. Slightly soluble in water, very slightly soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +165 to +178, in relation to the anhydrous substance. Determine in solution at 1% (w/v) in water.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. or if tests C. and D. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cefadroxil CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 235 nm to 340 nm, of the 0.002% (w/v) solution in citro-phosphate buffer pH 6.0, there is maximum absorption at 264 nm, identical to that observed in the spectrum of cefadroxil CRS, prepared identically.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, in *Related substances*. The main stain obtained with *Solution (4)* corresponds in position, color and intensity to the one obtained with *Solution (5)*. The test will only be valid if the chromatogram obtained with *Solution (4)* presents three clearly separated stains.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **A.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

pH (5.2.19). 4.0 to 6.0. Determine in aqueous suspension at 5% (w/v).

Light absorption. The absorption of the 0.02% (w/v) solution in citro-phosphate buffer pH 6.0, measured at 330 nm, is no more than 0.05 **(5.2.14)**.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of ethyl acetate, ethyl alcohol, water and formic acid (14:5:5:1) as mobile phase. Apply to the plate, separately, 2 µL of *Solutions (1)*, *(2)* and *(3)* and 4 µL of *Solution (4)* freshly prepared, described next.

Diluent: mixture of ethyl alcohol, water and 2.4 M hydrochloric acid (75:22:3).

Solution (1): dissolve an accurately weighed amount of the sample in *Diluent* to obtain a 25 mg/mL solution.

Solution (2): transfer 1 mL of *Solution (1)* to a 100 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Solution (3): dissolve an amount, accurately weighed, of 7-aminodesacetoxycephalosporanic acid and D-α-4-hydroxyphenylglycine in *Diluent* to obtain a 0.25 mg/mL solution of each substance.

Solution (4): mix 1 mL of *Solution (1)* with 1 mL of *Solution (3)*.

Solution (5): dissolve an accurately weighed amount of cefadroxil CRS in *Diluent* to obtain a 12.5 mg/mL solution.

Develop the chromatogram. Remove the plate, allow to air dry and nebulize with a 3% (w/v) ninhydrin solution in 4.55% (w/v) sodium metabisulfite. Allow the plate to air dry. The secondary stains obtained in the chromatogram with *Solution (1)*, corresponding to 7-aminodesacetoxycephalosporanic acid or to D-α-4- hydroxyphenylglycine, if present, are not more intense than the stains obtained in the chromatogram of *Solution (3)* (1%). Any stain obtained in the chromatogram with *Solution (1)*, except the main stain and the stains corresponding to 7-aminodesacetoxycephalosporanic acid or to D-α-4- hydroxyphenylglycine, is not more intense than the stain obtained in the chromatogram of *Solution (2)* (1%). The test will only be valid if the chromatogram obtained with *Solution (4)* presents three clearly separated stains.

Limit of N,N-dimethylaniline (5.3.2.13). No more than 20 ppm.

Water (5.2.20.1). Determine in 0.2 g of the sample. Between 4.0% and 6.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.5%.

ASSAY

Use one of the methods described below.

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 230 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Phosphate buffer pH 5.0: 0.05 M monobasic potassium phosphate, pH 5.0, adjusted with 2 M sodium hydroxide.

Mobile phase: mixture of *Phosphate buffer pH 5.0* and acetonitrile (96:4).

Sample solution: transfer 0.2 g of the sample, accurately weighed, to a 200 mL volumetric flask, using 120 mL of *Phosphate buffer pH 5.0*, and shake mechanically for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Use the solution on the same day.

Standard solution: transfer the equivalent to 25 mg of cefadroxil CRS to a 25 mL volumetric flask, add 15 mL of *Phosphate buffer pH 5.0* and shake mechanically for 15 minutes. Complete the volume with the same solvent and homogenize. Use the solution on the same day.

The number of theoretical plates must be no less than 1800. The tailing factor is no more than 2.2. The retention factor must be from 2 to 3.5. The relative standard deviation of the areas under the cefadroxil peak between the replicates must be no more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the sample potency, in µg of cefadroxil (C₁₆H₁₇N₃O₅S) in the sample from the potency of standard and responses obtained with the *Standard* and *Sample solutions*.

B. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: *Staphylococcus aureus* ATCC 6538 P.

Culture media: sterile saline solution for inoculum standardization, culture medium number 2 for base layer and culture medium number 1 for inoculum preparation.

Sample solutions: weigh, accurately, the equivalent to 50 mg of the sample and transfer quantitatively to a 100 mL volumetric flask using 60 mL of *Solution 1 (Potassium phosphate buffer 1%, sterile, pH 6.0)*. Leave in an ultrasonic bath for 15 minutes. Shake mechanically for 20 minutes and complete the volume with the same solvent. Homogenize and filter. Dilute, successively, to obtain concentrations of 40 µg/mL, 20 µg/mL and 10 µg/mL, using *Solution 1 (Potassium phosphate buffer 1%, sterile, pH 6.0)* as solvent.

Sample solutions: weigh, accurately, the equivalent to 25 mg of cefadroxil CRS and transfer quantitatively to a 50 mL volumetric flask using 30 mL of *Solution 1 (Potassium phosphate buffer*

1%, sterile, pH 6.0). Leave in an ultrasonic bath for 15 minutes. Shake mechanically for 20 minutes, complete the volume with the same solvent and homogenize. Dilute, successively, to obtain concentrations of 40 µg/mL, 20 µg/mL and 10 µg/mL, using *Solution 1 (Potassium phosphate buffer 1%, sterile, pH 6.0)* as solvent.

Procedure: add 20 mL of culture medium number 2 to each plate, wait until it solidifies, add 5 mL of 0.5% inoculum and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Calculate the potency of the sample, in µg of cefadroxil per milligram, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at temperature below 30 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

CEFADROXIL CAPSULES

Contains no less than 90.0% and no more than 120.0% the declared amount of cefadroxil ($C_{16}H_{17}N_3O_5S$).

IDENTIFICATION

A. Weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 20 mg of cefadroxil to a 100 mL volumetric flask using 60 mL of citro-phosphate buffer pH 6.0. Shake for 10 minutes and complete the volume with buffer. Homogenize and filter. Proceed as described in test **B.** of *Identification* from the *Cefadroxil* monograph.

B. Proceed as described in test **C.** of *Identification* from the *Cefadroxil* monograph. Prepare *Solution (1)* as described next.

Solution (1): weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Use an amount of powder equivalent to 20 mg of cefadroxil, dissolve in 5 mL of a mixture of methyl alcohol and citro-phosphate buffer pH 7.0 (1:1), homogenize and filter.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **A.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described on method **A.** from *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium and dilute in water until adequate concentration. Measure the absorbances at 263 nm (**5.2.14**) using the same solvent for zero adjustment. Calculate the amount of $C_{16}H_{17}N_3O_5S$ dissolved in the medium, comparing the readings obtained with that of the cefadroxil CRS solution at the concentration of 0.002% (w/v), prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{16}H_{17}N_3O_5S$ dissolve in 30 minutes.

PURITY TESTS

Water (5.2.20.1). No more than 7.0%

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in method **A.** of Assay in the *Cefadroxil* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 0.2 g of cefadroxil to a 200 mL volumetric flask, add 120 mL of *Phosphate buffer pH 5.0* and shake mechanically for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Use the solution on the same day.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of cefadroxil ($C_{16}H_{17}N_3O_5S$) in the capsules from the responses obtained with the *Standard solution* and with the *Sample solution*.

B. Proceed as described in method **B.** of Assay in the *Cefadroxil* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 0.125 g of cefadroxil to a 250 mL volumetric flask, using 150 mL of *Solution 1 (Potassium phosphate buffer 1%, sterile, pH 6.0)*. Leave in an ultrasonic bath for 15 minutes. Shake mechanically for 20 minutes and complete the volume with the same solvent. Homogenize and filter. Dilute, successively, to obtain concentrations of 40 µg/mL, 20 µg/mL and 10 µg/mL, and using the same solvent.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

CEFADROXIL TABLETS

Contains no less than 90.0% and no more than 120.0% the declared amount of cefadroxil ($C_{16}H_{17}N_3O_5S$). The tablets can be coated.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 20 mg of cefadroxil to a 100 mL volumetric flask, using 60 mL of citro-phosphate buffer pH 6.0. Shake for 10 minutes and complete the volume with buffer. Homogenize and filter. Proceed as described in test **B.** of *Identification* from the *Cefadroxil* monograph.

B. Proceed as described in test **C.** of *Identification* from the *Cefadroxil* monograph. Prepare *Solution (1)* as described next.

Solution (1): weigh and pulverize the tablets. Use an amount of powder equivalent to 20 mg of cefadroxil, dissolve in 5 mL of a mixture of methyl alcohol and citro-phosphate buffer pH 7.0 (1:1) and filter.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 500 mL volumetric flask with 300 mL of *Phosphate buffer pH 5.0* (described in method **A.** of *Assay* in the *Cefadroxil* monograph) and leave in ultrasonic bath for five minutes for the tablet disintegration. Shake mechanically for 15 minutes and complete the volume with the same solvent. Homogenize and filter. Transfer 10 mL of this solution to a 20 mL volumetric flask, complete the volume with *Phosphate buffer pH 5.0* and homogenize. Proceed as described in method **A.** from *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Apparatus: paddles, 50 rpm

Time: 30 minutes

Procedure: after the test, take an aliquot of the dissolution medium and dilute in water, until adequate concentration. Measure the absorbances at 263 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the amount of $C_{16}H_{17}N_3O_5S$ dissolved in the medium, comparing the readings obtained with that of the cefadroxil CRS solution at the concentration of 0.002% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{16}H_{17}N_3O_5S$ dissolve in 30 minutes.

PURITY TESTS

Water (5.2.20.1). No more than 8.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in method **A.** of Assay on the *Cefadroxil* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 0.2 g of cefadroxil to a 200 mL volumetric flask, add 120 mL of *Buffer pH 5.0* and shake mechanically for 15 minutes. Complete the volume with the same solvent and filter. Use the solution on the same day.

Procedure: inject, separately, 10 µL of each of the *Standard* and *sample solutions*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{16}H_{17}N_3O_5S$ in the tablets from the responses obtained for the *Standard* and *sample solutions*.

B. Proceed as described in method **B.** of Assay on the *Cefadroxil* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 0.125 g of cefadroxil to a 250 mL volumetric flask and add 150 mL of *Potassium phosphate buffer 1%, sterile, pH 6.0*. Leave in ultrasound for 15 minutes. Shake mechanically for 20 minutes and complete the volume with the same solvent. Homogenize and filter. Dilute to obtain concentrations of 10 µg/mL, 20 µg/mL and 40 µg/mL, using *Potassium phosphate buffer 1%, sterile, pH 6.0* as diluent.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

CEFADROXIL POWDER FOR ORAL SUSPENSION

Contains no less than 90.0% and no more than 120.0% of the declared amount of $C_{16}H_{17}N_3O_5S$. The powder for oral suspension is a mixture of cefadroxil monohydrate with one or more coloring, flavoring, buffering, sweetening and preservative agents.

IDENTIFICATION

A. Reconstitute the content of three flasks as indicated on the label and homogenize. Transfer an amount of oral suspension equivalent to 50 mg of cefadroxil to a 250 mL volumetric flask using 150 mL of citro-phosphate buffer pH 6.0. Shake for 10 minutes and complete the volume with the same solvent. Homogenize and filter. Proceed as described in test **B.** of *Identification* from the *Cefadroxil* monograph.

B. Proceed as described in test **C.** of *Identification* from the *Cefadroxil* monograph. Prepare *Solution (1)* as described next.

Solution (1): reconstitute the content of three flasks as indicated on the label and homogenize. Use an amount of oral suspension equivalent to 0.1 g of cefadroxil, dissolve in 25 mL of a mixture of methyl alcohol and citro-phosphate buffer pH 7.0 (1:1) and filter.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **A.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

pH (5.2.19). 4.5 to 6.0. Determine in the reconstituted suspension as indicated on the label.

Determination of weight (5.1.1). Complies with the test. Determine in powder before reconstituting.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described on method **A.** from *Assay*.

PURITY TESTS

Water (5.2.20.1). No more than 2.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in method **A.** of Assay in the *Cefadroxil* monograph. Prepare the *Sample solution* as described next.

Sample solution: reconstitute the content of three flasks as indicated on the label and homogenize. Transfer a volume of oral suspension equivalent to 0.25 g of cefadroxil to a 250 mL volumetric flask, add 150 mL of *Phosphate buffer pH 5.0* and shake mechanically for 15 minutes. Complete the volume with the same solvent and homogenize. Filter approximately 25 mL of this solution, in filter with porosity of 0.8 µm or smaller, and use the clear filtrate as sample solution. Use the solution on the same day.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of cefadroxil ($C_{16}H_{17}N_3O_5S$) in the oral suspension from the responses obtained with the *Standard solution* and with the *Sample solution*.

B. Proceed as described in method **B.** of Assay in the *Cefadroxil* monograph. Prepare the *Sample solution* as described next.

Sample solution: reconstitute the content of three flasks as indicated on the label and homogenize. Transfer a volume of oral suspension equivalent to 0.1 g of cefadroxil to a 200 mL volumetric flask, using 120 mL of *Solution 1 (Potassium phosphate buffer 1%, sterile, pH 6.0)*. Shake mechanically for 20 minutes and complete the volume with the same solvent. Homogenize and filter. Dilute, successively, to obtain concentrations of 40 µg/mL, 20 µg/mL and 10 µg/mL, using the same solvent.

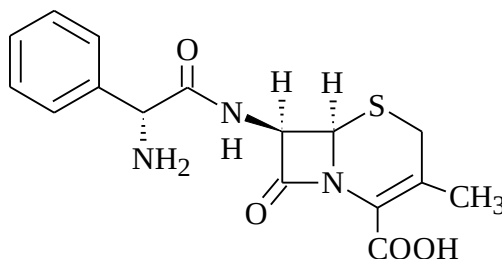
PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

CEFALEXIN
Cefalexinum



$C_{16}H_{17}N_3O_4S$; 347.39

cefalexin; 01826

(6*R*,7*R*)-7-[[[(2*R*)-2-amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2- carboxylic acid
[15686-71-2]

$C_{16}H_{17}N_3O_4S \cdot H_2O$; 365.40

cefalexin monohydrate; 01827

(6*R*,7*R*)-7-[[[(2*R*)-2-amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2- carboxylic acid hydrate (1:1)
[23325-78-2]

Contains no less than 950 µg and no more than 1030 µg of cefalexin ($C_{16}H_{17}N_3O_4S$) per milligram, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water, moderately soluble in methyl alcohol and practically insoluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +149 to +158, in relation to the anhydrous substance. Determine in 0.5% (w/v) solution, prepared in biphthalate buffer pH 4.4.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cefalexin CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of sample solution in water (1:50,000), there are maximum and minimum absorption points at the same wavelength as a cefalexin CRS solution, prepared identically. The absorptivity, calculated in relation to the anhydrous substance, at the wavelength of maximum absorption (about 262 nm), is no less

than 95% and no more than 104% of the one obtained with cefalexin CRS, prepared identically. Consider the declared potency of cefalexin CRS in the calculations.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

pH (5.2.19). 3.0 to 5.5. Determine in aqueous suspension with 50 mg/mL.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Eluent A: dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 1000 mL of water and 15 mL of triethylamine. Adjust pH to 2.5 ± 0.1 with phosphoric acid.

Eluent B: dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 300 mL of water and 15 mL of triethylamine. Adjust pH to 2.5 ± 0.1 with phosphoric acid. Add 350 mL of acetonitrile, 350 mL of methyl alcohol and homogenize.

Mobile phase: Adopt the gradient system described in the following table.

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0	100	0	equilibrium
0 – 1	100	0	isocratic
1 – 33.3	100 → 0	0 → 100	linear gradient
33.3 – 34.3	0	100	isocratic

Diluent: dissolve 18 g of monobasic sodium phosphate in 1000 mL of water.

Sample solution: transfer 25 mg of the sample, accurately weighed, to a 5 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Standard solutions: dissolve an amount of cefalexin CRS, accurately weighed, in the *Diluent* and dilute adequately to obtain solutions at 0.08 mg/mL and 0.16 mg/mL of cefalexin (C₁₆H₁₇N₃O₄S). Consider the declared potency of cefalexin CRS in the preparation of solutions.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under all peaks obtained. Build the analytical curve from the responses obtained for cefalexin with the *Standard solutions* versus its concentrations, calculated against the anhydrous substance, in mg/mL. Determine the concentration *C*, in mg/mL, of each related substance observed in the chromatogram obtained with *Sample solution*, considering the analytical curve obtained for cefalexin in the calculations. Calculate the percentage of each substance related to cefalexin by the formula:

$$\frac{500C}{A}$$

where *A* is the calculated amount of the anhydrous substance, in mg, of cefalexin taken to prepare the *Sample solution*. No more than 1.0% of any related substance. The sum of the amounts of all related substances is no more than 5.0%.

Water (5.2.20.1). The hydrated form has between 4.0% and 8.0%.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: prepare 1015 mL of a mixture of water, acetonitrile, methyl alcohol and triethylamine (850:100:50:15). Dissolve 1 g of sodium 1-pentanesulfonate in this mixture, adjust with phosphoric acid to pH 3.0 ± 0.1 and degas. Make adjustments if necessary.

Sample solution: transfer 0.1 g of the sample, accurately weighed, to a 100 mL volumetric flask, dissolve, complete the volume with water and homogenize. Transfer 10 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: dissolve an accurately weighed amount of cefalexin CRS in water and dilute adequately to obtain a 1 mg/mL stock solution. Transfer 10 mL to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content in µg of cefalexin ($C_{16}H_{17}N_3O_4S$) per mg of sample from the following formula:

$$100 \times \left(\frac{C \times P}{M} \right) \times \left(\frac{R_a}{R_p} \right)$$

where *C* is the concentration, in mg/mL, of cefalexin CRS in the stock solution used to prepare the *Standard solution*; *P* is the state potency of cefalexin, in µg/mg, of cefalexin CRS; *M* is the amount, in mg, of cefalexin taken to prepare the *Sample solution*; *R_a* and *R_p* are the areas under the peaks of the *Sample solution* and the *Standard solution*, respectively.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

CEFALEXIN TABLETS

Contains no less than 90.0% and no more than 120.0% of the declared amount of cefalexin ($C_{16}H_{17}N_3O_4S$). Cefalexin tablets are compounds of cefalexin anhydrous, monohydrate, or cefalexin hydrochloride with one or more diluent and lubricant agents that are adequate. The tablets can be coated.

IDENTIFICATION

The identification test A. can be omitted if tests B., C. and D. are carried out.

A. Remove any coating present on the tablets. Grind the tablets to a fine powder and dissolve an amount of powder equivalent to 0.5 g of cefalexin in 1 mL of water and 1.4 mL of *M* hydrochloric acid, add 0.1 g of activated charcoal, shake, filter and wash the filter with 1 mL of water. Slowly add to the filtrate a saturated sodium acetate solution until a precipitation occurs. Add 5 mL of methyl alcohol. Dry at a pressure not exceeding 7 kPa. In the infrared absorption spectrum (**5.2.14**) of the residue obtained, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cefalexin CRS, prepared identically.

B. Mix 20 mg of the residue obtained in test **A.** of *Identification* with 0.25 mL of a 1% (v/v) nitric acid solution in 80% (v/v) sulfuric acid. A yellow color develops.

C. Mix 20 mg of the residue obtained in test **A.** of *Identification* with 0.25 mL of a 1% (v/v) glacial acetic acid solution and add 0.1 mL of a 1% (w/v) cupric sulfate pentahydrate solution and 0.1 mL of 2 *M* sodium hydroxide. An olive green color develops.

D. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using non-bonded silica gel as support, and a mixture of 0.1 *M* citric acid, 0.1 *M* sodium phosphate dibasic and 6.7% (w/v) ninhydrin in acetone (60:40:1.5) as mobile phase. Before the test, put the plate in a tank with a mixture of *n*-hexane and tetradecane (95:5) with 1 cm of height, let the solvent run through the entire plate and let it evaporate. Apply, separately, to the plate 10 μ L of each solution described next.

Sample solution: weigh and pulverize the tablets. Dissolve an amount of powder in water to obtain an approximate concentration of 3 mg/mL of cefalexin.

Standard solution: prepare an aqueous solution with 3 mg/mL of cefalexin CRS.

Develop the chromatogram. Heat the plate at 110 °C for 10 minutes. The main stain obtained with the *Sample solution* corresponds in position, color and intensity to that obtained with the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). No more than 30 minutes.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described on method **A.** from *Assay*.

DISSOLUTION TEST (5.1.5)

Cefalexin

Dissolution medium: water, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute, if necessary, in water, until adequate concentration. Measure the absorbances at 262 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the amount of $C_{16}H_{17}N_3O_4S$ dissolved in the medium, comparing the readings obtained with that of the cefalexin CRS solution at the concentration of 0.002% (w/v), prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{16}H_{17}N_3O_4S$ dissolve in 30 minutes.

Cefalexin hydrochloride

Dissolution medium, Apparatus and Procedure: proceed as indicated for cefalexin.

Time: 45 minutes

Tolerance: no less than 75% (Q) of the declared quantity of $C_{16}H_{17}N_3O_4S$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as stationary phase. Before the test, put the plate in a tank with a mixture of *n*-hexane and tetradecane (95:5) with 1 cm of height, let the solvent run through the entire plate and let it evaporate. Develop the chromatography in the same direction as the impregnation. Prepare *Solution (1)* as described next.

Mobile phase: mixture of acetone, 7.2% (w/v) sodium phosphate dibasic dodecahydrate solution and 2.1% (w/v) citric acid solution (3:80:120).

Solution (1): weigh and pulverize the tablets. Dissolve an amount of powder equivalent to 0.25 g of cefalexin in 2 M hydrochloric acid and dilute to 10 mL with the same solvent. Homogenize and filter.

Solution (2): dilute the *Solution (1)* to 100 mL with 2 M hydrochloric acid.

Solution (3): solution with 0.025% (w/v) of 7-aminodesacetoxycephalosporanic acid in 2 M hydrochloric acid.

Solution (4): solution with 0.025% (w/v) of *D*- α -4- hydroxyphenylglycine in 2 M hydrochloric acid.

Solution (5): solution with 2.5% (w/v) of cefalexin, 0.025% (w/v) of 7-aminodesacetoxycephalosporanic acid and 0.025% (w/v) of *D*- α -4- hydroxyphenylglycine in 2 *M* hydrochloric acid.

Apply separately on the plate 5 μ L of each solution. Develop along 15 cm. Dry the plate at 90 °C for three minutes. Spray the hot plate with a 0.1% (w/v) ninhydrin solution in the *Mobile phase*. Heat the plate at 90 °C for 15 minutes and cool.

In the chromatogram obtained with *Solution (1)*, no stain corresponding to 7-aminodesacetoxycephalosporanic acid is more intense than the stain obtained with *Solution (3)*; no stain corresponding to *D*- α -4- hydroxyphenylglycine is more intense than the stain obtained with *Solution (4)*; no other secondary stain that appears between the main stain and the stains corresponding to 7-aminodesacetoxycephalosporanic acid and to *D*- α -4- hydroxyphenylglycine is more intense than the main stain in the chromatogram obtained with *Solution (2)*.

The test is not valid unless three clearly delimited stains appear in the chromatogram obtained with *Solution (5)*.

Water (5.2.20.1). No more than 9.0% for cefalexin tablets and 8% for cefalexin hydrochloride tablets.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter packed with silica chemically bonded to the octadecylsilane group (5 μ m); *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: use a mixture of water, acetonitrile, methyl alcohol and triethylamine (850:100:50:15). Dissolve 1 g of sodium 1-pentanesulfonate in this mixture and adjust pH to 3.0 ± 0.1 with phosphoric acid.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 0.25 mg of cefalexin to a 250 mL volumetric flask and add 100 mL of water. Shake mechanically for 30 minutes and complete the volume with the same solvent. Homogenize and filter. Transfer 25 mL of this solution to a 50 mL volumetric flask, complete the volume with water and homogenize.

Standard solution: dissolve, accurately, an amount of cefalexin CRS in water to obtain a concentration of 1 mg/mL of cefalexin (C₁₆H₁₇N₃O₄S). Transfer 25 mL of this solution to a 50 mL volumetric flask, complete the volume with water and homogenize.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of cefalexin ($C_{16}H_{17}N_3O_4S$) in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

B. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: *Staphylococcus aureus* ATCC 6538P.

Culture media: culture medium number 1 for microorganism maintenance; sterile saline solution for inoculum standardization; culture medium number 2 for base layer; and culture medium number 1 for inoculum preparation.

Sample solutions: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 250 mg of cefalexin to a 250 mL volumetric flask, using 200 mL of *Solution 1 (Potassium phosphate buffer 1%, sterile, pH 6.0)*. Shake for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Dilute, successively, to obtain concentrations of 2.5 µg/mL, 5 µg/mL and 10 µg/mL, using *Solution 1 (Potassium phosphate buffer 1%, sterile, pH 6.0)* as solvent.

Standard solutions: dissolve an amount, accurately weighed, of cefalexin CRS, in *Solution 1 (Potassium phosphate buffer at 1%, sterile, pH 6.0)* to obtain a 1 mg/mL solution. Dilute, successively, to obtain concentrations of 2.5 µg/mL, 5 µg/mL and 10 µg/mL, using *Solution 1 (Potassium phosphate buffer 1%, sterile, pH 6.0)* as solvent.

Procedure: add 20 mL of culture medium number 2 to each plate, wait until it solidifies, add 5 mL of 1% inoculum and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Calculate the potency of the sample, in µg of cefalexin per milligram, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from humidity and at temperature below 30 °C.

LABELING

Comply with current legislation.

CEFALEXIN POWDER FOR ORAL SUSPENSION

Contains no less than 90.0% and no more than 120.0% the declared amount of cefalexin ($C_{16}H_{17}N_3O_4S$). Cefalexin powder for oral suspension is a mixture of cefalexin with one or more buffering, coloring, flavoring, sweetening and preservative agents.

IDENTIFICATION

Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of 0.1 M citric acid, 0.1 M sodium phosphate dibasic and 6.7% (w/v) ninhydrin solution (60:40:1.5) as mobile phase. Beforehand, put the plate in a chromatographic chamber with a mixture of hexane and tetradecane (95:5) and allow this mixture to run along the entire extension of the plate. Remove the plate and allow it to air dry. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): reconstitute the powder as indicated on the label. Prepare a 3 mg/mL cefalexin solution in water.

Solution (2): dissolve an accurately weighed amount of cefalexin CRS in water to obtain a 3 mg/mL solution.

Develop the chromatogram. Remove the plate, dry at 110 °C for 10 minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test. Determine in powder before reconstituting.

pH (5.2.19). 3.0 to 6.0. Determine in the reconstituted suspension as indicated on the label.

PURITY TESTS

Determination of water (5.2.20.1). No more than 2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: Staphylococcus aureus ATCC 6538p.

Culture media: culture medium number 1 for microorganism maintenance; sterile saline solution for inoculum standardization; culture medium number 2 for base layer; and culture medium number 1 for inoculum preparation.

Sample solutions: reconstitute the suspension as indicated on the label. Transfer a volume of suspension equivalent to 200 mg of cefalexin to a 200 mL volumetric flask, complete the volume with *Solution 1 (Potassium phosphate buffer 1%, sterile, pH 6.0)*. Dilute to obtain concentrations of 4 µg/mL, 8 µg/mL and 16 µg/mL of cefalexin, using *Solution 1 (Potassium phosphate buffer at 1%, sterile, pH 6.0)* as diluent.

Standard solutions: dissolve an amount, accurately weighed, of cefalexin CRS, in *Solution 1 (Potassium phosphate buffer at 1%, sterile, pH 6.0)* to obtain a 1 mg/mL solution. Dilute to obtain concentrations of 4 µg/mL, 8 µg/mL and 16 µg/mL of cefalexin, using *Solution 1 (Potassium phosphate buffer at 1%, sterile, pH 6.0)* as diluent.

Procedure: add 20 mL of culture medium number 2 to a plate, wait until it solidifies, add 5 mL of 0.05% inoculum in culture medium number 1 and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Add to the cylinders 0.1 mL of the *Standard solution* and the *Sample solution* freshly prepared. Calculate the potency of the sample, in µg of cefalexin per milligram, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: dissolve 1 g of sodium 1-pentanesulfonate monohydrate in a mixture of water, acetonitrile, methyl alcohol and triethylamine (850:100:50:15). Adjust pH to 3.0 with phosphoric acid.

Sample solution: reconstitute the powder as indicated on the label. Transfer a volume of suspension equivalent to 200 mg of cefalexin to a 200 mL volumetric flask, complete the volume with water and homogenize. Transfer 10 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: dissolve an accurately weighed amount of cefalexin CRS in water to obtain a 1 mg/mL solution. Transfer 10 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

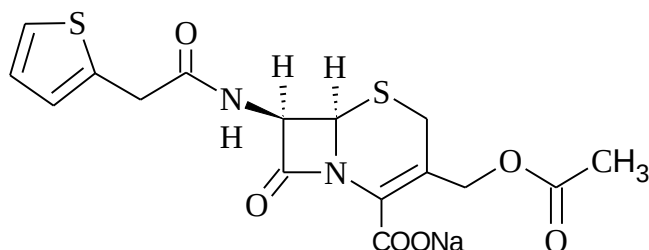
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of cefalexin (C₁₆H₁₇N₃O₄S) in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, at room temperature and protected from light.

LABELING

Comply with current legislation.

CEPHALOTHIN SODIUM*Cefalotinum natricum*C₁₆H₁₅N₂NaO₆S₂; 418.41

cephalothin sodium; 01836

Sodium salt of (6*R*,7*R*)-3-[(acetyloxy)-methyl]-8-oxo-7-[(2-thienylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

[58-71-9]

Contains no less than 850 µg of cephalothin (C₁₆H₁₆N₂O₆S₂) per milligram, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white, practically odorless crystalline powder.

Solubility. Freely soluble in water, in saline solution and in glucose solution. Insoluble in most organic solvents.

Physicochemical constants.

Specific optical rotation (5.2.8): +124 to +134, in relation to the desiccated substance. Determine in solution at 50 mg/mL in water.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cephalothin sodium CRS, prepared identically.

B. Complies with sodium ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.5 to 7.0. Determine in aqueous solution at 250 mg/mL.

Related substances. Proceed as described on the method from Assay.

Solution (1): transfer 1 mL of the *Standard solution*, described in Assay, to a 100 mL volumetric flask, dilute with *Mobile phase* and homogenize.

Solution (2): prepare as described on *Sample solution* in Assay.

Procedure: separately inject 20 µL of the *Solutions (1)* and *(2)*. For *Solution (2)*, record the chromatogram for no less than four times the retention time of the main peak. The area of any secondary peak obtained with *Solution (2)* is no more than the area under the main peak obtained with *Solution (1)* (1%). The sum of the areas under all the secondary peaks obtained with *Solution (2)* is no more than three times the area under the main peak obtained with *Solution (1)* (3%). Any peak obtained with *Solution (2)* with area lower than one tenth of the main peak in the chromatogram obtained with *Solution (1)* must be disregarded.

Loss on drying (5.2.9.1). Determine in 100 mg of the sample. Dry in oven at 60 °C for three hours, under pressure of no more than 5 mmHg, until constant weight. No more than 1.5%.

BIOLOGICAL SAFETY TEST

When indicated on the label that the substance is sterile, the sample complies with the Sterility and with the Bacterial endotoxin tests. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.13 EU/mg of cephalothin.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), at constant temperature of 40 °C; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: dissolve 17 g of sodium acetate in 790 mL of water and add 0.6 mL of glacial acetic acid. Adjust pH to 5.9 ± 0.1 with 0.1 M sodium hydroxide or glacial acetic acid. Add 150 mL of acetonitrile and 70 mL of ethyl alcohol and homogenize.

Sample solution: transfer 25 mg of the sample, accurately weighed, to a 25 mL volumetric flask, add 15 mL of *Mobile phase*, shake until dissolving, complete the volume with *Mobile phase* and homogenize.

Standard solution: dissolve an accurately weighed amount of cephalothin sodium CRS in *Mobile phase* and dilute adequately to obtain a solution at 1 mg/mL.

Resolution solution: heat in water bath for 10 minutes at temperature of 90 °C a portion of 5 mL of the *Standard solution*. Cool the solution and immediately inject in the chromatographic system.

Inject 20 µL replicates of the *Standard solution* and the *Resolution solution*. The resolution between the two main peaks obtained with the *Resolution solution* must be no less than 9.0. The tailing factor must be no less than 1.8 and the relative standard deviation of the area under the cephalothin peak between the replicates of *Standard solution* injections must be no more than 1.0%.

Procedure: inject 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of cephalothin (C₁₆H₁₆N₂O₆S₂) in µg per milligram in the sample from the content of the standard and the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

CEPHALOTHIN SODIUM POWDER FOR INJECTION SOLUTION

Contains no less than 90.0% and no more than 115.0% the declared amount of cephalothin sodium ($C_{16}H_{15}N_2NaO_6S_2$).

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B**, from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

pH (5.2.19). 6.0 to 8.5. Determine after reconstitution of the sample with the diluent.

Determination of weight (5.1.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Specific optical rotation (5.2.8): +124 to +134, in relation to the anhydrous substance and free from sodium bicarbonate. Determine in solution of cephalothin at 5% (w/v) in water.

Sodium bicarbonate. Dissolve, approximately, 1 g of powder for injection solution, accurately weighed, in 50 mL of water. Add 0.1% (w/v) methyl orange dissolved in water and titrate with 0.05 M sulfuric acid VS. Each mL of 0.05 M sulfuric acid VS is equivalent to 8.401 mg of $NaHCO_3$. Calculate the percentage of sodium bicarbonate and use the value obtained to calculate the *Specific optical rotation* in relation to the anhydrous base free from sodium bicarbonate.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Proceed as described in *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). No more than 0.13 EU/mg of cephalothin sodium.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh an amount of equivalent to 0.25 g of cephalothin sodium. Transfer to a 100 mL volumetric flask, complete the volume with water and homogenize. Dilute in the same solvent until concentration of 0.0025% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 285 nm, using water for zero adjustment. Calculate the amount of $C_{16}H_{15}N_2NaO_6S_2$ in the powder for injection solution, from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal

diameter, packed with silica chemically bonded to octadecylsilane group (5 μm), maintained at temperature of 40 °C; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: dissolve 17 g of sodium acetate in 790 mL of water, add 0.6 mL of glacial acetic acid and, if necessary, adjust pH to 5.9 ± 0.1 with glacial acetic acid or 0.1 M sodium hydroxide. Add 150 mL of acetonitrile, 70 mL of ethyl alcohol and homogenize.

Sample solution: reconstitute the content of a vial in ultrapure water, shake until complete dissolution, transfer the entire volume to a 200 mL volumetric flask, wash the vial with ultrapure water and transfer to the flask. Complete the volume and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize, obtaining a solution at 0.5 mg/mL of cephalothin sodium.

Standard solution: dissolve an accurately weighed amount of cephalothin sodium CRS in *Mobile phase* to obtain a final concentration of 0.5 mg/mL of cephalothin sodium.

Procedure: inject, separately, 10 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of cephalothin sodium ($\text{C}_{16}\text{H}_{15}\text{N}_2\text{NaO}_6\text{S}_2$) in the powder for injection solution, from the responses obtained with the *Standard solution* and the *Sample solution*.

C. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: *Staphylococcus aureus* ATCC 6538p.

Culture media: culture medium number 1 for microorganism maintenance and inoculum preparation; sterile saline solution, for inoculum standardization; and culture medium number 2, for base layer.

Sample solutions: reconstitute the injection solution as indicated on the label. Transfer a volume of the injection solution, accurately measured, to a volumetric flask, complete the volume with water and homogenize. Dilute in the same solvent until obtaining a 10 $\mu\text{g/mL}$ solution. Transfer aliquots of 6.4 mL, 10 mL and 15.6 mL of this solution to 100 mL flasks, complete the volume with *Solution 1* (*Potassium phosphate buffer at 1%, sterile, pH 6.0*) and homogenize. Solutions at 0.64 $\mu\text{g/mL}$, 1.0 $\mu\text{g/mL}$ and 1.56 $\mu\text{g/mL}$, respectively (A_1 , A_2 and A_3) are obtained.

Standard solutions: weigh 20 mg of cephalothin sodium CRS, transfer to a 20 mL volumetric flask and dissolve in water to obtain a 1 mg/mL solution. Dilute in the same solvent until obtaining a 10 $\mu\text{g/mL}$ solution. Transfer aliquots of 6.4 mL, 10 mL and 15.6 mL of this solution to 100 mL flasks, complete the volume with *Solution 1* (*Potassium phosphate buffer at 1%, sterile, pH 6.0*) and homogenize. Solutions at 0.64 $\mu\text{g/mL}$, 1.0 $\mu\text{g/mL}$ and 1.56 $\mu\text{g/mL}$, respectively (P_1 , P_2 and P_3) are obtained.

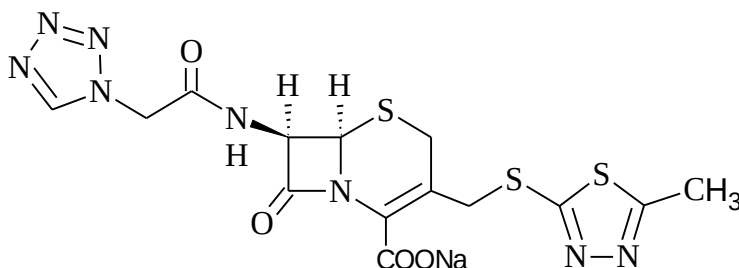
Procedure: pipette 20 mL of culture medium number 2 in each plate, wait until it solidifies, add 5 mL of 0.1% inoculum in culture medium number 1 and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Add to the cylinders 0.1 mL of the *Standard solution* and the *Sample solution* freshly prepared. Calculate the potency of the sample, in μg of cephalothin sodium per milligram, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, at temperature below 25 °C.

LABELING

Comply with current legislation.

CEFAZOLIN SODIUM*Cefazolinum natricum*C₁₄H₁₃N₈NaO₄S₃; 476.48

cefazolin sodium; 01846

Sodium salt of the (6*R*,7*R*)-3-[[[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanyl]methyl]-8-oxo-7-[[1*H*-tetrazol-1-yl]acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
[27164-46-1]

Contains no less than 95.0% and no more than 102.0% of cefazolin sodium (C₁₄H₁₃N₈NaO₄S₃), in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or nearly white, highly hygroscopic powder. Presents polymorphism.

Solubility. Very soluble in water and moderately soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): -10 to -24, in relation to the anhydrous substance. Determine in 5.5% (w/v) solution in 0.1 *M* sodium bicarbonate.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample submitted in advance to the procedure described next, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cefazolin CRS, prepared identically.

Procedure: dissolve 0.150 g of the sample in 5 mL of water, add 0.5 mL of acetic acid diluted at 12% (w/v), shake and allow to stand for 10 minutes in ice bath. Filter the precipitate and rinse with 1 mL to 2 mL of water. Dissolve in a mixture of 1 mL of water and 9 mL of acetone. Evaporate the solvent nearly to dryness and dry in oven at 60 °C for 30 minutes.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. The 5% (w/v) sample solution in water complies with sodium ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.0 to 6.0. Determine in aqueous solution at 10% (w/v).

Water (5.2.20.1). No more than 6%.

BIOLOGICAL SAFETY TESTS

When indicated on the label that the substance is sterile, the sample complies with the Sterility and with the Bacterial endotoxin tests. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.15 EU/mg of cefazolin sodium.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.8 mL/minute.

Buffer pH 3.6: transfer 0.9 g of sodium phosphate dibasic anhydrous and 1.298 g of monohydrate citric acid to a 1000 mL volumetric flask, dissolve in water, complete the volume with the same solvent and homogenize.

Buffer pH 7.0: transfer 5.68 g of sodium phosphate dibasic anhydrous and 3.63 g of monobasic potassium phosphate to a 1000 mL volumetric flask, dissolve in water, complete the volume with the same solvent and homogenize.

Mobile phase: mixture of *Buffer pH 3.6* and acetonitrile (8:2).

Internal standard solution: transfer 0.75 g of salicylic acid to a 100 mL volumetric flask, dissolve in 10 mL of methyl alcohol, complete the volume with *Buffer pH 7.0* and homogenize.

Standard solution: transfer, quantitatively, about 25 mg of cefazolin CRS to a 25 mL volumetric flask, complete the volume with *Buffer pH 7.0* and homogenize. Transfer 5 mL of this solution to a 100 mL volumetric flask, add 5 mL of the *Internal standard solution*, complete the volume with *Buffer pH 7.0* and homogenize.

Sample solution: transfer, quantitatively, about 26.2 mg of the sample (equivalent to 25 mg of cefazolin) to a 25 mL volumetric flask, complete the volume with *Buffer pH 7.0* and homogenize. Transfer 5 mL of this solution to a 100 mL volumetric flask, add 5 mL of the *Internal standard solution*, complete the volume with *Buffer pH 7.0* and homogenize.

The column efficiency must be no less than 1500 theoretical plates. The tailing factor for the cefazolin peak must be no more than 1.5. The relative standard deviation of the areas under the peaks of the replicates recorded must be no more than 2.0%. The relative retention time is approximately 1.3 for salicylic acid and 1.0 for cefazolin.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to cefazolin and to salicylic acid. Calculate the content of cefazolin sodium ($C_{14}H_{13}N_8NaO_4S_3$) in the sample from the responses obtained with the cefazolin/salicylic acid ratio, in the *Standard* and *sample solutions*.

PACKAGING AND STORAGE

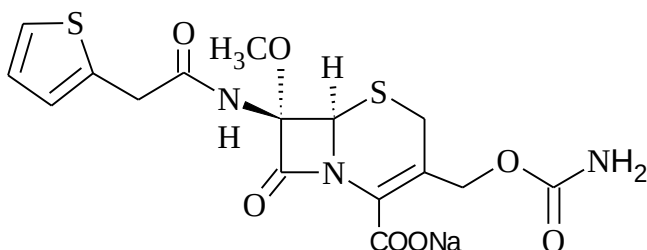
In tightly closed containers, between 2 °C and 8 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

CEFOXITIN SODIUM*Cefalotinum natricum*C₁₆H₁₆N₃NaO₇S₂; 449.43

cefoxitin sodium; 01883

Sodium salt of (6*R*,7*S*)-3-[[[(aminocarbonyl)oxy]methyl]-7-methoxy-8-oxo-7-[(2-thienylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1:1) [33564-30-6]

Contains no less than 927 µg and no more than 970 µg of cefoxitin (C₁₆H₁₇N₃O₇S₂) per milligram, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or nearly white, highly hygroscopic powder.

Solubility. Very soluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +206 to +214, in relation to the anhydrous substance. Determine in solution at 1% (w/v) in methyl alcohol.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of a 0.002% (w/v) solution in phosphate buffer pH 7.1, there are maximum absorption points only at the same wavelengths and with the same relative intensities as those observed in the cefoxitin sodium CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. The 5% (w/v) sample solution in water complies with sodium ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.2 to 7.0. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel F₂₅₄ as support, and a mixture of glacial acetic acid, water, acetone and ethyl acetate (10:10:20:50)

as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): transfer, quantitatively, approximately 0.25 g of the sample to a 10 mL volumetric flask, dissolve in water, complete the volume with the same solvent and homogenize.

Solution (2): transfer 0.5 mL of *Solution (1)* to a 100 mL volumetric flask, complete the volume with water and homogenize.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.5%).

Water (5.2.20.1). No more than 1%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.13 EU/mg of cefoxitin.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of water, acetonitrile and glacial acetic acid (84:16:1). Alternatively, use a mixture of water, acetonitrile and trifluoroacetic acid (84:16:1).

Sample solution: transfer, quantitatively, an amount of sample equivalent to approximately 0.15 g of cefoxitin to a 500 mL volumetric flask, dissolve in phosphate buffer pH 7.1, complete the volume with the same solvent and homogenize. Use this solution within five hours.

Standard solution: weigh, accurately, and dissolve in phosphate buffer pH 7.1 an amount of cefoxitin CRS sufficient to prepare a 0.3 mg/mL solution. Use this solution within 5 hours.

The column efficiency is no less than 2800 theoretical plates. The tailing factor for the cefoxitin peak is no more than 1.5. The relative standard deviation of the replicate areas of the recorded peaks is no more than 1.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of cefoxitin (C₁₆H₁₇N₃O₇S₂) in the cefoxitin sodium sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, between 2 °C and 8 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

CEFOXITIN SODIUM POWDER FOR INJECTION SOLUTION

Contains cefoxitin sodium equivalent to no less than 90.0% and no more than 120.0% of the declared amount of cefoxitin ($C_{16}H_{17}N_3O_7S_2$).

IDENTIFICATION

A. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

B. The 5% (w/v) sample solution in water complies with sodium ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test. Determine in the diluent vial.

pH (5.2.19). 4.2 to 7.0. Determine in aqueous solution at 1% (w/v).

Determination of weight (5.1.1). Complies with the test. Determine in the non-reconstituted powder.

PURITY TESTS

Water (5.2.20.1). No more than 1%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.13 EU/mg of cefoxitin.

ASSAY

Proceed as described in *Assay* in the *Cefoxitin sodium* monograph. Prepare the *Sample solution* as described next.

Sample solution: reconstitute the content of an ampoule vial in a volume of distilled water, accurately measured, corresponding to that indicated in the diluent vial. Transfer, quantitatively, the reconstituted solution to a volumetric flask with adequate capacity and dilute with water to obtain a solution at approximately 0.3 mg/mL of cefoxitin ($C_{16}H_{17}N_3O_7S_2$). Use this solution within five hours.

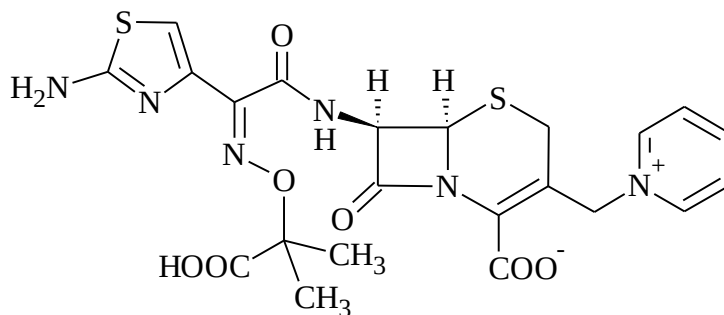
Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of cefoxitin ($C_{16}H_{17}N_3O_7S_2$) in the reconstituted injection solution from the responses obtained for the *Standard* and *sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, between 2 °C and 8 °C.

LABELING

Comply with current legislation.

CEFTAZIDIME PENTAHYDRATE*Ceftazidimum pentahydricum*C₂₂H₂₂N₆O₇S₂·5H₂O; 636.65

ceftazidime pentahydrate; 09370

Internal salt of 1-[[[(6*R*,7*R*)-7-[[[(2*Z*)-(2-amino-4-thiazolyl)][(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]pyridinium hydrate (1:5)
[78439-06-2]

Contains no less than 95% and no more than 102% of ceftazidime (C₂₂H₂₂N₆O₇S₂), in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water. Slightly soluble in methyl alcohol, practically insoluble in acetone and alcohol. Dissolves in acidic and alkaline solutions.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of ceftazidime CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **A.** from *Assay*, corresponds to the main peak of the *Standard solution*.

PURITY TESTS

Aspect of the preparation. The 0.5% (w/v) aqueous sample preparation is clear (5.2.25) and colorless (5.2.12).

Crystallinity. Suspend a few particles of the sample in mineral oil, transfer to a glass slide and examine on a microscope equipped with polarized light. The particles display birefringence, which is extinguished when moving the sample through micrometric adjustment.

Limit of pyridine. Proceed as described in *High performance liquid chromatography* (5.2.17.4), using a liquid chromatograph equipped with an ultraviolet detector at 255 nm; column with 250 mm of

length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute. Prepare the solutions immediately before use.

Mobile phase: mixture, filtered and degassed, of a solution with 28.8 g/L of ammonium phosphate dibasic, with pH adjusted in advance to 7.0 with ammonia, acetonitrile and water (8:24:68).

Sample solution: dissolve 0.5 g of the sample in phosphate buffer solution pH 7.0 at 10% (v/v) and dilute to 100 mL with the same solvent.

Standard solution: dissolve 1 g of pyridine in water and dilute to 100 mL with the same solvent. Dilute 5 mL of this solution to 200 mL with water. Add to 1 mL of the new solution 10 mL of phosphate buffer solution pH 7.0 and dilute to 100 mL with water.

Resolution solution: dilute 1 mL of the *Sample solution* to 200 mL with a phosphate buffer solution pH 7.0 at 10% (v/v). Add to 1 mL of this solution 20 mL of *Standard solution* and dilute to 200 mL with phosphate buffer solution pH 7.0 mL at 10% (v/v).

Inject 20 µL of the *Resolution solution*. The resolution between the peak of ceftazidime and of pyridine is no less than 7.0. The relative standard deviation of the area under the main peak obtained after six injections of 20 µL of the *Standard solution* is no more than 1%.

Procedure: inject, alternately, 20 µL of the *Sample solution* and the *Standard solution*, record the areas under the peaks for the *Standard solution* and the *Sample solution* and determine the amount of pyridine. No more than 500 ppm of pyridine.

Loss on drying (5.2.9.1). Determine in 0.2 g of the sample, in oven at 60 °C, under reduced pressure of no more than 5 mm of mercury, for three hours. Loss between 13% and 15%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.10 EU/mg of ceftazidime.

ASSAY

Use one of the methods described below.

A. Proceed as described for *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 245 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to hexylsilane or octylsilane group (5 µm); *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: dissolve 4.26 g of sodium phosphate dibasic and 2.73 g of monobasic potassium phosphate in water, in a 1000 mL flask, adjust, with water, to 980 mL, add 20 mL of acetonitrile and complete the volume with water. Homogenize. Adjust to pH 7.0 with phosphoric acid. Filter in membrane with 1 µm or smaller and degas. Make adjustments if necessary.

Sample solution: dissolve 25 mg of the sample, accurately weighed, in *Mobile phase*, complete the volume to 25 mL using the same solvent and homogenize.

Standard solution: dissolve 25 mg of ceftazidime CRS, accurately weighed, in *Mobile phase*, complete the volume to 25 mL using the same solvent and homogenize.

Resolution solution: dissolve 5 mg of ceftazidime delta-3-isomer CRS, accurately weighed, in 5 mL of the *Standard solution*.

Inject 20 µL of the *Resolution solution*. The resolution between ceftazidime and ceftazidime delta-3-isomer is no less than 1.0.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution*. Record the chromatograms and measure the areas under the peaks. Calculate the content of ceftazidime (C₂₂H₂₂N₆O₇S₂) in the sample from the responses obtained with the *Standard Solution* and the *Sample Solution*.

B. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: *Staphylococcus epidermidis* ATCC 12228

Culture media: culture medium number 2, for the base layer, and culture medium number 1, for the surface layer.

Sample solutions: prepare the stock solution in distilled water. Prepare solutions of sample diluted in *Phosphate buffer pH 6.0* in the concentrations equivalent to 100 µg/mL, 200 µg/mL and 400 µg/mL of ceftazidime.

Standard solutions: prepare the stock solution in distilled water. Prepare diluted solutions of ceftazidime CRS in *Phosphate buffer pH 6.0* in the concentrations of 100 µg/mL, 200 µg/mL and 400 µg/mL.

Procedure: add 21 mL of culture medium number 2 in each plate, wait until it solidifies, add 4 mL of culture medium number 1 with inoculum at 0.5% and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Add to the cylinders 0.2 mL of the freshly prepared solutions. Calculate the potency of the sample, in µg of ceftazidime per milligram, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

PACKAGING AND STORAGE

In hermetically closed containers. If targeted at production of injectable pharmaceutical dosage forms, it must be packaged in sterile containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

CEFTAZIDIME POWDER FOR INJECTION SOLUTION

Contains no less than 90% and no more than 105% of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) in relation to the desiccated substance free from sodium carbonate or arginine, and no less than 90% and no more than 120% of the declared amount of ceftazidime. Ceftazidime powder for injection solution is a sterile mixture of ceftazidime with sodium carbonate or arginine.

IDENTIFICATION

A. Proceed as described in method **B.** of *Identification* from the *Ceftazidime pentahydrate* monograph.

B. Dissolve the sample in *M* hydrochloric acid; there is effervescence. Bubble the gas produced in calcium hydroxide RS. A white precipitate is immediately formed.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.0 to 7.5, in a solution with 100 mg of ceftazidime per mL, constituted in the sealed container, taking care to eliminate the pressure inside the container during the reconstitution.

Determination of weight (5.1.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Content of sodium carbonate (if present).

Potassium chloride solution: dissolve 19.07 g of potassium chloride in water, transfer quantitatively to a 1000 mL volumetric flask, complete the volume with water and homogenize.

Standard solution: dissolve an amount of sodium chloride, previously desiccated at 105 °C for two hours, in water to obtain a solution with concentration of 14 µg/mL. Transfer 10 mL of this solution to a 100 mL volumetric flask, add 10 mL of *Potassium chloride solution*, complete volume with the same solvent and homogenize.

Sample solution: use stock solution, described in *Sample solution 1*, on method **A.** of *Assay*. Dilute, quantitatively, step by step, if necessary, with water, to obtain a solution with approximately 12.5 µg/mL of sodium carbonate. Transfer 10 mL of this solution to a 100 mL volumetric flask, add 10 mL of potassium chloride solution, complete volume with the same solvent and homogenize.

Blank solution: transfer 10 mL of *Potassium chloride solution* to a 100 mL volumetric flask, dilute with water to complete the volume and homogenize.

Procedure: determine the absorbances of the *Standard solution* and the *Sample solution* at 589 nm, using an atomic absorption spectrophotometer (**5.2.13.1**), equipped with sodium lamp and air-

acetylene flame, using the *Blank solution* for zero adjustment. Calculate the percentage of sodium carbonate, according to the formula:

$$\left(\frac{105,99}{116,88}\right) \times \left(\frac{0,1C}{M}\right) \times \left(\frac{Au}{As}\right)$$

where

105.99 = molar mass of sodium carbonate;

116.88 = double the molar mass of sodium chloride;

C = concentration, in µg/mL, of sodium chloride in the *Standard solution*;

M = amount, in mg, of ceftazidime powder for injection solution in each mL of the *Sample solution*, based on the amount used to prepare the stock solution and in the dilution;

Au and As = absorbances of the *Sample solution* and the *Standard solution*, respectively.

Use this percentage, corresponding to the anhydrous substance free from sodium carbonate, in the calculation of method **B**. from *Assay in Sample solution 1*.

Content of arginine (if present). Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph with ultraviolet detector at 206 nm; column with 250 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to dihydroxypropane(diol) group (3 µm to 10 µm) and a pre-column with 50 mm of length and 4.6 mm of internal diameter, packed with silica (30 µm to 50 µm); *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: dissolve 1.15 g of ammonium phosphate monobasic in approximately 800 mL of water. Adjust pH to 2.0 ± 0.1 with phosphoric acid and dilute to 1000 mL with water. Prepare a filtered and degassed mixture of acetonitrile and the solution described previously (750:250). Make adjustments, if necessary.

Standard solution: dissolve an amount of ceftazidime pentahydrate CRS and L-arginine CRS in water to obtain a solution with concentration of 0.2 mg/mL from each substance.

Sample solution: dissolve an amount of the sample in water to obtain a solution with concentration of 0.2 mg/mL of ceftazidime.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. The tailing factor is no more than 4.0. The resolution between the peak of ceftazidime and arginine is no less than 6.0. Calculate the amount of arginine in the sample, according to the formula:

$$100 \left(\frac{Cs}{Cu} \right) \times \left(\frac{ru}{rs} \right)$$

where

Cs = concentration, in mg/mL, of L-arginine CRS in the *Standard solution*;

Cu = concentration, in mg/mL, of ceftazidime powder for injection solution in the *Sample solution*;

ru and rs = areas under the peaks of arginine obtained for the *Sample solution* and the *Standard solution*, respectively.

Use this percentage, corresponding to the anhydrous substance free from arginine, in the calculation of method **A**. from Assay in *Sample solution 1*.

Limit of pyridine. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow of 1.6 mL/minute.

Mobile phase: mix acetonitrile, 0.25 M ammonium phosphate monobasic and water (300:100:600), adjusting pH to 7.0 ± 0.1 with ammonium hydroxide. Filter in membrane with pore size equal to or smaller than 1 µm of diameter and degas. Make adjustments if necessary.

Buffer pH 7.0: dissolve 5.68 g of sodium phosphate dibasic anhydrous and 3.63 g of monobasic potassium phosphate in water to complete to 1000 mL. Adjust pH with phosphoric acid, if necessary.

Standard solution: transfer about 250 mg of pyridine, accurately weighed, to a 100 mL volumetric flask and dilute in water. Immediately before the injection in the chromatograph, transfer 2 mL of this solution to a 200 mL volumetric flask and dilute with *Buffer pH 7.0* to obtain a solution with final concentration of 25 µg/mL of pyridine.

Sample solution: transfer about 660 mg of ceftazidime powder for injection solution, taken in advance from the ampoule vial and weighed, to a 100 mL volumetric flask and dilute with *Buffer pH 7.0*. This solution must be stored in a cold place and used within one hour.

Procedure: inject, separately, 10 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks of pyridine. The tailing factor is no more than 2.5. The relative standard deviation of the replicate areas under the recorded peaks is not more than 3%. Calculate the amount of pyridine in the sample, according to the formula:

$$10 \left(\frac{C}{W} \right) \times \left(\frac{ru}{rs} \right)$$

where

C = concentration, in µg/mL, of pyridine in the *Standard solution*;

W = weight, in mg, of ceftazidime powder for injection solution;

ru and rs = areas under the peak of pyridine obtained for the *Sample solution* and the *Standard solution*, respectively.

No more than 0.4% is found when the powder for injection solution has sodium carbonate; and no more than 0.3% when the powder for injection solution has arginine.

Loss on drying (5.2.9.1). Dry approximately 0.3 g of the sample, weighed in advance, in oven at 25 °C, under maximum pressure of 5 mmHg, for four hours. In the sample with arginine, the loss is no more than 12.5% of the weight. In the sample with sodium carbonate, the loss is no more than 13.5% of the weight. In the sample with arginine, use the percentage of loss, *m*, to calculate the mass of the desiccated substance free from arginine, in the result of *Sample solution 1*, in Assay. In the sample with sodium carbonate, heat the residue, under maximum pressure of 5 mmHg, at 100

°C for three hours, and calculate the total percentage of weight lost. Use this percentage, *m*, to calculate the mass of the substance desiccated and free from sodium carbonate, in the result of the *Sample solution 1*, obtained in the method **A.** of Assay.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Employ the *membrane filtration method*.

Bacterial endotoxins (5.5.2.2). No more than 0.10 EU/mg of ceftazidime.

ASSAY

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow of 2.0 mL/minute.

Buffer pH 7.0: dissolve 42.59 g of sodium phosphate dibasic anhydrous and 27.22 g of monobasic potassium phosphate in 900 mL of water. Adjust pH with phosphoric acid, if necessary. Complete the volume to 1000 mL and homogenize.

Mobile phase: mix 40 mL of acetonitrile and 200 mL of *Buffer pH 7.0* and dilute with water to obtain a final volume of 2 L. Filter with a filter of porosity of 1 µm or less and degas. Make adjustments if necessary.

Sample solution 1: transfer an amount of sample, accurately weighed, equivalent to 250 mg of ceftazidime, to a 250 mL volumetric flask. Dilute with water until completing the volume to obtain the stock solution. Protect this solution from light. Immediately before the injection in the chromatograph, transfer 5 mL of this solution to a 50 mL volumetric flask. Dilute with water to complete the volume and homogenize.

Sample solution 2 (used for single-dose containers): reconstitute the ceftazidime powder for injection solution in water, as specified on the medicine label. Remove the entire content from the container using a hypodermic needle. Dilute quantitatively the solution with water to obtain a final solution of 1 mg/mL. Protect this stock solution from light. Immediately before the injection in the chromatograph, transfer 5 mL of this solution to a 50 mL volumetric flask. Dilute with water to complete the volume and homogenize.

Sample solution 3 (used when the medicine label establishes the amount of ceftazidime in a volume of the reconstituted solution): reconstitute the ceftazidime powder for injection solution in a volume of water, accurately measured, corresponding to the volume of solvent specified in the medicine label. Dilute, quantitatively, a volume of this solution, accurately measured, in water, to obtain a solution at 1 mg/mL. Protect this stock solution from light. Immediately before the injection in the chromatograph, transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with water and homogenize.

Standard solution: transfer about 29 mg of ceftazidime pentahydrate CRS, accurately weighed, to a 25 mL volumetric flask, with 2.5 mL of *Buffer pH 7.0*; mix until full dissolution. Dilute with water to complete the volume and homogenize. Protect this stock solution from light. Immediately before

the injection in the chromatograph, transfer 5 mL of this stock solution to a 50 mL volumetric flask, complete the volume with water and homogenize. This solution has approximately 100 µg/mL of ceftazidime.

Resolution solution: prepare a solution of the delta 3 isomer of ceftazidime CRS at 0.1 mg/mL in *Buffer pH 7.0*. Immediately before the injection in the chromatogram, mix 1 mL of this solution with 8 mL of water and 1 mL of the stock solution used to prepare the *Standard solution* and homogenize.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution* and measure the areas under the peaks. For the *Sample solution 2* and the *Sample solution 3*, calculate the amount, in mg, of ceftazidime (C₂₂H₂₂N₆O₇S₂) in the sample analyzed, according to the formula:

$$C \times \left(\frac{ru}{rs} \right)$$

where

C = concentration, in µg/mL, of ceftazidime in the *Standard solution*;

ru and rs = areas under the peaks obtained with the *Sample solution* and the *Standard solution*, respectively.

For the *Sample solution 1*, calculate the amount of ceftazidime desiccated and free from sodium carbonate or arginine in a sample of ceftazidime powder for injection solution, according to the formula:

$$25\,000 \left[\frac{C}{W} (100 - m - s) \right] \times \left(\frac{ru}{rs} \right)$$

where

C = concentration, in µg/mL, of ceftazidime in the *Standard solution*;

W = amount, in mg, of ceftazidime powder for injection solution used to prepare the *Sample solution 1*;

m = amount of loss on drying;

s = percentage of sodium carbonate or arginine present in the sample;

ru and rs = areas under the peaks obtained with the *Sample solution* and the *Standard solution*, respectively.

Calculate the amount, in mg, of ceftazidime taken from the original container, or in the portion of the reconstituted solution, according to the formula:

$$\left(\frac{L}{D} \right) \times C \times \left(\frac{ru}{rs} \right)$$

where

L = labeled value, in mg, of ceftazidime in the container or in the volume of the reconstituted solution;

D = concentration, in $\mu\text{g/mL}$, of ceftazidime in the *Sample solution 2* or in the *Sample solution 3*, based on the labeled value of the container or on the portion of the reconstituted solution, respectively, and in the dilution.

B. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method. Reconstitute the content of 10 vials as indicated by the manufacturer.

Microorganism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 2, for the base layer, and culture medium number 1, for the surface layer.

Sample solutions: prepare the stock solution in distilled water. Prepare solutions of sample diluted in *Phosphate buffer pH 6.0* with concentrations equivalent to 100 $\mu\text{g/mL}$, 200 $\mu\text{g/mL}$ and 400 $\mu\text{g/mL}$ of ceftazidime.

Standard solutions: prepare the stock solution in distilled water. Prepare diluted solutions of ceftazidime CRS in *Phosphate buffer pH 6.0* in the concentrations of 100 $\mu\text{g/mL}$, 200 $\mu\text{g/mL}$ and 400 $\mu\text{g/mL}$.

Procedure: add 21 mL of culture medium number 2 in each plate, wait until it solidifies, add 4 mL of culture medium number 1 with inoculum at 0.5% and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Add to the cylinders 0.2 mL of the freshly prepared solutions. Calculate the potency of the sample, in μg of ceftazidime per milligram, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

PACKAGING AND STORAGE

In hermetically closed containers.

LABELING

Comply with current legislation.

KETOCONAZOLE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{26}H_{28}Cl_2N_4O_4$.

IDENTIFICATION

Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of *n*-hexane, ethyl acetate, methyl alcohol and glacial acetic acid (42:40:15:2:1) as mobile phase. Apply to the plate, separately, 10 μ L of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Dissolve an amount of sample equivalent to 50 mg of ketoconazole in chloroform, shake for about two minutes, complete the volume to 50 mL with the same solvent, homogenize and filter.

Solution (2): dissolve 10 mg of ketoconazole CRS in chloroform, complete the volume to 10 mL with the same solvent and homogenize.

Develop the chromatogram in non-saturated chamber. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot from the dissolution medium, filter and dilute in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 270 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_{26}H_{28}Cl_2N_4O_4$ dissolved in the medium, comparing the readings obtained with the one of the ketoconazole CRS solution at the concentration of 0.01% (w/v), prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{26}H_{28}Cl_2N_4O_4$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 225 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 µm to 10 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.5% (w/v) ammonium acetate (95:5).

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder, accurately weighed, equivalent to 0.1 mg of ketoconazole, to a 50 mL volumetric flask, add 30 mL of methyl alcohol and leave in ultrasonic bath for 30 minutes. Complete the volume with the same solvent, homogenize and filter. Dilute the filtrate until concentration of 0.1 mg/mL, using methyl alcohol as solvent.

Standard solution: dissolve an accurately weighed amount of ketoconazole CRS in methyl alcohol and dilute with the same solvent to obtain a 0.1 mg/mL solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₂₆H₂₈Cl₂N₄O₄ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

KETOCONAZOLE SHAMPOO

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{26}H_{28}Cl_2N_4O_4$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay* method, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 232 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at temperature of 40 °C; *Mobile phase* flow of 1.5 mL/minute.

Phosphate buffer pH 7.0: dissolve 2.8 g of sodium phosphate dibasic in 1000 mL of water. Adjust pH to 7.0 with phosphoric acid.

Mobile phase: prepare a mixture of acetonitrile, methyl alcohol, tetrahydrofuran and *Buffer phosphate pH 7.0* (390:95:15:500), adding 2.5 mL of a 25% (w/v) tetramethylammonium hydroxide solution in methyl alcohol. Filter and degas.

Sample solution: transfer an amount of shampoo, accurately weighed, equivalent to 20 mg of ketoconazole, to a 100 mL volumetric flask. Add 70 mL of methyl alcohol and leave in ultrasonic bath for 30 minutes to dissolve. Complete the volume with methyl alcohol, homogenize and filter in filter paper. Transfer 2.5 mL of this preparation to a 10 mL volumetric flask, add 1.5 mL of methyl alcohol, complete the volume with water and homogenize.

Standard solution: weigh, accurately, about 10 mg of ketoconazole CRS and transfer to a 100 mL volumetric flask. Add 70 mL of methyl alcohol and shake to dissolve. Complete the volume with methyl alcohol and homogenize. Transfer an aliquot of 5 mL to a 10 mL volumetric flask. Add 1 mL of methyl alcohol, complete the volume with water and homogenize.

The relative standard deviation of the replicate areas, of the standard solution, of the recorded peaks is no more than 2%.

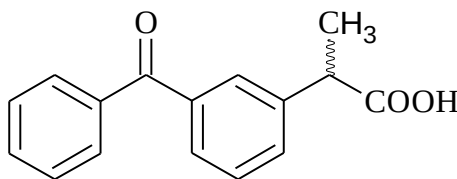
Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{26}H_{28}Cl_2N_4O_4$ in the sample, from the responses obtained with the *Standard* and *sample solutions*.

PACKAGING AND STORAGE

In hermetically closed containers and protected from excessive heat.

LABELING

Comply with current legislation.

KETOPROFEN***Ketoprofenum***C₁₆H₁₄O₃; 254.28

ketoprofen; 01960

3-benzoyl- α -methylbenzeneacetic acid

[22071-15-4]

Contains no less than 98.5% and no more than 101.0% of C₁₆H₁₄O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Practically insoluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 94 °C to 97 °C.

Specific optical rotation (5.2.8): -1 to +1, in relation to the desiccated substance. Determine in solution at 1% (w/v) in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ketoprofen CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 230 nm to 350 nm, of 0.001% (w/v) solution in ethyl alcohol, there is maximum absorption at 255 nm. The absorbance obtained at 255 nm is 0.615 to 0.680.

PURITY TESTS

Chromatographic purity. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 233 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); *Mobile phase* flow of 1.0 mL/minute.

Buffer pH 3.5: dissolve 68.0 g of monobasic potassium phosphate in 1000 mL of water and adjust the pH to 3.5 $\pm$ 0.1 with phosphoric acid.

Mobile phase: mixture of water, acetonitrile and *Buffer pH 3.5* (55:43:2).

Sample solution: dissolve an accurately weighed amount of the sample in *Mobile phase* to obtain a 200 µg/mL solution. Protect this solution from light.

Standard solution: dissolve an accurately weighed amount of ketoprofen CRS in *Mobile phase* to obtain a 200 µg/mL solution. Protect this solution from light.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is no less than 2250 theoretical plates. The tailing factor for the ketoprofen peak must be no more than 2.0. The relative standard deviation of the replicate areas of the recorded peaks is no more than 5.0%.

Procedure: inject, separately, 20 µL of *Sample solution* and *Standard solution*, record the chromatograms for no less than three times the retention time of ketoprofen, and measure the areas corresponding to the peaks of impurities. Calculate the percentage of each impurity present. No more than 0.2% of each individual impurity. The sum of all impurities is no more than 1.0% of the area under the ketoprofen peak obtained with the *Sample solution*.

Heavy metals (5.3.2.3). Use *Method II*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for four hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 0.2 g of the sample, previously desiccated, transfer to a 250 mL Erlenmeyer flask and dissolve in 25 mL of ethyl alcohol. Add 25 mL of water and 0.5 mL of phenol red TS. Titrate with 0.1 M sodium hydroxide VS. Alternatively, determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium hydroxide VS is equivalent to 25.428 mg of C₁₆H₁₄O₃.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

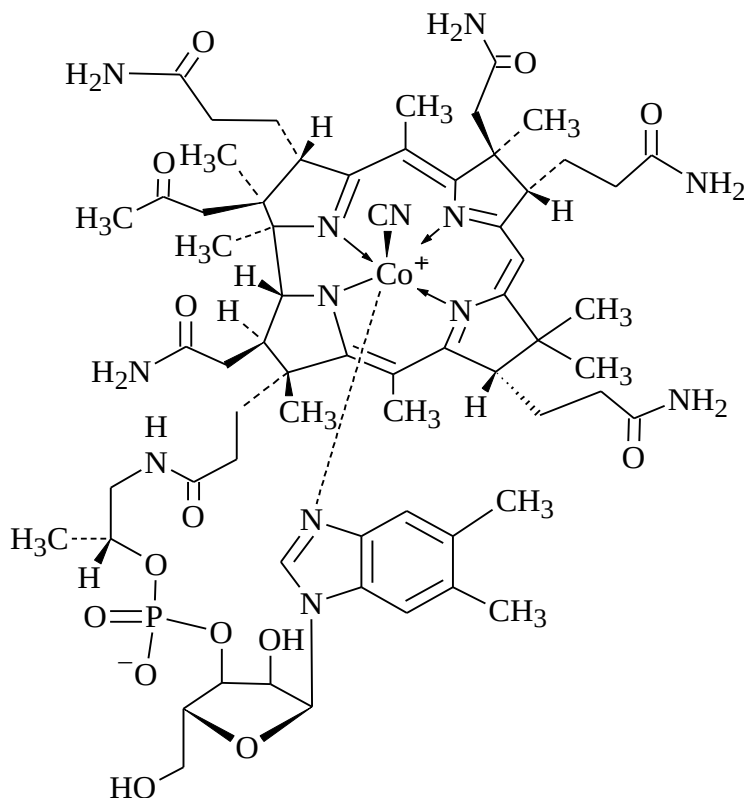
Comply with current legislation.

THERAPEUTIC CLASS

Anti-inflammatory.

CYANOCOBALAMIN

Cyanocobalaminum



$C_{63}H_{88}CoN_{14}O_{14}P$; 1355.39

cyanocobalamin; 01984

α -(5,6-Dimethylbenzimidazol-1-yl) cobamide cyanide

[68-19-9]

Contains no less than 96.0% and no more than 102.0% of $C_{63}H_{88}CoN_{14}O_{14}P$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline dark red powder or crystals.

Solubility. Moderately soluble in water and 96% ethyl alcohol.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 260 nm to 610 nm, of 0.0025% (w/v) solution in water, there are maximum absorption points at 278 nm, 361 nm and from 547 nm to 559 nm, identical to the ones observed in the spectrum of cyanocobalamin CRS solution, prepared identically.

B. Proceed protected from light, as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support, and a mixture of ammonia RS, methyl alcohol and methylene chloride (9:30:45) as mobile phase. Apply to the plate, separately, 10 μ L of each one of the freshly prepared solutions described below.

Solution (1): 2 mg/mL sample solution in mixture of ethyl alcohol and water (1:1).

Solution (2): 2 mg/mL solution of cyanocobalamin CRS in a mixture of ethyl alcohol and water (1:1).

Develop the chromatogram. Remove the plate and allow it to air dry. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 20.0 mg of the sample. Dry in oven at 105 °C, under reduced pressure, for two hours. No more than 12.0%.

Related substances. Proceed protected from light, as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 361 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 0.8 mL/minute.

Mobile phase: mixture of methyl alcohol and 1% (w/v) sodium phosphate dibasic solution adjusted to pH 3.5 with phosphoric acid (26.5:73.5). Use within 48 hours.

Solution (1): dissolve 10.0 mg of the sample in *Mobile phase*, dilute to 10 mL with the same solvent and homogenize. Use within one hour.

Solution (2): transfer 3.0 mL of *Solution (1)*, dilute to 100 mL with *Mobile phase* and homogenize. Use within one hour.

Solution (3): transfer 5.0 mL of *Solution (1)*, dilute to 50 mL with *Mobile phase* and homogenize. Transfer 1 mL of this solution to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Use within one hour.

Solution (4): dissolve 25.0 mg of the sample in 10 mL of water and heat slowly if necessary. Cool down. Add to this solution 5 mL of 0.1% (w/v) chloramine, 0.5 mL of 0.05 M hydrochloric acid and dilute to 25 mL with water. Homogenize the solution and wait five minutes. Dilute 1 mL of this solution to 10 mL with *Mobile phase* and inject immediately.

Procedure: inject, separately, 20 µL of each solution and record the chromatograms for no less than triple the retention time of the main peak. The sum of all areas under the secondary peaks obtained with *Solution (1)*, except that of the solvent peak, is no more than the area under the main peak obtained with *Solution (2)* (3%). Do not consider peaks with an area smaller than that presented by the main peak in the chromatogram obtained with *Solution (3)* (0.1%). The test is only valid if the chromatogram obtained with *Solution (4)* presents a resolution, between the two main peaks, of no less than 2.5 and if the signal/noise ratio is higher than 5 in the chromatogram with *Solution (3)*.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

When it is indicated on the label that the substance is sterile, the sample complies with the Bacterial endotoxin test and with the Sterility test. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.4 EU/ μ g of cyanocobalamin.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh, accurately, about 25 mg of the sample, transfer to a 100 mL volumetric flask and dissolve in water. Complete the volume with the same solvent and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with the same solvent and homogenize. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 361 nm, using water for zero adjustment. Calculate the content of $C_{63}H_{88}CoN_{14}O_{14}P$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vitamin.

CYANOCOBALAMIN INJECTION SOLUTION

Contains no less than 95.0% and no more than 115.0% the declared amount of $C_{63}H_{88}CoN_{14}O_{14}P$.

IDENTIFICATION

In the ultraviolet absorption spectrum and in the visible **(5.2.14)** in the range of 300 nm to 550 nm of the sample solution obtained in the *Assay*, there are maximum absorption points identical to those observed in the spectrum of the standard solution. The ratio between the absorbance values measured at 361 nm and 550 nm is comprised between 3.15 and 3.40.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.5 to 7.0.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.4 EU/ μ g of cyanocobalamin.

ASSAY

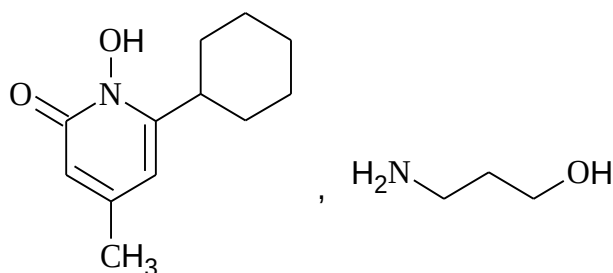
Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer a volume of injection solution equivalent to 0.3 mg of cyanocobalamin to a volumetric flask and dilute in water until concentration of 0.003% (w/v). Prepare a standard solution in the same conditions. Measure the absorbances of the solutions at 361 nm, using water for zero adjustment. Calculate the amount of $C_{63}H_{88}CoN_{14}O_{14}P$ in the injection solution from the readings obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In type I glass single-dose container, at room temperature and protected from light.

LABELING

Comply with current legislation.

CICLOPIROX OLAMINE*Ciclopirox olaminum*C₁₂H₁₇NO₂.C₂H₇NO; 268.36

ciclopirox olamine; 09336

6-Cyclohexyl-1-hydroxy-4-methyl-2(1*H*)-pyridinone with 2-aminoethanol (1:1)

[41621-49-2]

Contains no less than 97.5% and no more than 101.5% of ciclopirox olamine (C₁₂H₁₇NO₂.C₂H₇NO), in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or pale yellow crystalline powder.

Solubility. Slightly soluble in water, very soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of ciclopirox olamine CRS, prepared identically.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ as support, and a mixture of 13.5 *M* ammonia, water and ethyl alcohol (10:15:75) as mobile phase. Prepare two plates. Apply to each plate, separately, 10 µL of each of the freshly prepared solutions described next.

Solution (1): transfer 25 mg of the sample, accurately weighed, to a 10 mL volumetric flask and dissolve with methyl alcohol. Complete the volume with methyl alcohol and homogenize.

Solution (2): transfer 25 mg of ciclopirox olamine CRS to a 10 mL volumetric flask and dissolve with methyl alcohol. Complete the volume with methyl alcohol and homogenize.

Before developing the chromatogram, elute the plates with a mixture of 13.5 *M* ammonia, water and ethyl alcohol (10:15:75). The mixture must migrate to the top of the plate. Let the plates dry at room temperature for five minutes. After the application of *Solution (1)* and *Solution (2)*, develop the chromatograms. Remove the plate, allow to air dry for 10 minutes. Examine one of the plates under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. Nebulize the plate with 1% (w/v) ferric chloride in methyl alcohol. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. Nebulize the other plate with ninhydrin RS and

heat at 110 °C until stains appear. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

PURITY TESTS

pH (5.2.19). 8.0 to 9.0. Determine in aqueous solution at 1% (w/v).

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven, under reduced pressure. No more than 1.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Dissolve 0.2 g of the sample, previously desiccated, in 2 mL of methyl alcohol. Add 38 mL of water, shake and titrate with 0.1 M sodium hydroxide VS. Determine the endpoint potentiometrically. Carry out the blank and make the necessary corrections. Standardize the 0.1 M sodium hydroxide solution using 0.1 g of benzoic acid, weighed in advance, and conducting the titration in the conditions described earlier. Each mL of 0.1 M sodium hydroxide VS is equivalent to 12.212 mg of benzoic acid ($C_7H_6O_2$). Each mL of 0.1 M sodium hydroxide VS is equivalent to 26.84 mg of ciclopirox olamine ($C_{12}H_{17}NO_2.C_2H_7NO$).

B. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: Candida albicans ATCC 10231.

Culture media: sterile saline solution, for inoculum standardization, and culture medium number 19, for the inoculated layer.

Sample solutions: weigh, accurately, the equivalent to 25 mg of the sample and transfer to a 25 mL volumetric flask using dimethylsulfoxide. Complete the volume with the same solvent and homogenize. Dilute to obtain the concentrations of 56 µg/mL, 84 µg/mL and 126 µg/mL, using phosphate buffer pH 7.2, sterile, as solvent.

Standard solutions: weigh, accurately, the equivalent to 25 mg of ciclopirox olamine CRS and transfer to a 25 mL volumetric flask using dimethylsulfoxide. Complete the volume with the same solvent and homogenize. Dilute to obtain the concentrations of 56 µg/mL, 84 µg/mL and 126 µg/mL, using phosphate buffer pH 7.2, sterile, as solvent.

Procedure: add 8 mL of culture medium number 19, inoculated at 1% with the standardized microorganism suspension, to each plate, wait until it solidifies and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Calculate the potency of the sample in µg of ciclopirox olamine per milligram, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antifungal.

CICLOPIROX OLAMINE TOPICAL SOLUTION

Contains no less than 90.0% and no more than 110.0% than the declared amount of ciclopirox olamine ($C_{12}H_{17}NO_2 \cdot C_2H_7NO$).

IDENTIFICATION

In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of a 0.0008% (w/v) solution in methyl alcohol, there are maximum and minimum points, identical to the ones observed in the spectrum of ciclopirox olamine CRS, prepared identically.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: Candida albicans ATCC 10231.

Culture media: sterile saline solution, for inoculum standardization, and culture medium number 19 for the inoculated layer.

Phosphate buffer pH 7.2, sterile: mix 250 mL of 0.2 M monobasic potassium phosphate and 175 mL of 0.2 M sodium hydroxide. Complete the volume to 1000 mL with water and homogenize. Sterilize the solution for 20 minutes in autoclave at 121 °C.

Sample solutions: transfer, using a volumetric pipette, a volume equivalent to 25 mg of ciclopirox olamine to a 25 mL volumetric flask using dimethylsulfoxide. Complete the volume with dimethylsulfoxide and homogenize. Dilute to obtain the concentrations of 56 µg/mL, 84 µg/mL and 126 µg/mL, using *Phosphate buffer pH 7.2, sterile* as diluent.

Standard solutions: weigh, accurately, the equivalent to 25 mg of ciclopirox olamine CRS, transfer to a 25 mL volumetric flask and dissolve using dimethylsulfoxide. Complete the volume with the same solvent and homogenize. Dilute to obtain the concentrations of 56 µg/mL, 84 µg/mL and 126 µg/mL, using *Phosphate buffer pH 7.2, sterile* as diluent.

Procedure: add 8 mL of culture medium number 19, inoculated at 1% with the standardized microorganism suspension, to each plate, wait until it solidifies and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)* using cylinders. Calculate the amount, in µg of

ciclopirox olamine in the sample, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 300 nm; column with 250 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), capped, maintained at temperature of 30 °C; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of acetonitrile and water (50:50).

Sample solution: transfer a volume equivalent to 25 mg of ciclopirox olamine to a 25 mL volumetric flask. Dissolve with dimethylsulfoxide, complete the volume with the same solvent and homogenize.

Standard solution: transfer 25 mg of ciclopirox olamine CRS to a 25 mL volumetric flask. Dissolve with dimethylsulfoxide, complete the volume with the same solvent and homogenize.

Derivatization procedure: transfer, separately, 2 mL of the *Standard solution* to a 10 mL test tube. Add 1 mL of 0.1 M sodium hydroxide and 0.2 mL of dimethyl sulfate. Shake in vortex. Place in water bath at 37 °C, for 15 minutes. Add 0.2 mL of triethylamine. Shake in vortex. Dilute the resulting solution with *Mobile phase*, until concentration of 40 µg/mL. Repeat the same procedure using 2 mL of *Sample solution*.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution* after the derivatization process, record the chromatograms and measure the areas under the peaks. Calculate the amount of ciclopirox olamine (C₁₂H₁₇NO₂.C₂H₇NO) in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

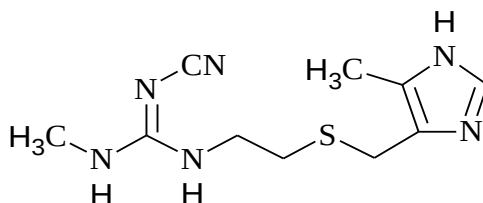
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CIMETIDINE

Cimetidinum



$C_{10}H_{16}N_6S$; 252.34

cimetidine; 02073

N-Cyano-*N'*-methyl-*N''*-[2-[[4-methyl-1*H*-imidazol-5-yl) methyl]thio]ethyl]guanidine
[51481-61-9]

Contains no less than 98.5% and no more than 101.5% of $C_{10}H_{16}N_6S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white powder.

Solubility. Slightly soluble in water, soluble in ethyl alcohol. Soluble in diluted mineral acids.

Physicochemical constants.

Melting range (5.2.2): 139 °C to 144 °C. If necessary, dissolve the substance in isopropyl alcohol, evaporate to dryness and determine the melting range again.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample desiccated at 105 °C, until constant weight, and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cimetidine CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of a 0.0012% (w/v) solution in 0.1 *M* hydrochloric acid, there is maximum absorption at 218 nm, identical to that observed in the spectrum of cimetidine CRS, prepared identically.

C. Proceed as described in *Related Substances*. The main stain obtained with *Solution (2)* is similar in position, color and intensity to the one obtained with *Solution (6)*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of 13.5 *M* ammonia, methyl alcohol and ethyl acetate (15:20:65) as

mobile phase. Saturate the chamber, for 15 minutes, with vapor from the mobile phase. Apply, separately, to the plate 5 µL of each solution described below.

Solution (1): dissolve 0.5 g of the sample in 10 mL of methyl alcohol.

Solution (2): dilute 1 mL of *Solution (1)* to 10 mL with methyl alcohol.

Solution (3): dilute 1 mL of *Solution (1)* to 100 mL with methyl alcohol and dilute 20 mL of this solution to 100 mL with methyl alcohol.

Solution (4): dilute 5 mL of *Solution (3)* to 10 mL with methyl alcohol.

Solution (5): dilute 5 mL of *Solution (4)* to 10 mL with methyl alcohol.

Solution (6): dissolve 10 mg of cimetidine CRS in 2 mL of methyl alcohol.

Develop the chromatogram. Remove the plate, dry in air stream, examine under ultraviolet light (254 nm) or leave under iodine vapor until obtaining maximum contrast of stains. Any secondary stain obtained with *Solution (1)* (5%) is not more intense than the main stain obtained with *Solution (3)* (0.001%), and no more than two stains can be more intense than the main stain obtained with *Solution (4)* (0.0005%). For the assay to be valid, the chromatogram obtained with *Solution (5)* must present a clearly visible stain.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105 °C, until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve, accurately, about 0.2 g of the sample in 60 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 25.234 mg of C₁₀H₁₆N₆S.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); *Mobile phase* flow of 1.2 mL/minute.

Mobile phase: mix 200 mL of methyl alcohol, 0.3 mL of phosphoric acid and complete the volume to 1000 mL with water.

Sample solution: dissolve an accurately weighed amount of the sample in a mixture of methyl alcohol and water (1:4). Leave in ultrasonic bath for 15 minutes, complete the volume with the same solvent and homogenize, obtaining a solution at 0.4 mg/mL. Carry out successive dilutions until concentration of 8 µg/mL, using *Mobile phase* as diluent.

Standard solution: dissolve an accurately weighed amount of cimetidine CRS in a mixture of methyl alcohol and water (1:4), to obtain a 0.1 mg/mL solution. Dilute with *Mobile phase* to obtain a 8 µg/mL solution.

The column efficiency must be no less than 1000 theoretical plates/meter. The relative standard deviation of the replicate areas of the recorded peaks is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₀H₁₆N₆S in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

H₂ receptor antagonist.

CIMETIDINE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{10}H_{16}N_6S$.

IDENTIFICATION

In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** of Assay, there is maximum absorption at 218 nm, identical to that observed in the spectrum of cimetidine CRS, prepared identically.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 15 minutes.

Procedure: take an aliquot from the dissolution medium, filter and, if necessary, dilute with 0.1 M sulfuric acid until adequate concentration. Measure the absorbances of the solutions at 218 nm **(5.2.14)**, using 0.1 M sulfuric acid for zero adjustment. Calculate the amount of cimetidine ($C_{10}H_{16}N_6S$) dissolved in the medium, comparing the readings obtained with that of the cimetidine CRS solution, at the concentration of 0.0005% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{10}H_{16}N_6S$ dissolve in 15 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.1 g of cimetidine to a 200 mL volumetric flask and add 50 mL of 0,1 M hydrochloric acid. Shake for 30 minutes, complete the volume with the same solvent and homogenize. Filter. Carry out successive dilutions to a concentration of

0.0012% (w/v), using 0.1 M hydrochloric acid as solvent. Prepare a standard solution in the same conditions. Measure the absorbances of the solutions at 218 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{10}H_{16}N_6S$ in the tablets, from the readings obtained.

B. Proceed as described in method **B.** of Assay in the *Cimetidine* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 40 mg of cimetidine to a 100 mL volumetric flask, add 20 mL of methyl alcohol and leave in ultrasonic bath for 15 minutes. Complete the volume with water, homogenize and filter. Carry out successive dilutions until concentration of 8 µg/mL, using *Mobile phase* as solvent.

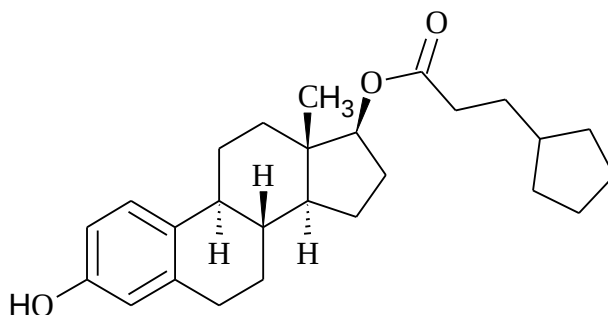
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the area under the peaks. Calculate the amount of $C_{10}H_{16}N_6S$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, at room temperature and protected from light.

LABELING

Comply with current legislation.

ESTRADIOL CYPIONATE*Estradioli cypionas* $C_{26}H_{36}O_3$; 396.57

estradiol cypionate; 03599

Estradiol 17 β -cyclopentanepropionate

[313-06-4]

Contains no less than 97.0% and no more than 103.0% of estradiol cypionate ($C_{26}H_{36}O_3$), in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Insoluble in water, soluble in ethyl alcohol and moderately soluble in vegetable oils.

Physicochemical constants.

Melting range (5.2.2): 149 °C to 153 °C.

Specific optical rotation (5.2.8): +39 to +44. Determine in solution at 20.0 mg/mL in dioxane.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of estradiol cypionate CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.010% (w/v) solution in ethyl alcohol, there are maximum and minimum points only at the same wavelengths of estradiol cypionate CRS, prepared identically. The absorbances of solutions at 280 nm differ no more than 3%, when calculated in relation to the desiccated substance.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C, for four hours. No more than 1.0%.

Residue by incineration (5.2.10). No more than 0.2%.

BIOLOGICAL SAFETY TEST

When it is indicated on the label that the substance is sterile, the sample complies with the Bacterial endotoxin test and with the Sterility test. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.84 EU/mg of estradiol cypionate.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 280 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: mixture of 2.7 g/L aqueous ammonium nitrate solution and acetonitrile (20:80).

Sample solution: use 10 mg of the sample, accurately weighed, and prepare the solution according to the procedure described in *Standard solution*.

Standard solution: transfer 10 mg of estradiol cypionate CRS, accurately weighed, to a 10 mL volumetric flask, complete the volume with tetrahydrofuran and homogenize.

Inject 10 µL replicates of the *Standard solution*.

The relative standard deviation of the areas of five replicates of the recorded peaks is no more than 1.5%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of estradiol cypionate (C₂₆H₃₆O₃) in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

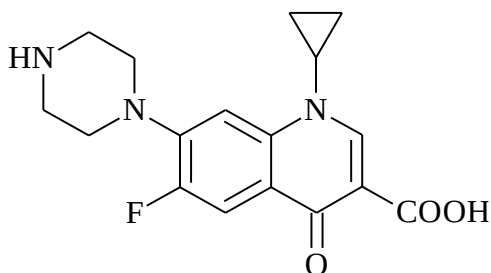
In tightly closed containers, resistant to light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Estrogen.

CIPROFLOXACIN*Ciprofloxacinum*C₁₇H₁₈FN₃O₃; 331.34

ciprofloxacin; 02137

1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4 dihydroquinoline-3-carboxylic acid
[85721-33-1]

Contains no less than 98.0% and no more than 102.0% of ciprofloxacin (C₁₇H₁₈FN₃O₃), in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Nearly white to light yellow, slightly hygroscopic crystalline powder.

Solubility. Practically insoluble in water. Soluble in diluted acetic acid, very slightly soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of ciprofloxacin CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Aspect of the preparation. The preparation at 2.5% (w/v) in 0.1 M hydrochloric acid is clear to slightly opalescent (5.2.25).

Related substances. Proceed as described on the method from *Assay*. Record the chromatograms and measure the areas under the peaks. Calculate the percentage of each impurity from ciprofloxacin present. No more than 0.2% for the ciprofloxacin ethylenediamine analog or any other individual impurity. Not more than 0.5% for total impurities.

Limit of fluoroquinolonic acid. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel F₂₅₄ as support and a mixture of methylene chloride, methyl alcohol, acetonitrile and ammonium hydroxide (4:4:1:2) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below:

Solution (1): transfer 5 mg of fluoroquinolonic acid to a 50 mL volumetric flask with 0.05 mL of 6 M ammonium hydroxide, complete o volume with water and homogenize.

Solution (2): transfer 2 mL of *Solution (1)* to a 10 mL volumetric flask, complete the volume with water and homogenize.

Solution (3): obtain a sample solution with concentration of 10 mg/mL of ciprofloxacin in 0.1 M acetic acid.

Put in an adequate chamber a container with ammonium hydroxide, along with the plate. After 15 minutes, transfer the plate to the chromatographic chamber. Develop the chromatogram. Remove the plate, allow to air dry for 15 minutes. Examine under ultraviolet light (254 nm). The secondary stain obtained with the *Solution (3)* has, as maximum, equal intensity and size in relation to the main stain obtained with *Solution (2)* (0.2%).

Sulfate. Prepare the *Standard solution* at 18.1 µg/mL of potassium sulfate in 30% (v/v) alcohol (10 µg/mL of sulfate). Prepare the *Sample solution* adding 0.5 g of ciprofloxacin in 5.0 mL of 2 M acetic acid and 15 mL of water. Transfer to two Nessler tubes 1.5 mL of the *Standard solution*, add, under continued agitation, 1 mL of 25% (w/v) barium chloride solution and wait one minute. Transfer to one of the tubes 15 mL of the *Standard solution* and 0.5 mL of 30% (v/v) acetic acid and shake. To the second tube, add 15 mL of the *Sample solution* and 0.5 mL of 30% (v/v) acetic acid and shake. The turbidity in the tube with the *Sample preparation* is, at maximum, equal to the one presented by the *Standard solution* (0.04%).

Chlorides (5.3.2.1). Proceed as described in *Limit test for chlorides*, except that the *Standard preparation* and *Sample preparation* must not be diluted to 50 mL. No more than 0.02% (200 ppm).

Sample preparation: dissolve 0.5 g of the sample in 30 mL of water and filter in chlorine-free paper filter. Transfer 15 mL of the filtrate to a 50 mL tube.

Standard preparation: prepare a solution at 8.2 µg/mL of sodium chloride (5 µg/mL of chloride). Transfer 10 mL to a second 50 mL tube, add 5.0 mL of water and shake.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 120 °C, under reduced pressure, for six hours. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%. When indicated for the preparation of oral suspension of ciprofloxacin, no more than 0.2%.

BIOLOGICAL SAFETY TESTS

When indicated on the label that the substance is sterile, the sample complies with the Sterility and with the Bacterial endotoxin tests. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test. Proceed as described in *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). No more than 0.5 EU/mg of ciprofloxacin.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 278 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 μ m); maintained at 30 °C, *Mobile phase* flow of 1.2 mL/minute.

Mobile phase: mixture of acetonitrile and 0.0125 M phosphoric acid with pH adjusted in advance to 2.5 ± 0.1 with triethylamine (13:87).

Sample solution: obtain a 0.1 mg/mL ciprofloxacin solution in *Mobile phase*.

Standard solution: obtain a 0.1 mg/mL ciprofloxacin CRS solution in *Mobile phase*.

Resolution solution: prepare a solution with ciprofloxacin ethylenediamine analog CRS at 250 μ g/mL in *Mobile phase*. Transfer 1 mL of the solution to a 10 mL volumetric flask, complete the volume with *Standard solution* and homogenize.

Inject 25 μ L of the *Resolution solution*. The column efficiency is no less than 2500 theoretical plates/meter. The relative retention times are approximately 0.7 for the ciprofloxacin ethylenediamine analog and 1.0 for ciprofloxacin. The tailing factor is no more than 2.5. The resolution between the peak of ciprofloxacin ethylenediamine analog and the peak of ciprofloxacin is no less than 5.0. The relative standard deviation of the replicate areas of the recorded peaks is no more than 2.0%.

Procedure: inject, separately, 25 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to ciprofloxacin. Calculate the content of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

CIPROFLOXACIN INJECTION SOLUTION

Contains no less than 90.0% and no more than 110.0% the declared amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$). Ciprofloxacin injection solution is a ciprofloxacin solution in water for injection, in 5% (w/v) glucose or 0.9% (w/v) sodium chloride solution, prepared with lactic acid.

IDENTIFICATION

Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel GF₂₅₄ as support and a mixture of methylene chloride, methyl alcohol, ammonium hydroxide and acetonitrile (4:4:2:1) as mobile phase. Apply, separately, to the plate, saturated in advance for 15 minutes in ammonia atmosphere, 10 µL of each of the freshly prepared solutions described below.

Solution (1): dilute a volume of injection solution in water to obtain a solution with concentration of approximately 0.05% (w/v) of ciprofloxacin.

Solution (2): ciprofloxacin hydrochloride CRS solution in water in the concentration of 0.05% (w/v). Develop the chromatogram. Remove the plate, allow to air dry and examine under ultraviolet light (254 nm and 366 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.5 to 4.6.

PURITY TESTS

Limit of ciprofloxacin ethylenediamine impurity. Proceed as described on method **B**. from Assay. Calculate the percentage of impurity, from the chromatogram obtained with the *Sample solution*, according to the formula:

$$100 \times [0,7 Ri / (0,7 Ri + Rc)]$$

where

0.7 = response factor between the impurity and ciprofloxacin;

Ri = response from the impurity peak;

Rc = response from the ciprofloxacin peak.

No more than 0.5%.

Limit of lactic acid. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with ultraviolet detector at 208 nm; column with 300 mm of length and 7.8 mm of internal diameter, packed with ion exchange resin made of styrene-divinylbenzene sulfonated copolymer in hydrogenated form (7 µm to 11 µm), maintained at 40 °C; *Mobile phase* flow of 0.6 mL/minute.

Mobile phase: mixture of 0.0025 M sulfuric acid and acetonitrile (85:15).

Sample solution: use the non-diluted injection solution.

Standard solution: 0.8 mg/mL sodium lactate CRS solution in water.

The column efficiency must be no less than 5000 theoretical plates/meter. The relative standard deviation of the replicate areas from the recorded peaks must be of no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount, in milligrams, of lactic acid (C₃H₆O₃) per milliliter of the injection solution, according to the expression:

$$\left(\frac{90,08}{112,07}\right) \times (C) \times \left(\frac{R_a}{R_p}\right)$$

where

90.08 and 112.07 = molar masses of lactic acid and sodium lactate, respectively;

C = concentration, in mg/mL, of the sodium lactate CRS solution;

R_a and R_p = responses from the peaks obtained with the *Sample solution* and the *Standard solution*, respectively.

The value obtained is between 0.288 mg and 0,352 mg of C₃H₆O₃ per mg of ciprofloxacin labeled.

Limit of glucose. Transfer 50 mL of the injection solution to a 100 mL volumetric flask. Add 0.2 mL of 6 M ammonium hydroxide, complete the volume with water and homogenize. Determine the rotation angle (α) in a 200 mm tube at 25 °C (**5.2.8**). The amount, in grams, of glucose monohydrate (C₆H₁₂O₆.H₂O) in 100 mL of injection solution is calculated according to the formula:

$$2 \times (1,0425 \times \alpha)$$

The value obtained is between 4.75 and 5.25 g of C₆H₁₂O₆.H₂O per 100 mL of injection solution.

Limit of sodium chloride. Transfer 10 mL of the injection solution to an Erlenmeyer flask, dilute with water to approximately 150 mL, add 1.5 mL of potassium chromate RS and titrate with 0.1 M silver nitrate VS. Each mL of 0.1 M silver nitrate VS is equivalent to 5.844 mg of sodium chloride. The value obtained is between 85.5 mg and 94.5 mg.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Use the *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). No more than 1.76 EU/mL of ciprofloxacin.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Dilute the injection solution in water, to obtain a concentration of approximately 0.0004% (w/v) of ciprofloxacin. Use ciprofloxacin hydrochloride CRS to prepare the standard solution, using the same solvent. The solutions must be maintained protected from light. Measure the absorbances of the resulting

solutions at 272 nm, using water for zero adjustment. Calculate the amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 278 nm; column with 250 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 μ m to 10 μ m), maintained at temperature of 30 °C; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: mixture of 0.025 M phosphoric acid with pH previously adjusted to 3.0 ± 0.1 with triethylamine and acetonitrile (87:13).

Sample solution: transfer a volume of the injection solution equivalent to 25 mg of ciprofloxacin to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: weigh, accurately, about 30 mg of ciprofloxacin hydrochloride CRS and transfer to a 100 mL volumetric flask, using *Mobile phase* as solvent, to obtain a concentration of 0.25 mg/mL of ciprofloxacin.

Resolution solution: dissolve in a portion of *Standard solution* an amount of impurity ciprofloxacin ethylenediamine CRS (1-cyclopropyl-6-fluoro-1,4-di-hydro-4-oxo-7-2[2-(amino)-3-quinoline carboxylic acid hydrochloride) to obtain a 0.25 mg/mL solution.

The column efficiency must be no less than 10 000 theoretical plates/meter. The relative retention times are of approximately 0.7 for the impurity from the *Resolution solution* and 1.0 for ciprofloxacin. The resolution between the impurity peak and the ciprofloxacin peak is no less than 6. The relative standard deviation of the replicate areas of the recorded peaks is no more than 1.5%.

Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

C. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 1 for microorganism maintenance; sterile saline solution, for inoculum standardization; and culture medium number 11, for base layer and inoculum preparation.

Sample solutions: transfer a volume of the injection solution with the equivalent to 20 mg of ciprofloxacin to a 50 mL volumetric flask, complete the volume with water and homogenize. Dilute to obtain concentrations of 2 μ g/mL, 4 μ g/mL and 8 μ g/mL, using *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Standard solutions: accurately weigh approximately 20 mg of ciprofloxacin CRS, transfer to a 50 mL volumetric flask, complete the volume with water and homogenize. Dilute to obtain concentrations of 2 μ g/mL, 4 μ g/mL and 8 μ g/mL, using *Solution 2 (0.1 M potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Procedure: add 20 mL of culture medium number 11 to a plate, wait until it solidifies, add 5 mL of 1% inoculum and proceed as described in *Microbiological assay by diffusion in agar (5.5.3.3.1)*, using cylinders. Add to the cylinders 0.1 mL of the freshly prepared solutions. Calculate the potency of the sample, in µg of ciprofloxacin per milligram, from the potency of standard and responses obtained with the *Standard solutions* and the *Sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, at room temperature and protected from light.

LABELING

Comply with current legislation.

CITALOPRAM TABLETS

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{20}H_{21}FN_2O$. The tablets can be coated.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of the 0.001% (w/v) citalopram solution in 0.1 M hydrochloric acid, there is maximum absorption at 239 nm, identical to that observed in the spectrum of citalopram CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* obtained in method **B.** from Assay corresponds to that of the main peak of the *Standard solution*.

C. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ as support and a mixture of water, butyl alcohol and acetic acid (15:12:3) as mobile phase. Prepare the mobile phase 24 hours beforehand. After discarding the organic layer, apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 10 mg of citalopram to a beaker, add 7 mL of water and submit to ultrasonic bath at room temperature for 10 minutes. Add 3 mL of the same solvent, shake and filter.

Solution (2): prepare a 1 mg/mL solution of citalopram CRS in water.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described on method **B.** from Assay. Prepare solution with final concentration of 40 µg/mL.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take a 10 mL aliquot from the dissolution medium and filter in quantitative filter. Proceed as described on method **B.** from Assay in the *Citalopram Hydrobromide* monograph, using the filtered aliquots as *Sample solution*. Calculate the amount of $C_{20}H_{21}FN_2O$ dissolved in the

medium, comparing the areas under the peaks obtained with the area under the peak of a citalopram CRS solution at the concentration of 22 µg/mL, prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{20}H_{21}FN_2O$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 10 mg of citalopram using 30 mL of 0.1 M hydrochloric acid to a 50 mL volumetric flask. Submit to ultrasonic bath for 10 minutes, complete the volume with the same solvent and homogenize. Filter. Dilute with the same solvent to obtain a final concentration of 0.001% (w/v). Prepare the standard solution in the same concentration using the same solvent. Measure the absorbances of the solutions at 239 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{20}H_{21}FN_2O$ in the tablets, from the responses obtained with the *Standard solution* and the *Sample solution*.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Proceed as described on method **B.** of Assay in the *Citalopram hydrobromide* monograph. Prepare the *Sample solution* as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 10 mg of citalopram to a 50 mL volumetric flask and add 40 mL of water, with pH adjusted to 1.85 with 10% (w/v) phosphoric acid. Submit to ultrasonic bath at room temperature for 10 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer a 5 mL aliquot to a 25 mL volumetric flask, complete the volume with water with pH adjusted to 1.85 with 10% (w/v) phosphoric acid and homogenize.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{20}H_{21}FN_2O$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with the current legislation.

CISPLATIN INJECTION SOLUTION

Contains no less than 90.0% and no more than 110.0% of the declared amount of $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 230 nm to 350 nm of the 0.1% (w/v) sample solution in water, there is maximum absorption at 300 nm, identical to that observed in the spectrum of cisplatin CRS solution, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.5 to 6.5.

PURITY TESTS

Limit of amminetrichloroplatinate. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 209 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to quaternary ammonium groups, strongly basic, for anion exchange (10 μm), maintained at room temperature; *Mobile phase* flow of 2 mL/minute.

Mobile phase: prepare a 0.04% (w/v) ammonium sulfate solution in water. The pH of this solution is 5.8 ± 0.1 . Adjust the ion strength, if necessary, to comply with the system suitability requirements. Degas and filter.

Solution (1): dilute the injection solution, if necessary, in 0.9% (w/v) sodium chloride solution, to obtain a 0.05% (w/v) cisplatin solution.

Solution (2): dissolve an amount, accurately weighed, of potassium amminetrichloroplatinate CRS in 0.9% (w/v) sodium chloride solution to obtain a 0.0015% (w/v) amminetrichloroplatinate solution.

Inject 20 μL replicates of the *Solution (2)*. The resolution between the sodium chloride peak and the amminetrichloroplatinate peak is no less than 2.0. The relative standard deviation of the replicate areas of the peaks obtained is no more than 2.0%.

Procedure: inject, separately, 20 μL of *Solution (1)* and of *Solution (2)* and record the chromatograms. The area under the peak corresponding to amminetrichloroplatinate obtained in the chromatogram of *Solution (1)* is not greater than the area under the main peak obtained in the chromatogram of *Solution (2)* (3%).

Limit of transplatin. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to cation exchange, strongly

acidic groups (10 µm), maintained at temperature of 45 °C and *Mobile phase* flow of 2.0 mL/minute for 30 minutes, 0.5 mL/minute for another 30 minutes, and 2 mL/minute again for 30 minutes.

Mobile phase: prepare a 2.5% (w/v) monobasic potassium phosphate solution in water and adjust the pH to 3.2 with phosphoric acid. Degas and filter.

Solution (1): 0.005% (w/v) transplatin CRS solution in 0.9% (w/v) sodium chloride solution.

Solution (2): 0.5% (w/v) thiourea solution in water.

Solution (3): 0.005% (w/v) cisplatin CRS solution in 0.9% (w/v) sodium chloride solution.

Solution (4): dilute the injection solution, if necessary, in 0.9% (w/v) sodium chloride solution to obtain a 0.05% (w/v) cisplatin solution.

Solution (5): transfer 10 mL of *Solution (1)* to a 50 mL volumetric flask. Add 25 mg of cisplatin CRS, accurately weighed. Add 25 mL of 0.9% (w/v) sodium chloride solution, shake for 30 minutes, complete the volume with the same solvent and homogenize.

Solution (6): mix 5 mL of *Solution (2)* with 5 mL of *M* hydrochloric acid and 10 mL of *Solution (4)*. Heat at 60 °C for one hour and cool.

Solution (7): mix 5 mL of *Solution (2)* with 5 mL of *M* hydrochloric acid and 10 mL of *Solution (5)*. Heat at 60 °C for one hour and cool.

Solution (8): mix 10 mL of *Solution (1)* with 10 mL of *Solution (3)*. Heat at 60 °C for one hour and cool.

Inject 10 µL replicates of the *Solution (7)* and of *Solution (8)*. The column efficiency, determined for the peak corresponding to transplatin in the *Solution (7)* chromatogram, is no less than 2500 theoretical plates. The retention times for transplatin and cisplatin, obtained in the *Solution (8)* chromatogram, are of approximately five minutes and nine minutes, respectively. If necessary, make adjustments in the composition of the *Mobile phase* and recondition the column. The resolution between cisplatin and transplatin, in the *Solution (8)* chromatogram, is no less than 1.7. The relative standard deviation of the replicate areas of the peaks obtained from the transplatin standard in the *Solution (7)* chromatogram is no more than 2.0%.

Procedure: inject, separately, 10 µL of *Solution (6)* and of *Solution (7)* and record the chromatograms. The area under the peak corresponding to transplatin obtained in the chromatogram of *Solution (6)* is not greater than the area under the transplatin peak obtained in the chromatogram of *Solution (7)* (2%).

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 2.0 EU/mg of cisplatin.

ASSAY

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to amino groups (10 µm), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: mixture of acetonitrile and water (90:10). Degas and filter.

Solution (1): dilute, if necessary, the injection solution in 0.9% (w/v) sodium chloride solution to obtain a 0.1% (w/v) cisplatin solution.

Solution (2): 0.1% (w/v) cisplatin CRS solution in 0.9% (w/v) sodium chloride solution.

Solution (3): 0.05% (w/v) cisplatin CRS solution and 0.005% (w/v) transplatin solution in 0.9% (w/v) sodium chloride solution.

Inject 10 µL of *Solution (3)*. The resolution between the peaks of cisplatin and transplatin is no less than 3.5. Inject 10 µL replicates of the *Solution (2)*. The relative standard deviation of the areas is no more than 2.0%.

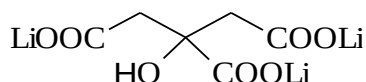
Procedure: inject, separately, 10 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$ in the injection solution from the responses obtained with *Solution (1)* and *Solution (2)*.

PACKAGING AND STORAGE

In tightly closed containers protected from light. It must not be refrigerated.

LABELING

Comply with current legislation.

LITHIUM CITRATE*Lithii citras*C₆H₅Li₃O₇; 209.92

lithium citrate; 09575

2-hydroxy-1,2,3-propanetricarboxylic acid lithium salt (3:1)

[919-16-4]

C₆H₅Li₃O₇.4H₂O; 281.98

lithium citrate tetrahydrate; 11385

Hydrated 2-hydroxy-1,2,3-propanetricarboxylic acid lithium salt (3:1:4)

[6080-58-6]

Contains no less than 98.0% and no more than 102.0% of C₆H₅Li₃O₇, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white fine crystalline powder.

Solubility. Freely soluble in water, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. Complies with citrate ion reactions **(5.3.1.1)**.

B. Dilute 3 mL of the preparation obtained in *Aspect of the preparation* in 10 mL of water. Add 3 mL of ferric potassium periodate RS A white or yellowish-white precipitate must be formed.

C. When moistened with hydrochloric acid and submitted to non-luminous flame, develops a red color.

PURITY TESTS

Aspect of the preparation. Weigh 10 g of the sample and dissolve in carbon dioxide-free water. Dilute to 100 mL with the same solvent. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

Acidity or alkalinity. Add 0.1 mL of phenolphthalein TS to 10 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.2 mL of 0.1 M hydrochloric acid or 0.2 mL of 0.1 M sodium hydroxide is necessary to promote the indicator turning.

Carbonates. Add, accurately, about 0.5 g of the sample in 5 mL of 6 M acetic acid. A slight effervescence is produced.

Oxalates. Weigh 0.5 g of the sample and dissolve in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of granulated zinc, and heat in water bath for one minute. Allow to stand for two minutes, transfer the liquid to a test tube with 0.25 mL of 1% (w/v) phenylhydrazine hydrochloride and heat until ebullition. Cool down quickly, transfer to a cylinder and add an equal volume of hydrochloric acid and 0.25 mL of potassium ferricyanide RS. Shake and allow to stand for 30 minutes. No pink color developed is more intense than that of the standard prepared in parallel in the same manner, using 4 mL of 0.005% (w/v) oxalic acid. No more than 0.03% (300 ppm).

Chlorides (5.3.2.1). Weigh 3.55 g of the sample and add 40 mL of water. Proceed as described in *Limit test for chlorides*. No more than 0.01% (100 ppm).

Sulfates (5.3.2.2). Weigh 2.4 g of the sample and add 40 mL of water. Proceed as described in *Limit test for sulfates*. No more than 0.05% (500 ppm).

Heavy metals (5.3.2.3). Dissolve 2 g of the sample in 2 mL of 0.1 M hydrochloric acid and dilute to 25 mL with water. No more than 0.001% (10 ppm).

Water (5.2.20.1). Determine in 0.1 g of the sample, leaving in agitation for 15 minutes before starting the titration. The tetrahydrate form must have between 24.0% and 27.0% of water.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Weigh, accurately, about 80 mg of the sample, add 50 mL of glacial acetic acid, heat until approximately 50 °C and cool. Add 0.25 mL of 1-naphtholbenzein TS and titrate with 0.1 M perchloric acid VS until the indicator turns from yellow to green. Each mL of 0.1 M perchloric acid VS is equivalent to 6.997 mg of C₆H₅Li₃O₇.

PACKAGING AND STORAGE

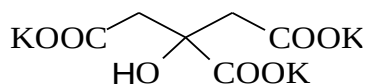
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antidepressant

POTASSIUM CITRATE*Lithii citras*C₆H₅K₃O₇; 306.39

potassium citrate; 02181

2-hydroxy-1,2,3-propanetricarboxylic acid potassium salt (3:1)

[866-84-2]

C₆H₅K₃O₇.H₂O; 324.41

potassium citrate monohydrate; 09373

Hydrated 2-hydroxy-1,2,3-propanetricarboxylic acid potassium salt (3:1:1)

[6100-05-6]

Contains no less than 99.0% and no more than 100.5% of C₆H₅K₃O₇, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, granular powder or colorless crystals.

Solubility. Very soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. The preparation obtained in *Aspect of the preparation* complies with the potassium ion reactions (5.3.1.1).

B. The preparation obtained in *Aspect of the preparation* complies with the citrate ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. Dissolve 10 g of the sample in carbon dioxide-free water, complete the volume to 100 mL with the same solvent and homogenize. The preparation obtained is clear (5.2.25) and colorless (5.2.12).

Alkalinity. The solution, of 1 g of the sample in 20 mL of water, is alkaline to litmus paper. Add to the solution 0.2 mL of 0.05 M sulfuric acid and one drop of phenolphthalein TS. The solution does not acquire a pink color.

Oxalates. Dissolve 0.5 g of the sample in 4 mL of water, add 3 mL of hydrochloric acid, 1 g of granulated zinc and heat in water bath for one minute. Allow to stand for two minutes, transfer the supernatant to a tube with 0.25 mL of 1% (w/v) phenylhydrazine chloride and heat until ebullition. Cool down quickly, transfer to a graduated vial, and add the same volume of hydrochloric acid and 0.25 mL of potassium ferricyanide RS. Homogenize and allow to stand for 30 minutes. Any pink color produced is not more intense than that of the standard preparation obtained in the same conditions, using 4 mL of 0.005% (w/v) oxalic acid. No more than 0.03% (300 ppm).

Sodium. Proceed as described in *Atomic emission spectrometry (5.2.13.2)*. Dissolve 1 g of the sample in water and dilute to 100 mL with the same solvent. Prepare the standard solution using standard sodium solution (200 ppm Na). Dilute if necessary. Measure the intensity of emission at 589 nm. No more than 0.3% (3000 ppm).

Tartrate. Add to 1 g of the sample 1.5 mL of water and 1 mL of 6 M acetic acid to a test tube. Scratch the tube wall with a glass rod. No crystalline precipitate is formed.

Calcium (5.3.2.7). Dilute 5 mL of the preparation obtained in *Aspect of the preparation* to 15 mL with diluted acetic acid and proceed as described in *Limit test for calcium*. Prepare the standard solution using a mixture of 5 mL of *Standard calcium solution (10 ppm)* and 10 mL of water. No more than 0.01% (100 ppm).

Chlorides (5.3.2.1). Determine in 7 g of the sample. No more than 0.005% (50 ppm).

Iron (5.3.2.1). Use 10 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.002% (20 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve 2 g of the sample in 25 mL of water and proceed as described in *Limit test for heavy metals*, without the need to adjust pH. No more than 0.001% (10 ppm).

Easily carbonizable substances. Add to 0.2 g of the powdered sample 10 mL of sulfuric acid and heat in water bath at 90 °C for one hour. Cool quickly. The color of the solution is not more intense than that of the mixture of 75 mL of the *Standard color solution SC F (5.2.12)* and 25 mL of 1% (w/v) hydrochloric acid, or the mixture of 15 mL of the *Standard color solution SC O* and 85 mL of 1% (w/v) hydrochloric acid.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 180 °C for four hours. Between 3% and 6%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve approximately 0.2 g of the sample, accurately weighed, in 25 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS, using two drops of methylrosanilinium chloride TS as indicator, until turning green. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 10.213 mg of C₆H₅K₃O₇.

PACKAGING AND STORAGE

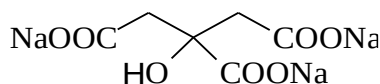
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiuro lithic, antacid.

SODIUM CITRATE*Natrii citras* $C_6H_5Na_3O_7$; 258.07

sodium citrate; 02182

2-hydroxy-1,2,3-propanetricarboxylic acid sodium salt (3:1)

[68-04-2]

 $C_6H_5Na_3O_7 \cdot 2H_2O$; 294.10

sodium citrate dihydrate; 02183

Hydrated 2-hydroxy-1,2,3-propanetricarboxylic acid sodium salt (3:1:2)

[6132-04-3]

Contains no less than 99.0% and no more than 100.5% of $C_6H_5Na_3O_7$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or white crystals.

Solubility. The hydrated form is freely soluble in water and very soluble in water in ebullition, insoluble in ethyl alcohol.

IDENTIFICATION

A. A 1:20 solution complies with sodium ion reactions (5.3.1.1).

B. A 1:20 solution complies with citrate ion reactions (5.3.1.1).

C. After incineration, it results in alkaline residue that effervesces when treated with diluted hydrochloric acid.

PURITY TESTS

Acidity or alkalinity. A solution of 1 g of the sample in 20 mL of sample is alkaline to litmus paper, but after the addition of 0.2 mL of 0.05 M sulfuric acid a pink color is not produced by one drop of phenolphthalein TS.

Heavy metals (5.3.2.3). Dissolve 2 g of the sample in 25 mL of water. No more than 0.001% (10 ppm).

Tartrate (5.3.1.1). Dissolve 1 g of the sample in 2 mL of water, add 1 mL of potassium acetate RS and 1 mL of 6 M acetic acid. Rub the tube wall with a glass rod; no crystalline precipitate is formed.

Loss on drying (5.2.9.1). Dry at 180°C for 18 hours. No more than 1% for the anhydrous form and no more than 10% to 13% for the hydrated form.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Weigh, accurately, about 350 mg of the sample, previously desiccated, transfer to a 250 mL beaker and dissolve in 100 mL of glacial acetic acid. Shake until dissolving completely. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 8.602 mg of $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$.

PACKAGING AND STORAGE

In hermetic containers.

LABELING

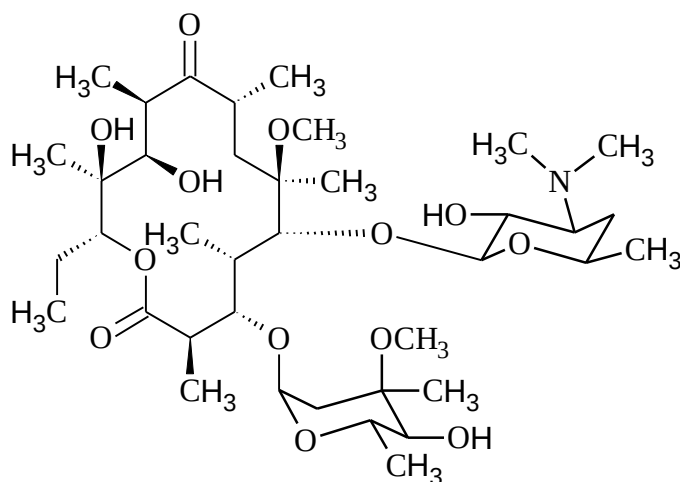
Comply with current legislation.

THERAPEUTIC CLASS

Systemic alkalinizing agent.

CLARITHROMYCIN

Clarithromycinum



$C_{38}H_{69}NO_{13}$; 747.95

clarithromycin; 02200

6-*O*-Methylethromycin

[81103-11-9]

Contains no less than 960 µg and no more than 1020 µg of clarithromycin ($C_{38}H_{69}NO_{13}$) per milligram, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Practically insoluble in water, moderately soluble in acetonitrile and slightly soluble in absolute ethyl alcohol and methyl alcohol. Slightly soluble in phosphate buffers with pH between 2.0 and 5.0.

Physicochemical constants.

Specific optical rotation (5.2.8): between -87 and -97, in relation to the anhydrous substance. Determine in solution at 1% (w/v) in chloroform, at 20 °C.

Melting range (5.2.2): 217 °C to 225 °C, with decomposition.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of clarithromycin CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

pH (5.2.19). 7.5 to 10.0. Determine in suspension at 0.2% (w/v) in a mixture of water and methyl alcohol (19:1).

Heavy metals (5.3.2.3). Use *Method II*. No more than 0.002% (20 ppm).

Water (5.2.20.1). No more than 2.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Moisten the sample with 2 mL of nitric acid and five drops of sulfuric acid. No more than 0.3%.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 210 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at temperature of 50 °C; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.067 M monobasic potassium phosphate (65:35). Adjust pH to 4.0 using phosphoric acid, if necessary.

Sample solution: transfer 50 mg of the sample to a 50 mL volumetric flask, add 35 mL of methyl alcohol, leave in ultrasonic bath for 30 minutes and complete the volume with the same solvent. Homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize, obtaining a solution at 0.2 mg/mL.

Standard solution: transfer 50 mg of clarithromycin CRS to a 50 mL volumetric flask, add 35 mL of methyl alcohol, leave in ultrasonic bath for 30 minutes and complete the volume with the same solvent. Homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize, obtaining a solution at 0.2 mg/mL.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the clarithromycin content ($C_{38}H_{69}NO_{13}$) per milligram of the sample from the content of the standard and the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

CLARITHROMYCIN TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of clarithromycin ($C_{38}H_{69}NO_{13}$). The tablets can be coated.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Proceed as described in *Assay*, using the solution described next as *Sample solution*. Transfer each tablet to a 250 mL volumetric flask, add 100 mL of 0.067 M monobasic potassium phosphate (if necessary, adjust pH to 4.0 with phosphoric acid) and wait until the total disintegration of the tablet. Add 130 mL of methyl alcohol and leave in ultrasonic bath for 30 minutes. Shake mechanically for 30 minutes. Complete the volume with methyl alcohol, homogenize and filter. Transfer a volume equivalent to 5 mg of the sample to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M sodium acetate buffer pH 5.0, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute, if necessary, in *Mobile phase*, to obtain a concentration of approximately 0.02% (w/v). Proceed as described in *Assay*. Calculate the amount of $C_{38}H_{69}NO_{13}$ dissolved in the medium, from the potency of clarithromycin CRS and the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{38}H_{69}NO_{13}$ dissolve in 30 minutes.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 110 °C, under reduced pressure, for three hours. No more than 6%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Assay* in the *Clarithromycin* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 50 mg of clarithromycin to a 50 mL volumetric flask, add 35 mL of methyl alcohol and leave in ultrasonic bath for 30 minutes. Shake mechanically for 30 minutes. Complete the volume with methyl alcohol. Homogenize and filter. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize, obtaining a solution at 200 µg/mL.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of clarithromycin ($C_{38}H_{69}NO_{13}$) in the tablets from the potency of clarithromycin CRS and the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation

CLARITHROMYCIN POWDER FOR ORAL SUSPENSION

Contains no less than 90.0% and no more than 115.0% of the declared amount of clarithromycin ($C_{38}H_{69}NO_{13}$). It may contain dispersing, diluent, preservative and flavoring agents.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to that from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test. Determine in the diluent vial.

pH (5.2.19). 4.0 to 5.4. Determine in the reconstituted suspension as indicated on the label.

Determination of weight (5.1.1). Complies with the test. Determine in the non-reconstituted powder.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for three hours. No more than 2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 210 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), maintained at 50 °C; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.067 M monobasic potassium phosphate (60:40). Adjust pH to 3.5 with phosphoric acid, if necessary.

Sample solution: reconstitute the suspension as described on the product label. Transfer a volume of suspension equivalent to 0.5 g of clarithromycin to a 250 mL volumetric flask with 100 mL of 0.067 M monobasic potassium phosphate. Shake mechanically for 30 minutes. Add 130 mL of methyl alcohol and leave in ultrasonic bath for 60 minutes, shaking regularly. Cool until room temperature. Complete the volume with methyl alcohol, homogenize and filter. Transfer 5 mL of the filtrate to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard stock solution: transfer 50 mg of clarithromycin CRS to a 25 mL volumetric flask, add 20 mL of methyl alcohol, leave in ultrasonic bath for 30 minutes, complete the volume with the same solvent and homogenize, to obtain a 2 mg/mL solution.

Standard solution: transfer 5 mL of the *Standard stock solution* to a 25 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

The column efficiency, determined from the responses obtained for clarithromycin with the *Standard solution*, is no less than 750 theoretical plates/column. The tailing factor is between 1.0 and 1.7 and the retention factor is between 2.5 and 6.0. The relative standard deviation of the replicate areas from the recorded peaks must be of no more than 2.0%.

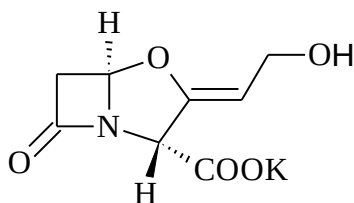
Procedure: inject, separately, 50 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of clarithromycin ($C_{38}H_{69}NO_{13}$) in the oral suspension reconstituted from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

POTASSIUM CLAVULANATE*Kalii clavulanas*C₈H₈KNO₅; 237.25

potassium clavulanate; 00137

(2*R*,3*Z*,5*R*)-3-(2-hydroxyethylidene)- 7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

potassium salt (1:1)

[61177-45-5]

Contains no less than 75.5% and no more than 92.0% of clavulanic acid (C₈H₈KNO₅), in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline, hygroscopic powder.

Solubility. Very soluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +53 to +63, in relation to the anhydrous substance. Determine in 2% (w/v) solution in carbon dioxide-free water.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of potassium clavulanate CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.1% (w/v) solution in phosphate buffer pH 7.0, there is maximum absorption at 278 nm. The absorbance at 278 nm is of approximately 0.40.

C. Complies with potassium ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5.5 to 8.0. Determine in 1% (w/v) solution in carbon dioxide-free water.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 230 nm; column with 100 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at temperature of 40 °C; *Mobile phase* flow of 1.0 mL/minute.

Eluent A: dissolve 7.8 g of monobasic sodium phosphate in 900 mL of water. Adjust the pH to 4.0 with phosphoric acid, complete the volume to 1000 mL with water and homogenize.

Eluent B: mixture of methyl alcohol and *Eluent A* (50:50).

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 4	100	0	isocratic
4 – 15	100 → 50	0 → 50	linear gradient
15 – 18	50	50	isocratic
18 – 24	50 → 100	50 → 0	linear gradient
24 – 39	100	0	isocratic

Solution (1): 10 mg/mL sample solution, in *Eluent A*.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with *Eluent A*.

Solution (3): dissolve 10 mg of lithium clavulanate CRS and 10 mg of amoxicillin trihydrate CRS in *Eluent A*, complete the volume to 100 mL with the same solvent and homogenize.

Procedure: inject separately 20 µL of each solution. Record the chromatograms and measure the areas under the peaks. The sum of the areas under all secondary peaks obtained with the *Solution (1)* is no more than double the area under the main peak, obtained with *Solution (2)* (2.0%), and no area under a peak is greater than that of the main peak obtained with *Solution (2)* (1.0%). Disregard peaks with areas below 0.05 times the area under the main peak obtained with *Solution (2)* (0.05%). The test will only be valid if the chromatogram obtained with *Solution (3)* presents a resolution between the peaks of clavulanate and amoxicillin of no less than 13.

Limit of aliphatic amines. Proceed as described in *Gas chromatography (5.2.17.5)*. Use a chromatograph equipped with a flame ionization detector; capillary column with 50 m of length and 0.53 mm of internal diameter, packed with polydimethylsiloxane, with film thickness of 5 µm; column temperature of 35 °C in the first seven minutes, 35 °C to 150 °C from seven minutes to 10.8 minutes, and 150 °C from 10.8 minutes to 25.8 minutes); injector temperature of 200 °C; detector temperature of 250 °C; use helium as carrier gas; 8.0 mL/minute carrier gas flow.

Internal standard solution: dissolve 50 µL of 3-methyl-2-pentanone in water and dilute to 100 mL with the same solvent.

Sample solution: transfer 1 g of the sample to a centrifuge tube and add 5 mL of *Internal standard solution*, 5 mL of 2 M sodium hydroxide, 10 mL of water, 5 mL of isopropyl alcohol and 5 g of sodium chloride. Shake vigorously for one minute and centrifuge for layer separation.

Standard solution: dissolve 80 mg of each one of the amines: 1,1-dimethylethylamine, diethylamine, tetramethylethylenediamine, 1,1,3,3-tetramethylbutylamine, *N,N'*-diisopropylethylenediamine and 2,2'-oxybis(*N,N*-dimethylethylamine) in 2 M hydrochloric acid and dilute to 200 mL with the same solvent. Transfer 5 g of the solution obtained to a centrifuge tube and add 5 mL of *Internal*

standard solution, 10 mL of 2 M sodium hydroxide, 5 mL of isopropyl alcohol and 5 g of sodium chloride. Shake vigorously for one minute and centrifuge for layer separation.

Inject, separately, 1 µL of the upper layer of the *Standard solution* and the *Sample solution*. The retention time of 3-methyl-2-pentanone is of approximately 11.4 minutes. The relative retention times for amines in relation to 3-methyl-2-pentanone are approximately 0.55 for 1,1-dimethylethylamine, 0.76 for diethylamine, 1.07 for tetramethylethylenediamine, 1.13 for 1,1,3,3-tetramethylbutylamine, 1.33 for *N,N'*-diisopropylethylenediamine and 1.57 for 2,2'-oxybis(*N,N*-dimethylethylamine).

Procedure: inject, separately, 1 µL of the upper layer of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. No more than 0.2% of aliphatic amines.

Water (5.2.20.1). Determine in 1 g of the sample. No more than 1.5%.

BIOLOGICAL SAFETY TESTS

When it is indicated on the label that the substance is sterile or when it is targeted at production of parenteral preparations, the sample complies with the tests.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.03 EU/mg of potassium clavulanate.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 300 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow of 1.6 mL/minute.

Buffer pH 4.4: dissolve 7.8 g of monobasic sodium phosphate in 900 mL of water. Adjust the pH to 4.4 ± 0.1 with phosphoric acid or 10 M sodium hydroxide, complete the volume to 1000 mL with water and homogenize.

Mobile phase: mixture of *Buffer pH 4.4* and methyl alcohol (95:5).

Sample solution: weigh, accurately, about 50 mg of the sample and transfer to a 200 mL volumetric flask. Dissolve in water, complete the volume with the same solvent and homogenize.

Standard solution: 0.25 mg/mL lithium clavulanate CRS solution in water.

Resolution solution: solution with 0.25 mg/mL lithium clavulanate CRS and 0.5 mg/mL amoxicillin trihydrate CRS in water.

Inject 20 µL replicates of the *Resolution solution*. The column efficiency is no less than 550 theoretical plates. The relative retention times are of approximately 0.5 for clavulanic acid and 1.0 for amoxicillin. The tailing factor is no more than 1.5. The resolution between clavulanic acid and

amoxicillin is no less than 3.5. The relative standard deviation of the replicate areas of the recorded peaks is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the area under the peaks. Calculate the content of clavulanic acid (C₈H₉NO₅) in the sample from the responses obtained with the *Standard solution* and with the *Sample solution*.

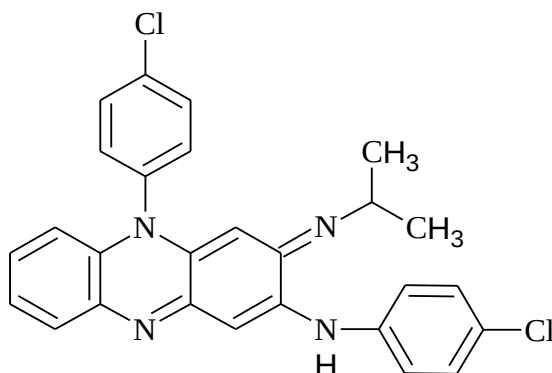
PACKAGING AND STORAGE

In hermetic containers, protected from light, at temperature between 2 °C and 8 °C.

THERAPEUTIC CLASS

Beta-lactamase inhibitor.

CLOFAZIMINE
Clofaziminum



$C_{27}H_{22}Cl_2N_4$; 473.40

clofazimine; 02268

N,5-Bis(4-chlorophenyl)-3,5-dihydro-3-[(1-methylethyl)imino]-2-phenazinamine

[2030-63-9]

Contains no less than 98.5% and no more than 101.5% of $C_{27}H_{22}Cl_2N_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Dark red crystalline powder.

Solubility. Insoluble in water and slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 217 °C to 219 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the 5% (w/v) sample solution in methylene chloride, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of clofazimine CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in 0.1 M methanolic hydrochloric acid, there are maximum and minimum points only at the same wavelengths of clofazimine CRS, prepared identically.

C. The main stain of the chromatogram from *Solution (1)*, obtained in *Related substances*, corresponds in position and color to the one obtained with *Solution (2)*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel HF₂₅₄ as support, and a mixture of methylene chloride and *n*-propanol (10:1) as mobile phase. Expose the plate to ammonia vapors for 30 minutes immediately before use, suspending the plate in a chamber with a surface layer of approximately 25 mL of freshly prepared 1% (v/v) ammonia solution, avoiding that the plate comes into contact with the liquid. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve an accurately weighed amount of the sample in methylene chloride to obtain a 50 mg/mL solution.

Solution (2): dissolve an accurately weighed amount of clofazimine CRS in methylene chloride, to obtain a 0.5 mg/mL solution.

Solution (3): dilute the *Solution (2)* in methylene chloride to obtain a 0.25 mg/mL solution.

Solution (4): dilute the *Solution (2)* in methylene chloride to obtain a 0.1 mg/mL solution.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram with *Solution (2)* (1.0%). The sum of the intensities of secondary stains obtained in the chromatogram with *Solution (1)* is no more than 2.0%.

Heavy metals (5.3.2.3). Proceed as described in *Method IV*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for three hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve about 0.3 g of the sample previously desiccated, accurately weighed, in 5 mL of chloroform. Heat carefully, if necessary. Add 20 mL of acetone and 5 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS and determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 47.340 mg of C₂₇H₂₂Cl₂N₄.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

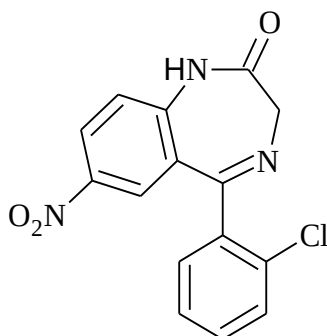
Comply with current legislation.

THERAPEUTIC CLASS

Leprostatic.

CLONAZEPAM

Clonazepamum



$C_{15}H_{10}ClN_3O_3$; 315.71

clonazepam; 02300

5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepin-2-one

[1622-61-3]

Contains no less than 98.0% and no more than 102.0% of $C_{15}H_{10}ClN_3O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Slightly yellowish crystalline powder.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol and in methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 237 °C to 240 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of clonazepam CRS, prepared identically.

PURITY TESTS

Limit of 2-bromo-2'-(2-chlorobenzoyl)-4'nitroacetanilide. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel as support and a mixture of acetone and *n*-heptane (3:2) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): 25 mg/mL sample solution in acetone.

Solution (2): 0.05 mg/mL 2-bromo-2'-(2-chlorobenzoyl)-4'nitroacetanilide (*impurity C* CRS) solution in acetone.

Develop the chromatogram. Remove the plate and allow to air dry. Nebulize with 2 *M* sulfuric acid and dry the plate in oven at 105 °C for 15 minutes. Pulverize the plate, successively, with 0.01 *M* sodium nitrate solution, 0.1% (w/v) ammonium sulfamate solution and *N*-(1-

naphthyl)ethylenediamine dihydrochloride RS solution. Allow the plate to air dry. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.2%).

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Buffer solution pH 8.0: dissolve 6.6 g of ammonium phosphate dibasic anhydrous in 900 mL of water, adjust pH to 8.0 ± 0.1 with *M* phosphoric acid or *M* sodium hydroxide, dilute to 1000 mL with water and homogenize.

Mobile phase: mixture of *Buffer solution pH 8.0*, methyl alcohol and tetrahydrofuran (60:52:13). Filter and degas.

Diluent: mixture of water, methyl alcohol and tetrahydrofuran (60:52:13).

Solution (1): transfer, quantitatively, approximately 25 mg of the sample to a 25 mL volumetric flask, complete the volume with methyl alcohol and homogenize. Carry out the dilution with the *Diluent*, to obtain a solution with 0.1 mg/mL.

Solution (2): transfer 10 mg of 3-amino-4-(2-chlorophenyl)-6-nitrocarbostyryl (*Clonazepam impurity A* CRS), 10 mg of 2-amino-2'-chloro-5-nitrobenzophenone (*Clonazepam impurity B*) and Clonazepam CRS to a 25 mL volumetric flask, complete the volume with methyl alcohol and homogenize. Transfer 1 mL to a 10 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Procedure: separately inject 50 µL of the *Solution (1)* and of *Solution (2)*. Record the chromatograms and measure the areas of all peaks. Calculate the percentage of each impurity in the sample, using the following formula:

$$100Pr_i / (r_c + \sum Pr_i)$$

where

P = 1.84 (relative response factor for 3-amino-4-(2-chlorophenyl)-6-nitrocarbostyryl (*Clonazepam impurity A* CRS)); 0.94 (relative response factor for 2-amino-2'-chloro-5-nitrobenzophenone (*Clonazepam impurity B*)) and 1 for the other impurities;

r_i = area under the peak of each impurity obtained from *Solution (1)*;

r_c = area under the clonazepam peak obtained from *Solution (1)*.

The area of individual peaks of 3-amino-4-(2-chlorophenyl)-6-nitrocarbostyryl (*Clonazepam impurity A* CRS) and 2-amino-2'-chloro-5-nitrobenzophenone (*Clonazepam impurity B*), obtained with *Solution (1)*, is no more than 0.2%. Neither the area of any individual impurity nor the sum of the areas under the secondary peaks obtained with *Solution (1)* is superior to 0.3%. Do not include solvent-related peaks in the calculations.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C, under reduced pressure, for four hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Dissolve 0.275 g of the sample previously desiccated in 50 mL of acetic anhydride and titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 31.57 mg of $C_{15}H_{10}ClN_3O_3$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use the method described in *Related substances*, with the following modifications.

Standard solution: transfer, quantitatively, approximately 25 mg of clonazepam CRS to a 25 mL volumetric flask, complete the volume with methyl alcohol and homogenize. Carry out the dilution with the *Diluent*, to obtain a solution at 0.1 mg/mL.

Sample solution: transfer, quantitatively, approximately 25 mg of the sample to a 25 mL volumetric flask, complete the volume with methyl alcohol and homogenize. Carry out the dilution with the *Diluent*, to obtain a solution at 0.1 mg/mL.

Resolution solution: transfer 10 mg of 3-amino-4-(2-chlorophenyl)-6-nitrocarbostyryl (*Clonazepam impurity A* CRS), 10 mg of 2-amino-2'-chloro-5-nitrobenzophenone (*Clonazepam impurity B*) and Clonazepam CRS to a 25 mL volumetric flask, complete the volume with methyl alcohol and homogenize. Transfer 1 mL to a 10 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Inject, separately, 50 μ L replicates of the *Resolution solution* and of *Solution (1)*. The relative retention times are 2.2, 2.5 and 1 for 3-amino-4-(2-chlorophenyl)-6-nitrocarbostyryl (*Clonazepam impurity A*); 2-amino-2'-chloro-5-nitrobenzophenone (*Clonazepam impurity B*) and Clonazepam CRS, respectively. The resolution between the peaks of 3-amino-4-(2-chlorophenyl)-6-nitrocarbostyryl (*Clonazepam impurity A*) and 2-amino-2'-chloro-5-nitrobenzophenone (*Clonazepam impurity B*) is no more than 2.0. The tailing factor is not more than 1.5 and the relative standard deviation of the replicate areas under the recorded peaks of clonazepam hydrochloride is not more than 2.0%.

Procedure: inject, separately, 50 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of clonazepam ($C_{15}H_{10}ClN_3O_3$) in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Benzodiazepine.

CLONAZEPAM TABLETS

Contains no less than 95.0% and no more than 110.0% of the declared amount of $C_{15}H_{10}ClN_3O_3$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 10 mg of clonazepam to a 125 mL separation funnel. Add 25 mL of water, shake for two minutes and extract with two portions of 40 mL of chloroform. Filter the organic extracts using anhydrous sodium sulfate, combine them and evaporate at room temperature, using nitrogen flow. Wash the residue with three portions of 10 mL of hexane. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the clonazepam CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 60 minutes.

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at temperature of 25 °C; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of water, methyl alcohol and acetonitrile (40:30:30).

Sample solution: after conducting the test, take aliquots from the dissolution medium.

Standard solution: dissolve an accurately weighed amount of clonazepam CRS in methyl alcohol, to obtain a 0.05 mg/mL solution. Dilute, successively, with dissolution medium, to obtain a concentration similar to that obtained in the dissolution tanks after the test.

Procedure: inject, separately, 100 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{15}H_{10}ClN_3O_3$ dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{15}H_{10}ClN_3O_3$ dissolve in 60 minutes.

PURITY TEST

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of ethyl acetate and carbon tetrachloride (1:1) as mobile phase. Apply to the plate, separately, 25 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Shake, for one minute, an amount of powder equivalent to 10 mg of clonazepam in 20 mL of acetone. Filter and evaporate to dryness. Dissolve the residue in 0.5 mL of acetone.

Solution (2): prepare a solution of 3-amino-4-(2-chlorophenyl)- 6-nitroquinolin-2(1*H*)-one CRS at 0.2 mg/mL in acetone.

Solution (3): prepare a solution of (2-amino-5-nitrophenyl) (2-chlorophenyl) methanone CRS at 0.2 mg/mL in acetone.

Develop the chromatogram. Remove the plate, allow to dry under a dry air stream and observe under ultraviolet light (254 nm). Nebulize the plate with 10% (v/v) sulfuric acid and heat at 105 °C for 15 minutes. Nebulize with 0.1% (w/v) sodium nitrite and dry under hot air stream. Nebulize with 0.5% (w/v) ammonium sulfamate and dry under hot air stream. Any stains obtained in the chromatogram with *Solution (1)*, different from the main stain, are not more intense than those obtained with *Solution (2)* (1.0%). Observe the reduction in the intensity of fluorescence in the plate. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solutions (2)* and *(3)*.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow of 0.6 mL/minute.

Ammonium phosphate buffer: transfer 6.6 g of ammonium phosphate dibasic to a 1000 mL volumetric flask and add 950 mL of water. Adjust the pH to 8.0 with 0.33 M phosphoric acid or M sodium hydroxide, complete the volume with water and homogenize.

Mobile phase: mixture of *Ammonium phosphate buffer*, methyl alcohol and tetrahydrofuran (60:52:13). Filter and degas.

Diluent: mixture of water, methyl alcohol and tetrahydrofuran (60:52:13).

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 5 mg of clonazepam to a 50 mL volumetric flask. Dissolve, initially, with 20 mL of methyl alcohol. Leave in ultrasonic bath for 15 minutes and complete the volume with *Diluent*. Homogenize and filter.

Standard solution: transfer 10 mg of clonazepam CRS to a 100 mL volumetric flask. Dissolve, initially, with 75 mL of methyl alcohol. Leave in an ultrasonic bath for approximately 15 minutes. Complete the volume with *Diluent* to obtain a 0.1 mg/mL solution. Homogenize and filter.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{15}H_{10}ClN_3O_3$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed amber glass containers, at temperature below 25 °C.

LABELING

Comply with current legislation.

CLONAZEPAM ORAL SOLUTION

Contains no less than 95% and no more than 115% of the declared amount of $C_{15}H_{10}ClN_3O_3$.

IDENTIFICATION

Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of toluene, ethyl acetate and anhydrous formic acid (60:40:5) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dilute in a centrifuge tube 2 mL of the sample with 10 mL of 0.1 M hydrochloric acid. Add 1 g of sodium chloride and shake until dissolution, for approximately two minutes. Add 5 mL of ethyl acetate, shake for three minutes and centrifuge for another three minutes. Use the organic phase.

Solution (2): dissolve 20 mg of clonazepam CRS in 20 mL of ethyl acetate.

Develop the chromatogram. Remove the plate, allow to dry under a dry air stream for 10 minutes and observe under ultraviolet light (254 nm). A reduction in the intensity of fluorescence in the plate is observed. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.6 to 4.1. Determine in solution at 50% (v/v) of the sample in water.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Assay* in the *Clonazepam tablets* monograph. Prepare the *Sample solution* as described next.

Sample solution: transfer to a 50 mL volumetric flask a volume of oral solution containing the equivalent to 5 mg of clonazepam, to obtain a 100 µg/mL solution. Dissolve, initially, in 20 mL of methyl alcohol, take to an ultrasonic bath for 15 minutes and complete the volume with *Diluent*. Homogenize and filter.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{15}H_{10}ClN_3O_3$ in the oral solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

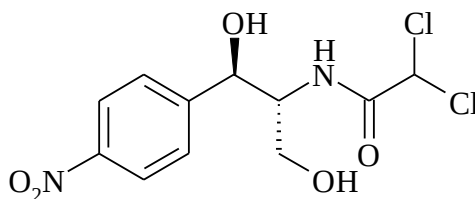
In tightly closed amber glass containers, at temperature below 25 °C.

LABELING

Comply with current legislation.

CHLORAMPHENICOL

Chloramphenicolum



$C_{11}H_{12}Cl_2N_2O_5$; 323.13

chloramphenicol; 02336

2,2-dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]acetamide

[56-75-7]

Contains no less than 97.0% and no more than 103.0% of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$).

DESCRIPTION

Physical characteristics. White, greyish-white or yellowish-white fine powder, or crystals in the shape of needles or elongated plates. Its solutions are practically neutral in litmus paper. Reasonably stable in neutral or moderately acidic solutions. It is dextrorotatory in ethylic solutions and levorotatory in ethyl acetate.

Solubility. Slightly soluble in water. Freely soluble in ethyl alcohol and propylene glycol.

Physicochemical constants.

Melting range (5.2.2): 149 °C to 153 °C.

Specific optical rotation (5.2.8): +17.0 to +20.0, in relation to the desiccated substance. Determine in a 50.0 mg/mL solution in ethyl alcohol anhydrous.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of chloramphenicol CRS, prepared identically.

B. The main stain of the chromatogram from *Sample solution*, obtained in *Related substances*, corresponds in position and size to the one obtained with *Solution (1)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay* method, corresponds to the one from the main peak of the *Standard solution*.

D. Transfer 50 mg of the sample to a porcelain crucible and add 0.5 g of sodium carbonate anhydrous. Heat in oven and open the flame for 10 minutes. Cool. Dissolve the residue with 5 mL of nitric acid and filter. Add 1 mL of water to 1 mL of the filtrate. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19) 4.5 to 7.5. Determine in aqueous suspension at 25 mg/mL.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and a mixture of water, methyl alcohol and chloroform (1:10:90) as mobile phase. Apply, separately, to the plate 1 µL and 20 µL of *Sample solution*, 1 µL of *Solution (1)* and 20 µL of *Solution (2)*, freshly prepared, described below.

Sample solution: 10.0 mg/mL sample solution in acetone.

Solution (1): dissolve an accurately weighed amount of chloramphenicol CRS in acetone to obtain a 10.0 mg/mL solution.

Solution (2): dilute the ideal amount of *Solution (1)* with acetone in volumetric flask to obtain a 50.0 µg/mL solution.

Develop the chromatogram along 15 cm. Remove the plate, allow to air dry and examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with 20 µL of the *Sample solution*, different from the main stain, is not more intense than the stain obtained with *Solution (2)* (0.5%).

Crystallinity. Suspend a few particles of the sample in mineral oil, transfer to a glass slide and examine on a microscope equipped with polarized light. The particles display birefringence, which is extinguished when moving the sample through micrometric adjustment.

Chlorides (5.3.2.1). Add to 1.00 g of the sample 20 mL of water and 10 mL of nitric acid. Shake for five minutes. Filter, in a filter washed in advance with 5 mL of water, until no opalescence is produced in 5 mL of the filtrate with addition of 0.1 mL of nitric acid and 0.1 mL of silver nitrate RS. A volume of 15 mL of the filtrate complies with the *Limit test for chlorides*. No more than 0.01% (100 ppm).

Loss on drying (5.2.9.1). Determine in 1.00 g of the sample. Dry at 105 °C. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 2.00 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TEST

When it is indicated on the label that the substance is sterile, the sample complies with the Pyrogen or Bacterial endotoxin test and with the Sterility test. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Pyrogen or Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test. Proceed as described in *Membrane filtration method*. Use 1 g of the sample.

Bacterial endotoxins (5.5.2.2). No more than 0.2 EU per mg of chloramphenicol.

Pyrogens (5.5.2.1). Complies with the test. Inject 2.5 mL/kg, using a 2 mg/mL chloramphenicol solution in water for injection.

ASSAY

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 280 nm; column with 100 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: prepare a mixture of water, methyl alcohol and glacial acetic acid (55:45:0.1). Make adjustments, if necessary.

Sample solution: transfer approximately 20 mg of the sample to a 100 mL volumetric flask, add *Mobile phase* until completing the volume and homogenize. Transfer 4.0 mL of this preparation to a 100 mL volumetric flask, dilute with *Mobile phase* and homogenize. Filter a portion of this preparation in a filter with porosity of 0.5 µm or less, and use the filtrate.

Standard solution: dissolve an accurately weighed amount of chloramphenicol CRS in *Mobile phase* and dilute adequately to obtain a solution at 80 µg/mL. Filter a portion of this preparation in a filter with porosity of 0.5 µm or less and use the filtrate.

Inject 10 µL replicates of the *Standard solution*. The column efficiency, determined with the main peak, is no less than 1800 theoretical plates; the tailing factor is no more than 2.0; and the relative standard deviation of the replicate areas under the recorded chloramphenicol peaks is no more than 1.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of chloramphenicol (C₁₁H₁₂Cl₂N₂O₅) in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers. If sterile, store in sterile, hermetically closed and sealed containers.

LABELING

Comply with current legislation. When targeted at parenteral use or other sterile pharmaceutical dosage forms, the label must declare that it is sterile or must be submitted to additional processing during the preparation of pharmaceutical dosage forms.

THERAPEUTIC CLASS

Antibiotic.

AMMONIUM CHLORIDE***Ammonii chloridum***

NH₄Cl; 53.49
ammonium chloride; 02362
Ammonium chloride
[12125-02-9]

Contains no less than 99.0% and no more than 100.5% of NH₄Cl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or colorless crystals.

Solubility. Freely soluble in water, soluble in glycerol, moderately soluble in ethyl alcohol.

IDENTIFICATION

A. The 0.1% (w/v) sample solution complies with ammonium ion reactions **(5.3.1.1)**.

B. The 0.1% (w/v) sample solution complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Dissolve 10 g of the sample in water, complete to 100 mL with the same solvent and homogenize. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

Acidity or alkalinity. Add 0.05 mL of methyl red TS to 10 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.5 mL of 0.01 M hydrochloric acid or 0.5 mL of 0.01 M sodium hydroxide is necessary to promote the indicator turning.

Bromides and iodides. Add to 10 mL of the preparation obtained in *Aspect of the preparation* 0.1 mL of hydrochloric acid and 0.05 mL of 2% (w/v) chloramine-T. After one minute, add 2 mL of chloroform and shake vigorously. The chloroform phase remains colorless.

Thiocyanate. Acidify 10 mL of the preparation obtained in *Aspect of the preparation* with hydrochloric acid and add a few drops of 9% (w/v) ferric chloride. Orangish-red color is not developed.

Calcium (5.3.2.7). Dilute 5 mL of the preparation obtained in *Aspect of the preparation* with 10 mL of water. No more than 0.02% (200 ppm).

Iron (5.3.2.4). Use *Method I*. Dilute 10 mL of the preparation obtained in *Aspect of the preparation* with 5 mL of water. Use *Standard iron solution (100 ppm Fe)*. No more than 0.002% (20 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Determine in 20 mL of the preparation obtained in *Aspect of the preparation*. No more than 0.001% (10 ppm).

Sulfates (5.3.2.2). Determine in 8 g of the sample. No more than 0.015% (150 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C, for two hours. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 2 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 1 g of the sample, dissolve in 20 mL of water and add a mixture of 20 mL of water and 5 mL of formaldehyde solution, neutralized in advance with the presence of phenolphthalein TS. After one to two minutes, titrate with *M* sodium hydroxide VS using phenolphthalein TS as indicator. Each mL of *M* sodium hydroxide VS is equivalent to 53.490 mg of NH₄Cl.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Systemic acidifier.

CALCIUM CHLORIDE DIHYDRATE

Calcii chloridum dihydricum

CaCl₂·2H₂O; 147.01
calcium chloride dihydrate; 02370
Calcium chloride dihydrate
[10035-04-8]

Contains no less than 97.0% and no more than 103.0% of CaCl₂·2H₂O.

DESCRIPTION

Physical characteristics. White crystalline powder, hygroscopic.

Solubility. Freely soluble in water, soluble in ethyl alcohol.

IDENTIFICATION

A. Dissolve 1 g of the sample in carbon dioxide-free water, complete to 10 mL with the same solvent and homogenize. The solution obtained complies with calcium ion reactions **(5.3.1.1)**.

B. Dissolve 1 g of the sample in carbon dioxide-free water, complete to 10 mL with the same solvent and homogenize. The solution obtained complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Dissolve 10 g of the sample in carbon dioxide-free water, complete to 100 mL with the same solvent and homogenize. The preparation obtained is clear **(5.2.25)** and not more stained than the mixture of 5 mL of the *Standard color solution SC F (5.2.12)* and 95 mL of 1% (w/v) hydrochloric acid.

Acidity or alkalinity. Add 0.1 mL of phenolphthalein TS to 10 mL of the freshly prepared preparation obtained in *Aspect of the preparation*. If the preparation acquires a pink color, it must become colorless by the addition of no more than 0.2 mL of 0.01 M hydrochloric acid. If no color appears, it must become pink by the addition of no more than 0.2 mL of 0.01 M sodium hydroxide.

pH (5.2.19). 4.5 to 9.2. Determine in aqueous solution at 5% (w/v).

Barium. Add 1 mL of calcium sulfate RS to 10 mL of the preparation obtained in *Aspect of the preparation*. After 15 minutes, any opalescence observed is not more intense than that of a mixture of 10 mL of the preparation obtained in *Aspect of the preparation* and 1 mL of water.

Iron, aluminum and phosphate. Dissolve 1 g of the sample in 20 mL of water. Add two drops of 3 M hydrochloric acid and one drop of phenolphthalein TS. Add, drop by drop, ammonium chloride - ammonium hydroxide RS, until a slight pink color appears, and add two drops in excess. Heat to boiling. No turbidity or precipitation occurs.

Magnesium and alkaline metals. Mix 20 mL of the preparation obtained in *Aspect of the preparation* and 80 mL of water. Add 2 g of ammonium chloride and 2 mL of ammonia RS. Heat to ebullition and add a hot solution of 5 g of ammonium oxalate in 75 mL of water. Allow to stand for four hours,

complete to 200 mL with water, homogenize and filter. Add to 100 mL of the filtrate 0.5 mL of sulfuric acid. Evaporate to dryness in water bath and incinerate at 600 °C until constant weight. The residue weight must be no more than 5 mg.

Aluminum (5.3.2.10). Add to 10 mL of the preparation obtained in *Aspect of the preparation* 2 mL of ammonium chloride RS, 1 mL of ammonia RS and boil the preparation. No turbidity or precipitation occurs. If used in the preparation of solutions for dialysis, dissolve 4 g of the sample in 100 mL of water. Add 10 mL of acetate buffer pH 6.0 and proceed as described in *Limit test for aluminum*. Use as standard solution a mixture of 2 mL of *Standard aluminum solution (2 ppm Al)*, 10 mL of acetate buffer pH 6.0 and 98 mL of water. For blank, use a mixture of 10 mL of acetate buffer pH 6.0 and 100 mL of water. No more than 0.0001% (1 ppm).

Arsenic (5.3.2.5). Use *Method I*. Determine in 1 g of the sample. No more than 0.0003% (3 ppm).

Iron (5.3.2.4). Use *Method I*. Use 20 mL of the preparation obtained in *Aspect of the preparation*. Use *Standard iron solution (100 ppm Fe)*. No more than 0.001% (10 ppm).

Sulfates (5.3.2.2). Determine in 4 g of the sample. No more than 0.03% (300 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Use 10 mL of the preparation obtained in *Aspect of the preparation*. Prepare the standard solution using *Standard lead solution (2 ppm Pb)*. No more than 0.002% (20 ppm).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 0.28 g of the sample, dissolve in 100 mL of water and proceed as described in *Complexometric titration for calcium (5.3.3.4)*, using 4 mL of 2 M sodium hydroxide. Each mL of 0.1 M disodium edetate VS is equivalent to 14.702 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation. The label must indicate whether it can be used in the preparation of solutions for dialysis.

THERAPEUTIC CLASS

Electrolytic replenisher. It can be used as diuretic, urine acidifier and antiallergic.

CALCIUM CHLORIDE HEXAHYDRATE*Calcii chloridum hexahydricum*CaCl₂·6H₂O; 219.07

calcium chloride hexahydrate; 02371

Calcium chloride hexahydrate

[7774-34-7]

Contains no less than 97.0% and no more than 103.0% of CaCl₂·6H₂O.

DESCRIPTION

Physical characteristics. Colorless crystals or colorless crystalline mass.**Solubility.** Very soluble in water and ethyl alcohol.**Physicochemical constants.***Melting temperature (5.2.2):* 30 °C.

IDENTIFICATION

A. Dissolve 15 g of the sample in carbon dioxide-free water, complete the volume to 100 mL with the same solvent and homogenize. The solution obtained complies with calcium ion reactions **(5.3.1.1)**.

B. The solution prepared identically to the solution from test **A.** of *Identification* complies with the chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Dissolve 15.0 g of the sample in carbon dioxide-free water, complete the volume to 100 mL with the same solvent and homogenize. The preparation obtained is clear **(5.2.25)** and presents less intense staining than the mixture of 5 mL of the *Standard color solution SC F* described in *Color of liquids (5.2.12)*.

Acidity or alkalinity. Add 0.1 mL of phenolphthalein TS to 10 mL of the freshly prepared preparation obtained in *Aspect of the preparation*. If the preparation acquires a pink color, it must become colorless by the addition of no more than 0.2 mL of 0.01 M hydrochloric acid. If no color appears, it must become pink by the addition of no more than 0.2 mL of 0.01 M sodium hydroxide.

Aluminum. Add to 10 mL of the preparation obtained in *Aspect of the preparation* 2 mL of ammonium chloride RS, 1 mL of 6 M ammonium hydroxide and boil the preparation; no turbidity or precipitation occurs. If used for preparation of solutions for dialysis, dissolve 6 g of the sample in 100 mL of water, add 10 mL of acetate buffer pH 6.0 and proceed as described in *Limit test for aluminum*. Use as standard solution a mixture of 2 mL of *Standard aluminum solution (2 ppm)*, 10 mL of acetate buffer pH 6.0 and 98 mL of water. For blank, use a mixture of 10 mL of acetate buffer pH 6.0 and 100 mL of water. No more than 0.0001% (1 ppm).

Barium. Add 1 mL of calcium sulfate RS to 10 mL of the preparation obtained in *Aspect of the preparation*. After 15 minutes, any opalescence observed is not more intense than that of a mixture of 10 mL of the preparation obtained in *Aspect of the preparation* and 1 mL of water.

Sulfates (5.3.2.2). Determine in 6 g of sample and proceed as described in *Limit test for sulfates*. No more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Use 10 mL of the preparation obtained in *Aspect of the preparation*. Prepare the standard solution using *Standard lead solution (10 ppm)*. No more than 0.002% (20 ppm).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 0.2 g of the sample, dissolve in 100 mL of water and proceed as described in *Complexometric titration for calcium (5.3.3.4)*, using 4 mL of 2 M sodium hydroxide. Each mL of 0.1 M disodium edetate VS is equivalent to 21.908 mg of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$.

PACKAGING AND STORAGE

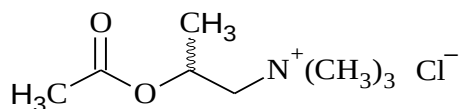
In tightly closed containers.

LABELING

Comply with current legislation. The label must indicate whether it can be used in the preparation of solutions for dialysis.

THERAPEUTIC CLASS

Electrolytic replenisher. It can be used as diuretic, urine acidifier and antiallergic.

METHACHOLINE CHLORIDE*Methacholini chloridum*C₈H₁₈ClNO₂; 195.69

methacholine chloride; 02401

2-(acetyloxy)-*N,N,N*-trimethyl-1-propanaminium chloride (1:1)

[62-51-1]

Contains no less than 98.0% and no more than 101.0% of C₈H₁₈ClNO₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or white or colorless crystals; very hygroscopic.

Solubility. Soluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): transfer 0.1 g of the sample to a glass beaker and dissolve in 3 mL of chloroform. Heat at 110 °C for one hour. Determine the melting range in the powder adhered to the beaker walls. Between 170 °C and 173 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of methacholine chloride CRS, prepared identically.

B. Prepare a 2% (w/v) solution of the sample. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

Acetylcholine chloride. Dissolve 10 mg of the sample in 100 mL of water. Add to 2 mL of this solution 3 mL of 20% (w/v) sodium perchlorate solution, shake and place under ice bath for five minutes. A precipitate is not formed.

Heavy metals (5.3.2.3). Use *Method IV*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 2 g of the sample. Dry in oven at 105 °C, for four hours. No more than 1.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.4 g of the sample, previously desiccated, and dissolve in a mixture of glacial acetic acid and mercury acetate RS (50:10). Titrate with 0.1 M perchloric acid VS, using methylrosanilinium chloride TS as indicator, until a green color appears. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 19.569 mg of $C_8H_{18}ClNO_2$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Cholinergic.

SODIUM CHLORIDE

Natrii chloridum

NaCl; 58.44
sodium chloride; 02421
Sodium chloride
[7647-14-5]

Contains no less than 99.0% and no more than 100.5% of NaCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, fine crystalline powder or colorless crystals.

Solubility. Freely soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. Complies with chloride ion reactions (5.3.1.1).

B. Complies with sodium ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. The preparation at 20% (w/v) in carbon dioxide-free water is clear (5.2.25) and colorless (5.2.12).

Acidity or alkalinity. No more than 0.5 mL of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is spent to neutralize 20 mL of the preparation described in *Aspect of the preparation*, using bromothymol blue TS as indicator.

Barium. Add to 5 mL of the preparation described in *Aspect of the preparation* 5 mL of water and 1 mL of M sulfuric acid. After 15 minutes, any opalescence observed is not more intense than that of a mixture of 5 mL of the preparation described in *Aspect of the preparation* and 7 mL of water.

Bromides. Add to 1.0 mL of the preparation described in *Aspect of the preparation* 4 mL of water, 2 mL of phenol red RS and 1 mL of 0.01% (w/v) chloramine-T, freshly prepared. Homogenize and allow to stand for two minutes. Add 0.15 mL of 0.1 M sodium thiosulfate, homogenize, complete the volume to 10 mL with water and homogenize. The absorbance of this solution (5.2.14), at 590 nm, using water for zero adjustment, is not greater than that of the standard solution, prepared in the same manner, using 2.5 mL of potassium bromide at 3 µg/mL. No more than 0.01% (100 ppm).

Ferricyanides. Dissolve 2 g of the sample in 6 mL of water. Add 0.5 mL of the mixture of 5 mL of 1% (w/v) ferrous ammonium sulfate in 0.05 M sulfuric acid solution and 95 mL of 1% (w/v) ferrous sulfate heptahydrate. A blue color is not developed.

Iodides. Moisten 5 g of the sample by the addition, drop by drop, of a freshly prepared mixture of 0.15 mL of sodium nitrite RS, 2 mL of 0.05 M sulfuric acid, 25 mL of starch TS and 25 mL of water. After five minutes, a blue color is not developed.

Nitrites. Add 10 mL of water to 10 mL of the preparation described in *Aspect of the preparation*. Measure the absorbance (**5.2.14**) of the preparation at 354 nm. The absorbance is no more than 0.01.

Potassium. *Required for sodium chloride targeted at preparation of solutions for parenteral use or solutions for hemodialysis.* Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*. Prepare solutions as described below.

Sample solution: dissolve 1 g of the sample in water and dilute to 100 mL with the same solvent.

Standard solution: dissolve an amount, accurately weighed, of potassium chloride, previously desiccated between 100 °C and 105 °C for three hours, to obtain a 0.1144% (w/v) solution in water. Dilute if necessary.

Measure the intensity of emission at 766.5 nm. No more than 0.05% (500 ppm).

Aluminum (5.3.2.10). *Required for sodium chloride targeted at preparation of solutions for hemodialysis.* Dissolve 20 g of the sample in 100 mL of water and add 10 mL of acetate buffer pH 6.0. Proceed as described in *Limit test for aluminum*. Use a mixture of 2 mL of *Standard aluminum solution (2 ppm)*, 10 mL of acetate buffer pH 6.0 and 98 mL of water as standard solution. Use a mixture of 10 mL of acetate buffer pH 6.0 and 100 mL of water as blank. No more than 0.00002% (0.2 ppm).

Arsenic (5.3.2.5). Use 15 mL of the preparation described in *Aspect of the preparation*. No more than 0.0001% (1 ppm).

Iron (5.3.2.4). Use 25 mL of the preparation described in *Aspect of the preparation*. No more than 0.0002% (2 ppm).

Phosphates (5.3.2.11). Use 2 mL of the preparation described in *Aspect of the preparation* and dilute with water to 100 mL. No more than 0.0025% (25 ppm).

Magnesium and alkaline earth metals (5.3.2.9). Use 10 g of sample. The volume of 0.01 M disodium edetate VS used is no more than 2.5 mL. No more than 0.01% (100 ppm), calculated as calcium.

Heavy metals (5.3.2.3). Use *Method I*. Determine in 20 mL of the preparation described in *Aspect of the preparation*. No more than 0.0005% (5 ppm).

Sulfates (5.3.2.2). Use 6 mL of the preparation described in *Aspect of the preparation*. No more than 0.025% (250 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C, for two hours. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve, accurately, about 50 mg of the sample in water and complete the volume to 50 mL with the same solvent. Titrate with 0.1 M silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 M silver nitrate VS is equivalent to 5.844 mg of NaCl.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Electrolytic replenisher.

SODIUM CHLORIDE INJECTION SOLUTION

Contains no less than 95.0% and no more than 105.0% the declared amount of NaCl. The solution does not contain antimicrobial agents.

IDENTIFICATION

A. Complies with sodium ion reactions (5.3.1.1).

B. Complies with chloride ion reactions (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.5 to 7.0.

PURITY TESTS

Iron (5.3.2.4). Use *Method III*. Dilute 5 mL of the injection solution to 45 mL with water and add 2 mL of hydrochloric acid. No more than 0.0002% (2 ppm).

Heavy metals (5.3.2.3). Transfer a volume of injection solution equivalent to 2 g of sodium chloride to an adequate container; if necessary, evaporate to a volume of 20 mL. Add 2 mL of *M* acetic acid, complete the volume to 25 mL with water and homogenize. Proceed as described on *Method I*, using 2 mL of the *Standard lead solution (10 ppm Pb)* in *Standard preparation* and in *Control preparation*. No more than 0.001% (10 ppm).

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.5 EU/mL, if the declared amount of sodium chloride is between 0.5% and 0.9%, and no more than 3.6 EU/mL, if the declared amount of sodium chloride is between 3.0% and 24.3%.

ASSAY

Transfer a volume of the injection solution with the equivalent to 90 mg of sodium chloride to an Erlenmeyer flask. Add water, if necessary, to obtain a volume of 10 mL. Add 10 mL of glacial acetic acid and 75 mL of methyl alcohol. Titrate with 0.1 *M* silver nitrate VS. Determine the endpoint using eosin Y TS as indicator, until a pink color appears. Each mL of 0.1 *M* silver nitrate VS is equivalent to 5.844 mg of NaCl.

PACKAGING AND STORAGE

In plastic or glass, preferably type I or type II, containers.

LABELING

Comply with current legislation.

ZINC CHLORIDE

Zinci chloridum

ZnCl₂; 136.32
zinc chloride; 02433
Zinc chloride
[7646-85-7]

Contains not less than 95.0% and not more than 100.5% of ZnCl₂.

DESCRIPTION

Physical-chemical characteristics. White or nearly white crystalline powder. Melting temperature (5.2.2): around 318 °C.

Solubility. Very soluble in water, freely soluble in ethyl alcohol and glycerol.

IDENTIFICATION

A. Dissolve 0.5 g of the sample in nitric acid RS and dilute to 10 mL with the same solvent. Complies with chloride ion reactions (5.3.1.1).

B. Add to 2 g of the sample 38 mL of carbon dioxide-free water. Drip hydrochloric acid RS until complete dissolution and dilute to 40 mL with carbon dioxide-free water. Complies with zinc ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.6 to 5.5. Determine in solution with 1 g of the sample in 9 mL of carbon dioxide-free water.

Aluminum, calcium, heavy metals, iron, magnesium. Add to 8 mL of the solution obtained on test B. of *Identification* 2 mL of concentrated ammonia solution and homogenize. The preparation is clear (5.2.25) and colorless (5.2.12). Add 1 mL of sodium phosphate dibasic dodecahydrate RS. The preparation remains clear for, at least, five minutes. Add 0.2 mL of sodium sulfide RS. A white precipitate is produced. The supernatant remains colorless.

Ammonia (5.3.2.6). Use 25 mg. No more than 0.04% (400 ppm).

Sulfates (5.3.2.2). Dissolve 0.8 g of the sample in 10 mL of water and proceed as described in *Limit test for sulfates*. No more than 0.03% (300 ppm).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.25 g of the sample in 5 mL of diluted acetic acid. Proceed as described in *Complexometric titrations (5.3.3.4)* for *Zinc*. Each mL of 0.1 M disodium edetate VS is equivalent to 13.632 mg of ZnCl_2 .

PACKAGING AND STORAGE

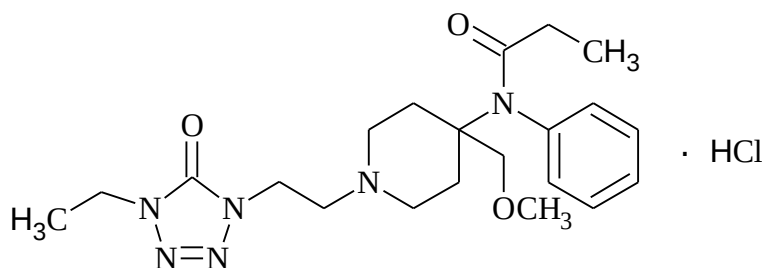
In tightly closed, non-metallic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Nutritional supplement.



This translation does not replace the portuguese version.

Chromatographic purity. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 235 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: mixture of 0.01 M tetrabutylammonium sulfate and acetonitrile (86:14).

Standard solution: dissolve an accurately weighed amount of alfentanil hydrochloride CRS in *Mobile phase* to obtain a final concentration of 0.54 mg/mL.

Sample solution: transfer about 54 mg of alfentanil hydrochloride, accurately weighed, to a 100 mL volumetric flask, dissolve, complete the volume with *Mobile phase* and homogenize.

Procedure: inject, separately, 25 µL of the *Standard solution* and the *Sample solution*, record the chromatogram and measure the areas under the peaks. Calculate the percentage of each impurity of the sample according to the formula:

$$\text{Porcentagem de impurezas} = 100 \times (r_i/r_s)$$

where r_i is the response for each impurity and r_s is the sum of the responses of all peaks. No more than 0.5% of any impurity is found. The sum of all impurities is no more than 1.0%.

Water (5.2.20.1). Use the *Direct method*. Determine in 500 mg of the sample. No more than 4.0%

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%

BIOLOGICAL SAFETY TEST

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

When it is indicated on the label that the substance is sterile, the sample complies with the Bacterial endotoxin test and with the Sterility test. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 18.38 EU/mg of alfentanil hydrochloride.

ASSAY

Weigh, accurately, about 350 mg of alfentanil hydrochloride, dissolve in 50 mL of a mixture of ethyl alcohol and water (1:4) and add 5 mL of 0.01 M hydrochloric acid. Titrate with 0.1 M sodium hydroxide VS, determining the endpoint potentiometrically. Read the volume added between the two inflection points. Carry out the blank test and make the necessary corrections.

Each mL of 0.1 M sodium hydroxide VS is equivalent to 45.30 mg of $C_{21}H_{32}N_6O_3 \cdot HCl$.

PACKAGING AND STORAGE

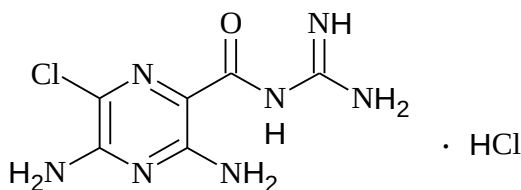
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Opioid analgesic.

AMILORIDE HYDROCHLORIDE***Amiloridi hidrokloridum***C₆H₈ClN₇O.HCl; 266.09

amiloride hydrochloride; 00672

3,5-Diamino-N-carbamimidoyl-6-chloropyrazine-2-carboxamide hydrochloride

[2016-88-8]

C₆H₈ClN₇O.HCl.2H₂O; 302.12

amiloride hydrochloride dihydrate; 10890

3,5-Diamino-N-carbamimidoyl-6-chloropyrazine-2-carboxamide hydrochloride dihydrate

[17440-83-4]

Contains no less than 98.0% and no more than 101.0% of C₆H₈ClN₇O.HCl, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Light yellow or greenish-yellow powder.

Solubility. Slightly soluble in water. Freely soluble in dimethylsulfoxide. Slightly soluble in isopropanol and absolute ethyl alcohol.

IDENTIFICATION

The identification test B. can be omitted if tests A. and C. are carried out. The Identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample, previously desiccated, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of amiloride hydrochloride CRS, prepared identically.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel R as support, and a mixture of ammonia RS, water and dioxane (6:6:88) as mobile phase. Apply, separately, to the plate 5 µL of each solution described below.

Solution (1): dissolve 40 mg of the sample in methyl alcohol, dilute to 10 mL with the same solvent and homogenize.

Solution (2): dissolve 40 mg of amiloride hydrochloride CRS in methyl alcohol, dilute to 10 mL with the same solvent and homogenize.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (365 nm). The main stain obtained in the *Solution (1)* chromatogram corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

Acidity. Dissolve 1.0 g of the sample in 100 mL of a mixture of methyl alcohol and water (1:1), and titrate with 0.1 M sodium hydroxide, determining the endpoint potentiometrically. No more than 0.3 mL of 0.1 M sodium hydroxide must be spent.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica gel chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of tetramethylammonium hydroxide, acetonitrile and water (5:250:745), adjust pH to 7.0 with phosphoric acid.

Solution (1): dissolve about 20 mg of the sample, accurately weighed, in 10 mL of a mixture of acetonitrile and water (1:3).

Solution (2): transfer 1 mL of *Solution (1)* to a 100 mL volumetric flask, dilute with the same solvent and homogenize.

Solution (3): transfer 1 mL of *Solution (2)* to a 10 mL volumetric flask, dilute with the same solvent and homogenize.

Solution (4): dissolve about 5 mg of amiloride impurity **A** CRS, accurately weighed, in 5 mL of a mixture of acetonitrile and water (1:3). Transfer 1 mL of this solution to a 100 mL volumetric flask, dilute with the same solvent and homogenize.

If necessary, adjust the proportion of acetonitrile in the *Mobile phase* so that the retention time of impurity **A** is between five and six minutes. If necessary, adjust the proportion of tetramethylammonium hydroxide, maintaining the pH at 7.0, so that the amiloride retention time is between nine and 12 minutes.

Procedure: inject, separately, 20 µL of *Solutions (1)*, (3) and (4) and record the chromatograms for no less than five times the retention time of amiloride.

System sensitivity: signal/noise ratio of no less than 5.0 for the amiloride peak obtained with *Solution (3)*.

Non-specific impurities: for each impurity peak obtained with *Solution (1)*, the area is of no more than 0.2 times the area under the impurity **A** peak obtained with *Solution (4)* (0.1%).

Total impurities: the sum of areas under the impurity peaks obtained with *Solution (1)* is no more than the area under the impurity **A** peak obtained with *Solution (4)* (0.5%).

Disregard: impurity peaks with areas lower than 0.1 times the area under the impurity **A** peak obtained with *Solution (4)* (0.05%).

Water (5.2.20.1). 11.0% to 13.0% for the dihydrate form.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve about 0.2 g of the sample, accurately weighed, in 50 mL of ethyl alcohol. Add 5 mL of 0.01 *M* hydrochloric acid and titrate with 0.1 *M* sodium hydroxide VS, determining the endpoint potentiometrically between the two inflection points. Each mL of 0.1 *M* sodium hydroxide VS is equivalent to 26.61 mg of $C_6H_8ClN_7O \cdot HCl$.

PACKAGING AND STORAGE

Store protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Diuretic.

AMILORIDE HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 0.1 g of hydrochlorothiazide to a beaker and dissolve in 50 mL of acetone. Filter and evaporate the filtrate to dryness. Dry the residue at 105 °C for one hour. In the infrared absorption spectrum **(5.2.14)** of the dry residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the hydrochlorothiazide CRS spectrum.

B. The retention time of the main peaks of the *Sample solution* chromatogram, obtained in the *Assay*, correspond to the ones from the main peaks of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, with *Dissolution medium*, until adequate concentration. Measure the absorbances of the solutions at 363 nm for amiloride hydrochloride and at 270 nm for hydrochlorothiazide **(5.2.14)**, using the same solvent for zero adjustment. Calculate the amount of $C_6H_8ClN_7O \cdot HCl$ and of $C_7H_8ClN_3O_4S_2$ dissolved in the medium, comparing the readings obtained with those of the amiloride hydrochloride CRS and hydrochlorothiazide CRS solutions of known concentrations prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_6H_8ClN_7O \cdot HCl$ and 75% (Q) of the declared quantity of $C_7H_8ClN_3O_4S_2$ dissolve in 30 minutes.

PURITY TESTS

Limit of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of dioxane and 3 M ammonium hydroxide (90:12) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): pulverize the tablets and dissolve an amount of powder equivalent to 17.5 mg of amiloride hydrochloride anhydrous in 10 mL of methyl alcohol and centrifuge.

Solution (2): dissolve 1 mg of methyl 3,5-diamino-6-chloropyrazine-2-carboxylate CRS in methyl alcohol and dilute to 100 mL with the same solvent.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (365 nm). Any stain corresponding to methyl 3,5-diamino-6-chloropyrazine-2-carboxylate obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (2)*.

Limit of 4-amino-6-chloro-1,3-benzenedisulfonamide. Proceed as described in *Assay*. Prepare the *Test solution* as described below.

Test solution: dissolve 1 mg of 4-amino-6-chloro-1,3-benzenedisulfonamide CRS in the mobile phase and dilute to 100 mL with the same solvent.

Inject, separately, 20 µL of the *Test solution* and 20 µL of the *Sample solution*, record the chromatograms and measure the areas under the peaks. The area under the peak related to 4-amino-6-chloro-1,3-benzenedisulfonamide obtained with the *Sample solution* is not greater than the area under the main peak obtained with the *Test solution*. No more than 1.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 286 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Phosphate buffer: dissolve 136 g of monobasic potassium phosphate in 800 mL of water, adjust pH to 3.0 with phosphoric acid and complete the volume to 1000 mL with water.

Mobile phase: mixture of water, methyl alcohol and *Phosphate buffer* (71:25:4).

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 5 mg of amiloride hydrochloride to a 50 mL volumetric flask. Add 15 mL of methyl alcohol and 2 mL of *M* hydrochloric acid. Leave in ultrasonic bath for 10 minutes, complete the volume with the water, homogenize and filter.

Standard solution: dissolve 20 mg of amiloride hydrochloride CRS in methyl alcohol and dilute to 20 mL with the same solvent. Transfer 10 mL of this solution to a 100 mL volumetric flask with, precisely, about 100 mg of hydrochlorothiazide CRS and 20 mL of methyl alcohol. Add 4 mL of *M* hydrochloric acid, complete the volume with water with water and homogenize.

Inject 20 µL replicates of the *Standard solution*. The relative retention times are approximately 0.7 for hydrochlorothiazide and 1 for amiloride hydrochloride. The resolution between the hydrochlorothiazide and amiloride peaks must be of no less than 2.0. The relative standard deviation of the replicate areas from the recorded peaks must be of no more than 2.0%.

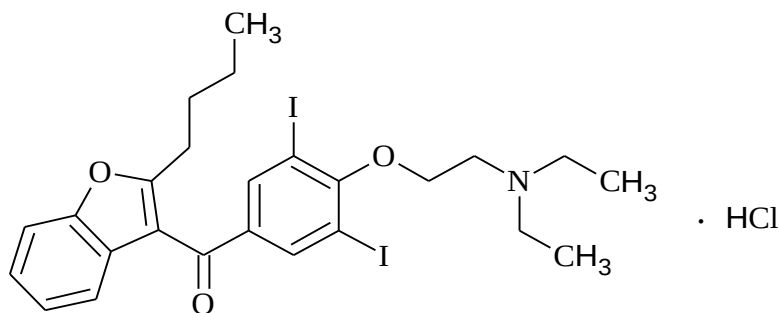
Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of amiloride hydrochloride ($C_6H_8ClN_7O \cdot HCl$) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

AMIODARONE HYDROCHLORIDE*Amiodaroni hydrochloridum* $C_{25}H_{29}I_2NO_3 \cdot HCl$; 681.77

amiodarone hydrochloride; 00700

(2-butyl-3-benzofuranyl)-[4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]-metanone hydrochloride (1:1)

[19774-82-4]

Contains no less than 98.5% and no more than 101.0% of $C_{25}H_{29}I_2NO_3 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white fine, crystalline powder.

Solubility. Practically insoluble in water, soluble in methyl alcohol, moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 159 °C to 163 °C, with decomposition.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of amiodarone hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.002% (w/v) solution in methyl alcohol, there are maximum and minimum points only at the same wavelengths of amiodarone hydrochloride CRS, prepared identically.

C. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

D. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. The 5% (w/v) preparation in methyl alcohol is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). 3.2 to 3.8. Determine in solution prepared as described next. Dissolve 1.25 g of the sample in water heated at 80 °C. Cool, complete the volume to 25 mL with water and homogenize.

Light absorption. The absorbance of the 0.002% (w/v) solution in methyl alcohol, measured at 242 nm, is between 1.03 and 1.05.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of formic acid, methyl alcohol and methylene chloride (5:10:85) as mobile phase. Apply to the plate, separately, 5 µL of each of the solutions, freshly prepared and kept protected from direct light, described below.

Solution (1): dissolve 1 g of the sample in methylene chloride, complete to 10 mL with the same solvent and homogenize, obtaining a 100 mg/mL solution.

Solution (2): dilute 0.5 mL of *Solution (1)* to 10 mL with methylene chloride, obtaining a 5 mg/mL solution.

Solution (3): dissolve 25 mg of amiodarone hydrochloride CRS in methylene chloride, complete to 5 mL with the same solvent and homogenize, obtaining a 5 mg/mL solution.

Solution (4): dilute 1 mL of *Solution (2)* to 10 mL with methylene chloride, obtaining a 0.5 mg/mL solution.

Solution (5): dilute 5 mL of *Solution (4)* to 10 mL with methylene chloride, obtaining a 0.25 mg/mL solution.

Solution (6): dissolve 10 mg of (2-chloroethyl) diethylamine hydrochloride in 50 mL of methylene chloride. Dilute 1 mL to 10 mL with methylene chloride, obtaining a 0.02 mg/mL solution.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (4)* (0.5%), and no more than one stain is more intense than that obtained in the chromatogram with *Solution (5)* (0.25%). Nebulize the plate with potassium iodobismuthate RS and then with 3% (w/v) hydrogen peroxide and examine immediately. Any stain corresponding to (2-chloroethyl)diethylamine hydrochloride obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (6)* (0.02%).

Volatile organic impurities. Proceed as described in *Gas chromatography (5.2.17.5)*. Use a chromatograph equipped with flame ionization detector; column with 30 m of length and 0.25 mm of internal diameter, with stationary phase of 35% of diphenylpolysiloxane (0.25 µm of thickness); column operated with the following programming: 40 °C, slope of 40 °C to 200 °C with heating rate of 15 °C per minute. Maintain the temperatures of the injector and the detector at 250 °C, respectively; use hydrogen as carrier gas, with pressure of 85 kPa in the column head.

Solution (1): transfer 0.3 g of the sample and 5 mL of dimethylsulfoxide to a 10 mL headspace sampling vial with 1 g of sodium sulfate anhydrous.

Solution (2): weigh 1 g of methylene chloride and dilute to 0.02% (w/v) (200 ppm) with dimethylsulfoxide. Transfer 5 mL of this solution to a 10 mL headspace sampling vial with 1 g of sodium sulfate anhydrous.

Solution (3): weigh 1 g of toluene and dilute to 0.02% (w/v) with dimethylsulfoxide. Transfer 5 mL of this solution to a 10 mL headspace sampling vial with 1 g of sodium sulfate anhydrous.

Cover the headspace sampling vials with polytetrafluoroethylene cap and aluminum seal. Heat the samples at 80 °C for 60 minutes.

Procedure: inject 1 µL of the vapor phase from each solution, record the areas of each peak. The retention time of toluene is approximately 2.5 minutes; the resolution between the methylene chloride and toluene peaks is no less than 2; the relative standard deviation of the replicate areas of the peaks recorded, for both solvents, is no more than 15%. The areas related to methylene chloride and toluene in the *Solution (1)* are not greater than the areas for the standards of methylene chloride from *Solution (2)* and toluene from *Solution (3)*.

Iodides. Prepare, simultaneously, the solutions described next.

Solution (1): add 1.5 g of the sample to 40 mL of water heated at 80 °C and shake until complete dissolution. Cool down to room temperature and dilute to 50 mL with water.

Solution (2): add to 15 mL of *Solution (1)* 1 mL of 0.1 M hydrochloric acid, 1 mL of 0.05 M potassium iodate and dilute to 25 mL with water. Allow to stand, protected from light, for four hours.

Solution (3): add to 15 mL of *Solution (1)* 1 mL of 0.1 M hydrochloric acid, 1 mL of 0.00882% (w/v) potassium iodide, 1 mL of 0.05 M potassium iodate and dilute to 25 mL with water. Allow to stand, protected from light, for four hours.

Measure the absorbances of *Solution (2)* and *Solution (3)* at 420 nm (5.2.14), using, for zero adjustment, a mixture of 15 mL of *Solution (1)* and 1 mL of 0.1 M hydrochloric acid, diluted to 25 mL with water. The absorbance of *Solution (2)* is not higher than half the absorbance of *Solution (3)*. No more than 0.015% (150 ppm).

Heavy metals (5.3.2.3). Use *Method IV*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry, under phosphorus pentoxide, in oven at 50 °C, under reduced pressure, not higher than 0.3 kPa, for four hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the following methods.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve about 0.5 g of the sample, accurately weighed, in 40 mL of glacial acetic acid. Add 10 mL of 5% (w/v) mercuric acetate in glacial acetic acid. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 68.177 mg of $C_{25}H_{29}I_2NO_3 \cdot HCl$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 50 mg of the sample and dissolve in methyl alcohol. Complete the volume to 100 mL with the same solvent and homogenize. Dilute, successively, in methyl alcohol until concentration of 0.0008% (w/v). Prepare an amiodarone hydrochloride CRS solution in the same concentration using the same solvent. Measure the absorbances of the resulting solutions at 242 nm, using methyl alcohol for zero adjustment. Calculate the content of $C_{25}H_{29}I_2NO_3 \cdot HCl$ in the sample from the readings obtained.

PACKAGING AND STORAGE

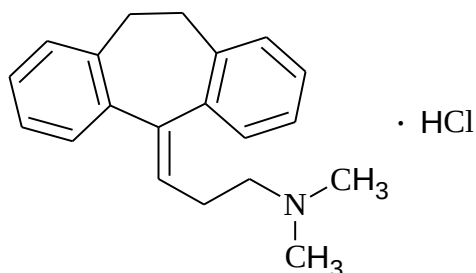
In tightly closed containers, protected from light at maximum temperature of 30 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-arrhythmic and antianginal.

AMITRIPTYLINE HYDROCHLORIDE*Amitriptylini hydrochloridum*C₂₀H₂₃N.HCl; 313.87

amitriptyline hydrochloride; 00712

3-(10,11-di-hydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-*N,N*-dimethyl-1-propanamine hydrochloride (1:1)

[549-18-8]

Contains no less than 99.0% and no more than 101.0% of C₂₀H₂₃N.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white powder or colorless crystals.

Solubility. Freely soluble in water and ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 195 °C to 199 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of amitriptyline hydrochloride CRS, prepared identically.

B. Proceed as described in *Ultraviolet absorption spectrophotometry*. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.001% (w/v) solution in methyl alcohol, there is maximum absorption at 239 nm, identical to that observed in the spectrum of amitriptyline hydrochloride CRS, prepared identically.

C. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 5.0 to 6.0. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and a mixture of chloroform, methyl alcohol and ammonium hydroxide (135:15:1)

as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): 40 mg/mL sample solution in methyl alcohol.

Solution (2): 0.8 mg/mL amitriptyline hydrochloride CRS solution in methyl alcohol.

Solution (3): dilute the *Solution (2)* in methyl alcohol to obtain a 0.4 mg/mL solution.

Solution (4): dilute the *Solution (2)* in methyl alcohol to obtain a 0.2 mg/mL solution.

Solution (5): dilute the *Solution (2)* in methyl alcohol to obtain a 0.16 mg/mL solution.

Solution (6): dilute the *Solution (2)* in methyl alcohol to obtain a 0.08 mg/mL solution.

Solution (7): dilute the *Solution (2)* in methyl alcohol until concentration of 0.04 mg/mL.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (4)* (0.5%). The sum of the intensities of all the secondary stains obtained with *Solution (1)* corresponds to no more than that obtained with *Solution (3)* (1%). Disregard any stain obtained in the chromatogram with *Solution (1)* that is less intense than the main stain obtained with *Solution (7)* (0.1%). Disregard any stains observed in the points of application of solutions.

Volatile organic impurities. Proceed as described in *Gas chromatography (5.2.17.5)*, using a chromatograph equipped with a flame ionization detector; fused silica column, with 30 m of length and 0.53 mm of internal diameter, packed with phenyl (5%) methyl (95%) polysiloxane, and silica pre-column with 5 m of length and 0.53 mm of internal diameter, deactivated with phenylmethylsiloxane. Maintain the column at 35 °C for five minutes, increase to 175 °C at 8 °C per minute, then to 260 °C at 35 °C per minute, and maintain for at least 16 minutes. Maintain the temperatures of the injector and the detector at 70 °C and 260 °C, respectively; use helium at linear speed of 35 cm/s as carrier gas.

Sample solution: dissolve, in organic material-free water, an accurately weighed amount of the sample, to obtain a 20 mg/mL solution.

Standard solution: prepare a solution, in organic material-free water, containing in each milliliter, 12 µg of methylene chloride, 1.2 µg of chloroform, 7.6 µg of dioxane and 1.6 µg of trichlorethylene. Prepare on the day of use.

Inject 1 µL replicates of the *Standard solution*. The resolution between the peaks of any two substances must be no less than 1.0. The relative standard deviation of the replicate areas of the recorded peaks is no more than 15.0%.

Procedure: inject, separately, 1 µL of the *Standard solution* and the *Sample solution*. Record the chromatograms and measure the areas under the peaks. The identity and response from each peak present in the chromatogram of the *Sample solution* can be related to the identity and response of the volatile organic impurities present in the chromatogram of the *Standard solution*. The amount

of each volatile organic impurity present in the chromatogram of the *Sample solution* does not exceed the limit prescribed on the following table.

<i>Volatile organic impurity</i>	<i>Limit (ppm)</i>
chloroform	60
dioxane	380
methylene chloride	600
trichlorethylene	80

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 1 g of the sample in 30 mL of glacial acetic acid, slightly heating, if necessary, and cool. Add 10 mL of mercury acetate RS. Titrate with 0.1 M perchloric acid VS, using methylrosanilinium chloride TS as indicator, until a green color appears. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 31.391 mg of C₂₀H₂₃N.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antidepressant.

AMITRIPTYLINE HYDROCHLORIDE CAPSULES

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{20}H_{23}N.HCl$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the *Sample solution* obtained in method **A.** from *Assay*, there are maximum and minimum points identical to those observed in the spectrum of the *Standard solution*.

B. The main stain of the chromatogram from *Solution (1)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. Weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Shake an amount of powder equivalent to 10 mg of amitriptyline hydrochloride with 5 mL of water. Filter. The solution complies with chloride ion reactions **(5.3.1.1)**.

E. Weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Shake an amount of powder equivalent to 0.1 g of amitriptyline hydrochloride with 10 mL of chloroform. Filter and reduce the volume of filtrate by evaporation. Precipitate by adding ethyl ether until producing turbidity. Allow to stand and filter. Dissolve 50 mg of the precipitate in 3 mL of water. Add 50 mL of 2.5% (w/v) quinhydrone in methyl alcohol. No red color develops for 15 minutes.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test. No more than 15 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each capsule to a 50 mL volumetric flask and add 35 mL of 0.1 M hydrochloric acid. Leave in ultrasonic bath for 20 minutes, occasionally shaking. Homogenize and filter. Transfer 5 mL of the filtrate to a 25 mL volumetric flask and complete the volume with 0.1 M hydrochloric acid. Transfer 5 mL of the resulting solution to a 50 mL volumetric flask and complete the volume with the same solvent, obtaining a 0.001% (w/v) solution. Proceed as described in method **A.** of *Assay* from "Prepare standard solution in the same concentration...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances at 239 nm **(5.2.14)**,

using the same solvent for zero adjustment. Calculate the amount of $C_{20}H_{23}N.HCl$ dissolved in the medium, comparing the readings obtained with that of the amitriptyline hydrochloride CRS solution, at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{20}H_{23}N.HCl$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of diethylamine, ethyl acetate and cyclohexane (3:15:85) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 20 mg of amitriptyline hydrochloride to a 5 mL volumetric flask and complete the volume with absolute ethyl alcohol. Shake for 10 minutes. Homogenize and filter.

Solution (2): transfer 20 mg of amitriptyline hydrochloride CRS to a 5 mL volumetric flask, complete the volume with absolute ethyl alcohol and homogenize, obtaining a 4 mg/mL solution.

Solution (3): transfer 1 mL of the *Solution (2)* to a 100 mL volumetric flask, complete the volume with absolute ethyl alcohol and homogenize, obtaining a 40 µg/mL solution.

Solution (4): transfer 2.5 mL of the *Solution (3)* to a 10 mL volumetric flask, complete the volume with absolute ethyl alcohol and homogenize, obtaining a 10 µg/mL solution.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than those obtained with *Solutions (3)* or *(4)* (1% and 0.25%). Nebulize the plate with potassium iodide and bismuth subnitrate RS. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain and stained by the visualization reagent, is not more intense than that obtained with *Solution (3)* (1%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 10 mg of amitriptyline hydrochloride to a 100 mL volumetric flask. Add 75 mL of 0.1 M hydrochloric acid, shake mechanically for 15 minutes and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent. Filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask and complete the volume with the same solvent, obtaining a 0.001%

(w/v) solution. Prepare a standard solution in the same concentration, using the same solvent. Determine the absorbances of the resulting solutions at 239 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{20}H_{23}N.HCl$ in the capsules, from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 2.0 mL/minute.

Triethylamine phosphate buffer: transfer 11.04 g of monobasic sodium phosphate and 3 mL of triethylamine to a 1000 mL volumetric flask and dissolve in 900 mL of water. Adjust the pH to 2.5 ± 0.5 with phosphoric acid, complete the volume with water and homogenize.

Mobile phase: mixture of *Triethylamine phosphate buffer* and acetonitrile (58:42).

Sample solution: weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Transfer an amount of powder equivalent to 50 mg of amitriptyline hydrochloride to a 50 mL volumetric flask, add 35 mL of *Mobile phase*, shake mechanically for 15 minutes and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 10 mL of the filtrate to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Standard solution: transfer 50 mg of amitriptyline hydrochloride CRS to a 50 mL volumetric flask, dilute in 35 mL of *Mobile phase*, complete the volume with the same solvent and homogenize. Transfer 10 mL of the solution to a 50 mL volumetric flask, complete the volume with the same solvent and homogenize, to obtain a 0.2 mg/mL amitriptyline hydrochloride solution.

The column efficiency must be no less than 800 theoretical plates. The tailing factor is no more than 2.5. The relative standard deviation of the replicate areas from the recorded peaks must be of no more than 2.0%.

Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{20}H_{23}N.HCl$ in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

AMITRIPTYLINE HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{20}H_{23}N.HCl$. The tablets can be coated.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from *Assay*, there are maximum and minimum points identical to those observed in the spectrum of the standard solution.

B. The main stain of the chromatogram from *Solution (1)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. Weigh and pulverize the tablets. Shake an amount of powder equivalent to 10 mg of amitriptyline hydrochloride with 5 mL of water. Filter. The solution complies with chloride ion reactions **(5.3.1.1)**.

E. Weigh and pulverize the tablets. Shake an amount of powder equivalent to 0.1 g of amitriptyline hydrochloride with 10 mL of chloroform. Filter and reduce the volume of filtrate by evaporation. Precipitate by adding ethyl ether until producing turbidity. Allow to stand and filter. Dissolve 50 mg of the precipitate in 3 mL of water. Add 50 mL of 2.5% (w/v) quinhydrone in methyl alcohol. No red color develops for 15 minutes.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test. No more than 15 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 50 mL volumetric flask and add 35 mL of 0.1 M hydrochloric acid. Leave in ultrasonic bath for 20 minutes, occasionally shaking. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of the filtrate to a 25 mL volumetric flask and complete the volume with 0.1 M hydrochloric acid. Transfer 5 mL of the resulting solution to a 50 mL volumetric flask and complete the volume with the same solvent, obtaining a 0.001% (w/v) solution. Proceed as described in method **A.** of *Assay* from "Prepare standard solution in the same concentration...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances at 239 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of C₂₀H₂₃N.HCl dissolved in the medium, comparing the readings obtained with that of the amitriptyline hydrochloride CRS solution, at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of C₂₀H₂₃N.HCl dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ as support, and a mixture of diethylamine, ethyl acetate and cyclohexane (3:15:85) as mobile phase. Apply to the plate, separately, 20 µL of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Transfer an amount of powder equivalent to 20 mg of amitriptyline hydrochloride to a 5 mL volumetric flask and complete the volume with absolute ethyl alcohol. Shake for 10 minutes. Homogenize and filter.

Solution (2): transfer 20 mg of amitriptyline hydrochloride CRS to a 5 mL volumetric flask, complete the volume with absolute ethyl alcohol and homogenize, obtaining a 4 mg/mL solution.

Solution (3): transfer 1 mL of the *Solution (2)* to a 100 mL volumetric flask, complete the volume with absolute ethyl alcohol and homogenize, obtaining a 40 mg/mL solution.

Solution (4): transfer 2.5 mL of the *Solution (3)* to a 10 mL volumetric flask and complete the volume with absolute ethyl alcohol, obtaining a 10 mg/mL solution.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than those obtained with *Solutions (3)* or *(4)* (1% and 0.25%). Nebulize the plate with potassium iodide and bismuth subnitrate RS. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main one and stained by the visualization reagent, is not more intense than that obtained with *Solution (3)* (1%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry* (**5.2.14**). Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 10 mg of amitriptyline hydrochloride to a

100 mL volumetric flask. Add 75 mL of 0.1 M hydrochloric acid, shake mechanically for 15 minutes and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent. Filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask and complete the volume with the same solvent, obtaining a 0.001% (w/v) solution. Prepare a standard solution in the same concentration, using the same solvent. Determine the absorbances of the resulting solutions at 239 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{20}H_{23}N.HCl$ in the tablets, from the readings obtained.

B. Proceed as described in method **B.** of *Assay* in the *Amitriptyline hydrochloride capsules* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize the tablets. Transfer an amount of powder equivalent to 50 mg of amitriptyline hydrochloride to a 50 mL volumetric flask, add 35 mL of *Mobile phase*, shake mechanically for 15 minutes and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 10 mL of the filtrate to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

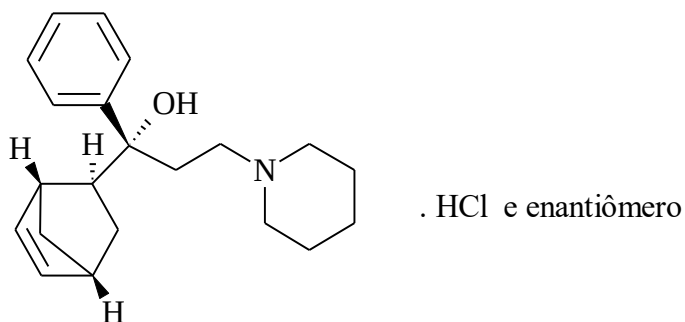
Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{20}H_{23}N.HCl$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

BIPERIDEN HYDROCHLORIDE*Biperideni hydrochloridum*C₂₁H₂₉NO.HCl; 347.93

biperiden hydrochloride; 01283

(1RS)-1-[(1RS,2SR,4RS)-bicyclo-[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride.

[1235-82-1]

Contains no less than 98.0% and no more than 101.0% of C₂₁H₂₉NO.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water and alcohol.

IDENTIFICATION

The identification test A. can be omitted if tests B., C. and D. are carried out. The identification tests B. and C. can be omitted if tests A. and D. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of biperiden hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.1% (w/v) sample solution in methyl alcohol, there is maximum absorption at 257 nm.

C. Dissolve 0.2 g of sample in 5 mL of phosphoric acid. A green color develops.

D. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel as support, and a mixture of methyl alcohol and ammonium hydroxide (100:1.5) as mobile phase. Apply, separately, to the plate 20 µL of each solution described below.

Solution (1): 10 mg/mL sample solution, in methyl alcohol.

Solution (2): 0.2 mg/mL biperiden hydrochloride CRS solution in methyl alcohol.

Solution (3): 0.1 mg/mL biperiden hydrochloride CRS solution in methyl alcohol.

Solution (4): 0.05 mg/mL biperiden hydrochloride CRS solution in methyl alcohol.

Solution (5): 0.01 mg/mL biperiden hydrochloride CRS solution in methyl alcohol.

Procedure: develop the chromatogram. Remove the plate and allow it to air dry. Expose the plate to iodine vapors for 10 minutes. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, has maximum intensity equal to the main stain obtained with *Solution (2)*. The sum of the impurities observed is no more than 2.0%.

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for three hours. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Dissolve 0.5 g of the sample in 80 mL of glacial acetic acid, slightly heating, if necessary, for dissolution. Cool down. Add one drop of methylrosanilinium chloride TS and 10 mL of mercury acetate RS. Titrate with 0.1 M perchloric acid VS until the color changes to emerald green. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 34.79 mg of C₂₁H₂₉NO.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anticholinergic.

BIPERIDEN HYDROCHLORIDE TABLETS

Contains no less than 93.0% and no more than 107.0% the declared amount of $C_{21}H_{29}NO \cdot HCl$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of isopropyl alcohol and 25% (v/v) ammonium hydroxide (95:5) as mobile phase. Apply separately to the plate 20 µL of each solution described below.

Solution (1): pulverize the tablets. Weigh an amount of powder equivalent to 10 mg of biperiden hydrochloride, add 5 mL of methylene chloride and homogenize. Filter and wash the residue with 5 mL of methylene chloride. Evaporate the filtrate. Dissolve the residue with 1 mL of methyl alcohol.

Solution (2): 10 mg/mL biperiden hydrochloride CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate, allow to air dry and examine under ultraviolet light (254 nm). Expose the plate for 10 minutes to iodine vapors in a closed chamber, saturated in advance, having iodine crystals on the background. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. Weigh and pulverize the tablets. Use an amount of powder equivalent to 10 mg of biperiden hydrochloride, add 10 mL of water and heat for 15 minutes. Filter. The filtrate complies with chloride ion reactions (5.3.1.1).

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 500 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Standard solution: dissolve an accurately weighed amount of biperiden hydrochloride CRS in methyl alcohol and dilute with the same solvent to obtain a concentration of approximately 1.0 mg/mL. Dilute, successively, with 0.01 M hydrochloric acid to obtain a concentration of 4 µg/mL.

Sample solution: use aliquots of the dissolution medium.

Procedure: after the test, take an aliquot of the dissolution medium and proceed as described in the method on *Assay*. Inject, separately, 50 µL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₂₁H₂₉NO.HCl dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: no less than 75% (Q) of the declared quantity of C₂₁H₂₉NO.HCl dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 205 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octylsilane group (5 µm), maintained at 25 °C; *Mobile phase* flow of 1.2 mL/minute.

Sodium phosphate buffer pH 2.5: dissolve 6.9 g of monobasic sodium phosphate in 500 mL of water. Add 1.011 g of sodium heptanesulfonate, complete the volume to 1000 mL with the same solvent and homogenize. If necessary, adjust pH to 2.5 with phosphoric acid.

Mobile phase: mixture of *Sodium phosphate buffer pH 2.5* and methyl alcohol (50:50).

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 2 mg of biperiden hydrochloride to a 20 mL volumetric flask. Add 10 mL of methyl alcohol and leave in ultrasonic bath for 20 minutes. Shake mechanically for 10 minutes and complete the volume with the same solvent. Homogenize and filter. Transfer 2 mL of the previous solution to a 10 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: accurately weigh 10 mg of biperiden hydrochloride CRS and transfer to a 10 mL volumetric flask. Complete the volume with methyl alcohol and homogenize. Transfer 0.2 mL of this solution to a 10 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

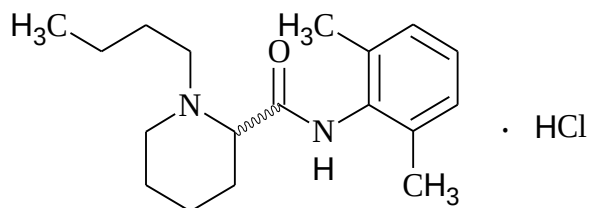
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₂₁H₂₉NO.HCl in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers at room temperature.

LABELING

Comply with current legislation.

BUPIVACAINE HYDROCHLORIDE***Bupivacaini hydrochloridum*** $C_{18}H_{28}N_2O \cdot HCl$; 324.89

bupivacaine hydrochloride; 01552

(RS)-1-butyl-N-(2,6-dimethylphenyl)piperidine-2-carboxamide hydrochloride

[18010-40-7]

 $C_{18}H_{28}N_2O \cdot HCl \cdot H_2O$; 342.91

bupivacaine hydrochloride monohydrate; 11219

(RS)-1-butyl-N-(2,6-dimethylphenyl)piperidine-2-carboxamide hydrochloride hydrate (1:1)

[73360-54-0]

Contains no less than 98.5% and no more than 101.5% of $C_{18}H_{28}N_2O \cdot HCl$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Soluble in water, freely soluble in alcohol.

Physicochemical constants.

Melting temperature (5.2.2): 254 °C, with decomposition.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the non-desiccated sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of bupivacaine hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.05% (w/v) sample solution in 0.1 M hydrochloric acid, there is maximum absorption at 271 nm.

C. Transfer to a separation funnel, quantitatively, about 50 mg of the sample and add 10 mL of water, alkalize with 6 M ammonium hydroxide and extract with 10 mL of ether. The aqueous phase complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). Between 4.5 and 6.0. Determine in aqueous solution at 1% (w/v).

Limit of residual solvent. Proceed as described in *Gas chromatography (5.2.17.5)*. Use a chromatograph equipped with flame ionization detector; column with 2 m of length and 4 mm of internal diameter, with stationary phase packed with ethylvinylbenzene and divinylbenzene copolymer, and an average pore diameter of 0.0075 µm. Use nitrogen as carrier gas, in a flow of 40 mL/minute. Maintain the column temperature at 175 °C and the injector and the detector temperatures at 200 °C and 280 °C, respectively.

Standard ethyl alcohol solution: pipette 2 mL of ethyl alcohol dihydrate to a 100 mL volumetric flask, complete the volume with water and homogenize. Transfer 2 mL of this solution to a 50 mL volumetric flask, complete the volume with water and homogenize. The solution contains 0.08% of ethyl alcohol.

Standard isopropyl alcohol solution: pipette 2 mL of isopropyl alcohol to a 1000 mL volumetric flask, complete the volume with water and homogenize. Transfer 2 mL of this solution to a 100 mL volumetric flask, complete the volume with water and homogenize. The solution contains 0.004% of isopropyl alcohol.

Test solution: transfer 1.0 mg of bupivacaine hydrochloride, accurately weighed, to a 25 mL volumetric flask, complete the volume with water and homogenize.

Inject 5 µL of the *Test solution*, the *Standard ethyl alcohol solution* and the *Standard isopropyl alcohol solution* in the gas chromatograph. Record the chromatograms and measure the areas under the peaks in the *Test solution*, the *Standard ethyl alcohol solution* and in the *Standard isopropyl alcohol solution*. Determine the content of ethyl alcohol and isopropyl alcohol in the *Test solution* from the responses obtained with the standard solutions. The sum of the content of ethyl alcohol and isopropyl alcohol is no more than 2%.

Water (5.2.20.1). Between 4.0% and 6.0%.

Heavy metals (5.3.2.3). Use *Method III*. Determine in 2.0 g of the sample. No more than 0.001% (10 ppm).

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

Chromatographic purity.

Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel as support and a mixture of chloroform and isopropylamine (99:1) as mobile phase. Apply, separately, to the plate 10 µL of each solution described below.

Solution (1): dissolve an amount of the sample, accurately weighed, in *Diluent* to obtain a 20 mg/mL solution.

Solution (2): dissolve an accurately weighed amount of bupivacaine hydrochloride CRS in *Diluent*, to obtain a 20 mg/mL solution.

Solution (3): dilute the *Solution (2)* in *Diluent* to obtain a 0.1 mg/mL solution.

Diluent: mixture of chloroform and isopropylamine (99:1).

Develop the chromatogram. Remove the plate and allow it to air dry. Expose the plate to iodine vapors for five minutes, remove and nebulize with 3.5 M sulfuric acid, until stains appear. The main stain of *Solution (1)* has the same R_f as the main stain of *Solution (2)*. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, has maximum intensity equal to the main stain obtained with *Solution (3)* (0.5%). The sum of the impurities observed is no more than 2.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.5 g of the sample in 80 mL of glacial acetic acid, slightly heating, if necessary, for dissolution. Cool down. Titrate with 0.1 M perchloric acid VS, determining the endpoint using one drop of methylrosanilinium chloride TS and 10 mL of mercury acetate RS until the color changes to emerald green. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 32.49 mg of $C_{18}H_{28}N_2O.HCl$.

PACKAGING AND STORAGE

Store protected from light, humidity, and direct natural light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anesthetic.

BUPIVACAINE HYDROCHLORIDE AND GLUCOSE INJECTION SOLUTION

Contains no less than 93.0% and no more than 107.0% of the declared amounts of bupivacaine hydrochloride ($C_{18}H_{28}N_2O.HCl$) and glucose ($C_6H_{12}O_6$). The injection solution can be prepared in water for injection or in another adequate solvent.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of butyl alcohol, water, ethyl alcohol and glacial acetic acid (6:2:1:1) as mobile phase. Apply to the plate, separately, 10 µL of the *Solution (1)*, *Solution (2)* and *Solution (4)*, and 1 µL of *Solution (1)* and *Solution (3)*, freshly prepared, as described below.

Solution (1): injection solution of bupivacaine hydrochloride and glucose.

Solution (2): bupivacaine hydrochloride CRS solution in water, in the concentration corresponding to that of the injection solution.

Solution (3): glucose CRS solution in water, in the concentration corresponding to that of the injection solution.

Solution (4): bupivacaine hydrochloride CRS solution using *Solution (3)* as diluent, to obtain a concentration corresponding to that of the injection solution.

Develop the chromatogram. Remove the plate and dry in circulating hot air. Examine the plate under ultraviolet light (254 nm). The position of the main stain obtained with *Solution (1)* corresponds to that of the main stains obtained with *Solution (2)* and *Solution (4)*. Nebulize with reagent naphthalenediol, heat at 90 °C for five minutes and examine the plate. The position of main brown stain, obtained with *Solution (1)*, corresponds to the one obtained with *Solution (3)*. Cool the plate, nebulize with iodoplatinate reagent and examine the plate. Bupivacaine is viewed as a violet blue stain in orange background, and the stain corresponding to glucose disappears gradually.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, on the method for determination of bupivacaine hydrochloride, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.0 to 6.5.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 2.5 EU/mL.

ASSAY

Bupivacaine hydrochloride. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 263 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 2.0 mL/minute.

Phosphate buffer pH 6.8: dissolve 1.94 g of potassium phosphate monobasic and 2.48 g of potassium phosphate dibasic in 1000 mL of water. Adjust the pH, if necessary, to 6.8 ± 0.05 with *M* potassium hydroxide or *M* phosphoric acid.

Mobile phase: mixture of acetonitrile and *Phosphate buffer pH 6.8* (65:35). Adjust the pH, if necessary, to 7.7 ± 0.02 with *M* phosphoric acid.

Sample solution: transfer a volume of the injection solution equivalent to 25 mg of bupivacaine hydrochloride to a 50 mL volumetric flask. Complete the volume with methyl alcohol and homogenize.

Standard solution: accurately weigh about 25 mg of bupivacaine hydrochloride CRS and transfer to a 50 mL volumetric flask. Dissolve in 5 mL of water, using ultrasonic bath, if necessary. Complete the volume with methyl alcohol and homogenize.

Inject 20 μ L replicates of the *Standard solution*. The relative standard deviation of the replicate areas of the recorded peaks is no more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{18}H_{28}N_2O.HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

Glucose. Measure the sample rotation angle, in an adequate tube **(5.2.8)**, using distilled water for zero adjustment. Calculate the amount of $C_6H_{12}O_6$, in each mL of the sample, using the formula:

$$\alpha \times 9,452 \times A$$

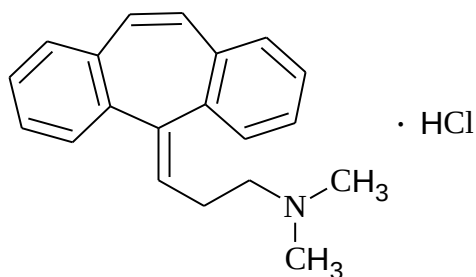
where α is the average reading obtained and A is the division of 200 by the tube length, in mm.

PACKAGING AND STORAGE

In single-dose, preferably type I glass, containers, protected from light.

LABELING

Comply with current legislation.

CYCLOBENZAPRINE HYDROCHLORIDE*Cyclobenzaprine hydrochloridum*C₂₀H₂₁N.HCl; 311.85

cyclobenzaprine hydrochloride; 02013

3-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-*N,N*-dimethyl-1:1-propanamine hydrochloride (1:1)
[6202-23-9]

Contains no less than 99.0% and no more than 101.0% of C₂₀H₂₁N.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder.

Solubility. Freely soluble in water, ethyl alcohol and methyl alcohol, moderately soluble in isopropyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 215 °C to 219 °C, and the range between the beginning and end of melting must not exceed 2 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cyclobenzaprine hydrochloride CRS, prepared identically.

B. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel GF₂₅₄ as support and a mixture of acetone, toluene and ammonium hydroxide (75:25:1) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 20 mg/mL sample solution in methyl alcohol.

Solution (2): 20 mg/mL cyclobenzaprine hydrochloride CRS solution in methyl alcohol.

Solution (3): dilute 0.1 mL of *Solution (1)* in 20 mL of methyl alcohol.

Develop the chromatogram. Remove the plate, allow to air dry for 15 minutes and observe under ultraviolet light (254 nm). The main stain of *Solution (1)* corresponds in position, color and intensity to that obtained with *Solution (2)*, and any other stain obtained from *Solution (1)* does not exceed in size or intensity the main stain obtained from *Solution (3)* (0.5%).

Heavy metals (5.3.2.3). Use *Method III*. Determine in 2 g of the sample. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample in oven at 105 °C, until constant weight. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh about 0.4 g of the sample, previously desiccated. Dissolve in 80 mL of glacial acetic acid and 15 mL of mercury acetate RS. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 31.185 mg of C₂₀H₂₁N.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Muscle relaxant.

CYCLOBENZAPRINE HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{20}H_{21}N.HCl$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer an amount of powder equivalent to 50 mg of cyclobenzaprine hydrochloride to a 25 mL beaker, add 10 mL of methylene chloride and shake to dissolve. Filter and evaporate until obtaining a volume of approximately 5 mL of filtrate. Transfer to a centrifuge tube and add 2 mL of ethyl ether. Evaporate by air stream to approximately 1 mL and shake until there is crystallization. Wash the crystals with portions of ethyl ether and dry at room temperature. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cyclobenzaprine hydrochloride CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 50 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and, if necessary, dilute in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 290 nm **(5.2.14)**, using the same solvent for zero adjustment. Calculate the amount of $C_{20}H_{21}N.HCl$ dissolved in the medium, comparing the readings obtained with that of the cyclobenzaprine hydrochloride CRS solution, in concentration similar to the one expected in the sample.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{20}H_{21}N.HCl$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 290 nm; column with 100 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: mixture of water, acetonitrile, methyl alcohol and methanesulfonic acid (48:28:24:0.2). Adjust pH to 3.6 with diethylamine.

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 10 mg of cyclobenzaprine hydrochloride to a 200 mL volumetric flask and add 150 mL of 0.1 M hydrochloric acid. Shake mechanically for 30 minutes. Complete the volume with the same solvent, obtaining a 50 µg/mL solution. Homogenize and filter.

Standard solution: dissolve an accurately weighed amount of cyclobenzaprine hydrochloride CRS in 0.1 M hydrochloric acid, to obtain a 50 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is no less than 1000 theoretical plates/meter. The relative standard deviation of the replicate areas of the recorded peaks is no more than 2.0%. The tailing factor of the main peak is no more than 2.0.

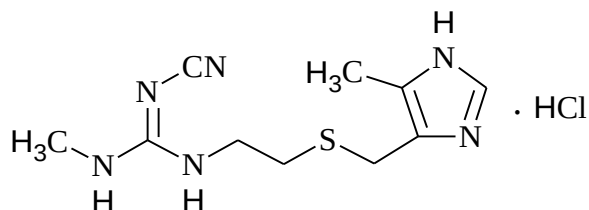
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₂₀H₂₁N.HCl in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CIMETIDINE HYDROCHLORIDE*Cimetidini hydrochloridum*C₁₀H₁₆N₆S.HCl; 288.80

cimetidine hydrochloride; 02074

N-Cyano-*N'*-methyl-*N''*-[2-[[[(5-methyl-1*H*-imidazol-4-yl) methyl]thio]ethyl]guanidine hydrochloride [70059-30-2]

Contains no less than 98.0% and no more than 102.0% of C₁₀H₁₆N₆S.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Slightly yellowish crystalline powder.

Solubility. Freely soluble in water, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cimetidine hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 220 nm to 400 nm, of 0.0014% (w/v) solution in 0.2 *M* sulfuric acid, there is maximum absorption at 218 nm. The absorbance at 218 nm is between 0.650 and 0.705.

C. Complies with chloride ion reactions (**5.3.1.1**).

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography* (**5.2.17.4**). Use a chromatograph equipped with ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μm), *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: transfer 940 mL of sodium 1-hexanesulfonate to a 1000 mL volumetric flask, add 240 mL of methyl alcohol, 0.3 mL of phosphoric acid, complete the volume with water and homogenize. Filter and degas. Make adjustments if necessary.

Solution (1): transfer, quantitatively, about 10 mg of the sample to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Solution (2): transfer 0.5 mL of *Solution (1)* to a 250 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Resolution solution: transfer 50 mg of cimetidine hydrochloride to a 10 mL volumetric flask and add 0.1 M hydrochloric acid to dissolve. Heat in water bath at 50 °C, for two minutes, and allow to cool. Complete the volume with the same solvent and homogenize. Dilute with *Mobile phase* until obtaining a solution with concentration of 2 µg/mL. If necessary, the heating time can be altered to obtain satisfactory responses from the analog amide peaks.

Inject, separately, 50 µL replicas of the *Resolution solution* and *Solution (2)*, record the chromatograms and measure the areas under the peaks. The resolution between the peaks of cimetidine and amide analogs, obtained in the chromatogram with the *Resolution solution*, is no less than 4. The column efficiency is no less than 2000 theoretical plates per meter; the retention factor is no less than 4.0, and the relative standard deviation of the areas of replicas of the recorded peaks is no more than 7.0%, in relation to the chromatogram obtained with *Solution (2)*.

Procedure: inject, separately, 50 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The individual area under the secondary peaks obtained with *Solution (1)* is no more than the area under the peak obtained with *Solution (2)* (0.2%). The sum of the areas under the secondary peaks obtained with *Solution (1)* is no more than five times the area under the peak obtained with *Solution (2)* (1.0%). Do not include solvent-related peaks in the calculations.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105 °C, for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

When it is indicated on the label that the substance is sterile, the sample complies with the Bacterial endotoxin test and with the Sterility test. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.5 EU/mg of cimetidine hydrochloride.

ASSAY

Dissolve 0.20 g of the sample, previously desiccated, in 5 mL of 0.01 M hydrochloric acid and add 50 mL of ethyl alcohol. Titrate with 0.1 M sodium hydroxide VS, determining the endpoint

potentiometrically between the two inflection points. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium hydroxide VS is equivalent to 28.88 mg of $C_{10}H_{16}N_6S.HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antihistamine H_2 .

CIMETIDINE HYDROCHLORIDE INJECTION SOLUTION

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{10}H_{16}N_6S$. Cimetidine hydrochloride injection solution is a sterile cimetidine hydrochloride solution in water for injection.

IDENTIFICATION

A. Dilute the injection solution, successively, in 0.1 M hydrochloric acid, to obtain a concentration of 0.0005% (w/v) of cimetidine. Use cimetidine hydrochloride CRS and the same solvent to prepare a standard solution in the same concentration of cimetidine. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, there are maximum and minimum points only at the same wavelengths observed in the spectrum of the standard cimetidine hydrochloride solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. The injection solution complies with chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.8 to 6.0.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 0.5 EU/mg of cimetidine hydrochloride.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer a volume of the injection solution equivalent to 0.1 g of cimetidine to a 200 mL volumetric flask, complete the volume with 0.1 M hydrochloric acid and homogenize. Dilute successively, with the same solvent to a concentration of 0.0005% (w/v). Prepare a standard solution in the same concentration and in the same solvent, using cimetidine hydrochloride CRS. Measure the absorbances of the solutions at 221 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{10}H_{16}N_6S$ in the injection solution from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph with an ultraviolet detector at 220 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 μ m to 10 μ m); *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: transfer 200 mL of methyl alcohol and 0.3 mL of phosphoric acid to a 1000 mL volumetric flask, complete with water, homogenize and filter.

Sample solution: transfer a volume of the injection solution equivalent to 0.25 g of cimetidine hydrochloride to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 1 mL of this solution to a 200 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: dissolve an accurately weighed amount of cimetidine hydrochloride CRS in a mixture of water and methyl alcohol (80:20), to obtain a 0.5 mg/mL solution. Transfer 2.5 mL of this solution to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Inject 50 µL replicates of the *Standard solution*. The column efficiency determined for the analyte peak is no less than 1000 theoretical plates. The retention factor is no less than 0.6. The relative standard deviation of the replicate areas of the recorded peaks is no more than 2.0%.

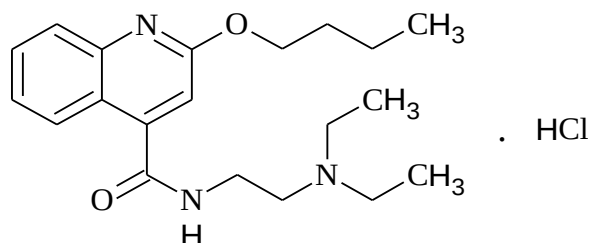
Procedure: inject, separately, 50 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₀H₁₆N₆S in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In type I glass single-dose container, at room temperature and protected from light.

LABELING

Comply with current legislation.

CINCHOCAINE HYDROCHLORIDE*Cinchocaini hydrochloridum*C₂₀H₂₉N₃O₂.HCl; 379.92

cinchocaine hydrochloride; 02092

2-butoxy-N-(2-diethylaminoethyl)quinoline-4-carboxamide hydrochloride

[61-12-1]

Contains no less than 97.0% and no more than 100.5% of C₂₀H₂₉N₃O₂.HCl, in relation to the desiccated base.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder, or colorless hygroscopic crystals. Agglomerates very easily.

Solubility. Very soluble in water and freely soluble in alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the non-desiccated sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of cinchocaine hydrochloride CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay* method, corresponds to the one from the main peak of the *Standard solution*.

C. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Heavy metals (5.3.2.3). Use *Method I*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 80 °C for five hours. No more than 2.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

Chromatographic purity. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel as support and a mixture of toluene, acetone, methyl alcohol and ammonium hydroxide

(50:30:5:1) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve an amount of the sample, accurately weighed, in chloroform, to obtain a 40 mg/mL solution.

Solution (2): dissolve an accurately weighed amount of cinchocaine hydrochloride CRS in chloroform, to obtain a 40 mg/mL solution.

Solution (3): dilute, quantitatively, a portion of *Solution (2)* in chloroform, to obtain a solution at 0.04 mg/mL (0.1%).

Solution (4): dilute, quantitatively, a portion of *Solution (2)* in chloroform, to obtain a solution at 0.12 mg/mL (0.3%).

Solution (5): dilute, quantitatively, a portion of *Solution (2)* in chloroform, to obtain a solution at 0.20 mg/mL (0.5%).

Develop the chromatogram. Remove the plate and allow it to air dry. Nebulize the plate with a 0.5% (w/v) potassium dichromate solution in diluted sulfuric acid (1:5). Heat at 140 °C for 10 minutes or until stains appear, and allow to cool. Examine under ultraviolet light (254 nm). The main stain obtained with the *Solution (1)* corresponds in position, color and intensity to the stain obtained with *Solution (2)*. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (5)* (0.5%). The sum of the amounts of impurities must be no more than 1.0%. Compare the secondary stains obtained with *Solution (1)* with the stains obtained with *Solution (3)*, *Solution (4)* and *Solution (5)* to estimate the amounts of impurities.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

When it is indicated on the label that the substance is sterile, the sample complies with the Bacterial endotoxin test and the Sterility test. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 35.7 EU/mg of cinchocaine hydrochloride.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph with an ultraviolet detector at 327 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Sodium acetate buffer pH 5.5: dissolve 0.2 g of sodium acetate in 250 mL of water, add 2 mL of triethylamine, adjust the pH to 5.5 ± 0.1 with acetic acid, complete the volume to 300 mL with water and homogenize.

Mobile phase: mixture of acetonitrile and *Sodium acetate buffer pH 5.5* (70:30).

Sample solution: transfer 25.0 mg of the sample, accurately weighed, to a 25 mL volumetric flask, add 15 mL of *Mobile phase*, leave in ultrasonic bath for 15 minutes, complete the volume with the same solvent and homogenize.

Standard solution: dissolve an accurately weighed amount of cinchocaine hydrochloride CRS in *Mobile phase* to obtain a 1.0 mg/mL solution and homogenize.

Inject 10 μ L replicates of the *Standard solution*. The column efficiency, measured for the cinchocaine hydrochloride peak, is no less than 2000 theoretical plates/meter. The tailing factor is no more than 2.0. The relative standard deviation of the replicate areas from the recorded peaks must be of no more than 1.5%.

Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{20}H_{29}N_3O_2 \cdot HCl$ in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

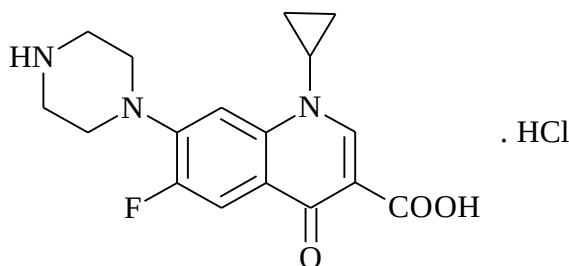
In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Local anesthetic.

CIPROFLOXACIN HYDROCHLORIDE*Ciprofloxacini hydrochloridum*C₁₇H₁₈FN₃O₃.HCl; 367.80

ciprofloxacin hydrochloride; 02138

1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4 dihydroquinoline-3-carboxylic acid
hydrochloride

[86483-48-9]

Contains no less than 98.0% and no more than 102.0% of C₁₇H₁₈FN₃O₃.HCl, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Light yellow, poorly hygroscopic, crystalline powder.

Solubility. Soluble in water, slightly soluble in methyl alcohol and very slightly soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the non-desiccated sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of ciprofloxacin hydrochloride CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay* method, corresponds to the one from the main peak of the *Standard solution*.

C. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 3.0 to 4.5. Determine in aqueous solution at 25 mg/mL.

Related substances. Proceed as described on the method from *Assay*. Record the chromatograms and measure the areas under the peaks. The areas under the peaks related to the ciprofloxacin ethylenediamine analog or any other impurity is no more than 0.2% of the total area under the peaks obtained.

Fluoroquinolonic acid. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and a mixture of methylene chloride, methyl alcohol, acetonitrile and

ammonium hydroxide (4:4:1:2) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below:

Solution (1): dissolve 5.0 mg of fluoroquinolonic acid CRS in 0.05 mL of 6 M sodium hydroxide, complete the volume to 50 mL with water and homogenize. Dilute 2.0 mL of this resulting solution to 10.0 mL with acetonitrile and homogenize.

Solution (2): obtain a sample solution with concentration of 10 mg/mL of ciprofloxacin hydrochloride in an acetonitrile:water solution (80:20).

Put in an adequate chamber a container with ammonium hydroxide, along with the plate. After 15 minutes, transfer the plate to the chromatographic chamber. Develop the chromatogram. Remove the plate, allow to air dry for 15 minutes. Examine under ultraviolet light (254 nm). The stain from *Solution (2)* with R_f corresponding to the main stain obtained with *Solution (1)* is, at maximum, equal in intensity and size to the main stain from *Solution (1)*.

Water (5.2.20.1). 4.7% to 6.7%.

Heavy metals (5.3.2.3). Use *Method I*. No more than 0.002% (20 ppm).

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 278 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at temperature of 30°C; *Mobile phase* flow of 1.2 mL/minute.

Mobile phase: mixture of acetonitrile and 0.0125 M phosphoric acid with pH adjusted in advance with triethylamine to 2.5 ± 0.1 (13:87).

Sample solution: obtain a 0.1 mg/mL ciprofloxacin hydrochloride solution in *Mobile phase*.

Standard solution: obtain a 0.1 mg/mL ciprofloxacin hydrochloride CRS solution in *Mobile phase*.

Resolution solution: prepare a solution with ciprofloxacin ethylenediamine CRS at 250 µg/mL in *Mobile phase*. Transfer 1.0 mL of the solution to a 10.0 mL volumetric flask, complete the volume with *Standard solution* and homogenize.

Inject 25 µL of the *Resolution solution*. The column efficiency is not less than 2500 theoretical plates/meter. The relative retention times are approximately 0.7 for ciprofloxacin ethylenediamine and 1.0 for ciprofloxacin. The tailing factor is no more than 2.5. The resolution between ciprofloxacin ethylenediamine and ciprofloxacin is no less than 5.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 25 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to ciprofloxacin

hydrochloride. Calculate the amount of $C_{17}H_{18}FN_3O_3 \cdot HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

CIPROFLOXACIN HYDROCHLORIDE TABLETS

Contains not less than 90.0% and not more than 110.0% of the declared amount of $C_{17}H_{18}FN_3O_3$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and a mixture of methylene chloride, methyl alcohol, ammonium hydroxide and acetonitrile (4:4:2:1) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.15 g of ciprofloxacin and transfer to a 100 mL volumetric flask. Transfer 70 mL of water, shake for about 20 minutes and complete the volume with the same solvent. Centrifuge and use the clear portion of the supernatant.

Solution (2): aqueous ciprofloxacin hydrochloride CRS solution with the equivalent to 0.15% (w/v) of ciprofloxacin.

Develop the chromatogram. Remove the plate and allow it to air dry for 15 minutes. Examine under ultraviolet light (254 nm and 336 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter immediately and dilute in water until adequate concentration. Measure the absorbances of the solutions at 272 nm (**5.2.14**), using water for zero adjustment. Calculate the amount of $C_{17}H_{18}FN_3O_3$ dissolved in the medium, comparing the readings obtained with that of the ciprofloxacin hydrochloride CRS solution, containing the equivalent to 0.0004% (w/v) of ciprofloxacin, prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{17}H_{18}FN_3O_3$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 1.25 g of ciprofloxacin and transfer to a 500 mL volumetric flask using 400 mL of water. Shake for 30 minutes, complete the volume and filter. Dilute successively to a concentration of 0.0004% (w/v), using water as solvent. Prepare a ciprofloxacin hydrochloride CRS solution in water with the same concentration of ciprofloxacin. Measure the absorbances of the resulting solutions at 272 nm, using water for zero adjustment. Calculate the amount of $C_{17}H_{18}FN_3O_3$ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 278 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 μ m to 10 μ m), maintained at temperature of 30 °C; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: mixture of 0.025 M phosphoric acid (pH adjusted in advance with triethylamine to 3.0 ± 0.1) and acetonitrile (85:15).

Sample solution: transfer five tablets to a 500 mL flask. Transfer about 400 mL of water and leave in ultrasonic bath for approximately 20 minutes, complete the volume with water and shake. Dilute successively to a concentration of 0.25 mg/mL of ciprofloxacin, using water as solvent, and filter.

Standard solution: accurately weigh an amount of ciprofloxacin hydrochloride CRS equivalent to 25 mg of ciprofloxacin, transfer to a 25 mL volumetric flask and complete the volume with water. Dilute successively in water to obtain a 0.25 mg/mL ciprofloxacin solution.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{17}H_{18}FN_3O_3$ in the tablets from the responses obtained for the *Standard solution* and the *Sample solution*.

C. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 1 for microorganism maintenance; sterile saline solution, for inoculum standardization; and culture medium number 11, for base layer and inoculum preparation.

Sample solution: transfer five tablets to a 500 mL flask. Add about 400 mL of water and leave in ultrasonic bath for approximately 20 minutes, complete the volume with water, shake and filter. Dilute in water to obtain concentrations of 4 µg/mL, 8 µg/mL and 16 µg/mL, using *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Standard solution: accurately weigh about 25 mg of ciprofloxacin CRS, transfer to a 20 mL volumetric flask, complete the volume with water and homogenize. Dilute in water to obtain concentrations of 4 µg/mL, 8 µg/mL and 16 µg/mL, using *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Procedure: add 20 mL of culture medium number 11 to each plate and wait until it solidifies. Add 5 mL of inoculum at 1% and proceed as described in *Microbiological assay of antibiotics by agar diffusion (5.5.3.3.1)*, using cylinders. Calculate the sample potency, in µg of ciprofloxacin per milligram, from the standard potency and the responses obtained with the *Standard solution* and with the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, at room temperature and protected from light.

LABELING

Comply with current legislation.

CIPROFLOXACIN HYDROCHLORIDE OPHTHALMIC DROPS

Contains no less than 90.0% and no more than 110.0% the declared amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and a mixture of methylene chloride, methyl alcohol, ammonium hydroxide and acetonitrile (4:4:2:1) as mobile phase. Apply to the plate, separately, 3 µL of each of the freshly prepared solutions described below.

Solution (1): dilute a volume of the sample in water, to obtain a 0.3% (w/v) ciprofloxacin solution.

Solution (2): aqueous ciprofloxacin hydrochloride CRS solution with the equivalent to 0.3% (w/v) of ciprofloxacin.

Develop the chromatogram. Remove the plate and allow it to air dry for 15 minutes. Examine under ultraviolet light (254 nm and 336 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **A.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.5 to 5.5.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Use the *Membrane filtration method*.

ASSAY

Use one of the methods described below.

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 280 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 µm to 10 µm), maintained at temperature of 30 °C; *Mobile phase* flow of 1.5 mL/minute.

Tetrabutylammonium phosphate buffer: prepare a 0005 M tetrabutylammonium phosphate solution with pH adjusted in advance with phosphoric acid to 2.0 ± 0.1 .

Mobile phase: mixture of *Tetrabutylammonium phosphate buffer* and methyl alcohol (75:25).

Sample solution: transfer a volume of the ophthalmic drops equivalent to 6 mg of ciprofloxacin to a 50 mL volumetric flask. Complete the volume with water and homogenize.

Standard solution: accurately weigh an amount of ciprofloxacin hydrochloride CRS, dissolve in water and dilute adequately to obtain a 0.12 mg/mL ciprofloxacin solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{17}H_{18}FN_3O_3$ in the ophthalmic drops from the responses obtained with the *Standard solution* and the *Sample solution*.

B. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 1 for microorganism maintenance; sterile saline solution, for inoculum standardization; and culture medium number 11, for base layer and inoculum preparation.

Sample solution: transfer a volume of the ophthalmic drops equivalent to 40 mg of ciprofloxacin to a 100 mL volumetric flask, complete the volume with water and shake. Dilute to obtain concentrations of 2 µg/mL, 4 µg/mL and 8 µg/mL, using *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Standard solution: accurately weigh about 25 mg of ciprofloxacin CRS, transfer to a 50 mL volumetric flask, complete the volume with water and homogenize. Dilute to obtain concentrations of 2 µg/mL, 4 µg/mL and 8 µg/mL of ciprofloxacin, using *Solution 2 (0.1 M Potassium phosphate buffer, sterile, pH 8.0)* as diluent.

Procedure: add 20 mL of culture medium number 11 to a plate, wait to solidify, add 5 mL of 1% inoculum and proceed as described in *Microbiological test of antibiotics by agar diffusion (5.5.3.3.1)*, adding 0.1 mL of the freshly prepared solutions to the cylinders. Calculate the potency of the ophthalmic drops, in µg of ciprofloxacin per milligram, from the standard potency and the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

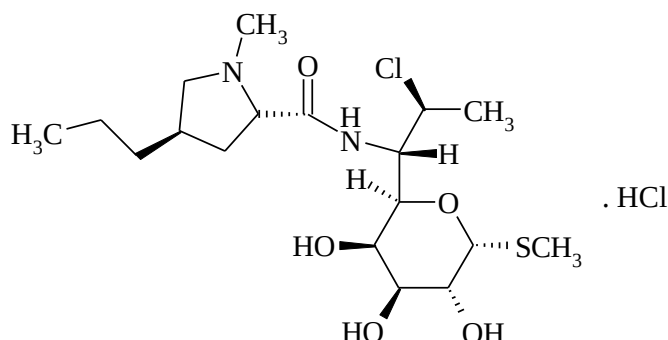
In tightly closed containers, at room temperature and protected from light.

LABELING

Comply with current legislation.

CLINDAMYCIN HYDROCHLORIDE

Clindamycini hydrochloridum



$C_{18}H_{33}ClN_2O_5S \cdot HCl$; 461.44

clindamycin hydrochloride; 02230

Methyl-7-chloro-6,7,8-trideoxy-6-[[[(2S,4R)-1-methyl-4-propyl-2-pyrrolidiny]carbonyl]-amino]-1-thio-L-threo-α-D-galacto-octopyranoside hydrochloride
[21462-39-5]

Contains no less than 91.0% and no more than 102.0% of $C_{18}H_{33}ClN_2O_5S \cdot HCl$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder. Stable at presence of air and light.

Solubility. Freely soluble in water and methyl alcohol, moderately soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +135 to +150, in relation to the anhydrous substance. Determine in solution at 0.04 g/mL in water.

IDENTIFICATION

The tests B. and C. can be omitted if the tests A. and D. are carried out. The test A. can be omitted if the tests B., C. and D. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of clindamycin hydrochloride CRS, prepared identically.

B. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel G as support, and a mixture of isopropanol, ammonium acetate RS and ethyl acetate (19:38:43) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 1 mg/mL sample solution in methyl alcohol.

Solution (2): 1 mg/mL clindamycin hydrochloride CRS solution in methyl alcohol.

Solution (3): 1 mg/mL clindamycin hydrochloride CRS and 1 mg/mL lincomycin hydrochloride CRS, each, solution in methyl alcohol.

Develop the chromatogram in 15 of the plate, allow to air dry. Spray a 0.1% (w/v) potassium permanganate solution. Examine under visible light. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. The test is only valid if the chromatogram obtained with *Solution (3)* presents two clearly separated main stains.

C. Dissolve 10 mg of the sample in 2 mL of hydrochloric acid RS and heat in water bath for three minutes. Add 3 mL of sodium carbonate solution RS in 1 mL of a 20 g/L sodium nitroprusside solution. A reddish-violet color develops.

D. Complies with chloride ion reactions (5.3.1.1). Transfer 0.1 g of the sample to a 10 mL volumetric flask and complete the volume with water.

PURITY TESTS

pH (5.2.19) 3.0 to 5.5. Determine in 100 mg/mL solution, in carbon dioxide-free water.

Related substances. Proceed as described in *Assay*. Prepare the *Test solution* as described next.

Test solution: use the *Sample solution* employed in *Assay*.

Procedure: inject, separately, 20 µL of each one of the solutions and record the chromatograms for no less than twice the retention time of the main peak. The relative retention time is about 0.4 for impurity **A**, 0.65 for impurity **B** and 0.8 for impurity **C**, having as reference the retention time of 10 minutes for clindamycin hydrochloride. For the chromatogram obtained with the *Test solution*, the limits of impurities in relation to the area under the main peak are: no more than 4.0% of impurity **C** and 2.0% of impurity **B**. No more than 1.0% for any other compound. The total of impurities is no more than 6.0%.

Water (5.2.20.1). 3.0% to 6.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.5%.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 210 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Phosphate buffer pH 7.5: weigh 6.8 g of monobasic sodium phosphate and dissolve in 950 mL of water. Adjust the pH to 7.5 with a 25% (w/v) potassium hydroxide solution and complete the volume to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer pH 7.5* and acetonitrile (55:45).

Sample solution: dissolve an accurately weighed amount of the sample in *Mobile phase* and dilute adequately to obtain a 1 mg/mL solution.

Standard solution: dissolve an accurately weighed amount of clindamycin hydrochloride CRS in the *Mobile phase* and dilute adequately to obtain a 1.0 mg/mL solution.

Inject six replicates of 20 µL of the *Standard solution*. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 0.85%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{18}H_{33}ClN_2O_5S.HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

CLINDAMYCIN HYDROCHLORIDE CAPSULES

Contains no less than 90.0% and no more than 110% of the declared amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the method on *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 210 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); *Mobile phase* flow of 1.5 mL/minute.

Phosphate buffer: dissolve 6.8 g of monobasic potassium phosphate in 950 mL of water, adjust pH to 7.5 with 25% (w/v) potassium hydroxide and dilute to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer* and acetonitrile (55:45). Make adjustments, if necessary.

Note: the reduction in the proportion of acetonitrile in the *Mobile phase* increases the resolution between the peaks related to 7-epiclindamycin and to clindamycin.

Solution (1): weigh the capsules, remove the content and weigh them again. Homogenize the content of the capsules. Accurately weigh an amount of powder equivalent to 50 mg of clindamycin to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Shake for 15 minutes. Homogenize and filter.

Solution (2): 1 mg/mL clindamycin hydrochloride CRS solution in *Mobile phase*.

Solution (3): dilute 2 mL of *Solution (1)* to 100 mL with *Mobile phase*.

Inject 20 μ L of *Solution (2)* and record the chromatogram for not less than twice the main peak retention time. The relative retention times are approximately 0.4 for lincomycin, 0.65 for clindamycin B, 0.8 for 7-epiclindamycin and 1.0 for clindamycin. The resolution between the peaks related to clindamycin B and to 7-epiclindamycin is no less than 2.4. The resolution between the peaks related to 7-epiclindamycin and to clindamycin is no less than 3.0.

Procedure: inject, separately, 20 μ L of *Solutions (1)* and *(3)*, record the chromatograms for not less than twice the main peak retention time and measure the areas under the peaks. The area of any secondary peak corresponding to clindamycin B in the chromatogram obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (3)* (2.0%). The area of any

secondary peak corresponding to 7-epiclindamycin in the chromatogram obtained with *Solution (1)* is not greater than twice the area under the main peak obtained with *Solution (3)* (4.0%). The sum of the areas of all secondary peaks obtained in the chromatogram with *Solution (1)*, except the main peak, is not greater than three times the area under the main peak obtained with *Solution (3)* (6.0%). Disregard peaks related to the solvent or with area below 0.025 times the area under the main peak obtained with *Solution (3)* (0.05%).

Water (5.2.20.1). No more than 7.0%

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 210 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 µm); *Mobile phase* flow of 1.5 mL/minute.

Phosphate buffer pH 7.5: weigh 6.8 g of monobasic sodium phosphate and dissolve in 950 mL of water. Adjust the pH to 7.5 with 25% (w/v) potassium hydroxide and complete the volume to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer pH 7.5* and acetonitrile (55:45). Make adjustments, if necessary.

Sample solution: weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Accurately weigh an amount of powder equivalent to 50 mg of clindamycin to a 50 mL volumetric flask and complete with *Mobile phase*. Shake for 15 minutes. Homogenize and filter.

Standard solution: dissolve an accurately weighed amount of clindamycin hydrochloride CRS in *Mobile phase* and dilute adequately to obtain a 1.0 mg/mL solution.

Inject 20 µL replicates of the *Standard solution* and record the chromatograms for not less than twice the main peak retention time. The resolution between the peaks related to clindamycin B and to 7-epiclindamycin is no less than 2.4. The resolution between the peaks related to 7-epiclindamycin and to clindamycin is no less than 3.0. The relative standard deviation of the replicate areas of the recorded peaks related to clindamycin is not more than 2.0%.

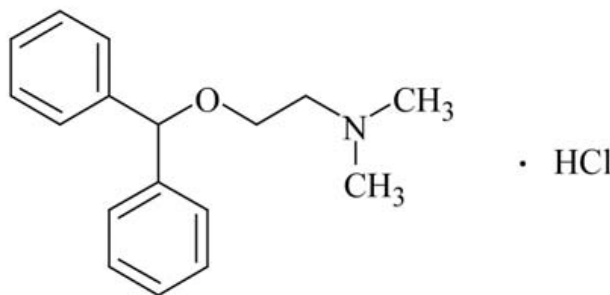
Procedure: inject, separately, 20 µL of *Standard solution* and *Sample solution*, record the chromatograms for no less than twice the retention time of the main peak and measure the areas under the peaks. Calculate the amount of $C_{18}H_{33}ClN_2O_5S$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

DIPHENHYDRAMINE HYDROCHLORIDE*Diphenhydramini hydrochloridum*C₁₇H₂₁NO.HCl; 291.82

diphenhydramine hydrochloride; 02979

2-diphenylmethoxy-*N,N*-dimethylethanamine hydrochloride (1:1)

[147-24-0]

Contains no less than 99.0% and no more than 101.0% of C₁₇H₂₁NO.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white odorless, crystalline powder.

Solubility. Freely soluble in water and ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 167 °C to 172 °C.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum **(5.2.14)** of the sample desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of diphenhydramine hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 350 nm, of the 0.05% (w/v) sample solution in ethyl alcohol, there is a maximum point at 253 nm, identical to the one observed in the spectrum of a similar diphenhydramine hydrochloride CRS solution.

C. The main stain of the chromatogram from *Solution (1)* obtained in *Related substances* corresponds in position, color and intensity to the one obtained with *Solution (3)*.

D. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. The 0.2% (w/v) aqueous solution and a solution five times more diluted are colorless (5.2.12). The 0.2% (w/v) aqueous solution is not more stained than the *Standard color solution SC G* (5.2.12).

pH (5.2.19). 4.0 to 6.0. Determine in aqueous solution at 5.0% (w/v).

Acidity or alkalinity. Dissolve 2.5 g of the sample in 50 mL of water. No more than 0.25 mL of 0.01 M hydrochloric acid is spent to neutralize 10 mL of the sample, using methyl red TS as an indicator. No more than 0.5 mL of 0.01 M sodium hydroxide is necessary to turn the solution color from pink to yellow.

Related substances. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel H as support and a mixture of diethylamine, methyl alcohol and chloroform (1:20:80) as mobile phase. Activate the plate at 105 °C, for one hour. Apply, separately, to the plate 5 µL of each solution described below.

Solution (1): 2% (w/v) sample solution in methyl alcohol.

Solution (2): 0.02% (w/v) sample solution in methyl alcohol.

Solution (3): 2% (w/v) diphenhydramine hydrochloride CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate, dry in air stream and nebulize with sulfuric acid. Heat at 120 °C, for 10 minutes, until stains appear. No stain obtained with *Solution (1)*, except for the main stain, is more intense than that obtained with *Solution (2)* (1.0%).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for three hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.25 g of the sample, previously desiccated, and dissolve in 20 mL of glacial acetic acid and 10 mL of mercury acetate RS. Transfer one drop of methylrosanilinium chloride TS and titrate with 0.1 M perchloric acid VS until the color turns greenish-blue. Carry out the blank test and make the necessary corrections. Alternatively, determine the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 29.182 mg of C₁₇H₂₁NO.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antihistamine.

DIPHENHYDRAMINE HYDROCHLORIDE TABLETS

Contains no less than 98.0% and no more than 100.5% of the declared amount of $C_{17}H_{21}NO \cdot HCl$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Use an amount of powder equivalent to 0.1 g of diphenhydramine hydrochloride. Add 1 mL of 0.1 M sulfuric acid and shake with three portions of 20 mL of ethyl ether. Discard the ethereal layer. Alkalinize the aqueous phase with 5 M sodium hydroxide and extract with two portions of 50 mL of *n*-heptane. Collect the organic extracts, wash them with 10 mL of water, filter over sodium sulfate anhydrous and evaporate the filtrate to dryness. Dissolve the residue in 1 mL of carbon disulfide. In the infrared absorption spectrum **(5.2.14)** of the residue there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of diphenhydramine hydrochloride CRS, prepared identically.

B. Weigh and pulverize the tablets. Homogenize an amount of powder equivalent to 0.1 g diphenhydramine hydrochloride with two portions of 15 mL of chloroform, filtering each portion. Evaporate the filtrate to dryness in a water bath. Dry the residue in oven at 80 °C, for one hour. The residue melts around 168 °C.

C. The precipitate obtained in test **B.** from *Identification* complies with chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium, filter and dilute, if necessary, with 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances at 254 nm **(5.2.14)**, using *dissolution medium* for zero adjustment. Calculate the amount of $C_{17}H_{21}NO \cdot HCl$ dissolved in the medium, comparing the readings obtained with that of the diphenhydramine hydrochloride CRS solution, at the same concentration and prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{17}H_{21}NO.HCl$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Related substances* in the *Diphenhydramine hydrochloride* monograph. Prepare *Solution (1)* and *Solution (2)* as described next.

Solution (1): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 50 mg of diphenhydramine hydrochloride and extract with three portions of 10 mL of chloroform. Filter, evaporate the combined extracts to dryness and dissolve the residue in 5 mL of chloroform.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with chloroform.

Water (5.2.20.1). Determine in 1.0 g of the sample. No more than 1.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.2 g of diphenhydramine hydrochloride and dissolve in 20 mL of acetic acid anhydrous and 10 mL of 6% (w/v) mercury acetate in glacial acetic acid. Titrate with 0.1 M perchloric acid VS, using methylrosanilinium chloride TS as indicator. Each mL of 0.1 M perchloric acid VS is equivalent to 29.180 mg of $C_{17}H_{21}NO.HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

DIPHENHYDRAMINE HYDROCHLORIDE ORAL SOLUTION

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{17}H_{21}NO.HCl$.

IDENTIFICATION

A. Transfer an amount of oral solution with 50 mg of diphenhydramine hydrochloride to a separation funnel. Add 0.5 mL of 2 M sulfuric acid and extract with three portions of 15 mL of ethyl ether, discarding the ethereal phases. In a second separation funnel, dissolve 50 mg of diphenhydramine hydrochloride CRS in 25 mL of water. Treat the solutions as follows: add 2 mL of M sodium hydroxide and extract with 75 mL of *n*-heptane. Wash the *n*-heptane extract with 10 mL of water, evaporate to dryness and dissolve the residue in 4 mL of chloroform. Filter if necessary to clarify the solution. Quickly determine the infrared absorption spectrum of the Standard solution and the Sample solution filtered, using chloroform as blank, in a 0.1 mm or 1 mm cell and between 7 μm and 15 μm . In the infrared absorption spectrum (**5.2.14**) of the sample, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of diphenhydramine CRS, prepared identically.

B. Evaporate to dryness 1 mL of *Solution (1)*. Dissolve the residue in 0.15 mL of water and add 2 mL of sulfuric acid. A yellow color is produced and, by adding 0.5 mL of nitric acid, it changes to red. Add 15 mL of water, cool, add 5 mL of chloroform and shake. The chloroform layer acquires a violet color.

Solution (1): acidify a volume of oral solution with 50 mg of diphenhydramine hydrochloride with 2 M hydrochloric acid and shake with three portions of 20 mL of ethyl ether. Discard the ethereal fraction. Extract with two portions of 20 mL of chloroform, dry the combined extracts under sodium sulfate anhydrous, evaporate the chloroform and dissolve the residue in 5 mL of chloroform.

C. Add excess M sodium hydroxide. There is release of ammonium vapors with characteristic odor, which can be recognized by the development of a red color when a filter paper is exposed to the vapors released.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.0 to 6.0.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel H as support and a mixture of methyl alcohol and chloroform (20:80) as mobile phase. Apply to the plate, separately, 5 μL of each of the freshly prepared solutions described below.

Solution (1): use the *Solution (1)* described on test **B.** of *Identification*.

Solution (2): dilute one volume of *Solution (1)* to 100 volumes with chloroform.

Develop the chromatogram. Remove the plate, allow to air dry and nebulize with diluted potassium iodobismuthate RS. Examine under visible light. No secondary stain obtained with *Solution (1)* is more intense than that obtained with *Solution (2)*.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Diphenhydramine hydrochloride

Acidify a volume of oral solution, accurately measured, containing 0.1 g of diphenhydramine hydrochloride with 2 M hydrochloric acid, shake and extract with three portions of 20 mL of ethyl ether. Discard the ethereal fractions. Alkalize the aqueous phase with 5 M sodium hydroxide and extract with four portions of 25 mL of ethyl ether. Wash the combined ether extracts with two portions of 5 mL of water. Extract the washing waters with 15 mL of ethyl ether, collect the ethereal extracts and evaporate to dryness. Dissolve the residue in 15 mL of 0.05 M sulfuric acid VS and titrate the excess acid with 0.1 M sodium hydroxide VS, using methyl red TS. Each mL of 0.05 M sulfuric acid VS corresponds to 29.180 mg of $C_{17}H_{21}NO \cdot HCl$.

Ammonium chloride

Conduct the ammonium chloride assay when it is present in the formulation. Contains no less than 95.0% and no more than 105.0% the declared amount of NH_4Cl .

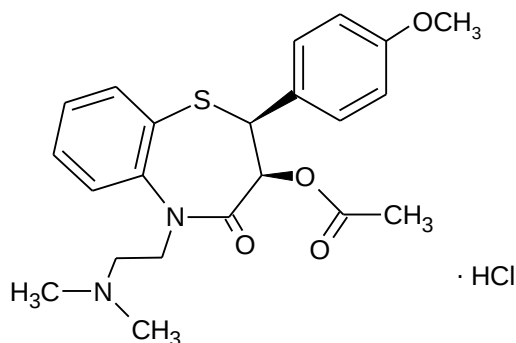
Dissolve one volume of the oral solution, accurately measured, in 20 mL of a 5.0% (w/v) ammonium chloride solution in water. Add a mixture of 5 mL of formaldehyde neutralized in advance in the presence of phenolphthalein TS and 20 mL of water. After one to two minutes, slowly titrate with M sodium hydroxide VS in the presence of 0.2 mL of the same indicator. Each mL of M sodium hydroxide VS corresponds to 53.490 mg of NH_4Cl . If the sample is stained, treat in advance with activated charcoal to remove the dye.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

DILTIAZEM HYDROCHLORIDE*Diltiazemi hydrochloridum*C₂₂H₂₆N₂O₄S.HCl; 450.98

diltiazem hydrochloride; 03038

(2S,3S)-3-acetyloxy-5-[2-(dimethylamino)ethyl]-2,3-di-hydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride
[33286-22-5]

Contains no less than 98.5% and no more than 101.0% of C₂₂H₂₆N₂O₄S.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Freely soluble in water and in methyl alcohol, slightly soluble in ethyl alcohol anhydrous.

Physicochemical constants.

Melting temperature (5.2.2): between 207.5 °C and 212.0 °C, with decomposition.

Specific optical rotation (5.2.8): +115 to +120, in relation to the desiccated substance. Dilute 5 mL of the preparation obtained in *Aspect of the preparation* to 25 mL with water.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of diltiazem hydrochloride CRS, prepared identically.

B. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Dissolve 1.0 g of the sample in 20 mL of carbon dioxide-free water. The preparation is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 4.3 to 5.3. Dissolve 1.0 g of the sample in carbon dioxide-free water.

Heavy metals (5.3.2.3). Use *Method I*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.400 g of the sample and dissolve in a mixture with 2 mL of formic acid anhydrous and 60 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 45.1 mg of $C_{22}H_{26}N_2O_4S \cdot HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-hypertensive, antianginal, anti-arrhythmic.

DILTIAZEM HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{22}H_{26}N_2O_4S.HCl$. The tablets can be coated.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay* method, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Grind each tablet individually and transfer, quantitatively, the powder to a 100 mL volumetric flask. Add 50 mL of methyl alcohol and leave in ultrasonic bath for 10 minutes. Complete the volume with water, homogenize and filter. Carry out successive dilutions until a concentration of 0.03 mg/mL, using a mixture of methyl alcohol and water (50:50) as solvent. Prepare a *Standard Solution* in the same conditions. Proceed as described in *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Apparatus: paddles, 75 rpm

Times: 30 minutes and three hours

Procedure: in the collecting times, take an aliquot of the dissolution medium, filter immediately and dilute, if necessary, in water until adequate concentration. Measure the absorbances at 237 nm (5.2.14), using *Dissolution medium* for zero adjustment. Calculate the amount of $C_{22}H_{26}N_2O_4S.HCl$ dissolved in the medium, comparing the readings obtained with that of the diltiazem hydrochloride CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: no less than 60% (Q) of the declared quantity of $C_{22}H_{26}N_2O_4S.HCl$ dissolve in 30 minutes and no less than 75% (Q) of the declared quantity of $C_{22}H_{26}N_2O_4S.HCl$ dissolve in three hours.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 240 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at temperature of 30 °C; *Mobile phase* flow of 1.0 mL/minute. Prepare the solutions immediately before use.

0.05% (v/v) trifluoroacetic acid solution in water: transfer 0.5 mL of trifluoroacetic acid to a 1000 mL volumetric flask with water, complete the volume with the same solvent and homogenize.

0.05% (v/v) trifluoroacetic acid solution in methyl alcohol: transfer 0.5 mL of trifluoroacetic acid to a 1000 mL volumetric flask with methyl alcohol, complete the volume with the same solvent and homogenize.

Mobile phase: mixture of the *0.05% (v/v) trifluoroacetic acid solution in methyl alcohol* and the *0.05% (v/v) trifluoroacetic acid solution in water* (56:44).

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 30 mg of diltiazem hydrochloride to a 100 mL volumetric flask, add 50 mL of methyl alcohol and leave in ultrasonic bath for 10 minutes. Complete the volume with water, homogenize and filter. Dilute, quantitatively, in a solution of methyl alcohol and water (50:50), to obtain a solution with concentration of 0.03 mg/mL.

Standard solution: accurately weigh an amount of diltiazem hydrochloride CRS, dissolve in a solution of methyl alcohol and water (50:50) and dilute adequately to obtain a 0.03 mg/mL solution.

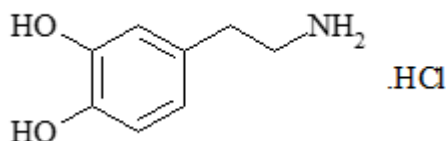
Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{22}H_{26}N_2O_4S.HCl$ in the tablets from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

DOPAMINE HYDROCHLORIDE*Dopamini hydrochloridum* $C_8H_{11}NO_2 \cdot HCl$; 189.64

dopamine hydrochloride; 03187

4-(2-aminoethyl)-1,2-benzenediol hydrochloride

[62-31-7]

Contains no less than 98.0% and no more than 102.0% of $C_8H_{11}NO_2 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, or off-white, crystalline powder.

Solubility. Freely soluble in water and soluble in ethyl alcohol, methyl alcohol and in alkali hydroxide solutions.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of dopamine hydrochloride CRS, prepared identically.

B. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Dissolve 0.1 g of sample in 5 mL of sulfuric acid. The preparation obtained is not more stained than the *Standard color solution SC A* **(5.2.12)**.

pH (5.2.19). 3.0 to 5.5. Determine in aqueous solution at 4.0% (w/v).

Acidity or alkalinity. Dissolve 0,5 g of the sample in 10 mL of water and add 0.1 mL of methyl red TS and 0.75 mL of 0.01 M sodium hydroxide VS. A yellow color develops. Add 1.5 mL of 0.01 M hydrochloric acid VS. A red color develops.

Related substances. Proceed as described in *High performance liquid chromatography* **(5.2.17.4)**. Use a chromatograph equipped with ultraviolet detector at 280 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane (5 μ m), maintained at room temperature; mobile phase flow of 1 mL/minute. Protect solutions from direct light.

Buffer: weigh 21 g of citric acid, dissolve in 200 mL of *M* sodium hydroxide, dilute to 1000 mL with water and homogenize. Transfer 600 mL of this solution to a 1000 mL volumetric flask, complete the volume with 0.1 *M* hydrochloric acid and homogenize.

Eluent A: weigh 1.08 g of sodium 1-octanesulfonate, dissolve in 880 mL of *Buffer* and add 50 mL of methyl alcohol and 70 mL of acetonitrile. Homogenize, filter and degas.

Eluent B: weigh 1.08 g of sodium 1-octanesulfonate, dissolve in 700 mL of *Buffer* and add 100 mL of methyl alcohol and 200 mL of acetonitrile. Filter and degas.

Mobile Phase gradient: adopt the gradient system described in the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 – 5	90	10	isocratic
5 – 20	90 → 40	10 → 60	linear gradient
20 – 25	40	60	isocratic

Sample solution: prepare, accurately, a sample solution in the concentration of 2 mg/mL using *Eluent A* as solvent.

Standard solution: Transfer 1 mL of the *Sample solution* to a 100 mL volumetric flask, complete the volume with *Eluent A* and homogenize. Transfer 1 mL of the previous solution to a 10 mL volumetric flask, complete the volume with the same diluent and homogenize.

Resolution solution: accurately weigh 10 mg of 3-*o*-methyl-dopamine hydrochloride and 10 mg of 4-*o*-methyl-dopamine hydrochloride, transfer to a 100 mL volumetric flask and complete with *Eluent A*. Homogenize. Transfer 6 mL of the previous solution to a 25 mL volumetric flask, complete the volume with the same solvent and homogenize.

Procedure: inject, separately, 10 µL of the *Standard solution*, the *Sample solution* and the *Resolution solution* and record the chromatograms. The resolutions between the peaks of 3-*o*-methyl-dopamine hydrochloride and 4-*o*-methyl-dopamine hydrochloride is no less than 5.0. The sum of the areas under the peaks obtained with the *Sample solution* is no more than twice the area under the main peak obtained with the *Standard solution* (0.2%). The area of any individual peak is no more than equal to the area under the main peak obtained with the *Standard solution* (0.1%). Any other impurity recorded in the chromatogram of the *Sample solution* has an area no more than 0.5 times the area obtained with the main peak of the *Standard solution* (0.05%).

Heavy metals (5.3.2.3). Proceed as described in *Thioacetamide reaction methods, Method 1*. Determine in 1.0 g of the sample. No more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Determine in 1.0 g of the sample. No more than 0.1%.

Loss on drying (5.2.9.1). Determine in 1.0 g of sample, at 105 °C, for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Accurately weigh 75 mg of the sample and dissolve in 5 mL of formic acid anhydrous and 25 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 18.964 mg of $C_8H_{11}NO_2$.

PACKAGING AND STORAGE

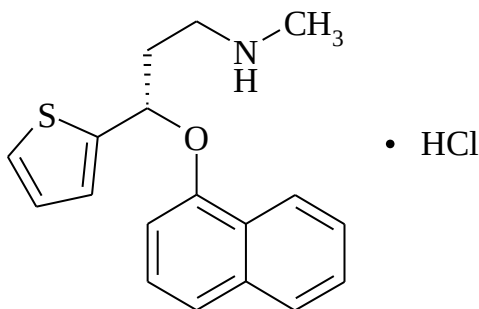
In tightly closed containers, protected from light, at room temperature.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Sympathomimetic.

DULOXETINE HYDROCHLORIDE*Duloxetini hydrochloridum*C₁₈H₁₉NOS.HCl; 333.88

duloxetine hydrochloride; 03263

(3S)-N-Methyl-3-(naphthalen-1-iloxy)-3 (thiophen-2-yl) propan-1-amine hydrochloride

[136434-34-9]

Contains no less than 97.5% and no more than 102.0% of C₁₈H₁₉NOS.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white powder.

Solubility. Moderately soluble in water, freely soluble in methyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +119 to +127, in relation to the desiccated substance. Determine in solution at 1% (w/v) in methyl alcohol.

IDENTIFICATION

The identification test B. can be omitted if test C. is carried out. The identification test C. can be omitted if test B. is carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of duloxetine hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 220 nm to 400 nm of the 0.0024% (w/v) sample solution in acetonitrile, there are maximum and minimum points only at the same wavelengths of a similar duloxetine hydrochloride CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method B. from Assay, corresponds to the one from the main peak of the *Standard solution*.

D. Complies with chloride ion reactions (5.3.1.1). Determine in solution at 0.5% (w/v) in methyl alcohol.

PURITY TESTS

Enantiomeric purity. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 230 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica gel OD for chiral separation (5 µm), maintained at temperature of 40 °C; *Mobile phase* flow of 1 mL/minute.

Mobile phase: dilute 2 mL of diethylamine to 1000 mL with a mixture of isopropyl alcohol and hexane (17:83) and homogenize.

Solution (1): accurately weigh 5 mg of the sample, dissolve in 50 mL of *Mobile phase*, dilute to 100 mL with the same solvent and homogenize.

Solution (2): transfer 1 mL of *Solution (1)* to a 200 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Solution (3): accurately weigh 5 mg of impurity A of duloxetine ((3R)-N-Methyl-3-(naphthalen-1-iloxy)-3 (thiophen-2-yl) propan-1-amine) and 5 mg of the sample, dissolve in 100 mL of *Mobile phase* and homogenize.

Inject 20 µL replicates of the *Solution (2)*. The signal-noise ratio is no less than 10 for duloxetine. Inject 20 µL replicates of the *Solution (3)*. The relative retention time between duloxetine (retention time of about seven minutes) and impurity **A** is of approximately 1.3. The resolution between the peaks of duloxetine and impurity **A** is not less than 3.0.

Procedure: inject, separately, 20 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under all peaks obtained. The area under the peak related to impurity **A** obtained with *Solution (1)* is no more than equal the area under the main peak obtained with *Solution (2)* (0.5%).

Related substances. Proceed as described on method **B.** from Assay. Prepare the *Sample solution* as described next.

Sample solution: accurately weigh 12 mg of the sample and transfer to a 100 mL volumetric flask, using a small amount of acetonitrile. Leave in ultrasonic bath for 15 minutes. Complete the volume with the *Mobile phase* and homogenize. Dilute, in *Mobile phase*, to obtain a concentration of 0.0012% (w/v).

Procedure: inject 20 µL of the *Sample solution*, record the chromatograms and measure the areas under all peaks obtained. The sum of the areas under the secondary peaks, except that related to the solvent, is no more than 0.4% of the area under the main peak. No area is greater than 0.2% of the area under the main peak. Disregard any areas under the peaks with area smaller than 0.05 times the area under the main peak. Do not include the areas under solvent-related peaks in the calculations.

Heavy metals (5.3.2.3). Determine in 2.5 g of the sample dissolved in methyl alcohol. Prepare the reference solution, using 2.5 mL of standard lead solution (10 ppm Pb) in methyl alcohol. Use *Method II*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for three hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh 0.12 g of the sample and transfer to a 100 mL volumetric flask. Dissolve with acetonitrile, complete the volume with the same solvent and homogenize. Leave in ultrasonic bath for 15 minutes, if necessary. Dilute, with the same solvent, to obtain a concentration of 0.0024% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 290 nm, using acetonitrile for zero adjustment. Calculate the amount of $C_{18}H_{19}NOS.HCl$ in the sample, from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 230 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at temperature of 25 °C; *Mobile phase* flow of 1 mL/minute.

Mobile phase: mixture of 50 mM monobasic phosphate buffer pH 6.0, with 0.3% (v/v) of triethylamine, and acetonitrile (60:40).

Sample solution: accurately weigh 24 mg of the sample and transfer to a 200 mL volumetric flask, using a small amount of acetonitrile. Leave in ultrasonic bath for 15 minutes. Dissolve, complete the volume with *Mobile phase* and homogenize. Dilute, successively, in *Mobile phase* until a concentration of 0.0012% (w/v).

Standard solution: accurately weigh 12 mg of duloxetine hydrochloride CRS and transfer to a 100 mL volumetric flask, using a small amount of acetonitrile. Dissolve, complete the volume with *Mobile phase* and homogenize. Dilute, in *Mobile phase*, until a concentration of 0.0012% (w/v).

The column efficiency for the duloxetine hydrochloride peak is no less than 5000 theoretical plates/meter. The tailing factor is no more than 2.0. The relative standard deviation of the replicate areas from the recorded peaks must be no more than 1.5%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₈H₁₉NOS.HCl in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antidepressant.

DULOXETINE HYDROCHLORIDE CAPSULES

Contains no less than 90.0% and no more than 110.0% of the declared amount of duloxetine ($C_{18}H_{19}NO_2$).

IDENTIFICATION

The identification test B. can be omitted if test C. is carried out. The identification test C. can be omitted if test B. is carried out.

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel 60 F₂₅₄ as support and a mixture of ethyl acetate, methyl alcohol and ammonium hydroxide (85:10:5) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the content of 10 capsules. Accurately weigh an amount of powder equivalent to 50 mg of duloxetine, transfer to a 50 mL volumetric flask and dissolve with 40 mL of acetonitrile. Leave in ultrasonic bath for 15 minutes at room temperature. Complete the volume with acetonitrile, homogenize and filter.

Solution (2): 1.12 mg/mL duloxetine hydrochloride CRS solution, equivalent to 1 mg/mL of duloxetine, in acetonitrile.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 220 nm to 400 nm of the sample solution obtained in method **A.** from *Assay*, there are maximum and minimum points only at the same wavelengths observed in the standard solution spectrum.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. Weigh and pulverize the content of 10 capsules. Accurately weigh an amount of powder equivalent to 25 mg of duloxetine to a 5 mL volumetric flask and complete the volume with methyl alcohol. Homogenize and filter. The resulting solution complies with chloride ion reactions (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test for *Enteric coated (gastro-resistant) capsules*.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Acid stage:

Dissolution medium: 0.1 M hydrochloric acid, 1000 mL.

Apparatus: baskets, 100 rpm.

Time: 120 minutes.

Standard solution: accurately weigh 11.2 mg of duloxetine hydrochloride CRS, equivalent to 10 mg of duloxetine, to a 100 mL volumetric flask, dissolve using phosphate buffer pH 6.8, complete the volume with phosphate buffer pH 6.8 and homogenize. Leave in ultrasonic bath, if necessary. Dilute, in *Mobile phase*, to obtain a concentration of 3 µg/mL of duloxetine.

Sample solution: after testing, use aliquots of the dissolution medium, filter and dilute, if necessary, in *Mobile phase*, to obtain a theoretical concentration of 3 µg/mL of duloxetine.

Procedure: after the test, take an aliquot of the dissolution medium, prepare the *Sample solution* and proceed as described in method **B.** of Assay. Inject, separately, 20 µL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₈H₂₉NOS dissolved in the medium, from the responses obtained with the *Standard solution* and the *Sample solution*.

Buffer pH 6.8 stage:

Dissolution medium: phosphate buffer pH 6.8, 1000 mL.

Apparatus: baskets, 100 rpm.

Time: 60 minutes (when the declared value is 60 mg, use the time of 90 minutes)

Standard solution: transfer, quantitatively, 11.2 mg of duloxetine hydrochloride CRS, equivalent to 10 mg of duloxetine, to a 100 mL volumetric flask, using phosphate buffer pH 6.8, complete the volume with phosphate buffer pH 6.8 and homogenize. Leave in ultrasonic bath, if necessary. Dilute, in *Mobile phase*, to obtain a concentration of 10 µg/mL of duloxetine.

Sample solution: after conducting the test, use aliquots of the dissolution medium, filter and dilute, if necessary, in *Mobile phase*, to obtain a theoretical concentration of 10 µg/mL of duloxetine.

Procedure: after the *Acid stage* test, transfer the baskets with the samples to tanks containing 1000 mL of phosphate buffer pH 6.8 and carry out the *Buffer pH 6.8 stage* test. After the test, take an aliquot from of dissolution medium, prepare the *Sample solution* and proceed as described in method **B.** from Assay. Inject, separately, 20 µL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₈H₂₉NOS dissolved in the medium, from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance:

Tolerance: no less than 10% (Q) of the declared quantity of C₁₈H₁₉NOS dissolve in 120 minutes.

Buffer pH 6.8 stage: no less than 75% (Q) of the declared quantity of C₁₈H₁₉NOS dissolve in the time specified.

Related substances. Proceed as described on method **B.** from Assay. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize the content of 20 capsules. Accurately weigh an amount of powder equivalent to 10 mg of duloxetine to a 100 mL volumetric flask, using a small amount of acetonitrile, and leave in ultrasonic bath for 15 minutes. Complete the volume with the *Mobile phase* and homogenize. Dilute in *Mobile phase*, until obtaining a concentration of 10 µg/mL of duloxetine.

Procedure: inject 20 µL of the *Sample solution*, record the chromatograms and measure the areas under all peaks obtained. The sum of the areas under the secondary peaks, except the one of the solvent peak, is no more than 0.4% of the area under the main peak. No area is greater than 0.2% of the area under the main peak. Disregard any peaks with area smaller than 0.05 times the area under the main peak. Do not include solvent-related peaks in the calculations.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize the content of 20 capsules. Transfer to a 100 mL volumetric flask an amount of powder equivalent to 10 mg of duloxetine, using 40 mL of acetonitrile. Leave in an ultrasonic bath for 15 minutes. Complete the volume to 100 mL with acetonitrile. Dilute, with the same solvent, to obtain a concentration of 20 µg/mL of duloxetine. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 290 nm, using acetonitrile for zero adjustment. Calculate the amount of duloxetine (C₁₈H₁₉NOS) in the capsules from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 230 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at temperature of 25 °C; *Mobile phase* flow of 1 mL/minute.

Mobile phase: mixture of 50 mM monobasic phosphate buffer pH 6.0, with 0.3% (v/v) of triethylamine, and acetonitrile (60:40).

Sample solution: weigh and pulverize the content of 20 capsules. Accurately weigh an amount of powder equivalent to 10 mg of duloxetine to a 100 mL volumetric flask using a small amount of acetonitrile and leave in ultrasonic bath for 15 minutes. Complete the volume with the *Mobile phase* and homogenize. Dilute, successively, in *Mobile phase*, until obtaining a concentration of 10 µg/mL of duloxetine.

Standard solution: accurately weigh 11.2 mg of duloxetine hydrochloride CRS, equivalent to 10 mg of duloxetine, transfer to a 100 mL volumetric flask, using phosphate buffer pH 6.8, complete the

volume with phosphate buffer pH 6.8, dissolve and homogenize. Leave in ultrasonic bath if necessary. Dilute, in *Mobile phase*, to obtain a concentration of 10 µg/mL of duloxetine.

The column efficiency for the duloxetine peak is no less than 5000 theoretical plates/meter. The tailing factor is no more than 2.0. The relative standard deviation of replicate areas from the recorded peaks must be no more than 1.5%.

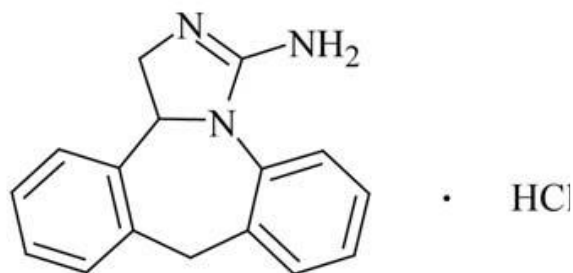
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of duloxetine (C₁₈H₁₉NOS) in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with the current legislation.

EPINASTINE HYDROCHLORIDE*Epinastini hydrochloridum*C₁₆H₁₅N₃.HCl; 285.77

epinastine hydrochloride; 03440

9,13b-dihydro-1*H*-dibenz[*c,f*]imidazo[1,5-*a*]azepin-3-amine hydrochloride (1:1)
[108929-04-0]

Contains no less than 98.5% and no more than 101.5% of C₁₆H₁₅N₃.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or pale yellow crystalline powder.

Solubility. Freely soluble in water.

Physicochemical constants.

Melting range (5.2.2): 273 °C to 275 °C.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of epinastine hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 300 nm, of the 0.025% (w/v) sample solution in 0.1 *M* hydrochloric acid, there is a maximum at 210 nm, identical to that observed in the spectrum of epinastine hydrochloride CRS solution, prepared identically.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak of the *Standard solution*.

D. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C until constant weight. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 207 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm) with deactivated base, maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of 0.3% (v/v) triethylamine, adjust pH to 4.0 with phosphoric acid, and methyl alcohol (60:40).

Sample solution: accurately weigh 25 mg of sample, transfer to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Transfer 4 mL of this solution to a 100 mL volumetric flask and complete the volume with *Mobile phase*, to obtain a 20 µg/mL solution.

Standard solution: accurately weigh 25 mg of epinastine hydrochloride CRS, transfer to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Transfer 4 mL of this solution to a 100 mL volumetric flask and complete the volume with *Mobile phase*, to obtain a 20 µg/mL solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₆H₁₅N₃.HCl in the sample from the responses obtained with the *Standard solution* and with the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antihistamine.

EPINASTINE HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{16}H_{15}N_3.HCl$. The tablets can be coated.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF254 as support and a mixture of water, butyl alcohol and glacial acetic acid (50:40:10) as mobile phase. Prepare the mobile phase 24 hours beforehand. Then, discard the lower layer. Apply to the plate, separately, 10 μL of each one of the freshly prepared solutions described below.

Solution (1): pulverize 20 tablets. Accurately weigh about 10 mg of epinastine hydrochloride and transfer to a 10 mL volumetric flask using 5 mL of methyl alcohol. Shake mechanically for 10 minutes, complete the volume with the same solvent and filter.

Solution (2): prepare the 1 mg/mL epinastine hydrochloride CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to that from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit dose (5.1.6). Complies with the test. Prepare the final solution with 20 $\mu g/mL$ of epinastine hydrochloride.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 60 rpm.

Time: 45 minutes.

Standard solution: dissolve in 0.1 M hydrochloric acid an accurately weighed amount of epinastine hydrochloride CRS to obtain a solution which concentration is $(L/900)$ mg/mL, where L is the declared amount, in milligrams, of epinastine hydrochloride per tablet.

Procedure: after the test, remove aliquot from the dissolution medium and immediately filter. Proceed as described in the *Assay* method in the *Epinastine hydrochloride* monograph. Inject, separately, 20 µL of the *Standard solution* and the *Sample solution*. Record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{16}H_{15}N_3.HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{16}H_{15}N_3.HCl$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in the *Assay* method on the *Epinastine hydrochloride* monograph. Prepare the *Sample* and *standard solutions* as described below.

Sample solution: pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 25 mg of epinastine hydrochloride and transfer to a 50 mL volumetric flask using 30 mL of methyl alcohol. Leave in ultrasonic bath at room temperature for 15 minutes and shake mechanically for 15 minutes. Complete the volume with the same solvent, shake and filter. Transfer an aliquot equivalent to 4 mL to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Standard solution: accurately weigh 25 mg of epinastine hydrochloride CRS, transfer to a 50 mL volumetric flask and add 30 mL of methyl alcohol. Shake mechanically for 15 minutes and complete the volume with the same solvent. Transfer an aliquot equivalent to 4 mL to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{16}H_{15}N_3.HCl$ in the tablets, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

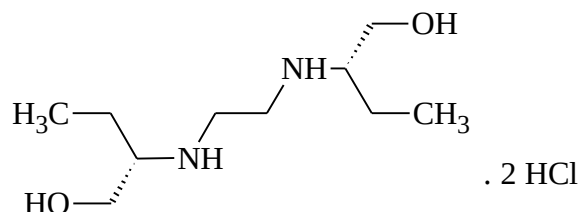
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

ETHAMBUTOL HYDROCHLORIDE

Ethambutoli hydrochloridum



$C_{10}H_{24}N_2O_2 \cdot 2HCl$; 277.23

ethambutol hydrochloride; 03642

(2*S*,2'*S*)-2,2'-(1,2-ethanediyl-di-imino)bis-1-butanol hydrochloride (2:1)

[1070-11-7]

Contains no less than 97.0% and no more than 101.0% of $C_{10}H_{24}N_2O_2 \cdot 2HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline, hygroscopic powder.

Solubility. Freely soluble in water, soluble in ethyl alcohol and methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 199 °C to 204 °C.

Specific optical rotation (5.2.8): +5.8 to +6.6, in relation to the anhydrous substance. Determine in solution at 10.0% (w/v).

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the desiccated sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of ethambutol hydrochloride CRS, prepared identically.

B. The main stain of the chromatogram from *Solution (2)*, obtained in *Limit of aminobutanol*, corresponds in position, color and intensity to the one obtained with *Solution (4)*.

C. The 10% (w/v) aqueous solution complies with the chloride ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 3.7 to 4.0. Determine in 2% (w/v) solution in carbon dioxide-free water.

Limit of aminobutanol. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of ammonium hydroxide, water and methyl alcohol (10:15:75) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described below.

Solution (1): 50 mg/mL sample solution in methyl alcohol.

Solution (2): 5 mg/mL sample solution in methyl alcohol.

Solution (3): 0.5 mg/mL aminobutanol CRS solution in methyl alcohol.

Solution (4): 5 mg/mL ethambutol hydrochloride CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate, allow to air dry and heat at 110 °C for 10 minutes. Cool and nebulize with ninhydrin RS. Heat the plate at 110 °C for five minutes. Any secondary stain, corresponding to aminobutanol, obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (3)* (1.0%).

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105 °C for three hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.2 g of the sample and dissolve in 100 mL of glacial acetic acid and 5 mL of mercury acetate RS. Titrate with 0.1 M perchloric acid VS, using methylrosanilinium chloride TS as indicator. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 13.862 mg of $C_{10}H_{24}N_2O_2 \cdot 2HCl$.

B. Accurately weigh 0.1 g of the sample and dissolve in 20 mL of a solution prepared as described: mixture of 4 mL of cupric sulfate RS with 70 mL of 2 M ammonium hydroxide, addition of 10 mL of M sodium hydroxide and dilution to 100 mL with water. After total dissolution, complete the volume to 25 mL with the same solvent. Prepare a standard solution in the same concentration, using the same solvent. Measure the rotation angle of the solutions in an adequate tube (**5.2.8**), using the same solvent for zero adjustment. Calculate the amount of $C_{10}H_{24}N_2O_2 \cdot 2HCl$ in the sample from readings of the angles obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial (tuberculostatic) agent.

ETHAMBUTOL HYDROCHLORIDE COATED TABLETS

Contains no less than 95.0% and no more than 105.0% of the declared amount of ethambutol hydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$). The tablets must be coated.

IDENTIFICATION

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of ethambutol hydrochloride and homogenize with 3 mL of methyl alcohol in a glass mortar. Add 5 mL of methyl alcohol, homogenize and filter. Collect the filtrate in a beaker with 100 mL of acetone. Shake the mixture and allow to crystallize for 15 minutes. Remove the supernatant and dry the crystals with compressed air. Proceed as described in test **A.** from *Identification* in the *Ethambutol hydrochloride* monograph.

B. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of ethambutol hydrochloride and shake with 10 mL of water. Add 2 mL of 1% (w/v) cupric sulfate and 1 mL of *M* sodium hydroxide. A blue color develops.

C. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 1 g of ethambutol hydrochloride and dissolve in 10 mL of water. The solution obtained complies with chloride ion reactions **(5.3.1.1)**.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **A.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: basket, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium, disregarding the initial 10 mL. Determine the amount of ethambutol dissolved as described on method **A.** in *Assay*. Prepare the *Standard solution* as described next.

Standard solution: accurately weigh 22.2 mg of ethambutol hydrochloride CRS and transfer to a 50 mL volumetric flask. Dissolve and complete the volume with the dissolution medium. Homogenize and filter.

Tolerance: not less than 75% (Q) of the declared quantity of $C_{10}H_{24}N_2O_2 \cdot 2HCl$ dissolve in 45 minutes.

PURITY TESTS

Limit of aminobutanol. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of concentrated ammonium hydroxide, water and methyl alcohol (10:15:75) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.5 g of ethambutol hydrochloride, add 7 mL of methyl alcohol and shake for five minutes. Complete the volume to 10 mL with the same solvent and filter, to obtain a 50 mg/mL solution.

Solution (2): 0.5 mg/mL aminobutanol CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate, allow to air dry and heat at 110 °C for 10 minutes. Cool and nebulize with ninhydrin RS. Heat at 110 °C for five minutes. Any secondary stain, corresponding to aminobutanol, obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (2)* (1.0%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 270 nm; column with 100 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); maintained at temperature of 40 °C; *Mobile phase* flow of 2.0 mL/minute.

Ammonium acetate buffer pH 5.0: qualitatively transfer 50 g of ammonium acetate and 0.2 g of copper acetate to a 1000 mL volumetric flask. Complete the volume with water, homogenize and adjust to pH 5.0 with glacial acetic acid.

Mobile phase: mixture of *Ammonium acetate buffer pH 5.0* and methyl alcohol (94:6).

Diluent: water and methyl alcohol (94:6).

Sample solution: weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 20 mg of ethambutol hydrochloride to a 50 mL volumetric flask and complete the volume with *Diluent*. Shake and filter. Dilute in *Mobile phase* to obtain a 0.4 mg/mL solution.

Standard solution: accurately weigh an amount of ethambutol hydrochloride CRS and dissolve in *Diluent*, to obtain a 0.4 mg/mL solution. Homogenize and filter.

Inject 20 μL replicates of the *Standard solution*. The column efficiency is no less than 2000 theoretical plates. The tailing factor is no more than 2.5. The relative standard deviation of the replicate areas from the recorded peaks must be no more than 2.0%.

Procedure: inject, separately, 20 μL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the area under the peaks. Calculate the amount of $\text{C}_{10}\text{H}_{24}\text{N}_2\text{O}_2 \cdot 2\text{HCl}$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

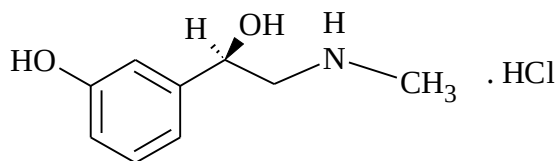
B. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.2 g of ethambutol hydrochloride and transfer to a separation funnel. Add 20 mL of 2 M sodium hydroxide solution and shake for five minutes. Extract with five portions of 25 mL of chloroform. Collect the chloroform layers in a beaker and evaporate in water bath until a volume of approximately 25 mL. Filter in paper to an Erlenmeyer flask. Wash the beaker and the filter paper with 100 mL of glacial acetic acid. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*, using as indicator 10 drops of 1-naphtholbenzein TS and as titrating agent 0.1 M VS perchloric acid. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 13.862 mg of $\text{C}_{10}\text{H}_{24}\text{N}_2\text{O}_2 \cdot 2\text{HCl}$ hydrochloride.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

PHENYLEPHRINE HYDROCHLORIDE*Phenylephrini hydrochloridum*C₉H₁₃NO₂.HCl; 203.65

phenylephrine hydrochloride; 03926

(1R)-2-methylamino-1-(3-hydroxyphenyl)ethanol hydrochloride

[61-76-7]

Contains no less than 98.5% and no more than 101.0% of C₉H₁₃NO₂.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Freely soluble in water and ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 140 °C to 145 °C.

Specific optical rotation (5.2.8): -43 to -47.0, in relation to the desiccated substance. Determine in aqueous solution at 2.0% (w/v).

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the desiccated sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of phenylephrine hydrochloride CRS, prepared identically.

B. Accurately weigh 10 mg of the sample, dissolve in 1 mL of water and add 50 µL of a 125 g/L copper sulfate solution and 1 mL of a 200 g/L sodium hydroxide solution. A violet color develops. Add 1 mL of ether and shake; the upper layer remains colorless.

C. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. The 2% (w/v) aqueous preparation is clear **(5.2.24)** and colorless **(5.2.12)**.

Acidity and alkalinity. Add to 10 mL of a 2% (w/v) aqueous solution 0.1 mL of a solution of methyl red TS and 0.2 mL of 0.01 M sodium hydroxide. The solution develops a yellow color. No more than 0.4 mL of 0.01 M hydrochloric acid must be spent for the solution to develop a red color.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 215 nm; column with 55 mm of length and 4.0 mm of internal diameter, packed with silica gel chemically bonded to octadecylsilane group (3 µm); maintained at temperature of 45 °C; *Mobile phase* flow of 1.5 mL/minute.

Buffer solution pH 2.8: weigh 3.25 g of sodium octanesulfonate monohydrate, dissolve in 1000 mL of water and adjust pH to 2.8 ± 0.1 with phosphoric acid RS.

Diluent: mixture of *Eluent A* and *Eluent B* (80:20 v/v).

Eluent A: mixture of acetonitrile and buffer solution pH 2.8 (10:90 v/v).

Eluent B: mixture of buffer solution pH 2.8 and acetonitrile (10:90 v/v).

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 - 3	93	7	isocratic
3 - 13	93 → 70	7 → 30	linear gradient
13 - 14	70 → 93	30 → 7	linear gradient

Sample solution: accurately weigh 50 mg of phenylephrine hydrochloride, transfer to a 50 mL volumetric flask, dissolve with 30 mL of *Diluent* and complete the volume with the same solvent.

Reference solution: transfer 5 mL of the *Sample solution* to a 100 mL volumetric flask and complete the volume with *Diluent*. Transfer 2 mL of this solution to a 100 mL volumetric flask and complete the volume with *Diluent*.

Resolution solution: dissolve the content of a vial of *Phenylephrine hydrochloride for identification of peak CRS* (with impurities C and E) in 2 mL of *Diluent*.

Inject 10 µL replicates of the *Resolution solution*. The retention time is about 2.8 minutes for phenylephrine and the relative retention times are about 1.3 for impurity C and 3.6 for impurity E. The tailing factor for the main peak is not higher than 1.9. Peak and valley ratio: minimum 5, where H_p = height above the peak baseline due to impurity C; H_v = height above the baseline from the lowest point of the curve that separates this top from the peak due to phenylephrine.

Procedure: inject, separately, 10 µL of the *Sample solution* and the *Reference solution*, record the chromatograms and measure the areas under the peaks. To calculate the amount of impurity C and E, multiply the area under the peak of each impurity by 0.5.

Impurity C and E: for each peak of impurity C and E obtained in the *Sample solution*, the area is not greater than the area under the main peak obtained with the *Reference solution* (0.1%).

Non-specific impurities: for each peak of impurity obtained in the *Sample solution*, the area is not greater than the area under the main peak obtained with the *Reference solution* (0.1%).

Total impurities: the sum of the areas under the impurity peaks obtained in the *Sample solution* is not greater than twice the area under the main peak obtained with the *Reference solution* (0.2%).

Disregard: impurity peaks with areas lower than 0.5 times the area under the main peak obtained with the *Reference solution* (0.05%).

Sulfates (5.3.2.2). Transfer 15 mL of a 2% (w/v) aqueous solution to a Nessler tube. Proceed as described in *Limit test for sulfates (5.3.2.2)*. No more than 0.05% (500 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of sample. Dry in oven at 105 °C for four hours. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh 0.15 g of the sample and dissolve in a mixture of 0.5 mL of 0.1 M hydrochloric acid and 80 mL of 96% ethyl alcohol. Titrate with 0.1 M ethylic sodium hydroxide VS, determining the endpoint potentiometrically between the two inflection points. Each mL of 0.1 M sodium hydroxide VS is equivalent to 20.37 mg of C₉H₁₃NO₂.HCl.

PACKAGING AND STORAGE

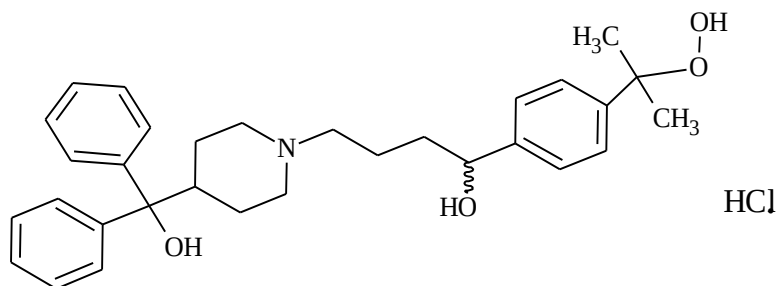
Store protected from light, humidity, and direct natural light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anticholinergic.

FEXOFENADINE HYDROCHLORIDE*Fexofenadini hydrochloridum*C₃₂H₃₉NO₄.HCl; 538.12

fexofenadine hydrochloride; 04038

4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidiny]butyl]- α,α -dimethyl-benzeneacetic acid hydrochloride (1:1)

[153439-40-8]

Contains no less than 98.0% and no more than 102.0% of C₃₂H₃₉NO₄.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or pale white crystalline powder.

Solubility. Moderately soluble in water, very soluble in ethyl alcohol and methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 195 °C to 197 °C.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are conducted.

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of fexofenadine hydrochloride CRS, prepared identically.

B. The ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 370 nm, of the 0.0014% (w/v) solution in ethyl alcohol, displays a characteristic shoulder at 220 nm, identical to the one observed in the spectrum of a similar fexofenadine hydrochloride CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Chlorides. Weigh 0.3 g of the sample and dissolve in 50 mL of methyl alcohol. Titrate potentiometrically with 0.1 M silver nitrate VS. Each mL of 0.1 M silver nitrate VS is equivalent to 3.545 mg of chloride. It must present a content of 6.45% to 6.75% of chloride, calculated over the anhydrous substance.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Water (5.2.20.1). No more than 0.5%.

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for two hours. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); maintained at temperature of 30 °C; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of 0.05 M ammonium acetate and acetonitrile (50:50). Adjust pH to 3.2 with M hydrochloric acid.

Sample solution: weigh 20 mg of the sample, transfer to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask and complete the volume with *Mobile phase*, to obtain a 40 µg/mL solution.

Standard solution: accurately weigh an amount of fexofenadine hydrochloride CRS and dissolve in *Mobile phase* to obtain a 40 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is no less than 2500 theoretical plates. The tailing factor is no more than 2.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{32}H_{39}NO_4.HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with the current legislation.

THERAPEUTIC CLASS

Antihistamine.

FEXOFENADINE HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{32}H_{39}NO_4 \cdot HCl$.

IDENTIFICATION

A. Weigh and pulverize a sufficient amount of tablets. Accurately weigh an amount of powder equivalent to about 60 mg of fexofenadine hydrochloride to a capped tube. Add 10 mL of the mixture of acetonitrile and methyl alcohol (10:1) and shake in vortex for one to two minutes until the sample disperses. Allow the solution to stand for 10 minutes or centrifuge for two to three minutes. Filter, using a 0.45 μm polytetrafluorethylene filter, to a 50 mL vial. Evaporate the solvent to about 0.5 mL using nitrogen flow with low heating (do not exceed 75 °C). Add 5 mL of water and five drops of diluted hydrochloric acid, shake and induce the precipitation. Introduce in ice bath for 30 minutes. Filter the solution to a sintered glass crucible with 10 μm to 15 μm . Dry the precipitate in oven at 105 °C for one hour. In the infrared absorption spectrum (**5.2.14**) of the residue obtained, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of fexofenadine CRS, prepared identically. To prepare the dispersion of potassium bromide with fexofenadine CRS, it is necessary to transfer about 60 mg of the standard with a capped tube and proceed as of "Add 10 mL of the mixture of acetonitrile and methyl alcohol (10:1)...".

B. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 14 mg of fexofenadine hydrochloride and transfer to a 100 mL volumetric flask using 60 mL of ethyl alcohol. Shake for 10 minutes and complete the volume with the same solvent. Homogenize and filter. Proceed as described in test **B.** of *Identification* from the *Fexofenadine hydrochloride* monograph.

C. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 10 mg of fexofenadine hydrochloride, shake with 10 mL of methyl alcohol, homogenize and filter. Proceed as described in test **C.** of *Identification* from the *Fexofenadine hydrochloride* monograph.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay* method, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: proceed as described in *Assay* in the *Fexofenadine hydrochloride* monograph. Prepare the *Sample solution* as described next.

Sample solution: after the test, take an aliquot of dissolution and dilute to a concentration close to that of the *Standard solution*, using *Mobile phase* as diluent.

Tolerance: no less than 70% (Q) of the declared quantity of $C_{32}H_{39}NO_4 \cdot HCl$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Assay* in the *Fexofenadine hydrochloride* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 40 mg of fexofenadine hydrochloride to a 200 mL volumetric flask, add 120 mL of *Mobile phase* and leave in ultrasonic bath for 15 minutes. Shake mechanically for 30 minutes, complete the volume with the same solvent, homogenize and filter. Transfer 5 mL to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

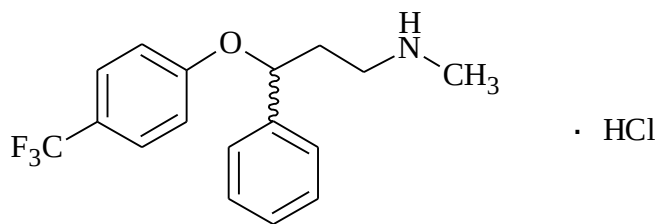
Procedure: inject, separately, 20 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the area under the peaks. Calculate the amount of $C_{32}H_{39}NO_4 \cdot HCl$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

FLUOXETINE HYDROCHLORIDE*Fluoxetini hydrochloridum*C₁₇H₁₈F₃NO.HCl; 345.79

fluoxetine hydrochloride; 04177

N-methyl-γ-[4-(trifluoromethyl)phenoxy] benzene propanamine hydrochloride (1:1)

[56296-78-7]

Contains no less than 98.5% and no more than 101.5% of C₁₇H₁₈F₃NO.HCl, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Moderately soluble in water, freely soluble in ethyl alcohol and methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 158.4 °C to 158.9 °C.

Specific optical rotation (5.2.8): -0.05 to +0.05. Determine in a 2% (w/v) solution in a mixture of water and methyl alcohol (15:85).

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the non-desiccated sample dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of fluoxetine hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm of the sample solution obtained in method A. from Assay, there are maximum and minimum points only at the same wavelengths observed in the spectrum of a similar fluoxetine hydrochloride CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method B. from Assay, corresponds to the one from the main peak of the *Standard solution*.

D. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.5 to 6.5. Determine in 1% (w/v) solution in carbon dioxide-free water.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 215 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm); maintained at temperature of 30 °C; *Mobile phase* flow of 1.5 mL/minute.

Phosphate buffer pH 3.6: weigh 3.395 g of tetrabutylammonium sulfate and 1.361 g of monobasic potassium phosphate, transfer to a 1000 mL volumetric flask, dissolve in 900 mL of water, adjust pH to 3.6 with ammonium hydroxide and complete the volume with water.

Mobile phase: mixture of *Phosphate buffer pH 3.6* and acetonitrile (78:22).

Solution (1): accurately weigh 30 mg of fluoxetine hydrochloride CRS, transfer to a 250 mL volumetric flask, dissolve in the *Mobile phase* and complete the volume with the same solvent. Transfer 5 mL of the resulting solution to a 50 mL volumetric flask and complete the volume with the same solvent. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with the same solvent, to obtain a 1.2 µg/mL solution.

Solution (2): accurately weigh 30 mg of the sample, transfer to a 25 mL volumetric flask and complete the volume with the *Mobile phase*. Homogenize.

Inject 20 µL replicates of the *Solution (1)*. The tailing factor is no more than 1.7. The relative standard deviation of the replicate areas from the recorded peaks must be no more than 10%.

Procedure: inject, separately, 20 µL of *Solution (1)* and of *Solution (2)* and record the chromatograms for no less than double the retention time of the main peak. Calculate the percentage of each impurity of *Solution (2)* from the formula: $0.1(r_i/r_p)$, where r_i is the response of the impurity peak on *Solution (2)* and r_p is the response of the peak related to fluoxetine in *Solution (1)*. No more than 0.15% of impurity with relative retention time of 0.88 and no more than 0.1% of another individual impurity. No more than 0.5% of total impurities.

Heavy metals (5.3.2.3). Use *Method III*. Determine in 0.67 g of the sample. Use standard lead solution 2 ppm. No more than 0.003% (30 ppm).

Water (5.2.20.1). No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh 75 mg of the sample, transfer to a 100 mL volumetric flask and dissolve in 0.1 M hydrochloric acid. Complete the volume with the same solvent. Dilute, successively, using 0.1 M hydrochloric acid, to a concentration of 0.0015% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 227 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{17}H_{18}F_3NO.HCl$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 227 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Triethylamine pH 6.0 buffer: transfer 10 mL of the triethylamine to a 1000 mL volumetric flask, add approximately 980 mL of water, adjust the pH to 6.0 with phosphoric acid and complete the volume with water.

Mobile phase: mixture of *Triethylamine pH 6.0 buffer*, tetrahydrofuran and methyl alcohol (6:3:1).

Sample solution: weigh, accurately, about 27.5 mg of the sample and transfer to a 25 mL volumetric flask. Dissolve with *Mobile phase* and complete the volume with the same solvent. Homogenize. Transfer 5 mL of the resulting solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Standard solution: accurately weigh about 27.5 mg of fluoxetine hydrochloride CRS and transfer to a 25 mL volumetric flask. Dissolve in *Mobile phase* and complete the volume with the same solvent. Homogenize. Transfer 5 mL of the resulting solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Inject 10 μ L replicates of the *Standard solution*. The tailing factor is no more than 2.0. The relative standard deviation of the replicate areas from the recorded peaks must be no more than 2.0%.

Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{17}H_{18}F_3NO.HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antidepressant.

FLUOXETINE HYDROCHLORIDE TABLETS

Contains fluoxetine hydrochloride equivalent to no less than 90.0% and no more than 110.0% of the declared amount of fluoxetine ($C_{17}H_{18}F_3NO$).

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 10 mg of fluoxetine to a beaker, dissolve 10 mL of ethyl alcohol and filter. Wash the container with 5 mL of the same solvent and evaporate the filtrate in water bath to residue. The residue obtained, desiccated at 60 °C, in oven under reduced pressure, for three hours complies with test **A.** of *Identification* from the *Fluoxetine hydrochloride* monograph.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from *Assay*, there are maximum and minimum points only at the same wavelengths observed in the standard solution spectrum.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 20 mg of fluoxetine, transfer to a 5 mL volumetric flask and complete the volume with water. Homogenize and filter. The resulting solution complies with chloride ion reactions (**5.3.1.1**).

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 100 mL volumetric flask. Add 70 mL of 0.1 M hydrochloric acid. Leave in ultrasonic bath for five minutes. Shake mechanically for 15 minutes, complete the volume with the same solvent, homogenize and filter. Dilute successively, in the same solvent, to a concentration of 0.0015% (w/v) of fluoxetine. Prepare a standard solution in the same concentration of fluoxetine ($C_{17}H_{18}F_3NO$), using fluoxetine hydrochloride CRS and the same solvent. Measure the absorbances of the resulting solutions at 227 nm (**5.2.14**), using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of fluoxetine ($C_{17}H_{18}F_3NO$) in each tablet from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances at 227 nm **(5.2.14)**, using the same solvent for zero adjustment. Calculate the amount of fluoxetine ($C_{17}H_{18}F_3NO$) dissolved in the medium, comparing the readings obtained with that of the fluoxetine hydrochloride CRS solution, at the concentration of 0.0015% (w/v) of fluoxetine ($C_{17}H_{18}F_3NO$), prepared in the same solvent.

Tolerance: not less than 70% (Q) of the declared quantity of fluoxetine ($C_{17}H_{18}F_3NO$) dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Related substances* from the *Fluoxetine hydrochloride* monograph. Prepare the *Solution (2)* as described next.

Solution (2): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 20 mg of fluoxetine, transfer to a 10 mL volumetric flask, add 8 mL of *Mobile phase*, leave in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Homogenize and filter.

Procedure: inject 20 μ L replicates of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of each impurity in the *Solution (2)* chromatogram, using the formula: $100 (r_i / r_t)$, where r_i is the response from each peak, excluding the peak related to fluoxetine, and r_t is the sum of the responses from all peaks, including the peak related to fluoxetine. Do not consider peaks related to the solvents. No more than 0.25% of individual impurity and 0.40% of total impurity.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 15 mg of fluoxetine ($C_{17}H_{18}F_3NO$) and transfer to a 100 mL volumetric flask. Add 70 mL of 0.1 M hydrochloric acid. Leave in an ultrasonic bath for 5 minutes. Shake mechanically for 15 minutes, complete the volume with the same solvent, homogenize and filter. Dilute successively, in the same solvent, to a concentration of 0.0015% (w/v) of fluoxetine. Prepare a standard solution in the same concentration of fluoxetine ($C_{17}H_{18}F_3NO$), using fluoxetine hydrochloride CRS and the same solvent. Measure the absorbances of the resulting solutions at 227 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of fluoxetine ($C_{17}H_{18}F_3NO$) in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Proceed as described in method **B.** of Assay on the *Fluoxetine hydrochloride* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 10 mg of fluoxetine ($C_{17}H_{18}F_3NO$), transfer to a 100 mL volumetric flask and add 70 mL of *Mobile phase*. Leave in an ultrasonic bath for 15 minutes. Shake mechanically for 15 minutes and complete the volume with the same solvent. Homogenize and filter.

Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of fluoxetine ($C_{17}H_{18}F_3NO$) in the tablets from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers protected from light.

LABELING

Comply with current legislation.

FLURAZEPAM HYDROCHLORIDE TABLETS

Contains no less than 92.5% and no more than 107.5% the declared amount of $C_{21}H_{23}ClFN_3O.HCl$.

IDENTIFICATION

Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of toluene, acetone and 25% (v/v) ammonium hydroxide (50:50:2) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): pulverize the tablets. Accurately weigh an amount of powder equivalent to 20 mg of flurazepam hydrochloride, transfer to a beaker, add 10 mL of methyl alcohol, shake for five minutes and filter.

Solution (2): 2 mg/mL flurazepam hydrochloride CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Proceed protected from light. Weigh individually and transfer each tablet to a 100 mL volumetric flask. Add 2 mL of water and leave in ultrasonic bath for five minutes. Add 80 mL of methyl alcohol and submit to ultrasonic bath for another five minutes. Shake for 10 minutes and complete the volume with methyl alcohol. Centrifuge and filter, if necessary. Dilute successively with 0.1 M methanolic sulfuric acid to obtain a concentration of 0.003% (w/v) of flurazepam hydrochloride. Prepare a standard solution as described on *Assay*. Measure the absorbances of the resulting solutions at 284 nm (**5.2.14**), using 0.1 M methanolic sulfuric acid for zero adjustment. Calculate the amount of $C_{21}H_{23}ClFN_3O.HCl$ in the tablets, from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 319$, at 284 nm, in 0.1 M methanolic sulfuric acid.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 20 minutes.

Procedure: after the test, take aliquots of the dissolution medium, filter using a membrane with porosity of 0.45 µm. Measure the absorbances of the solutions at 270 nm **(5.2.14)**, using water for zero adjustment. Calculate the amount of $C_{21}H_{23}ClFN_3O.HCl$ dissolved in the medium, comparing the readings obtained with that of the flurazepam hydrochloride CRS solution at the concentration of 0.0033% (w/v).

Tolerance: no less than 80% (Q) of the declared quantity of $C_{21}H_{23}ClFN_3O.HCl$ dissolve in 20 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

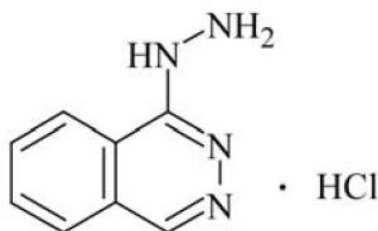
Conduct the assay protected from light. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.3 g of flurazepam hydrochloride and transfer to a 100 mL volumetric flask. Add 2 mL of water and leave in ultrasonic bath for five minutes. Add 80 mL of methyl alcohol and place in ultrasonic bath for another five minutes. Shake for 10 minutes and complete the volume with methyl alcohol. Centrifuge and filter, if necessary. Dilute successively with 0.1 M methanolic sulfuric acid to obtain a concentration of 0.003% (w/v) of flurazepam hydrochloride. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 284 nm **(5.2.14)**, using 0.1 M methanolic sulfuric acid for zero adjustment. Calculate the amount of $C_{21}H_{23}ClFN_3O.HCl$ in the tablets from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 319$, at 284 nm, in 0.1 M methanolic sulfuric acid.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

HYDRALAZINE HYDROCHLORIDE*Hydralazini hydrochloridum*

$C_8H_8N_4 \cdot HCl$; 196.64

hydralazine hydrochloride; 04647

1-hydrazinophththalazine hydrochloride (1:1)

[304-20-1]

Contains no less than 98.0% and no more than 102.0% of $C_8H_8N_4 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Soluble in water, slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting temperature (5.2.2): 275 °C, with decomposition.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated and dispersed in potassium chloride, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of hydralazine hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 230 nm to 350 nm, of a 0.002% (w/v) solution in water, there are maximum absorption points at 240 nm, 260 nm, 305 nm and 315 nm, identical to those observed in the spectrum of a similar hydralazine hydrochloride CRS solution. The absorbance values are of approximately 1.1, 1.1, 0.53 and 0.43, respectively.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method B. from Assay, corresponds to the one from the main peak of the *Standard solution*.

D. The 0.025% (w/v) aqueous sample solution complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 3.5 to 4.2. Determine in solution at 2% (w/v) in water.

Related substances. Proceed as described on method **B.** from *Assay*. Prepare the *Test solution* as described below.

Test solution: accurately weigh 25 mg of the sample to a 50 mL volumetric flask, dissolve in 30 mL of 0.1 M acetic acid and complete the volume with the same solvent.

Procedure: inject 20 µL of the *Test solution*, record the chromatograms and measure the areas under the peaks obtained. The sum of the areas under the secondary peaks, except that of the main peak, is not greater than 1.0% of the total area of the peaks obtained, including that of the main peak. Do not include solvent-related peaks in the calculations.

Heavy metals (5.3.2.3). Moisten the residue obtained in *Residue by incineration* with 2 mL of hydrochloric acid, evaporate, dry and add 20 mL of water. Proceed as described in *Limit test for heavy metals, Method I*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 110 °C for 15 hours, until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh, accurately, about 0.1 g of sample, transfer to a 250 mL Erlenmeyer flask with cap and dissolve in 25 mL of water. Add 35 mL of hydrochloric acid and cool down to room temperature. Add 5 mL of chloroform and titrate with 0.02 M potassium iodate VS. Near the endpoint, add the titrant drop by drop, shaking continuously until the purple color of the chloroform layer disappears. Alternatively, determine the endpoint potentiometrically, excluding the chloroform. Each mL of 0.02 M potassium iodate VS is equivalent to 3.933 mg of C₈H₈N₄.HCl.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 230 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to nitrile group (10 µm), maintained at room temperature; *Mobile phase* flow of 1 mL/minute.

Mobile phase: dissolve 1.44 g of sodium lauryl sulfate and 0.75 g of tetrabutylammonium bromide in 770 mL of water, add 230 mL of acetonitrile and homogenize. If necessary, adjust pH to 3.0 with 0.05 M sulfuric acid.

Sample solution: accurately weigh an amount of the sample and dissolve in 0.1 M acetic acid to obtain a 0.4 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with 0.1 M acetic acid and homogenize, obtaining a solution at 40 µg/mL.

Standard solution: accurately weigh an amount of hydralazine hydrochloride CRS and dissolve in 0.1 M acetic acid to obtain a 0.4 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with 0.1 M acetic acid and homogenize, obtaining a solution at 40 µg/mL.

Resolution solution: prepare a solution in 0.1 M acetic acid with approximately 0.25 mg of hydralazine hydrochloride CRS and 50 µg of phthalazine per milliliter. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with 0.1 M acetic acid and homogenize.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.65 for phthalazine and 1.0 for hydralazine hydrochloride. The resolution between the peaks of phthalazine and of hydralazine hydrochloride is no less than 4.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₈H₈N₄.HCl in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vasodilator.

HYDRALAZINE HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_8H_8N_4.HCl$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of hydralazine hydrochloride, transfer to a separation funnel and add 2 mL of 6 M ammonium hydroxide and 10 mL of water. Extract with two portions of 10 mL of chloroform. Collect the organic extracts and evaporate to dryness. The residue complies with test **A.** of *Identification* from the *Hydralazine hydrochloride* monograph.

B. In the ultraviolet absorption spectrum (**5.2.14**) in the range of 230 nm to 350 nm of the sample solution obtained in method **B.** from *Assay*, there are maximum absorption points at 240 nm, 260 nm, 305 nm and 315 nm, identical to those observed in the spectrum of the standard solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **C.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of hydralazine hydrochloride, transfer to a beaker, add 50 mL of a mixture of methyl alcohol and water (1:2), homogenize and filter. Concentrate the filtrate in water bath to 10 mL and allow to cool. Add to 5 mL of the concentrated solution 5 mL of 2% (w/v) 2-nitrobenzaldehyde in ethyl alcohol. Orange precipitate is produced.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Grind each tablet to a fine powder, transfer quantitatively to a 50 mL volumetric flask and add 25 mL of mixture of methyl alcohol and water (1:2). Proceed as described on method **B.** from *Assay*, starting from "Shake mechanically...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, with 0.01 M hydrochloric acid, until adequate concentration. Measure the absorbances of the solutions

at 260 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_8H_8N_4.HCl$ dissolved in the medium, comparing the readings obtained with that of hydralazine hydrochloride CRS solution at the concentration of 0.001% (w/v), prepared in 0.01 M hydrochloric acid.

Tolerance: no less than 60% (Q) of the declared quantity of $C_8H_8N_4.HCl$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.1 g of hydralazine hydrochloride, transfer to a beaker and add 25 mL of water. Add 35 mL of hydrochloric acid and cool down to room temperature. Shake, mechanically, for 15 minutes. Titrate with 0.02 M potassium iodate VS, with continuous agitation. Determine the endpoint potentiometrically. Each mL of 0.02 M potassium iodate VS is equivalent to 3.933 mg of $C_8H_8N_4.HCl$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 50 mg of hydralazine hydrochloride, transfer to a 50 mL volumetric flask and add 25 mL of a mixture of methyl alcohol and water (1:2). Shake, mechanically, for 10 minutes and complete the volume with the same solvent. Filter. Carry out successive dilutions to a concentration of 0.001% (w/v), using water as solvent. Prepare the standard solution in the same conditions. Measure the absorbances of the resulting solutions at 260 nm, using water for zero adjustment. Calculate the amount of $C_8H_8N_4.HCl$ in the tablets from the readings obtained.

C. Proceed as described in method **B.** of *Assay on the Hydralazine hydrochloride monograph*. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 20 mg of hydralazine hydrochloride to a 50 mL volumetric flask, add 40 mL of 0.1 M acetic acid and leave in ultrasonic bath for 15 minutes. Shake, mechanically, for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask and complete the volume with 0.1 M acetic acid, obtaining a 40 µg/mL solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_8H_8N_4.HCl$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

HYDRALAZINE HYDROCHLORIDE INJECTION SOLUTION

Contains no less than 90.0% and no more than 105.0% the declared amount of $C_8H_8N_4.HCl$.

IDENTIFICATION

The identification test A. can be omitted if tests B., C. and D. are carried out.

A. Transfer a volume of injection solution equivalent to 0.1 g of hydralazine hydrochloride to a separation funnel. Add 2 mL of 6 M ammonium hydroxide and 10 mL of water. Extract with two portions of 10 mL of chloroform. Collect the organic extracts and evaporate to dryness. The residue complies with the test **A.** of *Identification* from the *Hydralazine hydrochloride* monograph.

B. Dilute the injection solution in water, to a concentration of 0.002% (w/v). In the ultraviolet absorption spectrum (**5.2.14**) of the solution obtained, at the range of 230 nm to 350 nm, there are maximum absorption points at 240 nm, 260 nm, 305 nm and 315 nm.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **C.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. Transfer a volume of injection solution equivalent to 0.1 g of hydralazine hydrochloride to a beaker and add water, if necessary, to obtain a final volume of 10 mL. Transfer to 5 mL of the solution 5 mL of 2% (w/v) 2-nitrobenzaldehyde in ethyl alcohol. Orange precipitate is produced.

E. Dilute the injection solution in water, to a concentration of 0.025% (w/v). The solution obtained complies with chloride ion reactions (**5.3.1.1**).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.4 to 4.4.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 1.45 EU/mg of hydralazine hydrochloride.

ASSAY

Use one of the methods described below.

A. Transfer, to a 250 mL Erlenmeyer flask with cap, a volume of injection solution with the equivalent to 0.1 g of hydralazine hydrochloride, 25 mL of water, and proceed as described in method **A.** from *Assay* of the *Hydralazine hydrochloride* monograph as of "Add 35 mL of hydrochloric acid...". Each mL of 0.02 M potassium iodate VS is equivalent to 3.933 mg of $C_8H_8N_4.HCl$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer, to a 100 mL volumetric flask, a volume of the solution with the equivalent to 0.1 g of hydralazine hydrochloride and complete the volume with a mixture of methyl alcohol and water (1:2). Carry out successive dilutions to a concentration of 0.001% (w/v), using water as solvent. Prepare a standard solution in the same conditions. Measure the absorbances of the solutions at 260 nm, using water for zero adjustment. Calculate the amount of $C_8H_8N_4.HCl$ in the injection solution from the readings obtained.

C. Proceed as described in method **B.** of *Assay* on the *Hydralazine hydrochloride* monograph. Prepare the *Sample solution* as described next.

Sample solution: transfer to a 50 mL volumetric flask a volume of the injection solution with the equivalent to 20 mg of hydralazine hydrochloride, complete the volume with 0.1 M acetic acid and homogenize. Transfer 5 mL of the solution obtained to a 50 mL volumetric flask and complete the volume with 0.1 M acetic acid, obtaining a 40 µg/mL solution.

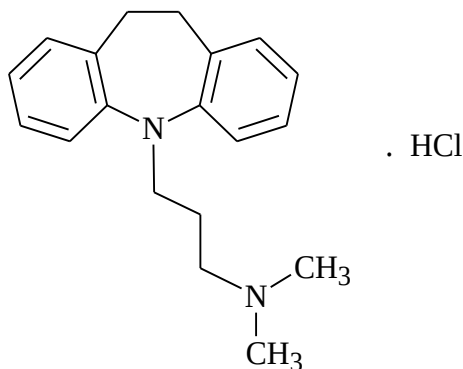
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_8H_8N_4.HCl$ in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

IMIPRAMINE HYDROCHLORIDE

$C_{19}H_{24}N_2 \cdot HCl$; 316.87

imipramine hydrochloride; 04837

10,11-di-hydro-*N,N*-dimethyl-5*H*-dibenz[*b,f*]azepine-5-propanamine hydrochloride (1:1)
[113-52-0]

Contains no less than 98.0% and no more than 102.0% of $C_{19}H_{24}N_2 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to slightly yellow crystalline powder.

Solubility. Freely soluble in water and ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 170 °C to 174 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of imipramine hydrochloride CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak of the *Standard solution*.

C. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 3.6 to 5.0. Determine in 10% (w/v) solution in carbon dioxide-free water.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of hydrochloric acid, water, glacial acetic acid and ethyl acetate

(5:5:35:55) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.25 g of the sample in methyl alcohol and complete to 10 mL with the same solvent.

Solution (2): dilute 1 mL of *Solution (1)* to 10 mL with methyl alcohol. Dilute 1 mL of the resulting solution to 50 mL with the same solvent.

Solution (3): dissolve 5 mg of iminodibenzyl in methyl alcohol and complete the volume to 100 mL with the same solvent.

Develop the chromatogram. Remove the plate and allow to air dry. Nebulize with a 0.5% (w/v) potassium dichromate solution in a mixture of water and sulfuric acid (4:1). Any secondary stain, corresponding to iminodibenzyl, obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (3)* (0.2%). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain and from iminodibenzyl, is not more intense than that obtained with *Solution (2)* (0.2%).

Heavy metals (5.3.2.3). Proceed as described in *Thioacetamide reaction methods, Method III*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 269 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); maintained at temperature of 40 °C; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: mixture of 0.06 M sodium perchlorate, acetonitrile and triethylamine (625:375:1). Adjust pH to 2.0 with perchloric acid.

Sample solution: dissolve an accurately weighed amount of the sample in a mixture of water and acetonitrile (5:3) to obtain a 0.3 mg/mL solution.

Sample solution: dissolve an accurately weighed amount of imipramine hydrochloride CRS in a mixture of water and acetonitrile (5:3) to obtain a 0.3 mg/mL solution.

Resolution solution: prepare a solution with imipramine hydrochloride CRS and desipramine hydrochloride CRS at 0.3 mg/mL, each, in a mixture of water and acetonitrile (5:3).

Inject 20 µL replicates of the *Resolution solution*. The resolution between the peaks of imipramine hydrochloride and of desipramine hydrochloride is no less than 1.3. The relative standard deviation of the replicate areas of the peaks recorded for imipramine hydrochloride is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₉H₂₄N₂. HCl in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antidepressant.

IMIPRAMINE HYDROCHLORIDE TABLETS

Contains imipramine hydrochloride equivalent to no less than 92.5% and no more than 107.5% of the declared amount of $C_{19}H_{24}N_2.HCl$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.25 g of imipramine hydrochloride and homogenize with 10 mL of chloroform. Filter and evaporate to reduce the volume. Add ethyl ether until a turbid solution is produced. Allow to stand. The precipitate, after filtration followed by recrystallization in acetone, presents a melting point around 172 °C.

B. Dissolve 5 mg of the crystals obtained on test **A.** of *Identification* in 2 mL of nitric acid. A blue color develops.

C. The crystals obtained on test **A.** of *Identification* respond to chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, with 0.01 M hydrochloric acid, until adequate concentration. Measure the absorbances at 250 nm **(5.2.14)**, using the same solvent for zero adjustment. Calculate the amount of $C_{19}H_{24}N_2.HCl$ dissolved in the medium, comparing the readings obtained with that of the imipramine hydrochloride CRS solution of known concentration, prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{19}H_{24}N_2.HCl$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Related substances* from the *Imipramine hydrochloride* monograph. Prepare *Solutions (1), (2) and (3)* as described next.

Solution (1): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.2 g of imipramine hydrochloride and add 30 mL of chloroform. Filter. Evaporate the filtrate to dryness. Dissolve the residue in 10 mL of methyl alcohol.

Solution (2): dilute 3 mL of *Solution (1)* to 100 mL with methyl alcohol. Dilute 1 mL of the resulting solution to 10 mL with the same solvent.

Solution (3): prepare a 60 µg/mL iminodibenzyl solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow it to air dry. Nebulize with 0.5% (w/v) potassium dichromate solution in sulfuric acid (1:4). The main stain obtained with *Solution (1)* presents a blue color. Any secondary stain, corresponding to iminodibenzyl, obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than the main stain obtained with *Solution (3)* (0.2%). Any secondary stain obtained different from the main stain is not more intense than the main stain obtained with *Solution (2)* (0.2%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

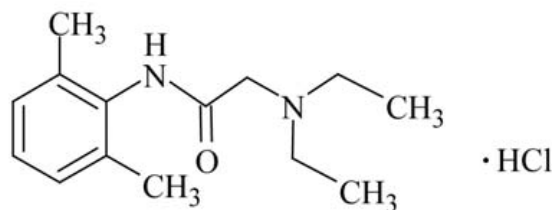
Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 50 mg of imipramine hydrochloride, transfer to a 100 mL volumetric flask and add 70 mL of 0.1 M hydrochloric acid. Shake, mechanically, for 40 minutes and complete the volume with the same solvent. Filter. Transfer 5 mL to a 100 mL volumetric flask and complete the volume with 0.1 M hydrochloric acid. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 250 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the content of C₁₉H₂₄N₂.HCl in the tablets from the readings obtained. Alternatively, calculate using $A(1\%, 1\text{cm}) = 264$, at 250 nm.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

LIDOCAINE HYDROCHLORIDE*Lidocaini hydrochloridum*C₁₄H₂₂N₂O.HCl; 270.80

lidocaine hydrochloride; 05314

2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide hydrochloride (1:1)

[73-78-9]

C₁₄H₂₂N₂O.HCl.H₂O; 288.81

lidocaine hydrochloride monohydrate; 11386

2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide hydrochloride hydrate (1:1:1)

[6108-05-0]

Contains no less than 97.5% and no more than 102.5% of C₁₄H₂₂N₂O.HCl, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Very soluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 74 °C to 79 °C, for the monohydrate form.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of lidocaine hydrochloride CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. Complies with chloride ion reactions (**5.3.1.1**).

PURITY TESTS

pH (5.2.19). 4.0 to 5.5. Determine in aqueous solution at 0.5% (w/v).

Limit of 2,6-dimethylaniline.

Test solution: accurately weigh 0.25 g of the sample, transfer to a 10 mL volumetric flask and complete the volume with methyl alcohol.

2,6-Dimethylaniline solution: accurately weigh 50 mg of 2,6-dimethylaniline, transfer to a 100 mL volumetric flask and complete the volume with methyl alcohol. Transfer 1 mL of this solution to a 100 mL volumetric flask and complete the volume with methyl alcohol.

Procedure: use three Nessler tubes and carry out the test as follows: put on the first tube 2 mL of the *Test solution*, on the second tube 1 mL of the *2,6-dimethylaniline solution* and 1 mL of methyl alcohol, and on the third tube 2 mL of methyl alcohol (blank). Add to each tube 1 mL of the 1% (w/v) *p*-dimethylaminobenzaldehyde solution in methyl alcohol, freshly prepared, and 2 mL of glacial acetic acid. Allow to stand for 10 minutes at room temperature. The intensity of the yellow color obtained in the *Test solution* tube must be between white and the yellow color obtained in the *2,6-dimethylaniline solution* (100 ppm).

Heavy metals (5.3.2.3). Use 1.0 g of sample. Proceed as described in *Limit test for heavy metals, Method III*. No more than 0.002% (20 ppm).

Sulfates. Weigh 0.2 g of the sample and dissolve in 20 mL of water and 2 mL of 3 M hydrochloric acid. Homogenize and add 1 mL of 12% (w/v) barium chloride. Concomitantly, prepare the reference standard by mixing 0.1 mL of 0.02 M sulfuric acid with 22 mL of water and 1 mL of 12% (w/v) barium chloride. Allow to stand for 10 minutes. The turbidity obtained by the sample is not more intense than the turbidity obtained by the reference standard. No more than 0.1%.

Water (5.2.20.1). 5.0% to 7.0%, for the monohydrate form.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

When the label indicates that the substance is sterile, it must comply with the sterility and with the bacterial endotoxins tests. When the label indicates that the substance must be sterilized during the production or the substance is targeted at production of sterile non-parenteral preparations, it must comply with the bacterial endotoxins test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 1.1 EU/mg of lidocaine hydrochloride.

ASSAY

Use one of the methods described below.

A. Accurately weigh 0.22 g of sample, transfer to a 125 mL Erlenmeyer flask and dissolve in 50 mL of ethyl alcohol. Add 5.0 mL of 0.01 M hydrochloric acid and titrate with 0.1 M sodium hydroxide VS, determining the endpoint potentiometrically between the two inflection points. Each mL of 0.1 M sodium hydroxide VS is equivalent to 27.08 mg of $C_{14}H_{22}N_2O \cdot HCl$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: homogenize 50 mL of glacial acetic acid and 930 mL of water. Adjust pH to 3.4 with M sodium hydroxide. Mix four volumes of this solution with one volume of acetonitrile. The lidocaine retention time must be between four and six minutes.

Sample solution: transfer 100 mg of the sample, accurately weighed, to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: 2.0 mg/mL lidocaine hydrochloride CRS solution in *Mobile phase*.

Resolution solution: prepare a 220 μ g/mL methylparaben solution using the *Mobile phase* as diluent. Mix 2 mL of this solution and 20 mL of the *Standard solution*.

Inject 20 μ L replicates of the *Resolution solution*. The resolution between the peaks of lidocaine and methylparaben is not less than 3. Inject 20 μ L replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is no more than 1.5%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{14}H_{22}N_2O \cdot HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

When the raw material is used in the process for manufacturing injection forms or other sterile pharmaceutical dosage forms, the label must indicate whether the product is sterile or must be sterilized during the process.

THERAPEUTIC CLASS

Local anesthetic.

LIDOCAINE HYDROCHLORIDE GEL

Contains no less than 95.0% and no more than 105.0% of the declared amount of $C_{14}H_{22}N_2O \cdot HCl$ in a hydrophilic, viscous and sterile base.

IDENTIFICATION

A. Accurately weigh an amount of gel equivalent to 80 mg of lidocaine hydrochloride and transfer to a test tube, add 4 mL of hydrochloric acid and heat in water bath for 10 minutes. Allow to cool, transfer to a separation funnel using 20 mL of water, add 5 M sodium hydroxide until there is full precipitation of the drug, and extract with two portions of 20 mL of chloroform. Collect the organic phases and filter with anhydrous sodium sulfate. Evaporate the filtrate in water bath to dryness, under nitrogen stream. In the infrared absorption spectrum **(5.2.14)** of the residue obtained, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of lidocaine CRS, prepared identically.

B. Dissolve 20 mg of the residue obtained on test **A.** of *Identification* in 1 mL of ethyl alcohol, add 0.5 mL of 10% (w/v) cobalt chloride, 0.5 mL of 5 M sodium hydroxide and shake for two minutes. A blueish-green precipitate is produced.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

PURITY TESTS

pH (5.2.19). 6.0 to 7.0.

Limit of 2,6-dimethylaniline. Accurately weigh an amount of gel equivalent to 25 mg of lidocaine hydrochloride anhydrous, homogenize with sufficient water to produce 5 mL and shake in a vortex shaker. Transfer to 2 mL of this mixture 1 mL of a freshly prepared 1% (w/v) *p*-dimethylaminobenzaldehyde solution in methyl alcohol. Shake in a vortex shaker and add 2 mL of glacial acetic acid. Allow to stand for 10 minutes at room temperature. Prepare a standard 2,6-dimethylaniline solution in the same conditions, using 2 mL of 0.0002% (v/v) 2,6-dimethylaniline in methyl alcohol, instead of the gel solution. The intensity of the yellow color obtained in the test solution tube is not more intense than that obtained in the standard 2,6-dimethylaniline solution.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

ASSAY

Accurately weigh an amount of gel equivalent to 20 mg of lidocaine hydrochloride and transfer to a separation funnel with 10 mL of water. Shake to dilute, add 1 mL of 6 M ammonium hydroxide and extract with four portions of 20 mL of chloroform. Collect the organic phases and evaporate in hot air stream. Add 25 mL of 0.005 M sulfuric acid VS before the last trace of chloroform is evaporated. Complete the evaporation of chloroform and titrate the excess acid with 0.01 M sodium hydroxide

VS. Determine the endpoint potentiometrically. Each mL of 0.005 M sulfuric acid VS is equivalent to 2.708 mg of $C_{14}H_{22}N_2O \cdot HCl$.

PACKAGING AND STORAGE

In tightly closed containers. Due to sterility, the cap must present a device indicating the tube opening.

LABELING

Comply with current legislation.

LIDOCAINE HYDROCHLORIDE POMADE

Contains no less than 95.0% and no more than 105.0% of the declared amount of $C_{14}H_{22}N_2O$ in hydrophilic base pomade.

IDENTIFICATION

Accurately weigh an amount of pomade equivalent to 300 mg of lidocaine and transfer to a separation funnel with 20 mL of water. Shake to dilute and extract with two portions of 30 mL of hexane. Wash the combined organic extracts with 10 mL of water and evaporate in hot air stream. Dry the residue using silica gel for 24 hours, under reduced pressure. In the infrared absorption spectrum (5.2.14) of the crystalline residue obtained in the drug extraction, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of standard lidocaine, prepared identically.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

PURITY TESTS

Limit of 2,6-dimethylaniline. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 230 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 0.8 mL/minute.

Buffer pH 8.0: transfer to a 0.685% (w/v) dibasic potassium phosphate solution a sufficient amount of 0.408% (w/v) monobasic potassium phosphate until pH 8.0.

Mobile phase: mixture of *Buffer pH 8.0* and methyl alcohol (35:65).

Solution (1): accurately weigh an amount of pomade with the equivalent to 50 mg of lidocaine, transfer to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 2 mL of this solution to a 20 mL volumetric flask and complete the volume with *Mobile phase*.

Solution (2): dissolve an accurately weighed amount of 2,6-dimethylaniline in methyl alcohol to obtain a 1 mg/mL solution. Dilute, successively, in *Mobile phase* until a concentration of 0.04 μ g/mL.

Solution (3): homogenize equal volumes of 0.1 mg/mL lidocaine CRS solution in *Mobile phase* and 0.05 g/mL 2,6-dimethylaniline solution in *Mobile phase*.

Inject 20 μ L replicates of *Solution (3)*. The relative retention times are approximately 1 for 2,6-dimethylaniline and 0.5 for lidocaine.

Procedure: inject, separately, 20 μ L of *Solution (1)* and 20 μ L of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The area under the peak related to 2,6-dimethylaniline, obtained with *Solution (1)*, is not greater than the area under the main peak obtained with *Solution (2)*. No more than 0.04% (400 ppm) in relation to the content of lidocaine.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Assay* in the *Lidocaine hydrochloride gel* monograph. Each mL of 0.005 M sulfuric acid VS is equivalent to 2.343 mg of C₁₄H₂₂N₂O.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

LIDOCAINE HYDROCHLORIDE INJECTION SOLUTION

Contains no less than 95.0% and no more than 105.0% of the declared amount of $C_{14}H_{22}N_2O.HCl$. Sterile lidocaine hydrochloride solution in water for injection.

IDENTIFICATION

A. Transfer a volume of injection solution with the equivalent to 0.1 g of lidocaine hydrochloride to a beaker and add a volume of 5 M sodium hydroxide until alkalinizing the solution. Filter and wash the residue with water. Dissolve the residue in 1 mL of ethyl alcohol, add 0.5 mL of 10% (w/v) cobalt chloride and shake for two minutes. A greenish blue precipitate is produced.

B. For a volume of injection solution with the equivalent to 0.1 g of lidocaine hydrochloride, transfer 10 mL of picric acid RS. The melting point of the precipitate obtained, after washing with water and drying at 105 °C, is around 229 °C.

C. Complies with chloride ion reactions (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.0 to 7.0.

PURITY TESTS

Limit of 2,6-dimethylaniline. In a volume of injection solution with the equivalent to 25 mg of lidocaine hydrochloride, add, if necessary, sufficient water to produce 10 mL. Add 2 M sodium hydroxide until alkalinizing the solution and extract with three portions of 5 mL of chloroform. Filter the combined organic extracts under sodium sulfate anhydrous and wash the filter with 5 mL of chloroform. Evaporate the filtrate to dryness under pressure of 2 kPa. Dissolve the residue in 2 mL of methyl alcohol, add 1 mL of 1% (w/v) *p*-dimethylaminobenzaldehyde in methyl alcohol, freshly prepared, and 2 mL of glacial acetic acid. Prepare a 2,6-dimethylaniline solution in the same manner using 10 mL of a 0.0001% (w/v) 2,6-dimethylaniline solution in methyl alcohol, instead of the injection solution. The intensity of the yellow color obtained in the solution tube with the sample must not be more intense than that obtained in the 2,6-dimethylaniline solution.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 1.1 EU/mg of lidocaine hydrochloride.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. In a volume of injection solution equivalent to 0.1 g of lidocaine hydrochloride, add 2 M sodium hydroxide until alkalinizing the solution and extract with three portions of 20 mL of chloroform. Wash each organic extract with

10 mL of water (always use the same 10 mL of water). Filter the combined organic elements extracted in a filter moistened with chloroform and wash the filter with 10 mL of chloroform. Collect the combined organic elements filtered and the washing elements. Titrate with 0.02 M perchloric acid VS. Determine the endpoint potentiometrically or using methylrosanilinium chloride TS as indicator. Each mL of 0.02 M perchloric acid VS is equivalent to 5.416 mg of $C_{14}H_{22}N_2O \cdot HCl$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained between 20 °C and 25 °C $\pm$ 1 °C of the temperature selected; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: homogenize 50 mL of glacial acetic acid and 930 mL of water. Adjust pH to 3.4 with M sodium hydroxide. Mix four volumes of this solution with one volume of acetonitrile. The composition of the *Mobile phase* can be adjusted so that the lidocaine peak as a retention time between 4 and 6 minutes.

Sample solution: transfer a volume of the injection solution equivalent to 0.1 g of lidocaine hydrochloride to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: transfer 42.5 mg of lidocaine CRS, accurately weighed, to a 25 mL volumetric flask and dissolve with heating, if necessary, in 0.5 mL of M hydrochloric acid. Complete the volume with *Mobile phase* to obtain a 1.7 mg/mL lidocaine solution.

Resolution solution: prepare a 0.22 mg/mL methylparaben solution using *Mobile phase* as diluent. Mix 2 mL of this solution and 20 mL of the *Standard solution*.

Inject 20 μ L replicates of the *Resolution solution*. The resolution between lidocaine and methylparaben is not less than 3. Inject 20 μ L replicates of the *Standard solution*. The relative standard deviation of the replicate areas of the recorded peaks is no more than 1.5%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{14}H_{22}N_2O \cdot HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

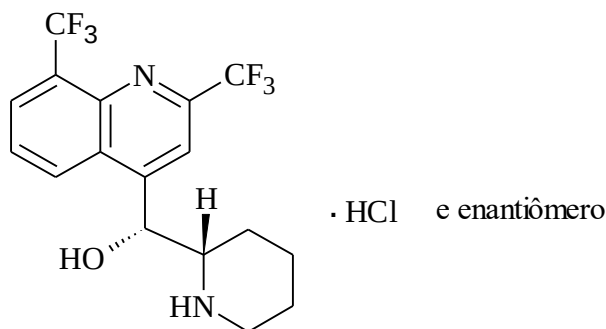
In type I glass containers, protected from light.

LABELING

Comply with current legislation.

MEFLOQUINE HYDROCHLORIDE

Mefloquini hydrochloridum



$C_{17}H_{16}F_6N_2O \cdot HCl$; 414.77

mefloquine hydrochloride; 05577

(αS)-*rel*- α -(2*R*)-2-piperidinyl-2,8-bis-(trifluoromethyl)-4-quinolinemethanol hydrochloride (1:1)
[51773-92-3]

Contains no less than 98.0% and no more than 102.0% of $C_{17}H_{16}F_6N_2O \cdot HCl$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or yellowish crystalline powder. Presents polymorphism.

Solubility. Slightly soluble in water, freely soluble in methyl alcohol, soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 259 °C to 260 °C.

Specific optical rotation (5.2.8): -0.2 to +0.2. Determine in solution at 5% (w/v) in methyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the non-desiccated sample dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of mefloquine hydrochloride CRS, prepared identically. If the spectra obtained are not identical, dissolve, separately, the standard and the sample in methyl alcohol and evaporate to dryness. Obtain new spectra with the residues.

B. Weigh 20 mg of the sample, add 0.2 mL of sulfuric acid. A blue fluorescence under ultraviolet light (365 nm) develops.

C. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph with an ultraviolet detector at 280 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 µm to 10 µm), capped; *Mobile phase* flow of 0.8 mL/minute. Balance the column for 30 minutes with a flow of 2 mL/minute.

Mobile phase: dissolve 1 g of tetraheptylammonium bromide in a mixture of methyl alcohol, 0.15% (w/v) sodium bisulfate and acetonitrile (2:4:4).

Solution (1): 4 mg/mL sample solution in *Mobile phase*.

Solution (2): transfer 1 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Solution (3): accurately weigh 8 mg of mefloquine hydrochloride CRS and 8 mg of quinidine sulfate and transfer to a 50 mL volumetric flask. Dissolve and complete the volume with *Mobile phase*. Transfer 5 mL to a 100 mL volumetric flask and complete the volume with the same solvent. Homogenize.

Inject 20 µL of *Solution (3)*. The relative retention times are approximately 0.5 for quinidine and 1.0 for mefloquine. The resolution between the mefloquine and quinidine peaks is no less than 8.5.

Procedure: inject, separately, 20 µL of *Solution (1)* and of *Solution (2)*, record the chromatograms for no less than 10 times the main peak retention time and measure the areas under the peaks. The area under any secondary peak with relative retention time of approximately 0.7 obtained with *Solution (1)* is not greater than twice the area under the main peak obtained with *Solution (2)* (0.2%). The area under any peak obtained with *Solution (1)*, except under the main peak and the peak with relative retention time of approximately 0.7, is not greater than the area under the main peak obtained with *Solution (2)* (0.1%). The sum of the areas under all peaks obtained with the *Solution (1)*, except the main peak, is not greater than five times the area under the main peak obtained with *Solution (2)* (0.5%). Disregard peaks with areas below 0.2 times the area under the main peak obtained with *Solution (2)*.

Heavy metals (5.3.2.3). Determine in 1.0 g of the sample. No more than 0.002% (20 ppm).

Water (5.2.20.1). Determine in 1.0 g of the sample. No more than 3.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 0.3 g of the sample in 15 mL of formic acid anhydrous and 40 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS

determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 41.477 mg of $C_{17}H_{16}F_6N_2O.HCl$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimalarial.

MEFLOQUINE HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{17}H_{16}F_6N_2O \cdot HCl$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 350 nm of the sample solution obtained in method **A.** from Assay, there are maximum and minimum points identical to those observed in the spectrum of the standard solution.

B. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.25 g of mefloquine hydrochloride, transfer to a beaker and add five drops of nitric acid. Shake with 50 mL of water and filter in filter paper for slow filtration. Add silver nitrate RS to the filtrate. A white caseous precipitate, insoluble in nitric acid, but soluble in a slight excess of 6 M ammonium hydroxide, is formed.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 100 rpm.

Time: 60 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute with *Dissolution medium* to adequate concentration. Measure the absorbances of the solutions at 283 nm **(5.2.14)** using *Dissolution medium* for zero adjustment. Calculate the amount of $C_{17}H_{16}F_6N_2O \cdot HCl$ dissolved in the medium, comparing the readings obtained with that of the mefloquine hydrochloride CRS solution, at the concentration of 0.006% (w/v), prepared in the same solvent. To ensure complete dissolution of the mefloquine hydrochloride CRS, it is possible to use up to 5% (v/v) of methyl alcohol in the first dilution.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{17}H_{16}F_6N_2O \cdot HCl$ dissolve in 60 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.1 g of mefloquine hydrochloride, transfer to a 100 mL volumetric flask and add 70 mL of methyl alcohol. Leave in ultrasonic bath for 10 minutes and complete the volume with methyl alcohol. Filter. Dilute, successively, in methyl alcohol until concentration of 0.004% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 283 nm, using methyl alcohol for zero adjustment. Calculate the amount of $C_{17}H_{16}F_6N_2O.HCl$ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with ultraviolet detector at 283 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m); maintained at temperature of 30 °C; *Mobile phase* flow of 1.0 mL/minute.

Buffer pH 3.5: weigh 6.80 g of monobasic potassium phosphate and transfer to a 1000 mL volumetric flask. Dissolve and complete the volume with water. Adjust pH to 3.5 with phosphoric acid.

Mobile phase: mixture of *Buffer pH 3.5* and methyl alcohol (40:60).

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 100 mg of mefloquine hydrochloride, transfer to a 100 mL volumetric flask, add approximately 80 mL of methyl alcohol and leave in ultrasound for 10 minutes. Complete the volume with the same solvent and filter, discarding the first 10 mL of the filtrate. Transfer 5 mL of the filtrate to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: accurately weigh about 10 mg of mefloquine hydrochloride CRS and transfer to a 100 mL volumetric flask. Add approximately 80 mL of *Mobile phase* and place in ultrasonic bath for 10 minutes. Complete the volume with the same solvent and homogenize.

Inject 20 μ L replicates of the *Standard solution*. The tailing factor is no more than 1.5. The standard deviation of the replicate areas of the recorded peaks is no more than 1.0%.

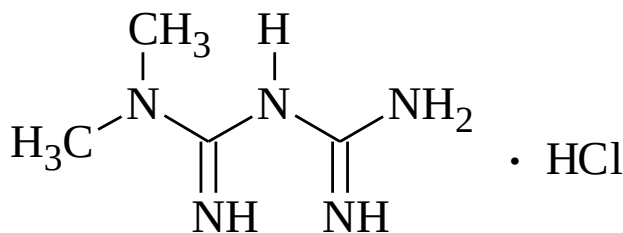
Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{17}H_{16}F_6N_2O.HCl$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

METFORMIN HYDROCHLORIDE*Metformini hydrochloridum*C₄H₁₁N₅.HCl; 165.62

metformin hydrochloride; 05782

N,N-dimethylimidodicarbonimidic diamide hydrochloride (1:1)

[1115-70-4]

Contains no less than 98.5% and no more than 101.0% of C₄H₁₁N₅.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Freely soluble in water, slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 222 °C to 226 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of metformin hydrochloride CRS, prepared identically.

B. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 218 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 µm to 10 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: dissolve 17 g of monobasic potassium phosphate in 1000 mL of water and adjust the pH to 3.0 ± 0.1 with phosphoric acid.

Solution (1): accurately weigh 20 mg of cyanoguanidine CRS, transfer to a 100 mL volumetric flask and complete the volume with water. Transfer 1 mL to a 200 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Solution (2): accurately weigh 500 mg of the sample, transfer to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Solution (3): dilute 1 mL of *Solution (2)* to 100 mL with *Mobile phase*. Transfer 1 mL to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Solution (4): accurately weigh 10 mg of melamine, dissolve in 90 mL of water, add 5 mL of *Solution (2)* and complete the volume to 100 mL with the same solvent. Dilute 1 mL of the solution obtained to 50 mL with *Mobile phase*.

Inject 20 µL of *Solution (4)*. The resolution between melamine and metformin hydrochloride must be greater than 10. The relative standard deviation of the replicate areas from the recorded peaks must be no more than 2.0%.

Procedure: inject, separately, 20 µL of *Solution (1)*, of *Solution (2)* and of *Solution (3)* and record the chromatograms for no less than double the retention time of the main peak. The peak obtained in *Solution (2)*, corresponding to cyanoguanidine, cannot be greater than 0.02%, compared to the peak obtained with *Solution (1)*. No individual impurity obtained with *Solution (2)* can be superior to 0.1%, compared to the peak obtained with *Solution (3)*. The sum of the areas of all peaks obtained with *Solution (2)*, except that of the solvent peak, is no greater than the area under the main peak, obtained with *Solution (3)* (0.5%). Do not consider peaks with area smaller than that presented by the main peak in the chromatogram obtained with *Solution (4)* (0.2%).

Heavy metals (5.3.2.3). Proceed as described in *Method I*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of sample. Dry in oven at 105°C for 5 hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Accurately weigh 60 mg of the sample previously desiccated, dissolve in 4 mL of formic acid anhydrous and add 50 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS. Determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 8.281 mg of C₄H₁₁N₅.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Oral hypoglycemic agent.

METFORMIN HYDROCHLORIDE TABLETS

Contains no less than 95.0% and no more than 105.0% the declared amount of $C_4H_{11}N_5.HCl$. The tablets can be coated.

IDENTIFICATION

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 20 mg of metformin hydrochloride, dissolve 20 mL of ethyl alcohol and shake. Filter, evaporate the filtrate to dryness in water bath and dry the residue at 105 °C for one hour. The residue complies with test **A.** of *Identification* from the *Metformin hydrochloride* monograph.

B. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 50 mg of metformin hydrochloride, transfer to a test tube and add 10 mL of water, homogenize and filter. The filtrate complies with chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 250 mL volumetric flask, add 150 mL of water, shake mechanically for 15 minutes and complete the volume with the same solvent. Filter. Transfer 5 mL of the filtrate to a 100 mL volumetric flask and complete the volume with water. Carry out successive dilutions to a concentration of 0.001% (w/v), using water as solvent. Prepare a standard solution in the same conditions. Measure the absorbances of the solutions at 232 nm **(5.2.14)**, using water for zero adjustment. Calculate the amount of $C_4H_{11}N_5.HCl$ in each tablet, from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 6.8, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute, if necessary, with water, until adequate concentration. Measure the absorbances at 233 nm **(5.2.14)**, using water for zero adjustment. Calculate the amount of $C_4H_{11}N_5.HCl$ dissolved in the medium, comparing the readings obtained with that of the metformin hydrochloride CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_4H_{11}N_5.HCl$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Except for the preparation of *Solution (2)*, proceed as described in *Related substances* in the *Metformin hydrochloride* monograph. Prepare the *Solution (2)* as described next.

Solution (2): Pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 500 mg of metformin hydrochloride, transfer to a 100 mL volumetric flask, dissolve with 60 mL of *Mobile phase*, shake for 15 minutes and complete the volume with the same solvent. Filter.

Procedure: inject, separately, 20 µL of the *Solution (1)*, *Solution (2)*, *Solution (3)* and *Solution (4)*, record the chromatograms and measure the areas of all peaks obtained. No more than 0.1% of individual impurity and no more than 0.6% of total impurity. Do not include solvent-related peaks in the calculations.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 100 mg of metformin hydrochloride, transfer to a 100 mL volumetric flask and add 70 mL of methyl alcohol. Shake mechanically for 15 minutes, complete the volume with the same solvent and filter. Transfer 10 mL of the filtrate to a 100 mL volumetric flask and complete the volume with water. Carry out successive dilutions to a concentration of 0.001% (w/v), using water as solvent. Prepare a standard solution in the same conditions. Measure the absorbances of the solutions at 232 nm, using water for zero adjustment. Calculate the amount of $C_4H_{11}N_5.HCl$ in the tablets, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

METOCLOPRAMIDE HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{14}H_{22}ClN_3O_2 \cdot HCl$.

IDENTIFICATION

The identification test A. can be omitted if tests B., C., D. and E. are carried out. The identification test B. can be omitted if tests A., C., D. and E. are carried out.

A. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from Assay, there are maximum absorption points identical to those observed in the spectrum of the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

C. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 10 mg of metoclopramide hydrochloride. Add 1 mL of water, shake mechanically and filter. Transfer to the filtrate 1 mL of 1% (w/v) *p*-dimethylaminobenzaldehyde in *M* hydrochloric acid. Orangish-yellow color develops.

D. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 10 mg of metoclopramide hydrochloride. Add 10 mL of hydrochloric acid SR, cool down to 0 °C and add 1 mL of 1% (w/v) sodium nitrite. A yellow precipitate develops. Add 1 mL of 2-naphthol RS. Orange red precipitate is produced.

E. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 10 mg of metoclopramide hydrochloride. Add 10 mL of water, shake and filter. The filtrate complies with chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 100 mL volumetric flask and proceed as described on method **A.** from Assay, as of "... add 70 mL of 0.1 M hydrochloric acid".

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: baskets, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot of the dissolution medium and dilute in water until adequate concentration. Measure the absorbances at 309 nm **(5.2.14)**, using water for zero adjustment. Calculate the amount of $C_{14}H_{22}ClN_3O_2.HCl$ dissolved in the medium, comparing the readings obtained with that of metoclopramide hydrochloride CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{14}H_{22}ClN_3O_2.HCl$ dissolve in 30 minutes.

PURITY TESTS

Water (5.2.20.1). No more than 3.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 10 mg of metoclopramide hydrochloride, transfer to a 100 mL volumetric flask and add 70 mL of 0.1 M hydrochloric acid. Leave in an ultrasonic bath for 15 minutes. Shake mechanically for 15 minutes, complete the volume with the same solvent, homogenize and filter. Dilute, successively, in the same solvent, to a concentration of 0.002% (w/v). Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 309 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{14}H_{22}ClN_3O_2.HCl$ in the tablets, from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 215 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: dissolve 2.7 g of sodium acetate in 500 mL of water. Add 500 mL of acetonitrile and 2 mL of 20% (w/v) tetramethylammonium hydroxide in methyl alcohol. Homogenize. Adjust pH to 6.5 with glacial acetic acid.

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 40 mg of metoclopramide hydrochloride, transfer to a 50 mL volumetric flask and add 35 mL of 0.01 M phosphoric acid. Leave in an ultrasonic bath for 15 minutes. Shake mechanically for 15 minutes, complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of the filtrate to a 100 mL volumetric flask and complete the volume with the same solvent.

Standard stock solution: accurately weigh 40 mg of metoclopramide hydrochloride CRS and transfer to a 50 mL volumetric flask. Dissolve in 0.01 M phosphoric acid and complete the volume with the same solvent, to obtain a 0.8 mg/mL solution.

Standard solution: transfer 5 mL of the *Standard stock solution* to a 100 mL volumetric flask and complete the volume with 0.01 M phosphoric acid, to obtain a 40 µg/mL solution.

Resolution solution: accurately weigh 12.5 mg of benzenesulfonamide and transfer to a 25 mL volumetric flask, dissolve in 15 mL of methyl alcohol and complete the volume with 0.01 M phosphoric acid. Transfer 5 mL of the resulting solution and 5 mL of the *Standard stock solution* to a 100 mL volumetric flask and complete the volume with 0.01 M phosphoric acid.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.7 for benzenesulfonamide and 1.0 for metoclopramide hydrochloride, with resolution of no less than 1.5. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{14}H_{22}ClN_3O_2.HCl$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

METOCLOPRAMIDE HYDROCHLORIDE INJECTION SOLUTION

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{14}H_{22}ClN_3O_2 \cdot HCl$.

IDENTIFICATION

The identification test A. can be omitted if tests B., C., D. and E. are carried out. The identification test B. can be omitted if tests A., C., D. and E. are carried out.

A. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from Assay, there are maximum absorption points identical to those observed in the spectrum of the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

C. Use a volume of injection solution equivalent to 10 mg of metoclopramide hydrochloride. Add 1 mL of water and shake. Add 1 mL of 1% (w/v) *p*-dimethylaminobenzaldehyde in *M* hydrochloric acid. Orangish-yellow color develops.

D. Use a volume of injection solution equivalent to 10 mg of metoclopramide hydrochloride. Add 10 mL of hydrochloric acid SR, cool down to 0 °C and add 1 mL of 1% (w/v) sodium nitrite. A yellow precipitate is produced. Add 1 mL of 2-naphthol RS. Orange red precipitate is produced.

E. Use a volume of injection solution equivalent to 10 mg of metoclopramide hydrochloride. Add 10 mL of water and shake. The resulting solution complies with chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 2.5 to 6.5.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 2.5 EU/mg of metoclopramide.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer a volume of the injection solution equivalent to 10 mg of metoclopramide hydrochloride to a 100 mL volumetric flask and complete the volume with 0.1 *M* hydrochloric acid. Homogenize. Dilute successively, with the same solvent, to a concentration of 0.002% (w/v). Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 309

nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{14}H_{22}ClN_3O_2.HCl$ in the injection solution from the readings obtained.

B. Proceed as described in method **B.** of *Assay* on the *Metoclopramide hydrochloride tablets* monograph. Prepare the *Sample solution* as described next.

Sample solution: transfer a volume of the injection solution equivalent to 40 mg of metoclopramide hydrochloride to a 100 mL volumetric flask and complete the volume with 0.01 M phosphoric acid. Homogenize. Transfer 5 mL to a 50 mL volumetric flask and complete the volume with the same solvent.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{14}H_{22}ClN_3O_2.HCl$ in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers protected from light.

LABELING

Comply with current legislation.

METOCLOPRAMIDE HYDROCHLORIDE ORAL SOLUTION

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{14}H_{22}ClN_3O_2 \cdot HCl$.

IDENTIFICATION

The identification test A. can be omitted if tests B., C., D. and E. are carried out. The identification test B. can be omitted if tests A., C., D. and E. are carried out.

A. In the visible absorption spectrum **(5.2.14)** in the range of 400 nm to 800 nm of the sample solution obtained in method **A.** from Assay, there are maximum absorption points identical to those observed in the spectrum of the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

C. Use a volume of oral solution equivalent to 10 mg of metoclopramide hydrochloride. Add 1 mL of water and shake. Add 1 mL of 1% (w/v) *p*-dimethylaminobenzaldehyde in *M* hydrochloric acid. Orangish-yellow color develops.

D. Use a volume of oral solution equivalent to 10 mg of metoclopramide hydrochloride. Add 10 mL of hydrochloric acid SR, cool down to 0 °C and add 1 mL of 1% (w/v) sodium nitrite. A yellow precipitate develops. Add 1 mL of 2-naphthol RS. Orange red precipitate is developed.

E. Use a volume of oral solution equivalent to 10 mg of metoclopramide hydrochloride. Add 10 mL of water and shake. The resulting solution complies with chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 2.0 to 5.5.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Proceed protected from direct light. Transfer a volume of oral solution equivalent to 10 mg of metoclopramide hydrochloride to a 100 mL volumetric flask and complete the volume with water. Homogenize. Transfer 5 mL to a 100 mL volumetric flask, add 5 mL of *M* hydrochloric acid and homogenize. Add 5 mL of 1% (w/v)

sodium nitrite, prepared extemporaneously. Mix and allow to stand for 10 minutes. Add 5 mL of 5% (w/v) ammonium sulfamate, prepared extemporaneously. Mix and allow to stand for 25 minutes. Add 5 mL of 0.5% (w/v) *N*-(1-naphthyl)ethylenediamine dihydrochloride in *M* hydrochloric acid, prepared extemporaneously, and homogenize. Complete the volume with water and allow to stand for five minutes, obtaining a 0.0005% (w/v) solution. Prepare a standard solution in the same conditions. Measure the absorbances of the resulting solutions at 534 nm, using water for zero adjustment. Calculate the amount of $C_{14}H_{22}ClN_3O_2.HCl$ in the oral solution from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 215 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: weigh 2.7 g of sodium acetate and dissolve in 600 mL of water. Add 400 mL of acetonitrile and 5 mL of 20% (w/v) tetramethylammonium hydroxide in methyl alcohol. Homogenize. Adjust pH to 6.5 with glacial acetic acid.

Sample solution: transfer a volume of the oral solution equivalent to 4 mg of metoclopramide hydrochloride to a 25 mL volumetric flask and complete the volume with 0.01 *M* phosphoric acid. Homogenize.

Standard stock solution: accurately weigh 40 mg of metoclopramide hydrochloride CRS and transfer to a 50 mL volumetric flask. Dissolve in 0.01 *M* phosphoric acid and complete the volume with the same solvent, to obtain a 0.8 mg/mL solution.

Standard solution: transfer 5 mL of the *Standard stock solution* to a 25 mL volumetric flask and complete the volume with 0.01 *M* phosphoric acid, to obtain a 0.16 mg/mL standard metoclopramide hydrochloride solution.

Resolution solution: accurately weigh 0.125 g of benzenesulfonamide and transfer to a 25 mL volumetric flask. Dissolve in 15 mL of methyl alcohol. Complete the volume with 0.01 *M* phosphoric acid. Transfer 15 mL of the previous solution and 5 mL of the *Standard stock solution* to a 250 mL volumetric flask and complete the volume with 0.01 *M* phosphoric acid. Homogenize.

Inject 20 μ L replicates of the *Resolution solution*. The relative retention time is approximately 0.2 for benzenesulfonamide and 1.0 for metoclopramide hydrochloride. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

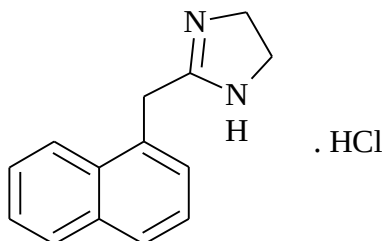
Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{14}H_{22}ClN_3O_2.HCl$ in the oral solution from the responses obtained with the *Standard* and *Sample solutions*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

NAPHAZOLINE HYDROCHLORIDE*Naphazolini hydrochloridum* $C_{14}H_{14}N_2 \cdot HCl$; 246.74

naphazoline hydrochloride; 06177

2-(naphthalene-1-ylmethyl)-4,5-dihydro-1H-imidazole hydrochloride

[550-99-2]

Contains no less than 98.0% and no more than 102.0% of $C_{14}H_{14}N_2 \cdot HCl$, in relation to the anhydrous base.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Freely soluble in water and soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 255 °C to 260 °C, with decomposition.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the non-desiccated sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of naphazoline hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 20 µg/mL sample solution in methyl alcohol, displays maximum absorption at 280 nm, identical to the one observed in the spectrum of a similar naphazoline hydrochloride CRS solution.

C. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 5.0 to 6.6. Determine in aqueous solution at 1.0% (w/v).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of methyl alcohol, glacial acetic acid and water (8:1:1) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): weigh 0.1 g of the sample, dissolve in methyl alcohol, complete the volume to 10 mL with the same solvent and homogenize.

Solution (2): weigh 5.0 mg of CRS, dissolve in methyl alcohol, complete the volume to 25 mL with the same solvent.

Develop the chromatogram. Remove the plate and allow it to air dry. Nebulize with metallic iodine. Any secondary stain in the chromatogram of *Solution (1)* is not more intense than that obtained with the chromatogram of *Solution (2)* (2.0%).

Loss on drying (5.2.9.1). Determine in 1.0 g of sample. Dry in oven at 105 °C for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.4 g of the sample, previously desiccated, dissolve in 50 mL of a mixture of acetic anhydride and glacial acetic acid (7:3). Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically between the two inflection points. Each mL of 0.1 M perchloric acid VS is equivalent to 24.67 mg of $C_{14}H_{14}N_2O \cdot HCl$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 280 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group, maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Buffer solution: weigh 3.0 g of monobasic potassium phosphate, transfer to a 1000 mL volumetric flask and dissolve in 800 mL. Add 3.0 mL of triethylamine, adjust pH with phosphoric acid to 3.0 and complete the volume with water.

Mobile phase: mixture of *Buffer solution* and acetonitrile (80:20).

Sample solution: accurately weigh 0.2 g of sample, transfer to a 200 mL volumetric flask, dissolve in water, complete the volume with the same solvent and homogenize. Transfer 5.0 mL of this solution to a 100 mL volumetric flask, dilute and complete the volume with water and homogenize, to obtain a 50 µg/mL naphazoline hydrochloride solution.

Standard solution: prepare, accurately, a 50 µg/mL naphazoline hydrochloride solution CRS in water.

Inject replicates of the *Standard solution*. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%. The capacity factor is no less than 2.0. The theoretical plates are no less than 1500. The tailing factor is no more than 2.0.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₄H₁₄N₂.HCl in the sample from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

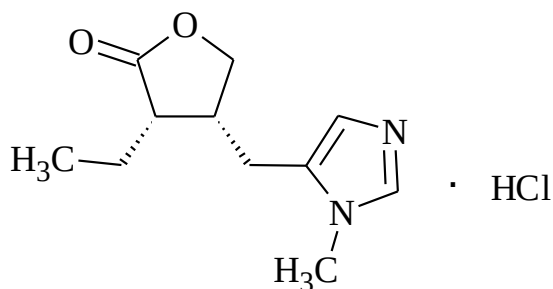
In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vasoconstrictor.

PILOCARPINE HYDROCHLORIDE*Pilocarpini hydrochloridum*C₁₁H₁₆N₂O₂.HCl; 244.72

pilocarpine hydrochloride; 07050

(3*S*,4*S*)-3-ethyl-dihydro-4-[(1-methyl-1*H*-imidazole- 5-yl)methyl]-2(3*H*)-furanone hydrochloride (1:1)
[54-71-7]

Contains no less than 98.5% and no more than 101.0% of C₁₁H₁₆N₂O₂.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or colorless crystals, hygroscopic.

Solubility. Soluble in water and in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 199 °C to 205 °C. The range between the beginning and the end of melting must not exceed 3 °C.

Specific optical rotation (5.2.8): +89 to +93, in relation to the desiccated substance. Determine in solution at 2.0% (w/v) in water.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out. The identification test B. can be omitted if tests A. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, previously desiccated, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of pilocarpine hydrochloride CRS, prepared identically.

B. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

C. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 3.5 to 4.5. Determine in 5% (w/v) solution in carbon dioxide-free water.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of concentrated ammonium hydroxide, methyl alcohol and methylene chloride (1:14:85) as mobile phase. Apply to the plate, separately, 25 µL of each of the freshly prepared solutions described below.

Solution (1): accurately weigh 0.25 g of the sample, transfer to a 5 mL volumetric flask, dissolve with methyl alcohol and complete the volume with the same solvent.

Solution (2): dilute 1 mL of *Solution (1)* to 10 mL with methyl alcohol.

Solution (3): accurately weigh 10 mg of pilocarpine hydrochloride CRS, transfer to a 2 mL volumetric flask, dissolve with methyl alcohol and complete the volume to 2 mL with the same solvent.

Solution (4): dilute 1 mL of *Solution (2)* to 10 mL with methyl alcohol.

Develop the chromatogram along 15 cm of the plate, allow the plate to dry between 100 °C and 105 °C for 10 minutes, allow to cool and nebulize with potassium iodobismuthate RS, and then with 3% (w/v) hydrogen peroxide. Examine under visible light. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (4)* (1.0%).

Iron (5.3.2.4). Determine in 20 mL of 5% (w/v) solution in carbon dioxide-free water. Prepare the standard using 0.1 mL of *Standard iron solution (100 ppm Fe)* and 5 mL of water. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Dry in oven between 100 °C and 105 °C for two hours. No more than 3.0%.

Residue by incineration (5.2.10). Determine in 0.5 g of the sample. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh 2 g of sample and dissolve in 60 mL of water. Titrate with *M* sodium hydroxide VS and determine the endpoint potentiometrically. Each mL of *M* sodium hydroxide VS is equivalent to 244.720 mg of C₁₁H₁₆N₂O₂.HCl.

PACKAGING AND STORAGE

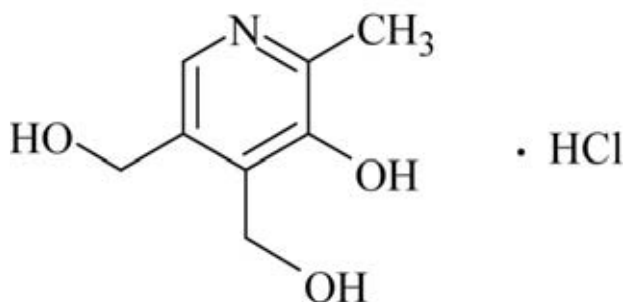
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-glaucoma.

PYRIDOXINE HYDROCHLORIDE*Pyridoxini hydrochloridum*C₈H₁₁NO₃.HCl; 205.64

pyridoxine hydrochloride; 07167

5-hydroxy-6-methyl-3,4-pyridinedimethanol hydrochloride (1:1)

[58-56-0]

Contains no less than 98.0% and no more than 102.0% of C₈H₁₁NO₃.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical-chemical characteristics. White or nearly white crystalline powder.

Solubility. Freely soluble in water, slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting point (5.2.2): Approximately 205 °C, with decomposition.

IDENTIFICATION

The identification test A. can be omitted if tests B., C. and D. are carried out. The identification tests B. and C. can be omitted if tests A. and D. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of pyridoxine hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 250 nm to 350 nm, of 0.001% (w/v) solution in 0.1 M hydrochloric acid, there are maximum points at 288 nm and 296 nm, with absorbance of 0.420 to 0.445. In the range of 220 nm to 350 nm, a solution prepared by diluting 1 mL of 0.1% (w/v) solution in 0.1 M hydrochloric acid to 100 mL with 0.025 M equimolar phosphate buffer, there are maximum points between 248 nm and 256 nm, and between 320 nm and 327 nm. The absorbances in each maximum point are, respectively, 0.175 to 0.195 and 0.345 to 0.365.

C. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (4)*.

D. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 2.4 to 3.0. Determine in 5.0% (w/v) sample solution in carbon dioxide-free water.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support and a mixture of acetone, carbon tetrachloride, tetrahydrofuran and 13.5 M ammonia (65:13:13:9) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described below.

Solution (1): 100 mg/mL aqueous sample solution.

Solution (2): 10 mg/mL aqueous sample solution.

Solution (3): 0.25 mg/mL aqueous sample solution.

Solution (4): 10 mg/mL aqueous pyridoxine hydrochloride solution CRS.

Procedure: develop the chromatogram. Remove the plate and allow it to air dry. Nebulize with a 5% (w/v) sodium carbonate solution in a mixture of water and ethyl alcohol (70:30). Dry in air stream and nebulize with a 0.1% (w/v) 2,6-dichloroquinone-4-chlorimide solution in ethyl alcohol. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (3)* (0.25%). Disregard stains that remain in the baseline.

Phenolic compounds. On a test tube, add 5 mg of the sample, 0.05 mL of 3 M hydrochloric acid, 1 mL of water and 1 mL of ferric chloride RS. Homogenize and add 1 mL of potassium ferricyanide RS. After 2 minutes, a blueish green color is not developed.

Limit of *N,N*-dimethylaniline. Accurately weigh 0.5 g of the sample, transfer to a 25 mL volumetric flask and dissolve with 20 mL of water using heating. Cool and transfer 2 mL of *M* acetic acid and 1 mL of 1% (w/v) sodium nitrite. Complete the volume with water and homogenize. The solution must not present a more intense color than the 0.001% (w/v) *N,N*-dimethylaniline solution (10 ppm) prepared in a similar manner.

Heavy metals (5.3.2.3). Use *Method I*. Determine in 20 mL of sample solution at 5.0% (w/v). No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Transfer to 0.4 g of the sample, accurately weighed, 30 mL of glacial acetic acid and 10 mL of mercury acetate RS. If necessary, leave in ultrasonic bath until completing the dissolution. Titrate with 0.1 M perchloric acid VS, using methylrosanilinium chloride TS as indicator. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 20.564 mg of $C_8H_{11}NO_3.HCl$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 280 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 to 10 μm), maintained at temperature of 25 °C; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: transfer to a 2 L volumetric flask 20 mL of glacial acetic acid, 1.2 g of sodium 1-hexanesulfonate and dilute with 1.4 L of water. Adjust the pH to 3.0 with glacial acetic acid or M sodium hydroxide. Add 470 mL of methyl alcohol, homogenize and complete the volume with water. Make adjustments, if necessary.

Internal standard solution: dissolve an amount of *p*-hydroxybenzoic acid in *Mobile phase* to obtain a concentration of 5 mg/mL.

Sample solution: transfer 50 mg of the sample, accurately weighed, to a 100 mL volumetric flask, dissolve and complete the volume with *Mobile phase*. Homogenize. Transfer 10 mL to a 100 mL volumetric flask, add 1 mL of the *Internal standard solution*, complete the volume with *Mobile phase* and homogenize.

Standard solution: accurately weigh 50 mg of pyridoxine hydrochloride CRS, transfer to a 100 mL volumetric flask, dissolve with mobile phase, complete the volume with the same solvent and homogenize. Transfer 10 mL to a 100 mL volumetric flask, add 1 mL of *Internal standard solution*, complete the volume with *Mobile phase* and homogenize.

Inject 20 μL replicates of the *Standard solution*. The resolution between the peaks corresponding to pyridoxine hydrochloride and to *p*-hydroxybenzoic acid is no less than 2.5. The relative standard deviation of the replicate areas of the recorded peaks is no more than 3.0%.

Procedure: inject, separately, 20 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to pyridoxine hydrochloride and to *p*-hydroxybenzoic acid. Calculate the amount of $C_8H_{11}NO_3.HCl$ in the sample from the responses obtained for the pyridoxine hydrochloride/*p*-hydroxybenzoic acid ratio with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vitamin supplement.

PYRIDOXINE HYDROCHLORIDE TABLETS

Contains no less than 95.0% and no more than 115.0% the declared amount of $C_8H_{11}NO_3 \cdot HCl$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of pyridoxine hydrochloride and dissolve in 50 mL of methyl alcohol shaking, mechanically, for 15 minutes. Filter, add sufficient 13.5 M ammonia to alkalize the filtrate and evaporate to dryness. Resume the residue with 15 mL of chloroform and filter. Drip to the filtrate 2 mL of acetyl chloride, transfer 8 mL of methyl alcohol and evaporate to dryness. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of pyridoxine hydrochloride CRS, prepared identically.

B. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 20 mg of pyridoxine hydrochloride, add 50 mL of 0.025 M phosphate buffer and shake for 15 minutes. Transfer to a 100 mL volumetric flask and complete the volume with 0.025 M phosphate buffer. In the ultraviolet absorption spectrum **(5.2.14)** of this solution, at the range of 230 nm to 350 nm, there are maximum absorption points at 254 nm and 324 nm.

C. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of pyridoxine hydrochloride, homogenize with 5 mL of water and filter to a test tube. Add two to three drops of ferric chloride RS. A reddish-orange color develops.

D. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 20 mg of pyridoxine hydrochloride, transfer to a beaker, homogenize with 50 mL of water and allow to stand to decantation. Transfer to 1 mL of the supernatant 10 mL of 5% (w/v) sodium acetate, 1 mL of water, 1 mL of 0.5% (w/v) 2-6-dichloroquinone-4-chlorimide in ethyl alcohol and homogenize. A blue color, with quick passage to brown, develops. Repeat the operation adding 1 mL of 0.3% (w/v) boric acid instead of water. A blue color is not produced.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 500 mL volumetric flask containing 300 mL of water, wait until disintegration and complete the volume with the same solvent. Filter discarding the first 25 mL of the filtrate. From the filtrate, carry out adequate dilutions with 1% (v/v) hydrochloric acid to obtain a concentration of 0.001% (w/v). Prepare a pyridoxine hydrochloride

CRS solution in the same concentration as the sample solution, using the same solvent. Determine the absorbances of the solutions at 290 nm (**5.2.14**), using 1% (v/v) hydrochloric acid for zero adjustment. Calculate the amount of $C_8H_{11}NO_3.HCl$ in each tablet, from the readings obtained.

DISSOLUTION TEST (**5.1.5**)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 290 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_8H_{11}NO_3.HCl$ dissolved in the medium, comparing the readings obtained with that of the pyridoxine hydrochloride CRS solution, at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_8H_{11}NO_3.HCl$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support and a mixture of acetone, carbon tetrachloride, tetrahydrofuran and 13.5 M ammonia (65:13:13:9) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 40 mg of pyridoxine hydrochloride, homogenize with 10 mL of water for 15 minutes, filter and use the filtrate.

Solution (2): dilute 1 mL of *Solution (1)* to 200 mL with water.

Procedure: develop the chromatogram. Remove the plate and allow it to air dry. Nebulize with 5% (w/v) sodium carbonate decahydrate in a mixture of ethyl alcohol and water (30:70). Dry in air stream and nebulize with 0.1% (w/v) 2-6-dichloroquinone-4-chlorimide in ethyl alcohol. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.5%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 25 mg of pyridoxine hydrochloride and add 50 mL of 0.1 M hydrochloric acid. Heat in water bath for 15 minutes, shaking occasionally. Cool, dilute to 100 mL with 0.1 M hydrochloric acid and filter, discarding the first 20 mL. Dilute 5 mL of the filtrate to 100 mL with 0.1 M hydrochloric acid. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances

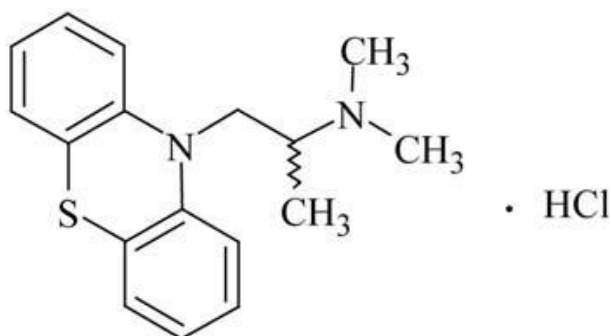
of the resulting solutions at 290 nm **(5.2.14)**, using 0.1 M hydrochloric acid for zero adjustment. Calculate the amount of $\text{C}_8\text{H}_{11}\text{NO}_3\cdot\text{HCl}$ in the tablets from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 430$, at 290 nm.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

PROMETHAZINE HYDROCHLORIDE*Promethazini hydrochloridum*C₁₇H₂₀N₂S.HCl; 320.88

promethazine hydrochloride; 07431

N,N,α-trimethyl-10*H*-phenothiazine-10-ethanamine hydrochloride (1:1)

[58-33-3]

Contains no less than 99.0% and no more than 101.0% of C₁₇H₂₀N₂S.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical-chemical characteristics. White to slightly yellow crystalline powder.

Solubility. Freely soluble in water and ethyl alcohol.

Physicochemical constants.

Melting point (5.2.2): Approximately 222 °C, with decomposition.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of promethazine hydrochloride CRS, prepared identically.

B. Complies with phenothiazine identification reactions (5.3.1.5).

C. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.0 to 5.0. Determine in a freshly prepared aqueous solution at 10.0% (w/v).

Related substances. Carry out the test for *Phenothiazine related substances* (5.3.1.6) using *Mobile phase B* and apply separately to the plate 10 µL of each one of the three solutions, freshly prepared, described next.

Solution (1): 2.0% (w/v) sample solution in a mixture of diethylamine and methyl alcohol (5:95).

Solution (2): 0.02% (w/v) isopromethazine hydrochloride CRS in the same solvent.

Solution (3): 0.01% (w/v) promethazine hydrochloride CRS in the same solvent.

Develop the chromatogram. Remove the plate and allow to air dry. No isopromethazine stain in the chromatogram obtained with *Solution (1)* is more intense than those obtained with *Solution (2)* (1%). No other secondary stain obtained with *Solution (1)* is more intense than those obtained with *Solution (3)* (0.5%).

Loss on drying (5.2.9.1). Determine in 1.0 g of sample. Dry in oven at 100 °C to 105 °C, until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh 0.25 g of the sample and dissolve in a mixture of 5 mL of 0.01 M hydrochloric acid and 50 mL of ethyl alcohol. Titrate with 0.1 M sodium hydroxide VS, determining the volume spent between the two inflection points potentiometrically. Each mL of 0.1 M sodium hydroxide VS corresponds to 32.088 mg of $C_{17}H_{20}N_2S.HCl$.

B. Accurately weigh about 700 mg of the sample and dissolve in a mixture of 75 mL of glacial acetic acid and 10 mL of mercury acetate RS solution. Add one drop of methylrosanilinium chloride TS and titrate with 0.1 M perchloric acid VS until there is a greenish-blue color. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 32.090 mg of $C_{17}H_{20}N_2S.HCl$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiemetic, antihistamine.

PROMETHAZINE HYDROCHLORIDE TABLETS

Contains no less than 95.0% and no more than 110.0% of the declared amount of $C_{17}H_{20}N_2.HCl$. The tablets must be coated.

IDENTIFICATION

A. Weigh and pulverize the tablets. Weigh an amount of powder equivalent to 40 mg of promethazine hydrochloride, add 10 mL of water and 2 mL of *M* sodium hydroxide, shake and extract with 15 mL of ethyl ether. Wash the ethereal extract with 5 mL of water, dry with sodium sulfate anhydrous, evaporate to dryness and dissolve the residue in 0.4 mL of chloroform. In the infrared absorption spectrum (**5.2.14**), there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of promethazine hydrochloride CRS, prepared identically.

B. Transfer 5 mL of sulfuric acid to the amount of tablet powder with 5 mg of promethazine hydrochloride. Allow to stand for five minutes. A red color develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 *M* hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium, filter immediately and dilute, if necessary, with 0.1 *M* hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 249 nm, using the same solvent for zero adjustment. Calculate the amount of $C_{17}H_{20}N_2S.HCl$ dissolved in the medium, comparing the readings obtained with that of promethazine hydrochloride CRS solution, prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{17}H_{20}N_2S.HCl$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described on *Study of impurities related to phenothiazines by thin layer chromatography (5.3.1.6)*, using *Mobile phase B* and applying to the plate, separately, 20 μL

of each one of the following solutions, prepared immediately before use. The operation under nitrogen atmosphere is not necessary.

Solution (1): extract an amount of powder from tablets equivalent to 0.1 g of promethazine hydrochloride with 10 mL of a mixture of diethylamine and methyl alcohol (5:95) and filter.

Solution (2): 0.01% (w/v) isopromethazine hydrochloride CRS solution in a mixture of diethylamine and methyl alcohol (5:95).

Solution (3): dilute one volume of *Solution (1)* to 200 volumes with a mixture of diethylamine and methyl alcohol (5:95).

Develop the chromatogram. Remove the plate and allow it to air dry. No stain corresponding to isopromethazine in the chromatogram obtained with *Solution (1)* is more intense than any other obtained with *Solution (2)* (1%). No other secondary stain is more intense than the stain obtained in the chromatogram with *Solution (3)* (0.5%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Conduct the assay protected from light. Pulverize 20 tablets. Weigh an amount of powder equivalent to 50 mg of promethazine hydrochloride, add 10 mL of 2 M hydrochloric acid, 200 mL of purified water, shake for 15 minutes, complete the volume to 500 mL with purified water and centrifuge 50 mL of the mixture. Add to 5 mL of the clear and light supernatant 10 mL of 0.1 M hydrochloric acid and complete the volume to 100 mL with purified water. Prepare a promethazine hydrochloride CRS solution in the same concentration, in 0.01 M hydrochloric acid. Measure the absorbances **(5.2.14)** of the resulting solutions at 249 nm, using 0.01 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{17}H_{20}N_2S.HCl$ in the tablets from the readings obtained with the standard and sample solutions.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

PROMETHAZINE HYDROCHLORIDE INJECTION SOLUTION

Contains no less than 95.0% and no more than 105.0% of the declared amount of $C_{17}H_{20}N_2 \cdot HCl$.

IDENTIFICATION

The test B can be omitted if the tests A. and C. are carried out.

A. Measure the volume of the injection solution equivalent to 25 mg of promethazine hydrochloride and complete to 50 mL with 0.5 M sulfuric acid. Dilute 5 mL to 100 mL with the same solvent. In the ultraviolet absorption spectrum (**5.2.14**), there are maximum and minimum points at the same wavelengths as the similar promethazine hydrochloride CRS solution.

B. Transfer, slowly, 2 mL of sulfuric acid to the volume of the solution with 5 mg of promethazine hydrochloride and allow to stand for five minutes. A red color is produced.

C. Complies with chloride ion reactions (**5.3.1.1**).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.0 to 6.0.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of diethylamine, hexane and acetone (15:45:50) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dilute a volume of injection solution with a mixture of diethylamine and methyl alcohol (5:95) until obtaining a 1.0% (v/v) promethazine hydrochloride solution.

Solution (2): dilute 1 mL of *Solution (1)* to 200 mL with the mixture of diethylamine and methyl alcohol (5:95).

Solution (3): prepare a 0.01% (v/v) isopromethazine hydrochloride CRS solution with the mixture of diethylamine and methyl alcohol (5:95).

Solution (4): prepare a 0.025% (v/v) promethazine sulfoxide CRS solution with the mixture of diethylamine and methyl alcohol (5:95).

Develop the chromatogram. Remove the plate and allow to air dry. Nebulize with 20% (v/v) perchloric acid. Heat the plate at 100 °C for five minutes. In the chromatogram obtained with *Solution (1)*, any stain corresponding to isopromethazine is not more intense than the main stain in the chromatogram obtained with *Solution (3)* (1.0%). Any stain corresponding to promethazine sulfoxide is not more intense than the stain in the chromatogram obtained with *Solution (4)* (2.5%), and any other secondary stain is not more intense than the stain in the chromatogram obtained with *Solution (2)* (0.5%). Disregard any stain remaining in the application line.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 5.0 EU/mg of promethazine hydrochloride.

ASSAY

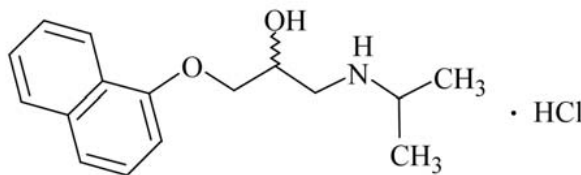
Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*, protecting from light. Transfer a volume of the injection solution equivalent to 25 mg of promethazine hydrochloride to a 100 mL volumetric flask and complete the volume with 0.01 M hydrochloric acid. Dilute 10 mL to 100 mL with 0.01 M hydrochloric acid and, then, dilute 10 mL to 50 mL with the same solvent, obtaining a concentration of 0.0005% (w/v). Prepare a standard solution in the same conditions. Measure the absorbances of the solution at 249 nm, using 0.01 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{17}H_{20}N_2S.HCl$ in the injection solution from the readings obtained.

PACKAGING AND STORAGE

In type I glass container, protected from light.

LABELING

Comply with current legislation.

PROPRANOLOL HYDROCHLORIDE*Propranololi hydrochloridum*C₁₆H₂₁NO₂.HCl; 295.80

propranolol hydrochloride; 07482

1-[(1-methylethyl)amino]-3-(1-naphthalenyloxy)-2-propanol hydrochloride (1:1)

[318-98-9]

Contains no less than 98.0% and no more than 101.5% of C₁₆H₂₁NO₂.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white powder, of crystalline or amorphous aspect.

Solubility. Soluble in water and ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 163 °C to 166 °C.

Specific optical rotation (5.2.8): -1.0 to +1.0. Determine in aqueous solution at 4.0% (w/v) of the desiccated substance.

IDENTIFICATION

The identification test A. can be omitted if tests B., C. and D. are carried out. The identification tests B. and C. can be omitted if tests A. and D. are carried out.

A. In the infrared absorption spectrum **(5.2.14)** of the sample, previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of propranolol hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.004% (w/v) solution in methyl alcohol, there are maximum absorption points at 290 nm, 306 nm and 319 nm. The absorbances are of approximately 0.84, 0.50 and 0.30, respectively.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF254 as support and a mixture of methyl alcohol and ammonia RS (99:1) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in methyl alcohol.

Solution (2): 10 mg/mL propranolol hydrochloride CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Nebulize the plate with a mixture of anisaldehyde, glacial acetic acid, methyl alcohol and sulfuric acid (0.5:10:85:5). Dry between 100 °C and 105 °C. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.2%).

D. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 5.0 to 6.0. Determine in aqueous solution at 1.0% (w/v).

Acidity and alkalinity. Weigh 0.2 g of the sample, dissolve in carbon dioxide-free water and complete to 20 mL with the same solvent. Add 0.2 mL of methyl red TS and 0.2 mL of 0.01 M hydrochloric acid. The solution is red. Add 0.4 mL of 0.01 M sodium hydroxide. The solution is yellow.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF254 as support and a mixture of toluene and methyl alcohol (90:10) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): 100 mg/mL sample solution in methyl alcohol.

Solution (2): 0.2 mg/mL sample solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Nebulize the plate with a mixture of anisaldehyde, glacial acetic acid, methyl alcohol and sulfuric acid (0.5:10:85:5). Dry between 100 °C and 105 °C. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.2%).

Heavy metals (5.3.2.3). No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C, for four hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.5 g of the sample and dissolve in a mixture of 50 mL of glacial acetic acid and 10 mL of mercury acetate RS. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically or using methylrosanilinium chloride TS as indicator. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 29.580 mg of $C_{16}H_{21}NO_2.HCl$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 290 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: weigh 0.5 g of sodium lauryl sulfate and dissolve in 18 mL of 0.15 M phosphoric acid, add 90 mL of acetonitrile, 90 mL of methyl alcohol and dilute with water to 250 mL.

Sample solution: accurately weigh 50 mg of the sample, transfer to a 50 mL volumetric flask, dissolve with 45 mL of methyl alcohol and leave in ultrasonic bath for five minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL to a 25 mL volumetric flask, complete the volume with methyl alcohol and homogenize.

Standard solution: accurately weigh 50 mg of propranolol hydrochloride CRS, transfer to a 50 mL volumetric flask, dissolve with methyl alcohol and complete the volume with the same solvent, to obtain a 1 mg/mL solution. Transfer 5 mL to a 25 mL volumetric flask, complete the volume with methyl alcohol and homogenize.

Resolution solution: prepare a 0.25 mg/mL procainamide hydrochloride solution in methyl alcohol. Transfer 5 mL of this solution and 5 mL of the *Standard solution* to a 25 mL volumetric flask. Complete the volume with methyl alcohol and homogenize.

Inject 20 μ L replicates of the *Resolution solution*. The resolution between the peaks corresponding to procainamide and to propranolol hydrochloride is no less than 2.0. The tailing factor corresponding to propranolol hydrochloride is no more than 3.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{16}H_{21}NO_2.HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-hypertensive, anti-arrhythmic, antianginal.

PROPRANOLOL HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{16}H_{21}NO_2 \cdot HCl$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Weigh an amount of powder equivalent to 0.1 g of propranolol hydrochloride, suspend in water and filter. Transfer the filtrate to a separation funnel, alkalize with *M* sodium hydroxide and extract with three volumes of 10 mL of ethyl ether. Wash the combined extracts with water until neutralizing. Filter over sodium sulfate anhydrous, evaporate the filtrate and dry the residue at reduced pressure at 50 °C for one hour. In the infrared absorption spectrum (5.2.14) of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of the residue obtained after identical treatment carried out with 0.1 g of propranolol hydrochloride CRS.

B. The melting point of the residue obtained on test **A.** of *Identification* is of approximately 94 °C (5.2.2).

C. Weigh and pulverize 20 tablets. Accurately weigh the equivalent to 20 mg of propranolol hydrochloride, transfer to a 50 mL volumetric flask using 15 mL of water and shake mechanically for 10 minutes. Add 25 mL of methyl alcohol and shake for 10 minutes, complete the volume with methyl alcohol, homogenize and filter. Use the solution at 0.004% (w/v) to proceed according to test **B.** of *Identification* in the *Propranolol hydrochloride* monograph.

D. Proceed as described in *Related substances*. The main stain obtained in the chromatogram with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (3)*.

E. Suspend in water an amount of powder from the tablets equivalent to 0.1 g of propranolol hydrochloride. Shake and filter in adequate filter paper. The filtrate complies with chloride ion reactions (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). No more than 30 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Weigh, individually, and transfer each tablet to a 100 mL volumetric flask, add 5 mL of 1% (v/v) hydrochloric acid and shake until the tablet disintegrates. Add 70 mL of methyl alcohol and submit to ultrasonic bath for one minute. Complete the volume with methyl alcohol, homogenize and filter in adequate filter paper, disregarding the first milliliters.

Dilute the filtrate to a concentration of 0.004% (w/v). Prepare a 0.004% (w/v) methanolic solution of propranolol hydrochloride CRS and measure the absorbances of the resulting solutions at 290 nm **(5.2.14)**, using methyl alcohol for zero adjustment. Calculate the individual amount of $C_{16}H_{21}NO_2.HCl$ in the tablets from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 1% (v/v) hydrochloric acid, 1000 mL.

Apparatus: basket, 100 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, with 1% (v/v) hydrochloric acid, until adequate concentration. Measure the absorbances at 289 nm **(5.2.14)**, using the same solvent for zero adjustment. Calculate the amount of $C_{16}H_{21}NO_2.HCl$ dissolved in the medium, comparing the readings obtained with that of the propranolol hydrochloride CRS solution at the concentration of 0.004% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{16}H_{21}NO_2.HCl$ dissolve in 30 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and a mixture of toluene, methyl alcohol, toluene and concentrated ammonia (80:20:1) as mobile phase. Apply, separately, to the plate respectively 100 µL, 20 µL and 100 µL of each solution described below.

Solution (1): 1% (w/v) propranolol hydrochloride CRS solution in methyl alcohol.

Solution (2): 0.02% (w/v) propranolol hydrochloride CRS solution in methyl alcohol.

Solution (3): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 50 mg of propranolol hydrochloride, transfer to a 5 mL volumetric flask, complete with methyl alcohol, homogenize and filter, obtaining a 1% (w/v) solution.

Develop the chromatogram. Remove the plate, allow to air dry, examine under ultraviolet light (254 nm) and mark the stains. Nebulize with a solution with of anisaldehyde, glacial acetic acid, methyl alcohol and sulfuric acid (0.5:10:85:5). Dry between 100 °C and 105 °C. Any secondary stain obtained in the chromatogram with *Solution (3)* is not greater or more intense than the stain obtained with *Solution (2)*.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 20 mg of propranolol hydrochloride, transfer to a 100 mL volumetric flask using 20 mL of water and shake mechanically for 10 minutes. Add 50 mL of methyl alcohol and shake for 10 minutes, complete the volume with methyl alcohol, homogenize and filter. Transfer, quantitatively, 10 mL of the filtrate to a 50 mL volumetric flask, completing the volume with methyl alcohol. Prepare a 0.004% (w/v) solution of propranolol hydrochloride CRS in methyl alcohol and measure the absorbances of the solutions at 290 nm, using methyl alcohol as blank. Calculate the amount of $C_{16}H_{21}NO_2.HCl$ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Proceed as described in method **B.** of Assay on the *Propranolol hydrochloride* monograph. Prepare the sample solution as described below.

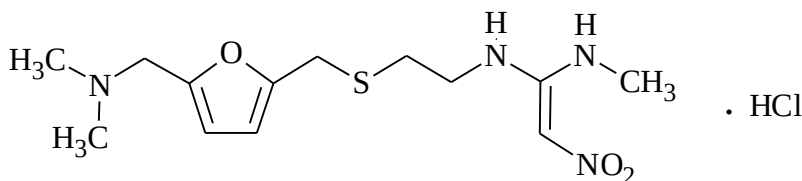
Sample solution: weigh and pulverize no less than 20 tablets. Accurately weigh an amount of powder equivalent to 50 mg of propranolol hydrochloride, transfer to a 50 mL volumetric flask using 40 mL of methyl alcohol, shake and leave in ultrasonic bath for five minutes. Complete the volume with methyl alcohol, homogenize and filter. Transfer 5 mL of the previous solution to a 25 mL volumetric flask, complete the volume with methyl alcohol, homogenize and filter in 0.45 µm membrane.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

RANITIDINE HYDROCHLORIDE*Ranitidini hydrochloridum*C₁₃H₂₂N₄O₃S.HCl; 350.86

ranitidine hydrochloride; 07639

N'-[2-[[[5-[(dimethylamino)methyl]-2-furyl]methyl]thio]ethyl]-*N*-methyl-2-nitro-1,1-ethenediamine hydrochloride (1:1)

[66357-59-3]

Contains no less than 98.5% and no more than 101.0% of C₁₃H₂₂N₄O₃S.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to pale yellow crystalline powder, sensitive to humidity. Presents polymorphism.

Solubility. Freely soluble in water and methyl alcohol, moderately soluble in ethyl alcohol. Freely soluble in acetic acid.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, previously desiccated at 60 °C under reduced pressure, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ranitidine hydrochloride CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in distilled water, there are maximum points at 229 nm and 315 nm. The ratio between the absorbance values measured at 229 nm and at 315 nm is comprised between 1.01 and 1.07.

C. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 4.5 to 6.0. Determine in 1.0% (w/v) solution in carbon dioxide-free water.

Heavy metals (5.3.2.3). Use *Method IV*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of sample. Dry in oven at 60 °C, under reduced pressure, for three hours. No more than 0.75%

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh about 0.28 g of the sample and dissolve in 35 mL of water. Titrate with 0.1 *M* sodium hydroxide VS, determining the endpoint potentiometrically. Each mL of 0.1 *M* sodium hydroxide VS is equivalent to 35.087 mg of $C_{13}H_{22}N_4O_3S.HCl$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh 50 mg of the sample, transfer to a 100 mL volumetric flask using 40 mL of water, shake and complete the volume with the same solvent. Dilute, successively, in water until concentration of 0.00125% (w/v). Prepare a standard solution in the same conditions using a sufficient amount of ranitidine hydrochloride CRS. Measure the absorbances of the resulting solutions at 314 nm, using water for zero adjustment. Calculate the amount of $C_{13}H_{22}N_4O_3S.HCl$ in the sample, from the readings obtained.

PACKAGING AND STORAGE

In hermetic containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

H₂ receptor antagonist.

RANITIDINE HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{13}H_{22}N_4O_3S$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from *Assay*, there are maximum absorption points at 229 nm and 315 nm, identical to those observed in the spectrum of the standard solution.

B. The retention time of the main peak obtained with the *Sample solution* obtained in method **B.** from *Assay* corresponds to that of the main peak of the *Standard solution*.

C. Pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.1 g of ranitidine, shake with 2 mL of water and filter. The filtrate complies with chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in water until adequate concentration. Measure the absorbances of the solutions at 314 nm **(5.2.14)**, using the same solvent for zero adjustment. Calculate the amount of $C_{13}H_{22}N_4O_3S.HCl$ dissolved in the medium, comparing the readings obtained with that of the ranitidine hydrochloride CRS solution at the concentration of 0.00125% (w/v), prepared with the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{13}H_{22}N_4O_3S$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. By Ultraviolet absorption spectrophotometry (5.2.14). Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.125 g of ranitidine, transfer to a 250 mL volumetric flask, dissolve with 150 mL of water, shake mechanically for 30 minutes and complete the volume with the same solvent. Filter. Dilute successively, until concentration of 0.00125% (w/v). Prepare the standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 314 nm, using water for zero adjustment. Calculate the amount of $C_{13}H_{22}N_4O_3S$ in the tablets from the readings obtained.

B. By High performance liquid chromatography (5.2.17.4). Use a chromatograph equipped with ultraviolet detector at 275 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1.7 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.1 M ammonium acetate (85:15).

Sample solution: Pulverize 20 tablets. Accurately weigh an amount of powder, transfer to an adequate volumetric flask using *Mobile phase*, shake, complete the volume with the same solvent and filter. Dilute the filtrate quantitatively with the *Mobile phase* until obtaining a solution with concentration similar to that of the *Standard solution*.

Standard solution: dissolve an accurately weighed amount of ranitidine hydrochloride CRS in *Mobile phase*, to obtain a 0.112 mg/mL solution, equivalent to 0.1 mg/mL of ranitidine.

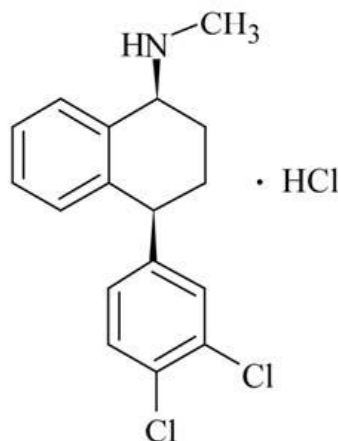
Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the area under the peaks. Calculate the amount of $C_{13}H_{22}N_4O_3S$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

SERTRALINE HYDROCHLORIDE*Sertralini hydrochloridum*C₁₇H₁₇Cl₂N.HCl; 342.69

sertraline hydrochloride; 07964

(1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride (1:1)

[79559-97-0]

Contains no less than 97.0% and no more than 102.0% of C₁₇H₁₇Cl₂N.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder. Presents polymorphism.

Solubility. Slightly soluble in water and in isopropyl alcohol. Moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 243 °C to 245 °C.

Specific optical rotation (5.2.8): +37.0 to +42.0, in relation to the desiccated substance. Determine in solution at 2.0% (w/v) in methyl alcohol.

IDENTIFICATION

The identification test A. can be omitted if tests B., C. and D. are carried out. The identification tests B. and C. can be omitted if tests A. and D. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of sertraline hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 400 nm of the sample solution obtained in method **B.** from Assay, there are maximum points at 266 nm, 274 nm and 282 nm, identical to those observed in the spectrum of the standard solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **C.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

D. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Heavy metals (5.3.2.3). Use *Method I*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 2.0 g of the sample, in oven at 105 °C, for two hours. No more than 1.0%.

Residue by incineration (5.2.10). No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.4 g of sample, transfer to a 250 mL Erlenmeyer flask and dissolve with 80 mL of glacial acetic acid. Add 10 mL of mercury acetate RS. Titrate with 0.1 M perchloric acid VS, using methylrosanilinium chloride TS as indicator, until the color changes to blueish green. Each mL of 0.1 M perchloric acid VS is equivalent to 34.269 mg of $C_{17}H_{17}Cl_2N.HCl$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh 0.5 g of the sample and transfer to a 200 mL volumetric flask. Transfer 100 mL of methyl alcohol and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Dilute in methyl alcohol until concentration of 0.025% (w/v). Prepare a standard solution in the same conditions using a sufficient amount of sertraline hydrochloride CRS. Measure the absorbances of the resulting solutions at 274 nm, using methyl alcohol for zero adjustment. Calculate the amount of $C_{17}H_{17}Cl_2N.HCl$ in the sample from the readings obtained.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 125 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at temperature of 30 °C; *Mobile phase* flow of 1.2 mL/minute.

Phosphate buffer pH 3.0: weigh 2.27 g of monobasic sodium phosphate, dissolve in 950 mL of water, adjust pH to 3.0 ± 0.1 with phosphoric acid and complete the volume to 1000 mL with water.

Mobile phase: mixture of acetonitrile, methyl alcohol and *Phosphate buffer pH 3.0* (30:60:10).

Sample solution: accurately weigh 50 mg of the sample, transfer to a 100 mL volumetric flask using 50 mL of methyl alcohol and leave in ultrasonic bath for 15 minutes. Complete the volume with methyl alcohol to obtain a 0.5 mg/mL solution.

Standard solution: dissolve an accurately weighed amount of sertraline hydrochloride CRS in methyl alcohol and dilute with the same solvent to obtain a 0.5 mg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The tailing factor is no more than 2.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₇H₁₇Cl₂N. HCl in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antidepressant.

SERTRALINE HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{17}H_{17}Cl_2N$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from Assay, there are maximum absorption points at 266 nm, 274 nm and 282 nm, identical to those observed in the spectrum of the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 100 mL volumetric flask and dissolve in 60 mL of methyl alcohol. Leave in ultrasonic bath for 20 minutes. After the full tablet disintegration, complete the volume with methyl alcohol, homogenize and filter. Carry out successive dilutions until an approximate concentration of 0.02% (w/v) of sertraline hydrochloride. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 274 nm **(5.2.14)**, using methyl alcohol for zero adjustment. Calculate the amount of $C_{17}H_{17}Cl_2N$ in each tablet from the responses obtained for the standard and sample solutions.

DISSOLUTION TEST (5.1.5)

Dissolution medium: sodium acetate buffer pH 4.5; 900 mL.

Apparatus: paddles; 75 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and filter. Measure the absorbances at 274 nm **(5.2.14)**, using the same solvent for zero adjustment. Calculate the amount of $C_{17}H_{17}Cl_2N$ dissolved in the medium, comparing the readings obtained with that of the sertraline hydrochloride CRS solution at the concentration of 0.005% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{17}H_{17}Cl_2N$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.5 g of sertraline hydrochloride and transfer to a 200 mL volumetric flask. Proceed as described on method **B.** of Assay from the *Sertraline hydrochloride* monograph, as of “Add 100 mL of methyl alcohol and leave in ultrasonic bath...”.

B. Proceed as described in method **C.** of Assay on the *Sertraline hydrochloride* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 50 mg of sertraline hydrochloride, transfer to a 100 mL volumetric flask using 50 mL of methyl alcohol and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, homogenize and filter.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{17}H_{17}Cl_2N$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers protected from light.

LABELING

Comply with current legislation.

SIBUTRAMINE HYDROCHLORIDE MONOHYDRATE CAPSULES

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{17}H_{26}ClN.HCl.H_2O$.

IDENTIFICATION

A. Filter part of the first solution of the sample obtained in *Assay*. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of the 0.001% (w/v) diluted sibutramine hydrochloride solution in methyl alcohol, displays maximum absorption at 223 nm, identical to the one observed in the spectrum of a similar sibutramine hydrochloride CRS solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to that from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 500 mL.

Apparatus: basket, 75 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter immediately and proceed as described in *Assay*, with the following changes.

Sample solution: after the test, take an aliquot from the dissolution medium and filter immediately.

Standard solution: accurately weigh 20 mg of sibutramine hydrochloride CRS, transfer to a 100 mL volumetric flask and complete the volume with methyl alcohol. Dilute, successively, until concentrations of 20 µg/mL (for capsules with concentration of 10 mg) or 30 µg/mL (for capsules with concentration of 15 mg) with the dissolution medium.

Inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{17}H_{29}Cl_2NO$ dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{17}H_{29}Cl_2NO$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 223 nm; column with 250 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 2 mL/minute.

Mobile phase: mixture of methyl alcohol, water and triethylamine (80:20:0.5). Adjust the mixture pH to 5.65 with phosphoric acid.

Standard solution: prepare a sibutramine hydrochloride CRS solution at 30 µg/mL in methyl alcohol.

Sample solution: weigh 20 capsules, remove the content and weigh them again. Homogenize the content of the capsules. Accurately weigh an amount of powder equivalent to 20 mg of sibutramine hydrochloride to a 100 mL volumetric flask and add 50 mL of methyl alcohol. Shake for 30 minutes, take to ultrasonic bath for another 20 minutes and complete the volume with the same solvent. Centrifuge a portion of the resulting solution for five minutes. Transfer 1.5 mL of the supernatant to a 10 mL volumetric flask and complete the volume with the same solvent.

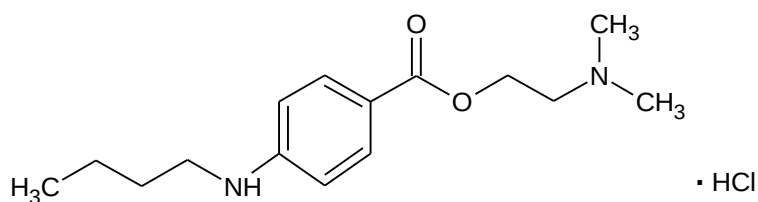
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₇H₂₉Cl₂NO in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with the current legislation.

TETRACAINE HYDROCHLORIDE*Tetracaini hydrochloridum* $C_{15}H_{24}N_2O_2 \cdot HCl$; 300.82

tetracaine hydrochloride; 08463

2-dimethylamino-ethyl 4-butylamino-benzoate hydrochloride

[136-47-0]

Contains no less than 99.0% and no more than 101.0% of $C_{15}H_{24}N_2O_2 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical-chemical characteristics. White or off-white crystalline powder. Hygroscopic.

Solubility. Freely soluble in water, soluble in 96% (v/v) ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): melts around 148 °C. Other two crystalline forms may appear, with melting point of 134 °C and 139 °C. The mixture of these forms melts between 134 °C and 147 °C.

IDENTIFICATION

The identification test B. can be omitted if tests A., C. and D. are carried out. The identification test A. can be omitted if tests B., C. and D. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of tetracaine hydrochloride CRS, prepared identically.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh, accurately, about 50 mg of tetracaine hydrochloride and dissolve in water. Complete the volume to 250 mL with the same solvent. Transfer 5.0 mL of this solution to a 100.0 mL volumetric flask, add 2 mL of *Phosphate buffer pH 6.0*, complete the volume with water and homogenize. Carry out the same procedure to prepare the *Standard solution*. The absorbances at 310 nm, calculated in the desiccated samples, do not differ more than 2.0% from each other.

C. Dissolve 1.0 g of tetracaine hydrochloride in 10 mL of water and add 1 mL of *ammonium thiocyanate RS*. A white crystalline precipitate is formed. Recrystallize the precipitate and dry at 80 °C for two hours. The solid obtained melts around 131 °C.

D. The solution of 100 mg of tetracaine hydrochloride in 5 mL of water complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. The tetracaine hydrochloride preparation at 10% (w/v) in carbon dioxide-free water is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 4.5 to 6.5. Determine in 1% (w/v) solution in carbon dioxide-free water.

Related substances. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel GF as support and mixture of chloroform, methyl alcohol and isopropylamine (98:7:2) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Test solution: 50 mg/mL aqueous tetracaine hydrochloride solution.

Standard solution: 0.2 mg/mL methanolic 4-(butylamino)benzoic acid solution.

Develop the chromatogram for three quarters of the plate height. Remove the plate, dry under a hot air stream and examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with the *Test solution*, different from the main stain, is not more intense than the main stain obtained with the *Standard solution* (0.4%) and the sum of the intensities of all secondary stains present is no more than 0.8%.

Heavy metals (5.3.2.3). Use tetracaine hydrochloride solution at 10% (w/v) in carbon dioxide-free water. Proceed as described in *Limit test for heavy metals – Method I* using *Standard lead solution* (1 ppm Pb). No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C until constant weight. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 250 mg of tetracaine hydrochloride in 50 mL of 96% (v/v) ethyl alcohol and add 5.0 mL of 0.01 M hydrochloric acid. Place in ultrasonic bath for about two minutes. Titrate with 0.1 M sodium hydroxide. Determine the endpoint potentiometrically between the two inflection points. Carry out the blank test and make corrections, if necessary. Each mL of 0.1 M sodium hydroxide is equivalent to 30.08 mg of tetracaine hydrochloride.

PACKAGING AND STORAGE

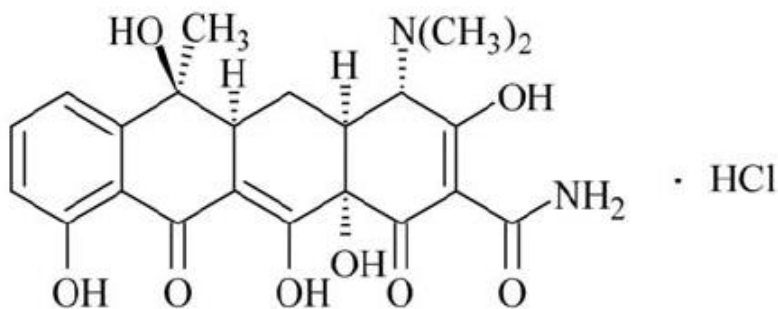
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Local anesthetic.

TETRACYCLINE HYDROCHLORIDE**Tetracyclini hydrochloridum**C₂₂H₂₄N₂O₈·HCl; 480.90

tetracycline hydrochloride; 08465

(4S,4aS,5aS,6S,12aS)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide hydrochloride (1:1)
[64-75-5]

Contains no less than 950 µg and no more than 1020 µg of C₂₂H₂₄N₂O₈·HCl per milligram, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow and slightly hygroscopic crystalline powder.

Solubility. Soluble in water and slightly soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): -240 to -255. Determine in solution at 1.0% (w/v) in 0.1 M hydrochloric acid.

IDENTIFICATION

The identification tests B. and C. can be omitted if tests A. and D. are carried out. The identification test A. can be omitted if tests B., C. and D. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the non-desiccated sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of tetracycline hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.002% (w/v) solution in 0.25 M sodium hydroxide, displays a maximum point at 380 nm. The absorbance at 380 nm, in relation to the desiccated substance, is 96% to 104% of the absorbance obtained with the tetracycline hydrochloride CRS solution prepared in the same conditions. The determination must be made six minutes after the final solution preparation.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **C.** of Assay, corresponds to the one from the main peak of the *Standard solution*.

D. Complies with chloride ion reactions (**5.3.1.1**).

PURITY TESTS

pH (5.2.19). 1.8 to 2.8. Determine in 1% (w/v) solution in carbon dioxide-free water.

Limit of 4-epianhydrotetracycline hydrochloride. Proceed as described on method **C.** from Assay. Prepare solutions as described below.

Solution (1): use the *Sample solution* described on method **C.** of Assay.

Solution (2): 2 µg/mL 4-epianhydrotetracycline hydrochloride CRS solution in *Mobile phase*.

Inject, separately, 20 µL of *Solutions (1) and (2)*, record the chromatograms and measure the areas under the peaks. The area of any peak corresponding to 4-epianhydrotetracycline in the chromatogram obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (2)*. No more than 2.0%.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.005% (50 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 60 °C, under reduced pressure of no more than 5 mmHg, for three hours, until constant weight. No more than 2.0%.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3.1)*, by the agar diffusion method.

Microorganism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 1 for microorganism maintenance; sterile saline solution, for inoculum standardization; and culture medium number 1, for base layer and for inoculum preparation.

Sample solution: accurately weigh 20 mg of the sample and transfer to a 100 mL volumetric flask using 70 mL of 0.01 M hydrochloric acid. Shake and complete the solution volume with the same solvent. Dilute to obtain concentrations of 2 µg/mL, 4 µg/mL and 8 µg/mL, using sterile water.

Standard solution: accurately weigh 20 mg of tetracycline hydrochloride CRS and transfer to a 100 mL volumetric flask using 70 mL of 0.01 M hydrochloric acid. Shake and complete the solution volume with the same solvent. Dilute to obtain concentrations of 2 µg/mL, 4 µg/mL and 8 µg/mL, using sterile water.

Procedure: add 20 mL of culture medium number 1 to each plate and wait until it solidifies. Add 5 mL of 2% inoculum and proceed as described in *Microbiological test of antibiotics by agar diffusion (5.5.3.3.1)*, adding 0.2 mL of the freshly prepared solutions to the cylinders.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Prepare a 0.05% (w/v) sample solution in 0.01 M hydrochloric acid. Prepare a standard solution in the same concentration, using the same solvent. Dilute 3 mL of each solution to 100 mL with 0.25 M sodium hydroxide. Homogenize and allow to stand for six minutes. Prepare a blank in parallel diluting 3 mL of 0.01 M hydrochloric acid to 100 mL with 0.25 M sodium hydroxide. Measure the absorbances of the solutions at 380 nm, using blank for zero adjustment. Calculate the amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ in the sample from the readings obtained.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 365 nm; column with 150 mm of length and 4.5 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μm), maintained at room temperature; *Mobile phase* flow of 0.8 mL/minute.

Mobile phase: mixture of 0.01 M oxalic acid, methyl alcohol and acetonitrile (70:20:10).

Sample solution: accurately weigh 50 mg of the sample, transfer to a 100 mL volumetric flask using 70 mL of *Mobile phase*, leave in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Transfer an aliquot equivalent to 5 mL to a 25 mL volumetric flask and complete the volume with the same solvent, obtaining a solution at 0.1 mg/mL.

Sample solution: accurately weigh 25 mg of tetracycline hydrochloride CRS, transfer to a 50 mL volumetric flask using 35 mL of *Mobile phase*, leave in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Transfer an aliquot equivalent to 5 mL to a 25 mL volumetric flask and complete the volume with the same solvent, obtaining a solution at 0.1 mg/mL.

Resolution solution: prepare, accurately, a solution with tetracycline hydrochloride CRS at 100 $\mu g/mL$ and 4-epianhydrotetracycline hydrochloride CRS at 25 $\mu g/mL$ using *Mobile phase* as solvent.

Inject 20 μL of the *Resolution solution*. The resolution between 4-epianhydrotetracycline and tetracycline is no less than 2. Inject 20 μL replicates of the *Standard solution*. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 20 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

TETRACYCLINE HYDROCHLORIDE CAPSULES

Contains no less than 90.0% and no more than 125.0% the declared amount of $C_{22}H_{24}N_2O_8 \cdot HCl$.

IDENTIFICATION

A. Homogenize the content of the capsules and accurately weigh an amount of powder equivalent to 25 mg of tetracycline hydrochloride, transfer 25 mL of methyl alcohol and allow to stand for 20 minutes. Filter and evaporate the filtrate in water bath to residue. In the infrared absorption spectrum **(5.2.14)** of the residue obtained, desiccated in oven at 60 °C under reduced pressure until constant weight and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as those observed in the tetracycline hydrochloride CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **C.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. Transfer to 2 mg of the residue obtained on test **A.** of *Identification* 5 mL of sulfuric acid. A reddish violet color is produced. Adding 2.5 mL of water to this solution turns the color yellow.

D. The residue obtained in test **A.** from *Identification* complies with chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). No more than 45 minutes.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described on method **A.** from *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 60 minutes (90 minutes for 500 mg capsules)

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in water until adequate concentration. Measure the absorbances of the solutions at 276 nm **(5.2.14)**, using water for zero adjustment. Calculate the amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ dissolved in the medium, comparing the readings obtained with that of the tetracycline hydrochloride CRS solution, at the concentration of 0.0015% (w/v), prepared in the same solvent.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{22}H_{24}N_2O_8 \cdot HCl$ dissolve in 60 minutes (90 minutes for 500 mg capsules).

PURITY TESTS

Limit of 4-epianhydrotetracycline hydrochloride. Proceed as described on method **C.** from Assay. Prepare the solutions as described below

Solution (1): use the sample solution described on method **C.** of Assay.

Solution (2): 10 µg/mL 4-epianhydrotetracycline hydrochloride CRS solution in *Mobile phase*.

Inject, separately, 20 µL of *Solutions (1) and (2)*, record the chromatograms and measure the areas under the peaks. The area of any peak corresponding to 4-epianhydrotetracycline in the chromatogram obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (2)*. No more than 3.0%.

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 60 °C, under reduced pressure of no more than 5 mmHg, for three hours and until constant weight. No more than 2.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method.

Microorganism: Staphylococcus epidermidis ATCC 12228.

Culture media: culture medium number 1 for microorganism maintenance; sterile saline solution, for inoculum standardization; and culture medium number 1, for base layer and inoculum preparation.

Sample solution: accurately weigh 20 mg of the sample and transfer to a 100 mL volumetric flask using 70 mL of 0.01 M hydrochloric acid. Shake and complete the solution volume with the same solvent. Dilute to obtain concentrations of 2 µg/mL, 4 µg/mL and 8 µg/mL, using sterile water.

Standard solution: accurately weigh 20 mg of tetracycline hydrochloride CRS and transfer to a 100 mL volumetric flask using 70 mL of 0.01 M hydrochloric acid. Shake and complete the solution volume with the same solvent. Dilute to obtain concentrations of 2 µg/mL, 4 µg/mL and 8 µg/mL, using sterile water.

Procedure: transfer 20 mL of culture medium number 1 to each plate and wait until it solidifies. Transfer 5 mL of 2% inoculum and proceed as described in *Microbiological test of antibiotics by agar diffusion (5.5.3.3)*, adding 0.2 mL of the freshly prepared solutions to the cylinders. Calculate the sample potency, in µg of tetracycline hydrochloride per milligram, from the standard potency and the responses obtained with the *Standard Solution* and the *Sample Solution*.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Prepare a 0.05% (w/v) sample solution in 0.01 M hydrochloric acid. Prepare a standard solution in the same concentration, using the same solvent. Dilute 3 mL of each solution to 100 mL with 0.25 M sodium hydroxide. Homogenize and allow to stand for six minutes. Prepare a blank in parallel diluting 3 mL of 0.01 M hydrochloric acid to 100 mL with 0.25 M sodium hydroxide. Measure the absorbances of the solutions at 380 nm, using blank for zero adjustment. Calculate the amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ in the capsules, in μg per milligram, from the potency of standard and from the readings obtained.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 365 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μm), maintained at room temperature, *Mobile phase* flow of 0.8 mL/minute.

Mobile phase: mixture of 0.01 M oxalic acid, methyl alcohol and acetonitrile (70:20:10).

Sample solution: accurately weigh 50 mg of the sample, transfer to a 100 mL volumetric flask using 70 mL of *Mobile phase*, leave in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Transfer an aliquot equivalent to 5 mL to a 25 mL volumetric flask and complete the volume with the same solvent, obtaining a solution at 0.1 mg/mL.

Sample solution: accurately weigh 25 mg of tetracycline hydrochloride CRS, transfer to a 50 mL volumetric flask using 35 mL of *Mobile phase*, leave in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Transfer an aliquot equivalent to 5 mL to a 25 mL volumetric flask and complete the volume with the same solvent, obtaining a solution at 0.1 mg/mL.

Resolution solution: prepare, accurately, a solution with tetracycline hydrochloride CRS at 100 $\mu g/mL$ and 4-epianhydrotetracycline hydrochloride at 25 $\mu g/mL$ using *Mobile phase* as solvent.

Inject 20 μL of the *Resolution solution*. The resolution between 4-epianhydrotetracycline and tetracycline is no less than 2. Inject 20 μL replicates of the *Standard solution*. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

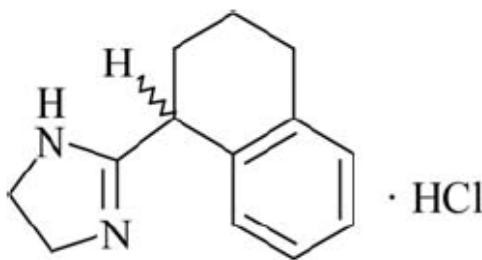
Procedure: inject, separately, 20 μL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{22}H_{24}N_2O_8 \cdot HCl$ in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

TETRYZOLINE HYDROCHLORIDE*Tetryzolini hydrochloridum*C₁₃H₁₆N₂.HCl; 236.74

tetryzoline hydrochloride; 08484

4,5-di-hydro-2-(1,2,3,4-tetrahydro-1-naphthalenyl)- 1*H*-imidazole hydrochloride (1:1)

[522-48-5]

Contains no less than 98.0% and no more than 100.5% of C₁₃H₁₆N₂.HCl, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white crystalline powder.

Solubility. Freely soluble in water and in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 253 °C to 259 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of tetryzoline hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of a 0.025% (w/v) solution in water, there are maximum points at 264 nm and 271 nm, identical to those observed in the spectrum of a similar tetryzoline hydrochloride CRS solution.

C. The 0.5% (w/v) aqueous solution complies with the chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Heavy metals (5.3.2.3). Use *Method I*. Weigh 0.4 g of the sample and dissolve in 23 mL of water and 2 mL of diluted acetic acid. No more than 0.005% (50 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C, for two hours. No more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 0.4 g of sample, transfer to a 250 mL Erlenmeyer flask and dissolve in 60 mL of glacial acetic acid, with heating, if necessary. Add 5 mL of acetic anhydride, 5 mL of mercury acetate RS and 1 drop of quinaldine red TS. Titrate with 0.1 M perchloric acid VS. Carry out the blank test and make the necessary correction. Each mL of 0.1 M perchloric acid VS is equivalent to 23.674 mg of $C_{13}H_{16}N_2 \cdot HCl$.

PACKAGING AND STORAGE

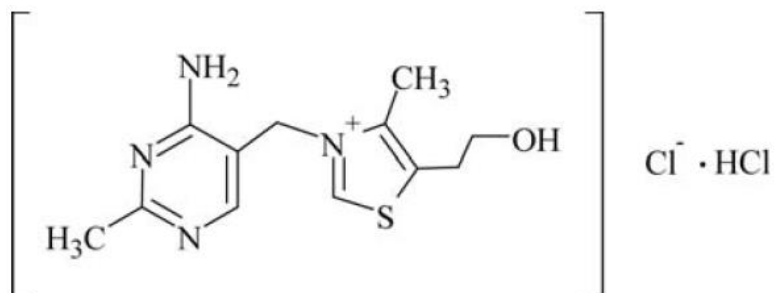
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Adrenergic (nasal).

THIAMINE HYDROCHLORIDE*Thiamini hydrochloridum*C₁₂H₁₇ClN₄OS.HCl; 337.27

thiamine hydrochloride; 08511

3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-5-(2-hydroxyethyl)-4-methyl-thiazolium chloride hydrochloride (1:1:1)

[67-03-8]

Contains no less than 98.0% and no more than 102.0% of C₁₂H₁₇ClN₄OS.HCl, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder or crystals. When exposed to air, quickly absorbs about 4% of water.

Solubility. Freely soluble in water, soluble in glycerol, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated at 105 °C for two hours, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of thiamine hydrochloride CRS, prepared identically.

B. Complies with chloride ion reactions (**5.3.1.1**).

PURITY TESTS

pH (5.2.19). 2.7 to 3.4. Determine in aqueous solution at 1.0% (w/v).

Light absorption. The absorbance of the aqueous solution at 10% (w/v), after filtration in sintered funnel with fine porosity, measured at 400 nm, does not exceed 0.025.

Chromatographic purity. Proceed as described on method **B.** of *Assay*, except for using *Mobile phase* flow of 0.75 mL/minute. Prepare the *Sample solution* as described next.

Sample solution: weigh 10 mg of the sample, transfer to a 10 mL volumetric flask and complete the volume with *Mobile phase*, to obtain a 1.0 mg/mL solution.

Procedure: inject 10 µL of *Sample solution* and record the chromatogram for no less than three times the main peak retention time. Measure the areas under the peaks. The sum of the areas under the secondary peaks is no more than 1.0% of total of areas from all peaks present in the chromatogram. Do not consider peaks related to the solvent.

Nitrate. Transfer 2 mL of sulfuric acid to 2 mL of 2% (w/v) aqueous solution, cool and transfer 2 mL of ferrous sulfate RS. No brown ring is produced in the junction of the two layers.

Sulfates (5.3.2.2). Determine in 0.5 g of the sample. No more than 0.03% (300 ppm).

Heavy metals (5.3.2.3). No more than 0.002% (20 ppm).

Water (5.2.20.1). Determine in 0.4 g of the sample. No more than 5.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.15 g of the sample and dissolve with 5 mL of formic acid anhydrous. Add 65 mL of glacial acetic acid and, with agitation, 10 mL of mercury acetate RS. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 16.864 mg of $C_{12}H_{17}ClN_4OS.HCl$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 150 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 µm to 10 µm), maintained at room temperature; *Mobile phase* flow of 1 mL/minute.

Solution A: sodium 1-octanesulfonate solution at 0.005 M in 1% (v/v) acetic acid.

Solution B: mixture of methyl alcohol and acetonitrile (3:2).

Mobile phase: mixture of *Solution A* and *Solution B* (60:40).

Internal standard solution: transfer 2 mL of methyl benzoate to a 100 mL volumetric flask, complete the volume with methyl alcohol and homogenize.

Sample solution: accurately weigh 0.2 g of the sample, transfer to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 10 mL of the solution prepared

in advance and 5 mL of the *Internal standard solution* to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: dissolve an accurately weighed amount of thiamine hydrochloride CRS in *Mobile phase* to obtain a 1 mg/mL solution. Transfer 20 mL of the solution prepared in advance and 5 mL of the *Internal standard solution* to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

The column efficiency for the peak corresponding to thiamine is no less than 1500 theoretical plates/meter. The tailing factor of the peak corresponding to thiamine is no more than 2.0. The resolution between the peaks corresponding to thiamine and to methyl benzoate is no less than 4.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to thiamine and to methyl benzoate. Calculate the amount of $C_{12}H_{17}ClN_4OS.HCl$ in the sample, from the responses obtained for the thiamine/methyl benzoate ratio with the *Standard solution* and with the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed non-metallic containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vitamin B complex.

THIAMINE HYDROCHLORIDE TABLETS

Contains no less than 92.5% and no more than 107.5% of the declared amount of $C_{12}H_{17}ClN_4OS.HCl$.

IDENTIFICATION

A. The retention time of the main peak obtained with the *Sample solution* obtained in the method from *Assay* corresponds to that of the main peak of the *Standard solution*.

B. Weigh and pulverize the tablets. Weigh an amount of powder equivalent to 10 mg of thiamine hydrochloride, dissolve in 10 mL of water and filter. Separate two portions of 2 mL of the filtrate. Transfer 1.27% (w/v) iodine solution to the first portion of the filtrate. A reddish brown precipitate is formed. Transfer mercury chloride RS to the second portion of the filtrate. A white precipitate that complies with the test for chlorides (**5.3.1.1.**) is produced.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, with *Dissolution medium* until adequate concentration. Measure the absorbances at 246 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_{12}H_{17}ClN_4OS.HCl$ dissolved in the medium, comparing the readings obtained with that of the thiamine hydrochloride CRS solution at the concentration of 0.002% (w/v), prepared with the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{12}H_{17}ClN_4OS.HCl$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 150 mg of thiamine and dissolve, with agitation, using 5 mL of formic acid anhydrous, 65 mL of glacial acetic acid and 10 mL of 6% (w/v) mercury acetate in glacial acetic acid. Titrate with 0.1 M perchloric acid VS. Determine the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS corresponds to 16.860 mg of $C_{12}H_{17}ClN_4OS.HCl$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 246 nm; column with 125 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m); *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: weigh 1 g of sodium heptanesulfonate and dissolve in a mixture of 180 mL of methyl alcohol and 10 mL of triethylamine. Complete the volume to 1000 with water and adjust the pH to 3.2 mL with phosphoric acid.

Standard solution: contains thiamine hydrochloride CRS at 0.06 mg/mL in 0.005 M hydrochloric acid.

Sample solution: for tablets with less than 10 mg of thiamine hydrochloride. Weigh and pulverize the tablets. Accurately weigh an amount equivalent to 6 mg of thiamine hydrochloride and dissolve in 5 mL of 0.1 M hydrochloric acid and 50 mL of water. Shake for 20 minutes, dilute to 100 mL with water and filter.

Sample solution: for tablets with 10 mg or more of thiamine hydrochloride. Weigh and pulverize the tablets. Accurately weigh an amount equivalent to 30 mg of thiamine hydrochloride and dissolve in 25 mL of 0.1 M hydrochloric acid and 250 mL of water. Shake for 20 minutes, dilute to 500 mL with water and filter.

Procedure: inject, separately, 20 μ L of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{12}H_{17}ClN_4OS.HCl$ in the tablets from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

Keep in non-metallic containers protected from light.

LABELING

Comply with current legislation.

THIAMINE HYDROCHLORIDE INJECTION SOLUTION

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{12}H_{17}ClN_4OS.HCl$. Sterile thiamine hydrochloride solution in water for injection.

IDENTIFICATION

The identification test B. can be omitted if tests A. and C. are carried out.

A. Transfer a volume of injection solution equivalent to 0.1 g of thiamine hydrochloride to 10 mL of distilled water and divided in four portions. Make each reaction react, separately, with 0.5 mL of mercuric chloride RS, iodine RS, potassium mercury iodide RS and trinitrophenol RS, producing, respectively, precipitates with white, brownish-red, light yellow and yellow color.

B. Dilute a portion of the injection solution with water for concentration of 10 mg/mL of thiamine hydrochloride. Add to 0.5 mL of this solution 5 mL of 0.5 M sodium hydroxide, then add 0.5 mL of potassium ferricyanide and 5 mL of isobutyl alcohol, shake vigorously for two minutes and allow to stand for 30 minutes. The alcoholic layer must present blue fluorescence, clearer when observed under ultraviolet light. This fluorescence disappears by acidification, returning by alkalinization of the mixture.

C. Complies with chloride ion reactions (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 2.5 to 4.5.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 3.5 EU/mg of thiamine hydrochloride.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: prepare a filtered and degassed mixture of 0.04 M monobasic potassium phosphate and methyl alcohol (55:45).

Internal standard solution: prepare a methylparaben solution in *Mobile phase* with concentration of 0.1 mg/mL.

Sample solution: transfer an equivalent volume of injection solution to obtain a solution with 0.5 mg/mL of thiamine hydrochloride. Transfer 10 mL of the resulting solution and 10 mL of the *Internal standard solution* to a 100 mL volumetric flask, complete the volume with *Mobile phase* and shake.

Standard solution: prepare a thiamine hydrochloride CRS solution in *Mobile phase* with concentration of 0.5 mg/mL. Transfer 10 mL of the resulting solution and 10 mL of the *Internal standard solution* to a 100 mL volumetric flask, complete the volume with *Mobile phase* and shake to obtain a solution with concentration of 50 µg/mL.

Inject 20 µL replicates of the *Standard solution*. The relative retention times are approximately 0.3 for thiamine and 1.0 for methylparaben. The resolution between the peaks of thiamine and methylparaben is no less than 6.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

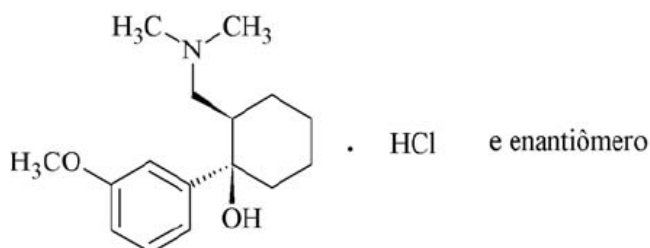
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{12}H_{17}ClN_4OS.HCl$ in each mL of the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELING

Comply with current legislation.

TRAMADOL HYDROCHLORIDE*Tramadoli hydrochloridum*C₁₆H₂₅NO₂.HCl; 299.84

tramadol hydrochloride; 08807

(1*R*,2*R*)-*rel*-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol hydrochloride (1:1)
[36282-47-0]

Contains no less than 99.0% and no more than 101.0% of C₁₆H₂₅NO₂.HCl, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Soluble in water, ethyl alcohol and methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 180 °C to 184 °C.

Specific optical rotation (5.2.8): -0.10 to +0.10. Determine in aqueous solution at 5% (w/v).

IDENTIFICATION

In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, presents maximum absorption only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of tramadol hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), at the range of 250 nm to 300 nm, of the 0.1 mg/mL solution, displays maximum absorption at 270 nm, identical to that observed at the tramadol hydrochloride CRS spectrum.

C. Complies with chloride ion reactions (5.3.1.1).

PURITY TESTS

Aspect of the preparation. The 5.0% (w/v) aqueous preparation is clear (5.2.25) and colorless (5.2.12).

Acidity or alkalinity. Weigh 1 g of the sample, dissolve in 20 mL of water and add 0.2 mL of methyl red TS and 0.2 mL of 0.01 M hydrochloric acid. A red color develops. Add 0.4 mL of 0.01 M sodium hydroxide. A yellow color develops.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, using a chromatograph equipped with an ultraviolet detector at 270 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octylsilane group (3 µm to 10 µm); *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: use a mixture of 0.2% (w/v) trichloroacetic acid and acetonitrile (705:295).

Sample solution: dissolve an accurately weighed amount of the sample in *Mobile phase*, to obtain a solution with 1.5 mg/mL.

Standard solution: dissolve an accurately weighed amount of tramadol hydrochloride CRS in *Mobile phase*, to obtain a solution with 0.003 mg/mL.

Resolution solution: accurately weigh 5 mg of tramadol hydrochloride related substance A CRS ((1*RS*, 2*SR*)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol) and transfer to a 100 mL volumetric flask along with 4 mL of the *Sample solution*. Complete the volume with *Mobile phase*.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. The relative retention time is 1.0 for tramadol hydrochloride (retention time: about five minutes) and approximately 0.85 for tramadol hydrochloride related substance A. The resolution between the peaks of tramadol hydrochloride and of tramadol hydrochloride related substance A is no less than 2.0. In the chromatogram obtained with the *Sample solution*, the areas under the peak of tramadol hydrochloride related substance A is not greater than the area obtained with the main peak of the *Standard solution* (0.2%). Any other impurity recorded in the chromatogram of the *Sample solution* does not have an area greater than 0.5 times the area obtained with the main peak of the *Standard solution* (0.1%).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve 1.0 g of the sample in water. Proceed as described in *Limit test for heavy metals*. No more than 0.002% (20 ppm).

Water (5.2.20.1). Determine in 1.0 g of the sample. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh 0.25 g of the sample and dissolve in 80 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 29.984 mg of C₁₆H₂₅NO₂.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Analgesic.

TRAMADOL HYDROCHLORIDE INJECTION SOLUTION

Contains no less than 95.0% and no more than 105.0% of the declared amount of $C_{16}H_{25}NO_2.HCl$. Sterile solution in water for injection.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)**, at the range of 250 nm to 300 nm of the sample solution obtained in *Assay*, there is maximum absorption at 270 nm, identical to that observed at the standard solution spectrum.

B. Complies with chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.5 to 6.3.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Use the direct inoculation or the membrane filtration method.

Bacterial endotoxins (5.5.2.2). No more than 7.0 EU/mL of tramadol hydrochloride.

ASSAY

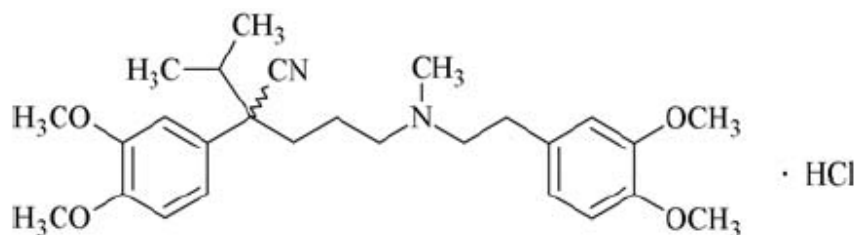
Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer a volume of injection solution equivalent to 50 mg of tramadol hydrochloride to a 100 mL volumetric flask and complete the volume with water. Transfer 10 mL to a 50 mL volumetric flask and complete the volume with water, obtaining a concentration of 0.01% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 270 nm, using water for zero adjustment. Calculate the amount of $C_{16}H_{25}NO_2.HCl$ in the injection solution from the readings obtained.

PACKAGING AND STORAGE

In type I glass containers, in a cool place and protected from light.

LABELING

Comply with current legislation.

VERAPAMIL HYDROCHLORIDE*Verapamili hydrochloridum*

$C_{27}H_{38}N_2O_4 \cdot HCl$; 491.06

verapamil hydrochloride; 09119

α -[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy- α -(1-methylethyl)-benzeneacetonitrile hydrochloride (1:1)
[152-11-4]

Contains no less than 99.0% and no more than 100.5% of $C_{27}H_{38}N_2O_4 \cdot HCl$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Soluble in water and slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 140 °C to 144 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of verapamil hydrochloride CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 210 nm to 340 nm, of the 0.002% (w/v) solution in 0.01 M hydrochloric acid, there are maximum points at 229 nm and 278 nm. The ratio between the absorbance values measured at 278 nm and 229 nm is 0.35 to 0.39.

C. Complies with chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 4.5 to 6.5. Determine in 5.0% (w/v) solution in carbon dioxide-free water.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 278 nm; column with 125 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 µm to 10 µm), maintained at room temperature; *Mobile phase* flow of 0.9 mL/minute.

Acetate buffer: aqueous solution of 0.015 M sodium acetate, with 3.3% (v/v) glacial acetic acid.

Mobile phase: mixture of *Acetate buffer*, acetonitrile and 2-aminoheptane (70:30:0.5).

Solution (1): 1.9 mg/mL sample solution in *Mobile phase*.

Solution (2): 5.6 µg/mL verapamil hydrochloride CRS solution in *Mobile phase*.

Solution (3): 9.4 µg/mL verapamil hydrochloride CRS solution in *Mobile phase*.

Resolution solution: accurately weigh a sufficient amount of verapamil hydrochloride CRS and α-[2-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]ethyl]-3,4-dimethoxy-α-(1-methylethyl)benzeneacetonitrile monohydrochloride (verapamil related compound B) CRS and dissolve in *Mobile phase* obtaining a resolution solution with known concentration of 1.9 and 1.5 mg/mL, respectively.

The relative retention times are about 0.88 for verapamil related compound B and 1.0 for verapamil. The resolution between the peak of verapamil related compound B and verapamil is no less than 1.5. The relative standard deviation for injection replicates is no more than 2.0%.

Procedure: inject, separately, 10 µL of each solution, record the chromatograms for no less than four times the main peak retention time and measure the areas under the peaks. The sum of the areas under the peaks, except the peak of verapamil, obtained with *Solution (1)*, is not greater than the area under the main peak obtained with *Solution (3)* (0.5%). The area of any individual peak obtained with *Solution (1)*, except the main peak, is not greater than the area under the main peak obtained with *Solution (2)* (0.3%).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for two hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.4 g of the sample and dissolve in 40 mL of glacial acetic acid, 5 mL of acetic anhydride and 10 mL of 6% (w/v) mercury acetate in glacial acetic acid. Titrate with 0.1 M perchloric acid VS determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 49.106 mg of C₂₇H₃₈N₂O₄.HCl.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-arrhythmic.

VERAPAMIL HYDROCHLORIDE TABLETS

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{27}H_{38}N_2O_4 \cdot HCl$.

IDENTIFICATION

The identification tests C. and D. can be omitted if tests A. and B. are carried out.

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 25 mg of verapamil hydrochloride, transfer to a separation funnel using 25 mL of water and shake for 30 minutes. Add 1 mL of *M* sodium hydroxide and extract with 25 mL of chloroform, shaking for 10 minutes. Filter in a filter with sodium sulfate anhydrous and evaporate to dryness. Dry at 105 °C for two hours. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of verapamil hydrochloride CRS, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of verapamil hydrochloride, transfer to a beaker using 10 mL of methylene chloride and homogenize. Filter, evaporate the filtrate to dryness and dissolve the residue in 10 mL of water. Transfer to 2 mL of the resulting solution 0.2 mL of 5% (w/v) mercury(II) chloride solution. A white precipitate is produced.

D. Transfer to 2 mL of the solution obtained on test **C.** of *Identification* 0.5 mL of 3 *M* sulfuric acid and 0.2 mL of 1.0% (w/v) potassium permanganate. A violet precipitate is produced and dissolves quickly, producing a pale yellow solution.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Use 900 mL of 0.1 *M* hydrochloric acid as disintegration medium. No more than 30 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 900 mL of 0.1 *M* hydrochloric acid.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, take an aliquot from the dissolution medium, filter and dilute, if necessary, in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances at 278 nm and at 300 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_{27}H_{38}N_2O_4$. HCl dissolved in the medium, through the difference between the measures at 278 nm and at 300 nm, comparing the readings obtained with that of the verapamil hydrochloride CRS solution, at the same concentration, prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{27}H_{38}N_2O_4$.HCl dissolve in 30 minutes.

PURITY TESTS

Chromatographic purity. Proceed as described on method **B.** from Assay. Prepare solutions as described below.

Solution (1): weigh and pulverize the tablets. Accurately weigh a sufficient amount of powder and transfer to a 25 mL volumetric flask to obtain a 1.9 mg/mL solution. Add 15 mL of *Mobile phase* and shake mechanically for 15 minutes. Complete the volume with *Mobile phase*, homogenize and filter.

Solution (2): dissolve an accurately weighed amount of verapamil hydrochloride CRS in *Mobile phase* to obtain a 5.6 µg/mL solution.

Solution (3): dissolve an accurately weighed amount of verapamil hydrochloride CRS in *Mobile phase* to obtain a 9.4 µg/mL solution.

Procedure: inject, separately, 10 µL of each solution. Record the chromatograms and measure the areas under the peaks. The sum of the areas under the peaks obtained with *Solution (1)*, except that of verapamil hydrochloride, is not greater than the area under the peak obtained with *Solution (3)* (0.5%). No peak presents an area greater than that of the peak obtained with *Solution (2)* (0.3%).

Water (5.2.20.1). No more than 4.0%

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 100 mg of verapamil hydrochloride, transfer to a 250 mL volumetric flask and dissolve in 0.01 M hydrochloric acid, with agitation. Complete the volume with the same solvent. Filter. Transfer 10 mL of the filtrate to a 100 mL volumetric flask and complete the volume with 0.01 M hydrochloric acid. Prepare a standard solution in the same conditions. Measure the absorbances of the solutions at 278 nm, using 0.01 M

hydrochloric acid for zero adjustment. Calculate the amount of $C_{27}H_{38}N_2O_4.HCl$ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 278 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μm), maintained at room temperature; *Mobile phase* flow of 0.8 mL/minute.

Acetate buffer: aqueous solution of 0.01 M sodium acetate with 3.3% (v/v) glacial acetic acid.

Mobile phase: mixture of *Acetate buffer*, acetonitrile and diethylamine (65:35:1). Filter and degas.

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 35 mg of verapamil hydrochloride, transfer to a 50 mL volumetric flask and add 30 mL of *Mobile phase*. Shake, mechanically, for 15 minutes. Complete the volume with *Mobile phase*, homogenize and filter, to obtain a 70 $\mu g/mL$ solution.

Standard solution: dissolve an accurately weighed amount of verapamil hydrochloride CRS in *Mobile phase* to obtain a 70 $\mu g/mL$ solution.

Resolution solution: dissolve an amount of verapamil hydrochloride CRS and α -[2-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]ethyl]-3,4-dimethoxy- α -(1-methylethyl)benzeneacetonitrile monohydrochloride (verapamil related compound B) CRS in *Mobile phase* to obtain a solution at 70 $\mu g/mL$ for each compound.

Inject 10 μL replicates of the *Resolution solution*. The relative retention times are about 0.88 for verapamil related compound B and 1.0 for verapamil hydrochloride. The resolution between the peaks of verapamil related compound B and verapamil hydrochloride is no less than 1.5. The standard deviation between injection replicates is no more than 2.0%.

Procedure: inject, separately, 10 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{27}H_{38}N_2O_4.HCl$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

VERAPAMIL HYDROCHLORIDE INJECTION SOLUTION

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{27}H_{38}N_2O_4 \cdot HCl$.

IDENTIFICATION

The identification tests C. and D. can be omitted if tests A. and B. are carried out.

A. Dilute a volume of sample with the equivalent to 10 mg of verapamil hydrochloride in 5 mL of 0.1 M hydrochloric acid, extract with 5 mL of ethyl ether, discard the extract and alkalinize the aqueous phase using potassium carbonate sesquihydrate. Extract with 5 mL of ethyl ether, filter the ethereal phase in sodium sulfate anhydrous and evaporate to dryness. In the infrared absorption spectrum **(5.2.14)** of the sample, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of verapamil hydrochloride CRS prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. Add 0.2 mL of 5% (w/v) mercury(II) chloride to a volume of injection solution with 5 mg of verapamil hydrochloride. A white precipitate is produced.

D. Add to a volume of injection solution with 5 mg of verapamil hydrochloride 0.5 mL of 3 M sulfuric acid and 0.2 mL of 1.0% (w/v) potassium permanganate. A violet precipitate is produced and dissolves quickly, forming an intense pale yellow solution.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test for small volume injection solutions.

pH (5.2.19). 4.0 to 6.5.

PURITY TEST

Related substances. Proceed as described on method **B.** from *Assay*. Prepare the Solutions as described next:

Solution (1): sample solution with 2.5 mg/mL of verapamil hydrochloride.

Solution (2): dissolve an accurately weighed amount of verapamil hydrochloride CRS in *Mobile phase* to obtain a solution with known concentration of 5.6 µg/mL.

Solution (3): dissolve an accurately weighed amount of verapamil hydrochloride CRS in *Mobile phase* to obtain a solution with known concentration of 9.4 µg/mL.

Procedure: inject, separately, 10 µL of *Solution (1)*, 10 µL of *Solution (2)* and 10 µL of *Solution (3)*. Record the chromatograms and measure the areas under the peaks. The sum of the responses from

peaks, except that of verapamil hydrochloride obtained in *Solution (1)*, is not greater than the response from the peak obtained with *Solution (3)* (0.5%); and no peak is greater than the response from the peak obtained with *Solution (2)* (0.3%).

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 16.7 EU/mg of verapamil hydrochloride.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer a volume of solution with 5 mg of verapamil hydrochloride to a 100 mL volumetric flask and dissolve with 0.01 M hydrochloric acid. Prepare a standard solution in the same conditions. Measure the absorbances of the solutions at 278 nm, using 0.01 M hydrochloric acid for zero adjustment. Calculate the amount of $C_{27}H_{38}N_2O_4 \cdot HCl$ in the tablets from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 118$, at 278 nm, in 0.01 M hydrochloric acid.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, using chromatograph equipped with detector at 278 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μm), maintained at room temperature; *Mobile phase* flow of 0.8 mL/minute.

Acetate buffer: aqueous solution of 0.015 M sodium acetate, with 3.3% (v/v) glacial acetic acid.

Mobile phase: prepare a mixture of *Acetate buffer*, acetonitrile and diethylamine (65:35:1). Filter and degas the mixture.

Sample solution: dilute the injection solution, accurately, if necessary, with *Mobile phase*, to obtain a 70 $\mu\text{g/mL}$ solution.

Standard solution: dissolve an accurately weighed amount of verapamil hydrochloride CRS in *Mobile phase* to obtain a known concentration of 70 $\mu\text{g/mL}$.

Resolution solution: dissolve an amount of verapamil hydrochloride CRS and α -[2-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]ethyl]-3,4-dimethoxy- α -(1-methylethyl)benzeneacetonitrile monohydrochloride (verapamil related compound B) CRS in *Mobile phase*, to obtain a solution of known concentration of 70 $\mu\text{g/mL}$ for each compound.

Inject 10 μL of the *Resolution solution* and record the responses from peaks. The relative retention times are approximately 0.88 for verapamil related compound B and 1.0 for verapamil hydrochloride CRS. The resolution between verapamil related compound B and verapamil hydrochloride CRS is no less than 1.5 and the standard deviation between injection replicates is no more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*. Calculate the amount of $C_{27}H_{38}N_2O_4 \cdot HCl$ in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

ALUMINUM CHLOROHYDRATE

Aluminium hydroxydatum chloratum

$\text{Al}_y(\text{OH})_{3y-z}\text{Cl}_z \cdot n\text{H}_2\text{O}$

aluminum hydrochloride; 02454

[1327-41-9]

aluminum chlorohydrate; 09695

[12042-91-0]

Aluminum chlorohydrates are hydrate polymeric compounds, encompassing a range of ratio between aluminum and chlorine atoms from 1.91:1 to 2.10:1. Contains no less than 90.0% and no more than 110.0% of the labeled amount of aluminum chlorohydrate anhydrous $[\text{Al}_y(\text{OH})_{3y-z}\text{Cl}_z]$.

IDENTIFICATION

The 10% (w/v) aqueous sample solution complies with aluminum and chloride ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 3.0 to 5.0. Determine in aqueous solution at 15.0% (w/v).

Aluminum content. Weigh 0.2 g of the sample, transfer to a 150 mL Erlenmeyer flask, add 20 mL of water and 5 mL of hydrochloric acid. Keep in ebullition for five minutes. Allow to cool. Then, add 25 mL of 0.1 M disodium edetate VS and adjust the pH to 4.7 ± 0.1 with ammonium hydroxide or M acetic acid. Add 20 mL of acetic acid-ammonium acetate buffer, 50 mL of ethyl alcohol and 5 mL of dithizone RS. The pH of this solution must be 4.7 ± 0.1 . Titrate with 0.1 M zinc sulfate VS until a pink-purple color appears. Carry out blank test. Each mL of 0.1 M disodium edetate VS consumed is equivalent to 2.698 mg of aluminum. Use the result obtained to calculate the atomic ratio of aluminum/chlorine.

Chloride content. Weigh 0.7 g of the sample, transfer to a 250 mL Erlenmeyer flask, add 100 mL of water and 10 mL of M nitric acid. Shake until dissolution and titrate with 0.1 M silver nitrate VS, determining the endpoint potentiometrically, using a silver-silver chloride electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 3.545 mg of chloride. Use the result obtained to calculate the atomic ratio of aluminum/chlorine.

Arsenic (5.3.2.5). Determine in 1.5 g of sample. No more than 0.0002% (2 ppm).

Iron (5.3.2.4). Use *Method I*. Weigh 0.667 g of the sample and add 40 mL of water. No more than 0.015% (150 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Weigh 1 g of the sample and dissolve with 40 mL of water. Adjust pH between 3.0 and 4.0 with 6 M ammonium hydroxide. Proceed as described in *Limit test for heavy metals* using 2 mL of the *Standard lead solution (10 ppm Pb)*. No more than 0.002% (20 ppm).

Aluminum/chlorine atomic ratio. Divide the percentage of aluminum found in test for *Aluminum content* by the percentage of chlorine found in the test for *Chlorine content* and multiply by 35.453/26.98. Where 35.453 is the atomic mass of chlorine and 26.98 is the atomic mass of aluminum. Between 1.90:1 and 2.10:1.

Water (5.2.20.1). No more than 18.0%

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Calculate the percentage of aluminum chlorohydrate, according to the formula:

$$Al\{[26.98x + [17.01(3x - 1)] + 35.453] / 26.98x\}$$

where

Al = percentage of aluminum found in the test for *Aluminum content*;

x = aluminum/chlorine atomic ratio found in the test for *Aluminum/chlorine atomic ratio*;

26.98 = aluminum atomic mass;

17.01 = hydroxide anion atomic mass;

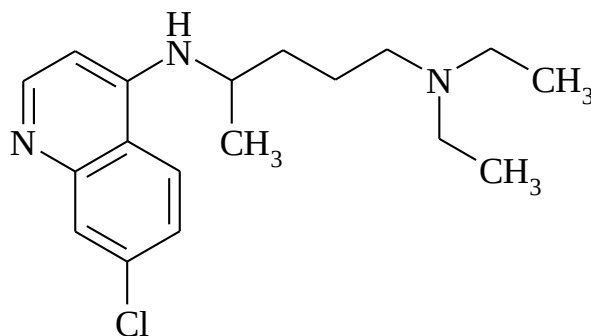
35.453 = chloride atomic mass.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

CHLOROQUINE*Chloroquine* $C_{18}H_{26}ClN_3$; 319.87

chloroquine; 02487

N4-(7-chloroquinolin-4-yl)-N1,N1-diethylpentane-1,4-diamine.

[54-05-7]

Contains no less than 98.0% and no more than 102.0% of $C_{18}H_{26}ClN_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish-white crystalline powder.

Solubility. Very slightly soluble in water. Soluble in diluted acids.

Physicochemical constants.

Melting range (5.2.2): 87 °C to 92 °C.

IDENTIFICATION

A. Weigh 25 mg of the sample, dissolve in 10 mL of water and 2 mL of 8.5% (w/v) sodium hydroxide. Shake twice with 20 mL of methylene chloride. Collect the organic phases and wash with 10 mL of water. Dry with sodium sulfate anhydrous, evaporate to dryness and dissolve the residue in 0.5 mL of methylene chloride. To prepare the standard, dissolve 40 mg of chloroquine phosphate CRS in 10 mL of water and follow the procedure described for preparing the sample. In the infrared absorption spectrum **(5.2.14)** of the sample solution, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of chloroquine phosphate CRS.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 10 µL/mL solution in 0.001% (v/v) hydrochloric acid, there are maximum and minimum points only at the same wavelengths of similar chloroquine CRS solution. The ratio between the absorbance values measured at 343 nm and 329 nm is comprised between 1.00 and 1.15.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample, in oven at 105 °C, for two hours. No more than 2%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.25 g of the sample, dissolve in 50 mL of glacial acetic acid and add 10 mg/mL methylrosanilinium chloride TS in acetic acid. Titrate with 0.1 M perchloric acid VS. The endpoint can also be determined potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 15.99 mg of C₁₈H₂₆ClN₃. Carry out the blank test and make corrections, if necessary.

PACKAGING AND STORAGE

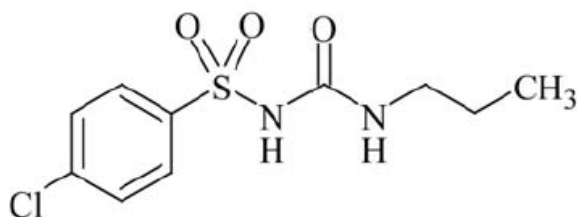
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimalarial.

CHLORPROPAMIDE*Chlorpropamidum*C₁₀H₁₃ClN₂O₃S; 276.74

chlorpropamide; 02505

4-Chloro-*N*-[(propylamino)carbonyl]benzenesulfonamide

[94-20-2]

Contains no less than 99.0% and no more than 100.5% of C₁₀H₁₃ClN₂O₃S in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder. Presents polymorphism.

Solubility. Practically insoluble in water, soluble in ethyl alcohol. Soluble in alkali hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 126 °C to 130 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the chlorpropamide CRS spectrum, prepared identically. If the spectra obtain show to be different, dissolve the standard and the sample in methylene chloride, evaporate to dryness and carry out new spectra using the residues.

B. Prepare a 0.01% (w/v) sample solution in methyl alcohol and dilute 10 mL from this solution in 100 mL of 0.01 *M* hydrochloric acid. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 220 nm to 350 nm, the sample solution displays maximum absorption at 232 nm, and the absorbance at 232 nm is 0.57 to 0.63.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ plate as support, and mixture of ammonia, cyclohexane, methyl alcohol and methylene chloride (11.5:30:50:100) as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.5 g of the sample in acetone and dilute to 10 mL with the same solvent.

Solution (2): dissolve 15 mg of 4-chlorobenzenesulfonamide (chlorpropamide impurity A) CRS in acetone and dilute to 100 mL with the same solvent.

Solution (3): dissolve 15 mg of 1,3-dipropylurea (chlorpropamide impurity B) CRS in acetone and dilute to 100 mL with the same solvent.

Solution (4): dilute 0.3 mL of *Solution (1)* to 100 mL with acetone.

Solution (5): dilute 5 mL of *Solution (3)* to 15 mL with acetone.

Solution (6): dissolve 0.1 g of the sample, 5 mg of 4-chlorobenzenesulfonamide (chlorpropamide impurity A) CRS and 5 mg of 1,3-dipropylurea (chlorpropamide impurity B) CRS in acetone and dilute to 10 mL with the same solvent.

Develop the chromatogram in 15 cm of plate. Dry the plate in oven at 110 °C for 10 minutes. Place at the bottom of the chromatographic chamber a mixture with one volume of hydrochloric acid, one volume of water and two volumes of a 5% (w/v) potassium permanganate solution. Close the chamber and wait 15 minutes. Put the plate in contact with chlorine vapor for two minutes. Remove the plate and put it in a place with cold air stream until the excess chlorine is removed and the coverage area below the application points does not present blue color with one drop of iodized starch RS1. Nebulize with iodized starch RS1. In the chromatogram obtained with *Solution (1)*, any stain corresponding to impurity A is not more intense than the stain obtained with *Solution (2)* (0.3%), any stain corresponding to impurity B is not more intense than that obtained in the chromatogram of *Solution (3)* (0.3%), any stain, in addition to the main stain, and any stain corresponding to impurities A and B is not more intense than the stain from the chromatogram obtained with *Solution (4)* (0.3%); no more than two stains are more intense than the stain obtained in the chromatogram from *Solution (5)* (0.1%). The test is not valid unless the chromatogram obtained with *Solution (6)* displays three clearly separate stains with approximate R_f values of 0.4 for chlorpropamide, 0.6 for impurity A and 0.9 for impurity B.

Heavy metals (5.3.2.3). Prepare a 10.0% (w/v) sample solution in acetone or dioxane with no less than 15% (v/v) of water. Proceed as described in *Method III*. Use *Standard lead solution (10 ppm Pb)*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of sample. Dry in oven between 100 °C and 105 °C until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.4%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh 0.25 g of the sample, dissolve in 50 mL of ethyl alcohol, neutralized in advance at the presence of phenolphthalein TS solution, and add 25 mL of water. Titrate with 0.1 M sodium hydroxide VS until a pink color is obtained. Each mL of 0.1 M sodium hydroxide VS is equivalent to 27.674 mg of $C_{10}H_{13}ClN_2O_3S$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 240 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: prepare a mixture of equal volume of diluted glacial acetic acid (1:100) and acetonitrile. Filter and degas the mixture.

Note: do not exceed the amount of 50% of acetonitrile. Make adjustments, if necessary.

Sample solution: accurately weigh 50 mg of the sample, transfer to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 10 mL of this solution to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: accurately weigh an amount of chlorpropamide CRS, dissolve in *Mobile phase* and dilute adequately with *Mobile phase*, to obtain a 0.05 mg/mL solution.

Inject 20 μ L replicates of the *Standard solution*. The retention time is about 2.2 minutes for chlorpropamide. The tailing factor is no more than 1.5 and the relative standard deviation of the injection replicates is no more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{10}H_{13}ClN_2O_3S$ in the sample from the responses obtained with the *Sample solution* and the *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Hypoglycemic agent.

CHLORPROPAMIDE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{10}H_{13}ClN_2O_3S$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 1 g of chlorpropamide, dissolve in 4 mL of acetone, filter and evaporate to dryness. In the infrared absorption spectrum (**5.2.14**) of the residue obtained, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of chlorpropamide CRS, prepared identically.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ as support and a mixture of methylene chloride, methyl alcohol, cyclohexane and ammonium hydroxide (100:50:30:10) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): homogenize an amount of powder from the tablet, equivalent to 100 mg of chlorpropamide, with 20 mL of *M* hydrochloric acid and extract with 50 mL of chloroform. Filter the solution and wash the cotton with chloroform in a beaker. Evaporate the chloroform in a water bath to dryness. Dry the residue at 105 °C for one hour. Prepare from the residue obtained a 1 mg/mL solution in acetone.

Solution (2): prepare a 1 mg/mL solution of chlorpropamide CRS in acetone.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 60 minutes.

Procedure: after the test, take an aliquot from the dissolution medium, filter and dilute, if necessary, with 0.1 *M* hydrochloric acid until adequate concentration. Measure the absorbances at 230 nm,

using the same solvent for zero adjustment. Calculate the amount of $C_{10}H_{13}ClN_2O_3S$ dissolved in the medium, comparing the readings obtained with that of chlorpropamide CRS solution of known concentration, prepared in 0.1 M hydrochloric acid.

Note: a volume of ethyl alcohol that does not exceed 10% (v/v) can be added to the preparation of the standard solution to dissolve chlorpropamide CRS.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{10}H_{13}ClN_2O_3S$ dissolve in 60 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.25 g of chlorpropamide, homogenize with 40 mL of methyl alcohol for 20 minutes, complete the volume to a 50 mL volumetric flask with the same solvent. Filter and dilute to a concentration of 0.001% (w/v) with 0.1 M hydrochloric acid. Prepare a chlorpropamide CRS solution in the same concentration. Measure the absorbances of the solutions at 232 nm. Calculate the amount of $C_{10}H_{13}ClN_2O_3S$ in the tablets from the readings obtained. Alternatively, it is possible to use $A(1\%, 1\text{ cm}) = 598$, at 232 nm.

B. Proceed as described in method **B.** of Assay in the *Chlorpropamide* monograph. Prepare the *Sample solution* as described next.

Sample solution: Pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 50 mg of chlorpropamide and transfer to a 100 mL volumetric flask using 75 mL of *Mobile phase*. Homogenize for 10 minutes and complete the volume with the *Mobile phase*. Filter, discarding the first 10 mL of the filtrate. Pipette 10 mL of the filtrate and place on a second 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

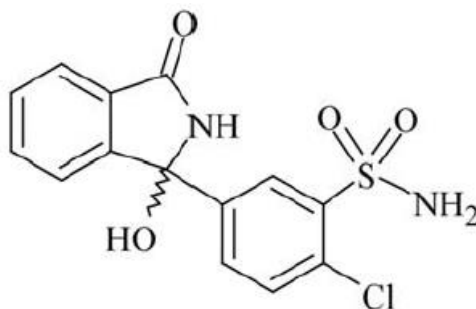
Procedure: inject, separately, 20 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{10}H_{13}ClN_2O_3S$ in the sample from the responses obtained with the *Sample solution* and the *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CHLORTALIDONE*Chlortalidonum*C₁₄H₁₁ClN₂O₄S; 338.77

chlortalidone; 02510

2-Chloro-5-(2,3-dihydro-1-hydroxy-3-oxo-1H-isoindol-1-yl)-benzenesulfonamide

[77-36-1]

Contains no less than 98.0% and no more than 102.0% of C₁₄H₁₁ClN₂O₄S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish-white powder. Presents polymorphism.

Solubility. Practically insoluble in water, soluble in methyl alcohol, slightly soluble in ethyl alcohol. Soluble in diluted alkali hydroxide solutions.

Physicochemical constants.

Specific optical rotation (5.2.8): -0.15 to +0.15. Determine in solution at 1.0% (w/v) in methyl alcohol.

IDENTIFICATION

The identification tests B. and C. can be omitted if test A. is carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wave numbers and with the same relative intensities as the ones observed in the spectrum of chlortalidone CRS, prepared identically. If the spectra obtain show to be different, dissolve the standard and the sample in methyl alcohol, evaporate to dryness and carry out new spectra using the residues.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 230 nm to 340 nm, of 0.01% (w/v) sample solution in ethyl alcohol, there are maximum and minimum points only at the same wavelengths of a similar chlortalidone CRS solution. The ratio between the absorbance values measured at 284 nm and 275 nm is comprised between 0.73 and 0.88.

C. The stain in the chromatogram from *Solution (3)*, obtained in *Related substances* corresponds in position, color and intensity to the one in the chromatogram from *Solution (4)*.

PURITY TESTS

Acidity. Shake 1 g of the sample in 50 mL of the mixture of water and carbon-dioxide free water (1:1) using heating. Allow to cool. No more than 0.75 mL of 0.1 M sodium hydroxide is spent to neutralize, determining the endpoint potentiometrically.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of toluene, xylene, ammonium hydroxide, dioxane and isopropyl alcohol (5:10:20:30:30) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): accurately weigh 200 mg of the sample, dissolve in a mixture of water and acetone (1:4) and dilute to 5 mL with the mobile phase.

Solution (2): accurately weigh 20 mg of 2-(4-chloro-3-sulfamoylbenzoyl)-benzoic acid CRS and 20 mg of chlortalidone SQR, dissolve in a mixture of water and acetone (1:4) and dilute to 50 mL with the mobile phase.

Solution (3): transfer 1 mL of *Solution (1)* to a 200 mL volumetric flask and complete the volume with a mixture of water and acetone (1:4).

Solution (4): 0.2 mg/mL chlortalidone CRS solution in a mixture of water and acetone (1:4).

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm). Any stain corresponding to 2-(4-chloro-3-sulfamoylbenzoyl)-benzoic acid obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (2)* (1.0%). Any secondary stain, different from the main stain and from the to 2-(4-chloro-3-sulfamoylbenzoyl)-benzoic acid stain obtained in the chromatogram with *Solution (1)*, is not more intense than that obtained with *Solution (3)* (0.5%). The test will only be valid if the chromatogram obtained with *Solution (2)* presents two clearly separated stains.

Chlorides (5.3.2.1). Pulverize and weigh 0.3 g of the sample. Add 30 mL of water, shake for five minutes and filter. Use 10 mL of the filtrate to carry out the *Limit test for chlorides*. Prepare the standard of chlorides. No more than 350 ppm (0.035%).

Heavy metals (5.3.2.3). Use *Method IV*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 2.0 g of the sample. Dry in oven at 105 °C for four hours. No more than 0.4%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh 0.2 g of the sample and dissolve 50 mL of acetone. Titrate with 0.1 M tetrabutylammonium hydroxide VS under nitrogen atmosphere. Determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M tetrabutylammonium hydroxide VS is equivalent to 33.877 mg of $C_{14}H_{11}ClN_2O_4S$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1 mL/minute.

Mobile phase: mixture of 0.01 M dibasic ammonia phosphate and methyl alcohol (3:2). Adjust pH to 5.5 ± 0.1 with phosphoric acid.

Internal standard solution: accurately weigh an amount of 2,7-naphthalenediol, dissolve in methyl alcohol and dilute quantitatively to obtain a 1 mg/mL solution.

2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid solution: accurately weigh an amount of 2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid CRS, dissolve in methyl alcohol and dilute quantitatively to obtain a 5 μ g/mL solution.

Sample solution: accurately weigh 50 mg of the sample, dissolve in methyl alcohol and dilute to 50 mL with the same solvent. Transfer 5 mL of this solution to a 50 mL volumetric flask. Add 5 mL of the *Internal standard solution* and 10 mL of methyl alcohol. Complete the volume with water and homogenize.

Standard solution: accurately weigh an amount of chlortalidone CRS, dissolve in methyl alcohol and dilute quantitatively to obtain a 1 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask. Add 5 mL of the *Internal standard solution* and 10 mL of the *2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid solution*. Complete the volume with water and homogenize.

Inject 25 μ L replicates of the *Standard solution*. The relative retention time are approximately 0.5 for 2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid, 0.8 for chlortalidone and 1.0 for 2,7-naphthalenediol. The resolution between the peaks of chlortalidone and of 2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid and between the peaks of chlortalidone and of 2,7-naphthalenediol is no less than 1.5. The tailing factor for the peaks of chlortalidone and of 2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid is no more than 2.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{14}H_{11}ClN_2O_4S$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Diuretic.

CHLORTALIDONE TABLETS

Contains no less than 92.0% and no more than 108.0% of the declared amount of $C_{14}H_{11}ClN_2O_4S$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of chlortalidone, transfer to a beaker and dissolve in 10 mL of acetone. Heat in a water bath for five minutes. Allow to cool and filter. Transfer 20 mL of water to the filtrate and heat in water bath for five minutes. Softly apply an air stream over the solution to remove the acetone. Allow to cool in ice bath, filter and dry the crystals at 105 °C for four hours. The dry residue complies with test **A.** of *Identification* from the *Chlortalidone* monograph.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** of *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 60 minutes.

Procedure: after the test, take an aliquot of the dissolution medium and dilute, if necessary, with water, until adequate concentration. Measure the absorbances of the solutions at 275 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_{14}H_{11}ClN_2O_4S$ dissolved in the medium, comparing the readings obtained with that of the chlortalidone CRS solution at the concentration of 0.5% (w/v) in methyl alcohol. Carry out successive dilutions of this solution in water up to an adequate concentration.

Tolerance: no less than 70% (Q) of the declared quantity of $C_{14}H_{11}ClN_2O_4S$ dissolve in 60 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel 60 F₂₅₄ as support and a mixture of toluene, xylene, ammonium hydroxide, dioxane and isopropyl alcohol (5:10:20:30:30) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 100 mg of chlortalidone and dissolve 5 mL of ethyl alcohol. Leave in ultrasonic bath for 15 minutes and centrifuge. Use the clear supernatant.

Solution (2): Transfer 1 mL of *Solution (1)* to a 200 mL volumetric flask and complete the volume with ethyl alcohol.

Solution (3): accurately weigh an amount of 2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid CRS, dissolve in ethyl alcohol and dilute to obtain a 0.02% (w/v) solution in the same solvent.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). Any stain corresponding to 2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (3)* (1.0%). Any other secondary stain obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (2)* (0.5%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 100 mg of chlortalidone, add 30 mL of methyl alcohol and boil under reflux for five minutes. Shake mechanically for 15 minutes. Allow to cool and filter. Wash the residue with methyl alcohol, add the washings to the filtrate and dilute to 100 mL with the same solvent. Transfer 5 mL of this solution and 2 mL of *M* hydrochloric acid to a 50 mL volumetric flask and complete the volume with methyl alcohol. Measure the absorbance of the resulting solution at 275 nm, using methyl alcohol for zero adjustment. Calculate the amount of $C_{14}H_{11}ClN_2O_4S$ in the tablets, from the readings obtained. Alternatively, calculate using $A(1\%, 1\text{ cm}) = 57.4$, at 275 nm.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, according to method **B.** of Assay from the *Chlortalidone* monograph. Prepare the *Standard* and *Sample solutions* as described below.

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 100 mg of chlortalidone and transfer to a 100 mL volumetric flask. Add 50 mL of methyl alcohol and shake mechanically for 30 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask with 5 mL of the *Internal standard solution* and 10 mL of methyl alcohol. Complete the volume with water and homogenize.

Standard solution: prepare as indicated in the *Chlortalidone* monograph. Replace the 2-(4-chloro-3-sulfamoylbenzoyl)benzoic acid solution with 10 mL of methyl alcohol.

Inject 25 μ L replicates of the *Standard solution*. The resolution between the peaks of chlortalidone and of 2,7-naphthalenediol must be no less than 1.5. The tailing factor for the peaks of chlortalidone

and of 2.7-naphthalenediol is no more than 2.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 25 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₄H₁₁ClN₂O₄S in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

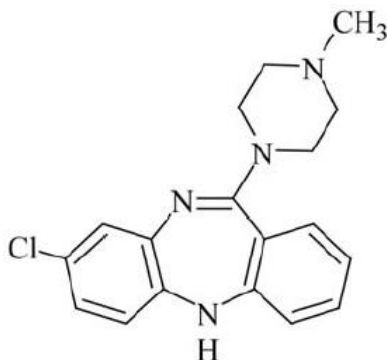
In tightly closed containers.

LABELING

Comply with current legislation.

CLOZAPINE

Clozapinum



C₁₈H₁₉ClN₄; 326.82

clozapine; 02540

8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[*b,e*][1,4] diazepine
[5786-21-0]

Contains no less than 99.0% and no more than 101.0% of C₁₈H₁₉ClN₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow crystalline powder.

Solubility. Practically insoluble in water, soluble in ethyl alcohol. Soluble in diluted acetic acid.

Physicochemical constants.

Melting range (5.2.2): 182 °C to 186 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, previously desiccated, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the clozapine CRS spectrum, prepared identically.

B. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (1)*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel 0.25 mm as support, and a mixture of chloroform and methyl alcohol (3:1) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.1 g of the sample in ethyl alcohol and complete to 10 mL with chloroform.

Solution (2): accurately weigh 2.5 mg of clozapine CRS and transfer to a 25 mL volumetric flask. Dissolve in chloroform and complete the volume with the same solvent.

Solution (3): transfer 3 mL of *Solution (2)* to a 10 mL volumetric flask and complete the volume with chloroform. Percentage for comparison with the sample: 0.3%.

Solution (4): transfer 1 mL of *Solution (2)* to a 5 mL volumetric flask and complete the volume with chloroform. Percentage for comparison with the sample: 0.2%.

Solution (5): transfer 1 mL of *Solution (2)* to a 10 mL volumetric flask and complete the volume with chloroform. Percentage for comparison with the sample: 0.1%.

Solution (6): transfer 1 mL of *Solution (2)* to a 20 mL volumetric flask and complete the volume with chloroform. Percentage for comparison with the sample: 0.05%.

Develop the chromatogram. Remove the plate and allow to air dry. Examine under ultraviolet light (254 nm) and compare the intensity of any secondary stain observed in the chromatogram from *Solution (1)* with that of the main stain of the chromatogram from *Solution (2)*. Any stain from the chromatogram of *Solution (1)* with R_f value of 0.82 corresponds to 11,11-(piperazine-1,4-diyl)bis(8-chloro-5H-dibenzo[b,e][1,4]diazepine); R_f of 0.67 corresponds to 8-chloro-5,10-dihydro-11H-dibenzo[b,e][1,4]diazepine-11-one and R_f of 0.10 corresponds to 8-chloro-11-(1-piperazine-1-yl)-5H-dibenzo[b,e][1,4]diazepine) or more intense than the *Solution (3)*, *Solution (4)* and *Solution (5)*, respectively. Any other secondary stain from the chromatogram of *Solution (1)* is not greater or more intense than the main stain obtained with *Solution (5)*. The sum of the intensity of all secondary stains obtained with *Solution (1)* corresponds to no more than 0.6%.

Heavy metals (5.3.2.3). Use *Method III*. Prepare the standard with 2 mL of solution at 10 ppm of lead (Pb). No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven between 100 °C and 105 °C for four hours, until constant weight. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Weigh, accurately, about 0.1 g of sample, transfer to a 250 mL Erlenmeyer flask and dissolve in 50 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS. Determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 16.341 mg of C₁₈H₁₉ClN₄.

PACKAGING AND ASSAY

In tightly closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antipsychotic.

CLOZAPINE TABLETS

Contains no less than 90% and no more than 110% of the declared amount of $C_{18}H_{19}ClN_4$.

IDENTIFICATION

A. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (1)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay* method, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 1000 mL volumetric flask. Add 640 mL of methyl alcohol, leave in ultrasonic bath for 10 minutes and complete the volume with water. Proceed as described in the method from *Assay* as of "Homogenize..."

DISSOLUTION TEST (5.1.5)

Dissolution medium: acetate buffer pH 4.0, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium, filter and dilute, if necessary, with *Dissolution medium* until adequate concentration. Measure the absorbances of the solutions at 290 nm (5.2.14), using the same solvent for zero adjustment. Calculate the amount of $C_{18}H_{19}ClN_4$ dissolved in the medium, comparing the readings obtained with that of the clozapine CRS solution at the concentration of 1.11% (w/v), prepared in the same solvent.

Tolerance: no less than 85% (Q) of the declared quantity of $C_{18}H_{19}ClN_4$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel 0.25 mm as support, and a mixture of *n*-heptane, chloroform, absolute ethyl alcohol and ammonium hydroxide (30:30:30:1) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions, described next.

Diluent solution: prepare a mixture of chloroform and methyl alcohol (4:1).

Solution (1): weigh and pulverize 20 tablets. Accurately weigh the equivalent to 125 mg of clozapine, transfer to a 25 mL volumetric flask and dissolve using 20 mL of *Diluent solution*. Shake, mechanically, for 15 minutes and complete the volume with *Diluent solution*. Filter and homogenize.

Solution (2): 5 mg/mL clozapine CRS sample solution in *Diluent solution*.

Solution (3): transfer 1 mL of *Solution (2)* to a 200 mL volumetric flask and complete the volume with chloroform. Percentage for comparison with the sample: 0.5%.

Solution (4): transfer 1 mL of *Solution (2)* to a 250 mL volumetric flask and complete the volume with chloroform. Percentage for comparison with the sample: 0.4%.

Solution (5): transfer 3 mL of *Solution (2)* to a 1000 mL volumetric flask and complete the volume with chloroform. Percentage for comparison with the sample: 0.3%.

Solution (6): transfer 1 mL of *Solution (2)* to a 500 mL volumetric flask and complete the volume with chloroform. Percentage for comparison with the sample: 0.2%.

Solution (7): transfer 1 mL of *Solution (2)* to a 1000 mL volumetric flask and complete the volume with chloroform. Percentage for comparison with the sample: 0.1%.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light in short wavelength and compare the intensity of any secondary stain observed in the chromatogram from *Solution (1)* with that of the main stain of the chromatogram from *Solutions (2), (3), (4), (5), (6) and (7)*. No other secondary stain from the chromatogram of *Solution (1)* is larger or more intense than the main stain obtained in the chromatogram of *Solution (3)* (0.5%); and the sum of the intensities of all secondary stains obtained from *Solution (1)* corresponds to no more than 2.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 257 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of methyl alcohol, water and triethylamine (800:200:0.75).

Sample solution: weigh and pulverize no less than 20 tablets. Accurately weigh an amount of powder equivalent to 125 mg of clozapine, transfer to a 1000 mL volumetric flask and dissolve in 640 mL of methyl alcohol. Leave in ultrasonic bath for 10 minutes, complete the volume with water and homogenize, obtaining a solution at 0.125 mg/mL.

Standard solution: accurately weigh an amount of clozapine CRS and dissolve in methyl alcohol to obtain a 0.125 mg/mL solution. The final composition of the solvent methyl alcohol:water must be of approximately 8:2.

Resolution solution: accurately weigh 10 mg of clozapine and transfer to an adequate container. Add 5 mL of 0.1 M hydrochloric acid and heat for two hours at 90 °C. Transfer this solution to a 100 mL volumetric flask, using 15 mL of water, and complete the volume with methyl alcohol. Homogenize. Transfer 10 mL of this preparation and 10 mL of the *Standard solution* to an adequate container and homogenize.

Inject 10 µL replicates of the *Standard solution*. The column efficiency must be no less than 1500 theoretical plates, and the relative standard deviation for the replicates is no more than 2.0%. Inject 10 µL of the *Resolution solution*. The resolution between clozapine and any other peak must be no less than 1.5.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of clozapine ($C_{18}H_{19}ClN_4$) in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

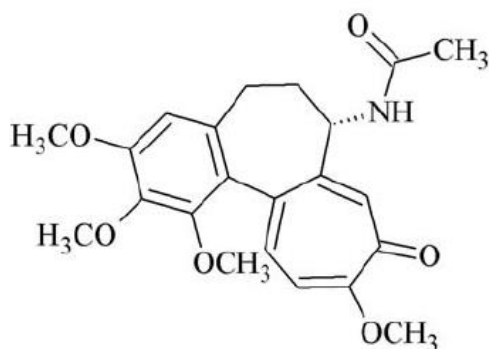
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

COLCHICINE

Colchicinum



$C_{22}H_{25}NO_6$; 399.44

colchicine; 02567

N-[(7*S*)-5,6,7,9-Tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[*a*]heptalen-7-yl]acetamide
[64-86-8]

Contains no less than 94.0% and no more than 101.0% of $C_{22}H_{25}NO_6$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Pale yellow crystalline or amorphous powder.

Solubility. Soluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 142 °C to 150 °C (amorphous powder); 157 °C (crystal).

Specific optical rotation (5.2.8): -240 to -250, determined in 1% (w/v) solution in ethyl alcohol, in relation to the anhydrous substance.

IDENTIFICATION

A. Dissolve the sample in 0.5 mL of chloroform, disperse in potassium bromide, homogenize and evaporate the solvent first in an air stream and then by heating at 80 °C for 60 minutes. In the infrared absorption spectrum (5.2.14) of the sample, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of colchicine CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of the 0.001% (w/v) solution in ethyl alcohol, there are maximum points at 243 nm and 350 nm, identical to the ones observed in the spectrum of a similar colchicine CRS solution.

PURITY TESTS

Aspect of the preparation. The preparation at 0.5% (w/v) in water is clear **(5.2.25)**, not presenting a color more intense **(5.2.12)** than a solution prepared by diluting 8.5 mL of the *Color reference solution SC O* in 91.5 mL of 1% (w/v) hydrochloric acid.

Acidity or alkalinity. Add to 10 mL of the 0.5% (w/v) sample solution in water 0.1 mL of bromothymol blue TS. There is no change in color or production of a green color. No more than 0.1 mL of 0.01 M sodium hydroxide is necessary for the indicator to turn blue.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel HF₂₅₄ as support, and a mixture of concentrated ammonia, chloroform and acetone (1:25:50) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in chloroform.

Solution (2): dilute 2 mL of *Solution (1)* to 100 mL with chloroform.

Solution (3): dilute 5 mL of *Solution (2)* to 10 mL with chloroform.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained with *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram of *Solution (2)* (2%), and no more than one stain is more intense than that obtained in the chromatogram of *Solution (3)* (1%).

Ethyl acetate (5.2.33). No more than 6% (w/w).

Water (5.2.20.1). Determine in 0.5 g of sample. No more than 2.0%.

Residue by incineration (5.2.10). Determine in 0.5 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.25 g of the sample, dissolve in a mixture of 10 mL of acetic anhydride and 20 mL of toluene and heat, if necessary. Titrate with 0.1 M perchloric acid VS and determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 39.940 mg of C₂₂H₂₅NO₆.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (3 µm to 10 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of 0.5 M monobasic potassium phosphate, water and methyl alcohol (4.5:45:53). Adjust pH to 5.50 ± 0.05 with phosphoric acid.

Sample solution: 6 µg/mL solution of sample in a mixture of methyl alcohol and water (1:1). Prepare immediately before use.

Standard solution: 6 µg/mL colchicine CRS solution in a mixture of methyl alcohol and water (1:1). Prepare immediately before use.

The column efficiency is no less than 4500 theoretical plates/meter. The retention time for colchicine is between 5.5 and 9.5. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{22}H_{25}NO_6$ from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antigout agent.

COLCHICINE TABLETS

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{22}H_{25}NO_6$.

IDENTIFICATION

Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 20 mg of colchicine and disperse with 20 mL of water. Filter. Transfer the filtrate to a separation funnel. Extract with 30 mL of chloroform. Evaporate the chloroform, to residue, under mild heat. Proceed as described on test **A.** of *Identification* in the *Colchicine* monograph using the residue obtained.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 500 mL.

Apparatus: basket, 100 rpm.

Time: 30 minutes.

Procedure: carry out the test, with agility, protected from light. After the test, take an aliquot of the dissolution medium and filter immediately. Dilute, if necessary, with water, until adequate concentration. Proceed as described in method **B.** of *Assay* on the *Colchicine* monograph.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{22}H_{25}NO_6$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in method **B.** of *Assay* on the *Colchicine* monograph. Prepare the *Sample solution* as described next.

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.6 mg of colchicine and transfer to a 100 mL volumetric flask using 50 mL of a mixture of methyl

alcohol and water (1:1). Shake mechanically for 15 minutes and complete the volume with the same solvent. Prepare immediately before use, protected from light.

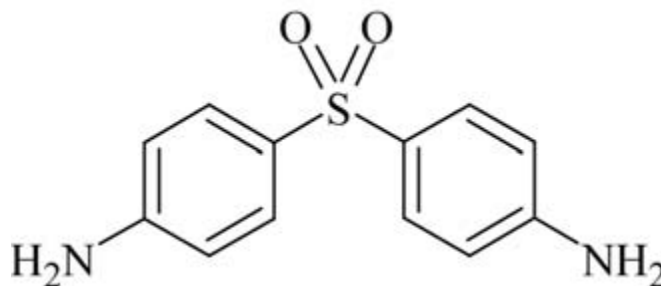
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₂₂H₂₅NO₆ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

DAPSONE***Dapsonum*** $C_{12}H_{12}N_2O_2S$; 248.30

dapsone; 02686

4,4'-Sulfonylbis-benzenamine

[80-08-0]

Contains no less than 99.0% and no more than 101.0% of $C_{12}H_{12}N_2O_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or slightly yellow crystalline powder.

Solubility. Practically insoluble in water, moderately soluble in ethyl alcohol. Freely soluble in diluted mineral acids.

Physicochemical constants.

Melting range (5.2.2): 175 °C to 181 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of dapsone CRS, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of a 0.0005% (w/v) solution in methyl alcohol, there are maximum points at 260 nm and 295 nm and minimum points at 232 nm and 268 nm, identical to the ones observed in the spectrum of a similar dapsone CRS solution.

C. Proceed as described in *Related substances*. The main stain obtained in the chromatogram with *Solution (4)* corresponds in position, color and intensity to the one obtained with *Solution (5)*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support and a mixture of chloroform and acetone (1:1) as mobile phase. Apply to the plate, separately, 10 µL of each of the following solutions, prepared in methyl alcohol.

Solution (1): 1% (w/v) sample solution.

Solution (2): 0.01% (w/v) sample solution.

Solution (3): 0.002% (w/v) sample solution.

Solution (4): 0.1% (w/v) sample solution.

Solution (5): 0.1% (w/v) dapsone CRS solution.

Develop the chromatogram. Remove the plate, dry at room temperature and nebulize first with 0.5% (w/v) sodium nitrite solution in 0.1 M hydrochloric acid. Wait five minutes and nebulize with N-(1-naphthyl)ethylenediamine dihydrochloride RS. Any stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than the stain obtained in the chromatogram with *Solution (2)* (1.0%), and no more than any two of these stains are more intense than the stain obtained in the chromatogram with *Solution (3)* (0.2%).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at temperature between 100 °C and 105 °C for three hours. No more than 1.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Diazotization titrations (5.3.3.1)*, use *Method 1* or *Method 2*. Accurately weigh 0.25 g of the sample. Each mL of 0.1 M sodium nitrite VS is equivalent to 12.415 mg of C₁₂H₁₂N₂O₂S.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh 50 mg of the sample, dissolve with 40 mL of ethyl alcohol and submit to ultrasonic bath for 10 minutes. Shake for 30 minutes and complete the volume to 100 mL with the same solvent. Dilute, successively, in ethyl alcohol until concentration of 0.0005% (w/v). Prepare a standard dapsone CRS solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 295 nm using ethyl alcohol for zero adjustment. Calculate the amount of C₁₂H₁₂N₂O₂S in the sample from the readings obtained.

PACKAGING AND STORAGE

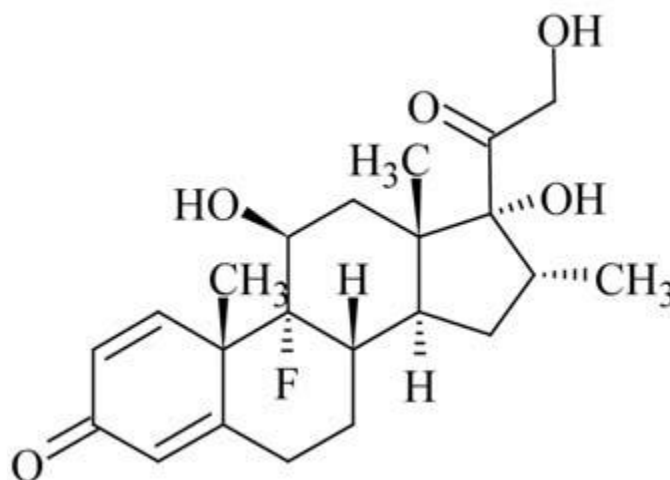
In tightly closed and opaque containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Chemotherapeutic.

DEXAMETHASONE*Dexamethasonum*C₂₂H₂₉FO₅; 392.46

dexamethasone; 02817

(11 β ,16 α)-9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione

[50-02-2]

Contains no less than 97.0% and no more than 102.0% of C₂₂H₂₉FO₅, calculated in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting temperature (5.2.2): 255 °C, with decomposition.

Specific optical rotation (5.2.8): +72 to +80, in relation to the desiccated substance. Determine in solution at 1.0% (w/v) in dioxane.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of dexamethasone CRS, prepared identically.

B. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel with a fluorescence indicator with maximum intensity at 254 nm as support, and a mixture of butyl alcohol saturated with water, toluene and ethyl ether (5:10:85) as mobile phase. Apply to the plate, separately, 5 μ L of each of the freshly prepared solutions described below.

Solution (1): prepare a 1 mg/mL sample solution in a mixture of methyl alcohol and methylene chloride (1:9).

Solution (2): prepare a 1 mg/mL dexamethasone CRS solution in a mixture of methyl alcohol and methylene chloride (1:9).

Solution (3): prepare a 1 mg/mL betamethasone CRS solution in *Solution (2)*.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. Nebulize with ethanolic sulfuric acid solution. Heat at 120 °C for 10 minutes or until stains appear and allow to cool. Examine under visible light and ultraviolet light at 365 nm. The main stain obtained with *Solution (1)* corresponds in position, color under visible light, fluorescence under ultraviolet 365 nm light and dimensions to the main stain obtained with *Solution (2)*. The test is only valid if *Solution (3)* presents two stains that cannot be entirely separated.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with phenyl group chemically bonded to porous silica particle (5 µm to 10 µm), maintained at room temperature; *Mobile phase* flow of 1 mL/minute.

Formate buffer pH 3.6: weigh 1.32 g of ammonium formate, transfer to a 1000 mL volumetric flask, add water and shake until dissolution. Adjust with formic acid to pH 3.6.

Mobile phase: mixture of *Formate buffer pH 3.6* and acetonitrile (67:33). Make adjustments if necessary.

Solution (1): accurately weigh 180 mg of the sample, transfer to a volumetric flask and dilute with acetonitrile. Transfer 33 mL of the solution to a 100 mL volumetric flask, complete the volume with *Formate buffer pH 3.6* and homogenize.

Procedure: inject 10 µL of *Solution (1)*, record the chromatograms and measure the areas under all the peaks obtained. The individual area under the secondary peaks, except the main peak, is no more than 1% of the total area of the peaks obtained. The sum of all secondary peaks, except the main peak, is no more than 2% of the total area of the peaks obtained. Do not include solvent-related peaks in the calculations. The column efficiency is no less than 5000 theoretical plates.

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105 °C for three hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 0.25 g of the sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. By Ultraviolet absorption spectrum (5.2.14). Accurately weigh 0.1 g of the sample and dissolve in ethyl alcohol. Complete the volume to 100 mL with the same solvent. Transfer 2.0 mL of this solution to a 100 mL volumetric flask and complete the volume with ethyl alcohol. Prepare a standard dexamethasone solution in ethyl alcohol, in the same final concentration. Measure the absorbances of the resulting solutions at 238.5 nm, using ethyl alcohol for zero adjustment. Calculate the amount of $C_{22}H_{29}FO_5$ in the sample from the readings obtained.

B. By High performance liquid chromatography (5.2.17.4). Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 μ m), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: prepare adequately a solution of methyl alcohol and water (75:25).

Sample solution: accurately weigh 30 mg of the sample, transfer to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: accurately weigh an amount of dexamethasone CRS and dissolve in methyl alcohol to obtain a 1 mg/mL solution. Transfer 3 mL of this solution to a 10 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 0.3 mg/mL solution.

Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{22}H_{29}FO_5$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation

THERAPEUTIC CLASS

Anti-inflammatory.

DEXAMETHASONE ELIXIR

Contains no less than 90.0% and no more than 110.0% the declared amount of $C_{22}H_{29}FO_5$.

IDENTIFICATION

Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel as support, and a mixture of chloroform, acetone and glacial acetic acid (80:40:1) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): sample solution with 0.1 mg/mL of dexamethasone.

Solution (2): 0.1 mg/mL dexamethasone CRS solution in a mixture of methylene chloride and methyl alcohol (1:1).

Develop the chromatogram. Remove the plate. Evaporate the solvent. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 2.7 to 4.0.

Determination of alcohol (5.3.3.8). Between 3.8% and 5.7%. *N*-propyl alcohol as internal standard.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: prepare adequately a solution of methyl alcohol and water (75:25).

Sample solution: sample solution with 0.1 mg/mL of dexamethasone, using *Mobile phase* as diluent.

Standard solution: accurately weigh dexamethasone CRS and dissolve in *Mobile phase*, to obtain a concentration of 0.1 mg/mL.

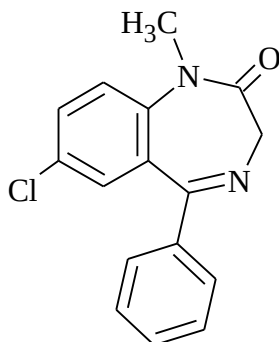
Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₂₂H₂₉FO₅ in the sample, from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation

DIAZEPAM**Diazepamum**

$C_{16}H_{13}ClN_2O$; 284.74

diazepam; 02904

7-chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepine-2-one.

[439-14-5]

Contains no less than 99.0% and no more than 101.0% of $C_{16}H_{13}ClN_2O$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Very slightly soluble in water, soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 131 °C to 135 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the diazepam CRS spectrum, prepared identically.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of ethyl acetate and hexane (1:1) as mobile phase. Apply to the plate, separately, 5 μ L of each of the freshly prepared solutions described below.

Solution (1): dissolve an accurately weighed amount of the sample in ethyl alcohol, to obtain a solution with concentration of 1 mg/mL.

Solution (2): dissolve an accurately weighed amount of diazepam CRS in ethyl alcohol, to obtain a solution with concentration of 1 mg/mL.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

PURITY TESTS

Related substances. Proceed protected from light, as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm), maintained at 30 °C; *Mobile phase* flow of 1 mL/minute.

Mobile phase: mixture of 0.025 M monobasic potassium phosphate (pH 5.0, adjusted with sodium hydroxide RS), methyl alcohol and acetonitrile (44:34:22).

Solution (1): accurately weigh 25 mg of the sample, dissolve in 0.5 mL of acetonitrile and dilute to 50 mL with *Mobile phase*.

Solution (2): transfer 1 mL of *Solution (1)*, dilute to 100 mL with *Mobile phase* and homogenize. Dilute 1 mL of the solution to 10 mL with *Mobile phase* and homogenize.

Resolution solution: dissolve the content of a vial of *diazepam for system suitability* (containing impurities A (7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one (nordazepam)), B (2-chloro-N-(4-chloro-2-benzoylphenyl)-N-methylacetamida) and E (6-chloro-1-methyl-4-phenylquinazolin-2-(1H)-one)) to 1 mL with *Mobile phase*.

Inject 20 µL replicates of the *Resolution solution*. Considering the peak related to diazepam with retention time of approximately nine minutes, the relative retention times are about 0.7 for impurity E, 0.8 for impurity A and 1.3 for impurity B. The test is only valid if the chromatogram presents resolution between the peaks of impurities E and A of no less than 2.5, and between the peaks of impurity A and diazepam of no less than 6.0.

Procedure: inject, separately, 20 µL of *Solution (1)* and *Solution (2)*, record the chromatograms for no less than four times the retention time of the main peak and measure the areas under the peaks. Calculate the percentage of each impurity reported. Use 1.3 as correction factor for calculating the impurities B and E. The areas corresponding to impurities A, B and E obtained with *Solution (1)* are no more than equal to the areas under the main peak obtained with *Solution (2)* (0.1%). No area of secondary peak obtained with *Solution (1)*, except that of the solvent peak, is greater than the area under the main peak obtained with *Solution (2)* (0.1%). The sum of all areas under the secondary peaks obtained with *Solution (1)*, except that of the solvent peak, is not greater than twice the area under the main peak obtained with *Solution (2)* (0.2%). Do not consider peaks with areas obtained with *Solution (1)* smaller than 0.5 time the area under the main peak obtained with *Solution (2)* (0.05%).

Heavy metals (5.3.2.3). Use *Method III*. Determine in 1.0 g of the sample. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of sample. Dry in oven at 60 °C, under vacuum, for four hours. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.2 g of the sample and dissolve in 50 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 28.47 mg of $C_{16}H_{13}ClN_2O$.

PACKAGING AND STORAGE

Store protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Benzodiazepine.

DIAZEPAM TABLETS

Contains no less than 92.5% and no more than 107.5% of the declared amount of $C_{16}H_{13}ClN_2O$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 230 nm to 350 nm of the sample solution obtained in method **A.** from Assay, there are maximum absorption points at 242 nm and 284 nm, identical to the ones observed in the spectrum of the standard solution.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and a mixture of ethyl acetate and hexane (50:50) as mobile phase. Apply, separately, to the plate 2 µL of the solutions described below.

Solution (1): accurately weigh an amount of powder from tablets sufficient to prepare a solution with 0.02% (w/v) of diazepam, shake with ethyl alcohol and filter.

Solution (2): accurately prepare a 0.02% (w/v) diazepam CRS solution in ethyl alcohol.

Develop the chromatogram. Remove the plate, dry at room temperature and examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 100 mL volumetric flask, add 5 mL of water, shake and wait 15 minutes until its disintegration. Proceed as described in method **A.** of Assay, as of "Add 70 mL of 0.1 M hydrochloric acid...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid, until adequate concentration. Measure the absorbances at 284 nm **(5.2.14)**,

using the same solvent for zero adjustment. Calculate the amount of $C_{16}H_{13}ClN_2O$ dissolved in the medium, comparing the readings obtained with that of diazepam CRS solution at the concentration of 0.0005% (w/v) or 0.001% (w/v), according to the dose of drug in the tablet, prepared in 0.1 M hydrochloric acid.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{16}H_{13}ClN_2O$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and a mixture of ethyl acetate and hexane (50:50) as mobile phase. Apply to the plate, separately, 20 µL of *Solution (1)* and 5 µL of *Solution (2)* freshly prepared, described below.

Solution (1): accurately weigh an amount of powder from tablets corresponding to 50 mg of diazepam, shake with 5 mL of ethyl alcohol and filter.

Solution (2): dilute one volume of *Solution (1)* to 50 volumes with ethyl alcohol.

Develop the chromatogram. Remove the plate, dry at room temperature and examine under ultraviolet light (254 nm). No secondary stain obtained with *Solution (1)* is more intense than the main stain obtained with *Solution (2)* (2%).

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh quantity of powder equivalent to 10 mg of diazepam, transfer to a 100 mL volumetric flask, add 5 mL of water, homogenize and allow to stand for 15 minutes. Add 70 mL of 0.1 M hydrochloric acid, shake, mechanically, for 15 minutes and complete the volume with the same solvent. Filter. Carry out successive dilutions to a concentration of 0.002% (w/v), using 0.1 M hydrochloric acid as solvent. Prepare a standard solution in the same conditions. Measure the absorbances of the solutions at 284 nm, using 0.1 M hydrochloric acid for zero adjustment. Carry out the reading of solutions within 30 minutes. Calculate the amount of $C_{16}H_{13}ClN_2O$ in the tablets, from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); maintained at temperature of 30 °C; *Mobile phase* flow of 0.8 mL/minute.

Mobile phase: mixture of water, acetonitrile and methyl alcohol (4:4:2).

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 10 mg of diazepam, transfer to a 50 mL volumetric flask using 40 mL of *Mobile phase* and leave in ultrasonic bath for five minutes. Shake, mechanically, for five minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 20 µg/mL solution.

Standard solution: accurately weigh an amount of diazepam CRS and dissolve in *Mobile phase* to obtain a 0.2 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with the *Mobile phase*, obtaining a 20 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is no less than 5000 theoretical plates. The tailing factor is no more than 2.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₆H₁₃ClN₂O in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

DIAZEPAM INJECTION SOLUTION

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{16}H_{13}ClN_2O$.

IDENTIFICATION

The identification test C. can be omitted if tests A. and B. are carried out. The identification test A. can be omitted if tests B. and C. are carried out.

A. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from Assay, displays maximum absorption at 368 nm and with the same relative intensity as the ones observed in the spectrum of the standard solution.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support and a mixture of chloroform and methyl alcohol (10:1) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): dilute the injection solution in methyl alcohol to obtain a 1 mg/mL solution.

Solution (2): accurately weigh an amount of diazepam CRS and dissolve in methyl alcohol, to obtain a solution with concentration of 1 mg/mL.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (365 nm). Nebulize with 10% (w/v) sulfuric acid in absolute ethyl alcohol and heat the plate at 105 °C for 10 minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to that from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 6.2 to 7.0.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). No more than 11.6 EU/mg of diazepam.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer a volume of injection solution equivalent to 10 mg of diazepam to a separation funnel and add 20 mL of

phosphate buffer pH 7.0. Extract with four portions of 20 mL of chloroform, passing the extracts in 5 g of sodium sulfate anhydrous. Collect the extracts in a 100 mL volumetric flask and complete the volume with chloroform. Homogenize. Evaporate an aliquot of 10 mL, under nitrogen stream, to dryness. Dissolve the residue with 25 mL of 0.05 M methanolic sulfuric acid. Prepare the standard solution in the same conditions as the sample solution. Measure the absorbance of the sample solution and the standard solution at 368 nm, using 0.05 M methanolic sulfuric acid for zero adjustment. Calculate the amount of $C_{16}H_{13}ClN_2O$ in the injection solution from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 151$, at 368 nm.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μm); *Mobile phase* flow of 1.4 mL/minute.

Mobile phase: prepare a filtered and degassed solution of methyl alcohol and water (65:35).

Internal standard solution: prepare a *p*-tolualdehyde solution with 0.3 $\mu\text{L/mL}$ in methyl alcohol.

Sample solution: transfer, accurately, a volume of the injection solution equivalent to 10 mg of diazepam to a 50 mL volumetric flask. Transfer 10 mL of the *Internal standard solution* to the *Sample solution*, dilute with methyl alcohol to the final volume and homogenize.

Standard solution: accurately weigh an amount of diazepam CRS, dissolve in methyl alcohol and dilute with the same solvent to obtain a 1 mg/mL solution. Transfer 5.0 mL of this solution and 5 mL of the *Internal standard solution* to a 25 mL volumetric flask, complete the volume with methyl alcohol and homogenize.

Inject 10 μL replicates of the *Standard solution*. The tailing factor for the diazepam peak is no more than 2.5 and the resolution between the peaks of *p*-tolualdehyde and diazepam is no less than 3.5. The standard deviation for the injection replicates is no more than 2.0%.

Procedure: inject, separately, 10 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. The relative retention times are approximately 0.5 for *p*-tolualdehyde and 1.0 for diazepam. Calculate the amount of $C_{16}H_{13}ClN_2O$ in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution* through the formula:

$$50 \times \frac{C}{V} \times \frac{Ru}{Rs}$$

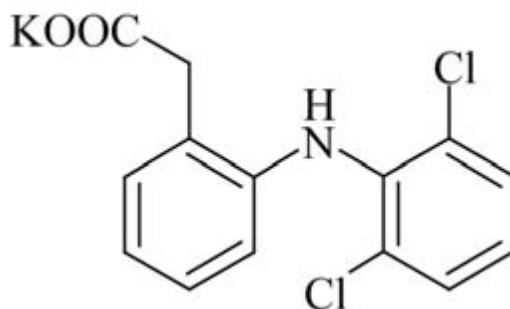
where *C* is the concentration in mg/mL of diazepam CRS in the *Standard solution*, *V* is the volume in mL of the injection solution taken, and *Ru* and *Rs* are the ratios of responses from the peaks of diazepam to *p*-tolualdehyde obtained from the *Sample solution* and the *Standard solution*, respectively.

PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELING

Comply with current legislation

DICLOFENAC POTASSIUM*Diclofenacum kalicum* $C_{14}H_{10}Cl_2KNO_2$; 334.24

diclofenac potassium; 02929

2-[(2,6-dichlorophenyl)amino]benzeneacetic acid potassium salt (1:1)

[15307-81-0]

Contains no less than 99.0% and no more than 101.0% of $C_{14}H_{10}Cl_2KNO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or slightly yellow, mildly hygroscopic crystalline powder.

Solubility. Moderately soluble in water, freely soluble in methyl alcohol, soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 295 °C to 300 °C, with decomposition.

IDENTIFICATION

The identification tests B. and C. can be omitted if tests A. and D. are carried out. The identification test A. can be omitted if tests B., C. and D. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of diclofenac potassium CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 350 nm, of 0.001% (w/v) solution in 0.1 M potassium hydroxide, there are maximum points at 218 nm and 275 nm, identical to those observed in the spectrum of similar diclofenac potassium CRS solution.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of ammonium hydroxide, methyl alcohol and ethyl acetate (10:10:80) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): accurately weigh 25 mg of sample, dissolve in methyl alcohol and complete the volume to 5 mL with the same solvent.

Solution (2): accurately weigh 25 mg of diclofenac potassium CRS, dissolve in methyl alcohol and complete the volume to 5 mL with the same solvent.

Solution (3): dilute 10 mg of indomethacin CRS with *Solution (2)* and complete the volume to 2 mL with methyl alcohol.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. The test will only be valid if the chromatogram obtained with *Solution (3)* presents two clearly separated stains.

D. Accurately weigh 0.5 g of sample and dissolve in 10 mL of water. Add 2 mL of diluted hydrochloric acid, shake for one hour and filter under reduced pressure. Neutralize with 5 M sodium hydroxide. Complies with the potassium ion reaction 2 **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. The preparation at 5% (w/v) in methyl alcohol is clear **(5.2.25)** and colorless **(5.2.12)** and the ultraviolet absorbance of the solution, determined at 440 nm, is not superior to 0.05.

pH (5.2.19). 7.0 to 8.5. Determine in aqueous solution at 1.0% (w/v).

Related substances. Proceed as described on method **B.** from *Assay*. Prepare *Solutions (1)* and *(2)* as described below.

Diluent: mixture of water and methyl alcohol (30:70).

Solution (1): accurately weigh 50 mg of sample, transfer to a 100 mL volumetric flask and complete the volume with *Diluent*.

Solution (2): transfer 2 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with *Diluent*. Transfer 1 mL of this solution to a 10 mL volumetric flask and complete the volume with the same solvent.

Procedure: inject, separately, 20 µL of *Solutions (1)* and *(2)*, record the chromatograms and measure the areas under the peaks. No secondary peak obtained with *Solution (1)* presents an area greater than the area under the main peak obtained in the chromatogram of *Solution (2)* (0.2%). The sum of all areas of all peaks, except the main peak, in the chromatogram of *Solution (1)* is not greater than 2.5 times the area under the main peak, obtained in the chromatogram of *Solution (2)* (0.5%). In the chromatogram from *Solution (1)*, disregard any peak which area is smaller than 0.25 times the area under the main peak obtained in the chromatogram from *Solution (2)*.

Heavy metals (5.3.2.3). Weigh 2.0 g of the sample. Incinerate between 500 °C and 600 °C. If the residue does not show to be completely white after the incineration, add a sufficient amount of hydrogen peroxide to dissolve. Heat until complete evaporation. Repeat the procedure until obtaining a completely white residue and proceed as described on *Method III*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven between 100 °C and 105 °C for three hours. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.3 g of sample and dissolve in 30 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS and determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 33.424 mg of C₁₄H₁₀Cl₂KNO₂.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow of 1.0 mL/minute.

Phosphate buffer pH 2.5: homogenize equal volumes of 0.05% (w/v) phosphoric acid and 0.08% (w/v) monobasic sodium phosphate. If necessary, adjust pH to 2.5 with phosphoric acid.

Mobile phase: mixture of *Phosphate buffer pH 2.5* and methyl alcohol (30:70).

Diluent: mixture of water and methyl alcohol (30:70).

Sample solution: accurately weigh an amount of the sample and dissolve in *Diluent* to obtain a 40 µg/mL solution.

Standard solution: accurately weigh an amount of diclofenac potassium CRS and dissolve in *Diluent* to obtain a 40 µg/mL solution.

Resolution solution: accurately weigh 2.0 mg of diethyl phthalate, 10.0 mg of diclofenac potassium CRS and 1.0 mg of 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one (*Impurity A*), transfer to a 200 mL volumetric flask, complete volume with *Diluent* and homogenize.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are about 0.5 for diethyl phthalate, 0.7 for *Impurity A* and 1 for diclofenac potassium. The resolution between diethyl phthalate and *Impurity A* is no less than 4.0; and between *Impurity A* and diclofenac potassium is no less than 6.5. The relative standard deviation of the areas of replicates under the peaks recorded is no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₄H₁₀Cl₂KNO₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-inflammatory.

DICLOFENAC POTASSIUM TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{14}H_{10}Cl_2KNO_2$. The tablets can be coated with sugar or film. In this case, they must not undergo friability and hardness tests.

IDENTIFICATION

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.15 g of diclofenac potassium, dissolve with 0.5 mL of glacial acetic acid and 15 mL of methyl alcohol. Leave in ultrasonic bath for 15 minutes and shake for another minute. Filter and collect the filtrate with 15 mL of water. Filter the solid formed under reduced pressure, wash with four portions of 5 mL of water and dry at 105 °C for two to three hours. Accurately weigh 50 mg of diclofenac potassium CRS, dissolve in 5 mL of methyl alcohol, 0.5 mL of glacial acetic acid, 15 mL of water and shake. Filter the solid formed under reduced pressure, wash with four portions of 5 mL of water and dry at 105 °C for two to three hours. In the infrared absorption spectrum **(5.2.14)** of the residue obtained, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the diclofenac potassium CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 350 nm of the sample solution obtained in method **A.** from *Assay*, there are maximum points at 218 nm and 275 nm, identical to those observed in the spectrum of the standard solution.

C. Proceed as described in test **C.** of *Identification* on the *Diclofenac potassium* monograph. Prepare *Solution (1)* as described next.

Solution (1): Pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 50 mg of diclofenac potassium, transfer to a 10 mL volumetric flask and add 7 mL of methyl alcohol. Leave in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Homogenize and filter.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 6.8 ± 0.05 , 900 mL

Apparatus: paddles; 40 rpm

Time: 60 minutes

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, with phosphate buffer pH 6.8 ± 0.05 , to adequate concentration. Measure the absorbances at 276 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_{14}H_{10}Cl_2KNO_2$ dissolved in the medium, comparing the readings obtained with the diclofenac potassium CRS solution at the concentration of 0.005% (w/v), prepared in phosphate buffer pH 6.8 ± 0.05 .

Tolerance: no less than 80% (Q) of the declared quantity of $C_{14}H_{10}Cl_2KNO_2$ dissolve in 60 minutes.

PURITY TESTS

Related substances. Proceed as described in method **B.** of Assay on the *Diclofenac potassium* monograph. Prepare *Solutions (1)* and *(2)* as described below.

Diluent: mixture of water and methyl alcohol (30:70).

Solution (1): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 50 mg of diclofenac potassium, transfer to a 50 mL volumetric flask and add 30 mL of *Diluent*. Leave in ultrasonic bath for 15 minutes and complete the volume with the same solvent, to obtain a solution at 1 mg/mL. Homogenize and filter.

Solution (2): transfer 2 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with *Diluent*. Transfer 1 mL of this solution to a 10 mL volumetric flask and complete the volume with the *Diluent*, to obtain a 2 µg/mL solution. Homogenize.

Procedure: inject, separately, 20 µL of *Solutions (1) and (2)*, record the chromatograms and measure the areas under the peaks. No secondary peak obtained with *Solution (1)* must present an area greater than the area under the main peak obtained in the chromatogram of *Solution (2)* (0.2%). The sum of all areas of all peaks, except the main peak, in the chromatogram of *Solution (1)* must not be greater than 2.5 times the area under the main peak, obtained in the chromatogram of *Solution (2)* (0.5%). In the chromatogram from *Solution (1)*, disregard any peak which area is smaller than 0.25 times the area under the main peak obtained in the chromatogram from *Solution (2)*.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 50 mg of diclofenac potassium, transfer to a 100 mL volumetric flask and add 70 mL of 0.1 M sodium hydroxide. Leave in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Filter. Transfer 5 mL of the

filtrate to a 50 mL volumetric flask, complete the volume with the same solvent and homogenize, to obtain a 50 µg/mL solution. Prepare a standard solution in the same conditions. Measure the absorbances of the solutions at 276 nm, using 0.1 M sodium hydroxide for zero adjustment. Calculate the amount of $C_{14}H_{10}Cl_2KNO_2$ in the tablets, from the readings obtained.

B. Proceed as described in method **B.** of Assay on the *Diclofenac potassium* monograph. Prepare the *Sample solution* as described below:

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 25 mg of diclofenac potassium, transfer to a 25 mL volumetric flask and add 15 mL of *Diluent*. Leave in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Transfer 1 mL of this solution to a 25 mL volumetric flask and complete the volume with the *Diluent*, to obtain a 40 µg/mL solution. Homogenize.

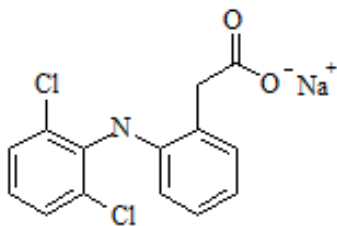
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{14}H_{10}Cl_2KNO_2$ in the tablets, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

DICLOFENAC SODIUM*Diclofenacum natricum* $C_{14}H_{10}Cl_2NNaO_2$; 318.13

diclofenac sodium; 02930

Sodium 2-[2-(2,6-dichloroanilino)phenyl]acetate

[15307-79-6]

Contains no less than 99.0% and no more than 101.0% of $C_{14}H_{10}Cl_2NNaO_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder. Hygroscopic.

Solubility. Freely soluble in methyl alcohol, soluble in ethyl alcohol and moderately soluble in water.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the diclofenac sodium CRS spectrum, prepared identically.

B. Weigh 10 mg of the sample and dissolve in 10 mL of ethyl alcohol R. Transfer to 1 mL of the solution obtained 0.2 mL of a solution prepared in advance with equal amounts of 0.6% potassium ferricyanide and 0.9% ferric chloride. Allow to stand protected from light for five minutes. Add 3 mL of a 1.0% hydrochloric acid solution and allow to stand protected from light for another 15 minutes. A blue color appears and precipitate is formed.

C. The preparation obtained in *Aspect of the preparation* at 5% (w/v) complies with sodium ion reactions **(5.3.1.1)**.

PURITY TESTS

Aspect of the preparation. Prepare a 5% (w/v) sample solution in methyl alcohol. The preparation obtained is clear **(5.2.25)** and the absorptivity **(5.2.14)** at 440 nm is no more than 0.05.

Related substances. Proceed as described in *High performance liquid chromatography* **(5.2.17.4)**. Use a chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m); maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Phosphate buffer pH 2.5: homogenize equal volumes of 0.01 M phosphoric acid and 0.01 M monobasic sodium phosphate. If necessary, adjust the pH to 2.5 ± 0.2 with an appropriate component.

Mobile phase: mixture of methyl alcohol and phosphate buffer pH 2.5 (70:30), filtered and degassed.

Diluent solution: mixture of methyl alcohol and water (70:30).

Standard solution: prepare, accurately, a 0.75 mg/mL solution of diclofenac impurity A CRS in methyl alcohol. Dilute an adequate amount of this solution in *Diluent solution* to obtain a solution at 1.5 µg/mL.

Resolution solution: prepare, accurately, a solution in *Diluent solution* with 20 µg of diethyl phthalate, 7.5 µg of diclofenac impurity A CRS and 0.75 mg of diclofenac CRS per mL.

Sample solution: prepare, accurately, a solution at 0.75 mg/mL of diclofenac sodium in *Diluent solution*.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*. Record all chromatograms for a period corresponding to 2.5 times the diclofenac CRS retention time. Calculate the percentage of diclofenac impurity A CRS in relation to diclofenac sodium, through this formula:

$$10(C/W)(r_u/r_s)$$

where C is the concentration in µg/mL of diclofenac impurity A CRS in the standard *Solution*; W is the amount in mg of diclofenac sodium in the *Sample solution*; and r_u and r_s are the responses obtained for the peaks of diclofenac impurity A CRS in the *Sample solution* and in the *Standard solution*, respectively. The percentage found is no more than 0.2%. Calculate the percentage for any other impurity present through this formula:

$$10(C/W)(r_i/r_s)$$

Where r_i is the response obtained for the impurity in the *Sample solution* and the other terms are the same described previously. The percentage found is no more than 0.2%. The sum of all percentages obtained is no more than 0.5%.

pH (5.2.19). 7.0 to 8.5. Determine in solution at 1.0% (w/v).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry at 105 °C for three hours. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 0.25 g of the sample previously desiccated and dissolve in 50 mL of glacial acetic acid. Titrate with 0.1 *M* perchloric acid and determine the endpoint potentiometrically. Carry out the blank test and make corrections, if necessary. Each mL of 0.1 *M* perchloric acid is equivalent to 31.81 mg of diclofenac sodium.

PACKAGING AND STORAGE

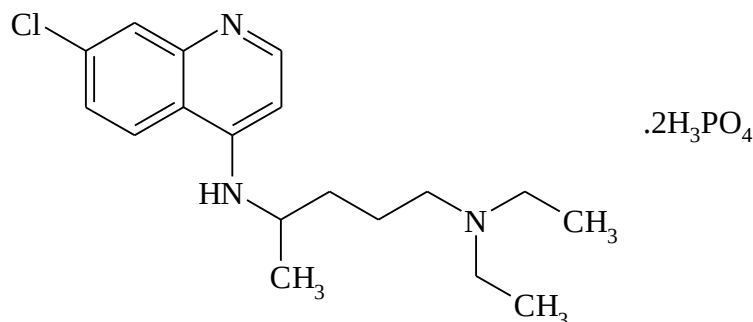
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-inflammatory.

CHLOROQUINE DIPHOSPHATE*Cloroquini diphosphas* $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$; 515.87

chloroquine diphosphate; 02489

*N*⁴-(7-chloro-4-quinolinyl)-*N*¹,*N*¹-diethyl-1,4-pentanediamine bis(dihydrogene phosphate)
[50-63-5]

Contains no less than 98.0% and no more than 102.0% of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or nearly white hygroscopic, crystalline powder. Presents polymorphism.

Solubility. Freely soluble in water, very slightly soluble in ethyl alcohol and methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 193 °C to 195 °C for one of the polymorphs and 215 °C to 218 °C for the other polymorph.

IDENTIFICATION

The identification tests B. and C. can be omitted if tests A. and D. are carried out. The identification test A. can be omitted if tests B., C. and D. are carried out.

A. Weigh 0.1 g of the sample, transfer to a separation funnel with 10 mL of water. Transfer 2 mL of 2 M sodium hydroxide and extract with two portions of 20 mL of methylene chloride. Combine the organic extracts, wash with water, dry with sodium sulfate anhydrous and evaporate to dryness. In the infrared absorption spectrum (5.2.14) of the residue, dissolved in 2 mL of methylene chloride, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of chloroquine diphosphate CRS, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm of the sample solution obtained in method B. from Assay, there are maximum and minimum points only at the

same wavelengths of similar chloroquine diphosphate CRS solution. The ratio between the absorbance values measured at 343 nm and 329 nm is comprised between 1.00 and 1.15.

C. Weigh 25 mg of the sample, dissolve in 20 mL of water and add 8 mL of 1% (w/v) picric acid. A yellow precipitate is formed. Wash the precipitate, successively, with water, ethyl alcohol and methylene chloride. Allow to dry. The residue melts between 206 °C and 209 °C.

D. Weigh 0.1 g of the sample, dissolve in 10 mL of water, add 2 mL sodium of 2 M sodium hydroxide and extract with two portions of 20 mL of methylene chloride. The aqueous layer, acidified with nitric acid, complies with the phosphate ion reaction 2 (**5.3.1.1**).

PURITY TESTS

pH (5.2.19). 3.8 to 4.3. Determine in 10.0% (w/v) solution in carbon dioxide-free water.

Loss on drying (5.2.9.1). Determine in 1 g of sample, in oven at 105 °C, for 16 hours. No more than 2.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.2 g of the sample and dissolve in 50 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 25.794 mg of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh 0.1 g of the sample and transfer to a 100 mL volumetric flask. Add 5 mL of water and dissolve. Complete the volume with 0.1% (w/v) hydrochloric acid. Dilute, successively, in 0.1% (w/v) hydrochloric acid, to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 343 nm, using 0.1% (w/v) hydrochloric acid for zero adjustment. Calculate the amount of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ in the sample, from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimalarial.

CHLOROQUINE DIPHOSPHATE TABLETS

Contains no less than 93.0% and no more than 107.0% of the declared amount of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of chloroquine diphosphate, transfer to a separation funnel and dissolve in 10 mL of water. Transfer 2 mL of 2 M sodium hydroxide and extract with two portions of 20 mL of chloroform. Combine the organic extracts, wash with water, dry with sodium sulfate anhydrous and evaporate to dryness. In the infrared absorption spectrum (**5.2.14**) of the residue, dissolved in 2 mL of chloroform, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of chloroquine diphosphate CRS, prepared identically.

B. Pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.1 g of chloroquine diphosphate and transfer to a 100 mL volumetric flask. Add 70 mL of water, leave in ultrasonic bath for 10 minutes and complete the volume with the same solvent (use this solution also on tests **C.** and **D.** of *Identification*). Filter. Dilute, successively, with water until concentration of 0.001% (w/v). In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the resulting solution, there are maximum and minimum points only at the same wavelengths of similar chloroquine diphosphate CRS solution. The ratio between the absorbance values measured at 343 nm and 329 nm is comprised between 1.00 and 1.15.

C. Add 5 mL of picric acid RS1 to 20 mL of the first solution obtained on test **B.** of *Identification*. A yellow precipitate is formed. Filter and wash the precipitate with water until the last washing water is colorless. Dry over silica gel. The residue obtained melts between 205 °C and 210 °C.

D. The solution obtained in test **B.** of *Identification* complies with the phosphate ion reactions (**5.3.1.1**).

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute with water until adequate concentration. Measure the absorbances of the solutions at 343 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the amount of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ dissolved in the medium, comparing the readings obtained with that of the chloroquine diphosphate CRS solution at the concentration of 0.002% (w/v), prepared in the same solvent.

Tolerance: no less than 75% (Q) of the declared quantity of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.5 g of chloroquine diphosphate and dissolve with 20 mL of *M* sodium hydroxide. Transfer, quantitatively, to a 250 mL separation funnel and extract with four portions of 25 mL of chloroform. Combine the chloroform extracts and evaporate in water bath to the volume of 10 mL. Add 40 mL of acetic anhydride and titrate with 0.1 *M* perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 *M* perchloric acid VS is equivalent to 25.794 mg of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$.

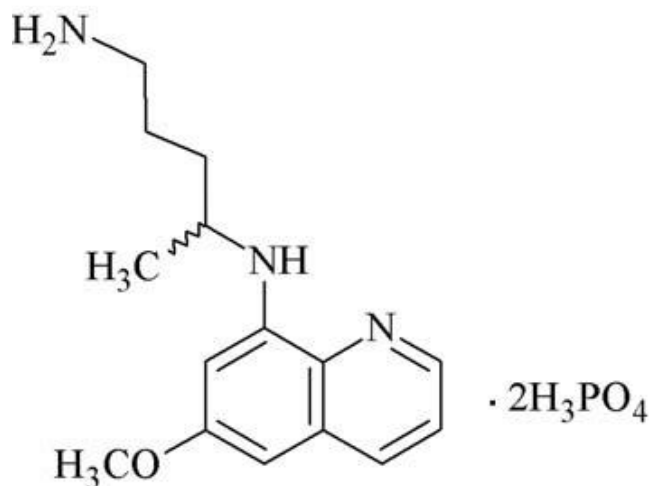
B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.8 g of chloroquine diphosphate, transfer to a 200 mL volumetric flask and add 100 mL of water. Shake mechanically for 10 minutes and complete the volume with the same solvent. Homogenize. Filter, discarding the first 50 mL of the filtrate. Transfer 50 mL of the filtrate to a separation funnel and add 5 mL of 6 *M* ammonium hydroxide. Shake and extract with five portions of 25 mL of chloroform. Collect the chloroform extracts and wash with 10 mL of water. Wash the aqueous phase with 10 mL of chloroform. Evaporate the combined chloroform extracts in water bath to the volume of 10 mL. Add 50 mL of 0.1% (v/v) hydrochloric acid and continue evaporating until the odor of chloroform is no longer perceptible. Transfer the resulting solution to a 200 mL volumetric flask, washing the vial walls with 0.1% (v/v) hydrochloric acid, and complete the volume with the same solvent. Dilute, successively, in 0.1% (v/v) hydrochloric acid, to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using 0.1% (v/v) hydrochloric acid as solvent. Measure the absorbances of the resulting solutions at 343 nm, using the same solvent for zero adjustment. Calculate the amount of $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ in the tablets from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at controlled temperature.

LABELING

Comply with current legislation.

PRIMAQUINE DIPHOSPHATE*Primaquini diphosphas*

$C_{15}H_{21}N_3O \cdot 2H_3PO_4$; 455.34

primaquine diphosphate; 07367

*N*4-(6-methoxy-8-quinolinyl)-1,4-pentanediamine phosphate (2:1)

[63-45-6]

Contains no less than 98.0% and no more than 102.0% of $C_{15}H_{21}N_3O \cdot 2H_3PO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Orange crystalline powder.

Solubility. Soluble in water, practically insoluble in ethyl alcohol.

Physical-chemical characteristics.

Melting range (5.2.2): 197 °C to 198 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the primaquine diphosphate CRS spectrum, prepared identically.

B. The residue obtained by sample ignition complies with phosphate ion reactions (5.3.1.1), but the precipitate obtained with the addition of silver nitrate RS is white, and the one obtained with the addition of ammonium molybdate RS is yellow.

PURITY TESTS

pH (5.2.19). 2.5 to 3.5. Determine in aqueous solution at 1.0% (w/v).

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 261 nm; column with 200 mm of length and 4.6 mm of internal diameter, packed with silica gel (10 µm), maintained at room temperature; *Mobile phase* flow of 3 mL/minute.

Mobile phase: mixture of *n*-hexane, chloroform, methyl alcohol and concentrated ammonia solution (45:45:10:0.1).

Solution (1): accurately weigh 50 mg of primaquine diphosphate CRS, transfer to a 5 mL volumetric flask in water and complete the volume with the same solvent. Transfer to 1 mL of this solution 0.2 mL of concentrated ammonia solution and homogenize with 10 mL of the *Mobile phase*. Use the lower clear layer.

Solution (2): accurately weigh 50 mg of the sample, transfer to a 5 mL volumetric flask in water and complete the volume with the same solvent. Transfer to 1 mL of this solution 0.2 mL of concentrated ammonia solution and homogenize with 10 mL of the *Mobile phase*. Use the lower clear layer.

Solution (3): dilute 3 mL of *Solution (2)* to 100 mL with the *Mobile phase*.

Solution (4): dilute 1 mL of *Solution (2)* to 10 mL with the *Mobile phase*. Dilute 1 mL of the resulting solution to 50 mL with the *Mobile phase*.

Procedure: inject, separately, 20 µL of each solution and record the chromatograms for no less than twice the retention time of the main peak. The sum of the areas of all peaks obtained with *Solution (2)*, except that of the solvent peak, is no greater than the area under the main peak, obtained with *Solution (3)* (3%). Do not consider peaks with area smaller than that presented by the main peak in the chromatogram obtained with *Solution (4)* (0.2%). The test is only valid if the chromatogram obtained with *Solution (1)* presents, before the main peak, a peak with area of approximately 6% of the primaquine peak; the resolution, between the two peaks is no less than 2.0 and, in the chromatogram obtained with *Solution (4)*, the signal/noise ratio is higher than 5.

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C, for two hours. No more than 1.0%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh 0.2 g of the sample and dissolve in 40 mL of glacial acetic acid, moderately heating. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 22.767 mg of C₁₅H₂₁N₃O.2H₃PO₄.

PACKAGING AND STORAGE

In hermetically closed amber vials, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimalarial.

PRIMAQUINE DIPHOSPHATE TABLETS

Contains no less than 93.0% and no more than 107.0% of the declared amount $C_{15}H_{21}N_3 \cdot O \cdot 2H_3PO_4$.

IDENTIFICATION

A. Pulverize the tablets. Accurately weigh an amount of powder with the equivalent to 60 mg of primaquine and transfer to a separation funnel. Add 10 mL of water, 2 mL of 2 M sodium hydroxide and extract with two portions of 20 mL of chloroform, shaking for 10 minutes. Filter in a filter with sodium sulfate anhydrous, evaporate to dryness and dissolve the residue in 2 mL of chloroform. In the infrared absorption spectrum (**5.2.14**) of the solution obtained, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of primaquine diphosphate CRS, prepared identically.

B. Pulverize the tablets. Accurately weigh an amount of powder with the equivalent to 25 mg of primaquine diphosphate, dissolve in 10 mL of water and filter. The filtrate, after neutralization with 2 mL of 2 M nitric acid, complies with the phosphate ion reactions (**5.3.1.1**).

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium, filter and proceed as described in *High performance liquid chromatography (5.2.17.4)*, using a chromatograph equipped with an ultraviolet detector at 254 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with octadecylsilane groups chemically bonded to porous silica or ceramic particles (3 mm to 10 mm); *Mobile phase* flow of 2.0 mL/minute.

Aqueous sodium 1-pentanesulfonate solution: weigh 961 mg of sodium 1-pentanesulfonate and dissolve using 1 mL of glacial acetic acid to 400 mL of water. Homogenize.

Mobile phase: filtered and degassed mixture of methyl alcohol and *Aqueous sodium 1-pentanesulfonate solution* (60:40).

Sample solution: after the test, take an aliquot from the dissolution medium, filter and dilute, if necessary, with dissolution medium, until adequate concentration.

Standard solution: accurately weigh an amount of primaquine diphosphate CRS and dilute in 0.1 M hydrochloric acid to obtain a solution at 0.003% (w/v).

Procedure: inject, separately, 20 mL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{15}H_{21}N_3O \cdot 2H_3PO_4$ dissolved in the medium from the responses obtained with the *Standard* and *sample solutions*.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{15}H_{21}N_3O \cdot 2H_3PO_4$ dissolve in 45 minutes.

PURITY TESTS

Water (5.2.20.1). No more than 4%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.15 mg of primaquine diphosphate and dissolve in 20 mL of water. Transfer, quantitatively, to a 250 mL separation funnel. Add 5 mL of 2 M sodium hydroxide and extract with four portions of 25 mL each of chloroform. Combine the chloroform extracts and evaporate to a volume of approximately 10 mL. Add 40 mL of glacial acetic acid and titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 22.768 mg of $C_{15}H_{21}N_3O \cdot 2H_3PO_4$.

PACKAGING AND STORAGE

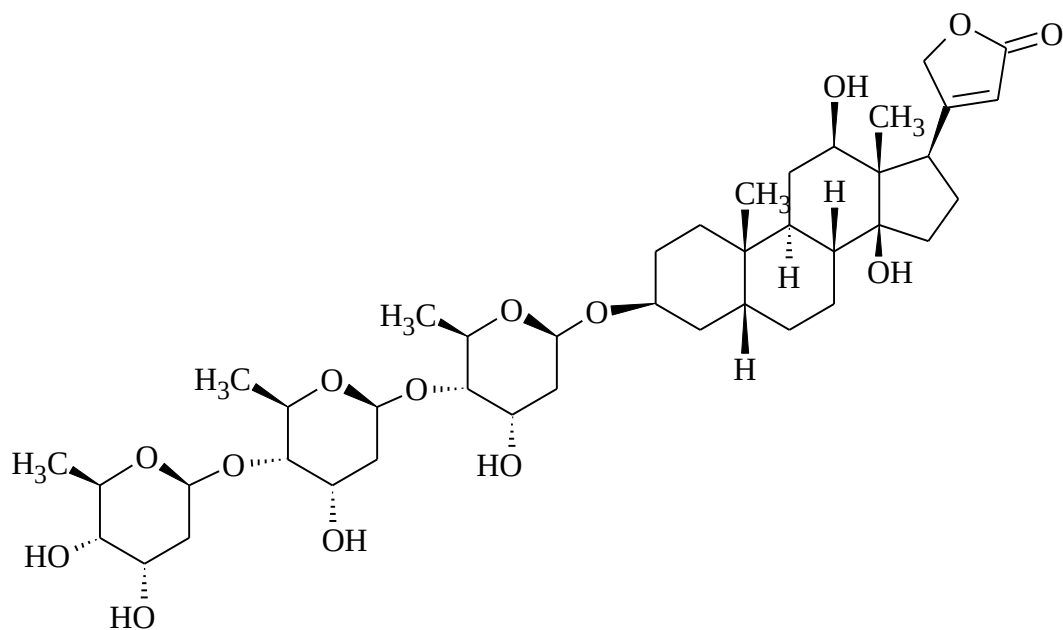
In hermetically closed containers and protected from light.

LABELING

Comply with current legislation.

DIGOXIN

Digoxinum



$C_{41}H_{64}O_{14}$; 780.94

digoxin; 03010

(3 β ,5 β ,12 β)-3-[(O-2,6-Dideoxy- β -D-ribo-hexopyranosyl-(1 $\rightarrow$ 4)-O-2,6-dideoxy- β -D-ribo-hexopyranosyl-(1 $\rightarrow$ 4)-2,6-dideoxy- β -D-ribo-hexopyranosyl)oxy]-12,14-dihydroxy-card-20(22)-enolide

[20830-75-5]

Contains no less than 95.0% and no more than 103.0% of $C_{41}H_{64}O_{14}$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white powder or crystals.

Solubility. Practically insoluble in water, soluble in a mixture of ethyl alcohol and water and in pyridine.

Physicochemical constants.

Specific optical rotation (5.2.8): +10.0 to +13.0, in relation to the desiccated substance. Determine in solution at 2.0% (w/v) in pyridine anhydrous.

IDENTIFICATION

The identification test A. can be omitted if tests B., D. and E. are carried out. The identification test A. can be omitted if tests C., D. and E. are carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated in oven under reduced pressure at 105 °C for one hour and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the digoxin CRS spectrum, prepared identically.

B. The main stain of the chromatogram from *Solution (1)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. Accurately weigh 0.5 mg of sample and dissolve in 0.2 mL of 60% (v/v) ethyl alcohol. Add 0.1 mL of 2% (w/v) 3,5-dinitrobenzoic acid in ethyl alcohol and 0.1 mL of sodium hydroxide RS. A violet color develops.

E. Accurately weigh 0.5 mg of the sample, dissolve in 1 mL of glacial acetic acid, slightly heat, wait to cool and transfer 0.05 mL of ferric chloride RS. Add 1 mL of sulfuric acid, avoiding to homogenize the two phases. A brown ring develops in the interface, which must change to green. Soon after, the superior phase must change to blue.

PURITY TESTS

Aspect of the preparation. The preparation at 0.5% (w/v) in mixture of methyl alcohol and methylene chloride (1:1) is clear (**5.2.25**) and colorless (**5.2.12**).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using reverse phase silica gel, chemically bonded to octadecylsilane group, as support, and a mixture of methyl alcohol and water (7:3) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in mixture of chloroform and methyl alcohol (2:1).

Solution (2): 10 mg/mL solution of digoxin CRS in a mixture of chloroform and methyl alcohol (2:1).

Solution (3): 0.3 mg/mL solution of gitoxin CRS in a mixture of chloroform and methyl alcohol (2:1).

Develop the chromatogram. Remove the plate and allow it to air dry. Nebulize with trichloroacetic-chloramine-T acid RS. Heat in oven at 110 °C for 10 minutes and examine under ultraviolet light (365 nm). Any secondary stain obtained in the chromatogram of *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (3)* (3.0%).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample, in oven under reduced pressure at 105 °C, for one hour. No more than 1.0%.

Residue by incineration (5.2.10). Determine in the sample submitted to the test of *loss on drying*. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 40 mg of the sample and dissolve in ethyl alcohol. Heat if necessary and complete the volume to 50 mL with the same solvent. Dilute, successively, in ethyl alcohol until concentration of 0.004% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Transfer to 5 mL of each diluted solution 3 mL of alkaline sodium picrate RS and allow to stand, for 30 minutes, protected from light. Measure the absorbances of the sample and standard solutions at 495 nm, using a mixture of 5 mL of ethyl alcohol and 3 mL of alkaline sodium picrate RS for zero adjustment. Calculate the amount of $C_{41}H_{64}FO_{14}$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 218 nm; column with 250 mm of length and 4.2 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group; *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: mixture of water and acetonitrile (70:30).

Sample solution: accurately weigh 20 mg of the sample and transfer to a 50 mL volumetric flask. Add 35 mL of mixture of ethyl alcohol and water (1:1) and leave in ultrasonic bath for 30 minutes. Complete the volume and homogenize. Dilute with the same solvent, to obtain a 40 µg/mL solution.

Standard solution: accurately weigh 20 mg of digoxin CRS and transfer to a 50 mL volumetric flask. Add 35 mL of mixture of ethyl alcohol and water (1:1) and leave in ultrasonic bath for 30 minutes. Complete the volume and homogenize. Dilute with the same solvent, to obtain a 40 µg/mL solution.

The column efficiency must be no less than 4800 theoretical plates/meter. The tailing factor of the digoxin peak must be no more than 2.0. The relative standard deviation of the replicate areas from the recorded peaks must be no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{41}H_{64}FO_{14}$ in the sample, from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Cardiotonic glycoside.

DIGOXIN TABLETS

Contains not less than 90.0% and not more than 110.0% of the declared amount of $C_{41}H_{64}O_{14}$.

IDENTIFICATION

A. Proceed as described in *Related substances* in the *Digoxin* monograph, using the following solutions.

Solution (1): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.5 mg of digoxin and transfer to a centrifuge tube using 2 mL of a mixture of chloroform and methyl alcohol (2:1). Shake for 10 minutes and centrifuge. Decant and use the clear supernatant.

Solution (2): 0.25 mg/mL digoxin CRS solution in a mixture of chloroform and methyl alcohol (2:1).

Develop the chromatogram. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 10 mL volumetric flask containing 7 mL of a mixture of ethyl alcohol and water (1:1) and wait for the tablet to completely disintegrate. Leave in ultrasonic bath for 30 minutes and complete the volume with the same diluent. Homogenize and filter. Proceed as described in method **B.** from *Assay*. Prepare *Standard solution* in the same concentration as the *Sample solution*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid; 500 mL

Apparatus: baskets, 120 rpm

Time: 60 minutes

Standard solution: accurately weigh 25 mg of digoxin CRS, transfer to a 500 mL volumetric flask and dissolve with a small amount of ethyl alcohol. Complete the volume with 80% (v/v) ethyl alcohol and homogenize. Transfer an aliquot of 10 mL of this solution to a 100 mL volumetric flask and complete the volume with 80% (v/v) ethyl alcohol. Transfer aliquots of this solution to a 50 mL

volumetric flask to prepare a standard curve equivalent to 20%, 40%, 60%, 80% and 100% of the declared amount of digoxin in 500 mL and complete the volume with the *Dissolution medium*.

Procedure: after the test, take an aliquot from the dissolution medium, filter in filter with porosity inferior to 0.8 mm and discard the first 10 mL. Transfer, to individual vials with cap, in duplicate, 1 mL of the sample solution, 1 mL of the standard curve solution and 1 mL of the *Dissolution medium* to prepare the blank and add, quickly, the following reagents: 1 mL of 0.2% (w/v) ascorbic acid solution in methyl alcohol, 5 mL of hydrochloric acid and 1 mL of methanolic hydrogen peroxide. Shake after adding each reagent. Close the vials and after two hours measure the fluorescence of the solutions at excitation wavelength of 372 nm and emission wavelength of 485 nm. To verify the stability of the fluorometer, repeat the fluorescence reading in the standard curve solutions. Correct the readings by the blank and analyze the results plotting the standard fluorescence curve due to the percentage of dissolution.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{41}H_{64}O_{14}$ dissolve in 60 minutes. If there is the need to carry out the step E_2 (5.1.5), the acceptance criterion of the average of 12 units is equal to or greater than Q and no unit presents result below Q - 5%.

Attention. The dissolution tanks must be washed, successively, before the test with hydrochloric acid, water and ethyl alcohol and carefully dried. Such precautions are taken to prevent contaminations by metallic particles coming from cleaning materials.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 1.25 mg of digoxin, add 3 mL of water and shake. Allow to stand for 10 minutes and shake occasionally. Add 25 mL of glacial acetic acid, shake for one hour and filter. Transfer 4 mL of the filtrate and 1 mL of dimethyl sulfoxide to a 25 mL volumetric flask. Complete the volume with xanthidrol reagent, homogenize and allow to stand, protected from light, for four hours. Prepare standard solution in the same conditions, using the same solvents. Prepare the blank using the same solvents. Measure the absorbances of the resulting solutions at 545 nm, using blank for zero adjustment. Calculate the amount of $C_{41}H_{64}Cl_{14}$ in the tablets, from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 218 nm; column with 250 mm of length and 4.2 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group; *Mobile phase* flow of 2.0 mL/minute.

Mobile phase: mixture of water and acetonitrile (70:30).

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 1 mg of digoxin and transfer to a 25 mL volumetric flask. Add 15 mL of the mixture of ethyl alcohol and water (1:1) and leave in ultrasonic bath for 30 minutes. Complete the volume and homogenize, to obtain a 40 µg/mL solution.

Standard solution: accurately weigh 20 mg of standard digoxin and transfer to a 50 mL volumetric flask. Add 35 mL of mixture of ethyl alcohol and water (1:1) and leave in ultrasonic bath for 30 minutes. Complete the volume with the same solvent and homogenize. Dilute, successively, with the same solvent to obtain a 40 µg/mL solution.

The column efficiency must be no less than 4800 theoretical plates/meter. The tailing factor of the digoxin peak must be no more than 2. The relative standard deviation of the replicate areas from the recorded peaks must be no more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₄₁H₆₄O₁₄ in the tablets, from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

DIGOXIN ORAL SOLUTION

Contains no less than 90% and no more than 105% of the declared amount of $C_{41}H_{64}O_{14}$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using reverse phase silica gel plate, chemically bonded to octadecylsilane group, with 0.25 mm of thickness, as support, and a mixture of methyl alcohol and water (7:3) as mobile phase. Apply to the plate, separately, 10 μ L of each of the freshly prepared solutions described below.

Solution (1): transfer a volume of oral solution equivalent to 0.5 mg of digoxin and 5 mL of water to a separation funnel. Extract with three portions of 10 mL of chloroform. Combine the organic extracts and evaporate to dryness in water bath with the help of air stream for drying. In the case of remaining traces of water or propylene glycol, dry under reduced pressure at 100 °C for 30 minutes. Dissolve the residue in 2 mL of a mixture of chloroform and methyl alcohol (2:1).

Solution (2): accurately weigh an amount of digoxin CRS in a mixture of methyl alcohol and water (7:3) and dilute with the same solvent to obtain a 0.25 mg/mL solution.

Develop the chromatogram along 15 cm. Remove the plate and allow it to air dry. Nebulize with freshly prepared trichloroacetic-chloramine-T acid RS. Heat in oven at 110 °C for 10 minutes and examine under ultraviolet light (365 nm). The main stain obtained with *Solution (1)* corresponds in position to that obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 218 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 μ m to 10 μ m), maintained at temperature of 40°C; *Mobile phase* flow of 0.5 mL/minute.

Mobile phase: mixture of water, acetonitrile and isopropyl alcohol (70:27.5:2.5).

Sample solution: transfer accurately a volume of the oral solution, equivalent to 0.5 mg of digoxin, to a 25 mL volumetric flask. Complete the volume with 42% (w/w) ethyl alcohol and homogenize.

Standard solution: accurately weigh an amount of dexamethasone digoxin CRS and dissolve in 42% (w/w) ethyl alcohol to obtain a 20 µg/mL solution.

Inject 10 µL replicates of the *Standard solution*. The tailing factor is no more than 2.0. The relative standard deviation of the replicate areas of the recorded peaks is not more than 2.0%.

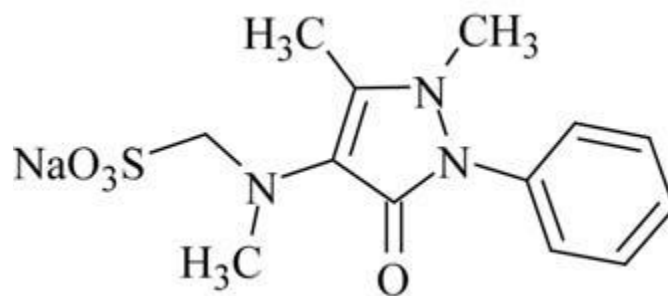
Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₄₁H₆₄O₁₄ in the oral solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

DIPYRONE MONOHYDRATE*Dipyronum monohydricum*C₁₃H₁₆N₃NaO₄S.H₂O; 351.35

dipyrone monohydrate; 09564

1-[(2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1H-pyrazol-4-yl)methylamino] methanesulfonic acid sodium salt hydrate (1:1:1)

[5907-38-0]

Contains no less than 99.0% and no more than 100.5% of C₁₃H₁₆N₃NaO₄S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Off-white crystalline powder.

Solubility. Soluble in water and methyl alcohol, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the dipyrone CRS spectrum, prepared identically.

B. The 5% aqueous sample solution complies with sodium ion reactions (**5.3.1.1**).

PURITY TESTS

Aspect of the preparation. Weigh 2.5 g of the sample, dissolve in carbon dioxide-free water and complete the volume to 50 mL with the same solvent. The preparation is clear (**5.2.25**). Immediately after the preparation, compare 5 mL of the sample solution with 5 mL of the *Color standard solution*, described next. The color is not more intense than that of the color standard solution (**5.2.12**).

Color standard solution: homogenize 0.75 mL of *Solution (1)*, 0.25 mL of *Solution (2)*, 0.25 mL of *Solution (3)* and 48.75 mL of *Solution (4)*.

Solution (1): weigh 4.51 g of ferric chloride, dissolve in 3.2 mL of *M* hydrochloric acid and complete the volume with water to 100 mL.

Solution (2): weigh 6.5 g of cobalt chloride, dissolve in 3 mL of 6 M hydrochloric acid and complete the volume with water to 100 mL.

Solution (3): weigh 6.242 g of cupric sulfate pentahydrate, dissolve in water and complete the volume to 100 mL.

Solution (4): 1% (w/v) hydrochloric acid.

Acidity or alkalinity. Add 0.1 mL of phenolphthalein TS to 5 mL of the preparation obtained in *Aspect of the preparation*. The preparation color does not change. The indicator turning pink consumes no more than 0.1 mL of 0.02 M sodium hydroxide in relation to the blank.

Chloroform-soluble impurities. Weigh 1 g of sample, add 10 mL of chloroform and allow to stand for 30 minutes. Filter and wash twice with 5 mL of chloroform. Evaporate in water bath and dry at 105 °C until constant weight. No more than 0.5%.

Heavy metals (5.3.2.3). Proceed as described in *Method I*. No more than 0.002% (20 ppm).

Sulfates (5.3.2.2). No more than 0.1% (1000 ppm).

Loss on drying (5.2.9.1). Determine in 0.25 g of the sample. Dry in oven at 105 °C until constant weight. No less than 4.9% and no more than 5.3%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh 0.35 g of sample and dissolve in 50 mL of water. Add 3 mL of 6% (v/v) acetic acid and titrate with 0.05 M iodine VS at temperature below 20 °C, using starch TS. Carry out the blank test and make the necessary corrections. Each mL of 0.05 M iodine VS is equivalent to 16.67 mg of $C_{13}H_{16}N_3NaO_4S$ or to 17.57 mg of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Analgesic and antipyretic.

DIPYRONE MONOHYDRATE TABLETS

Contains no less than 95.0% and no more than 105.0% of the declared amount of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$.

IDENTIFICATION

A. Weigh and pulverize 20 tablets. Weigh an amount of powder equivalent to 0.5 g of dipyrone monohydrate and add a few drops of concentrated hydrogen peroxide. A blue color develops and will quickly disappear, turning into intense red (strongly exothermal reaction).

B. Mix 0.5 g of powder from tablets with a few drops of 10% (w/v) potassium persulfate. An intense yellow color is developed after five minutes of reaction.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit dose (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 500 mL.

Apparatus: paddles, 50 rpm

Time: 45 minutes

Procedure: after the test, take an aliquot of the dissolution medium, filter and dilute in 0.1 M hydrochloric acid, until adequate concentration. Measure the absorbances of the solutions at 258 nm, using the same solvent for zero adjustment. Calculate the amount of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ dissolved in the medium, comparing the readings obtained with that of the dipyrone CRS solution at known concentration, prepared in the same solvent.

Tolerance: not less than 70% (Q) of the declared quantity of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh and pulverize 20 tablets. Weigh an amount of powder equivalent to 0.35 g of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ and transfer, quantitatively, to an Erlenmeyer flask. Add 25 mL of water, 5 mL of glacial acetic acid and shake until homogeneous dispersion. Titrate with 0.05 M iodine VS at temperature below 15 °C, using 1 mL of starch TS as indicator. Each mL of 0.05 M iodine VS is equivalent to 17.57 mg of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

DIPYRONE MONOHYDRATE ORAL SOLUTION

Contains no less than 95.0% and no more than 110.0% of the declared amount of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$.

IDENTIFICATION

A. Transfer to 2 mL of the oral solution 2 mL of 30% (w/w) hydrogen peroxide. A blue color is developed and quickly disappears, turning into an intense red.

B. Transfer to 2 mL of the oral solution 2 mL of 10% (w/v) potassium persulfate. An intense yellow color develops.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.5 to 7.0.

Drip test (5.1.8). Dipyrone oral solution packaged in containers with the integrated measuring device complies with the test.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

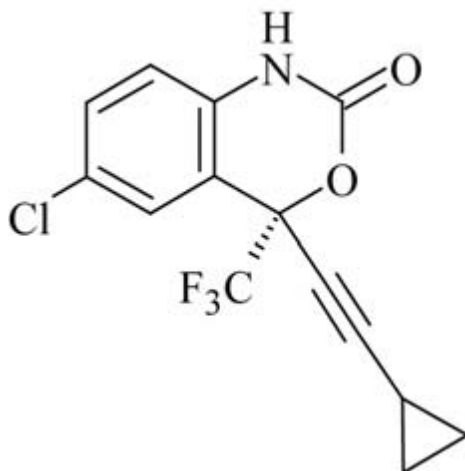
Transfer volume of the oral solution corresponding to 5 g of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ to a 200 mL volumetric flask. Complete the volume with water and homogenize. Transfer 10 mL of the solution, 50 mL of water, 5 mL of glacial acetic acid to an Erlenmeyer flask and homogenize. Titrate with 0.05 M iodine VS at temperature below 15 °C, using starch TS as indicator. Each mL of 0.05 M iodine VS is equivalent to 17.57 mg of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

EFAVIRENZ*Efavirenzum*C₁₄H₉ClF₃NO₂; 315.67

efavirenz; 03308

(4S)-6-Chloro-4-(2-cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one
[154598-52-4]

Contains no less than 98.0% and no more than 102.0% of C₁₄H₉ClF₃NO₂ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, soluble in methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 136 °C to 141 °C.

Specific optical rotation (5.2.8): -86 to -98, in relation to the desiccated substance. Determine in solution at 0.3% (w/v) in methyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the efavirenz CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 350 nm, of the 0.001% (w/v) solution in methyl alcohol, there are maximum points at 206 nm, 247 nm and 293 nm, identical to the ones observed in the spectrum of a similar efavirenz CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to that from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with ultraviolet detector at 250 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to cyan group (5 µm), maintained at temperature of 30 °C; *Mobile phase* flow of 1.5 mL/minute.

Note: trifluoroacetic acid must be preferably used up to six months after opening the vial.

Eluent (A): mixture of water, methyl alcohol and trifluoroacetic acid (90:10:0.05).

Eluent (B): mixture of water, methyl alcohol and trifluoroacetic acid (10:90:0.05).

Mobile phase: use the elution gradient described next:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 16	60 → 50	40 → 50	linear gradient
16 – 23	50 → 35	50 → 65	linear gradient
23 – 28	35 → 30	65 → 70	linear gradient
28 – 29	30 → 20	70 → 80	linear gradient
29 – 31	20	80	isocratic
31 – 32	20 → 60	80 → 40	linear gradient

Balance the column in the initial conditions for 30 minutes. Carry out a blank run using the gradient described before injecting *Solution (1)*, *Solution (2)* and *Solution (3)*. At the end of each run, rebalance the column for, at least, eight minutes before starting the new run.

Diluent: mixture of water and acetonitrile (1:1).

Solution (1): 500 µg/mL sample solution in *Diluent*.

Solution (2): 500 µg/mL efavirenz CRS solution in *Diluent*.

Solution (3): dilute *Solution (2)* with *Diluent* to obtain a 1.25 µg/mL efavirenz CRS solution.

Inject 35 µL replicates of *Solution (2)*. The column efficiency is no less than 30 000 theoretical plates/meter. Inject 35 µL replicates of *Solution (3)*. The relative standard deviation of the replicate areas of the recorded peaks is no more than 5.0%. Inject 35 µL replicates of *Solution (1)*. The relative retention times are approximately 0.93 for (4S)-6-chloro-4-[(1-E)-cyclopropylethenyl]-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one (*trans*-alkene impurity), if present, and 1.0 for efavirenz. The resolution between the peaks is no less than 1.7.

Procedure: inject, separately, 35 µL of *Solution (1)* and of *Solution (3)*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of the *trans*-alkene impurity, if present, in the sample according to the formula:

$$1.1 \times 100 \times (C_{S3} \cdot At / C_{S1} \cdot Ae)$$

where

1.1 = quantification factor for *trans*-alkene impurity;

C_{S1} = concentration of the sample, in mg/mL, on *Solution (1)*;

C_{S3} = concentration of efavirenz CRS, in mg/mL, on *Solution (3)*;

At = area under the peak corresponding to the *trans*-alkene impurity in the chromatogram obtained with *Solution (1)*;

Ae = area under the peak corresponding to efavirenz in the chromatogram obtained with *Solution (3)*.

No more than 0.15% of *trans*-alkene impurity. The sum of the areas of all peaks obtained with *Solution (1)*, except the ones corresponding to efavirenz and to the *trans*-alkene impurity, is not greater than the area under the main peak obtained with *Solution (3)* (0.5% from other impurities). Do not consider peaks related to the solvent.

Heavy metals (5.3.2.3). Use *Method III*. No more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 2.0 g of the sample. Dry in oven at 60 °C, under reduced pressure, for three hours. No more than 1.0%.

Water (5.2.20). No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.2%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh 50 mg of sample and dissolve in methyl alcohol. Complete the volume to 50 mL with the same solvent. Dilute, successively, in methyl alcohol until concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting sample and standard solutions at 247 nm, using methyl alcohol for zero adjustment. Calculate the amount of $C_{14}H_9ClF_3NO_2$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 252 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at room temperature; *Mobile phase* flow of 1.0 mL/minute.

Mobile phase: mixture of acetonitrile, water and orthophosphoric acid (70:30:0.1).

Diluent: use the *Mobile phase*.

Sample solution: accurately weigh 40 mg of the sample, transfer to a 100 mL volumetric flask, complete the volume with *Diluent* and homogenize. Transfer 5 mL of this solution to a 100 mL volumetric flask and complete the volume with *Diluent*, obtaining a 20 µg/mL solution.

Standard solution: accurately weigh 40 mg of efavirenz CRS, transfer to a 100 mL volumetric flask, complete the volume with *Diluent* and homogenize. Transfer 5 mL of this solution to a 100 mL volumetric flask and complete the volume with *Diluent*, obtaining a 20 µg/mL solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₄H₉ClF₃NO₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antirretroviral.

EFAVIRENZ TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{14}H_9ClF_3NO_2$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Weigh an amount of powder equivalent to 0.3 g of efavirenz and homogenize with 10 mL of ethyl ether for one minute. Filter in sintered glass funnel, applying vacuum, if necessary. Wash with two portions of 5 mL of ethyl ether and combine the ethereal extracts in a 50 mL beaker. Evaporate under air stream at room temperature. Dry the residue in oven at 60 °C for 30 minutes and cool at room temperature. Add 3 mL of heptane, heat in water bath at 70 °C and dissolve the residue using a spatula. Cover the beaker mouth with watch glass, cool in ice bath at -10 °C for five minutes and allow to stand at room temperature for 25 minutes. Filter under vacuum, wash the residue with three portions of 2 mL of heptane and divide the residue finely using a spatula, maintaining vacuum for 10 minutes. Dry in oven at 80 °C, under reduced pressure, for six hours. The residue complies with test **A.** of *Identification* from the *Efavirenz* monograph.

B. The residue obtained in test **A.** of *Identification* melts around 138 °C.

C. Weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.1 g of efavirenz and transfer to a 100 mL volumetric flask. Add 70 mL of methyl alcohol. Leave in ultrasonic bath for five minutes and complete the volume with the same solvent. Filter, discarding the first milliliters of the filtrate, and dilute with methyl alcohol to concentration of 0.002% (w/v). In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 350 nm, there are maximum points at 206 nm, 247 nm and 293 nm, identical to those observed in the spectrum of similar efavirenz CRS solution.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test. No more than 60 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 1% (w/v) sodium lauryl sulfate, 900 mL.

Apparatus: paddles; 100 rpm.

Time: 45 minutes.

Procedure: after the test, take an aliquot from the dissolution medium and dilute, if necessary, with dissolution medium until adequate concentration. Measure the absorbances at 247 nm **(5.2.14)**, using *Dissolution medium* for zero adjustment. Calculate the amount of $C_{14}H_9ClF_3NO_2$ dissolved in the medium, comparing the readings obtained with that of the efavirenz CRS solution at the concentration of 0.0012% (w/v) with *Dissolution medium*.

Tolerance: no less than 80% (Q) of the declared quantity of $C_{14}H_9ClF_3NO_2$ dissolve in 45 minutes.

PURITY TEST

Related substances. Proceed as described in *Related substances* in the *Efavirenz* monograph. Prepare *Solution (1)* as described next.

Solution (1): weigh and pulverize the tablets. Accurately weigh an amount of powder equivalent to 0.25 g of efavirenz and transfer to a 100 mL volumetric flask. Add 70 mL of *Diluent* and leave in ultrasonic bath for five minutes. Complete the volume with the same solvent, homogenize and allow to stand for 15 minutes. Filter and, if necessary, dilute with the same solvent to obtain a 250 µg/mL solution. No more than 0.15% of *trans*-alkene impurity and 1.0% of other impurities.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 0.15 g of efavirenz, transfer to a 100 mL volumetric flask and add 70 mL of absolute ethyl alcohol. Leave in ultrasonic bath for five minutes. Filter, if necessary, and dilute to a concentration of 0.0075% (w/v) using the same solvent. Prepare a standard solution in the same conditions. Measure the absorbances of the resulting solutions at 293 nm using absolute ethyl alcohol for zero adjustment. Calculate the amount of $C_{14}H_9ClF_3NO_2$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Proceed as described in method **B.** of Assay in the *Efavirenz* monograph. Prepare the *Sample solution* as described below:

Sample solution: weigh and pulverize 20 tablets. Accurately weigh an amount of powder equivalent to 40 mg of efavirenz, transfer to a 100 mL volumetric flask, add 40 mL of *Diluent* and leave in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL to a 100 mL volumetric flask and complete the volume with *Diluent*, obtaining a 20 µg/mL solution.

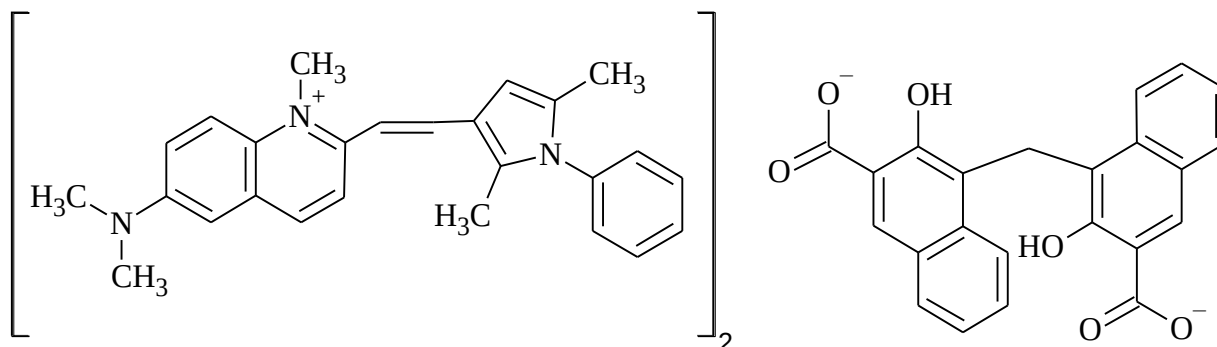
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₄H₉ClF₃NO₂ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

PYRVINIUM EMBONATE*Pyrvinii embonas* $(C_{26}H_{28}N_3)_2 \cdot C_{23}H_{14}O_6$; 1151.39

pyrvinium embonate; 03346

6-(Dimethylamino)-2-[2-(2,5-dimethyl-1-phenyl-1*H*-pyrrol-3-yl)ethenyl]-1-methyl-quinoline 4,4'-methylenebis[3-hydroxy-2-naphthalenecarboxylate] (1:2)
[3546-41-6]

Contains no less than 96.0% and no more than 104.0% of $(C_{26}H_{28}N_3)_2 \cdot C_{23}H_{14}O_6$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Light orange or orangey-red to nearly black crystalline powder.

Solubility. Practically insoluble in water, very slightly soluble in methyl alcohol. Freely soluble in glacial acetic acid.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the pyrvinium embonate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), at the range of 200 nm to 800 nm, of the sample solution obtained in Assay, there are maximum absorbance points around 358 nm and 505 nm. The ratio between the absorbance values measured is between 1.93 nm and 2.07.

PURITY TESTS

Water (5.2.20). Determine in 0.2 g of the sample, employing a mixture of 10 mL of methyl alcohol and 10 mL of chloroform as solvent. No more than 6.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. No more than 0.5%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Note: use vials with low actinism for the solutions, and also protect them from exposure to bright light. Carry out the assay without prolonged interruptions.

Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Accurately weigh about 0.25 g of the sample and dissolve in 125 mL of glacial acetic acid. Complete the volume to 250 mL with methyl alcohol and homogenize. Dilute, successively, in methyl alcohol until concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvents. Measure the absorbances of the standard solution and the sample solution at 505 nm, using methyl alcohol for zero adjustment. Calculate the amount of $(C_{26}H_{28}N_3)_2.C_{23}H_{14}O_6$ in the sample from the readings obtained.

PACKAGING AND STORAGE

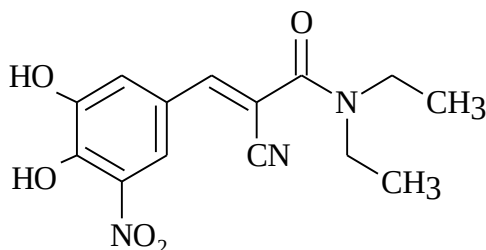
In hermetic, opaque containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anthelmintic (enterobioasis).

ENTACAPONE***Entacaponum***

$C_{14}H_{15}N_3O_5$; 305.29

entacapone; 03415

(2*E*) -2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N*, *N*-diethylprop-2-enamide

[130929-57-6]

Contains no less than 98.0% and no more than 102.0% of $C_{14}H_{15}N_3O_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Greenish-yellow or yellow powder. Presents polymorphism.

Solubility. Practically insoluble in water, moderately soluble in methyl alcohol, slightly soluble in ethyl alcohol.

IDENTIFICATION

The identification test B. can be omitted if test C. is carried out. The identification test C. can be omitted if test B. is carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the entacapone CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 220 nm to 500 nm of the sample solution obtained in method **A.** from Assay, there are maximum and minimum points identical to those observed in the spectrum of the standard solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described on method **B.** from Assay. Prepare the *Sample solution* as described next.

Solution (1): use the *Sample solution* obtained on method **B.** of Assay.

Procedure: inject 20 µL of *Solution (1)*, record the chromatograms and measure the areas under all the peaks obtained. The sum of the areas under the secondary peaks, except that under the solvent peak, is no more than 0.2% of the area under the main peak. No area is greater than 0.15% of the area under the main peak. Disregard any peaks with area smaller than 0.05 times the area under the main peak. Do not include solvent-related peaks in the calculations.

Heavy metals (5.3.2.3). Dissolve 2 g of the sample in a mixture of dimethylformamide and methyl alcohol (25:75 v/v). Prepare the reference solution, using 2 mL of standard lead solution (10 ppm Pb) in a mixture of dimethylformamide and methyl alcohol (25:75 v/v). After the filtration, wash the filter with at least 20 mL of methyl alcohol. Proceed as described in *Method II*. No more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 60 °C, under reduced pressure. No more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. No more than 0.1%.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 100 mg of the sample and dissolve in acetonitrile. Complete the volume to 100 mL with the same solvent. Dilute successively, with the same solvent, to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 305 nm, using acetonitrile for zero adjustment. Calculate the content of C₁₄H₁₅N₃O₅ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 305 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at temperature of 25 °C; *Mobile phase* flow of 2 mL/minute.

Mobile phase: mixture of water pH 3.0, adjusted in advance with 10% (v/v) phosphoric acid, and acetonitrile (65:35).

Sample solution: transfer, quantitatively, approximately 50 mg of the sample to a 100 mL volumetric flask, complete the volume with methyl alcohol and homogenize. Transfer 2 mL of this solution to a 50 mL volumetric flask and complete the volume with methyl alcohol, to obtain a 20 µg/mL entacapone solution.

Standard solution: dissolve an accurately weighed amount of entacapone CRS in methyl alcohol to obtain a 0.5 mg/mL solution. Transfer 2 mL of this solution to a 50 mL volumetric flask, complete the volume with methyl alcohol and homogenize, obtaining a solution at 20 µg/mL.

Inject 20 µL replicates of the *Standard solution*. The column efficiency for the entacapone peak is no less than 2000 theoretical plates/meter. The tailing factor is no more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is no more than 1.5%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₄H₁₅N₃O₅ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Catechol-*o*-methyltransferase (COMT) inhibitor.

ENTACAPONE TABLETS

Contains no less than 90.0% and no more than 110.0% of the declared amount of $C_{14}H_{15}N_3O_5$. The tablets must be coated.

IDENTIFICATION

The identification test A. can be omitted if test B. is carried out. The identification test B. can be omitted if test A. is carried out.

A. The ultraviolet absorption spectrum (**5.2.14**), in the range of 220 nm to 400 nm of the *Sample solution* obtained in method **A.** from *Assay*, displays maximum and minimum points only at the same wavelengths observed in the *Standard solution* spectrum.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF254 as support and a mixture of isobutanol, methyl alcohol, water and anhydrous formic acid (667:95:95:143) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Place in ultrasonic bath, for 15 minutes, an amount of powder equivalent to 10 mg of entacapone with 10 mL of methyl alcohol and filter.

Solution (2): 1 mg/mL entacapone CRS solution in methyl alcohol.

Develop the chromatogram. Remove the plate and allow it to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: acetate buffer pH 5.3, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Standard solution: transfer, quantitatively, about 11 mg of entacapone CRS to a 50 mL volumetric flask. Add 5 mL of methyl alcohol, leave in ultrasonic bath for 30 minutes and complete the volume with acetate buffer pH 5.3 solution. Dilute with methyl alcohol to obtain a 44 µg/mL entacapone solution.

Sample solution: after testing, use aliquots from the dissolution medium, filter and dilute, if necessary, in methyl alcohol, to obtain a 44 µg/mL entacapone solution.

Procedure: after the test, take an aliquot of the dissolution medium, prepare the *Sample solution* and proceed as described in method **B.** of Assay. Inject, separately, 20 µL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₄H₁₅N₃O₅ dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: no less than 80% (Q) of the declared quantity of C₁₄H₁₅N₃O₅ dissolve in 30 minutes.

Related substances. Proceed as described on method **B.** from Assay. Prepare *Solution (1)* as described next.

Solution (1): Use the *Sample solution* obtained on method **B.** of Assay.

Procedure: inject 20 µL of *Solution (1)*, record the chromatograms and measure the areas under all the peaks obtained, except the ones coming from the solvent. The sum of the areas under the secondary peaks, except the ones under the solvent peak and under the placebo components, is no more than 0.2% of the area under the main peak. No area is greater than 0.1% of the area under the main peak. Disregard any peaks with area smaller than 0.03 times the area under the main peak. Do not include in the calculations the peaks related to the solvent, with the same procedure as the product sample solution.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 100 mg of entacapone to a 100 mL volumetric flask and add 60 mL of acetonitrile. Leave in ultrasonic bath, at room temperature, for 15 minutes and shake mechanically for another 15 minutes. Complete the volume with acetonitrile, homogenize and filter. Dilute successively, with the same solvent, to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 305 nm, using acetonitrile for zero adjustment. Calculate the amount of C₁₄H₁₅N₃O₅ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with ultraviolet detector at 305 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at temperature of 25 °C; *Mobile phase* flow of 2 mL/minute.

Mobile phase: mixture of water pH 3.0, adjusted in advance with 10% (v/v) phosphoric acid, and acetonitrile (65:35).

Sample solution: weigh and pulverize 20 tablets. Transfer an amount of powder equivalent to 50 mg of entacapone to a 100 mL volumetric flask and add 60 mL of methyl alcohol. Leave in ultrasonic bath, at room temperature, for 15 minutes and shake mechanically for 15 minutes. Complete the volume with methyl alcohol and homogenize. Transfer 2 mL of this solution to a 50 mL volumetric flask and complete the volume with methyl alcohol to obtain a 20 µg/mL entacapone solution.

Standard solution: dissolve an accurately weighed amount of entacapone CRS in methyl alcohol to obtain a 0.5 mg/mL solution. Transfer 2 mL of this solution to a 50 mL volumetric flask and complete the volume with methyl alcohol, obtaining a 20 µg/mL solution.

The column efficiency for the entacapone peak is no less than 10 000 theoretical plates/meter. The tailing factor is no more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is no more than 1.5%.

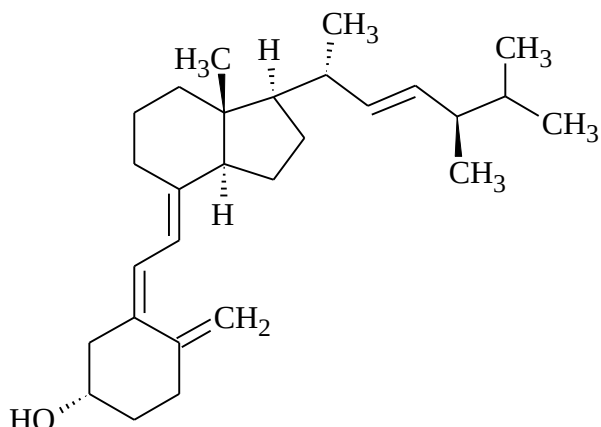
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of C₁₄H₁₅N₃O₅ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

ERGOCALCIFEROL*Ergocalciferolum*C₂₈H₄₄O; 396.65

ergocalciferol; 03477

(1*S*,3*Z*)-4-Methylene-3-[(2*E*)-2-[(1*R*,3*aS*,7*aR*)-octahydro-7*a*-methyl-1-[(1*R*,2*E*,4*R*)-1,4,5-trimethyl-2-hexen-1-yl]-4*H*-inden-4-ylidene]etihiydene]cyclohexanol
[50-14-6]

Contains no less than 97.0% and no more than 103.0% of C₂₈H₄₄O.

DESCRIPTION

Physical characteristics. White to yellowish-white powder.

Solubility. Practically insoluble in water, soluble in ethyl alcohol and in fatty oils.

Physicochemical constants.

Melting range (5.2.2): 115 °C to 119 °C.

Specific optical rotation (5.2.8): +103 to +106. Determine in solution at 1.5% (w/v) in ethyl alcohol. Carry out the reading within 30 minutes after the solution was prepared.

IDENTIFICATION

Note: carry out the analyses protected from direct light and use amber glassware.

The identification tests **B.**, **C.** and **D.** can be omitted if test **A.** is carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ergocalciferol CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of the 0.001% (w/v) solution in ethyl alcohol, there is a maximum point at 265 nm, identical to the one observed in the spectrum of similar ergocalciferol CRS solution.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support and a mixture of cyclohexane and ethyl ether (1:1) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below. Carry out the analysis protected from light.

Solution (1): prepare a solution of squalane (1:100) in chloroform, with 50 mg of the sample per mL.

Solution (2): prepare a solution of squalane (1:100) in chloroform, with 50 mg of ergocalciferol CRS per mL.

Solution (3): prepare a solution of squalane (1:100) in chloroform, with 100 µg of ergosterol per mL.

Develop the chromatogram. Remove the plate and allow it to air dry. Nebulize the plate with 2% acetyl chloride solution in antimony chloride RS. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to that obtained with *Solution (2)*, and can present a violet stain below the ergocalciferol stain. The color of the violet stain is less intense than that of the chromatogram obtained with the ergosterol solution.

D. Dissolve 5 mg of the sample in 5 mL of chloroform and add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid. Shake vigorously. A bright red color develops and quickly turns violet, then blue and, finally, green.

PURITY TESTS

Reducing substances. Dissolve 0.1 g of the sample in ethyl alcohol and complete the volume to 10 mL with the same solvent. Add 0.5 mL of tetrazolium blue solution (1:200) in ethyl alcohol and 0.5 mL of 10% (v/v) tetramethylammonium hydroxide in ethyl alcohol. Allow the mixture to stand for five minutes and then add 1 mL of glacial acetic acid. Carry out a blank test using 10 mL of ethyl alcohol. Determine the solution absorbance at 525 nm. The absorbance is lower than that obtained with a solution with 0.2 g of hydroquinone per mL of ethyl alcohol, prepared in the same manner.

BIOLOGICAL SAFETY TESTS

Count of the total amount of mesophilic microorganisms (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica (5 µm), maintained at room temperature; *Mobile phase* flow of 2 mL/minute.

Mobile phase: *n*-amyl alcohol and hexane (3:997).

Sample solution: transfer 10 mg of the sample, accurately weighed, to a 100 mL volumetric flask, dissolve in 10 mL of toluene and complete the volume with *Mobile phase*.

Standard solution: dissolve an accurately weighed amount of ergocalciferol CRS in 10 mL of toluene and complete the volume with *Mobile phase* to obtain a 100 µg/mL solution.

Resolution solution: dissolve 1 mg of cholecalciferol in 5 mL of *Mobile phase*. Heat for 45 minutes in water bath at 90 °C, with reflux, and allow to cool.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.4 for pre-cholecalciferol, 0.5 for *trans*-cholecalciferol and 1.0 for cholecalciferol. The resolution between pre-cholecalciferol and *trans*-cholecalciferol is no less than 1.0. The relative standard deviation of the replicate areas under the cholecalciferol peaks is no more than 1.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₈H₄₄O in the sample from the responses obtained with the *Standard solution* and with the *Sample solution*.

PACKAGING AND STORAGE

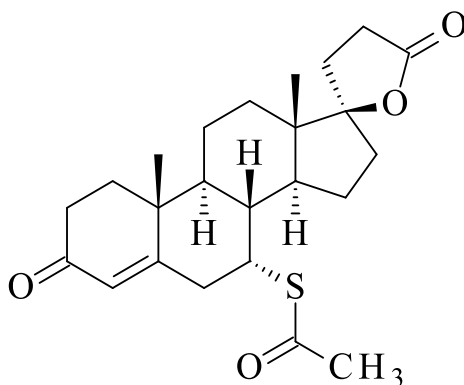
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vitamin.

SPIRONOLACTONE*Spironolactonum*C₂₄H₃₂O₄S; 416.57

spironolactone; 03561

γ-Lactone of (7α,17α)-7-(acetylthio)-17-hydroxy-3-oxopregn-4-ene-21-carboxylic acid

[52-01-7]

Contains no less than 97.0% and no more than 103.0% of C₂₄H₃₂O₄S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Light beige to yellowish-brown crystalline powder. Stable on air. Presents polymorphism.

Solubility. Practically insoluble in water, soluble in absolute ethyl alcohol, slightly soluble in methyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): between -33 and -37, in relation to the desiccated substance. Determine in solution at 1% (w/v) in chloroform.

Melting range (5.2.2): 198 °C to 207 °C, with decomposition. Occasionally, it may show preliminary melting at around 135 °C followed by resolidification.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the previously desiccated sample in a 5% (w/v) solution in chloroform, there are maximum absorption points only at the same wavelengths and with the same relative intensities as those observed in the spironolactone CRS spectrum, identically prepared. If the spectrum of the sample is not identical to that of the standard, dissolve the sample and the standard separately in methyl alcohol, evaporate to dryness and repeat the test with the residues.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from Assay, there are maximum and minimum points only at the same wavelengths of similar spironolactone CRS solution. The absorptivities, calculated in the maximum absorbance wavelength of approximately 238 nm, do not differ more than 3%.

PURITY TESTS

Related substances. Proceed as described in *Thin Layer Chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and butyl acetate as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.2 g of the sample in ethyl alcohol and complete to 10 mL with the same solvent.

Solution (2): dilute 0.5 mL of *Solution (1)* to 50 mL with ethyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Spray with sulfuric acid/methyl alcohol RS, heat plate at 105 °C for 10 minutes and examine immediately. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram with *Solution (2)* (1,0%).

Mercapto compounds. Shake 2 g of the sample with 30 mL of water and filter. Then add 3 mL of starch TS to 15 mL of the filtrate, and titrate with 0.005 M VS iodine. Carry out a blank for the necessary correction. Not more than 0.10 mL of 0.005 M VS iodine is consumed.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for two hours. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 50 g of the sample and dissolve in methyl alcohol. Complete the volume to 250 mL with the same solvent. Dilute, successively, in methyl alcohol to concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 238 nm, using methyl alcohol for zero adjustment. Calculate the content of C₂₄H₃₂O₄ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 230 nm; column with 150 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of methyl alcohol and water (60:40).

Sample solution: transfer approximately 50 mg of the sample to a 100 mL volumetric flask and complete the volume with a mixture of acetonitrile and water (50:50). Homogenize. Transfer 2 mL of this solution to a 10 mL volumetric flask and complete the volume with a mixture of acetonitrile and water (50:50), obtaining a 100 µg/mL solution.

Standard solution: Dissolve an accurately weighed quantity of spironolactone CRS in a mixture of acetonitrile and water (50:50) to obtain a 500 µg/mL solution. Dilute successively in a mixture of acetonitrile and water (50:50) to obtain a 100 µg/mL solution.

Procedure: Inject, separately, 20 µL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₄H₃₂O₄ in the sample from the responses obtained with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

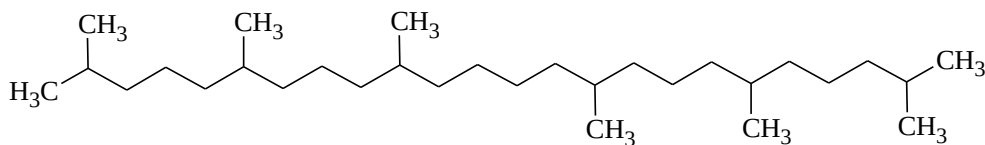
Comply with current legislation.

THERAPEUTIC CLASS

Diuretic.

SQUALANE

Squalanum



C₃₀H₆₂; 422.81

squalane; 09701

2,6,10,15,19,23-Hexamethyltetracosane

[111-01-3]

DESCRIPTION

Physical characteristics. Oily, clear and colorless liquid.

Solubility. Practically insoluble in water and slightly soluble in ethyl alcohol. Miscible with water.

Physicochemical constants.

Relative density (5.2.5): 0.807 to 0.810.

Refractive index (5.2.6): 1.4510 to 1.4525.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed among potassium bromide plate, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the squalane CRS spectrum, prepared identically.

PURITY TESTS

Acidity value (5.2.29.7). Not more than 0.2.

Saponification value (5.2.29.8). Not more than 2.0.

Iodine value (5.2.29.10). Not more than 4.0.

Chromatographic purity Proceed as described in *Gas chromatography (5.2.17.5)*. Use a chromatograph equipped with a flame ionization detector; capillary column 30 m in length and 0.25 mm in internal diameter, packed with polydimethylsiloxane, with film thickness of 0.25 µm; column temperature from 60 °C to 290 °C (60 °C held for 3 minutes, increased to 290 °C to 6 °C per minute); injector temperature 280 °C and detector temperature 300 °C; use nitrogen as carrier gas; 2 mL/minute carrier gas flow.

Solution (1): prepare a 1.5% (w/v) sample solution.

Solution (2): prepare a 1.5% (w/v) squalane CRS solution.

Procedure: inject, separately, 1 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The sum of the areas under the secondary peaks obtained in the chromatogram with *Solution (1)*, except that under the main peak, is not more than 3.0% of the total area under the peaks obtained. Do not include solvent-related peaks in the calculations.

PACKAGING AND STORAGE

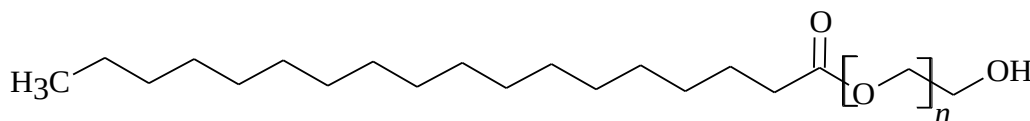
In tightly closed containers, protected from light and at temperature between 8 °C and 15 °C.

LABELING

Comply with current legislation. Specify the origin (vegetable or animal) on the label.

CATEGORY

Adjuvant.

MACROGOL STEARATE*Macrogoli stearas*C₁₈H₃₆O₂·(C₂H₄O)_n; 05475 α -(1-Oxo-octadecyl)- ω -hydroxypoly(oxy-1,2-ethanediyl)

[9004-99-3]

DESCRIPTION**Physical characteristics.** Scaly white solid.**Solubility.** Moderately soluble in water, soluble in ethyl alcohol, ethyl ether and acetone and insoluble in mineral and vegetable oils.**IDENTIFICATION**

In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the polyoxyl 40 stearate CRS spectrum, prepared identically.

PURITY TESTS**Congealing temperature (5.2.4)** Not less than 37,0% and not more than 47,0%.**Acidity value (5.2.29.7).** Not more than 2.0.**Saponification value (5.2.29.8).** Between 25 and 35.**Hydroxyl value (5.2.29.12).** Between 25 and 40.

Free polyethylene glycols. Accurately weigh 6 g of sample and transfer to a 500 mL separating funnel containing 50 mL of ethyl acetate. Dissolve completely and add 50 mL of a 29% sodium chloride solution (w/v), shake vigorously for two minutes and allow to stand for 15 minutes. If separation is incomplete, carefully insert the separating funnel into a steam bath at short intervals. Repeat this procedure as many times as necessary to ensure complete phase separation. Cool and separate the lower, aqueous phase into a second 500 mL separating funnel, extract the upper phase again with 50 mL of a 29% (w/v) sodium chloride solution, repeating the procedure described above. To the second separating funnel containing the aqueous phases add 50 mL of ethyl acetate, shake vigorously for two minutes and allow to stand for 15 minutes. Separate the lower aqueous phase into a third 500 mL separatory funnel and extract with two 50 mL portions of chloroform, shaking for two minutes at a time. Repeat the steam bath procedure to obtain complete phase separation. Transfer the chloroform portions to a 150 mL beaker and evaporate on the steam bath until apparently dry. Add 15 mL of chloroform to the residue and filter, collecting the filtrate in a 150 mL beaker. Wash the filter with small portions of chloroform, collecting in the same 150 mL beaker as

the filtrate was collected, and evaporate until the odor of chloroform or ethyl acetate is no longer noticed. Dry at a temperature of 60 °C in oven, under reduced pressure, for one hour. Cool in desiccator and weigh. The sample contains not less than 17.0% and not more than 27.0% of free polyethylene glycols.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20). Not more than 3.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

In tightly closed containers protected from light.

LABELING

Comply with current legislation.

CATEGORY

Pharmacotechnical adjuvant, surfactant.

ZINC STEARATE***Zinci stearas***

(C₁₇H₃₅CO₂)₂Zn; 632.34

zinc stearate; 10665

[557-05-1]

It contains not less than 10.0% and not more than 12.0% of zinc.

DESCRIPTION

Physical characteristics. White, amorphous, light powder, free from lumpy particles.

Solubility. Practically insoluble in water and in ethyl alcohol.

Physicochemical constants.

Solidification temperature (5.2.29.3): not less than 54 °C. Determine the residue obtained in the preparation of *Solution (1)*, described in *Preparation aspect*.

IDENTIFICATION

It meets zinc ion reactions (5.3.1.1). Determine in 1 mL of *Solution (1)*, obtained in *Preparation aspect*.

PURITY TESTS

Preparation aspect. Prepare *Solution (1)* as described below. *Solution (1)* is not more stained than the *Standard solution SC F colored (5.2.12)*.

Solution (1): Dissolve 5 g of the sample in 50 mL of ethyl ether and 40 mL of nitric acid 7.5% (v/v). Heat under reflux until complete dissolution, then allow to cool. Separate the aqueous phase, shake the ethereal phase with 4 mL of water and repeat the procedure. Combine the aqueous phases, wash with 15 mL of ethyl ether and complete volume to 50 mL with water. Evaporate the ethereal phase and place the residue to dryness in oven at 105 °C.

Fatty acid preparation aspect. Dissolve 0.5 g of the residue obtained in the preparation of *Solution (1)* in *Preparation aspect* in 10 mL of chloroform. The preparation is not more stained than the 12.5% (v/v) solution of *Ferric Chloride Base Solution (5.2.12)* in 100 mL of 1% HCl.

Acidity and alkalinity. Heat to boiling for one minute, accurately weighed 1 g of sample dissolved in 5 mL of ethyl alcohol and 20 mL of water. Allow to cool and filter. Not more than 0.3 mL of 0.1 M sodium hydroxide is spent to neutralize 10 mL of the filtrate, using phenol red TS as an indicator. Not more than 0.3 mL of 0.1 M hydrochloric acid is spent to neutralize 10 mL of the filtrate, using the same indicator.

Acidity value (5.2.29.7). 195 to 210. Determine in 0.2 g of the residue obtained in the preparation of the *Solution (1)* in *Preparation aspect*.

Cadmium. Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*. Use *Method II*. Use spectrophotometer equipped with flame fed by mixture of air-acetylene, cadmium hollow cathode lamp and select the emission line at 228.8 nm. Not more than 0.0005% (5 ppm).

Sample solution: dilute 20 mL of *Solution (1)*, obtained in *Preparation aspect* in 50 mL of 3.5% (v/v) solution of nitric acid in water.

Standard solution: Prepare the reference solutions using a 1000 ppm cadmium stock solution. Dilutions should be made in nitric acid 3.5% (v/v).

Lead. Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*. Use *Method II*. Use spectrophotometer equipped with flame fed by mixture of air-acetylene, lead hollow cathode lamp and select the emission line at 283.3 nm. Not more than 0.0025% (25 ppm).

Sample solution: use *Solution (1)*, obtained in *Preparation aspect*.

Standard solution: prepare the reference solutions using a 1000 ppm lead stock solution. Dilutions should be made in nitric acid 3.5% (v/v).

Chlorides (5.3.2.1). With an aliquot of 14 mL of *Solution (1)*, obtained in *Preparation aspect*, proceed as described in *Limit Test for Chlorides*, using 1 mL of 0.01 M hydrochloric acid for the *Standard Preparation*. Not more than 0.025% (250 ppm).

Sulfates (5.3.2.2). With an aliquot of 2 mL of *Solution (1)*, obtained in *Preparation aspect*, proceed as described in *Limit Test for sulfates*, using 2,5 mL of 0.005 M sulfuric acid for the *Standard Preparation*. Not more than 0.6% (6000 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample after drying in oven at a temperature between 100 °C and 105 °C, until constant weight. Not more than 6.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 1 g of the sample and dissolve in 50 mL of sulfuric acid 0.05 M. Boil this solution for 10 minutes or until a clear layer of fatty acids is formed, adding water if necessary to maintain the solution original volume. Cool and filter. Carefully wash the filter and the flask with water until the last washing is not acidic to the litmus paper. Add the washing waters to the filtrate. Proceed as described in *Complexometric titrations (5.3.3.4)* for *Zinc*. Each mL of 0.05 M disodium edetate EDTA VS is equivalent to 3.268 g of Zn.

PACKAGING AND STORAGE

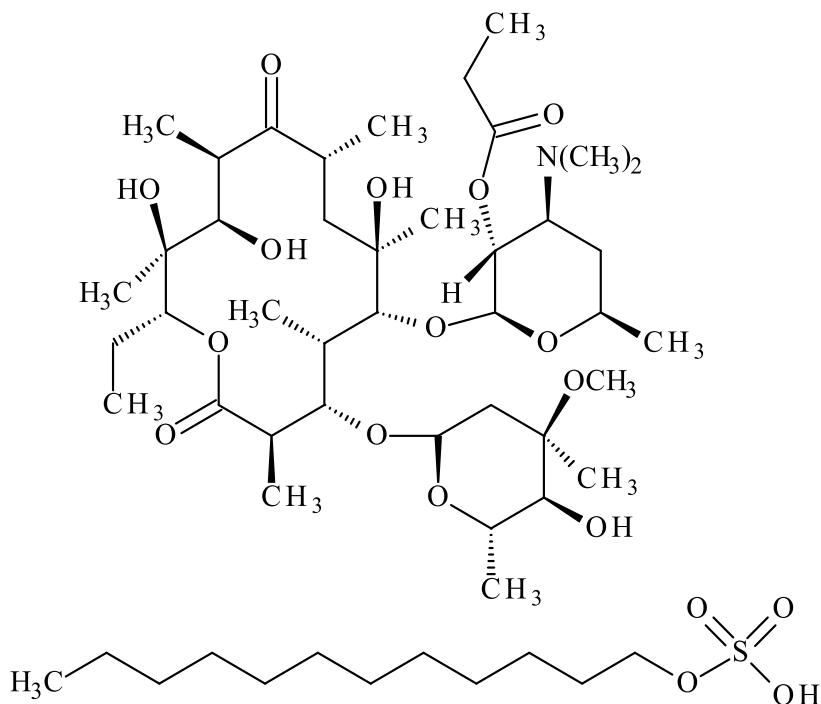
In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Pharmacotechnical adjuvant.

ERYTHROMYCIN ESTOLATE**Erythromycini estolas**

$C_{40}H_{71}NO_{14} \cdot C_{12}H_{26}O_4S$; 1056.39

Erythromycin estolate; 03494

Erythromycin 2'-propanoate dodecyl sulfate (1:1)
[3521-62-8]

It has a potency of not less than 610 IU of erythromycin estolate ($C_{40}H_{71}NO_{14} \cdot C_{12}H_{26}O_4S$) per milligram in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Practically insoluble in water and soluble in ethyl alcohol. Practically insoluble in diluted hydrochloric acid.

Physicochemical constants.

Melting range (5.2.2): 135 °C to 138 °C, with decomposition.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the erythromycin estolate CRS spectrum, prepared identically.

B. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

PURITY TESTS

pH (5.2.19). 4,5 to 7,0. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a 15% (w/v) ammonium acetate mixture with pH adjusted to 7.0; ethyl alcohol and chloroform (1:15:85) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): 4 mg/mL sample solution in acetone.

Solution (2): dilute 2.5 mL of *Solution (1)* in 10 mL of acetone.

Solution (3): 1 mg/mL solution of erythromycin estolate CRS in acetone.

Solution (4): dissolve 10 mg of erythromycin estolate CRS and 10 mg of erythromycin ethylsuccinate in 10 mL of acetone.

Solution (5): 80 µg/mL solution of erythromycin CRS in acetone.

Develop chromatogram. Remove plate and allow to air dry. Spray with anisaldehyde RS and heat at 110 °C for five minutes. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (5)* (2.0%). The test is only valid if, in the chromatogram obtained with *Solution (4)*, there are two clearly separated stains.

Water (5.2.20.1). Use 20 mL of methyl alcohol containing 10% of imidazole in the titration flask. Not more than 4.0%.

Residue by incineration (5.2.10). Determine in 0.5 g of the sample. Not more than 0.5%.

ASSAY

Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)* by agar diffusion using cylinders.

Microorganism: *Kocuria rhizophila* ATCC 9341.

Culture media: culture medium number 1 for microorganism maintenance, sterile saline solution for inoculum standardization, culture medium number 11 for base layer and for inoculum preparation.

Sample solution: Dissolve an amount of sample equivalent to 50 mg of erythromycin in 20 mL of methyl alcohol. Dilute to 50 mL with *sterile 0.1 M Potassium Phosphate Buffer, pH 8.0 (Solution 2)*. Keep at 60°C for three hours. Filter. Dilute to obtain concentrations of 0.30 µg/mL, 0.60 µg/mL and 1.2 µg/mL using *sterile 0.1 M Potassium Phosphate Buffer, pH 8.0 (Solution 2)*.

Standard solution: accurately weigh about 50 mg of erythromycin estolate CRS and quantitatively transfer to a 50 mL volumetric flask with the aid of 20 mL of methyl alcohol. Shake, complete the volume with *sterile 0,1 M Potassium Phosphate Buffer, pH 8.0 (Solution 2)*. Dilute to obtain concentrations of 0.30 µg/mL, 0.60 µg/mL and 1.2 µg/mL using *sterile 0.1 M Potassium Phosphate Buffer, pH 8.0 (Solution 2)*.

Procedure: add 20 mL of medium number 11 to each plate, wait to solidify, add 5 mL of 1.5% inoculum and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*, adding to the cylinders 0.2 mL of the freshly prepared solutions. Calculate the sample potency, in IU of erythromycin estolate per milligram, from the standard potency and the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In hermetically closed containers, protected from light and at temperature below 30 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

ERYTHROMYCIN ESTOLATE TABLETS

It contains erythromycin estolate equivalent to not less than 90.0% and not more than 120.0% of the stated quantity of erythromycin ($C_{37}H_{67}NO_{13}$). The tablets must be coated.

IDENTIFICATION

Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel (0.25 mm) as support, and a mixture of methyl alcohol and chloroform (85:15) as mobile phase. Apply to the plate, separately, 3 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. From the powder, prepare a solution equivalent to 20 mg/mL of erythromycin in methyl alcohol.

Solution (2): use erythromycin estolate CRS to obtain a 20 mg/mL solution of erythromycin in methyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Spray with a mixture of ethyl alcohol, anisaldehyde and sulfuric acid (90:5:5). Heat the plate at 100 °C for 10 minutes. The main stain obtained with the *Solution (1)*, of black to purple in color, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Not more than 30 minutes. Use simulated gastric fluid as disintegration medium.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Water (5.2.20.1). Use 20 mL of methyl alcohol containing 10% of imidazole in the titration flask. Not more than 5.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Microbiological Assay of Antibiotics* (5.5.3.3) and as described in *Microbiological Assay by agar diffusion* (5.5.3.3.1) using cylinders. Prepare *Sample solution* as described below.

Sample Solution: weigh and pulverize the tablets. Use an amount of powder equivalent to 0.5 g of erythromycin and transfer to a 500 mL volumetric flask. Dilute in 200 mL of methyl alcohol and shake

mechanically for 10 minutes. Add 100 mL of *sterile 0.1 M Potassium Phosphate Buffer, pH 8.0 (Solution 2)* and shake mechanically for 10 minutes. Complete the volume with the same solvent and filter.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

ERYTHROMYCIN ESTOLATE ORAL SUSPENSION

Erythromycin estolate oral suspension is a mixture of erythromycin estolate with one or more coloring, flavoring, buffering, sweetening and preservative agents. It contains erythromycin estolate equivalent to not less than 90.0% and not more than 115.0% of the stated quantity of erythromycin ($C_{37}H_{67}NO_{13}$).

IDENTIFICATION

Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel (0.25 mm) as support and a mixture of methyl alcohol and chloroform (85:15) as mobile phase. Apply to the plate, separately, 3 μ L of each of the freshly prepared solutions described below.

Solution (1): transfer volume of oral suspension equivalent to 20 mg of erythromycin to separating funnel. Add 15 mL of 0.02 M sodium hydroxide and shake. Add 2 g of sodium chloride and 25 mL of chloroform and shake for three minutes. Separate the chloroform phase by passing it through a small quantity of anhydrous sodium sulfate, previously washed with chloroform. Collect the chloroform extract. Wash the sodium sulfate with an additional 5 mL of chloroform. Evaporate the organic phase to dryness on a rotary evaporator. Dissolve the residue in 1 mL of methyl alcohol.

Solution (2): Transfer a quantity of erythromycin estolate CRS equivalent to 20 mg of erythromycin to a separating funnel and proceed with extraction as described for *Solution (1)*.

Develop chromatogram. Remove the plate, allow to air dry and spray with a mixture of ethyl alcohol, anisaldehyde and sulfuric acid (90:5:5). Heat the plate at 100 °C for 10 minutes. The main stain obtained with the *Solution (1)*, of black to purple in color, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.5 to 6.5.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Microbiological Assay of Antibiotics* (5.5.3.3), using cylinders.

Microorganism: Kocuria rhizophila ATCC 9341.

Standard solution: dissolve an accurately weighed quantity of erythromycin CRS in methyl alcohol to obtain a 10 mg/mL solution. Quantitatively dilute with *sterile 0.1 M Potassium Phosphate Buffer, pH 8.0 (Solution 2)* to a concentration of 1 mg/mL. Dilute successively with *sterile 0.1 M Potassium*

phosphate buffer, pH 8.0 (Solution 2) to obtain solutions in the concentration range suitable for the standard curve.

Sample solution: transfer volume of the oral suspension, free of bubbles, equivalent to 0.25 g of erythromycin, to a 250 mL volumetric flask. Add 100 mL methyl alcohol and shake for 10 minutes. Complete the volume with *sterile 0.1 M Potassium Phosphate Buffer, pH 8.0 (Solution 2)* and heat to 60 °C for three hours, cool and filter. Dilute successively with *sterile 0.1 M Potassium phosphate buffer, pH 8.0 (Solution 2)* to obtain solutions in the concentration range suitable for the standard curve.

Procedure: add 20 mL of medium base number 11 to each plate, allow to solidify, add 5 mL of inoculum number 11 and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*. Calculate the quantity, in mg of erythromycin ($C_{37}H_{67}NO_{13}$), in the oral suspension from the standard potency and the responses obtained for the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

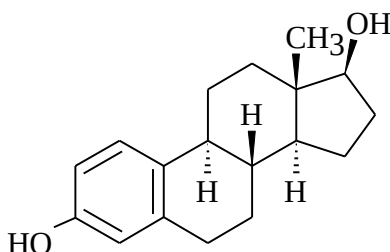
In tightly closed containers.

LABELING

Comply with current legislation.

ESTRADIOL

Estradiolum



$C_{18}H_{24}O_2$; 272.38

estradiol; 03595

(17 β)-Estra-1,3,5(10)-triene-3,17-diol

[50-28-2]

It contains not less than 97.0% and not more than 103.0% of $C_{18}H_{24}O_2$ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White to yellowish-white powder.

Solubility. Practically insoluble in water, freely soluble in ethyl alcohol, soluble in dioxane, moderately soluble in vegetable oil. Soluble in alkali hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 173 °C to 179 °C.

Specific optical rotation (5.2.8): +76 to +83. Determine in solution at 1% (w/v) in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the estradiol CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.005% (w/v) solution in ethyl alcohol, there is a maximum point at 280 nm, identical to the one observed in the spectrum of similar estradiol CRS solution.

PURITY TESTS

Water (5.2.20.1). Not more than 3.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 205 nm; 300 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: acetonitrile and water (55:45).

Internal Standard solution: dissolve an accurately weighed quantity of ethylparaben in methyl alcohol to obtain a 0.60 mg/mL solution.

Sample solution: transfer 100 mg of the sample, accurately weighed, to a 250 mL volumetric flask and complete the volume with methyl alcohol. Transfer 10 mL to a 200 mL volumetric flask and add 5 mL of the *Internal Standard Solution*, complete the volume with water and homogenize.

Standard solution: dissolve an accurately weighed quantity of estradiol CRS and estrone CRS in methyl alcohol to obtain a 0.4 mg/mL and 0.24 mg/mL solution, respectively. Transfer 10 mL of this solution and 5 mL of the *Internal Standard Solution* to a 200 mL volumetric flask. Add 100 mL of methyl alcohol and complete the volume with water, obtaining a 20 µg/mL solution of estradiol CRS.

Inject 20 µL replicates of the *Standard solution*. Retention times are about 0.7 for ethylparaben, 1.0 for estradiol and 1.3 for estrone. The resolution between estrone and estradiol is not less than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₈H₂₄O₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

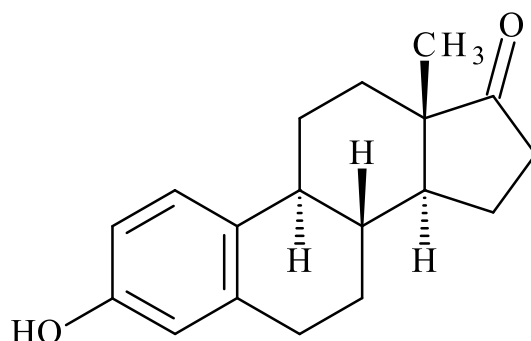
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Hormone.

ESTRONE*Estronum*C₁₈H₂₂O₂; 270.37

estrone; 03630

3-Hydroxyestra-1,3,5(10)-trien-17-one

[53-16-7]

It contains not less than 97.0% and not more than 103.0% of C₁₈H₂₂O₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to yellowish white powder or small white to yellowish white crystals.

Solubility. Practically insoluble in water, soluble in ethyl alcohol, in methyl alcohol and in vegetable oils. Slightly soluble in fixed alkali hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 258 °C to 262 °C.

Specific optical rotation (5.2.8): +158 to +165, in relation to the desiccated substance. Determine in solution at 1% (w/v) in dioxane.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the estrone CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.005% (w/v) solution in ethyl alcohol heated in water bath and cooled at room temperature, there are maximum points identical to the ones observed in the spectrum of similar estrone CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1 g of sample, in oven, at 105 °C, for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 280 nm; 150 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: acetonitrile and 0.05 M monobasic potassium phosphate (50:50).

Sample solution: dissolve an accurately weighed quantity of the sample in methyl alcohol to obtain a 0.2 mg/mL solution. Transfer 5 mL of this solution to a 25 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 40 µg/mL solution.

Standard solution: dissolve an accurately weighed quantity of estrone CRS in methyl alcohol to obtain a 0.2 mg/mL solution. Transfer 5 mL of this solution to a 25 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 40 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency must be not less than 1500 theoretical plates/meter. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₈H₂₂O₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

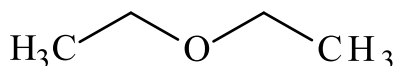
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Hormone.

ETHYL ETHER*Aether ethylicus*

C₄H₁₀O; 74.12
ethyl ether; 03663
1,1'-Oxybisethane
[60-29-7]

DESCRIPTION

Physical characteristics. Clear, colorless, volatile, flammable liquid.

Solubility. Soluble in water, miscible with ethyl alcohol, methylene chloride and fatty oils.

Physicochemical constants.

Relative density (5.2.5). 0.714 to 0.716.

Distillation range (5.2.3). Note: Do not distill if the sample does not meet the peroxide test. The sample fully distills between 34 °C and 35 °C. Carry out the test using an appropriate heating device. Proceed with caution, avoid heating the flask above the liquid level.

IDENTIFICATION

A. It meets the *Relative Density* test (5.2.5).

B. It meets the *Determination of distillation range* test (5.2.3).

PURITY TESTS

Acidity. In a flask with a ground cap, introduce 10 mL of ethyl alcohol, 2 mL of distilled water, 0.5 mL of phenolphthalein TS and add 0.02 M sodium hydroxide solution until a persistent pink color is obtained after shaking for 30 seconds. Add accurately weighed 25 mL of sample, cap and shake carefully. Add 0.02 M sodium hydroxide solution until pink color persists after shaking for 30 seconds. Not more than 0.4 mL of 0.02 M sodium hydroxide should be used to neutralize the ethyl ether.

Peroxides. In a 25 mL graduated cylinder with a ground-glass cap, introduce 10 mL of the sample and 1 mL of a freshly prepared 10% (w/v) potassium iodide solution and shake. The mixture should be protected from light for one hour. Liquids should be colorless.

Non-volatile residue. Evaporate 50 mL of the sample, spontaneously, in a previously tared porcelain capsule. Dry the residue in oven at 105 °C for one hour, allow to cool and weigh. The residue weight must not exceed 1 mg (0.003%).

Aldehydes. To a separating funnel, transfer 20 mL of the sample and add 7 mL of the mixture of 1 mL of alkaline mercuric potassium iodide RS and 17 mL of saturated sodium chloride solution. Close and shake vigorously for 10 seconds. Allow to stand for a minute. The aqueous layer must not show turbidity.

Foreign odor. On an 80 mm diameter filter paper disk, apply 5 ml of the sample and allow to spontaneously evaporate. After sample volatilization, foreign odor to ethyl ether should not be observed.

PACKAGING AND STORAGE

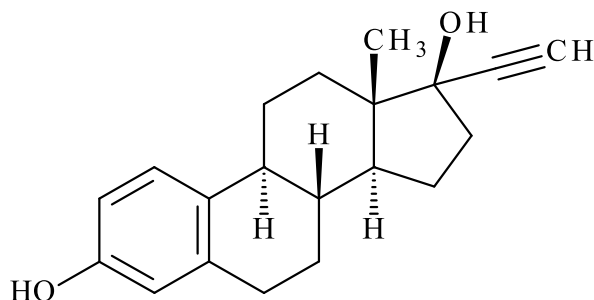
In tightly closed containers, protected from light and at temperature between 8 °C and 15 °C.

LABELING

The label must indicate the name and concentration of the non-volatile antioxidant eventually used.

CATEGORY

Solvent.

ETHINYLESTRADIOL*Ethinylestradiolum*C₂₀H₂₄O₂; 296,40

ethinylestradiol; 03699

(17 α)-19-Norpregna-1,3,5(10)-trien-20-yne-3,17-diol

[57-63-6]

It contains not less than 97.0% and not more than 102.0% of C₂₀H₂₄O₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or slightly yellowish crystalline powder. Presents polymorphism.

Solubility. Practically insoluble in water, soluble in dioxane and in ethyl alcohol. Soluble in diluted alkaline solutions.

Physicochemical constants.

Melting range (5.2.2): 180 °C to 186 °C. It has a polymorphic structure with melting range from 142 °C to 146 °C.

Specific optical rotation (5.2.8): –28.0 to –29.5, in relation to the desiccated substance. Determine in solution at 0,4% (w/v) in pyridine. Pyridine must be colorless and come from a freshly opened flask.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ethinylestradiol CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.005% (w/v) solution in ethyl alcohol, there is a maximum point at 281 nm, identical to the one observed in the spectrum of similar ethinylestradiol CRS solution.

C. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

PURITY TESTS

Preparation aspect. The 2% (w/v) preparation in ethyl alcohol is clear **(5.2.25)**.

Related substances. Proceed as described in *Thin layer chromatography* **(5.2.17.1)**, using silica gel G as support, and mixture of ethyl alcohol and toluene (10:90), as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Diluent: mixture of methyl alcohol and chloroform.

Solution (1): dissolve 0.2 g of the sample in mixture of methyl alcohol and chloroform (10:90) and complete to 10 mL with the same solvent.

Solution (2): dilute 0.5 mL of *Solution (1)* to 10 mL with a mixture of methyl alcohol and chloroform (10:90).

Solution (3): dissolve 25 mg of ethinylestradiol CRS in a mixture of methyl alcohol and chloroform (10:90) and complete to 25 mL with the same solvent.

Solution (4): dissolve 10 mg of estrone CRS in a mixture of methyl alcohol and chloroform (10:90) and complete to 10 mL with the same solvent. Dilute 2 mL of this solution to 10 mL with the same solvent.

Solution (5): dilute 1 mL of *Solution (2)* to 5 mL with a mixture of methyl alcohol and chloroform (10:90).

Develop chromatogram. Remove the plate, allow to air dry and heat at 110°C for 10 minutes. Spray the hot plate with methanolic sulfuric acid RS. Heat plate again at 110 °C for 10 minutes and examine under ultraviolet light (365 nm). Any secondary stain, corresponding to estrone, obtained in the chromatogram with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (4)* (1.0%). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain and from that corresponding to estrone, is not more intense than that obtained in the chromatogram with *Solution (5)* (1.0%).

Loss on drying (5.2.9.1). Determine in 0,5 g of sample, in oven, at 105 °C, for three hours. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh 0.2 g of the sample. Dissolve in 40 mL of tetrahydrofuran and add 5 mL of 10% silver nitrate (w/v). Titrate with 0.1 M sodium hydroxide VS, determining the endpoint

potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium hydroxide VS is equivalent to 29.640 mg of $C_{20}H_{24}O_2$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh, accurately, about 50 mg of the sample, dissolve in ethyl alcohol and complete the volume to 100 mL with the same solvent. Dilute with ethyl alcohol until concentration of 0.01% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 281 nm, using ethyl alcohol for zero adjustment. Calculate the content of $C_{20}H_{24}O_2$ in the sample from the readings obtained.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 280 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (3 μ m to 10 μ m), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of acetonitrile and water (1:1).

Sample solution: quantitatively transfer about 25 mg of the sample to a 25 mL volumetric flask, dissolve and complete the volume with *Mobile Phase*. Transfer 10 mL to a 50 mL volumetric flask, dilute and complete the volume with *Mobile Phase*.

Standard solution: quantitatively dissolve about 10 mg of ethinylestradiol CRS in *Mobile phase* and dilute to 50 mL with the same solvent.

Procedure: inject, separately, 25 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{20}H_{24}O_2$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

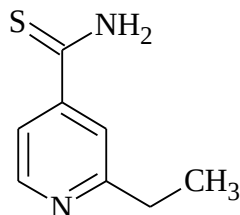
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Contraceptive.

ETHIONAMIDE***Ethionamidum*** $C_8H_{10}N_2S$; 166.24

ethionamide; 03704

2-Ethyl-4-pyridinecarbothioamide

[536-33-4]

It contains not less than 98.0% and not more than 102.0% $C_8H_{10}NO_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow crystalline powder or small yellow crystals.

Solubility. Practically insoluble in water, soluble in methyl alcohol, moderately soluble in ethyl alcohol, slightly soluble in propylene glycol.

Physicochemical constants.

Melting range (5.2.2): 158 °C to 164 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, desiccated for 18 hours over silica gel and under reduced pressure, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ethionamide CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 220 nm to 350 nm of the sample solution obtained in method **B.** from Assay, there are maximum and minimum points only at the same wavelengths of similar ethionamide CRS solution.

PURITY TESTS

pH (5.2.19). 6.0 to 7.0. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of chloroform and methyl alcohol (90:10) as mobile phase. Separately apply to the plate 10 µL of each solution, described below.

Solution (1): 20 mg/mL sample solution in acetone.

Solution (2): 0.1 mg/mL sample solution in acetone.

Solution (3): 0.04 mg/mL sample solution in acetone.

Develop chromatogram. Remove plate, allow to air dry, examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.5%), and not more than a secondary stain obtained with *Solution (1)* is more intense than that obtained with *Solution (3)* (0.2%).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 0.25 g of the sample in 50 mL of glacial acetic acid and titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 16.624 mg of C₈H₁₀N₂S.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 50 g of the sample and dissolve in methyl alcohol. Complete the volume to 100 mL with the same solvent. Dilute, successively, in methyl alcohol until concentration of 0.001% (w/v). Prepare a standard solution of ethionamide CRS in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 290 nm, using methyl alcohol for zero adjustment. Calculate the content of C₈H₁₀N₂S in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, in cool place.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial (tuberculostatic).

ETHIONAMIDE TABLETS

It contains not less than 95.0% and not more than 110.0% of the declared quantity of $C_8H_{10}N_2S$. Tablets must be coated (sugar coated).

IDENTIFICATION

A. Weigh and pulverize the tablets. Shake the quantity of powder equivalent to 0.125 g of ethionamide with 25 ml of ethyl ether for two or three minutes. Filter and evaporate the filtrate at room temperature. Dry the residue over silica gel, under reduced pressure. In the infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ethionamide CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), at the range of 220 nm to 350 nm, of the sample solution obtained in *Assay*, there is maximum absorption at 290 nm, identical to that observed at the standard solution spectrum.

C. Weigh and pulverize the tablets. Shake the quantity of powder equivalent to 1 g of ethionamide with 50 ml of methyl alcohol and filter using slow filter paper. Evaporate the filtrate on steam bath until dryness. The residue obtained melts between 155°C and 164°C.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Use 0.1 M hydrochloric acid as disintegration medium. Not more than 30 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 100 mL volumetric flask containing 60 mL of methyl alcohol and wait for the tablet to completely disintegrate. Place in ultrasonic bath for 10 minutes, shake mechanically for 15 minutes and complete the volume with the same solvent. Filter, discarding the first 20 mL of the filtrate. Carry out successive dilutions up to a concentration of 0.001% (w/v), using methyl alcohol as solvent. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 290 nm (**5.2.14**), using methyl alcohol for zero adjustment. Calculate the quantity of $C_8H_{10}N_2S$ in the tablets from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium and dilute, if necessary, with 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 274 nm (**5.2.14**) using the same solvent for zero adjustment. Calculate the quantity of $C_8H_{10}N_2S$

dissolved in the medium, comparing the readings obtained with the ethionamide CRS solution at the concentration of 0.001% (w/v), prepared in 0.1 M hydrochloric acid.

Tolerance: not less than 75% (Q) of the stated quantity of $C_8H_{10}N_2S$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

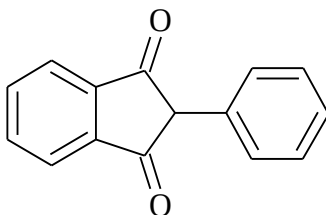
Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer a quantity of powder equivalent to 50 mg of ethionamide to a 100 mL volumetric flask and add 80 mL of methyl alcohol. Place in ultrasonic bath for 10 minutes, shake mechanically for 15 minutes and complete the volume with the same solvent. Filter, discarding the first 20 mL of the filtrate. Transfer 2 mL of the filtrate to a 100 mL volumetric flask, complete the volume with methyl alcohol and homogenize. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 290 nm, using methyl alcohol for zero adjustment. Calculate the quantity of $C_8H_{10}N_2S$ in the tablets from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

PHENINDIONE*Phenindionum* $C_{15}H_{10}O_2$; 222.24

phenindione; 03938

2-Phenyl-1*H*-indene-1,3(2*H*)-dione

[83-12-5]

It contains not less than 98.0% and not more than 100.5% of $C_{15}H_{10}O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder, or creamy, white or slightly yellowish crystals.

Solubility. Insoluble in water and slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 148 °C to 151 °C, with decomposition.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the phenindione CRS spectrum, prepared identically.

B. Dissolve 0.1 g of the sample in 30 mL of ethyl alcohol, with the aid of heating, if necessary. Cool, transfer to a 50 mL volumetric flask and complete the volume with the same solvent. From this solution and, using 0.1 *M* sodium hydroxide, dilute until obtaining a solution at 0.0004% (w/v) of the sample. In the ultraviolet absorption spectrum **(5.2.14)** of this solution, in the range of 200 nm to 400 nm, there are maximum and minimum points at the same wavelengths and with the same intensities as those observed with the phenindione CRS, identically prepared.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel F₂₅₄ as support, and 0.02% (w/v) butylhydroxytoluene in a mixture of ethyl acetate and glacial acetic acid (20:4) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in methylene chloride.

Solution (2): dilute 1 mL of *Solution (1)* to 50 mL with methylene chloride.

Solution (3): dilute 2.5 mL of *Solution (2)* to 10 mL with methylene chloride.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (2.0%). At maximum, a secondary stain obtained in the chromatogram with *Solution (1)* is more intense than the main stain obtained with *Solution (3)* (0.5%).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for two hours. Not more than 1.0%.

Residue by incineration (5.2.10). Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

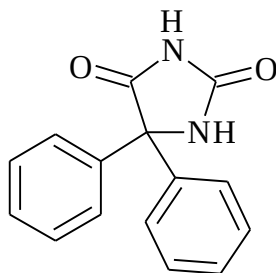
Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh, accurately, about 50 mg of the sample, dissolve in 0.1 M sodium hydroxide and dilute to 250 mL with the same solvent. Dilute, successively, in 0.1 M sodium hydroxide until concentration of 0.0004% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 278 nm, using 0.1 M sodium hydroxide for zero adjustment. Calculate the content of C₁₅H₁₀O₂ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

THERAPEUTIC CLASS

Anticoagulant.

PHENYTOIN*Phenytoinum* $C_{15}H_{12}N_2O_2$; 252.27

phenytoin, 03953

5,5-Diphenyl-2,4-imidazolidinedione

[57-41-0]

It contains not less than 98.0% and not more than 102.0% of $C_{15}H_{12}N_2O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, soluble in hot ethyl alcohol, slightly soluble in cold ethyl alcohol. Dissolves in diluted alkali hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 295 °C to 298 °C, with decomposition.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the phenytoin CRS spectrum, prepared identically.

PURITY TESTS

Acidity or alkalinity. Accurately weigh 1 g of the sample. Add 45 mL of water and heat to boiling for two minutes. Cool and filter. Then rinse the filter with carbon dioxide-free water. Collect the washing waters in a 50 mL volumetric flask and complete the volume with carbon dioxide-free water. Homogenize. Pipet 10 mL of the obtained solution into an Erlenmeyer flask. Add 0.15 mL of methyl red TS. Titrate with 0.01 M hydrochloric acid until red color. Not more than 0.5 mL of the titrant is used for the indicator changeover. Pipet 10 mL of the initial solution into another Erlenmeyer flask. Add 0.15 mL of bromothymol blue TS. Titrate with 0.01 M sodium hydroxide until blue color. Not more than 0.5 mL of the titrant is used for the indicator changeover.

Related substances. Proceed as described in *Assay*. Prepare the solutions as described below at the moment of use.

Solution (1): prepare a 1 mg/mL sample solution in *Diluent*. Place in ultrasonic bath, if necessary.

Solution (2): prepare a solution at 1 µg/ml of phenytoin CRS, 5 µg/ml of impurity A (2,2-diphenylglycine), 9 µg/ml of impurity B (2,2-diphenyl-2-ureidoacetic acid) and 1 µg/ml of benzophenone in *Diluent*.

Inject 20 µL replicates of the *Solution (2)*. The signal-noise ratio in relation to the phenytoin peak is greater than 10 and the relative standard deviation of the replicate areas under the recorded peaks is not more than 5.0%. Relative retention times are about 0.14 for impurity A, 0.53 for impurity B, 1.0 for phenytoin and 2.11 for benzophenone.

Procedure: inject, separately, 20 µL of the *Solution (2)* and of *Solution (1)*, record the chromatograms and measure the areas under the peaks. The areas under the peaks related to impurities A, B and benzophenone, obtained with *Solution (2)*, are not greater than the areas under the respective peaks obtained with *Solution (1)* (impurity A 0.5%, impurity B 0.9% and benzophenone 0.1%). The sum of the areas under all other peaks obtained with *Solution (2)*, except the solvent peaks, is not greater than the area under the phenytoin peak of *Solution (1)* (0.1%). Do not consider the peaks with an area less than 0.5 times that presented by the phenytoin peak obtained with *Solution (2)* (0.05%). The sum of all impurities, except benzophenone, is not more than 0.9%.

Heavy metals (5.3.2.3). Use *Method III*. Determine in 2 g of the sample. Use 2 mL of *Standard lead solution (10 ppm Pb)*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105 °C until constant weight. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; 150 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (3 µm), kept at room temperature; *Mobile phase* flow rate of 1,0 mL/minute.

Eluent A: 0.68% (w/v) monobasic potassium phosphate solution. Adjust pH to 2.5 with phosphoric acid.

Eluent B: mixture of methyl alcohol and acetonitrile (60:40).

Mobile phase: Adopt the gradient system described in the following table.

<i>Time (minutes)</i>	<i>Eluent (A) (%)</i>	<i>Eluent (B) (%)</i>	<i>Elution</i>
0 - 23	60	40	isocratic
23 - 38	60 → 42	40 → 58	linear gradient
38 - 45	42 → 30	58 → 70	linear gradient
45 - 50	30	70	isocratic
50 - 51	30 → 60	70 → 40	linear gradient
51 - 55	60	40	isocratic

Diluent: mixture of *Eluent B* and water (1:1).

Sample solution: prepare sample solution at 0.2 mg/mL in *Diluent*. Place in ultrasonic bath, if necessary.

Standard solution: prepare 0.2 mg/mL solution of phenytoin CRS in *Diluent*. Place in ultrasonic bath, if necessary.

Inject 20 µL replicates of the *Standard solution*. The tailing factor in relation to the phenytoin peak is not more than 1.5 and the relative standard deviation of the replicate areas under the recorded peaks is not more than 0.73%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₅H₁₂N₂O₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anticonvulsant.

PHENYTOIN TABLETS

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $C_{15}H_{12}N_2O_2$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.05 M tris buffer pH 9.0, 900 mL.

Apparatus: paddles, 100 rpm.

Time: 120 minutes.

Procedure: proceed as described in Assay. Immediately after the test, remove aliquot from the dissolution medium and filter, discarding the first milliliters. Pipet 10 mL of the filtrate and transfer to a 50 mL volumetric flask. Complete the volume with the *Mobile phase* and homogenize.

Standard solution: accurately weigh about 75 mg of phenytoin CRS, transfer to a 25 mL volumetric flask, dissolve in methyl alcohol and complete volume with the same solvent. Pipet 1 mL of this solution and transfer to a 50 mL volumetric flask, completing the volume with *Dissolution medium*. Pipet 10 mL of the above solution and dilute to 25 mL with *Mobile Phase*.

Tolerance: not less than 70% (Q) of the stated quantity of $C_{15}H_{12}N_2O_2$ dissolve in 120 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of water, methyl alcohol, acetonitrile, 1% triethylamine (v/v) and acetic acid (500:270:230:5:1).

Sample solution: weigh and pulverize 20 tablets. Transfer a quantity of powder equivalent to 50 mg of phenytoin to a 100 mL volumetric flask, add approximately 70 mL of *Mobile Phase* and place in ultrasonic bath for 10 minutes. Complete the volume with the same solvent and homogenize.

Standard solution: weigh, accurately, about 50 mg of phenytoin CRS and transfer to a 100 mL volumetric flask. Dissolve in not more than 5 mL of methyl alcohol using ultrasonic bath. Complete the volume with the *Mobile phase* and homogenize.

Inject 25 µL replicates of the *Standard solution*. The column efficiency must be not less than 6500 theoretical plates/meter. The tailing factor is not more than 1.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 25 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₅H₁₂N₂O₂ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

PHENYTOIN SODIUM INJECTION SOLUTION

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $C_{15}H_{11}N_2NaO_2$.

IDENTIFICATION

A. The main stain of the chromatogram from *Solution (1)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. It meets sodium ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 10.0 to 12.3.

PURITY TESTS

Limit for Benzophenone and Benzyl. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of dioxane and hexane (30:75) as mobile phase. Before testing, wash the plate with the mobile phase and allow to air dry. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): dilute the volume of the injection solution with methyl alcohol to obtain a 20 mg/mL sodium phenytoin solution.

Solution (2): 20 mg/mL solution of sodium phenytoin CRS in methyl alcohol.

Solution (3): 0.1 mg/mL of benzophenone in ethyl alcohol.

Solution (4): 0.1 mg/mL of benzyl in ethyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any stain corresponding to benzophenone or benzyl obtained in the chromatogram with *Solution (1)* is not more intense than the stains obtained in the chromatogram with *Solution (3)* and *Solution (4)* (0.5%).

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 0.35 EU/mg sodium phenytoin.

ASSAY

Proceed as described in *High Performance liquid chromatography* (5.2.17.4). Use chromatograph equipped with ultraviolet detector at 254 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of methyl alcohol and water (55:45).

Sample solution: transfer a volume of the injection solution equivalent to 0.25 g of sodium phenytoin to a 100 mL volumetric flask. Complete the volume with the *Mobile phase* and homogenize. Transfer 5 mL to a 50 mL volumetric flask. Complete the volume with the *Mobile phase* and homogenize.

Standard solution: dissolve an accurately weighed quantity of sodium phenytoin CRS in the *Mobile phase* and dilute to obtain a 0.25 mg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

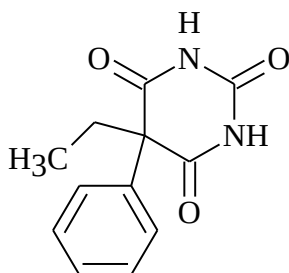
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $C_{15}H_{11}N_2NaO_2$ injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In type I glass containers, protected from light, at a temperature below 25 °C.

LABELING

Comply with current legislation.

PHENOBARBITAL***Phenobarbitalum***

$C_{12}H_{12}N_2O_3$; 232.24

phenobarbital; 03960

5-Ethyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione

[50-06-6]

It contains not less than 99.0% and not more than 101.0% of $C_{12}H_{12}N_2O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or colorless crystals.

Solubility. Very slightly soluble in water, freely soluble in ethyl alcohol. Soluble in diluted carbonates and hydroxides.

Physicochemical constants.

Melting range (5.2.2): 174 °C to 178 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the phenobarbital CRS spectrum, prepared identically.

B. The relation between the retention times of the main peak and the internal standard peak in the *Sample solution* chromatogram, obtained in method **C.** of Assay, corresponds to the relation between the retention times of the main peak and the internal standard peak in the *Standard solution* chromatogram.

PURITY TESTS

Preparation aspect. The 10% (w/v) preparation in a mixture of 2 *M* sodium hydroxide and water (2:3) is clear **(5.2.25)**.

Acidity. Boil 1 g of the sample in 50 mL of water for two minutes. Cool and filter. Add, to 10 mL of the filtrate, 0.15 mL of methyl red TS. The solution turns yellow-orangish. Titrate with 0.1 M sodium hydroxide VS. Not more than 0.1 mL of 0.1 M sodium hydroxide VS is required to produce a clear yellow color.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); *Mobile phase* flow rate of 1.0 mL/minute.

Acetate buffer pH 4.5: dissolve about 6.6 g of sodium acetate trihydrate and 3 mL of glacial acetic acid in 1000 mL of water and adjust, if necessary, with glacial acetic acid to pH 4.5 ± 0.1 .

Mobile phase: mixture of *Acetate buffer pH 4.5* and methyl alcohol (60:40).

Solution (1): Dissolve 0.125 g of the sample in 5 mL of methyl alcohol and dilute to 25 mL with the *Mobile Phase*.

Solution (2): mix 1 mL of *Solution (1)*, 20 mL of methyl alcohol and dilute to 100 mL with *Mobile Phase*. Mix 1.0 mL of this solution with 2 mL of methyl alcohol and dilute to 10 mL with *Mobile Phase*.

Solution (3): dissolve 5 mg of impurity A ((5RS)-5-ethyl-2,6-diimino-5-phenyltetrahydropyrimidine-4-(1H)-one), 5 mg of impurity B ((5RS)-5-ethyl-6-imino-5-phenyldihydropyrimidine-2,4(1H,3H)-dione) in 2 mL of methyl alcohol and dilute to 10 mL with *Mobile phase*. Mix 1 mL of this solution with 20 mL of methyl alcohol and dilute to 100 mL with *Mobile Phase*.

Inject 20 μ L replicates of the *Solution (3)*. Relative retention times are about 0.2 for impurity A, 0.3 for impurity B and 1.0 for phenobarbital. The resolution between the peaks of impurities A and B is not less than 1.5.

Procedure: separately inject 20 μ L of the *Solution (1)* and of *Solution (2)*. Record the chromatograms and measure the areas under the peaks. The area under the peak corresponding to impurity A must not be greater than 1.5 times the area under the peak corresponding to the same impurity obtained with *Solution (3)* (0.15%). The area under the peak corresponding to impurity B must not be greater than the area under the peak corresponding to the same impurity obtained with *Solution (3)* (0.1%). The area under the peaks of other impurities separately must not be greater than the area under the main peak obtained with *Solution (2)* (0.1%) and the sum of the other impurities is not more than 0.2%. Disregard peaks that correspond to half the area under the main peak obtained with *Solution (2)* (0.05%).

Loss on drying. (5.2.9.1). Dry in oven at 105 °C for two hours. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh approximately 0.4 g of sample, transfer to a 125 mL erlenmeyer flask and dissolve in 50 mL of ethyl alcohol, previously neutralized with 0.1 M sodium hydroxide VS, using six drops of thymolphthalein TS. Titrate with 0.1 M sodium hydroxide VS. Each mL of 0.1 M sodium hydroxide VS is equivalent to 23.224 mg of $C_{12}H_{12}N_2O_3$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 50 mg of sample, transfer to a 50 mL volumetric flask, dissolve in 5 mL of ethyl alcohol and complete the volume with borate buffer pH 9.6. Dilute successively to a concentration of 0.001% (w/v) using the same solvent. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbance of the resulting solutions at 240 nm, using borate buffer pH 9.6 for zero adjustment. Calculate the content of $C_{12}H_{12}N_2O_3$ in the sample from the readings obtained.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to the octadecylsilane group (5 μ m); *Mobile phase* flow rate of 1.2 mL/minute.

Acetate buffer pH 4.5: dissolve about 6.6 g of sodium acetate trihydrate and 3 mL of glacial acetic acid in 1000 mL of water and adjust, if necessary, with glacial acetic acid to pH 4.5 ± 0.1 .

Mobile phase: mixture of *Acetate buffer pH 4.5* and methyl alcohol (56:44).

Diluent: mixture of *Acetate buffer pH 4.5* and methyl alcohol (1:2).

Internal Standard solution: dissolve an accurately weighed quantity of caffeine CRS in *Diluent* to obtain a 0.6 mg/mL solution.

Sample solution: quantitatively transfer about 60 mg of the sample to a 100 mL volumetric flask. Add 10 mL of *Internal Standard Solution* and approximately 60 mL of *Diluent*. Place in ultrasonic bath for 15 minutes. Complete the volume with *Diluent*.

Sample solution: quantitatively transfer about 60 mg of phenobarbital CRS to a 100 mL volumetric flask. Add 10 mL of *Internal Standard Solution* and approximately 60 mL of *Diluent*. Place in ultrasonic bath for 15 minutes and complete the volume with *Diluent* to obtain a solution containing 0.6 mg/mL of phenobarbital.

Inject 20 μ L replicates of the *Standard solution*. The relative retention times are approximately 0.4 for caffeine and 1.0 for phenobarbital. The tailing factor is not more than 2.0. The resolution between phenobarbital and caffeine is not less than 1.2. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to phenobarbital and

caffeine. Calculate the content of $C_{12}H_{12}N_2O_3$ in the sample from the responses obtained for the phenobarbital/ caffeine ratio with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anticonvulsant, hypnotic, sedative.

PHENOBARBITAL TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{12}H_{12}N_2O_3$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer a quantity of powder equivalent to 60 mg of phenobarbital to a beaker, add 50 mL of chloroform, homogenize and filter. Evaporate to dryness. Dry the residue in oven at 105 °C for two hours. The residue meets the **A.** test *Identification* of the *Phenobarbital* monograph.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from *Assay*, there are maximum and minimum points identical to those observed in the spectrum of the standard solution.

C. The relation between the retention times of the main peak and the internal standard peak in the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the relation between the retention times of the main peak and the internal standard peak in the *Standard solution* chromatogram.

D. The residue obtained in the test **A.** from *Identification* (**5.2.2**) melts between 174 °C and 178 °C.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 100 mL volumetric flask containing 5 mL of water and wait for the tablet to completely disintegrate. Add 5 mL of ethyl alcohol and 60 mL of borate buffer pH 9.6. Place in ultrasonic bath for 15 minutes. Shake, mechanically, for 15 minutes, complete the volume with the buffer. Proceed as described in method **A.** of *Assay* from "Homogenize and filter".

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium and dilute with borate buffer pH 9.6 to adequate concentration. Measure the absorbances of the solutions at 240 nm (**5.2.14**) using the same solvent for zero adjustment. Calculate the quantity of $C_{12}H_{12}N_2O_3$ dissolved in the

medium, comparing the readings obtained with the phenobarbital CRS solution at the concentration of 0.001% (w/v), prepared in borate buffer pH 9.6

Tolerance: not less than 75% (Q) of the stated quantity of $C_{12}H_{12}N_2O_3$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. By Ultraviolet absorption spectrophotometry (5.2.14). Weigh and pulverize 20 tablets. Transfer a quantity of powder equivalent to 50 mg of phenobarbital to a 50 mL volumetric flask, dissolve in 5 mL of ethyl alcohol and add 35 mL of borate buffer pH 9.6. Place in ultrasonic bath for 15 minutes and complete the volume with the buffer. Homogenize and filter. Dilute successively, with the same solvent, to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbance of the resulting solutions at 240 nm wavelength, using borate buffer pH 9.6 for zero adjustment. Calculate the quantity of $C_{12}H_{12}N_2O_3$ in the tablets from the readings obtained.

B. By High performance liquid chromatography (5.2.17.4) Proceed as described in method C. from Assay in the *Phenobarbital* monograph. Prepare *Sample solution* as described below.

Sample Solution: weigh and pulverize 20 tablets. Transfer a quantity of powder equivalent to 60 mg of phenobarbital to a 100 mL volumetric flask, add 10 mL of *Internal standard solution* and 60 mL of *Diluent*. Place in ultrasonic bath for 15 minutes. Shake, mechanically for 15 minutes, complete the volume with the *Diluent*, homogenize and filter.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to phenobarbital and caffeine. Calculate the content of $C_{12}H_{12}N_2O_3$ in the tablets from the responses obtained for the phenobarbital/ caffeine ratio, with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

PHENOBARBITAL ORAL SOLUTION

It contains not less than 90.0% and not more than 105.0% of the stated quantity of $C_{12}H_{12}N_2O_3$. It contains appropriate stabilizing and alkalizing agents.

IDENTIFICATION

A. Transfer volume of the oral solution, equivalent to 0.4 g of phenobarbital, to a 125 mL separating funnel containing 20 mL of water, add 5 mL of sodium hydroxide *M*, homogenize and extract with two 10 mL portions of chloroform, discarding the organic layer. Add 5 mL of 3 *M* hydrochloric acid and extract with two 25 mL portions of chloroform, filter, collecting the organic extracts in a beaker. Evaporate to dryness. Dry the residue in oven at 105 °C for two hours. The residue meets the **A.** test *Identification* of the *Phenobarbital* monograph.

B. The relation between the retention times of the main peak and the internal standard peak in the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the relation between the retention times of the main peak and the internal standard peak in the *Standard solution* chromatogram.

C. The residue obtained in the test **A.** in *Identification* (5.2.2) melts between 174 °C and 178 °C.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 9.2 to 10.2.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in method **C.** from *Assay* in the *Phenobarbital* monograph. Prepare *Sample solution* as described below.

Sample solution: transfer a volume of the oral solution equivalent to 0.6 g of phenobarbital to a 100 mL volumetric flask and complete the volume with the *Diluent*. Transfer 10 mL to a 50 mL volumetric flask, add 4 mL of the *Internal Standard Solution* and complete the volume with the *Diluent*.

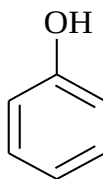
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to phenobarbital and caffeine. Calculate the content of $C_{12}H_{12}N_2O_3$ in the oral solution from the responses obtained for the phenobarbital/caffeine ratio, with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

PHENOL
Phenolum

C₆H₆O; 94.11
phenol; 03968
Phenol
[108-95-2]

It contains not less than 99.0% and not more than 100.5% of C₆H₆O in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Colorless acicular crystals or white crystalline mass, corrosive, irritating to mucous membranes and skin, deliquescent. Darkens when exposed to air and light.

Solubility. Soluble in water, very soluble in ethyl alcohol, glycerol and fixed and volatile oils.

Physicochemical constants.

Congearing temperature (5.2.4): not less than 39 °C.

IDENTIFICATION

A. To 5 mL of a 2% (w/v) aqueous sample solution, add one drop of 5% (w/v) aqueous ferric chloride solution. A violet color develops. Add 10 mL of ethyl alcohol at 90% (v/v). The color turns yellow.

B. Add bromine water RS to a 1% (w/v) aqueous sample solution. A white precipitate is produced which dissolves immediately but becomes permanent after addition of excess reagent.

PURITY TESTS

Preparation aspect. The preparation of 1 g of the sample in 15 mL of water is clear **(5.2.25)**.

Acidity. To 5 mL of a solution of 1 g of the sample in 15 mL of water, add a drop of methyl orange TS. Yellow color is produced.

Water (5.2.20.1). Not more than 0.5%.

Residue by evaporation. Accurately weigh about 5 g of the sample, evaporate in water bath and dry at 105 °C for one hour. The residue mass is not more than 2.5 mg (0.05%).

ASSAY

Weigh, accurately, about 0.2 g of the sample, dissolve in water and complete the volume to 100 mL with the same solvent. Transfer 25 mL of the solution to a capped Erlenmeyer flask and add 50 mL of 0.05 M bromine VS and 5 mL of hydrochloric acid. Cap, shake occasionally for 20 minutes and keep protected from light for 15 minutes. Add 5 mL of 20% potassium iodide solution (w/v) and shake slightly. Titrate with 0.1 M sodium thiosulfate VS. Add 3 mL of starch TS solution and 10 mL of chloroform when the solution is slightly yellowish. Continue the titration with vigorous agitation until the blue color disappears. Carry out the blank test and make the necessary corrections. Each mL of 0.05 M bromine VS is equivalent to 1.569 mg of C₆H₆O.

PACKAGING AND STORAGE

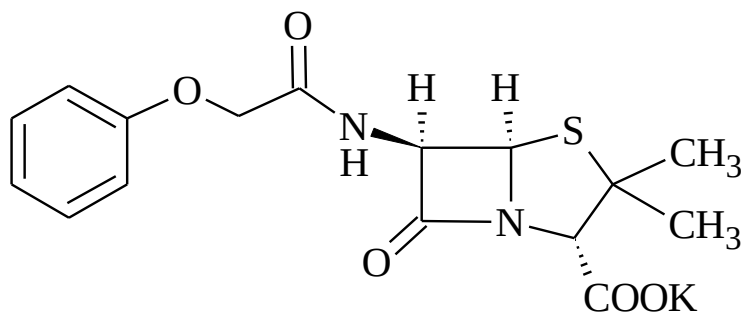
In hermetic containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Antiseptic, preservative and antipruritic.

PHENOXYMETHYLPENICILLIN POTASSIUM*Phenoxymethylpenicillinum kalicum*C₁₆H₁₇KN₂O₅S; 388.48

phenoxymethylpenicillin potassium; 03996

Acid potassium salt (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic (1:1)

[132-98-9]

It has a content of not less than 1380 IU and not more than 1610 IU of phenoxymethylpenicillin (C₁₆H₁₈N₂O₅S) per milligram, in relation to the desiccated substance.

DESCRIPTION**Physical characteristics.** White crystalline powder.**Solubility.** Very soluble in water, slightly soluble in ethyl alcohol.**Physicochemical constants.**

Specific optical rotation (5.2.8): +220 to +235, in relation to the desiccated substance. Determine in 1% (w/v) solution in carbon dioxide-free water.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the phenoxymethylpenicillin potassium CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **A.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. It meets potassium ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.0 to 7.5. Determine in aqueous solution at 3% (w/v).

Limit for Phenoxyacetic acid Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute. Prepare solutions as described below.

Phosphate buffer pH 6.6: transfer 250 mL of 0.2 M monobasic potassium phosphate and 82 mL of 0.2 M sodium hydroxide to a 1000 mL volumetric flask. Complete the volume with water and homogenize.

Mobile phase: mixture of water, acetonitrile and glacial acetic acid (65:35:1). Make the necessary adjustments.

Solution (1): 0.1 mg/mL phenoxyacetic acid solution in *Phosphate Buffer pH 6.6*.

Solution (2): 20 mg/mL sample solution in *Phosphate Buffer pH 6.6*. Use the solution on the same day.

Inject 20 μ L replicates of the *Solution (1)*. The tailing factor is not more than 1.5 and the relative standard deviation of the replicate areas under the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Not more than 0.5% of phenoxyacetic acid.

limit for 4-Hydroxyphenoxymethylpenicillin. Use the chromatogram obtained in the *Assay* and calculate the percentage of 4-hydroxyphenoxymethylpenicillin in the sample using the formula: $100rh/rs$, where rh is the area under the peak of 4-hydroxyphenoxymethylpenicillin and rs is the sum of the areas under the peaks of 4-hydroxyphenoxymethylpenicillin and phenoxymethylpenicillin. Not more than 5.0%.

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105°C. Not more than 1.5%.

ASSAY

Use one of the methods described below.

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water, acetonitrile and glacial acetic acid (650:350:5.75).

Sample solution: dissolve an accurately weighed quantity of the sample in *Mobile phase* to obtain a 2.5 mg/mL solution.

Standard solution: dissolve an accurately weighed quantity of phenoxymethylpenicillin potassium CRS in the *Mobile phase* to obtain a 2.5 mg/mL solution.

Resolution solution: prepare a *Mobile phase solution* containing approximately 2.5 mg of potassium benzylpenicillin and 2.5 mg of phenoxymethylpenicillin per milliliter.

Inject 10 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.8 for benzylpenicillin and 1.0 for phenoxymethylpenicillin. The resolution between the phenoxymethylpenicillin and benzylpenicillin peaks is not less than 3.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: Inject 10 µL of the *Standard Solution* and the *Sample Solution* separately, record the chromatograms and measure the areas under the largest phenoxymethylpenicillin peak and any peak with a retention time relative to the main phenoxymethylpenicillin peak of approximately 0.4. Calculate the UI content of phenoxymethylpenicillin (C₁₆H₁₈N₂O₅S) per milligram of the sample from the standard content and the responses obtained for the sum of the areas under the peaks obtained with the *Standard Solution* and the *Sample Solution*.

B. Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)* by agar diffusion using cylinders.

Microorganism: *Staphylococcus aureus* ATCC 6538P.

Culture media: culture medium number 1, for microorganism maintenance and inoculum preparation; culture medium number 2, for base layer.

Sample solution: Dissolve accurately weighed quantity of sample in *sterile 1% Potassium Phosphate Buffer, pH 6.0 (Solution 1)* to obtain a 100 IU/mL solution. Dilute to obtain concentrations of 0.2 UI/mL, 0.4 UI/mL and 0.8 UI/mL, using *1% Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* as diluent.

Standard solution: Dissolve accurately weighed quantity of phenoxymethylpenicillin potassium in *sterile 1% Potassium Phosphate Buffer, pH 6.0 (Solution 1)* to obtain a 100 IU/mL solution. Dilute to obtain concentrations of 0.2 UI/mL, 0.4 UI/mL and 0.8 UI/mL, using *1% Potassium phosphate buffer, sterile, pH 6.0 (Solution 1)* as diluent.

Procedure: add 21 mL of culture medium number 2 to each plate, wait to solidify, add 4 mL of 1% inoculum and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*, adding 0.2 mL of the freshly prepared solutions to the cylinders. Calculate the sample potency, in IU of phenoxymethylpenicillin per milligram, from the standard potency and the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In hermetic containers and protected from light.

LABELING

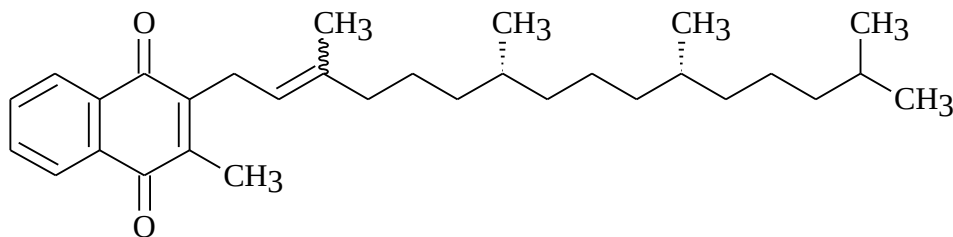
Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

PHYTOMENADIONE

Phytomenadionum



$C_{31}H_{46}O_2$; 450.70

phytomenadione, 04060

2-Methyl-3-[(2*E*,7*R*,11*R*)-3,7,11,15-tetramethyl-2-hexadecen-1-yl]-1,4-naphthalenedione
[84-80-0]

Phytomenadione is a mixture of *E* and *Z* isomers that contains not less than 97.0% and not more than 103.0% of $C_{31}H_{46}O_2$. It contains not more than 21.0% of the *Z* isomer.

DESCRIPTION

Physical characteristics. Clear yellow to amber liquid, very viscous, oily. It is stable in air but decomposes on exposure to light.

Solubility. Practically insoluble in water, soluble in absolute ethyl alcohol and vegetable oils, moderately soluble in ethyl alcohol.

Physicochemical constants.

Refractive index (5.2.6): 1.523 to 1.526.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample thin film, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the phytomenadione CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in *n*-hexane, there are maximum and minimum points only at the same wavelengths as the phytomenadione CRS solution, prepared identically.

PURITY TESTS

Acidity or alkalinity. The 5% (v/v) solution in absolute ethyl alcohol is neutral to litmus paper.

Menadione. Mix approximately 20 mg of the sample with 0.5 mL of a mixture of equal volumes of ammonia RS and methyl alcohol. Add one drop of ethyl cyanoacetate and shake gently. No purple or blue color develops.

Limit for (Z)-phytomenadione. Proceed as described in Assay. Calculate the (Z)-phytomenadione content in the sample using the formula:

$$100 \times a_z / (a_z + a_e)$$

where

a_z = area under the peak of (Z)-phytomenadione obtained with the *Sample solution*;

a_e = area under the peak of (E)-phytomenadione obtained with the *Sample solution*;

Not more than 21.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Protect solutions from exposure to direct light. Use a chromatograph equipped with an ultraviolet detector at 254 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica (5 µm), kept at 30 °C; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of *n*-hexane and *n*-amyl alcohol (2000:1.5).

Internal Standard solution: dissolve an accurately weighed quantity of cholesteryl benzoate in *Mobile phase* to obtain a 2.5 mg/mL solution.

Sample solution: quantitatively transfer about 60 mg of the sample to a 50 mL volumetric flask, dissolve in 20 mL of *Mobile Phase*. Complete the volume with the same solvent and homogenize. Transfer 4 mL of the solution to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 10 mL of the solution obtained and 7 mL of the *Internal Standard solution* to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Standard solution: quantitatively transfer about 60 mg of the phytomenadione CRS to a 50 mL volumetric flask and dissolve in 20 mL of *Mobile Phase*. Complete the volume with the same solvent and homogenize. Transfer 4 mL of the solution to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 10 mL of the solution obtained and 7 mL of the *Internal Standard solution* to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Inject 50 µL replicates of the *Standard solution*. The relative retention times are about 0.7 for cholesteryl benzoate, 0.9 for (Z)-phytomenadione and 1.0 for (E)-phytomenadione. The resolution between (E)-phytomenadione and (Z)-phytomenadione is not less than 1.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 50 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the C₃₁H₄₆O₂ content in the sample from the responses obtained for the ratio ((Z)-phytomenadione area + (E)-phytomenadione area) / cholesterol benzoate area, with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

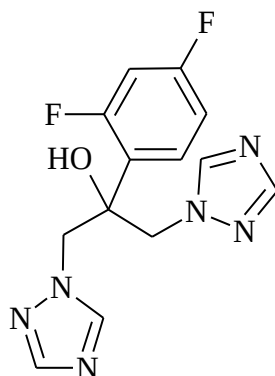
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-hemorrhagic.

FLUCONAZOLE**Fluconazolum**C₁₃H₁₂F₂N₆O; 306.27

fluconazole; 04109

α -(2,4-Difluorophenyl)- α -(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-1,2,4-triazol-1-ethanol
[86386-73-4]

It contains not less than 98.0% and not more than 102.0% of C₁₃H₁₂F₂N₆O, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white powder. Presents polymorphism.

Solubility. Slightly soluble in water, freely soluble in methyl alcohol, soluble in ethyl alcohol, moderately soluble in isopropyl alcohol. Soluble in hydrochloric acid *M* and sodium hydroxide *M*.

Physicochemical constants.

Melting range (5.2.2): 138 °C to 140 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the fluconazole CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.02% (w/v) solution in 0.1 *M* sodium hydroxide, there is a maximum point at 261 nm, identical to that observed in the spectrum of similar fluconazole CRS solution.

PURITY TESTS

Preparation aspect. The 5% (w/v) preparation in methyl alcohol is clear **(5.2.25)** and colorless **(5.2.12)**.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 260 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm); kept at 40 °C, *Mobile phase* flow rate of 1 mL/minute. Prepare solutions as described below.

Mobile phase: mixture of acetonitrile and 0.63 g/L of ammonium formate solution (14:86).

Solution (1): Dissolve accurately weighed quantity of sample in *Mobile Phase*. Place in ultrasonic bath, if necessary, and dilute adequately to obtain a 10 mg/mL solution.

Solution (2): dilute 5 mL of *Solution (1)* to 100 mL using *Mobile phase*. Dilute 1 mL of the solution to 10 mL using *Mobile phase*.

Solution (3): Dissolve 5 mg of fluconazole for CRS peak identification (containing impurity A) in *Mobile Phase*. Place in ultrasonic bath, if necessary, and dilute to 10 mL using *Mobile Phase*.

Solution (4): dissolve accurately weighed quantity of fluconazole impurity B CRS in *Mobile Phase*. Place in ultrasonic bath, if necessary, and dilute adequately to obtain a 0.3 mg/mL solution.

Solution (5): dissolve an accurately weighed quantity of fluconazole impurity C CRS in *Mobile phase* and dilute adequately to obtain a solution at 0.1 mg/mL. Mix 1 mL of this solution with 1 mL of *Solution (1)* and dilute to 10 mL with *Mobile Phase*.

Inject replicates of *Solution (5)* The resolution between the peaks of impurity C and of fluconazole is not less than 3.0.

Procedure: inject, separately, 20 µL of *Solution (1)*, *Solution (2)*, *Solution (3)*, *Solution (4)* and *Solution (5)*, record the chromatograms for 3.5 times the fluconazole retention time and measure the areas under the peaks. Identify impurities A, B and C with the chromatograms obtained with *Solutions (3)*, *(4)* and *(5)*, respectively. The retention time for the fluconazole peak is about 11 minutes. The relative retention time of impurities B, A and C is 0.4, 0.5 and 0.8, respectively. The area under the peak corresponding to impurity A in the chromatogram of *Solution (1)* is not more than 0.8 times the area under the main peak obtained with *Solution (2)* (0.4%). The area under the peak corresponding to impurity B is not greater than the area under the main peak obtained with *Solution (4)* (0.3%). The area under the peak corresponding to impurity C is not greater than the area under the main peak obtained with *Solution (5)* (0.1%). The peak of each unspecified impurity in the chromatogram of *Solution (1)* is not more than 0.2 times the area under the main peak obtained with *Solution (2)* (0.1%). The sum of the areas under all peaks is not more than 1.2 times the area under the main peak obtained with *Solution (2)* (0.6%). Disregard peaks with areas below 0.1 times the area under the main peak obtained with *Solution (2)* (0.05%).

Iron (5.3.2.4). Dissolve 0.5 g of the sample in 5 mL of ethyl alcohol. Add 5 mL of water, homogenize and proceed as described in *Limit Test for Iron, Method III*. Not more than 0.002% (20 ppm).

Heavy metals (5.3.2.3). Use the residue obtained in the *Residue test by incineration* and proceed as described in *Method III*, starting from "add 4 ml of 6 M hydrochloric acid...". Use 2.5 mL of *Standard lead solution (10 ppm Pb)* for *Standard preparation*. Not more than 0.0025% (25 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh about 0.1 g of the sample and dissolve in 50 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS. Determine the endpoint potentiometrically or using methylrosanilinium chloride TS as indicator. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 15.314 mg of $C_{13}H_{12}F_2N_6O$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antifungal.

FLUCONAZOLE CAPSULES

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{13}H_{12}F_2N_6O$.

IDENTIFICATION

A. Shake the quantity of powder equivalent to 10 mg of fluconazole with 20 mL of methyl alcohol. Filter and evaporate the filtrate to dryness. The residue meets the test **A.** in *Identification* of the *Fluconazole* monograph.

B. Transfer a quantity of powder equivalent to 0.1 g of fluconazole to a 100 mL volumetric flask. Add 70 mL of 0.1 M hydrochloric acid. Place in ultrasonic bath for 10 minutes, complete the volume with the same solvent, homogenize and filter. Dilute with 0.1 M hydrochloric acid to a concentration of 0.02% (w/v). In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of the 0.02% (w/v) solution in 0.1 M hydrochloric acid, there is a maximum point at 261 nm, identical to that observed in the spectrum of similar fluconazole CRS solution.

C. Dissolve the amount of powder equivalent to 0.25 g of the sample in 5 mL of acetone and filter. Add, under agitation, five to eight drops of 5% chromic acid (w/v). Precipitate is produced in five seconds.

D. Add 3 mL of 1% ferric chloride (w/v) in glacial acetic acid to a quantity of powder equivalent to 50 mg of fluconazole and shake. Add sulfuric acid M carefully through the tube walls. The color should change to orangish and yellow.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described in method **A.** from *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and measure the absorbances of the solutions at 261 nm (**5.2.14**), using 0.1 M hydrochloric acid for zero adjustment. Calculate the quantity of $C_{13}H_{12}F_2N_6O$ dissolved in the medium, comparing the readings obtained with that of the fluconazole CRS solution at the concentration of 0.02% (w/v), prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{13}H_{12}F_2N_6O$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh 20 capsules, remove contents and weigh again. Homogenize the contents of the capsules. Transfer a quantity of powder equivalent to 0.1 g of fluconazole to a 100 mL volumetric flask. Add 70 mL of 0.1 M hydrochloric acid. Place in ultrasonic bath for 10 minutes, complete the volume with the same solvent, homogenize and filter. Dilute with 0.1 M hydrochloric acid to a concentration of 0.02% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 261 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the quantity of $C_{13}H_{12}F_2N_6O$ in the capsules from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 260 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 μ m); kept at 30 °C, Mobile phase flow rate of 0.8 mL/minute.

Mobile phase: mixture of water and acetonitrile (78:22).

Sample solution: Weigh the capsules, remove contents and weigh again. Homogenize the contents of the capsules. Quantitatively transfer quantity of powder equivalent to 50 g of fluconazole to a 100 mL volumetric flask. Add 70 mL of *Mobile phase* and place in ultrasonic bath for 10 minutes. Complete the volume with the same solvent, homogenize and filter.

Standard solution: dissolve an accurately weighed quantity of fluconazole CRS in *Mobile phase* to obtain a 0.5 mg/mL solution.

Inject 20 μ L replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{13}H_{12}F_2N_6O$ in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

FLUNITRAZEPAM TABLETS

It contains not less than 92.5% and not more than 107.5% of the stated quantity of $C_{16}H_{12}FN_3O_3$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the sample solution obtained in *Assay*, there are maximum and minimum points identical to those observed in the spectrum of the standard solution.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of nitromethane, ethyl ether, *n*-heptane and 25% ammonia (v/v) (30:60:15:2.5) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Use a quantity of powder equivalent to 20 mg of flunitrazepam and add 20 ml of methyl alcohol, shake and filter.

Solution (2): 1 mg/mL flunitrazepam CRS solution in methyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Spray with 10% sodium hydroxide solution (w/v). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Weigh, individually, and transfer each tablet to a centrifuge tube, add 5 mL of water and place in ultrasonic bath until the tablet disintegrates. Add 25 mL of methyl alcohol, place in ultrasonic bath for 3 minutes and shake for 10 minutes. Centrifuge for 10 minutes, filter the supernatant through a membrane with porosity of 0.45 µm. Dilute, successively, in methyl alcohol until concentration of 0.0016% (w/v). Proceed as described in *Assay*, starting from "Prepare standard solution...". Calculate the quantity of $C_{16}H_{12}FN_3O_3$ in each tablet from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: simulated gastric fluid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use chromatograph equipped with ultraviolet detector at 279 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm); *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: transfer 670 mL of 0.05 M monobasic potassium phosphate to a 1000 ml volumetric flask and complete the volume with acetonitrile. Adjust the pH of the solution to 6.2 with 0.25 M potassium hydroxide solution.

Sample solution: after testing, remove a sufficient aliquot of the dissolution medium, filter through a 0.45 µm membrane, cool and dilute in the *Dissolution Medium*, if necessary, to a concentration of 1.1 µg/mL.

Standard solution: accurately weigh about 22 mg of flunitrazepam CRS, transfer to a 250 mL volumetric flask, add 100 mL of methyl alcohol and place in ultrasonic bath until complete dissolution. Complete the volume with the same solvent and homogenize. Dilute successively in the *Dissolution Medium* to obtain a 1.1 µg/mL solution.

Procedure: inject, separately, 100 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₆H₁₂FN₃O₃ dissolved in the medium, from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 85% (Q) of the stated quantity of C₁₆H₁₂FN₃O₃ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh and pulverize 20 tablets. Quantitatively transfer quantity of powder equivalent to 16 mg of flunitrazepam to a 100 mL volumetric flask. Add 10 mL of water and shake until complete dissolution. Complete the volume with methyl alcohol, shake on a magnetic stirrer for 20 minutes and filter through a 0.45 µm membrane. Dilute, successively, in methyl alcohol until concentration of 0.0016% (w/v). Prepare a standard solution in the same concentration, using methyl alcohol as solvent. Measure the absorbances of the resulting solutions at 309 nm (5.2.14), using methyl alcohol for zero adjustment. Calculate the quantity of C₁₆H₁₂FN₃O₃ in the tablets from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

FLUNITRAZEPAM INJECTION SOLUTION

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $C_{16}H_{12}FN_3O_3$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the sample solution obtained in *Assay*, there are maximum and minimum points identical to those observed in the spectrum of the standard solution.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel 60 GF₂₅₄ as support, and a mixture of chloroform, methyl alcohol and ammonium hydroxide 25% (v/v) (90:10:1) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below. Proceed protected from direct light.

Solution (1): dilute the volume of the injection solution in ethyl alcohol to obtain a concentration of about 0.5 mg/mL.

Solution (2): 0.5 mg/mL flunitrazepam CRS solution in ethyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Spray with potassium iodide and bismuth subnitrate RS. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. Other stains may appear due to the presence of excipients.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.9 to 4.7.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Pyrogens (5.5.2.1). Complies with the test. Inject 0.5 mL/kg, using flunitrazepam at 0.2 mg/mL in saline solution.

ASSAY

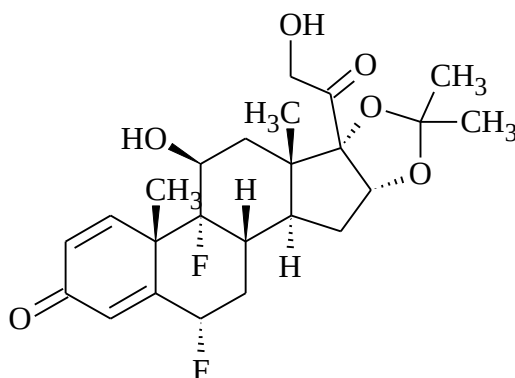
Transfer a volume of the injectable solution, equivalent to 0.2 g of flunitrazepam to a 200 mL volumetric flask, dissolve and complete the volume with methyl alcohol. Dilute, successively, in methyl alcohol to concentration of 0.0015% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 309 nm (**5.2.14**), using methyl alcohol for zero adjustment. Calculate the quantity of $C_{16}H_{12}FN_3O_3$ in the injection solution from the readings obtained.

PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELING

Comply with current legislation.

FLUOCINOLONE ACETONIDE*Fluocinoloni acetonidum*C₂₄H₃₀F₂O₆; 452.49

fluocinolone acetonide; 04159

(6 α ,11 β ,16 α)-6,9-Difluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-pregna-1,4-diene-3,20- dione
[67-73-2]

It contains not less than 98.0% and not more than 102.0% of C₂₄H₃₀F₂O₆, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder. Presents polymorphism.

Solubility. Practically insoluble in water, soluble in ethyl alcohol and in methyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +98 to +108, in relation to the desiccated substance. Determine in solution at 1% (w/v) in methyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the fluocinolone acetonide CRS spectrum, prepared identically. If differences are observed, dissolve the sample and standard separately in ethyl alcohol, evaporate the solvent and plot new spectra with the residues.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed protected from direct light. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 238

nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute. Prepare the solutions described below.

Mobile phase: mixture of acetonitrile and water (45:55).

Solution (1): 2,5 mg/mL sample solution in acetonitrile.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with acetonitrile.

Solution (3): Dissolve 2.5 mg of fluocinolone acetonide CRS and 2.5 mg of triamcinolone acetonide in 45 mL of acetonitrile. Complete the volume to 100 mL with water and shake.

Inject 20 µL replicates of the *Solution (3)*. The relative retention times are about 0.85 for triamcinolone acetonide and 1.0 for fluocinolone acetonide. The resolution between fluocinolone acetonide and triamcinolone acetonide on the chromatogram is not less than 3.0.

Procedure: separately inject 20 µL of the *Solution (1)* and of *Solution (2)*. Record the chromatograms for not less than four times the peak retention time for fluocinolone acetonide and measure the area under the peaks. The sum of the areas under the secondary peaks obtained with the *Solution (1)*, except that under the main peak, is not more than 2.5 times the area under the main peak obtained with *Solution (2)* (2.5%). Any secondary peak obtained with the *Sample solution* must be greater than the main peak obtained with *Solution (2)* (1.0%). Not more than a secondary peak obtained with *Solution (1)* has an area greater than half the area under the main peak obtained with *Solution (2)* (0.5%) Disregard peaks with areas below 0.05 times the area under the main peak obtained with *Solution (2)* (0.05%).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for three hours. Not more than 1.0% for the anhydrous form and not more than 8.5% for the hydrated form.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 100 mm long and 4.5 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of water, acetonitrile and tetrahydrofuran (77:13:10).

Sample solution: transfer 20 mg of the sample, accurately weighed, to a 100 mL volumetric flask, dissolve in 23 mL of a mixture of acetonitrile and tetrahydrofuran (13:10), complete the volume with water and homogenize.

Standard solution: transfer 20 mg of fluocinolone acetonide CRS, accurately weighed, to a 100 mL volumetric flask, dissolve in 23 mL of a mixture of acetonitrile and tetrahydrofuran (13:10), complete the volume with water and homogenize.

Inject 20 µL replicates of the *Standard solution*. The column efficiency must be not less than 3000 theoretical plates. The relative standard deviation of the replicate areas under the peaks recorded is not more than 3.0%. The *Mobile Phase* flow should be adjusted so that the fluocinolone acetonide peak retention time is between 9 and 13 minutes.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{24}H_{30}F_2O_6$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

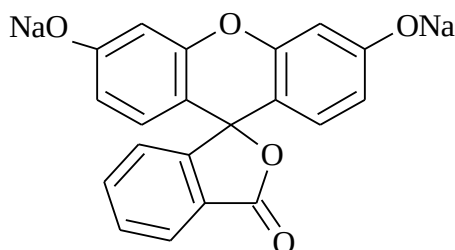
Comply with current legislation. The label should indicate whether the fluocinolone acetonide form is anhydrous or hydrated.

THERAPEUTIC CLASS

Steroidal anti-inflammatory.

FLUORESCEIN SODIUM

Fluoresceinum natricum



$C_{20}H_{10}Na_2O_5$; 376.27

fluorescein sodium; 04167

Sodium salt of 3',6'-dihydroxyspiro[isobenzofuran-1(3*H*),9'-(9*H*)xanthen]-3-one (2:1)
[518-47-8]

It contains not less than 95.0% and not more than 103.0% of $C_{20}H_{10}Na_2O_5$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Fine, red-orangish, hygroscopic powder.

Solubility. Freely soluble in water, moderately soluble in ethyl alcohol.

IDENTIFICATION

A. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

B. Calcine 0.1 g of the sample in a porcelain crucible. Dissolve the residue in 5 mL of water and filter. The solution meets sodium ion reactions (**5.3.1.1**).

PURITY TESTS

pH (5.2.19). 7.0 to 9.0. Determine in aqueous solution at 2% (w/v).

Related substances. Proceed as described in *Assay*. Prepare solutions as described below.

Solution (1): quantitatively transfer about 0.1 g of the sample to a 100 mL volumetric flask. Complete the volume with *Diluent* and homogenize.

Solution (2): transfer 5 mL of the *Standard solution* obtained in *Assay* to a 20 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Solution (3): solution containing 0.005 mg/mL of resorcinol CRS, 0.005 mg/mL of phthalic acid CRS and 0.005 mg/mL of fluorescein related compound C CRS (2-(2,4-dihydroxybenzoyl)benzoic acid) in *Diluent*.

Inject 20 μ L replicates of the *Solution (3)*. The resolution between phthalic acid and resorcinol is not less than 1.5.

Procedure: inject, separately, 20 μ L of each of the solutions, record the chromatograms and measure the areas under the peaks. The relative retention times for fluorescein, whose retention time is about 15 minutes, are about 0.42 for resorcinol, 0.48 for phthalic acid and 0.86 for fluorescein related compound C. Determine the percentage of each of the impurities found in *Solution (1)*, where the individual content of resorcinol, phthalic acid and fluorescein related compound C is not more than 0.5%, in relation to fluorescein sodium. The percentage for any other individual impurity is not more than 0.1% and not more than 0.5% for the sum of all impurities present. Do not consider solvent-related peaks or those with area less than 0.05% the area under the fluorescein peak. The test will only be valid if the resolution between resorcinol and phthalic acid peaks in the *Standard Solution (2)* is greater than 1.5.

Acriflavin. Dissolve 10 mg of the sample in 5 mL of water and add some drops of 10% (w/v) aqueous sodium salicylate solution. Precipitate is not formed.

Zinc. Dissolve 0.1 g of the sample in 10 mL of saturated sodium chloride solution. Add 2 mL of 3 M hydrochloric acid, shake vigorously and filter. Add 1 mL of potassium ferricyanide RS. No turbidity is produced.

Water (5.2.20.1). Not more than 17.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; 250mm long, 4.6 mm internal diameter column, packed with silica gel chemically bonded to an octylsilane group (5 μ m); kept at 35 °C°, *Mobile phase* flow rate of 1 mL/minute.

Eluent A: Dissolve 0.61 g of monobasic potassium phosphate in 1000 mL of water and adjust the pH to 2.0 with phosphoric acid. Degas and filter.

Eluent (B): acetonitrile.

Diluent: mixture of *Eluent A* and *Eluent B* (70:30).

Mobile phase gradient: adopt the gradient system described on the following table:

Time (minutes)	Eluent A (%)	Eluent B (%)	Elution
0 – 20	85 → 20	15 → 80	linear gradient
20 – 29	20	80	isocratic

29 – 30	20 → 85	80 → 15	linear gradient
30 – 35	85	15	isocratic

Sample solution: quantitatively transfer about 0.1 g of the sample to a 100 mL volumetric flask. Complete the volume with *Diluent* and homogenize. Transfer 5 mL of this solution to a 250-mL volumetric flask, complete the volume with *Diluent* and homogenize.

Standard solution: dissolve 110 mg of diacetyl fluorescein CRS, equivalent to 100 mg of fluorescein sodium, in a mixture of 10 mL of ethyl alcohol and 2 mL of 2.5 M sodium hydroxide. Heat in boiling water bath for 20 minutes, with agitation. Cool, transfer to a 100 mL volumetric flask and complete the volume with water to obtain a 1 mg/mL fluorescein sodium solution. Transfer 5 mL of this solution to a 250-mL volumetric flask, complete the volume with *Diluent* and homogenize.

Inject 20 µL replicates of the *Standard solution*. The tailing factor is not more than 1.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₀H₁₀Na₂O₅ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Diagnosis agent.

SODIUM FLUORIDE

Natrii fluoridum

NaF; 41.99
sodium fluoride; 04170
Sodium fluoride
[7681-49-4]

It contains not less than 98.0% and not more than 102.0% of NaF, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder.

Solubility. Soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. Transfer 1 g of the sample to a platinum crucible, in a hood, and add 15 mL of sulfuric acid. Cover with a clean, polished piece of glass and heat in water bath for an hour. Remove the glass lid, rinse with water and dry. The surface of the glass is marked.

B. The 4% (w/v) solution meets sodium ion reactions (5.3.1.1).

PURITY TESTS

Acidity or alkalinity. Dissolve 2 g of the sample in 40 mL of water in a platinum capsule, add 10 mL of saturated potassium nitrate solution, cool the solution to 0 °C and add three drops of phenolphthalein TS. If the solution turns pink, not more than 0.5 mL of 0.05 M sulfuric acid is required for indicator changeover. If the solution remains colorless, not more than 2 mL of 0.1 M sodium hydroxide is required for a persistent pink color to develop for 15 seconds.

Fluorosilicate. Heat the neutralized solution obtained in *Acidity or alkalinity* to boiling and titrate, while still hot, with 0.1 M sodium hydroxide VS until a permanent pink color. Not more than 1.5 mL of 0.1 M sodium hydroxide VS is required.

Chlorides (5.3.2.1). Dissolve 0.3 g of the sample in 20 mL of water and add 0.2 g of boric acid, 1 mL of nitric acid RS and 1 mL of 0.1 M silver nitrate. Any resulting turbidity is not more intense than that of a standard prepared with 0.1 mL of 0.01 M hydrochloric acid, using the same reagents. Not more than 0.012% (120 ppm).

Heavy metals (5.3.2.3). Transfer 1 g of the sample to a platinum capsule or crucible, add 1 mL of water and 3 mL of sulfuric acid. Heat, in a hood, at as low a temperature as possible, until all the sulfuric acid has been expelled. Dissolve the residue in 20 mL of water and neutralize with ammonium hydroxide, using phenolphthalein TS. Add 1 mL of glacial acetic acid, dilute with water to 45 mL and filter. Proceed as described in *Method I* using 30 mL of the filtrate. Not more than 0.003% (30 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample, in oven at 150 °C, for four hours. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 80 mg of the sample, add 5 ml of acetic anhydride, 20 mL of glacial acetic acid and heat gently until complete dissolution. Allow to cool and add 20 mL of dioxane. Add three drops of methylrosanilinium chloride TS and titrate with 0.1 M perchloric acid VS until emerald green color. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 4.199 mg of NaF.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Dental prophylaxis.

SODIUM FLUORIDE ORAL SOLUTION

It contains not less than 90.0% and not more than 110.0% the stated quantity of NaF.

IDENTIFICATION

A. Transfer 0.1 mL of the oral solution to a test tube, add 0.1 mL of a mixture of alizarin TS and 0.1% (w/v) zirconyl nitrate in 7 M hydrochloric acid (1:1). A yellow color develops.

B. If necessary, reduce the volume of the oral solution by heating in water bath to a volume containing approximately 10 mg of sodium per milliliter. The resulting solution meets sodium ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.5 to 7.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Carry out a potentiometric determination with an ion-selective electrode for fluoride.

Buffer solution: to 500 mL of water, add 57 mL of glacial acetic acid, 58 g of sodium chloride and 4 g of 1,2-cyclohexylene-dinitrile-tetraacetic acid (CDTA). In a cold water bath, slowly add, under agitation, concentrated sodium hydroxide solution RS until pH between 5.3 and 5.5. Transfer to a 1000-mL volumetric flask, complete the volume with water and homogenize.

Sample solution: sample dilution in water (1:100).

Standard solution: prepare the fluoride reference stock solution at 100 mg/L in water, using analytical grade sodium fluoride. Plot the analytical curve with the fluoride reference solutions at the following concentrations: 0.25 mg/L; 0.5 mg/L; 1.0 mg/L; 2.5 mg/L and 5.0 mg/L.

Procedure: for every 10 mL of *Sample Solution* or *Standard Solution*, add 10 mL of *Buffer Solution*. Under magnetic agitation, measure the potentials of the solutions. Plot a graph relating the fluoride concentrations of the standards on the ordinate (on a logarithmic scale, $\log_{10}$) with the measurements of potential differences on the abscissa (on a normal scale). Determine the fluoride concentration in mg/L. Each mg of fluoride is equivalent to 2.211 mg of NaF.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

TIN FLUORIDE

Stannosi fluoridum

SnF₂; 156.71
tin fluoride; 09827
Tin fluoride
[7783-47-3]

It contains not less than 71.2% of tin ion (Sn²⁺) and not less than 22.3% and not more than 25.5% of fluoride in relation to the desiccated substance.

DESCRIPTION

Physicochemical characteristics. White powder. Melting point (5.2.2): around 213 °C.

Solubility. Freely soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. Dissolve 0.25 g of sample in 25 mL of water. In a test tube, add 2 mL of calcium chloride RS to 5 mL of the sample solution. A white precipitate of calcium fluoride is formed.

B. Use the same sample solution as in test **A.** in *Identification*. Add two drops of 0.1 M silver nitrate to two drops of the sample solution. A black precipitate is formed.

C. Add two drops of mercuric chloride RS to one drop of the sample solution from the identification test **A.** A white precipitate is formed. With the addition of an excess of the sample solution, a black precipitate is formed.

PURITY TESTS

pH (5.2.19). 2.8 to 3.5. Determine in freshly prepared 0.4% (w/v) solution.

Water-insoluble substances. Transfer 5 g of sample to a beaker, add 100 mL of water and shake for three minutes or until complete dissolution. Filter in a crucible, with pore size between 10 and 16 µm, of known mass and previously tared. Wash with 1% ammonium fluoride solution (w/v) and water. Dry the residue for four hours at 105 °C, cool and weigh. The residue weight does not exceed 0.2%.

Antimony.

Sample solution: transfer 1 g of stannous fluoride to a 50 mL volumetric flask, dissolve in 6 M hydrochloric acid and complete to a volume of 50 mL with the same solvent.

Standard solution: transfer 55 mg of antimony and potassium tartrate to a 200 mL volumetric flask, dissolve in water and complete to 200 mL with the same solvent. Transfer 5 mL of this solution to a 500 mL volumetric flask, dissolve in 6 M hydrochloric acid and complete the volume with the same solvent.

Rhodamine B solution: Dissolve 20 mg of rhodamine B in 200 mL of 0.5 M hydrochloric acid.

Pipet 5 mL of *Sample Solution* and *Standard Solution* into distinct separating funnels, add 15 mL of hydrochloric acid, 1 g of ceric sulfate and allow to stand for five minutes, shaking occasionally. Add 500 mg of hydroxylamine hydrochloride and shake for one minute. Transfer 15 mL of isopropyl ether to the solution, shake for 30 seconds, add 7 mL of water and shake again. Cool in water bath at room temperature for 10 minutes, shake for 30 seconds, allow to stand until phase separation occurs and discard the aqueous phase. Add 20 mL of *Rhodamine B Solution*, shake for 30 seconds and discard the aqueous phase. Decant the ether phase and, if necessary, centrifuge to obtain a clear solution. Determine the absorbance of the solutions obtained from the *Sample Solution* and *Standard Solution* at a wavelength of not more than 550 nm. The absorbance of the *Sample Solution* is not greater than the absorbance of the *Standard Solution* (0.005%). Carry out blank test.

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105 °C for four hours. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Tin ion.

0.1 M Potassium iodate iodide solution VS: in a 1000 mL volumetric flask, dissolve 3.567 g of potassium iodate, previously dried at 110 °C to constant weight, in 200 mL of water containing 1 g of sodium hydroxide and 10 g of potassium iodide. Complete the volume with water. Standardize this solution by titration using analytical grade metallic tin (99.5% purity) and dissolve in hydrochloric acid. Each mL of 0.1 M potassium iodate iodide is equivalent to 5.936 mg of Sn²⁺.

Accurately weigh approximately 250 mg of stannous fluoride and dissolve in 300 mL of heated (freshly boiled) 3 M hydrochloric acid. Rotate the flask containing the sample while passing an inert gas (free of oxygen) flowing over the surface of the liquid. Cool at room temperature. Add 5 mL of potassium iodide RS, 3 mL of starch TS and titrate with *0.1 M Potassium iodate-iodide solution VS* in inert atmosphere. Each mL of 0.1 M potassium iodate-iodide is equivalent to 5.936 mg of Sn²⁺.

Fluoride ion.

Note: Except *Buffer Solution*, prepare and store all solutions in plastic containers.

Buffer solution: dissolve 57 mL of glacial acetic acid, 58 g of sodium chloride and 4 g of 1,2-cyclohexylene-dinitrile-tetraacetic acid in 500 mL of water. Adjust pH to 5.25± 0.25 with sodium hydroxide and complete the volume to 1000 mL with water.

Sample solution: transfer 100 mg of tin fluoride into a 250-mL volumetric flask. Add 50 mL of water, shake vigorously for five minutes and complete volume to 250 mL with water. Transfer 10 mL of this solution to a 50-mL volumetric flask and adjust the volume with water.

Standard Solution: Dissolve an exact quantity of sodium fluoride CRS in water to obtain a 0.42 mg/mL solution. Each mL of this solution (*Standard Solution A*) contains 0.19 mg of fluoride ion ($10^{-2} M$). Transfer 25 mL of this solution to a 250-mL volumetric flask, adjusting the volume with water. This solution, *Standard Solution B*, contains 19 µg of fluoride ion per mL ($10^{-3} M$). Transfer 25 mL of this solution to a 250-mL volumetric flask and adjust the volume with water. This solution, *Standard Solution C*, contains 1,9 µg of fluoride ion per mL ($10^{-4} M$).

Pipet 20 mL of each *Standard Solution A*, *B* and *C*, transfer to separate plastic beakers and add 20 mL of the *Buffer Solution* to each flask. Determine the potential of each *Standard Solution* and *Sample Solution*, in mV, using an ion selective electrode for fluoride. During measurements, immerse the electrode in the solution under magnetic agitation and wait until equilibrium is reached to record the potential value. Wash the electrode with water between measurements and dry carefully to avoid damage to the electrode solid membrane. Draw an analytical curve by plotting a log chart of the fluoride ion concentration (in µg/mL) versus the potential (in mV). Calculate the fluoride ion concentration in the sample solution by interpolating the potential value recorded on the analytical curve for the *Sample solution*. The percentage of fluoride ion is calculated by the formula: $125C/W$, where C is the concentration of fluoride ion determined in the sample in µg/mL and W is the used mass of the sample, in mg.

PACKAGING AND STORAGE

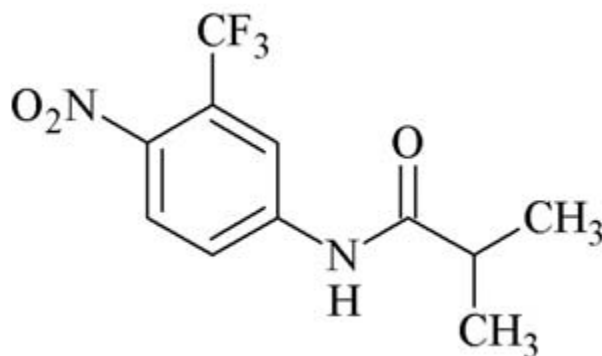
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Prophylactic to tooth decay.

FLUTAMIDE*Flutamidum*C₁₁H₁₁F₃N₂O₃; 276.21

flutamide; 04220

2-Methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide

[13311-84-7]

It contains not less than 98.0% and not more than 101.0% of C₁₁H₁₁F₃N₂O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow crystalline powder.

Solubility. Practically insoluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 110 °C to 114 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the flutamide CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 240 nm; column with 250 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute. Prepare solutions as described below.

Mobile phase: mixture of water and acetonitrile (50:50).

Solution (1): dissolve an accurately weighed quantity of the sample in *Mobile phase* to obtain a 1 mg/mL solution.

Solution (2): dilute 1 mL of *Solution (1)* to 50 mL using *Mobile phase*. Dilute 2 mL of the solution to 20 mL with the *Mobile phase*.

Solution (3): prepare a solution containing 0.04 mg/mL of impurity C of flutamide CRS (N-(4-nitro-3-(trifluoromethyl)phenyl)propanamide) and 0.04 mg/mL of flutamide CRS in *Mobile phase*. Transfer 1 mL of this solution to a 20 mL volumetric flask and complete with *Mobile phase*, obtaining a 2 µg/mL solution of flutamide impurity C and of flutamide.

Inject 20 µL replicates of the *Solution (3)*. The relative retention times are approximately 1.0 for flutamide and 0.72 for flutamide impurity C. The tailing factor is not more than 2.0. The resolution between flutamide and flutamide impurity C is not less than 10.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.5%.

Procedure: inject, separately, 20 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The area under the peak corresponding to impurity C in the chromatogram of *Solution (1)* is not more than 1.5 times the area under the main peak obtained with *Solution (2)* (0.3%). No other individual impurity can have an area under the peak greater than the area under the main peak obtained with *Solution (2)* (0.2%). The sum of the areas under all the secondary peaks, except that under the solvent peak, is not more than 2.5 times the area under the main peak obtained with *Solution (2)* (0.5%). Disregard peaks with areas below 0.25 times the area under the main peak obtained with *Solution (2)* (0.05%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 240 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water and acetonitrile (50:50).

Sample solution: dissolve an accurately weighed quantity of the sample in *Mobile phase* to obtain a 0.5 mg/mL solution.

Standard solution: dissolve an accurately weighed quantity of flutamide CRS in *Mobile phase* to obtain a 0.5 mg/mL solution.

Resolution Solution: prepare *Mobile Phase* solution containing approximately 0.5 mg of *o*-flutamide CRS per milliliter. Transfer 1 mL of this solution and 1 mL of the *Standard solution* to a 50 mL volumetric flask and complete with *Mobile phase*, obtaining a 0.01 mg/mL solution of *o*-flutamide and of flutamide.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are approximately 1.0 for flutamide and 1.4 for *o*-flutamide. The tailing factor is not more than 2.0. The resolution between *o*-flutamide and flutamide is not less than 6.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.5%.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{11}H_{11}F_3N_2O_3$ in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiandrogen.

FLUTAMIDE TABLETS

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $C_{11}H_{11}F_3N_2O_3$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of chloroform and ethyl acetate (3:1) as mobile phase. Apply to the plate, separately, 20 μ L of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Transfer a quantity of powder equivalent to 15 mg of flutamide to a 10 mL volumetric flask, complete volume with a mixture of chloroform and methyl alcohol (5:1).

Solution (2): dissolve about 15 mg of flutamide CRS in 10 mL of a mixture of chloroform and methyl alcohol (5:1).

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The stain related to the flutamide obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 3% (w/v) aqueous sodium lauryl sulfate solution, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute, if necessary, in 3% aqueous solution of sodium lauryl sulfate (w/v) until adequate concentration. Measure the absorbances of the solutions at 306 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the quantity of $C_{11}H_{11}F_3N_2O_3$ dissolved in the medium, comparing the readings obtained with the flutamide CRS solution at the concentration of 0.0025% (w/v), prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated quantity of $C_{11}H_{11}F_3N_2O_3$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 300 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of 0.05 M monobasic potassium phosphate and methyl alcohol (30:70).

Sample solution: weigh and pulverize 20 tablets. Transfer a quantity of powder equivalent to 125 mg of flutamide to a 100 mL volumetric flask and add 60 mL of a mixture of methyl alcohol and water (95:5). Shake mechanically for 30 minutes and complete the volume with methyl alcohol, homogenize and filter. Transfer 10 mL of the filtrate to a 50 mL volumetric flask and complete the volume with methyl alcohol to obtain a 0.25mg/mL flutamide solution.

Standard solution: dilute an accurately weighed quantity of flutamide CRS in methyl alcohol to obtain a 0.25 mg/mL solution.

Inject 10 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

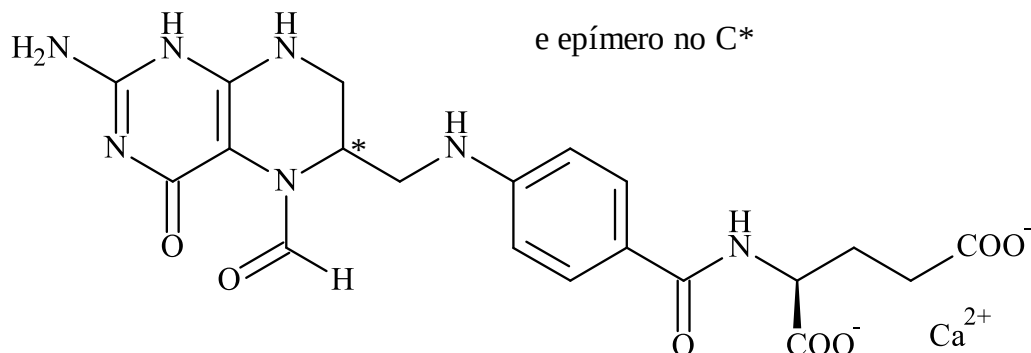
Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₁H₁₁F₃N₂O₃ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CALCIUM FOLINATE*Calcii folinas*C₂₀H₂₁CaN₇O₇; 511.50

calcium folinate; 00197

Calcium salt of acid *N*-[4-[[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridiny)methyl]amino]benzoyl]-L-glutamic (1:1)
[1492-18-8]

It contains not less than 95.0% and not more than 105.0% of C₂₀H₂₁CaN₇O₇, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Amorphous or crystalline powder, white or yellowish, hygroscopic.

Solubility. Moderately soluble in water, practically insoluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +14,4 to +18,0, in relation to the anhydrous substance. Determine in a 2.5% (w/v) solution in carbon dioxide-free water.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the calcium folinate CRS spectrum, prepared identically.

B. It meets the calcium ion reaction 2 (5.3.1.1).

PURITY TESTS

Preparation aspect. The 2.5% (w/v) aqueous preparation is clear (5.2.25) and yellow. Measure the solution absorbance at 420 nm, using water for zero adjustment. The absorbance must not be greater than 0.60.

pH (5.2.19). 6.8 to 8.0. Determine in aqueous solution at 2.5% (w/v).

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 280 nm; 250mm long, 4.0 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at 40 °C; *Mobile phase* flow rate of 1.0 mL/minute. Prepare the solutions described below.

Mobile phase: mix 220 mL of methyl alcohol and 780 mL of a solution containing 2.0 mL of 40% (w/v) tetrabutylammonium hydroxide solution and 2.2 g of dibasic sodium phosphate. Adjust mobile phase pH to 7.8 with phosphoric acid.

Solution (1): dissolve quantity, accurately weighed, of the sample in *Diluent* to obtain a solution at 1.0 mg/mL.

Solution (2): dissolve accurately weighed quantity of calcium folinate CRS in *Mobile Phase* to obtain a 1.0 mg/mL solution.

Solution (3): dilute 1 mL of *Solution (2)* with water to 100 mL .

Solution (4): dissolve accurately weighed quantity of formylfolic acid CRS (impurity D) in *Mobile Phase* to obtain a 0.1 mg/mL solution. Dilute 1 mL of the solution to 10 mL with water.

Solution (5): dilute 1 mL of *Solution (4)* with water to 10 mL .

Solution (6): dilute 5 mL of *Solution (4)* with *Solution (3)* to 10 mL .

Inject 10 µL replicates of the *Solution (6)*. The resolution between calcium folinate and impurity D is not less than 2.2. Record chromatograms for not less than 2.5 times the retention time of calcium folinate.

Procedure: inject, separately, 10 µL of the *Solution (1)* and of *Solution (3),(4), (5) and (6)*, record the chromatograms and measure the areas under the peaks. The area under the peak corresponding to impurity D must not be greater than the area under the main peak obtained with *Solution (4)* (1.0%); impurities A, B, C, E, F, G: the area under the peak corresponding to each impurity must not be greater than the area under the main peak obtained with *Solution (3)* (1.0%). The sum of impurities, except for D, must be not more than 2.5 times the area under the main peak obtained with *Solution (3)* (2.5%); do not consider peaks with areas smaller than that under the main peak obtained with *Solution (5)* (0.1%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.005% (50 ppm).

Water (5.2.20.3). Determine in 0,1 g of the sample. Not more than 17.0%.

BIOLOGICAL SAFETY TESTS

When it is indicated on the label that the substance is sterile, the sample complies with the Bacterial endotoxin test and with the Sterility test. When it is indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 0.5 EU/mg of calcium folinate.

ASSAY

Note: Proceed protected from direct light. Carry out the assay without prolonged interruption.

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mix 835 mL of water, 125 mL of acetonitrile and 15 mL of 25% (w/v) tetrabutylammonium hydroxide solution in methyl alcohol. Adjust pH to 7.5 ± 0.1 with 0.2 M monobasic sodium phosphate and complete the volume to 1000 mL with water.

Diluent: mix 900 mL of water and 15 mL of 25% (w/v) tetrabutylammonium hydroxide solution in methyl alcohol. Adjust pH to 7.5 ± 0.1 with 0.2 M monobasic sodium phosphate and complete the volume to 1000 mL with water.

Sample solution: dissolve an accurately weighed quantity of the sample in *Diluent* to obtain a 0.2 mg/mL solution.

Standard solution: dissolve an accurately weighed quantity of calcium folinate CRS in *Diluent* to obtain a 0.2 mg/mL solution.

Resolution solution: transfer 17.5 mg of folic acid to a 100 mL volumetric flask, dissolve in *Diluent* and complete the volume with the same solvent. Transfer 5 mL to a 25 mL volumetric flask and complete the volume with *Standard solution*. Homogenize.

Inject 15 µL replicates of the *Resolution solution*. The relative retention times are approximately 1.0 for calcium folinate and 1.6 for folic acid. The resolution between acid folic and calcium folinate is not less than 3.6. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 15 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{20}H_{21}CaN_7O_7$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antidote to folic acid antagonists.

ALUMINUM PHOSPHATE***Aluminii phosphas***

AlPO_4 ; 121.95

aluminum phosphate; 00200

Aluminum salt of phosphoric acid (1:1)

[7784-30-7]

$\text{AlPO}_4 \cdot \frac{1}{3}\text{H}_2\text{O}$; 127.96

aluminum phosphate terhydrate; 11401

Aluminum salt of phosphoric acid hydrate (3:3:1)

[1117729-44-8]

It contains not less than 94.0% and not more than 102.0% of AlPO_4 , in relation to the incinerated substance.

DESCRIPTION

Physical characteristics. White or off-white powder.

Solubility. Very slightly soluble in water, practically insoluble in ethyl alcohol. Soluble in diluted solutions of alkali hydroxides and in diluted mineral acid solutions.

IDENTIFICATION

A. The preparation obtained in *Preparation aspect* meets the aluminum ion reactions **(5.3.1.1)**.

B. The preparation obtained in *Preparation aspect* meets the phosphate ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Transfer 2 g of the sample to a 100 mL volumetric flask, dissolve in 50 mL of hydrochloric acid RS and complete the volume with the same solvent. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). 5.5 to 7.2. Weigh 2.0 g of the sample, add 50 mL of water, under vigorous agitation for five minutes, and determine the pH.

Neutralizing capacity. Pass a sufficient quantity of the sample through a 75 μm sieve opening. To 0.5 g of the sieved sample, add 30 mL of 0.1 M hydrochloric acid, previously heated to 37 °C. Keep this mixture at 37°C for 30 minutes, shaking continuously. After 30 minutes, the pH **(5.2.19)** of the mixture at 37°C is between 2.0 and 2.5.

Chlorides (5.3.2.1). Dissolve 54,4 mg of the sample in 30 mL of 20% nitric acid (w/v) and filter. Use 15 mL of this solution and 1 mL of 0.01 M hydrochloric acid Standard solution. Not more than 1.3% (13 000 ppm).

Soluble Phosphates. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Accurately weigh about 5 g of the sample and dissolve in 150 mL of water. Shake for two hours. Filter and wash with 50 mL of water. Collect the filtrate in a 250 mL volumetric flask and complete

the volume with water. Transfer 10 mL of this solution to a 100 mL volumetric flask and adjust the volume with water, obtaining *Solution (1)*. In parallel, transfer 2.86 g of monobasic potassium phosphate for a 100 mL volumetric flask, dissolve in water and complete the volume with the same solvent, obtaining *Solution (2)*. Transfer 1 mL of *Solution (2)* to a 5-mL volumetric flask and complete the volume with water, obtaining *Solution (3)*. Transfer 3 mL of *Solution (2)* to a 5-mL volumetric flask and complete the volume with water, obtaining *Solution (4)*. Transfer 5 mL of each solution obtained to a 25 mL volumetric flask, add 4 mL of *M* sulfuric acid, 1 mL of ammonium molybdate RS, 5 mL of water and 2 mL of a solution containing 0.1 g of 4-sulfate methylaminophenol, 0.5 g of anhydrous sodium sulfite, 20 g of sodium metabisulfite in 100 mL with water. Shake and allow to stand for 15 minutes. Complete the volume with water, homogenize and allow to stand for another 15 minutes. Measure the absorbances of the resulting solutions at 730 nm. Calculate the concentration of PO_4^{3-} in *Solution (1)* from the calibration curve prepared with *Solutions (2), (3) and (4)*. Not more than 1.0%.

Sulfates (5.3.2.2). Dissolve 0.4 g of the sample and filter into a 25 mL volumetric flask, complete the volume and homogenize. Use 12.5 mL of this solution and 2.5 mL of 0.005 *M* sulfuric acid Standard solution. Not more than 0.6% (6000 ppm).

Arsenic (5.3.2.5). Determine in 3 g of the sample. Proceed as described in *Spectrophotometric Method, Method I*, and use 1 mL of Arsenic Standard solution. Not more than 0.0001% (1 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve 2 mg of the sample in 20 mL of hydrochloric acid RS. Use 10 mL of this solution. Not more than 0.002% (20 ppm).

Loss on ignition (5.2.9.2). Determine in 1 g of the sample. Incinerate at 800°C until constant weight. Between 10.0 and 20.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh approximately 0.4 g of the previously incinerated sample, dissolve in 10 mL of diluted hydrochloric acid and dilute to 100 mL with water. To 10 mL of this solution, add 10 mL of 0.1 *M* disodium edetate VS and 30 mL of a mixture of ammonium acetate RS and diluted acetic acid (1:1). Boil for three minutes, allow to cool. Add 25 mL of ethyl alcohol and 1 mL of fresh-prepared solution of 0.025% (w/v) dithizone in ethyl alcohol. Titrate excess 0.1 *M* disodium edetate VS with 0.1 *M* zinc sulfate VS until the color changes to pink. Each mL of 0.1 *M* disodium edetate VS is equivalent to 12.195 g of AlPO_4 .

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antacid.

AMMONIUM PHOSPHATE DIBASIC

Ammonii hydrogenophosphas

$(\text{NH}_4)_2\text{HPO}_4$; 132.06

ammonium phosphate dibasic; 04277

Ammonium salt of phosphoric acid (2:1)

[7783-28-0]

It contains not less than 96.0% and not more than 102.0% of $(\text{NH}_4)_2\text{HPO}_4$.

DESCRIPTION

Physical characteristics. White or off-white powder or crystals.

Solubility. Freely soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. The 5% (w/v) solution meets ammonium ion reactions **(5.3.1.1)**.

B. The 5% (w/v) solution meets phosphate ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.9.1). 7.6 to 8.2. Determine in aqueous solution at 1% (w/v).

Arsenic (5.3.2.5). Use *Spectrophotometric method, Method I*. Determine in 1 g of the sample. Not more than 0.0003% (3 ppm).

Chlorides (5.3.2.1). Determine in 1.22 g of the sample. Not more than 0.03% (300 ppm).

Sulfates (5.3.2.2). Determine in 0.8 g of the sample. Not more than 0.15% (1500 ppm).

Heavy metals (5.3.2.3). Dissolve 2 g of the sample in 25 mL of water. Not more than 0.001% (10 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0,6 g of the sample in 40 mL of water. Titrate with 0.05 M sulfuric acid VS, determining the endpoint potentiometrically at pH 4.6. Each mL of 0.05 M sulfuric acid VS is equivalent to 13.206 mg of $(\text{NH}_4)_2\text{HPO}_4$.

PACKAGING AND STORAGE

In tightly closed, non-metallic containers.

LABELING

Comply with current legislation.

CATEGORY

Buffering agent, sequestering agent.

CALCIUM PHOSPHATE DIBASIC DIHYDRATE*Calcii hydrogenophosphas dihydricus*CaHPO₄·2H₂O; 172.09

calcium phosphate dibasic dihydrate; 04278

Calcium salt of phosphoric acid hydrate (1:1:2)

[7789-77-7]

It contains not less than 98.0% and not more than 100.5% of CaHPO₄·2H₂O.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Practically insoluble in water and in ethyl alcohol. Soluble in diluted solutions of hydrochloric acid and nitric acid, slightly soluble in diluted acetic acid.

IDENTIFICATION

A. Dissolve approximately 0.1 g of the sample, using heating, in a mixture of 5 mL of 3 *M* hydrochloric acid and 5 mL of water. Add, under agitation, dropwise 2.5 mL of 6 *M* ammonium hydroxide and add 5 mL of ammonium oxalate RS. A white precipitate is produced.

B. Dissolve 0.1 g of the sample in 5 mL of diluted nitric acid. Heat the solution to 70 °C, add 2 mL of freshly prepared ammonium molybdate solution RS. A yellow precipitate of ammonium phosphomolybdate is produced.

PURITY TESTS

Barium. Dissolve 2.5 mg of the sample in 20 mL of hydrochloric acid RS and filter, if necessary. Add ammonia RS until a precipitate is formed. Add hydrochloric acid RS until the precipitate dissolves and complete the volume to 50 mL with water. To 10 mL of this solution, add 0.5 mL of diluted sulfuric acid RS. To another 10 mL aliquot of the same solution, add 0.5 mL of water. After 15 minutes, any opalescence in the acid-added aliquot is not more intense than that obtained with the water-added aliquot.

Carbonates. Mix 0.5 g of the sample with 5 mL of carbon dioxide-free water and add 1 mL of hydrochloric acid. Effervescence is not observed.

Monocalcium and tricalcium phosphates. Dissolve 2 g of the sample in 30 mL of *M* hydrochloric acid VS, add 20 mL of water and 0.05 mL of methyl orange TS. Titrate the excess *M* hydrochloric acid VS with *M* sodium hydroxide VS. The consumption of *M* hydrochloric acid VS is between 11 mL and 12.5 mL.

Acid-insoluble substances. Heat 5 g of the sample with a mixture of 40 mL of water and 10 mL of hydrochloric acid, until maximum solubilization, and complete the volume to 100 mL with water. If an insoluble residue appears, filter on quantitative filter paper, wash with hot water, until the reaction for chlorides is negative. Incinerate the residue and filter paper at 600 ± 50 °C for one hour. Not more than 0.2%.

Arsenic (5.3.2.5). Dissolve 2.5 g of the sample in 20 mL of hydrochloric acid RS. Filter, if necessary, and add ammonia RS until a precipitate is formed. Add hydrochloric acid RS until the precipitate dissolves and complete the volume to 50 mL with water. Use 20 mL of this solution and proceed as described in *Visual method*. Not more than 0.0002% (2 ppm).

Chlorides (5.3.2.1). Dissolve 1.42 g of the sample in a mixture with 3 mL of nitric acid and 20 mL of water. Dilute to 50 mL with water. Use 5 mL of this solution. Not more than 0.25% (2500 ppm).

Iron (5.3.2.4). Dissolve 2.5 g of the sample in 20 mL of hydrochloric acid RS. Filter, if necessary, and add ammonia RS until a precipitate is formed. Add hydrochloric acid RS until the precipitate dissolves and complete the volume to 50 mL with water. Use 5 mL of this solution and proceed as described in *Method I*. Use 1 mL of *Standard iron solution (100 ppm Fe)*. Not more than 0.04% (400 ppm).

Heavy metals (5.3.2.3). Dissolve, as much as possible, by heating, 1.3 g of the sample in 3 mL of 3 M hydrochloric acid, cool and dilute to 50 mL with water. Determine in 25 mL of this solution. Not more than 0.003% (30 ppm).

Sulfates (5.3.2.2). Dissolve 0.8 g of the sample in 20 mL of hydrochloric acid RS. Filter, if necessary, and add ammonia RS until a precipitate is formed. Add hydrochloric acid RS until the precipitate dissolves and complete the volume to 50 mL with water. Use 15 mL of this solution. Not more than 0.5% (5000 ppm).

(5.2.9.1) Loss on ignition (5.2.9.2). Determine in 1 g of the sample. Incinerate between 800°C and 825°C until constant weight. Between 24.5 and 26.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.3 g of the sample, dissolve in a mixture with 2.5 mL of hydrochloric acid RS and 5 mL of water. Add 25 mL of 0.1 M disodium edetate VS and dilute to 200 mL with water. Neutralize with ammonium hydroxide and add 10 mL of ammonium chloride buffer solution pH 10.7 and approximately 50 mg of eriochrome T black. Titrate excess disodium edetate with 0.1 M zinc sulfate VS until violet color. Carry out blank test. Each mL of 0.1 M disodium edetate VS is equivalent to 17.209 mg of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$.

PACKAGING AND STORAGE

In tightly closed, non-metallic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Calcium supplement.

CALCIUM PHOSPHATE TRIBASIC***Tricalcii phosphas***

$\text{Ca}_5(\text{OH})(\text{PO}_4)_3$; 502.31

calcium phosphate tribasic; 00203

calcium hydroxyphosphate

[12167-74-7]

Tribasic calcium phosphate consists of a variable mixture of calcium phosphates. It contains not less than 35.0% and not more than 40.0% of Ca (40.08).

DESCRIPTION

Physical characteristics. White, tasteless powder.

Solubility. Practically insoluble in water and in ethyl alcohol. Soluble in diluted hydrochloric acid and nitric acid solutions. Practically insoluble in acetic acid.

IDENTIFICATION

A. Dissolve 0.1 g of the sample in 5 mL of diluted nitric acid solution at 25% (v/v). The solution meets phosphate ion reactions **(5.3.1.1)**.

B. It meets calcium ion reactions **(5.3.1.1)**.

PURITY TESTS

Barium. Weigh 0.5 g of the sample. Add 10 mL of water, heat, add hydrochloric acid dropwise until solution is obtained and add two drops of excess acid. Filter and add 1 mL of 1% aqueous potassium sulfate solution (w/v) to the filtrate. The solution obtained must remain clear.

Acid-insoluble substances. Dissolve 5 g of the sample in a mixture with 10 mL of hydrochloric acid and 30 mL of water. Filter, wash the residue with water and dry in oven at 105 °C until constant weight. The residue mass is not more than 10 mg (0.2%).

Arsenic (5.3.2.5). Add 0,75 g of the sample in 20 mL of water. Add hydrochloric acid until complete dissolution and proceed as described in *Visual method*. Not more than 0.0004% (4 ppm).

Chlorides (5.3.2.1). Add 0.25 g of the sample in 10 mL of water. Add 1 mL of nitric acid, shake until dissolved and dilute to 40 mL with water. Proceed as described in *Limit test for chlorides*. Not more than 0.14% (1400 ppm).

Iron (5.3.2.4). Dissolve 2.5 g of the sample in 20 mL of diluted hydrochloric acid. Filter if the preparation is not clear. Add diluted ammonia, slowly, until a precipitate is formed. Dissolve the precipitate with diluted hydrochloric acid and complete the volume to 50 mL with water. Use 5 mL of the solution obtained and proceed as described in *Method I*. Use 1 mL of *Standard iron solution* (100 ppm Fe). Not more than 0.04% (400 ppm).

Heavy metals (5.3.2.3). Dissolve 0.6667 g of the sample in 10 mL of 10% (w/v) hydrochloric acid and heat in water bath for five minutes. Dilute with water to 25 mL and proceed as described in *Method I*. Not more than 0.003% (30 ppm).

Sulfates (5.3.2.2). Add 0.15 g of the sample in 10 mL of water. Add 1 mL of hydrochloric acid until fully dissolved and dilute to 40 mL with water. Not more than 0.8% (8000 ppm).

Loss on ignition (5.2.9.2). Determine in 1 g of the sample. Incinerate in muffle furnace at 800 ± 25 °C until constant weight. Not more than 8.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.2 g of the sample in a mixture with 1 mL of hydrochloric acid RS and 5 mL of water. Add 25 mL of 0.1 M disodium edetate and complete the volume to 200 mL with water. Adjust the solution pH to 10.0 with ammonia and add 10 mL of ammonium chloride buffer pH 10.0. Use eriochrome black T TS as indicator. Titrate excess 0.1 M disodium edetate with 0.1 M zinc sulfate VS until the color changes from blue to violet. Each mL of 0.1 M disodium edetate is equivalent to 4.008 mg of Ca.

PACKAGING AND STORAGE

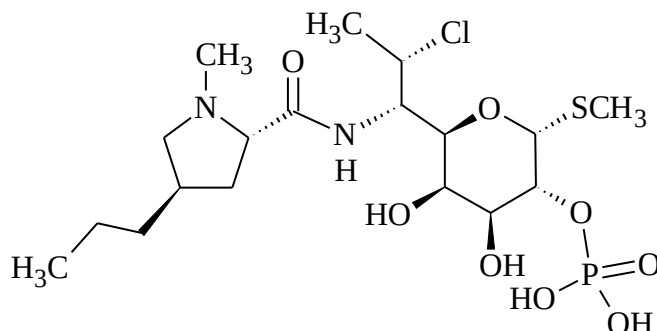
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Supplement and pharmacotechnical adjuvant.

CLINDAMYCIN PHOSPHATE*Clindamycini phosphas* $C_{18}H_{34}ClN_2O_8PS$; 504.96

clindamycin phosphate; 02232

2-(Dihydrogenphosphate) of 7-chloro-6,7,8-trideoxy-6-[[[(2S,4R)-1-methyl-4-propyl-2-pyrrolidinyl]carbonyl]amino]-1-methylthio-L-threo- α -D-galacto-octopyranoside
[24729-96-2]

It contains not less than 95.0% and not more than 100.5% of $C_{18}H_{34}ClN_2O_8PS$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white powder, slightly hygroscopic. Presents polymorphism.

Solubility. Freely soluble in water, very slightly soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +115 to +130, in relation to the anhydrous substance. Determine in solution at 1% (w/v) in water.

IDENTIFICATION

A. In the infrared absorption spectrum(5.2.14)of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the clindamycin phosphate CRS spectrum, prepared identically. If the spectrum of the sample is not identical to that of the standard, dissolve the sample and the standard separately in water, evaporate to dryness under reduced pressure, dry at 105 °C for two hours and repeat the test with the residues.

B. Heat 0.1 g of the sample under reflux with a mixture of 5 mL of concentrated sodium hydroxide solution RS and 5 mL of water for 90 minutes. Cool. Add 5 mL of nitric acid. Extract with three 15-mL portions of methylene chloride. Filter the aqueous layer. The filtrate meets phosphate ion reactions (5.3.1.1).

PURITY TESTS

Preparation aspect. Dissolve 1 g of the sample in water, with heating if necessary. Cool and complete the volume to 25 mL with water. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). 3.5 to 4.5. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 210 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at 30 °C; *Mobile phase* flow rate of 1.1 mL/minute.

Eluent A: mixture of acetonitrile and 1.36% (w/v) monobasic sodium phosphate buffer(21:79) pH 6.0 adjusted with 45% w/v potassium hydroxide solution.

Eluent B: mixture of acetonitrile and 1.36% (w/v) monobasic sodium phosphate buffer (60:40) pH 6.0 adjusted with 45% w/v potassium hydroxide solution.

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 - 13	100	0	isocratic
13 - 18	100 → 50	0 → 50	linear gradient
18 - 39	50	50	linear gradient

Solution (1): dissolve 30 mg of the sample in *Eluent A* and dilute to 10 mL with the same solvent.

Solution (2): dissolve 30 mg of clindamycin phosphate CRS in *Eluent A* and dilute to 10 mL with the same solvent.

Solution (3): dilute 1 mL of *Solution (1)* to 20 mL with *Eluent A*. Dilute 1 mL of this solution to 10 mL with *Eluent A*.

Solution (4): Dissolve 3.0 mg of clindamycin phosphate for the CRS system suitability (containing impurities B, E, F, G, I, J, K and L) and dilute to 10 ml in *Eluent A*.

Inject, separately, 20 µL replicates of *Solutions (2)* and *(4)*. The retention times for clindamycin phosphate, whose retention time is about twelve minutes, are about 0.15 for impurity F (2-lincomycin phosphate (methyl 6,8-dideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-2-O-phosphono-1-thio-D-erythro-a-D-galacto-octopyranoside)), 0.19 for impurity G (2,4-phosphatidyl lincomycin (methyl 6,8-dideoxy-2,4-O-(hydroxyphosphoryl)-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]1-thio-D-erythro-a-D-galacto-octopyranoside)), 0.34 for impurity I (clindamycin 2,4-bisphosphate (methyl 7-chloro-6,7,8-trideoxy-6)-[[[(2S,4R)-4-ethyl-1-methylpyrrolidin-2-yl]carbonyl]amino]-2,4-di-O-phosphono-thio-L-threo-a-D-galacto-octopyranoside)), 0.45 for impurity B (2-clindamycin B phosphate (methyl 7-chloro-6,7,8-trideoxy-6-[[[(2S,4R)-4-ethyl-1-methylpyrrolidine-2-yl]carbonyl]amino]-2-O-phosphono-thio-L-threo-a-D-galacto-octopyranoside)), 0.64 for impurity L (2-phosphate 7-epiclindamycin (methyl 7-chloro-6, 7,8-trideoxy-6- [[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-2-O-phosphono-1-thio-D-erythro-a-D-galacto-octopyranoside)), 1.20 for impurity J (propylidene analogue of clindamycin 2-phosphate (methyl 7-chloro-6,7,8-trideoxy-6-

[[[(2S)-1-methyl-4-propylidenepyrrolidin-2-yl]carbonyl] amino]-2-O-phosphono-thio-L-threo-a-D-galacto-octopyranoside)), 1.73 for impurity E (clindamycin (methyl 7-chloro-6,7,8-trideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl]amino]-1-thio-L-threo-a-D-galacto-octopyranoside)) and 1.9 for impurity K (pyrophosphate of diclindamycin (2,2'-oxybis(hydroxyphosphoryl)bis[methyl-7-chloro-6,7,8-trideoxy-6-[[[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl] carbonyl]amino]-1-thio-L-threo-a-D-galacto-octopyranoside]])). The resolution between the impurity G and impurity F peaks is not less than 2.0.

Procedure: separately inject 20 µL of the *Solutions (1)* and *(3)*. Record the chromatograms and measure the areas under the peaks. Determine the percentage of each of the impurities found in the *Sample Solution* in relation to the concentration of clindamycin phosphate in *Solution (3)*. Not more than 1.0% of impurity B, 0.5% of impurity E, 0.5% of impurity F, 0.2% of impurity G, 0.2% of impurity I, 0.2% of impurity J and 0.2% of impurity K. Not more than 0.1% for another individual impurity. Not more than 2.0% of the total impurities found. Disregard peaks with area smaller than 0.05 times the area under the main peak obtained in *Solution (3)*.

Water (5.2.20.1). Determine in 0.25 g of the sample. Not more than 6.0%.

BIOLOGICAL SAFETY TESTS

When it is indicated on the label that the substance is sterile, the sample complies with the Sterility and with the Bacterial endotoxin tests. When it is indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test. Use the *Membrane Filtration Method*.

Bacterial endotoxins (5.5.2.2). Not more than 0.6 EU/mg of clindamycin phosphate.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 210 nm; 250mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to an octylsilane group (5 µm to 10 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mix 200 mL of acetonitrile and 800 mL of 1.36% monobasic potassium phosphate (w/v), previously adjusted to pH 2.5 with phosphoric acid.

Sample solution: transfer 75 mg of the sample, accurately weighed, to a 25 mL volumetric flask, complete the volume with *Mobile phase* and mix.

Standard solution: dissolve an accurately weighed quantity of clindamycin phosphate CRS in the *Mobile phase* and dilute adequately to obtain a solution at 3 mg/mL.

Resolution Solution: dissolve 5 mg of lincomycin hydrochloride CRS and 15 mg of clindamycin hydrochloride CRS in 5 mL of the *Standard Solution* and complete the volume to 100 mL with *Mobile Phase*.

Inject 20 µL replicates of the *Resolution solution*. The assay is valid only if the lincomycin hydrochloride peak is separated from the solvent peak. The resolution between the clindamycin

phosphate and clindamycin hydrochloride peaks is not less than 6.0. The tailing factor for the clindamycin phosphate peak is not more than 1.5.

Inject 20 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₈H₃₄ClN₂O₈PS in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation. When the substance is intended for the production of parenteral preparations, the label must indicate whether the product is sterile or whether it must be sterilized during the process.

THERAPEUTIC CLASS

Anti-bacterial; antiprotozoal.

CLINDAMYCIN PHOSPHATE INJECTION SOLUTION

It contains not less than 90.0% and not more than 105.0% of the stated quantity of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of methyl alcohol, toluene and 18 M ammonia (70:30:1.5), as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): transfer volume of the injection solution, equivalent to 50 mg of clindamycin phosphate to a 10 mL volumetric flask, complete the volume with methyl alcohol and homogenize.

Solution (2): 5 mg/mL solution of clindamycin phosphate CRS in methyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Spray plate with diluted potassium iodobismuthate RS. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.5 to 7.0.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 0.58 EU/mg of clindamycin phosphate.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 210 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mix 250 mL of acetonitrile and 750 mL of 1.36% monobasic potassium phosphate (w/v), previously adjusted to pH 2.5 with phosphoric acid.

Sample solution: dilute volume of the injection solution in the *Mobile Phase* to obtain a 0.15 mg/mL clindamycin solution.

Standard solution: 0.18 mg/mL clindamycin phosphate CRS solution in the *Mobile Phase*.

Resolution solution: solution containing 0.12 mg/mL of lincomycin hydrochloride CRS and 0.24 mg/mL of clindamycin phosphate CRS in the *Mobile Phase*.

Inject 20 µL replicates of the *Resolution solution*. The resolution between the lincomycin hydrochloride and clindamycin phosphate peaks is not less than 7.7. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

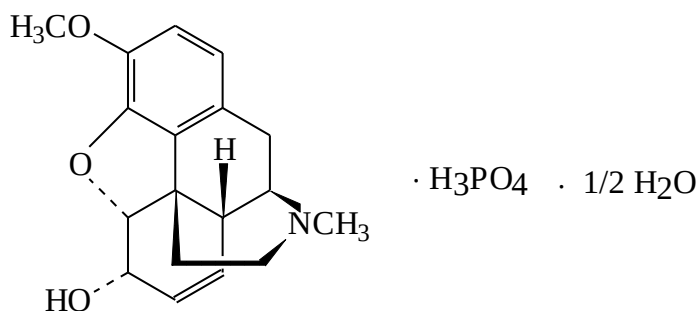
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{18}H_{33}ClN_2O_5S$ in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*. Each mg of clindamycin phosphate ($C_{18}H_{34}ClN_2O_8PS$) is equivalent to 0.8416 mg of clindamycin ($C_{18}H_{33}ClN_2O_5S$).

PACKAGING AND STORAGE

In type I glass containers, protected from light, at temperatures between 8 °C and 30 °C.

LABELING

Comply with current legislation.

CODEINE PHOSPHATE*Codeini phosphas*

$C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2} H_2O$; 406.37

codeine phosphate hemihydrate; 10802

(5 α ,6 α)-7,8-Didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol phosphate.

[41444-62-6]

It contains not less than 99.0% and not more than 101.5% of $C_{18}H_{21}NO_3H_3PO_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Freely soluble in water and slightly soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): -98 to -102, in relation to the desiccated substance. Determine in aqueous solution at 2% (w/v).

IDENTIFICATION

The identification test A. can be omitted if tests B., C. and D. are carried out. The identification tests B. and D. can be omitted if tests A. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the codeine phosphate CRS spectrum, prepared identically.

B. Dilute 25 mL of a sample solution at 0.04% (w/v) in water, in a 100 mL volumetric flask, add 10 mL of *M* sodium hydroxide and complete the volume with water. In the ultraviolet absorption spectrum (5.2.14), at the range of 250 nm to 350 nm, there is a maximum peak at 284 nm. The absorbance at 284 nm is of approximately 0.38.

C. It meets with phosphate reactions (5.3.1.1).

D. It meets alkaloids reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.0 to 5.0. Determine in aqueous solution at 4% (w/v).

Acidity. Dissolve 0.1 g of the sample in 20 mL of water and titrate with 0.01 M sodium hydroxide VS to pH 5.4, determined with the aid of a potentiometer. Not more than 1 mL of 0.01 M sodium hydroxide VS is required to promote the pH shift.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of absolute ethyl alcohol, cyclohexane and ammonium hydroxide (72:30:6) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): solution at 40 mg/mL of the sample in absolute ethyl alcohol.

Solution (2): dilute 2 mL of *Solution (1)* to 100 mL with absolute ethyl alcohol.

Solution (3): dilute 1 mL of *Solution (1)* to 100 mL with absolute ethyl alcohol.

Develop chromatogram. Remove the plate, allow to dry in the development chamber. Pulverize the plate with a solution of 3 mL of 10% chloroplatinic acid (v/v), 97 mL of water and 100 mL of 6% (w/v) potassium iodide. Examine the plate. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (2.0%) and no other stain is more intense than that obtained with *Solution (3)* (1.0%).

Morphine limit. Dissolve 0.05 g of potassium ferrocyanide in 10 mL of water and add one drop of ferric chloride RS and 1 mL of the 1% (w/v) sample solution in water. Blue color is not immediately produced.

Chlorides. Acidify 10 mL of the sample solution at 1% (w/v) in water with nitric acid and add some drops of 0.1 M silver nitrate. No opalescence is immediately produced.

Sulfates. To 10 mL of the 1% (w/v) sample solution in water, add some drops of 12% (w/v) barium chloride. No turbidity is immediately produced.

Loss on drying (5.2.9.1). Determine in 1.5 g of sample. Dry in oven at 105 °C until constant weight. Not less than 1.5% and not more than 3.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.4.5)*. Dissolve 0.35 g of the previously desiccated sample in 10 mL of acetic anhydride and 20 mL of dioxane. Titrate with 0.1 M perchloric acid VS, using 0.05 mL of methylrosaniline chloride TS as indicator. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 39.737 mg of $C_{18}H_{21}NO_3 \cdot H_3PO_4$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Opioid receptor agonist; analgesic.

SODIUM PHOSPHATE DIBASIC***Natrii hydrogenophosphas***

Na_2HPO_4 ; 141.96

sodium phosphate dibasic; 00207

Sodium salt of phosphoric acid (2:1)

[7558-79-4]

$\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$; 159.97

sodium phosphate dibasic monohydrate; 11657

Disodium salt of phosphoric acid monohydrate (2:1:1)

[118830-14-1]

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$; 177.99

sodium phosphate dibasic dihydrate; 00209

Disodium salt of phosphoric acid dihydrate (2:1:2)

[10028-24-7]

$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$; 268.07

sodium phosphate dibasic heptahydrate; 00211

Disodium salt of phosphoric acid heptahydrate (2:1:7)

[7782-85-6]

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$; 358.14

sodium phosphate dibasic dodecahydrate; 00210

Sodium salt of phosphoric acid dodecahydrate (2:1:12)

[10039-32-4]

Dibasic sodium phosphate is anhydrous or contains one, two, seven or twelve molecules of water of hydration. It contains not less than 98.0% and not more than 100.5% of Na_2HPO_4 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Anhydrous: Colorless or white powder, hygroscopic. Dihydrate: white or off-white powder, or colorless crystals. Heptahydrate: Colorless or white granular salt which presents efflorescence in hot, dry air. Dodecahydrate: colorless, transparent, highly efflorescent crystals.

Solubility. Anhydrous and dihydrate: Soluble in water, practically insoluble in ethyl alcohol. Heptahydrate: Freely soluble in water, very slightly soluble in ethyl alcohol. Dodecahydrate: very soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. The 3% (w/v) solution meets phosphate ion reactions **(5.3.1.1)**.

B. The 3% (w/v) solution meets sodium ion reactions **(5.3.1.1)**.

PURITY TESTS

Insoluble substances. Dissolve quantity of sample equivalent to 5 g of Na_2HPO_4 in 100 mL of hot water, filter in a tared Gooch crucible, wash the residue with hot water and dry at 105 °C for two hours. The residue mass is not more than 20 mg (0.4%).

Arsenic (5.3.2.5). Use *Spectrophotometric method, Method I*. Determine in a solution containing the equivalent of 187.5 mg of Na_2HPO_4 in 35 mL of water. Not more than 0.0016% (16 ppm).

Chlorides (5.3.2.1). Determine in sample quantity equivalent to 0.6 g of Na_2HPO_4 . Not more than 0.06% (600 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve sample quantity equivalent to 2.1 g of Na_2HPO_4 in 50 mL of water and use 24 mL of the obtained solution for *Sample preparation*. Not more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Determine in sample quantity equivalent to 0.6 g of Na_2HPO_4 . Not more than 0.2% (2000 ppm).

Loss on drying (5.2.9.1). Dry in oven at 130 °C until constant weight. Not more than 5.0% for the anhydrous form; between 10.3% and 12.0% for the monohydrate form; between 18.5% and 21.5% for the dihydrate form; between 43.0% and 50.0% for the heptahydrate form and between 55.0% and 64.0% for the dodecahydrate form.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh sample quantity equivalent to 1 g of Na_2HPO_4 and dissolve in 40 mL of water. Add, using a volumetric pipette, 15 mL of hydrochloric acid *M*. Titrate potentiometrically with *M* sodium hydroxide VS to an inflection point close to pH 4.0 and record the volume used. Continue titration until the second inflection point, close to pH 8.8. Carry out a blank test, transferring 15 mL of *M* hydrochloric acid with a volumetric pipette and 40 mL of water to an Erlenmeyer flask and titrating potentiometrically with *M* sodium hydroxide VS. The difference between the volume of *M* sodium hydroxide VS used in the blank and the volume used in the sample titration to the pH 4.0 inflection point is *Volume A*. The difference between the volume of *M* sodium hydroxide VS used between the pH 4.0 and pH 8.8 inflection points is *Volume B*. If *Volume A* is equal to or less than *Volume B*, each mL of *Volume A* of *M* sodium hydroxide VS is equivalent to 141.960 mg of Na_2HPO_4 . If *Volume A* is equal to or less than *Volume B*, each mL of $(2 \text{ Volume B}) - \text{Volume A}$ of *M* sodium hydroxide VS is equivalent to 141.960 mg of Na_2HPO_4 .

PACKAGING AND STORAGE

In tightly closed, non-metallic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Adjuvant.

MONOBASIC SODIUM PHOSPHATE

Natrii dihydrogenophosphas

NaH_2PO_4 ; 119,98
monobasic sodium phosphate; 00212
Sodium salt of phosphoric acid (1:1)
[7558-80-7]

$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; 137.99
monobasic sodium phosphate monohydrate; 09331
Disodium salt of phosphoric acid monohydrate (1:1:1)
[10049-21-5]

$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$; 156.01
monobasic sodium phosphate dihydrate; 00213
Calcium salt of phosphoric acid hydrate (1:1:2)
[13472-35-0]

Monobasic sodium phosphate is anhydrous or contains one or two molecules of water of hydration. It contains not less than 98.0% and not more than 103.0% of NaH_2PO_4 , in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Crystalline powder or colorless or white crystals and slightly deliquescent.

Solubility. Freely soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. The 5% (w/v) aqueous solution meets phosphate ion reactions (5.3.1.1).

B. The 5% (w/v) aqueous solution meets sodium ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 4.1 to 4.5. Determine in a solution containing the equivalent of 1 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 20 mL of water.

Insoluble substances. Dissolve sample quantity equivalent to 10 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 100 mL of hot water, filter in a tared Gooch crucible, wash the residue with hot water and dry at 105 °C for two hours. The residue mass is not more than 20 mg (0.2%).

Aluminum, calcium and related elements. The solution containing the equivalent to 1 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 10 mL of water does not present turbidity when the medium is made slightly alkaline with 6 M ammonium hydroxide, using pink litmus paper.

Arsenic (5.3.2.5). Use *Spectrophotometric method, Method I*. Determine in a solution containing the equivalent to 0.375 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 35 mL of water. Not more than 0.0008% (8 ppm).

Chlorides (5.3.2.1). Determine in sample quantity equivalent to 2.5 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. Not more than 0.014% (140 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve sample quantity equivalent to 1 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 20 mL of water, add 1 mL of 3 *M* hydrochloric acid and complete the volume to 25 mL with water. Not more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Determine in sample quantity equivalent to 0.8 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. Not more than 0.15% (1500 ppm).

Water (5.2.20.1). Not more than 2.0% for the anhydrous form; between 10.0% and 15.0% for the monohydrate form and between 18.0% and 26.5% for the dihydrate form.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately dissolve about 2.5 g of the sample in 10 mL of cold water and add 20 mL of cold saturated sodium chloride solution. Titrate with *M* sodium hydroxide VS, using phenolphthalein TS as indicator and keeping the solution temperature between 10 and 15 °C during the titration. Each mL of *M* sodium hydroxide VS is equivalent to 119.980 mg of NaH_2PO_4 .

PACKAGING AND STORAGE

In tightly closed, non-metallic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Adjuvant.

SODIUM PHOSPHATE ORAL SOLUTION

Sodium phosphate oral solution is a solution containing sodium phosphate dibasic and sodium phosphate monobasic or sodium phosphate dibasic and phosphoric acid in purified water. It contains, in 100 mL, not less than 16.2 g and not more than 19.8 g of dibasic sodium phosphate heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) and not less than 43.2 g and not more than 52.8 g of monobasic sodium phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$).

IDENTIFICATION

A. It meets sodium ion reactions (5.3.1.1).

B. It meets phosphate ion reactions (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

Relative density (5.2.5). 1.333 to 1.366.

pH (5.2.19). Between 4.4 and 5.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

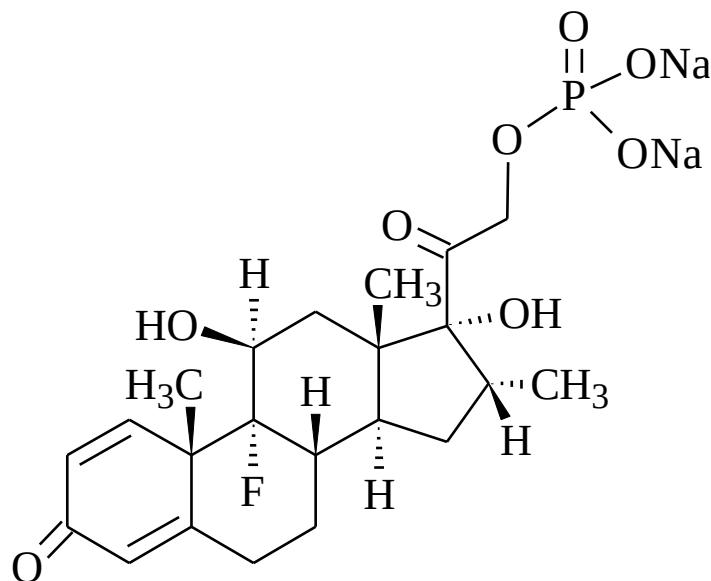
Pipet 25 mL of this solution, transfer to a 500 mL volumetric flask and complete the volume with water. Transfer 25 mL from this solution to a 250 mL beaker, add 15 mL of 0.5 M sodium hydroxide and 75 mL of water. Titrate the excess base, potentiometrically, with 0.5 M hydrochloric acid VS to the first inflection point (at pH close to 9.2). The volume of 0.5 M hydrochloric acid used is *Volume A*. Continue titration until the second inflection point (close to pH 4.4) and record *Volume B*. For blank determination, transfer 15 mL of 0.5 M sodium hydroxide into a 250 mL beaker, add 100 mL of water and titrate immediately with 0.5 M hydrochloric acid VS. Record the volume of 0.5 M hydrochloric acid VS used (*Volume C*), in mL. Each mL of the volume (C-A) of 0.5 M hydrochloric acid is equivalent to 68.995 mg of monobasic sodium phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) and each mL of the volume of (BC) of 0.5 M hydrochloric acid VS is equivalent to 134.035 mg of dibasic sodium phosphate heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$).

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

DEXAMETHASONE DISODIUM PHOSPHATE*Dexamethasoni natrii phosphas*C₂₂H₂₈FN₂O₈P; 516.41

dexamethasone disodium phosphate; 02821

(11 β ,16 α)-9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphono-oxy)-pregna-1,4-diene-3,20-dione sodium salt (2: 1)

[2392-39-4]

It contains not less than 97.0% and not more than 102.0% of C₂₂H₂₈FN₂O₈P, in relation to the anhydrous substance, ethyl alcohol-free.

DESCRIPTION

Physical characteristics. White powder, very hygroscopic. Presents polymorphism.

Solubility. Freely soluble in water, very slightly soluble in ethyl alcohol and in dioxane.

Physicochemical constants.

Specific optical rotation (5.2.8): +75 to +83, in relation to the anhydrous substance and ethyl alcohol-free. Determine in solution at 1% (w/v) in water.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the dexamethasone disodium phosphate CRS spectrum, prepared identically. If the spectrum obtained in the solid state presents some differences, dissolve the substance to be examined and the dexamethasone disodium phosphate CRS, separately, in a minimum volume of ethyl alcohol, evaporate and dry in water bath. Record new spectra using the residues.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel HF₂₅₄ as support, and a mixture of chloroform, methyl alcohol and water (180:15:1) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): weigh 20 mg of the sample in a centrifuge tube. Add 5 mL of alkaline phosphatase solution, shake vigorously and allow to stand for 30 minutes. Add 5 mL of ethyl acetate, shake vigorously, centrifuge and use the upper layer.

Solution (2): 3 mg/ml solution of dexamethasone CRS in ethyl acetate.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. Dissolve 2 mg in 2 mL of sulfuric acid ad mix. A yellowish brown color develops within five minutes. Add 10 mL of water and mix. The color disappears and the preparation remains clear.

D. Incinerate the sample as described in *Residue by incineration (5.2.10)*. The residue obtained meets sodium ion and phosphate ion reactions (5.3.1.1).

PURITY TESTS

Preparation aspect. The preparation at 5% (w/v) carbon dioxide-free water is clear.(5.2.25).

pH (5.2.19). 7.5 to 10.5. Determine in 1% (w/v) solution in carbon dioxide-free water.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 125mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to an octylsilane group (5 µm), kept at 30 °C; *Mobile phase* flow rate of 1 mL/minute.

Eluent A: dissolve 2.1 g of ammonium acetate in 650 mL of water, adjust the pH to 3.8 with acetic acid and add 350 mL of methyl alcohol.

Eluent B: dissolve 2.1 g of ammonium acetate in 300 mL of water, adjust the pH to 4.0 with glacial acetic acid and add 700 mL of methyl alcohol.

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 - 3,5	90	10	isocratic
3.5 - 23.5	90 → 60	10 → 40	linear gradient
23.5 - 34.5	60 → 5	40 → 95	linear gradient
34.5 - 50	5	95	isocratic

Solution (1): dissolve quantity, accurately weighed, of the sample in *Eluent A* to obtain a solution at 1.0 mg/mL.

Solution (2): transfer 1 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with *Eluent A*. Dilute 1 mL of this solution to 10 mL with the same solvent.

Solution (3): dissolve 2 mg of dexamethasone disodium phosphate CRS and 2 mg of betamethasone sodium phosphate (impurity B) in *Eluent A* and dilute to 100 mL with the same solvent.

Solution (4): dissolve 20 mg of dexamethasone disodium phosphate for identification of CRS peaks (containing impurities A, C, D, E, F and G) in *Eluent A* and dilute to 2 mL with the same solvent.

Inject, separately, 20 µL replicates of *Solutions (3)* and *(4)*. The relative retention times for dexamethasone disodium phosphate, whose retention time is about twenty-two minutes, are about 0.5 for impurity C, about 0.6 for impurity D, about 0.8 for impurity E, about 0.92 for impurity F, about 0.95 for impurity B (betamethasone phosphate or 9-fluoro-11b,17-dihydroxy-16b-methyl-3,20-dioxopregna-1,4 dihydrogen phosphate -diene-21-yl), about 1.37 for impurity A (dexamethasone or 9-fluoro-11b,17,21-trihydroxy-16a-methylpregna-1,4-diene-3,20-dione) and about 1.41 for impurity G (9-fluoro-11b,17-dihydroxy-16a-methyl-3-oxoandrosta-1,4-dien-17b-carboxylic acid). For each impurity C, D, E or F, one or more diastereoisomers of (9-fluoro-11b,17a-dihydroxy-16-methyl-3,17-dioxo-D-homo-androsta-1,4-dien dihydrogenphosphate) -17a-yl)-methyl (stereochemistry undefined in C16 and C17a) or (9-fluoro-11b,17-dihydroxy-16a-methyl-3,17a-dioxo-D-homo-androsta-1,4-dihydrogenphosphate) dien-17-yl)methyl (stereochemistry undefined in C17) may be present. The resolution between betamethasone sodium phosphate and dexamethasone disodium phosphate is not less than 2.0.

Procedure: Inject 20 µL of *Solution (1)* and *Solution (2)* separately and record the chromatograms for not less than twice the retention time of the main peak. The area under the peak corresponding to impurity A in the chromatogram of *Solution (1)* is not greater than five times the area under the main peak obtained with *Solution (2)* (0.5%). The area under the peak corresponding to impurity G in the chromatogram of *Solution (1)* is not greater than three times the area under the main peak obtained with *Solution (2)* (0.3%). The area under the peak corresponding to each of the impurities B, C, D, E and F in the chromatogram of *Solution (1)* is not greater than twice the area under the main peak obtained with *Solution (2)* (0.2%). The sum of the areas under all peaks obtained with the *Solution (1)*, except that under the solvent peak and that under the main peak, is not greater than ten times the area under the main peak obtained with *Solution (2)* (1.0%). Disregard peaks with areas below the half of the area under the main peak obtained with *Solution (2)* (0.05%).

Ethyl alcohol limit. Proceed as described in *Gas chromatography (5.2.17.5)*. Use gas chromatograph equipped with flame ionization detector, using capillary column with 1 m of length and 3.2 mm of internal diameter, packed with copolymer ethylvinylbenzene and divinylbenzene with film thickness of 150 µm to 180 µm; column temperature of 150°C, injector temperature at 250°C and detector temperature at 280 °C. Use nitrogen as carrier gas; flow rate of 30 mL/minute.

Internal standard solution: dilute 1 mL of *n*-propyl alcohol in 100 mL of water.

Solution (1): dissolve 0.5 g of sample in 5 mL of *Internal Standard Solution* and dilute to 10 mL with water.

Solution (2): dilute 1 mL of ethyl alcohol to 100 mL with water. Transfer 2 mL of this solution to a 10 mL volumetric flask, add 5 mL of the *Internal Standard Solution* and complete the volume with water.

Procedure: inject, separately, 2 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the ethyl alcohol content in the sample from the responses obtained for the ethyl alcohol / *n*-propyl alcohol ratio with *Solution (1)* and *Solution (2)*. Not more than 1.5% (w/w) of ethyl alcohol.

Phosphate ion limit. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Dissolve, accurately weighed, about 50 g of the sample in a mixture of 10 mL of water and 5 mL of sulfuric acid *M*. Heat if necessary. Add 1 mL of 5% (w/v) ammonium molybdate solution in 0.05 *M* sulfuric acid and 1 mL of 4-methylaminophenol sulfate RS. Complete the volume to 25 mL with water, homogenize and allow to stand for 30 minutes. Prepare standard solution at the same concentration, using 5 mL of 0.01433% (w/v) monobasic potassium phosphate solution, in place of the sample, and the same solvents. Measure the absorbances of the resulting solutions at 730 nm, using water for zero adjustment. The absorbance of the sample solution is not greater than the absorbance of the standard solution. Not more than 1.0%.

Water (5.2.20.1). Determine in 0.2 g of sample. Not more than 10.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as per *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 0.1 g of the sample and dissolve in water. Complete the volume to 100 mL with the same solvent. Dilute, successively, in water until concentration of 0.002% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 241 nm, using water for zero adjustment. Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 150 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (7 µm), kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mix 2 mL of phosphoric acid with 520 mL of water. Adjust temperature to 20°C and adjust pH to 2.6 with sodium hydroxide solution. Mix this solution with 36 mL of tetrahydrofuran and 364 mL of methyl alcohol.

Sample solution: dissolve 30 mg of the sample in *Mobile phase* and dilute to 50 mL with the same solvent. Dilute 5 mL of this solution to 50 mL with *Mobile phase*.

Standard solution: dissolve 30 mg of dexamethasone disodium phosphate CRS in *Mobile phase* and dilute to 50 mL with the same solvent. Dilute 5 mL of this solution to 50 mL with the same solvent.

Resolution solution: dissolve 2 mg of dexamethasone CRS (impurity A) and 2 mg of dexamethasone disodium phosphate CRS in 2 mL of tetrahydrofuran and dilute to 100 mL with *Mobile Phase*. Dilute 5 mL of this solution to 50 mL with *Mobile phase*.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are 1.0 for dexamethasone disodium phosphate, whose retention time is about eight minutes, and 2.0 for dexamethasone. The resolution between dexamethasone and dexamethasone disodium phosphate is not less than 6.0.

Procedure: Inject 20 µL of *Sample solution* and *Standard solution* separately and record the chromatograms for not less than three times the retention time of the main peak and measure the areas under the peaks. Calculate the content of $C_{22}H_{28}FNa_2O_8P$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

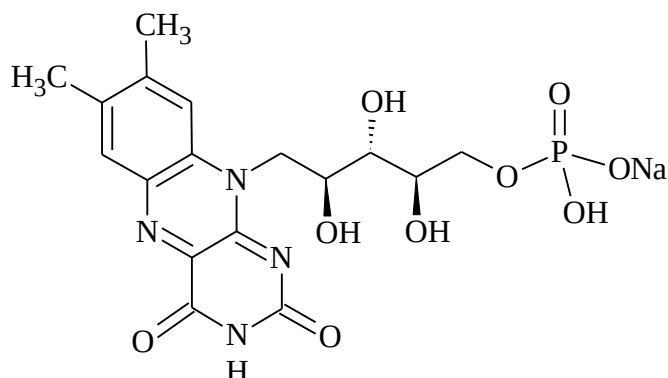
In hermetically closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Steroidal anti-inflammatory.

RIBOFLAVIN SODIUM PHOSPHATE***Riboflavini natrii phosphas***C₁₇H₂₀N₄NaO₉P; 478.33

riboflavin sodium phosphate; 07703

Riboflavin 5'-(dihydrogen phosphate) sodium salt (1:1)

[130-40-5]

C₁₇H₂₀N₄NaO₉P.2H₂O; 514.36

riboflavin sodium phosphate dihydrate; 11222

Riboflavin 5'-(dihydrogen phosphate) monosodium salt dihydrate

[6184-17-4]

Mixture containing riboflavin 5'-(sodium hydrogen phosphate) as the main component and other sodium riboflavin monophosphates. It contains not less than 73.0% and not more than 79.0% of riboflavin (C₁₇H₂₀F₄O₆), in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder, yellow or orange-yellowish, hygroscopic.

Solubility. Soluble in water, very slightly soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +38 to +43. Determine in solution at 1.2% (w/v) in 5 M hydrochloric acid.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 230 nm to 350 nm, of 0,001% (w/v) solution in citro-phosphate buffer pH 7.0, there is a maximum point at 266 nm. The absorbance at 266 nm is between 0.58 and 0.64.

B. The retention time of the main peak from the *Solution (1)* chromatogram, obtained in *Related substances*, corresponds to the one from the main peak of the *Solution (3)*.

C. Transfer 10 mg of the sample to a 100 mL volumetric flask, dissolve in sodium hydroxide RS and complete the volume with the same solvent. Expose 1 mL of this solution to ultraviolet light (254 nm) for five minutes. Acidify the solution with acetic acid, using blue litmus paper as indicator. Shake with 2 mL of methylene chloride. The lower phase of the mixture presents yellow fluorescence.

D. To 0.5 g of the sample, add 10 mL of nitric acid and evaporate in water bath to dryness. Heat the residue until it turns white, dissolve in 5 mL of water and filter. The filtrate meets sodium ion reactions (5.3.1.1) and phosphate ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 5.0 to 6.5. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed protected from direct light, as described in *High performance liquid chromatography* (5.2.17.4). Use chromatograph equipped with ultraviolet detector at 266 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 2 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.735%(w/v) monobasic potassium phosphate (15:85).

Solution (1): quantitatively transfer about 0.1 g of the sample to a 100 mL volumetric flask, dissolve in 50 mL of water and complete the volume with *Mobile Phase*. Transfer 4 mL of this solution to a 25 mL volumetric flask and complete the volume with *Mobile phase*.

Solution (2): quantitatively dissolve approximately 60 mg of riboflavin CRS in 1 mL of hydrochloric acid and dilute to 250 mL with water. Transfer 4 mL of this solution to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Solution (3): quantitatively dissolve approximately 0,1 mg of riboflavin sodium phosphate CRS in 50 mL of water and dilute to 100 mL with *Mobile phase*. Transfer 4 mL of this solution to a 25 mL volumetric flask and complete the volume with *Mobile phase*.

Inject 100 µL replicates of the *Solution (1)* and of *Solution (3)*. Record the chromatogram until complete elution of the peak corresponding to riboflavin. The relative retention times are about 0.2 for riboflavin 3',4'-diphosphate; 0.3 for riboflavin 3',5'-diphosphate; 0.5 for riboflavin 4',5'-diphosphate; 0.7 for riboflavin 3'-monophosphate; 0.9 for riboflavin 4'-monophosphate; 1.0 for riboflavin 5'-monophosphate; and 2.0 for riboflavin. The resolution between riboflavin 4'-monophosphate and riboflavin and 5'-monophosphate in the chromatogram obtained with *Solution (3)* is not less than 1.5. The relative standard deviation of the replicate areas under the peak for riboflavin 5'-monophosphate is not more than 1.5%

Procedure: inject, separately, 100 µL of the *Solution (1)*, *Solution (2)* and *Solution (3)*, record the chromatograms and measure the areas under the peaks. Calculate the content of free riboflavin and riboflavin diphosphates in the sample from the areas under the peaks in the chromatogram obtained with *Solutions (1)*, (2) and (3). Not more than 6.0% of free riboflavin and 6.0% of riboflavin diphosphates, in relation to the desiccated substance.

Lumiflavin limit. Shake 35 mg of the sample with 10 mL of freshly prepared methylene chloride for five minutes and filter. The absorbance (5.2.14) of the resulting solution, at 440 nm, using methylene chloride for zero adjustment, is not more than 0.025.

Free phosphate limit. Dissolve 0.3 g of the sample in water, dilute to 100 mL with the same solvent. Transfer 10 mL of this solution to a 50 mL volumetric flask. Add 10 mL of acid ammonium molybdate solution and 5 mL of 10% ferrous sulfate (w/v) in 0.075 M sulfuric acid. Homogenize. Prepare standard solution similarly using 10 mL of 0.0044% (w/v) monobasic potassium phosphate, 10 mL of acidic ammonium molybdate solution and 5 mL of 10% (w/v) ferrous sulfate in 0.075 M sulfuric acid. Measure the absorbances of the resulting solutions at 700 nm **(5.2.14)**. Use mixture of 10 mL of water, 10 mL of acid ammonium molybdate solution and 5 mL of 10% (w/v) ferrous sulfate in 0.075 M sulfuric acid for zero adjustment. The absorbance of the sample solution is not greater than the absorbance of the standard solution. Not more than 1.0%.

Heavy metals (5.3.2.3). Transfer 2 g of the sample to a silica crucible, add dropwise 2 mL of nitric acid and 0.25 mL of sulfuric acid. Carefully heat until white smoke appears and the sample ignites. Cool. Extract the residue with two 2 mL portions of hydrochloric acid. Evaporate extracts to dryness. Dissolve the residue with 2 mL of diluted acetic acid and dilute to 20 mL with water. Proceed as described in *Method I*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample, in oven at 105 °C, under reduced pressure, for five hours. Not more than 8.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 25.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed protected from direct light, as described in *Visible absorption spectrophotometry (5.2.14)*. Accurately weigh about 0.1 g of the sample and dissolve in 150 mL of water. Add 2 mL of glacial acetic acid and dilute to 1000 mL with water. Transfer 10 mL of this solution to a 50 mL volumetric flask, add 3.5 mL of 1.4% (w/v) sodium acetate and complete the volume with water. Record the solution absorbance at 444 nm. Use a mixture of water and 1.4% (w/v) sodium acetate (93:7) for zero adjustment. Calculate the content of $C_{17}H_{20}N_4O_6$ in the sample considering $A(1\%, 1\text{ cm}) = 328$, at 444 nm.

B. Proceed protected from direct light, as described in *Fluorescence spectrophotometry (5.2.15)*. Quantitatively dissolve about 50 mg of the sample in 20 mL of pyridine and 75 mL of water, and dilute to 1000 mL with water. Likewise, prepare riboflavin CRS standard solution at a concentration of 35 µg/mL. Transfer 10 mL of each solution to 1000 mL volumetric flasks. Add 0.05 M sulfuric acid until pH adjusts between 5.9 and 6.1 (approximately 4 mL) and complete the volume with water. Measure the fluorescence intensities of the resulting solutions in a fluorimeter, at an excitation wavelength of 440 nm and emission of 530 nm. Calculate the content of $C_{17}H_{20}N_4O_6$ in the sample from the readings obtained.

PACKAGING AND STORAGE

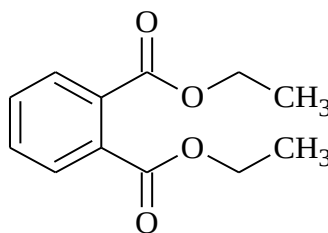
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vitamin B component.

ETHYL PHTHALATE*Ethylis phthalas*

C₁₂H₁₄O₄; 222.24

ethyl phthalate; 09828

1,2-benzenedicarboxylic acid 1,2-diethyl ester

[84-66-2]

It contains not less than 99.0% and not more than 101.0% of C₁₂H₁₄O₄, in relation to the anhydrous substance.

DESCRIPTION

Physicochemical characteristics. Colorless or slightly yellowish oily liquid. Boiling temperature (5.2.3): approximately 295 °C.

Solubility. Practically insoluble in water; miscible with ethyl alcohol.

Physicochemical constants.

Relative density (5.2.5): 1.117 to 1.121. Determine at 20 °C.

Refractive index (5.2.6): 1.500 to 1.505. Determine at 20 °C.

IDENTIFICATION

In the infrared absorption spectrum (5.2.14) of the sample, dispersed among sodium chloride plates, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ethyl phthalate CRS spectrum, prepared identically.

PURITY TESTS

Preparation aspect. The examined preparation is clear (5.2.25) and not more stained than the solution described below (5.2.12). Mix 24 mL of *Ferric chloride base solution*, 6 mL of *Cobalt chloride base solution* and 70 mL of 1% (w/v) hydrochloric acid. Transfer 12.5 mL of this solution to a 100 mL volumetric flask and complete the volume with 1% (w/v) hydrochloric acid.

Acidity. Dissolve 20 g of the sample in 50 mL of previously neutralized ethyl alcohol. Add 0.2 mL of phenolphthalein TS and titrate with 0.1 M sodium hydroxide. Not more than 0.1 mL of 0.1 M sodium hydroxide is used to neutralize the solution.

Related substances. Proceed as described in *Gas chromatography (5.2.17.5)*. Use chromatograph equipped with flame ionization detector, 2 m long and 2 mm internal diameter glass column, with stationary phase filled with silanized diatomaceous earth for gas chromatography, packed with 3% polymethylphenylsiloxane, particles of 150 µm to 180 µm, and nitrogen for chromatography as carrier gas, with flow rate of 30 mL/minute. Keep the column temperature at 150°C and the injector and the detector temperature at 225°C.

Internal standard solution: dissolve 60 mg of naphthalene in methylene chloride and dilute to 20 mL with the same solvent.

Solution (1): dissolve 1.0 g of the sample in methylene chloride and dilute to 20 mL with the same solvent.

Solution (2): dissolve 1.0 g of the sample in methylene chloride, add 2 mL of the *Internal standard solution* and dilute to 20 mL with the same solvent.

Solution (3): to 1 mL of the *Solution (1)*, add 10 mL of the *Internal standard solution* and dilute to 100 mL with methylene chloride.

Separately inject 1 µL of each of the solutions into the gas chromatograph. Elute for three times the ethyl phthalate retention time. The expected elution order is naphthalene followed by ethyl phthalate. The resolution between the ethyl phthalate and naphthalene peaks in the chromatogram obtained with *Solution (1)* is greater than 10. In the chromatogram obtained with *Solution (1)*, no peak should have the same retention time as that obtained with the *Internal Standard Solution*.

Procedure: inject, separately, 1 µL of the *Solution (3)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the ratio - between the area under the ethyl phthalate peak and the area under the naphthalene peak obtained in the chromatogram with *Solution (3)*; calculate the ratio between the sum of the areas under any peaks, except that under the main peak and under the internal standard, and the area under the naphthalene peak in the chromatogram obtained with *Solution (2)*. The ratio is not more than 1.0%.

Water (5.2.20.1). Determine in 5 g of the sample. Not more than 0.2%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Accurately weigh about 0.75 g of the sample and transfer to a 250 mL Erlenmeyer. Add 25 mL of 0.5 M ethanolic potassium hydroxide VS and some glass beads. Heat in water bath, under reflux, for one hour. Add 1 mL of phenolphthalein TS and titrate immediately with 0.5 M hydrochloric acid VS. Carry out the blank test and make the necessary corrections. Calculate the volume of 0.5 M ethanolic potassium hydroxide VS used in the saponification. Each mL of 0.5 M ethanolic sodium hydroxide VS is equivalent to 55.560 mg of C₁₂H₁₄O₄.

PACKAGING AND STORAGE

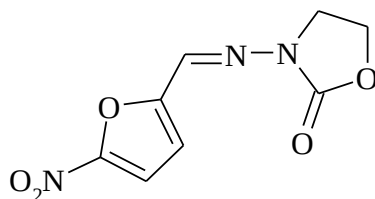
In tightly closed, fully filled containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Pharmaceutical adjuvant.

FURAZOLIDONE***Furazolidonum*** $C_8H_7N_3O_5$; 225.16

furazolidone; 04343

3-[[[5-nitro-2-furanyl]-methylene]amino]-2-oxazolidone

[67-45-8]

It contains not less than 97.0% and not more than 103.0% of $C_8H_7N_3O_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow crystalline powder.

Solubility. Practically insoluble in water and in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the furazolidone CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in water, there are maximum and minimum points only at the same wavelengths as similar furazolidone CRS solution.

C. Add approximately 50 mg of the sample to 10 mL of a freshly prepared mixture of dimethylformamide and 0.5 M ethanolic potassium hydroxide (90:10). The solution develops a purple color, changing to an intense blue color immediately. After 10 minutes, it develops purple color again.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 100 °C for one hour. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. Not more than 0.25%.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). Quantitatively transfer about 0.1 g of the sample to a 250 mL volumetric flask, dissolve in dimethylformamide and complete the volume with the same solvent. Transfer 5 mL to a 250 mL volumetric flask, complete the volume with water and homogenize. Prepare a standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 367 nm, using mixture of dimethylformamide and water (1:50) for zero adjustment. Calculate the content of $C_8H_7N_3O_5$ in the sample from the readings obtained.

PACKAGING AND STORAGE

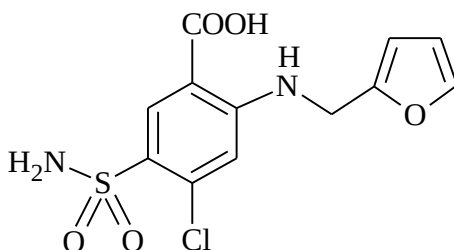
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-bacterial; antiprotozoal.

FUROSEMIDE***Furosemidum***

$C_{12}H_{11}ClN_2O_5S$; 330.75

furosemide; 04361

5-(Aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid

[54-31-9]

It contains not less than 98.0% and not more than 101.0% of $C_{12}H_{11}ClN_2O_5S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, soluble in methyl alcohol, moderately soluble in ethyl alcohol. Freely soluble in aqueous solutions of alkali hydroxides.

IDENTIFICATION

The Identification test A. can be omitted if tests B. and C. are carried out. The Identification tests B. and C. can be omitted if test A. is carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the furosemide CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of 0.0005% (w/v) solution in 0.1 M sodium hydroxide, there are maximum points at 228 nm, 271 nm and 333 nm, identical to those observed in the spectrum of similar furosemide CRS solution. The absorbances of solutions at 271 nm do not differ by more than 3% when calculated in relation to the desiccated substance.

C. The retention time of the main peak from the *Solution (1)* chromatogram, obtained in *Related substances*, corresponds to the one from the main peak of the *Solution (4)*.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 238 nm; column with 250 mm of length and 4,6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μ m),

kept at room temperature; *Mobile phase* flow rate of 1 mL/minute. Protect solutions from direct light.

Mobile phase: dissolve 2.0 g of monobasic potassium phosphate and 2.5 g of cetrimide in 700 mL of water, adjust pH to 7.0 with ammonia and add 300 mL of propyl alcohol.

Solution (1): dissolve 50 mg of the sample in *Mobile phase* and dilute to 50 mL with the same solvent.

Solution (2): Dissolve 2 mg of related substance A in *Mobile Phase*, add 2.0 mL of *Solution (1)* and dilute to 20 mL with the same solvent. Dilute 0,5 mL of this solution to 20 mL with *Mobile phase*.

Solution (3): dilute 1 mL of *Solution (1)* to 100 mL with *Mobile phase*. Dilute 1 mL of this solution to 10 mL with *Mobile phase*.

Solution (4): Dissolve 2 mg of furosemide for CRS peak identification (containing impurities C and D) in 2 mL of *Mobile Phase*.

Inject, separately, 20 µL replicates of *Solutions (2), (3) and (4)*. The retention times for furosemide, whose retention time is about nine minutes, are about 0.5 for impurity C (2-amino-4-chloro-5-sulfamoylbenzoic acid), about 0.8 for impurity A (2-chloro-4-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid) and about 1.5 for impurity D (2,4-bis[(furan-2-ylmethyl)amino acid]-5-sulfamoylbenzoic). The resolution between the furosemide peak and impurity A is not less than 4.0. The signal-noise ratio is not less than 40 for the main peak obtained with *Solution (3)*.

Procedure: Inject, separately, 20 µL replicates of *Solutions (1) and (3)*. Record the chromatograms and measure the areas under the peaks. For limits determination purposes, use the following correction factors as multipliers of the areas corresponding to the respective impurities: 1.4 for impurity C and 2.0 for impurity D. The area under the peak corresponding to impurity C, obtained with *Solution (1)*, is not more than twice the area under the main peak obtained with *Solution (3)* (0.2%). The area under the peak corresponding to impurity D, obtained with *Solution (1)*, is not more than 1.5 times the area under the main peak obtained with *Solution (3)* (0.15%). The area under the peak corresponding to each of the other impurities, obtained with *Solution (1)*, is not more than equal to the area under the main peak obtained with *Solution (3)* (0,1%). The total of impurities is not more than five times the area under the main peak of the chromatogram obtained with *Solution (3)* (0.5%). Disregard peaks with area smaller than 0.5 times the area under the main peak obtained with *Solution (3)* (0,05%).

Chlorides (5.3.2.1). To 1 g of the sample, add a mixture of 0.2 mL of nitric acid and 30 mL of water. Shake for five minutes, allow to stand for 15 minutes and filter. Use 15 mL of the filtrate. Not more than 0.02% (200 ppm).

Sulfates (5.3.2.2). To 2 g of the sample, add a mixture of 0.2 mL of acetic acid and 30 mL of water. Shake for five minutes, allow to stand for 15 minutes and filter. Use 15 mL of the filtrate. Not more than 0.03% (300 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Determine in 1 g of sample. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Dry in oven at 105 °C for three hours. Not more than 1.0%.

Residue by incineration (5.2.10). Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.25 g of the sample in 20 mL of dimethylformamide, add 0.2 mL of 1% (w/v) bromothymol blue solution in dimethylformamide and titrate with 0.1 M sodium hydroxide VS until blue color. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium hydroxide VS is equivalent to 33.075 mg of $C_{12}H_{11}ClN_2O_5S$.

PACKAGING AND STORAGE

In opaque, tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Diuretic.

FUROSEMIDE TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{12}H_{11}ClN_2O_5S$.

IDENTIFICATION

In the ultraviolet absorption spectrum **(5.2.14)**, at the range of 220 nm to 320 nm, of the final solution obtained in Assay, there are maximum absorption points at 228 nm and 271 nm.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Weigh each tablet separately and pulverize. Quantitatively transfer to a 100 mL volumetric flask, add 0.1 M sodium hydroxide, shake, complete the volume with the same solvent and homogenize. Filter, transfer 1 mL of the filtrate to a 50 mL volumetric flask and complete the volume with the same solvent. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 271 nm **(5.2.14)**, using 0.1 M sodium hydroxide for zero adjustment. Calculate the quantity of $C_{12}H_{11}ClN_2O_5S$ in each tablet from the readings obtained. Alternatively, calculate using $A(1\%, 1\text{cm}) = 580$, at 271 nm.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 5.8, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 60 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute with phosphate buffer pH 5.8 to adequate concentration. Measure the absorbances of the solutions at 271 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the quantity of $C_{12}H_{11}ClN_2O_5S$ dissolved in the medium, comparing the readings obtained with the furosemide CRS solution at the concentration of 0.0008% (w/v) prepared with the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{12}H_{11}ClN_2O_5S$ dissolve in 60 minutes.

PURITY TESTS

Free primary aromatic amines. Pulverize the tablets and weigh an amount of powder equivalent to 0.1 g of furosemide. Transfer to a 25 mL volumetric flask, with the aid of methyl alcohol. Shake, complete the volume with methyl alcohol, homogenize and filter.

Pipet 1 mL of filtrate, transfer to a 25 mL volumetric flask, add, with agitation, 3 mL of dimethylformamide, 12 mL of distilled water and 1 mL of hydrochloric acid M. Cool and add 1 mL of sodium nitrite 0.5% (w/v), with agitation. Allow to stand for 5 minutes. Add 1 mL of sulfamic acid 2.5% (w/v) with agitation and allow to stand for 3 minutes. Then, add 1 mL of N-(1-naphthyl) ethylenediamine dihydrochloride solution 0.5% (w/v) and dilute to 25 mL with distilled water. In parallel, carry out a blank test, replacing 1 mL of the filtrate with 1 mL of methanol. Immediately carry out the reading of the absorbance, at 530 nm. The absorbance obtained is not more than 0.20.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh and pulverize 20 tablets. Transfer a quantity of powder, equivalent to 0.2 g of furosemide, to a 500 mL volumetric flask with the aid of 300 mL of 0.1 M sodium hydroxide. Shake for 10 minutes. Complete the volume with the same solvent, homogenize and filter. Dilute 5 mL of the filtrate to 250 mL with 0.1 M sodium hydroxide and homogenize. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 271 nm (**5.2.14**), using 0.1 M sodium hydroxide for zero adjustment. Calculate the quantity of $C_{12}H_{11}ClN_2O_5S$ in the tablets from the readings obtained. Alternatively, calculate using $A(1\%, 1\text{cm}) = 580$, at 271 nm.

PACKAGING AND STORAGE

In opaque, tightly closed containers.

LABELING

Comply with current legislation.

FUROSEMIDE INJECTION SOLUTION

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{12}H_{11}ClN_2O_5S$.

IDENTIFICATION

A. Transfer volume of the injection solution containing the equivalent to 20 mg of furosemide to a 100 mL volumetric flask, complete the volume with water and homogenize. Dilute 5 mL of the solution to 100 mL with 0.1 M sodium hydroxide. In the ultraviolet absorption spectrum **(5.2.14)** of the solution obtained, in the range of 220 nm to 340 nm, there are maximum points at 228 nm and 271 nm, identical to those observed in the spectrum of similar furosemide CRS solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 8.0 to 9.3.

PURITY TESTS

Free primary aromatic amines. Transfer volume of the injection solution containing the equivalent to 40 mg of furosemide to a 10 mL volumetric flask, complete the volume with methyl alcohol, homogenize and filter. Proceed as described in *Free primary aromatic amines* assay, in the *Furosemide* monograph, from "Pipet 1 mL of filtrate...". The absorbance obtained is not more than 0.20.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 3.6 EU/mg of furosemide.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Pipet volume of the injection solution containing the equivalent to 20 mg of furosemide, transfer to a 100 mL volumetric flask, complete the volume with water and homogenize. Dilute 5 mL to 100 mL with 0.1 M sodium hydroxide and homogenize. Prepare a standard solution in the same concentration, using 0.1 M sodium hydroxide solution. Measure the absorbances of the resulting solutions at 271 nm, using 0.1 M sodium hydroxide for zero adjustment. Calculate the quantity of $C_{12}H_{11}ClN_2O_5S$ in the injection solution from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 580$, at 271 nm, in 0.1 M sodium hydroxide.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Protect solutions from exposure to light. Use chromatograph equipped with ultraviolet detector at 272 nm; 150mm

long, 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm); kept at 30 °C, *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water, tetrahydrofuran and glacial acetic acid (70:30:1).

Diluent: dilute 22 mL of glacial acetic acid in a mixture of water and acetonitrile (50:50), complete the volume to 1000 mL and homogenize.

Sample solution: transfer volume of the injection solution containing the equivalent to 20 mg of furosemide to a 50 mL volumetric flask, complete the volume with *Diluent* and homogenize. Transfer 5 mL of the solution to a 50 mL volumetric flask, complete the volume with the same solvent and homogenize.

Standard solution: dissolve an accurately weighed quantity of furosemide CRS in the *Diluent* and dilute successively to obtain a 40 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

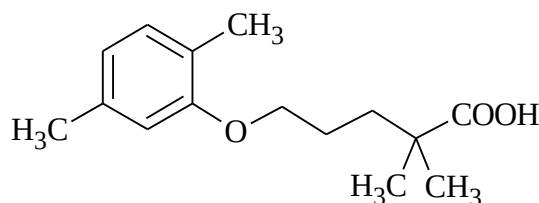
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₂H₁₁ClN₂O₅S in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In type I glass containers, protected from light.

LABELING

Comply with current legislation.

GEMFIBROZIL***Gemfibrozilum***C₁₅H₂₂O₃; 250.33

gemfibrozil; 04421

5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid

[25812-30-0]

It contains not less than 98.0% and not more than 102.0% of C₁₅H₂₂O₃, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline, waxy powder.

Solubility. Practically insoluble in water, freely soluble in methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 58 °C to 61 °C.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the gemfibrozil CRS spectrum, prepared identically.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 276 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: transfer 10 mL of glacial acetic acid and 750 mL of methyl alcohol to a 1000 mL volumetric flask. Complete the volume with water and homogenize.

Solution (1): Dissolve accurately weighed quantities of Gemfibrozil CRS, 5-[2,5-dimethyl-4-(1-propenyl)phenoxy]-2,2-dimethylpentanoic acid (Impurity A) CRS and 2,5-dimethylphenol in *Mobile phase*, to obtain a solution with concentrations around 0.2 mg/mL, 0.05 mg/mL and 0.05 mg/mL, respectively.

Solution (2): quantitatively transfer about 10 mg of gemfibrozil CRS and 10 mg of Impurity A CRS to a 100 mL volumetric flask, dissolve in 50 mL of methyl alcohol, complete the volume with the same solvent and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Solution (3): quantitatively transfer about 0.1 g of the sample to a 10 mL volumetric flask. Dissolve in *Mobile phase*, complete the volume with the same solvent and homogenize.

Inject 100 µL replicates of the *Solution (1)*. The relative retention times are about 0.35 for 2,5-dimethylphenol, 1.0 for gemfibrozil, and 2.1 for Impurity A. The relative standard deviation of the replicate areas under the recorded peaks is not more than 3.0%.

Procedure: Inject 100 µL of *Solutions (2)* and *(3)* separately, record the chromatograms for not less than three times the gemfibrozil peak retention time and measure the areas under the peaks. The area under the peak corresponding to impurity A in the chromatogram of *Solution (3)* is not greater than the area under the peak corresponding to impurity A obtained with *Solution (2)* (0.1%). The area under the peak corresponding to 2,5-dimethylphenol in the chromatogram obtained with *Solution (3)* is not greater than the area under the peak corresponding to 2,5-dinitrofen obtained with *Solution (2)* (0.1%). No other peak in the chromatogram obtained with *Solution (3)* has an area greater than the area obtained with the impurities already described (0.1%). The sum of the areas under all peaks obtained with the *Solution (3)*, except that under the solvent peak and that under the main peak, is not greater than five times the area under the main peak obtained with *Solution (2)* (0.5%). Disregard peaks with areas below 0.5 times the area under the main peak obtained with *Solution (2)* (0.05%).

Heavy metals (5.3.2.3). Determine in 1 g of sample using *Method III*. Not more than 0.002% (20 ppm).

Water (5.2.20.1). Determine in 1.5 g of the sample. Not more than 0.25%.

Residue by incineration (5.2.10). Determine in 2 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 276 nm; column with 300 mm in length and 3.9 in internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile Phase* flow of 0.8 mL/minute.

Mobile phase: transfer 10 mL of glacial acetic acid and 800 mL of methyl alcohol to a 1000 mL volumetric flask. Complete the volume with water and homogenize.

Sample solution: weigh, accurately, about 25 mg of the sample and transfer to a 25 mL volumetric flask. Dissolve in methyl alcohol and complete the volume with the same solvent. Homogenize.

Transfer 5 mL of this solution to a 25 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Standard solution: weigh, accurately, about 25 mg of gemfibrozil CRS and transfer to a 25 mL volumetric flask. Dissolve in methyl alcohol and complete the volume with the same solvent. Homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Resolution solution: prepare 0.2 mg/mL Gemfibrozil solution and 0.05 mg/mL of 2,5-Dimethylphenol solution in *Mobile Phase*.

Inject 10 µL replicates of the *Resolution solution*. The resolution between gemfibrozil and 2,5-dimethylphenol is not less than 8.0. Inject 10 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₅H₂₂O₃ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

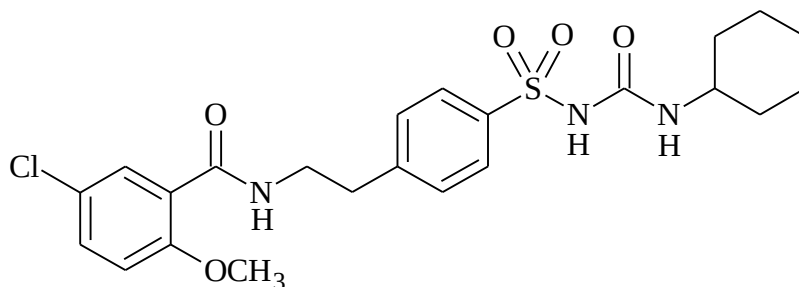
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antilipemic.

GLIBENCLAMIDE***Glibenclamidum***

$C_{23}H_{28}ClN_3O_5S$; 494.00

glibenclamide; 04451

5-Chloro-*N*-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-2-methoxybenzamide
[10238-21-8]

It contains not less than 98.0% and not more than 102.0% of $C_{23}H_{28}ClN_3O_5S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder. Presents polymorphism.

Solubility. Practically insoluble in water, slightly soluble in methyl alcohol and in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 169 °C to 174 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, previously desiccated, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the glibenclamide CRS spectrum, prepared identically. If the spectra obtained are different, moisten the sample and the CRS separately in methyl alcohol, pulverize, dry between 105 °C and 110 °C and obtain new spectra with the residues.

B. The retention time of the main peak from the *Solution (3)* chromatogram, obtained in *Related substances*, corresponds to the one from the main peak of the *Solution (1)*.

C. Mix 0.2 g of the sample with 0.25 g of anhydrous sodium carbonate and 0.25 g of anhydrous potassium carbonate. Incinerate the mixture for 10 minutes, cool, add 10 mL of hot water to the residue, shake for one minute and filter. The filtrate meets the chloride ions and sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. The 1% (w/v) sample preparation in ethyl alcohol, prepared with the aid of heating, is clear **(5.2.25)** and colorless **(5.2.12)**.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 230 nm; 100mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (3 µm) with inactivated base, kept at 35 °C; *Mobile phase* flow rate of 0.8 mL/minute.

Eluent A: mix 20 mL of triethylamine solution at 101.8 g/L, freshly distilled and previously adjusted to pH 3.0 with phosphoric acid, with 50 mL of acetonitrile. Dilute with water to 1000 mL and homogenize.

Eluent B: mixture of *Eluent A*, water and acetonitrile (20:65:915).

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 15	45	55	isocratic
15 – 30	45 → 5	55 → 95	linear gradient
30 – 40	5	95	isocratic
40 – 41	5 → 45	95 → 55	linear gradient
41 – 55	45	55	isocratic

Solution (1): accurately dissolve about 10 mg of glibenclamide CRS, about 5 mg of impurity A (5-chloro-2-methoxy-N-(2-(4-sulfamoylphenyl)ethyl)benzamide) of glibenclamide CRS and 5 mg impurity B (methyl((4-(2-((5-chloro-2-methoxybenzoyl)amino)ethyl)phenyl)sulfonyl)carbamate) from glibenclamide CRS in methyl alcohol and dilute to 100 mL with the same solvent. Transfer 5 mL to a 20 mL volumetric flask, complete the volume with methyl alcohol and shake.

Solution (2): dissolve, accurately, about 25 mg of the sample in methyl alcohol, dilute to 10 mL with the same solvent and shake.

Solution (3): dilute 2 mL of *Solution (2)* to 100 mL with methyl alcohol. Transfer 5 mL to a 50 mL volumetric flask, complete the volume with methyl alcohol and shake.

Solution (4): accurately dissolve approximately 5 mg of gliclazide CRS in methyl alcohol, add 2 mL of *Solution (2)* and complete the volume to 100 mL with the same solvent. Transfer 1 mL to a 10 mL volumetric flask, complete the volume with the same solvent and shake.

The relative retention times corresponding to glibenclamide (retention time = about five minutes) are about 0.5 for impurity A, 0.6 for impurity B and 1.0 for glibenclamide. The resolution between glibenclamide and gliclazide is not less than 5.0.

Procedure: inject, separately, 10 µL of each of the freshly prepared solutions, record the chromatograms and measure the areas under the peaks. The area under the peak of impurity A obtained with *Solution (2)* is not greater than the area under the peak obtained with *Solution (1)* (0.5%). The area under the peak of impurity B obtained with *Solution (2)* is not greater than the area under the peak obtained with *Solution (1)* (0.5%). Any area under secondary peak obtained with *Solution (2)* is not greater than the area under the main peak of *Solution (3)* (0.2%). Only two areas under the secondary peaks obtained with *Solution (2)*, evaluated separately and excluding the peaks

of impurities A and B, may present an area greater than half the area under the main peak of *Solution (3)* (0.1%). The sum of the areas under all the peaks obtained with *Solution (2)*, except that of glibenclamide, is not greater than 2.5 times the area under the main peak obtained with *Solution (3)* (0.5%). Disregard peaks with areas below 0.25 times the area under the main peak in the chromatogram obtained with *Solution (3)* (0.05%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for six hours. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh about 0.5 mg of the sample and dissolve in 100 mL of heated ethyl alcohol, neutralized in advance with 0.1 M sodium hydroxide VS. Titrate with 0.1 M sodium hydroxide VS using phenolphthalein TS as indicator. Each mL of 0.1 M sodium hydroxide VS is equivalent to 49.400 mg of $C_{23}H_{28}ClN_3O_5S$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 230 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μ m); *Mobile phase* flow rate of 1.5 mL/minute.

Phosphate buffer pH 3.0: dissolve 1.36 g of monobasic potassium phosphate in 900 mL of water, adjust pH to 3.0 ± 0.1 with phosphoric acid RS and dilute to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer pH 3.0* and acetonitrile (45:55).

Sample solution: quantitatively transfer about 22 mg of the sample to a 100 mL volumetric flask, add 70 mL of methyl alcohol and place in ultrasonic bath for 20 minutes. Complete the volume with the same solvent and homogenize. Dilute successively with phosphate buffer pH 7.3 to concentration of 5.5 μ g/mL.

Standard solution: quantitatively transfer about 22 mg of glibenclamide CRS to a 100 mL volumetric flask, add 70 mL of methyl alcohol and place in ultrasonic bath for 20 minutes. Complete the volume with the same solvent and homogenize. Dilute with phosphate buffer pH 7.3 to concentration of 5.5 μ g/mL.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₃H₂₈ClN₃O₅S in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and excessive heat.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Oral hypoglycemic agent.

GLIBENCLAMIDE TABLETS

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_{23}H_{28}ClN_3O_5S$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Mix in a mortar quantity of powder equivalent to 20 mg of glibenclamide with 20 mL of a mixture of methylene chloride and acetone (2:1). Filter, evaporate the filtrate to dryness at room temperature and dry in oven at 105 °C for two hours. In the infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the glibenclamide CRS spectrum, prepared identically.

B. Weigh and pulverize the tablets. Transfer a quantity of powder equivalent to 20 g of glibenclamide to a 200 mL volumetric flask. Add 4 mL of 0.5 M hydrochloric acid and shake. Add 100 mL of methyl alcohol, place in ultrasonic bath for 15 minutes and shake mechanically for 15 minutes. Complete the volume with methyl alcohol and homogenize. Filter. In the ultraviolet absorption spectrum (**5.2.14**) of the solution obtained at the range of 230 nm to 350 nm, there are maximum peaks at 275 nm and 300 nm.

C. The main stain of the chromatogram from *Solution (1)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Not more than 15 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to 25 mL volumetric flask. Add 2.5 mL of water and wait for the tablet to completely disintegrate. Add 15 mL of methyl alcohol, place in ultrasonic bath for 20 minutes. Complete the volume with methyl alcohol and homogenize. Filter. Proceed as described in *Assay*.

DISSOLUTION TEST (5.1.5)

Note: before testing, the dissolution medium must be heated to 41 °C and deaerated using a vacuum filtration system with vigorous agitation. Keep agitation for about five minutes after the end of filtration in the system, still under vacuum. Filter aliquots of the dissolution medium using a membrane with porosity of 0.45 µm, compatible with the dissolution medium.

Dissolution medium: phosphate buffer pH 7.3; 900 mL.

Apparatus: paddles, 75 rpm.

Time: 60 minutes.

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use chromatograph equipped with ultraviolet detector at 230 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm); *Mobile phase* flow rate of 1.5 mL/minute.

Phosphate buffer pH 3.0: dissolve 1.36 g of monobasic potassium phosphate in 900 mL of water, adjust pH to 3.0 ± 0.1 with phosphoric acid and dilute to 1 000 mL with water.

Mobile phase: mixture of *Phosphate buffer pH 3.0* and acetonitrile (45:55).

Sample solution: after testing, remove aliquot from the dissolution medium and filter.

Standard solution: quantitatively transfer about 22 mg of glibenclamide CRS to a 100 mL volumetric flask, add 70 mL of methyl alcohol and place in ultrasonic bath for 20 minutes. Complete the volume with the same solvent and homogenize. Dilute successively with phosphate buffer pH 7.3 to concentration of 5.5 µg/mL.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{23}H_{28}ClN_3O_5S$ dissolved in the medium, from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 70% (Q) of the stated quantity of $C_{23}H_{28}ClN_3O_5S$ dissolve in 60 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel GF₂₅₄ as support, and a mixture of chloroform, cyclohexane, ethyl alcohol and glacial acetic acid (45:45:5:5) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Shake in a mortar quantity of powder equivalent to 40 mg of glibenclamide with 20 mL of a mixture of methylene chloride and acetone (2:1) and filter. Evaporate the filtrate to dryness, at a temperature not exceeding 40 °C, under reduced pressure. Dissolve the residue in 4 mL of mixture of chloroform and methyl alcohol (1:1).

Solution (2): 0.24 mg/mL solution of glibenclamide CRS in a mixture of chloroform and methyl alcohol (1:1).

Solution (3): 10 mg/mL solution of glibenclamide CRS in a mixture of chloroform and methyl alcohol (1:1).

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (2.4%).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 300 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm); *Mobile phase* flow rate of 1.5 mL/minute.

Phosphate buffer pH 3.0: dissolve 1.36 g of monobasic potassium phosphate in 900 mL of water, adjust pH to 3.0 ± 0.1 with phosphoric acid and dilute to 1000 mL with water.

Mobile phase: mixture of *Phosphate buffer pH 3.0* and acetonitrile (47:53).

Sample solution: weigh and pulverize 20 tablets. Quantitatively transfer quantity of powder equivalent to about 5 mg of glibenclamide to a 25 mL volumetric flask, add 15 mL of a mixture of methyl alcohol and water (10:1) and place in ultrasonic bath for 20 minutes. Complete the volume with the same solvent, homogenize and filter.

Standard solution: quantitatively transfer about 20 mg of glibenclamide CRS to a 100 mL volumetric flask, add 70 mL of mixture of methyl alcohol and water (10:1) and place in ultrasonic bath for 20 minutes. Complete the volume with the same solvent and homogenize.

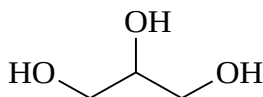
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{23}H_{28}ClN_3O_5S$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

GLYCEROL*Glycerolum*

C₃H₈O₃; 92.09
glycerol; 04469
1,2,3-Propanetriol
[56-81-5]

It contains not less than 98.0% and not more than 101.0% of C₃H₈O₃, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Syrupy, colorless or almost colorless liquid, clear, hygroscopic.

Solubility. Miscible with water and with ethyl alcohol; practically insoluble in benzene, chloroform, petroleum ether, fatty oils and essential oils.

Physicochemical constants.

Relative density (5.2.5): 1.25 to 1.26.

IDENTIFICATION

Mix 1 mL of sample and 0.5 mL of nitric acid. Add 0.5 mL of 10.6% potassium dichromate (w/v). On the contact surface, a blue ring develops which, for 10 minutes, does not diffuse into the lower layer.

PURITY TESTS

Preparation aspect. Dilute 25 g of the sample to 50 mL with carbon dioxide-free water. The preparation is clear **(5.2.25)**. Dilute 10 mL of the solution obtained to 25 mL with water. The preparation is colorless **(5.2.12)**.

Limit of chlorinated compounds. In a round-bottomed flask adapted to a condenser, add 5 g of the sample and 15 mL of morpholine. Heat gently under reflux for three hours. Wash the condenser with 10 mL of water. Collect the washing water in the flask. Transfer to a Nessler tube. Acidify with nitric acid R, add 0.5 mL of 0.5 M silver nitrate and dilute to 50 mL with water. Shake. Prepare standard, in a Nessler tube, using 15 mL of morpholine, 10 mL of water and 0.4 mL of 0.01 M hydrochloric acid. Proceed as described for sample preparation from "Acidify...". Any turbidity produced in the sample preparation must not be more intense than that obtained with the standard preparation. Not more than 0.003% (30 ppm).

Acrolein, glucose and ammonia compounds. Mix 5 mL of the sample and 5 mL of 10% potassium hydroxide (w/v). Heat at 60°C for five minutes. Ammonia vapors are not released. Red color is not developed.

Other reducing substances. Mix 5 mL of sample with 5 mL of 10% ammonium hydroxide (w/v) and heat at 60°C for five minutes. Quickly add 0.5 mL of 0.1 M silver nitrate, keeping the pipette tip above the tube, dropping the solution directly into the solution without touching the tube walls. Shake and keep in a dark place for five minutes. The solution does not darken.

Fatty acids and esters. Mix 50 g of the sample with 100 mL of hot, freshly boiled water. Add 1 mL of phenolphthalein TS and neutralize with 0.1 M sulfuric acid. Add 15 mL of 0.2 M sodium hydroxide. Heat under reflux for five minutes, cool and titrate with 0.1 M sulfuric acid VS. Carry out blank test using 140 mL of freshly boiled water. The difference between titrations is not more than 1.6 mL.

Sucrose. To 4 mL of the sample add 6 mL of 0.5 M sulfuric acid. Heat for one minute, cool and neutralize with sodium hydroxide RS, using litmus paper as indicator. Add 5 mL of alkaline cupric tartrate RS and heat to boiling for one minute. Red-orangish precipitate is not formed.

Chlorides. To 10 mL of 10% (w/v) sample solution add 0.25 mL of nitric acid RS and 0.5 mL of 0.1 M silver nitrate. Shake. Turbidity is not formed.

Sulfates. To 10 mL of 10% (w/v) sample solution add three drops of hydrochloric acid RS and five drops of barium chloride RS. Turbidity is not formed.

Arsenic (5.3.2.5). Proceed as described in *Visual method*. Not more than 0.00015% (1.5 ppm).

Heavy metals (5.3.2.3). Mix 4 g of the sample with 2 mL of 0.1 M hydrochloric acid and dilute with water to 25 mL. Not more than 0.0005% (5 ppm).

Water (5.2.20.1). Determine in 1 g of the sample. Not more than 2.0%.

Residue by incineration (5.2.10). Determine in 5 g of the sample. Not more than 0.01%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 0.1 g of sample, transfer to a 250 mL Erlenmeyer flask and dissolve in 45 mL of water. Add 25 mL of a mixture of 0.1 M sulfuric acid and 2.14% (w/v) sodium periodate (1:20) and allow to stand for 15 minutes, protected from light. Add 5 mL of 50% (w/v) ethylene glycol and allow to stand for 20 minutes, protected from light.. Titrate with 0.1 M sodium hydroxide VS using 0.5 mL of phenolphthalein TS. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium hydroxide VS is equivalent to 9.209 mg of C₃H₈O₃.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Humectant, solvent.

GLYCEROL SUPPOSITORIES

It contains not less than 75.0% and not more than 90.0% of the stated quantity of $C_3H_8O_3$. Contains sodium stearate.

IDENTIFICATION

A. Under heating, dissolve 12 suppositories in 125 mL of water. Cool, add 1.5 mL of hydrochloric acid and transfer the mixture to a 250 mL separating funnel. Extract with 75 mL of hexane, discard the aqueous layer and collect the organic layer in a beaker. Evaporate in water bath until dryness. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the stearic acid CRS spectrum, prepared identically.

B. Dissolve 1 g of sodium borate decahydrate in 100 mL of water, add 25 drops of phenolphthalein TS and homogenize. In a test tube containing 0.5 mL of this solution, add two drops of a previously melted suppository. The intense pink color is completely discolored. When the solution is heated, the pink color reappears.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

PURITY TESTS

Water (5.2.20.1). Not more than 15.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

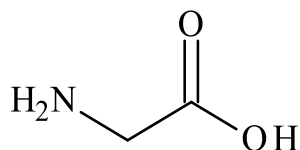
Weigh the quantity of suppositories equivalent to 0.25 g of glycerin, dissolve in water, complete the volume to 250 mL and filter. Transfer 5 mL of this solution to an Erlenmeyer flask, add 50 mL of a reagent prepared by mixing 40 mL of 5% sulfuric acid (v/v) and 60 mL of 0.1% potassium periodate (w/v) acidified with three to five drops of sulfuric acid. Heat the solution in water bath for 15 minutes, cool to room temperature and add 1 g of potassium iodide. Allow to stand for five minutes. Titrate with 0.02 M sodium thiosulfate VS, using starch TS as indicator, which should be added very close to the changeover. Carry out the blank test and make the necessary corrections. Each mL of 0.02 M sodium thiosulfate VS is equivalent to 0.4605 mg of $C_3H_8O_3$.

PACKAGING AND STORAGE

In hermetic, opaque containers.

LABELING

Comply with current legislation.

GLYCINE
Glycinum

C₂H₅NO₂; 75.07

glycine; 04472

Glycine

[56-40-6]

It contains not less than 98.5% and not more than 101.5% of C₂H₅NO₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder. Presents polymorphism.

Solubility. Freely soluble in water and very slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 232 °C to 236 °C, with decomposition.

IDENTIFICATION

The infrared absorption spectrum **(5.2.14)** of the sample desiccated at 105 °C, for two hours, presents maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the glycine CRS spectrum, prepared identically. If the spectra obtained are different, dissolve the sample and the CRS separately in a minimum quantity of ethyl alcohol 60% v/v, evaporate to dryness and obtain new spectra.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 210 nm; 250mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at 25 °C; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: dissolve 1.4 g of sodium pentanesulfonate in 900 mL of water, adjust pH to 2.2 with phosphoric acid and dilute to one liter with water.

Diluent: dilute 1.5 mL of phosphoric acid in 500 mL of *Mobile Phase*.

Solution (1): dissolve 200 mg of the sample in *Diluent* and dilute to 20 mL with the same solvent.

Solution (2): dilute 1 mL of sample solution to 100 mL with *Diluent*. Dilute 1 mL of this solution to 10 mL with *Diluent*.

Solution (3): Dissolve 80 mg of iminodiacetic acid (impurity A, 2,2'-iminodiacetic acid) and 80 mg of the sample in *Diluent* and dilute to 50 mL with the same solvent.

Solution (4): dissolve 50 mg of glycine anhydride (impurity B, piperazine-2,5-dione), 50 mg of diglycine (impurity H, 2-[(2-aminoacetyl)amino]acetic acid) and 50 mg of triglycine (Impurity I, 2-[[[2(aminoacetyl)amino]acetyl]amino]acetic acid) in *Diluent* and dilute to 50 mL with the same solvent. Dilute 1 mL of this solution to 100 mL with *Diluent*.

Inject, separately, 10 µL replicates of *Solutions (3) and (4)*. The glycine retention time is about 5.5 minutes. The glycine retention times are about 0.7 for impurity A, about 0.75 for impurity B, about 1.7 for impurity H, and about 2.0 for impurity I. The resolution between the peaks of impurity A and glycine is not less than 5.0.

Procedure: inject 10 µL replicates of the *Solutions (1), (2) and (4)*, record the chromatograms and measure the areas under the peaks. The sum of the areas under all the secondary peaks obtained with *Solution (1)* is not greater than twice the area under the main peak obtained with *Solution (2)* (0.20%). The areas under the peaks of impurities B, H and I in the chromatogram obtained with *Solution (1)* are not greater than the areas under the respective peaks in the chromatogram obtained with *Solution (4)* (0.10% for each impurity). The area under the peak of any other impurity not specified in the chromatogram obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (2)* (0.10%). Disregard peaks with area equal to or smaller than 0.5 times the area under the main peak in the chromatogram obtained with *Solution (2)* (0,05%).

Preparation aspect. The 10% (w/v) solution in freshly boiled and cooled water is clear **(5.2.25)** and colorless **(5.2.12)**.

Chlorides (5.3.2.1). Proceed as described in *Limit test for chlorides*. Determine in 5 g of sample. Not more than 0.007% (70 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Prepare 20 mL of a 10% (w/v) solution of the sample in water and proceed according to *Limit Test for Heavy Metals*. Use *Standard lead solution (1 ppm Pb)*. Not more than 0.001% (10 ppm).

Sulfates (5.3.2.2.). Dissolve 4 g of the sample in 40 mL of water and proceed as described in *Limit test for sulfates*, using 0,65 mL of the 0.005 M standard sulfuric acid solution. Not more than 0.0065% (65 ppm).

Loss on drying (5.2.9). Determine in 1 g of sample. Dry in oven at 105 °C for two hours. Not more than 0.2%.

Sulfated ashes (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.15 g of the sample and dissolve in 100 mL of glacial acetic acid, heat gently to facilitate dissolution. Add two drops of methylrosanilinium chloride TS and titrate with 0.1 M perchloric acid VS until blue color turns greenish-blue. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 7.507 mg of $C_2H_5NO_2$.

PACKAGING AND STORAGE

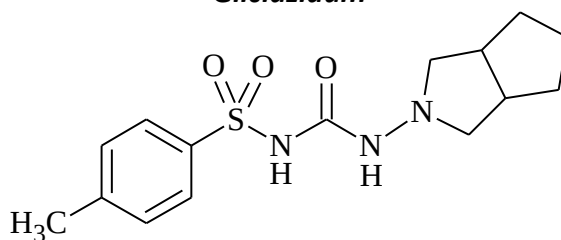
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Non-essential amino acid.

GLICLAZIDE**Gliclazidum**C₁₅H₂₁N₃O₃S; 323.41

gliclazide; 04474

N-[[(Hexahydrocyclopenta[*c*]pyrrole-2(1*H*)-yl)amino]carbonyl]-4-methylbenzenesulfonamide
[21187-98-4]

It contains not less than 98.0% and not more than 101.0% of C₁₅H₂₁N₃O₃S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol.

IDENTIFICATION

In the infrared absorption spectrum (5.2.14) of the sample, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the gliclazide CRS spectrum, prepared identically.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Prepare solutions just before use. Use chromatograph equipped with ultraviolet detector at 235 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to an octylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 0.9 mL/minute.

Mobile phase: mixture of triethylamine, trifluoroacetic acid, acetonitrile and water (0.1:0.1:45:55).

Solution (1): quantitatively dissolve about 50 mg of the sample in 23 mL of acetonitrile and dilute to 50 mL with water.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with mixture of acetonitrile and water (45:55). Dilute 10 mL of the resulting solution to 100 mL with the same diluent.

Solution (3): quantitatively dissolve about 5 mg of the sample and 15 mg of 1-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(2-methylphenyl)sulfonyl]urea CRS in 23 mL of acetonitrile and dilute to 50 mL with water. Dilute 5 mL of the resulting solution to 100 mL with mixture of acetonitrile and water (45:55).

Solution (4): quantitatively dissolve about 10 mg of 1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(2-methylphenyl)sulfonyl]urea CRS in 45 mL of acetonitrile and dilute to 100 mL with water. Dilute 1 mL of the resulting solution to 100 mL with mixture of acetonitrile and water (45:55).

Inject 20 µL of *Solution (3)*. The resolution between the peaks of 1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(2-methylphenyl)sulfonyl]urea and gliclazide is not less than 1.8.

Procedure: separately inject 20 µL of the solutions (1), (2) and (4). Record *Solution (1)* chromatograms for not less than twice the gliclazide retention time and measure the areas under the peaks. In the chromatogram obtained with *Solution (1)*, the area under the peak of 1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(2-methylphenyl)sulfonyl]urea is not greater than the area under the peak obtained with *Solution (4)* (0.1%). The areas under all peaks obtained with *Solution (1)*, except that under the main peak and under the peak of 1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(2-methylphenyl)sulfonyl]urea are not greater than the area under the main peak obtained with *Solution (2)* (0.1%). The sum of the areas under all peaks obtained with *Solution (1)*, except that under the main peak and under the peak of 1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(2-methylphenyl)sulfonyl]urea is not greater than twice the area under the main peak obtained with *Solution (2)* (0.2%). Disregard peaks with areas below 0.2 times the area under the main peak obtained with *Solution (2)* (0.02%).

Heavy metals (5.3.2.3). Use *Method IV*. Not more than 0.001% (10 ppm). Prepare the standard lead solution at a concentration of 10 ppm Pb.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven between 100 °C and 105 °C for two hours. Not more than 0.25%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.25 mg of the sample previously desiccated and dissolve in 50 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS and determine the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 32.341 mg of C₁₅H₂₁N₃O₃S.

PACKAGING AND STORAGE

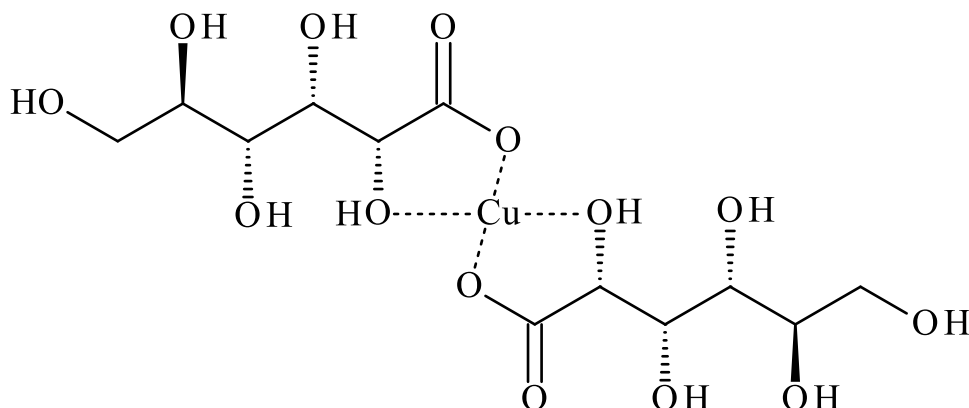
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Oral antidiabetic.

COPPER GLUCONATE*Cupri gluconas*C₁₂H₂₂CuO₁₄; 453.84

copper gluconate; 04479

Bis(D-gluconate-κO1,κO2)-copper

[527-09-3]

It contains not less than 98.0% and not more than 102.0% of C₁₂H₂₂CuO₁₄.

DESCRIPTION

Physical characteristics. Fine, greenish-blue powder.

Solubility. Freely soluble in water and insoluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 155 °C to 157 °C.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel as support, and a mixture of alcohol, ethyl acetate, ammonium hydroxide and water (50:10:10:30), as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in water, heating to 60 °C in water bath if necessary.

Solution (2): 10 mg/mL potassium gluconate solution.

TLC visualization reagent: dissolve 2.5 g of ammonium molybdate in 50 mL of *M* sulfuric acid in a 100 mL volumetric flask, add 1.0 g of ceric sulfate, shake to dissolve and complete the volume with *M* sulfuric acid.

Develop the chromatogram and allow the mobile phase to run three-quarters of the plate length. Remove the plate, dry at 110 °C for 20 minutes. Cool, spray with the *TLC visualization reagent* and heat at 110 °C for 10 minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. To 5 mL of slightly heated aqueous 10% copper gluconate solution, add 0.7 mL of glacial acetic acid and 1 mL of freshly distilled phenylhydrazine. Heat in water bath for 30 minutes and cool. When scraping a glass rod along the inner walls of the tube, small precipitated crystals are formed.

C. It meets copper ion reactions **(5.3.1.1)**.

PURITY TESTS

Reducing substances limit. Weigh 1 g of the sample, dissolve in 10 mL of water and add 25 mL of alkaline cupric citrate RS. Cap the flask, boil gently for five minutes and cool quickly to room temperature. Add 25 mL of 0.6 *M* acetic acid, 10 mL of 0.1 *M* iodine VS and 10 mL of 3 *M* hydrochloric acid. Titrate with 0.1 *M* sodium thiosulfate VS, add 3 mL of starch TS near the endpoint. Carry out the blank test and make the necessary corrections. Each mL of 0.1 *M* SV sodium thiosulfate solution VS is equivalent to 2.7 mg of reducing substances (expressed as dextrose). Not more than 1.0%.

Lead. Proceed as described in *Method II of Atomic absorption spectrometry (5.2.13.1.4)*. Use a spectrophotometer equipped with graphite furnace, hollow lead cathode lamp and select the emission line at 283.3 nm. Use the following temperature schedule, with argon flow of three liters per minute, 70 °C for 10 seconds, 90 °C for 60 seconds, 120 °C for 15 seconds, 250 °C for five seconds (no gas flow) , 250 °C for 10 seconds, 250 °C for two seconds (no gas flow), and 2000 °C for 3.2 seconds.

Standard solution: transfer 10 mL of lead SRA to a 100 mL volumetric flask. Add 40 mL of water and 5 mL of nitric acid. Complete the volume with water and mix. Transfer 0.4 mL of this solution to a second volumetric flask. Add 50 mL of water and 1 mL of nitric acid. Complete the volume with water and mix. This solution contains 0,04 µg/mL of lead.

Sample solution: quantitatively transfer about 4 g of copper gluconate to a 100 mL volumetric flask. Add 50 mL of water and 5 mL of nitric acid. Place in ultrasonic bath until the substance dissolves. Complete the volume with water and mix. Transfer 4 mL of this solution to a second 100 mL volumetric flask. Add 50 mL of water and 1 mL of nitric acid. Complete the volume with water and mix.

Blank solution: transfer 1.2 mL of nitric acid to a 100 mL volumetric flask. Complete the volume with water and mix.

Prepare analytical solutions from the *Sample solution*, *Standard solution* and *Blank solution* in the following proportions, by volume: 10:0:10 (0 µg/ml lead); 10:4:6 (0.008 µg/mL of lead); 10:7:3 (0.014 µg/mL of lead) and 10:10:0 (0.020 µg/mL of lead). Inject 20 µL of the blank solution and of analytical solutions separately. Determine the absorbance at a temperature of 2000 °C. Calculate the lead concentration in the *Sample solution*. Not more than 25 ppm.

Arsenic (5.3.2.5). Weigh 1 g of the sample and proceed according to *Method I of Limit test for arsenic*. Not more than 0.0003% (3 ppm).

Chlorides (5.3.2.1). Dissolve 0,5 g of the sample in 40 mL of boiling water and proceed as described in *Limit test for chlorides*. Not more than 0.07% (700 ppm).

Sulfates (5.3.2.2). Dissolve 2.4 g of the sample in 40 mL of boiling water and proceed as described in *Limit test for sulfates*. Not more than 0.05% (500 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 1.5 g of the sample and dissolve in 100 mL of water. Add 2 mL of glacial acetic acid and 5 g of potassium iodide. Titrate with 0.1 M sodium thiosulfate VS until a light yellow color is formed. Add 2 g of ammonium thiocyanate. Mix and add 3 mL of starch TS. Continue titration until color changeover. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium thiosulfate VS is equivalent to 45.384 mg of $_{12}H_{22}CuO_{14}$.

PACKAGING AND STORAGE

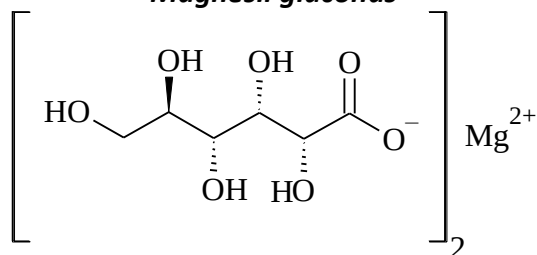
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Food supplement.

MAGNESIUM GLUCONATE*Magnesii gluconas* $\text{C}_{12}\text{H}_{22}\text{MgO}_{14}$; 414.60

magnesium gluconate; 04480

Magnesium salt of D-glyconic acid (1:2)

[3632-91-5]

 $\text{C}_{12}\text{H}_{22}\text{MgO}_{14} \cdot 2\text{H}_2\text{O}$; 450.63

magnesium gluconate dihydrate; 11388

Magnesium salt of D-glyconic acid hydrate (1:2:2)

[59625-89-7]

It contains not less than 98.0% and not more than 102.0% of $\text{C}_{12}\text{H}_{22}\text{MgO}_{14}$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White powder or granule or colorless crystals; hygroscopic.

Solubility. Freely soluble in water and slightly soluble in ethyl alcohol.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel as support, and a mixture of alcohol, ethyl acetate, ammonium hydroxide and water (50:10:10:30), as mobile phase. Apply to the plate, separately, 5 μL of each one of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in water, heating to 60 °C in water bath if necessary.

Solution (2): 10 mg/mL potassium gluconate solution.

TLC visualization reagent: dissolve 2.5 g of ammonium molybdate in 50 mL of *M* sulfuric acid in a 100 mL volumetric flask, add 1.0 g of ceric sulfate, shake to dissolve and complete the volume with *M* sulfuric acid.

Develop the chromatogram and allow the mobile phase to run three-quarters of the plate length. Remove the plate, dry at 110 °C for 20 minutes. Cool, spray with the *TLC visualization reagent* and heat at 110 °C for 10 minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. To 5 mL of warmed aqueous 10% magnesium gluconate solution, add 0.7 mL of glacial acetic acid and 1 mL of freshly distilled phenylhydrazine. Heat in water bath for 30 minutes and cool. When scraping a glass rod along the inner walls of the tube, small precipitated crystals are formed.

C. It meets magnesium ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 6.0 to 7.8. Determine in aqueous solution at 5% (w/v).

Reducing substances limit. Accurately weigh 1 g of the sample, dissolve in 20 mL of hot water, cool and add 25 mL of alkaline cupric citrate RS. Cap the flask, boil gently for five minutes and cool quickly to room temperature. Add 25 mL of 2 M acetic acid, 10 mL of 0.1 M iodine VS and 10 mL of 3 M hydrochloric acid. Titrate with 0.1 M sodium thiosulfate VS, add 3 mL of starch SI near the endpoint. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M SV sodium thiosulfate solution VS is equivalent to 2.7 mg of reducing substances (expressed as dextrose). Not more than 1.0%.

Arsenic (5.3.2.5). Use *Method II*. Dissolve 1.0 g of sample in 35 mL of water. Proceed according to *Limit test for arsenic*. Not more than 0.0003% (3 ppm).

Chlorides (5.3.2.1). Weigh 0.7 g of sample and proceed as described in *Limit test for chlorides*. Not more than 0.05% (500 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve 1.0 g of the sample in 10 mL of water, add 6 mL of 3.0 M hydrochloric acid and complete the volume to 25 mL with water. Proceed according to the *Limit test for heavy metals*. Not more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Dissolve 2.4 g of the sample in 40 mL of boiling water and proceed as described in *Limit test for sulfates*. Not more than 0.05% (500 ppm).

Water (5.2.20.1). Use the *Indirect Method*. Not more than 12.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 800 g of the sample and dissolve in 20 mL of water. Add 5 mL of ammonium chloride RS and 0.1 mL of eriochrome black T TS. Titrate with 0.05 M disodium edetate VS until the color turns blue. Each mL of 0.05 M disodium edetate is equivalent to 20.730 mg of $C_{12}H_{22}MgO_{14}$.

PACKAGING AND STORAGE

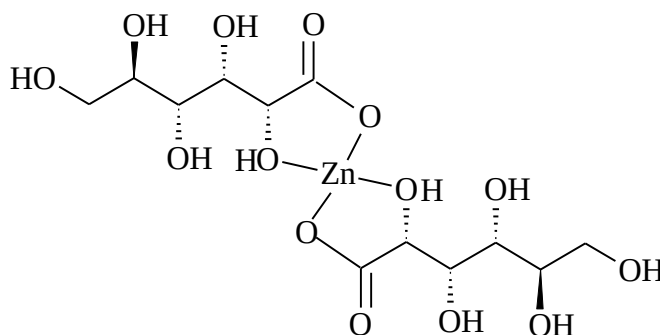
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Food supplement.

ZINC GLUCONATE*Zinci gluconas*C₁₂H₂₂O₁₄Zn; 455.68

zinc gluconate; 09453

(T-4)-Bis(D -gluconate-κO1,κO2)-zinc

[4468-02-4]

It contains not less than 97.0% and not more than 102.0% of C₁₂H₂₂O₁₄Zn, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline or granular powder.

Solubility. Freely soluble in water and very slightly soluble in ethyl alcohol.

IDENTIFICATION

It meets zinc ion reactions **(5.3.1.1)**. Carry out tests 1 and 2.

PURITY TESTS

pH (5.2.19). 5.5 to 7.5. Determine in aqueous solution at 1% (w/v).

Volatile organic impurities limit. Proceed as described in *Gas chromatography (5.2.17.5)*. Use chromatograph equipped with flame ionization detector, using a mixture of nitrogen, synthetic air and hydrogen (1:1:10) as auxiliary gases to the detector flame; capillary column 30 m in length and 0.53 mm in internal diameter, packed with stationary phase bonded to 5% phenylpolysiloxane and 95% to methylpolysiloxane, with a film thickness of 5 µm; column temperature from 35 °C to 260 °C (35 °C held for five minutes, increased to 175 °C to 8 °C per minute, increased to 260 °C to 35 °C per minute and held at this temperature for not less than 16 minutes), injector temperature of 70 °C and detector temperature of 260 °C; use helium as carrier gas; carrier gas flow rate of 1.0 mL/minute.

Sample solution: accurately weigh approximately 1 g of sample and dissolve in 50 mL of organic compounds-free water.

Standard solution: prepare a solution, in water free of organic compounds, containing in each mL, 10 µg of methylene chloride, 1 µg of chloroform, 2 µg of benzene, 2 µg of dioxane and 2 µg of trichlorethylene.

Procedure: inject separately 1 µL of the *Sample solution* and the *Standard solution* in the gas chromatograph. Obtain the chromatograms and measure the areas under the peaks. Identify, based on the retention time, any peaks present in the *Sample Solution* chromatogram. The presence and identification of the peaks in the chromatogram must be established by comparing the chromatograms of the *Sample Solution* and the *Standard Solution*. Not more than 2 ppm of benzene, 50 ppm of chloroform, 100 ppm of dioxane, 500 ppm of methylene chloride and 80 ppm of trichlorethylene.

Reducing substances limit. Weigh 1 g of the sample in a 250 mL erlenmeyer, dissolve in 10 mL of water and add 25 mL of alkaline cupric citrate RS. Cap the erlenmeyer, boil gently for five minutes and cool quickly to room temperature. Add 25 mL of 6 M acetic acid, 10 mL of 0.1 M iodine VS and 10 mL of 3 M hydrochloric acid. Titrate with 0.1 M sodium thiosulfate VS, add 3 mL of starch RS close to the endpoint. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium thiosulfate solution VS is equivalent to 2.7 mg of reducing substances (expressed as dextrose). Not more than 1.0%.

Cadmium. Proceed as described in *Atomic absorption spectrophotometry (5.2.13.2)*. Use *Method II*. Use spectrophotometer equipped with flame fed by mixture of air-acetylene, cadmium hollow cathode lamp and select the emission line at 228.8 nm.

Sample solution: dissolve 10 g of the sample in 50 mL of water.

Standard solution: transfer 137,2 mg of cadmium nitrate to a 1000 mL volumetric flask, dissolve in water and complete the volume with the same solvent. Homogenize. Transfer 25 mL of this solution and 1 mL of hydrochloric acid to a 100 mL volumetric flask. Complete the volume with water and homogenize. This solution contains 12.5 µg/mL of cadmium.

Procedure: Use three 25 mL volumetric flasks. The *Standard Solution* will not be added to the first one. Add 2 mL and 4 mL of the *Standard Solution* to the other two flasks. In each flask, add 5 mL of the *Sample Solution*, complete with water and mix. These solutions contain, respectively, 0 µg/mL; 1 µg/mL and 2 µg/mL of cadmium from the *Standard Solution*. Plot the absorbance values of the *Sample Solution* versus cadmium concentration, in µg/mL, of the *Standard Solutions*, obtain the line that best fits the three points and extrapolate the line until it intercepts the concentration axis. From the intercept, determine the quantity, in µg, of cadmium in each mL of the *Sample solution* (the one to which the *Standard solution* has not been added). Prepare blank in parallel using water. Calculate the quantity, in ppm, of cadmium from the readings obtained. Not more than 0.0005% (5 ppm).

Lead. Proceed as described in *Atomic absorption spectrophotometry (5.2.13.2)*. Use *Method I*. Use spectrophotometer equipped with flame fed by mixture of air-acetylene, lead hollow cathode lamp and select the emission line at 283.3 nm.

Ascorbic acid-sodium iodide solution: in a 200 mL volumetric flask, dissolve 20 g of ascorbic acid and 38.5 g of sodium iodide in water. Complete the volume with the same diluent and homogenize.

Trioctylphosphine oxide solution: in a 100 mL volumetric flask, dissolve 5 g of trioctylphosphine oxide in methyl isobutyl ketone. Complete with the same diluent and homogenize. **Note:** Caution! This solution may cause eye and skin irritation!

Standard solution: transfer 5 mL of *Ammonium sulfate stock solution (5.3.2.3)* to a 100 mL volumetric flask and complete the volume with water. To a 50 mL volumetric flask, transfer 2 mL of this solution, 10 mL of 9 M hydrochloric acid, 20 mL of *Ascorbic acid-sodium iodide solution* and 5 mL of *Trioctylphosphine oxide solution*. Shake for 30 seconds and allow to stand to separate the phases. Complete the volume with water to bring the organic phase to the top of the flask, transfer to a separating funnel, allow the phases to separate and proceed with the separation. The organic phase is the one containing the *Standard Solution* (2.0 µg/mL).

Sample solution: to a 50 mL volumetric flask, transfer 1 g of zinc gluconate, 10 mL of hydrochloric acid, about 10 mL of water, 20 mL of *Ascorbic acid-sodium iodide solution* and 5 mL of *Trioctylphosphine oxide solution*. Shake for 30 seconds and allow to separate the phases. Add water to bring the organic phase to the top of the flask, transfer to a separating funnel. Shake again and proceed to separation. The organic phase is the one that contains the sample to be analyzed.

Blank solution: To a 50 mL volumetric flask, transfer 10 mL of 9 M hydrochloric acid, 20 mL of *Ascorbic acid-sodium iodide solution* and 5 mL of *Trioctylphosphine oxide solution*. Shake for 30 seconds and allow to stand to separate the phases. Complete the volume with water to bring the organic phase to the top of the flask, allow the phases to separate and proceed with the separation. The organic phase is the one that contains the blank.

Procedure: measure the absorbances of the *Standard solution*, *Sample solution* and *Blank solution* for zero adjustment. Calculate the quantity, in ppm, of lead from the readings obtained. Not more than 0.001% (10 ppm).

Arsenic (5.3.2.5). Use *Method I*. Weigh 1 g of sample and proceed as described in *Limit test for arsenic*. Not more than 0.0003% (3 ppm).

Chlorides (5.3.2.1). Dissolve 0.7 g of the sample in 40 mL of boiling water and proceed as described in *Limit test for chlorides*. Not more than 0.05% (500 ppm).

Sulfates (5.3.2.2). Dissolve 2.4 g of the sample in 40 mL of boiling water and proceed as described in *Limit test for sulfates*. Not more than 0.05% (500 ppm).

Water (5.2.20.1). Use the *Indirect Method*. Not more than 11.6%.

BIOLOGICAL SAFETY TESTS.

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.7 g of the sample and dissolve in 100 mL of water. Add approximately 5 mL of ammonium chloride buffer pH 10.7 and 0.1 mL of eriochrome black T TS and titrate with 0.05

M disodium edetate VS. Titrate until a blue color is formed, corresponding to the titration endpoint. Each mL of 0.05 *M* disodium edetate VS is equivalent to 22.784 mg of $C_{12}H_{22}O_{14}Zn$.

PACKAGING AND STORAGE

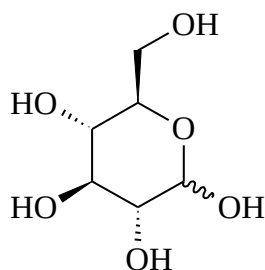
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Food supplement.

GLUCOSE*Glucosum* $C_6H_{12}O_6$; 180.16

glucose; 04485

D-Glucose

[50-99-7]

 $C_6H_{12}O_6 \cdot H_2O$; 198.17

glucose monohydrate; 04486

 α -D-Glucose monohydrate (1:1)

[14431-43-7]

It contains not less than 99.0% and not more than 101.5% of $C_6H_{12}O_6$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Colorless crystals or crystalline white powder, odorless, with sweet flavor.

Solubility. Freely soluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +52,5 to +53,5, in relation to the desiccated substance. Determine in 10% (w/v) solution in 0.012 M ammonium hydroxide.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of *n*-propyl alcohol, ethyl acetate and water (70:20:10) as mobile phase. Apply to the plate, separately, 2 μ L of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.1 g of the sample in water and complete the volume to 10 mL.

Solution (2): dissolve 0.1 g of glucose CRS in water and complete the volume to 10 mL.

Develop the chromatogram, allowing the solvent front to ascend 17 cm above the application line, remove the plate from the chamber and air dry. Spray with 0.2% sodium periodate solution (w/v). Air dry the plate for 15 minutes and spray with a 2% (w/v) 4,4-methylenebis-*N,N*-dimethylaniline solution in a mixture of 20 volumes of glacial acetic acid and 80 volumes of acetone. The main stain

obtained with *Solution (1)* chromatogram corresponds in position, color and intensity to the one obtained with *Solution (2)* chromatogram.

B. Dissolve 0.1 g of the sample in 10 mL of water. Add 3 mL of alkaline cupric tartrate RS and heat. A red precipitate is produced.

PURITY TESTS

Solution aspect. Dissolve 12.5 g of the sample in water and complete the volume to 25 mL. The solution obtained is not more intensely colored (**5.2.12**) than a solution prepared by mixing 1 mL of cobalt chloride RS, 3 mL of ferric chloride RS and 2 mL of cupric sulfate RS in water sufficient for 10 mL, diluting, then 1.5 mL of this solution with water to obtain 25 mL. Carry out the comparison on a white background in Nessler tubes.

Acidity. Dissolve 5 g of the sample in 50 mL of carbon dioxide-free water, add phenolphthalein TS and titrate with 0.02 M sodium hydroxide VS until pink color. Not more than 0.3 mL of the titrant is used for the neutralization.

Soluble starch and sulfites. Dissolve 1 g of the sample in 10 mL of water and add one drop of 0.1 M iodine VS. The solution turns yellowish and does not develop blue color.

Less soluble sugars and dextrins. Dissolve 1 g of the powdered sample in 30 mL of ethyl alcohol at 90% (v/v) and heat, under agitation, in a flask equipped with a reflux column. After cooling, the preparation remains clear.

Arsenic (5.3.2.5). Determine in 3 g of sample. Not more than 0.0001% (1 ppm).

Chlorides (5.3.2.1). Determine in 2 g of sample. Not more than 0.018% (180 ppm).

Heavy metals (5.3.2.3). Carry out the test in 4 g of sample. Not more than 0.0005% (5 ppm).

Sulfates (5.3.2.2). Determine in 2 g of sample. For the *Standard Preparation* use 1 mL of 0.005 M sulfuric acid. Not more than 0.025% (250 ppm).

Water (5.2.20.1). Determine in 0.5 g of the sample. Not more than 1.0% for anhydrous glucose and between 7.0% and 9.5% for monohydrate glucose.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 0.1 g of sample and dissolve in 50 mL of water, in an erlenmeyer flask with a ground cap. Add 25 mL of 0.05 M iodine VS and 10 mL of 5% sodium carbonate solution (w/v). Homogenize and allow to stand for 20 minutes, protected from light. Add 15 mL of dilute hydrochloric acid and titrate excess iodine with 0.1 M sodium thiosulfate VS, using starch TS as

indicator. Carry out the blank test and make the necessary corrections. Each mL of 0.05 M iodine VS is equivalent to 9.008 mg of $C_6H_{12}O_6$ and to 9.909 mg of $C_6H_{12}O_6 \cdot H_2O$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at room temperature.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Sweetener, energizer, excipient.

GLUCOSE INJECTION SOLUTION

It contains not less than 95.0% and not more than 105.0% the stated quantity of $C_6H_{12}O_6$. The glucose injection solution is a sterile, colorless solution of anhydrous glucose or glucose monohydrate in water for injections. It does not contain antimicrobial agents.

IDENTIFICATION

A. Heat a portion of the injection solution f with alkaline cupric tartrate RS. A red precipitate is produced.

B. The solution obtained in Assay is dextrorotatory (5.2.8).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

Contamination by particles (5.1.7). Use *Method I* of *Sub-Visible Particles (5.1.7.1)*. Complies with *Test A* or *Test B*, depending on the volume of the containers.

pH (5.2.19). 3.2 to 6.5. Determine in a solution containing the equivalent of 5% (w/v) of $C_6H_{12}O_6$. Dilute the sample with water for injections if necessary. Add 0.3 mL of saturated potassium chloride solution for each 100 mL of solution.

PURITY TESTS

5-Hydroxymethylfurfural and related substances. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Dilute volume of the injection solution containing the equivalent to 1 g of $C_6H_{12}O_6$ to 250 mL with water. The absorbance at 284 nm is not more than 0.25.

Heavy metals (5.3.2.3). Transfer a volume of the injection solution equivalent to 4 g of glucose to a suitable container and adjust the volume to 25 mL by evaporation or addition of water, as required. Not more than 0.0005% (5 ppm).

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 0.25 EU/ml of injection solution. Dilute in water for injections, if required, to a concentration of 5% (w/v) of $C_6H_{12}O_6$.

ASSAY

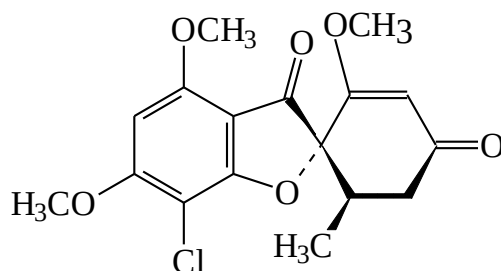
Proceed as described in *Determination of optical rotation and of specific optical rotation (5.2.8)*. Quantitatively transfer the volume of the injection solution containing between 2 g and 5 g of $C_6H_{12}O_6$ to a 100 mL volumetric flask, add 0.2 mL of 5 M ammonia and complete the volume with water. Homogenize and allow to stand for 30 minutes. Determine optical rotation in a 2 dm tube. The rotation angle obtained, multiplied by 0.9477, represents the mass, in grams, of $C_6H_{12}O_6$ present in the volume used.

PACKAGING AND STORAGE

In tightly closed, single-dose, plastic or glass containers, preferably type I or II.

LABELING

Comply with current legislation.

GRISEOFULVIN***Griseofulvinum***

$C_{17}H_{17}ClO_6$; 352.77

griseofulvin; 04536

Spiro[benzofuran-[2](3*H*), 1'-2cyclohexene]-3,4'-dione, 7-chloro-2,4,6-trimethoxy-6'-methyl-, (1'*S-trans*)-7-chloro-2',4,6-trimethoxy-6 β '-methylspiro(benzofuran-2(3*H*), 1'-[2]cyclohexene)-3,4'dione [126-07-8]

It presents a potency of not less than 900 μ g of $C_{17}H_{17}ClO_6$ per milligram.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Practically insoluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 217 °C to 224 °C.

Specific optical rotation (5.2.8): +348 to +364. Determine in solution at 1% (w/v) in dimethylformamide.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the griseofulvin CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Heavy metals (5.3.2.3). Proceed as described in *Thioacetamide reaction methods, Method III*. Not more than 0.0025% (25 ppm).

Loss on drying (5.2.9.1). Determine in 0.1 g of sample. Dry in oven at 105°C. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.2%.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). Quantitatively transfer about 0,08 mg of the sample to a 100 mL volumetric flask, dissolve, complete the volume with ethyl alcohol and homogenize. Dilute, with the same solvent to obtain a concentration of 0.008% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 290 nm, using ethyl alcohol for zero adjustment. Calculate the content of $C_{17}H_{17}ClO_6$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use a chromatograph equipped with an ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μ m); *Mobile phase* flow rate of 1.6 mL/minute.

Mobile phase: mixture of water and acetonitrile (60:40).

Sample solution: quantitatively transfer about 25 mg of the sample to a 25 mL volumetric flask, dissolve using 15 mL of methyl alcohol, complete the volume with the same solvent and homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer, from this solution, 2 mL to a 10 mL volumetric flask, complete the volume with *Mobile phase* and homogenize to obtain a 40 μ g/mL solution.

Standard solution: dissolve an accurately weighed quantity of griseofulvin CRS in *Mobile phase* to obtain a 40 mg/mL solution.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{17}H_{17}ClO_6$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

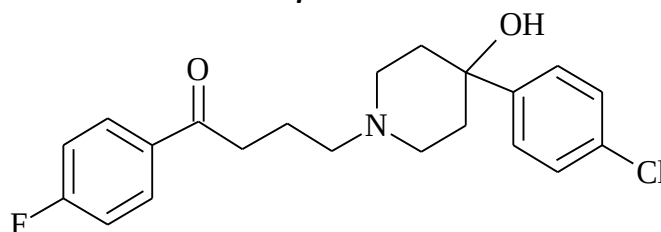
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antifungal.

HALOPERIDOL*Haloperidolum*C₂₁H₂₃ClFNO₂; 375.87

haloperidol; 04589

4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone
[52-86-8]

It contains not less than 98.0% and not more than 102.0% of C₂₁H₂₃ClFNO₂ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white powder, micro-crystalline or amorphous.

Solubility. Insoluble in water, freely soluble in methyl alcohol and slightly soluble in ethyl alcohol. Freely soluble in diluted acids.

Physicochemical constants.

Melting range (5.2.2): 148 °C to 151 °C. Determine in a sample dried in oven at 105 °C for one hour.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample desiccated at 105 °C and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the haloperidol CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Related substances*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Preparation aspect. Dissolve 0.2 g of the sample in 20 mL of 1% lactic acid (v/v). Place in ultrasonic bath, if required, until complete dissolution. The preparation is clear **(5.2.25)** and does not stain more than *SC F Color Standard Solution (5.2.12)* diluted to 2.5% (v/v) in water.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 230 nm; 100mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (3 µm); *Mobile phase* flow rate of 1.5 mL/minute.

Eluent A: 17 g/L aqueous tetrabutylammonium hydrogen sulfate solution.

Eluent (B): acetonitrile.

Mobile Phase gradient: adopt the gradient system described on the following table:

Time (minutes)	Eluent (A) (%)	Eluent (B) (%)	Elution
0 – 2	90	10	isocratic
2 – 17	90 → 50	10 → 50	linear gradient
17 - 22	50	50	isocratic

Note: Prepare the solutions immediately before use and protected from light.

Solution (1): dissolve 100 mg of the sample in methyl alcohol and dilute to 10 mL with the same solvent.

Solution (2): Dissolve 10 mg haloperidol for CRS system suitability (containing impurities B, 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(2-fluorophenyl)butan-1-one, and D, 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-[4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]phenyl]butan-1-one) in 1.0 mL of methyl alcohol.

Solution (3): dilute 1 mL of *Sample solution* to 100 mL with methyl alcohol. Dilute 1 mL of this solution to 10 mL with the same solvent.

Solution (4): dissolve 10 mg of haloperidol for identification of CRS peaks (containing impurities G and H, undetermined structures) in 1 mL of methyl alcohol.

Inject, separately, 10 µL replicates of *Solution (2)*. The resolution between the haloperidol and impurity B peaks is not less than 3.0. Use the chromatogram provided with haloperidol for CRS system suitability and the chromatogram obtained with *Solution (2)* to identify the peaks of impurities B and D; use the chromatogram provided with haloperidol to identify CRS peaks and the chromatogram obtained with *Solution (4)* to identify the peaks of impurities G and H. The relative retention times corresponding to haloperidol, whose retention time is about eight minutes, is about 0.9 for impurity B, about 1.6 for impurity D, about 1.8 for impurity G, and about 2.0 for impurity H.

Procedure: inject, separately, 10 µL of the *Solution (1)* and of *Solution (3)*, record the chromatograms and measure the areas under the peaks. Determine the percentage of each of the impurities found in the *Solution (1)* in relation to the haloperidol concentration in *Solution (3)*. To calculate impurity B, multiply the area under the peak by 0.7. Not more than 0.5% of impurity D, 0.3% of impurity B, 0.15% of impurity G and 0.15% of impurity H. Not more than 0.1% for other individual impurities. Not more than 1.0% of the total impurities found. Disregard peaks with area smaller than 0.5 times the area under the main peak obtained in *Solution (3)* chromatogram.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

When indicated on the label that the substance is sterile, the sample complies with the Sterility and with the Bacterial endotoxin tests. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 71.4 EU/mg of haloperidol.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Accurately weigh about 0.3 g of the sample and dissolve in 30 mL of glacial acetic acid. Add five drops of 1-naphtholbenzene TS and titrate with 0.1 M perchloric acid VS until color turns from yellow-orangish to green. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 37.587 mg of $C_{21}H_{23}ClFNO_2$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250mm long, 4.0 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 μ m); kept at 30 °C, mobile phase flow rate of 1 mL/minute.

Mobile phase: mixture of methyl alcohol, 0.05 M monobasic potassium phosphate, tetrahydrofuran and triethylamine (50:47:3:0.3). Adjust pH to 3.5 ± 0.1 with phosphoric acid RS.

Sample solution: dissolve an accurately weighed quantity of the sample in *Mobile phase* to obtain a 10 μ g/mL solution.

Standard solution: dissolve an accurately weighed quantity of haloperidol CRS in *Mobile phase* to obtain a 10 μ g/mL solution.

Inject 20 μ L replicates of the *Standard solution*. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{21}H_{23}ClFNO_2$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antipsychotic.

HALOPERIDOL TABLETS

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_{21}H_{23}ClFNO_2$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer a quantity of powder equivalent to 10 mg of haloperidol to a separating funnel, add 10 mL of water and 1 mL of *M* sodium hydroxide. Extract with 10 mL of water-saturated chloroform. Filter and evaporate to dryness. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the haloperidol CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 50 mL volumetric flask. Add 30 mL of hot methyl alcohol, and place in ultrasonic bath for 15 minutes. Shake mechanically for 15 minutes, complete the volume with methyl alcohol and filter. Dilute, if required, with methyl alcohol to obtain a 0.002% (w/v) solution. Prepare a haloperidol CRS solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 245 nm **(5.2.14)**, using methyl alcohol for zero adjustment. Calculate the quantity of $C_{21}H_{23}ClFNO_2$ in each tablet from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: simulated gastric fluid (without enzyme), 900 mL.

Apparatus: baskets, 100 rpm.

Time: 60 minutes.

Procedure: proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250mm long, 4.0 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 μ m); kept at 30 °C, *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.05 *M* monobasic potassium phosphate (60:40). Adjust the mixture pH to 4.0 ± 0.1 with phosphoric acid or diluted sodium hydroxide.

Sample solution: after testing, remove aliquot from the dissolution medium, filter and dilute, if required, with dissolution medium, to obtain a solution with concentration of about 1.11 µg/mL.

Sample solution: quantitatively transfer about 27,75 mg of haloperidol CRS to a 250 mL volumetric flask. Dissolve in 5 mL of methyl alcohol, complete the volume with dissolution medium and homogenize. Dilute this solution, with dissolution medium, to obtain a solution with an approximate concentration of 1.11 µg/mL.

Inject 100 µL replicates of the *Standard solution*. The tailing factor is not more than 2. The relative standard deviation of the replicate areas under the peaks recorded is not more than 3.0%.

Procedure: inject, separately, 100 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₂₁H₂₃ClFNO₂ dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 80% (Q) of the stated quantity of C₂₁H₂₃ClFNO₂ dissolve in 60 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of chloroform, glacial acetic acid and methyl alcohol (80:10:10) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Shake the quantity of powder equivalent to 10 mg of haloperidol with 10 mL of chloroform. Filter and evaporate the residue to dryness. Dissolve the residue with 1 mL of chloroform.

Solution (2): dilute 0.25 mL of *Solution (1)* to 25 mL with chloroform.

Solution (3): dilute 5 mL of *Solution (2)* to 10 mL with chloroform.

Develop chromatogram. Remove the plate, allow to air dry and spray with potassium iodobismutate RS. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (1.0%), and only one stain is more intense than that obtained with *Solution (3)* (0.5%).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250mm long and 4.0 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm); kept at 30 °C, *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.05 M monobasic potassium phosphate (60:40). Adjust the mixture pH to 4.0 ± 0.1 with phosphoric acid or diluted sodium hydroxide.

Sample solution: weigh and pulverize 20 tablets. Transfer a quantity of powder equivalent to 5 g of haloperidol to a 50 mL volumetric flask. Add 30 mL of *Mobile phase* and place in ultrasonic bath for 30 minutes. Shake mechanically for 30 minutes. Complete the volume with the *Mobile phase*, homogenize and filter. Transfer 5 mL to a 50 mL volumetric flask and complete the volume with *Mobile Phase*.

Standard solution: Weigh, accurately, about 25 mg of haloperidol CRS and transfer to a 250 mL volumetric flask. Dissolve in 5 mL of methyl alcohol, complete the volume with *Mobile phase* and homogenize. Dilute this solution, with *Mobile phase*, to obtain a solution with an approximate concentration of 10 µg/mL.

Inject 20 µL replicates of the *Standard solution*. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 3.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{21}H_{23}ClFNO_2$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

HALOPERIDOL INJECTION SOLUTION

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_{21}H_{23}ClFNO_2$. The injection solution may contain lactic acid and suitable preservatives.

IDENTIFICATION

In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the sample solution obtained in *Assay*, there are maximum points at 245 nm, identical to those observed in the spectrum of the standard solution.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.0 to 3.8.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 71.4 EU/mg of haloperidol.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Quantitatively transfer sample volume equivalent to 10 mg of haloperidol to a separating funnel and add 20 mL of 5% hydrochloric acid (v/v). Extract with four 25 mL portions of ethyl ether. Combine the ethyl phases and add, to the aqueous phase, three 5 mL portions of diluted hydrochloric acid (1 in 20). Transfer the aqueous phase to a 50 mL volumetric flask, complete the volume with 5% (v/v) hydrochloric acid and shake. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with methyl alcohol. Prepare a haloperidol CRS solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 245 nm, using 5 mL of 5% hydrochloric acid (v/v) in 50 mL of methyl alcohol for zero adjustment. Calculate the quantity of $C_{21}H_{23}ClFO_2$ in the injection solution from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

HALOPERIDOL ORAL SOLUTION

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_{21}H_{23}ClFNO_2$. The oral solution may contain lactic acid and suitable preservatives.

IDENTIFICATION

A. Transfer volume of oral solution equivalent to 10 mg of haloperidol to separating funnel. Add 1 mL of *M* sodium hydroxide, homogenize and extract with a 10 mL portion of chloroform. Discard the aqueous phase. Evaporate the extract to dryness. The residue meets the test **A.** in *Identification* of the *Haloperidol* monograph.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.1.19). 2.5 to 3.5.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Quantitatively transfer sample volume equivalent to 10 mg of haloperidol to a separating funnel and add 20 mL of diluted hydrochloric acid (1 in 20). Extract with four 25 mL portions of ethyl ether. Combine the ethyl phases and add, to the aqueous phase, three 5 mL portions of diluted hydrochloric acid (1 in 20). Transfer the aqueous phase to a 50 mL volumetric flask, complete the volume with diluted hydrochloric acid (1 in 20) and shake. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with methyl alcohol. Prepare a haloperidol CRS solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 245 nm, using 5 mL of diluted hydrochloric acid (1 in 20) in 50 mL of methyl alcohol for zero adjustment. Calculate the quantity of $C_{21}H_{23}ClFNO_2$ in the oral solution from the readings obtained.

B. Proceed as described in method **B.** of *Assay* in the *Haloperidol* monograph. Prepare *Sample solution* and *Resolution solution* as described below.

Sample solution: transfer a volume of the oral solution equivalent to 10 g of haloperidol to a 100 mL volumetric flask and complete the volume with *Mobile* phase. Transfer 5 mL to a 50 mL volumetric flask and complete the volume with *Mobile Phase*.

Resolution solution: prepare solution in *Mobile phase* containing approximately 10 µg of haloperidol CRS, 3 µg of methylparaben and 3 µg of propylparaben per milliliter.

Inject, separately, 20 µL replicates of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. The tailing factor for the haloperidol peak is not more than 2.0. The relative standard deviation of the replicate areas under the haloperidol peaks recorded is not more than 2.0%. The resolution between the haloperidol peaks and the methylparaben and propylparaben peaks is not less than 2.0.

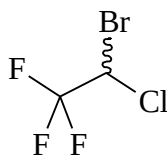
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₁H₂₃ClFNO₂ in the oral solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at room temperature.

LABELING

Comply with current legislation.

HALOTHANE***Halothanum***

C₂HBrClF₃; 197.38

halothane; 04596

2-Bromo-2-chloro-1,1,1-trifluoroethane

[151-67-7]

DESCRIPTION

Physical characteristics. Dense, colorless, mobile, non-flammable liquid, it produces a burning sensation.

Solubility. Slightly soluble in water, miscible in ethyl alcohol and fixed oils.

IDENTIFICATION

A. To 5 mL of the sample add 5 mL of sulfuric acid. The acid forms a layer on the sample (differentiation of chloroform and trichlorethylene).

B. To 0.3 mL of the sample contained in a 12 x 75 mm borosilicate glass test tube, add a clean sodium fragment of about 8 mm in diameter and allow to stand for a few minutes. Hold the tube in an upright position and heat gently with a microburner until the metal melts and the reaction starts. Then remove the heat source and cool the tube. Cautiously add 2 mL of water and allow the reaction to complete. Filter the solution and add 0.5 mL of glacial acetic acid to the filtrate. Add two drops of this solution to a mixture of 0.1 mL of freshly prepared alizarin TS and 0.1 mL of zirconyl nitrate RS. There is a change in color from red to yellow.

PURITY TESTS

Acidity or alkalinity. Shake 20 mL of the sample in 20 mL of carbon dioxide-free water for three minutes and allow the layers to separate. The aqueous layer requires not more than 0.1 mL of 0.01 M sodium hydroxide or not more than 0.6 mL of 0.01 M hydrochloric acid for neutralization, using bromocresol purple TS as indicator.

Chloride and bromide. Shake 25 mL of the sample in 25 mL of water for five minutes and allow the liquids to fully separate. Remove the aqueous layer and, to 10 mL of it, add one drop of nitric acid and five drops of silver nitrate RS. Opalescence should not be produced.

Thymol.

Buffer solution: use borate buffer pH 8.0.

Chlorimide solution: dissolve 100 mg of 2,6-dibromoquinone-4-chlorimide in 25 mL of absolute ethyl alcohol. The solution must be freshly prepared.

Thymol standard solution: prepare 0.1 mg/mL of thymol solution in 0.25 M sodium hydroxide.

Thymol Standard Curve: pipet 1 mL, 3 mL and 5 mL of *Thymol Standard Solution* respectively into three 100 mL volumetric flasks and add, if necessary, 0.25 M sodium hydroxide to complete the volume to 5 mL. Add 5 mL of 0.25 M sodium hydroxide in a fourth flask to prepare the blank. Add 10 mL of *Buffer Solution* to each flask, shake gently and add 1 mL of *Chlorimide Solution*. Allow to stand for exactly 15 minutes, add 3 mL of 0.25 M sodium hydroxide solution and complete the volume with water. With an appropriate spectrophotometer, measure the absorbances of the solutions containing thymol and that of the blank at 590 nm. Plot the readings graph and calculate the best adjustment curve.

Procedure: place 2 mL of the sample, accurately measured, in a 100 mL volumetric flask containing 5 mL of 0.25 M sodium hydroxide solution and shake gently. Evaporate the halothane under a nitrogen stream and add 10 mL of *Buffer Solution* and 1 mL of *Chlorimide Solution*. Shake gently and allow to stand for exactly 15 minutes, add 3 mL of 0.25 M sodium hydroxide solution and complete the volume with water. Read the absorbance of the resulting solution and calculate the percentage of thymol in the halothane weight used, using the *Thymol standard curve* as reference. It contains not less than 0.008% and not more than 0.012% of thymol in weight as stabilizer.

Residue by evaporation. Evaporate 50 mL of the sample, in a tared capsule, in water bath until dryness. Dry the residue in oven at 105 °C for two hours. The residue weight must not exceed 1 mg.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

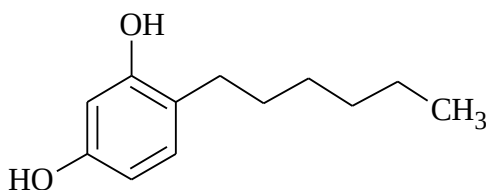
In tightly closed containers and protected from light.

LABELING

Comply with legislation in force.

THERAPEUTIC CLASS

General anesthetic (inhalation).

HEXYLRESORCINOL*Hexylresorcinolum* $C_{12}H_{18}O_2$; 194.27

hexylresorcinol; 04636

4-Hexyl-1,3-benzenediol

[136-77-6]

It contains not less than 98.0% and not more than 101.0% of $C_{12}H_{18}O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White and slightly yellowish acicular crystals or slabs or clumps of acicular masses or crystalline powder.

Solubility. Very slightly soluble in water, freely soluble in ethyl alcohol, glycerol, methyl alcohol and fixed oils.

Physicochemical constants.

Melting range (5.2.2): 62 °C to 67 °C.

IDENTIFICATION

A. To 10 mL of 1% (w/v) of sample solution in ethyl alcohol, add two drops of ferric chloride RS, green color is formed.

B. To 1 mL of saturated sample solution, add 1 mL of nitric acid RS. Light red color is formed.

C. To 1 mL of saturated sample solution, add 1 mL of 0.1 M bromine. A yellow flaky precipitate is produced which dissolves by the addition of 2 mL of ammonia RS and a yellow solution is formed.

PURITY TESTS

Acidity. Dissolve 0.25 g of the sample in 500 mL of distilled water. Not more than 1.0 mL of 0.02 M sodium hydroxide is used to neutralize, using phenol red TS as indicator.

Resorcin and other phenols. Shake approximately 1 g of the sample with 50 mL of water for a few minutes. Filter. Add three drops of ferric chloride RS to the filtrate. It should not develop red or blue color.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh approximately 70 mg to 100 mg of the sample, previously desiccated on silica gel for four hours, and dissolve in 10 mL of methyl alcohol in a 250 mL iodine flask. Add 30 mL of 0.1 M bromine VS, then quickly add 5 mL of hydrochloric acid, placing stopper immediately. Cool under running water at room temperature. Shake vigorously for five minutes and allow to stand for five minutes. Add 6 mL of potassium iodide RS around the stopper and carefully loosen the stopper. Then close hermetically and shake gently. Add 1 mL of chloroform and titrate the iodine released with 0.05 M sodium thiosulfate VS, adding 3 mL of starch TS when the endpoint approaches. Carry out blank test. Each mL of 0.1 M bromine VS is equivalent to 4.857 mg of $C_{12}H_{18}O_2$.

PACKAGING AND STORAGE

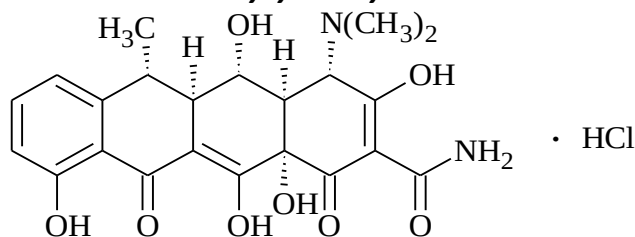
In hermetically closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anthelmintic (nematodes and trematoids).

DOXYCYCLINE HYCLATE*Doxycyclini hyclas*C₂₂H₂₄N₂O₈; 444.44

doxycycline; 03217

(4*S*, 4*aR*, 5*S*, 5*aR*, 6*R*, 12*aS*)-4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12, 12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
[564-25-0]

C₂₂H₂₄N₂O₈.HCl.½C₂H₆O.½H₂O; 512.94

doxycycline hyclate; 03222

(4*S*, 4*aR*, 5*S*, 5*aR*, 6*R*, 12*aS*)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-hydrochloride pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide ethanolated hydrate (2:2:1:1)
[24390-14-5]

It contains the equivalent of not less than 800 µg and not more than 920 µg of doxycycline (C₂₂H₂₄N₂O₈) per milligram.

DESCRIPTION

Physical characteristics. Yellow crystalline powder, hygroscopic.

Solubility. Freely soluble in water and in methyl alcohol, moderately soluble in ethyl alcohol. Soluble in alkali hydroxide solutions.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the doxycycline hyclate CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. To 2 mL of the sample add 5 mL of sulfuric acid. Yellow color is produced.

D. It meets chloride ion reactions (**5.3.1.1**).

PURITY TESTS

pH (5.2.19). 2.0 to 3.0. Determine in aqueous solution at 1% (w/v).

Light absorption. Dissolve 25 mg in a mixture of *M* hydrochloric acid and methyl alcohol (1:99) and dilute to 25 mL with the same solvent mixture. Dilute 1 mL of this solution to 100 mL with the mixture of *M* hydrochloric acid and methyl alcohol (1:99). Proceed with reading one hour after preparing the solution. The solution absorbance of the anhydrous, ethyl alcohol-free substance, measured at 349 nm, is between 0.300 and 0.335.

Optical rotation (5.2.8). -105° to -120°. Dissolve 0.25 g of the sample in a mixture of *M* hydrochloric acid and methyl alcohol (1:99) and dilute to 25 mL with the same solvent. Proceed with reading within five minutes after preparation.

Related substances. Proceed as described in *Assay*. Prepare solutions as described below.

Solution (1): use the *Sample Solution*, prepared as described in *Assay*.

Solution (2): dissolve accurately weighed quantity of methacycline hydrochloride CRS with *Diluent* and quantitatively dilute to obtain a solution with known concentration of 1.2 mg/mL.

Solution (3): use the *Standard Solution*, prepared as described in *Assay*.

Solution (4): transfer 2 mL of the *Solution (3)* and 2 mL of *Solution (2)* to a 100 mL volumetric flask, dilute with *Diluent* to complete the volume and homogenize. This solution contains approximately 0.024 mg of doxycycline hyclate CRS and methacycline hydrochloride CRS per mL.

Solution (5): use the *Resolution Solution*, prepared as described in *Assay*.

Inject 20 µL replicates of the *Solution (5)*. The relative retention times are about 0.4 for 4-epidoxycycline (major degradation product), 0.6 for methacycline, and 1.0 for doxycycline. The resolution between 4-doxycycline and epidoxycycline peaks is not less than 3.0. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of *Solution (4)* and of *Solution (1)*, record the chromatograms for a period of time corresponding to 1.7 times the doxycycline retention time and measure the areas under the peaks. Calculate the methacycline percentage according to the formula.

$$10\,000 (C_M / W)(r_u / r_M)$$

where

C_M = concentration, in mg/mL, of methacycline hydrochloride CRS in *Solution (4)*;

W = weight, in mg, of doxycycline hyclate in *Solution (1)*;

r_u = area under the metacycline peak in the *Solution (1)* chromatogram;

r_M = area under the metacycline peak in the *Solution (4)* chromatogram .

Not more than 2.0% of methacycline is found. Calculate the percentages of other related substances present in the sample according to the formula:

$$10\,000 (C_s / W)(r_i / r_s)$$

where

C_s = concentration, in mg/mL, of doxycycline hyclate CRS in *Solution (4)*;

W = weight, in mg, of doxycycline hyclate in *Solution (1)*;

r_i = area under the peak of each related substance in the chromatogram in *Solution (1)*;

r_s = area under the doxycycline peak in the *Solution (4)* chromatogram.

Not more than 0.5% of some impurity eluted before the methacycline is found; not more than 2.0% of 6-epidoxycycline is found and not more than 0.5% of some impurity eluted after the doxycycline main peak is found.

Light absorbing impurities. Dissolve 0,10 g of the sample in a mixture of M hydrochloric acid and methyl alcohol (1:99) and dilute to 10 mL with the same solvent mixture. Proceed with reading one hour after preparing the solution. The solution absorbance, measured at 490 nm, of the anhydrous, ethyl alcohol-free substance, is not more than 0.7.

Heavy metals (5.3.2.3). Proceed as described in *Thioacetamide reaction methods, Method IV*. Not more than 0.005% (50 ppm).

Water (5.2.20.1). 1.4 to 2.8.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.4%.

BIOLOGICAL SAFETY TESTS

When indicated on the label that the substance is sterile, the sample complies with the Sterility and with the Bacterial endotoxin tests. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test. Use the *Membrane Filtration Method*.

Bacterial endotoxins (5.5.2.2). Not more than 1.14 EU/mg of doxycycline.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 270 nm; 250 mm long and 4.6 mm internal diameter column, packed with styrene divinylbenzene spherical copolymer (5 μ m), kept at $(60 \pm 1)^\circ\text{C}$; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: dissolve 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.5 g of tetrabutylammonium sulfate, and 0.4 g of disodium edetate in 850 mL of water in a 1000 mL volumetric flask. Add 60 g of *tert*-butyl alcohol with the aid of water, complete the volume with water and adjust pH to 8.0 ± 0.1 with M sodium hydroxide. Filter and de-aerate the solution before use. The decrease in the proportion of *tert*-butyl alcohol results in an extension of the doxycycline retention time and improves the separation of doxycycline from its related substances.

Diluent: 0.01 M hydrochloric acid.

Sample solution: quantitatively transfer about 120 mg of the sample to a 100 mL volumetric flask. Dissolve and complete the volume with *Diluent*. Homogenize and filter.

Standard solution: quantitatively transfer about 12 mg of doxycycline hyclate CRS to a 10 mL volumetric flask. Add 6 mL of *Diluent*, shake for five minutes or until dissolved, complete the volume with *Diluent* and homogenize.

Resolution solution: prepare 6 mg/mL doxycycline hyclate solution CRS using *Diluent*. Transfer 5 mL to a 25 mL volumetric flask, heat on steam bath for 60 minutes and evaporate to dryness on a heating plate, taking care not to incinerate the residue. Dissolve the residue and complete the volume with *Diluent*. Homogenize and filter. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline and doxycycline. When stored in a refrigerator, this solution can be used for 14 days.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are about 0.4 for 4-epidoxycycline (major degradation product), 0.7 for 6-epidoxycycline and 1.0 for doxycycline. The resolution between doxycycline and 4-epidoxycycline peaks is not less than 3.0. The tailing factor for the doxycycline peak is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of *Standard Solution* and of *Sample solution*, record the chromatograms for a period of time corresponding to 1.7 times the doxycycline retention time and measure the areas under the peaks. Calculate the doxycycline content ($C_{22}H_{24}N_2O_8$) in the sample, in µg per milligram, from the content of the standard and the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

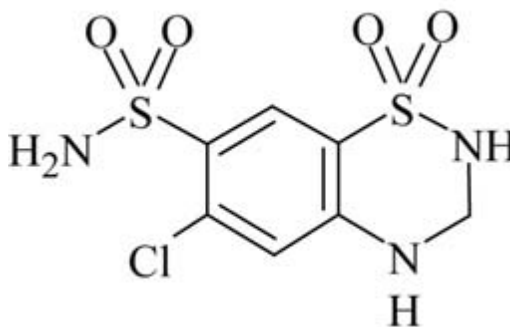
In tightly closed containers, protected from light.

LABELING

Comply with current legislation. When the substance is intended for the production of parenteral preparations, the label must indicate whether the product is sterile or whether it must be sterilized during the process.

THERAPEUTIC CLASS

Anti-bacterial; antiparasitic (plague, trachoma and malaria).

HYDROCHLOROTHIAZIDE*Hydrochlorothiazidum*C₇H₈ClN₃O₄S₂; 297.74

hydrochlorothiazide; 04652

6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide
[58-93-5]

It contains not less than 98.0% and not more than 102.0% of C₇H₈ClN₃O₄S₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Very slightly soluble in water, slightly soluble in ethyl alcohol. Freely soluble in sodium hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 266 °C to 270 °C, with decomposition.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the hydrochlorothiazide CRS spectrum, prepared identically.

B. Determine the absorbance (5.2.14) of the following solutions:

Solution (1): dissolve 50 g of sample in 10 mL of 0.1 M sodium hydroxide solution and complete the volume to 100 mL with water. Dilute 10 mL of this solution to 100 mL with 0.01 M sodium hydroxide solution. In the ultraviolet absorption spectrum (5.2.14), at the range of 200 nm to 400 nm, there is a maximum peak at 323 nm. The absorbance at 323 nm is between 0.450 and 0.475.

Solution (2): dilute 2 mL of the *Solution (1)* to 100 mL with 0.01 M sodium hydroxide. In the ultraviolet absorption spectrum (5.2.14), at the range of 200 nm to 400 nm, there is a maximum peak at 273 nm. The absorbance at 273 nm is between 0.0505 and 0.0530.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Acidity or alkalinity. Shake 0.5 g of the sample with 25 mL of water for two minutes and filter. To 10 mL of this solution, add 0.2 mL of 0.01 M sodium hydroxide and five drops of methyl red TS. The solution presents yellow color. Not more than 0.4 mL of 0.01 M hydrochloric acid is required to turn the indicator color to pink.

Chlorides (5.3.2.1). Dissolve 1 g of the sample in 25 mL of acetone and complete the volume to 30 mL with water. 15 mL of this solution must meet the *Limit test for chlorides*. Use as *Standard preparation* 10 mL of standard chloride solution (5 ppm Cl) added with 5 mL of acetone solution in water (85:15). Not more than 100 ppm.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105 °C for one hour. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250 mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 2.0 mL/minute.

Mobile phase: mixture of 0.1 M potassium phosphate solution and acetonitrile (9:1). Degas, adjust pH to 3.0 and filter.

Sample solution: quantitatively transfer approximately 30 mg of sample to a 200 mL volumetric flask, dissolve in a volume of acetonitrile not exceeding 10% of the flask capacity, complete the volume with *Mobile Phase* and homogenize.

Standard solution: dissolve an accurately weighed quantity of hydrochlorothiazide CRS in *Mobile phase* to obtain a 0.15 mg/mL solution. A volume of acetonitrile not greater than 10% of the total volume of the solution can be used to dissolve the CRS.

Resolution solution: dissolve an accurately weighed quantity of hydrochlorothiazide CRS and chlorothiazide in *Mobile phase* to obtain a 0.15 mg/mL solution of each.

Inject 20 µL replicates of the *Resolution solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.5%. The relative retention times are about 0.8 for chlorothiazide and 1 for hydrochlorothiazide and the resolution between hydrochlorothiazide and chlorothiazide is not less than 2.0.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_7H_8ClN_3O_4S_2$ from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, at temperature between 15 °C and 25 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Diuretic.

HYDROCHLOROTHIAZIDE TABLETS

It contains not less than 93.0% and not more than 107.0% the stated quantity of $C_7H_8ClN_3O_4S_2$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Shake the quantity of powder equivalent to 10 mg of hydrochlorothiazide, with 20 mL of acetone. Filter and evaporate the filtrate to dryness. The residue meets the test **A.** in *Identification* of the *Hydrochlorothiazide* monograph.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of ethyl acetate and isopropyl alcohol (85:15) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Shake the quantity of powder equivalent to 10 mg of hydrochlorothiazide, with 10 mL of acetone. Filter.

Solution (2): 1 mg/mL of hydrochlorothiazide CRS solution in acetone.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute, if necessary, with 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 272 nm (**5.2.14**) using the same solvent for zero adjustment. Calculate the quantity of $C_7H_8ClN_3O_4S_2$ dissolved in the medium, comparing the readings obtained with the hydrochlorothiazide CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: not less than 60% (Q) of the stated quantity of $C_7H_8ClN_3O_4S_2$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Shake the quantity of powder equivalent to 30 mg of hydrochlorothiazide, with 50 mL of 0.1 M sodium hydroxide for 20 minutes. Dilute to 100 mL with the same solvent. Homogenize and filter. Dilute with water until concentration of 0.0015% (w/v). Prepare standard solution in the same conditions, using the same solvents. Measure the absorbances at 273 nm, using water for zero adjustment. Calculate the quantity of $C_7H_8ClN_3O_4S_2$ in the tablets from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 520$, at 273 nm.

B. Proceed as described in *Assay* in the monograph *Hydrochlorothiazide*. Prepare the sample solution as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer a quantity of powder equivalent to 30 mg of hydrochlorothiazide to a 200 mL volumetric flask, add 20 mL of *Mobile Phase* and place in ultrasonic bath for five minutes. Add 20 mL of acetonitrile and place in ultrasonic bath for five minutes. Add 50 mL of *Mobile Phase* and shake mechanically for 10 minutes. Complete the volume with *Mobile phase*, homogenize and filter, discarding the first 10 mL of the filtrate.

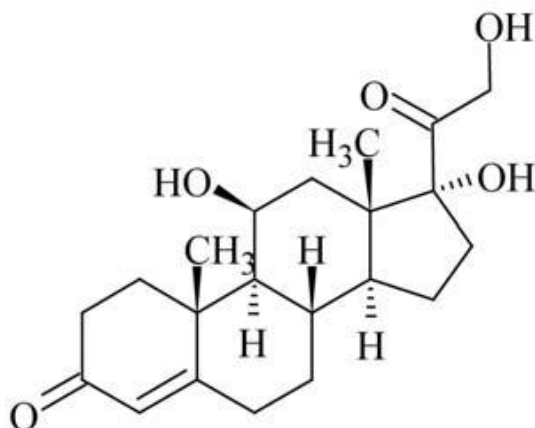
Procedure: inject, separately, 20 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_7H_8ClN_3O_4S_2$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

HYDROCORTISONE***Hydrocortisonum***

$C_{21}H_{30}O_5$; 362.47

hydrocortisone; 04664

(11 β)-11,17,21-Trihydroxypregn-4-ene-3,20-dione

[50-23-7]

It contains not less than 97.0% and not more than 102.0% of $C_{21}H_{30}O_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Physicochemical constants.

Melting range (5.2.2): 214 °C to 215 °C, with decomposition.

Optical rotation (5.2.8): +150° to +156°, in relation to the desiccated substance. Determine in solution at 1% (w/v) in dioxane.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the desiccated sample dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the hydrocortisone CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.0002% (w/v) solution in ethyl alcohol, there are maximum points identical to the ones observed in the spectrum of similarly prepared hydrocortisone CRS solution.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G₆₀ as support, and a mixture of chloroform and ethyl alcohol (85:15) as mobile phase. Separately apply to the plate 10 μ L of each solution, described below.

Solution (1): dissolve about 20 mg of the sample in 10 mL of mixture of chloroform-methyl alcohol (9:1).

Solution (2): pipet 1 mL of *Solution (1)* into a 50 mL volumetric flask and complete the volume with mixture of chloroform and methyl alcohol (9:1).

Develop chromatogram. Remove plate, allow to air dry, examine under ultraviolet light (254 nm). No secondary stain obtained with *Solution (1)* is more intense than the main stain obtained with *Solution (2)* (2.0%).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105 °C for three hours. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 100 mg of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Quantitatively transfer 20 mg of sample to a 100 mL volumetric flask, complete the volume with ethyl alcohol and shake. Transfer 5 mL of this solution to a 100 mL volumetric flask and complete the volume with ethyl alcohol. Prepare a hydrocortisone CRS solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 242 nm, using ethyl alcohol for zero adjustment. Calculate the quantity of $C_{21}H_{30}O_5$ from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 μ m); *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water, acetonitrile and methyl alcohol (50:25:25).

Diluent: mixture of methyl alcohol and water (1;1).

Internal standard solution: prepare 1 mg/mL propylparaben solution in methyl alcohol.

Sample solution: weigh, accurately, about 50 mg of the sample and transfer to a 50 mL volumetric flask. Dissolve and dilute with methyl alcohol until complete the volume and homogenize. Transfer 2.0 mL of this solution and 2.0 mL of the *Internal Standard Solution* to a 50 mL volumetric flask. Complete the volume with *Diluent* and homogenize.

Standard solution: weigh, accurately, about 25 mg of hydrocortisone CRS and transfer to a 25 mL volumetric flask. Dissolve in methyl alcohol, complete the volume and homogenize to obtain a solution with an approximate concentration of 1 mg/mL. transfer 2.0 mL of this solution and 2.0 mL

of *Internal standard solution* to a 50 mL volumetric flask. Complete the volume with *Diluent* and homogenize.

Inject 10 µL replicates of the *Standard solution*. The column efficiency must be not less than 3000 theoretical plates for hydrocortisone. The relative retention times are approximately 1.0 for hydrocortisone and 1.8 for propylparaben. The tailing factor is not more than 1.2. The resolution between propylparaben and hydrocortisone is not less than 9.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks corresponding to hydrocortisone and to propylparaben. Calculate the content of C₂₁H₃₀O₅ from the responses obtained for the hydrocortisone/ propylparaben ratio with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

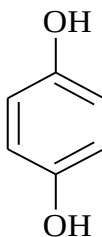
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-inflammatory.

HYDROQUINONE*Hydrochinonum*

C₆H₆O₂; 110.11
hydroquinone; 09457
1,4-Benzenediol
[123-31-9]

It contains not less than 99.0% and not more than 100.5% of C₆H₆O₂, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline crystals that turn dark when exposed to air.

Solubility. Soluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 170 °C to 171 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the hydroquinone CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range from 200 nm to 400 nm, of sample solution at 0,0025% (w/v) prepared in methyl alcohol, there are maximum absorption points only on the same wavelengths as the ones observed in the spectrum of similar hydroquinone CRS solution.

PURITY TESTS

Water (5.2.20.1). Determine in 3 g of the sample. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.25 g of the sample and dissolve in a mixture of 100 mL of water and 0,05 mL *M* sulfuric acid (10:1) and add 3 mL of diphenylamine RS. Titrate with 0.05 *M* ceric sulfate VS until red color develops. Each mL of 0.05 *M* ceric ammonium sulfate VS is equivalent to 5.506 mg of $C_6H_6O_2$.

PACKAGING AND STORAGE

In containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Depigmenting.

HYDROXOCOBALAMIN INJECTION SOLUTION

Hydroxocobalamin injection solution contains not less than 95% and not more than 115% of the declared quantity of $C_{62}H_{89}CoN_{13}O_{15}P$.

IDENTIFICATION

Dilute 3 mL of the solution for injection to 100 mL using pH 4.0 buffer described below: dissolve 2.61 g of sodium acetate and 20.5 g of sodium chloride in 5.25 mL of glacial acetic acid and water sufficient to complete 1500 mL of solution. If necessary, adjust pH. In the ultraviolet and visible absorption spectrum **(5.2.14)** there are maximum points at (352 ± 1) nm and at (528 ± 2) nm. The ratio between the absorbance values measured at 352 nm and 528 nm ranges between 2.7 and 3.3.

CHARACTERISTICS

pH (5.2.19). 3.5 to 5.0.

Determination of volume (5.1.2). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Use the *Membrane Filtration Method*.

Bacterial endotoxins (5.5.2.2). Not more than 0.4 EU/ μ g of hydroxocobalamin.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*.

Borate buffer pH 9.3: dissolve 28.3 g of sodium tetraborate and 402 mg of boric acid in water sufficient to complete 1500 mL of solution and homogenize. If necessary, adjust pH.

Sample solution: transfer an accurately measured volume of the injection solution, equivalent to approximately 5 mg of hydroxocobalamin, to a 50 mL volumetric flask containing 25 mL of borate buffer pH 9.3. Add 5 mL of potassium cyanide solution (1:10 000). Allow to stand for 30 minutes at room temperature and dilute with *Borate buffer pH 9.3* up to the indicated volume and homogenize. Transfer 15 mL of this solution to a second 50 mL volumetric flask, dilute with *Borate buffer pH 9.3* up to the indicated volume and homogenize.

Standard solution: dissolve, in *Borate buffer pH 9.3*, a sufficient quantity of cyanocobalamin CRS, accurately weighed, and quantitatively dilute, step by step if necessary, to obtain a solution at 30 μ g/mL.

Procedure: determine the absorbances of the solutions in 1 cm cuvettes, at a maximum absorption wavelength of about 361 nm, in an appropriate spectrophotometer, using *Borate buffer pH 9.3* as a blank. Calculate the quantity, in mg of $C_{62}H_{89}CoN_{13}O_{15}P$, in each mL of the injection solution, according to the formula:

$$\frac{1346,38}{1355,39} \times \frac{0,1667 \times C}{V} \times \frac{A_u}{A_s}$$

where

1346.38 and 1355.39 = are the molar masses of hydroxocobalamin and cyanocobalamin, respectively;

C = concentration in $\mu\text{g/mL}$ of the cyanocobalamin CRS solution in the preparation of the *Standard Solution*;

V = volume in mL of the injection solution used;

A_u and A_s = absorbances of the *Sample solution* and the *Standard solution*, respectively.

PACKAGING AND STORAGE

In type I glass for single or multiple dose containers, protected from light.

LABELING

Comply with current legislation.

ALUMINUM HYDROXIDE***Aluminii hydroxidum***

$\text{Al}(\text{OH})_3$; 78.00

Al_2O_3 ; 101.96

aluminum hydroxide; 04694

Aluminum hydroxide

[21645-51-2]

It contains the equivalent to not less than 47.0% and not more than 60.0% of Al_2O_3 .

DESCRIPTION

Physical characteristics. White or off-white powder, amorphous.

Solubility. Practically insoluble in water. Soluble in acid and alkaline hydroxide solutions.

IDENTIFICATION

A. Mix 15 mg of the sample in 2 mL of water and 0.5 mL of 2 M hydrochloric acid. Filter. Add 0.5 mL of thioacetamide RS. Precipitate is not formed. Add 2 M sodium hydroxide, dropwise. A white gelatinous precipitate is formed which dissolves in excess 2 M sodium hydroxide. Gradually add ammonium chloride RS. A white gelatinous precipitate is produced.

B. The preparation obtained in *Preparation aspect* meets the aluminum ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 2.5 g of the sample in 15 mL of hydrochloric acid RS, heat in water bath and complete the volume to 100 mL with water. The preparation obtained is not more opalescent than the *Reference suspension II* **(5.2.25)** and is not more intensely stained than the *Color reference solution* **(5.2.12)**, described below.

Color reference solution: prepare a mixture of *Ferric chloride base solution*, *Cobalt chloride base solution* and *Cupric sulfate base solution* ((96:2:2) **(5.2.12)**). Transfer 1.5 mL of this solution to a 100 mL volumetric flask and complete the volume with 1% (w/v) hydrochloric acid.

Alkalinity. Shake approximately 1 g of the sample in 20 mL of carbon dioxide-free water for one minute. Filter. Add 0.1 mL of phenolphthalein TS to 10 mL of the filtrate. If pink color develops, not more than 0.3 mL of 0.1 M hydrochloric acid is required to neutralize 10 mL of the filtrate.

Neutralizing capacity. Shake 0.5 g of the sample in 100 mL of water kept at 37 °C throughout the test period. Add 100 mL of 0.1 M hydrochloric acid at 37 °C and shake continuously. The pH of the solution after 10 minutes, 15 minutes and 20 minutes is not less than 1.8, 2.3 and 3.0, respectively. In no time is it greater than 4.5. Add 10 mL of 0.5 M hydrochloric acid at 37 °C and shake continuously for one hour. Add 0.1 M sodium hydroxide at 37 °C until pH 3.5. Not more than 35 mL of 0.1 M sodium hydroxide is required.

Arsenic (5.3.2.5). Use *Visual Method*. Use 30 mL of the preparation obtained in *Preparation aspect*. Not more than 0.0004% (4 ppm).

Chlorides (5.3.2.1). Proceed as described in *Limit Test for Chlorides*, except that the *Standard Preparation* and *Sample Preparation* must not be diluted to 50 mL. Not more than 1.0% (10 000 ppm).

Sample preparation: dissolve 0.106 g of the sample, under heating, in 10 mL of 2 M nitric acid and complete the volume to 100 mL with water. Transfer 10 mL of the obtained solution to a suitable tube and dilute to 25 mL with water

Standard preparation: Transfer 0.3 mL of 0.01 M hydrochloric acid to a suitable tube and dilute to 25 mL with water

Heavy metals (5.3.2.3). Dissolve 0.33 g of the sample in 10 mL of 3 M hydrochloric acid with the aid of heating, filter if necessary and dilute to 25 mL with water. Not more than 0.006% (60 ppm).

Sulfates (5.3.2.2). Dilute 4 mL of the preparation obtained in *Preparation aspect* to 100 mL with water. Use 15 mL of this preparation. For the *Standard Preparation* use 0,3 mL of 0.005 M sulfuric acid. Not more than 1.0% (10 000 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Complexometric titrations (5.3.3.4)* for *Aluminum*. Weigh, accurately, about 0.8 g of the sample and dissolve in 10 mL of hydrochloric acid RS, heating in water bath. Allow to cool and dilute to 50 mL with water. To 10 mL of this solution, add ammonia RS until a precipitate starts to form. Add sufficient volume of hydrochloric acid RS required to dissolve the precipitate and dilute to 20 mL with water. Each mL of 0.1 M disodium edetate VS is equivalent to 5.098 mg of Al_2O_3 .

PACKAGING AND STORAGE

In tightly closed containers, at temperature below 30 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antacid.

ALUMINUM HYDROXIDE CHEWABLE TABLETS

It contains not less than 90.0% and not more than 110.0% the stated quantity of $\text{Al}(\text{OH})_3$. Chewable tablets may contain sweetening agents.

IDENTIFICATION

A. Weigh and pulverize the tablets. Use a quantity of powder equivalent to about 15 mg of aluminum hydroxide, add 2 mL of water and 0.5 mL of 2 M hydrochloric acid. Filter. Add 0.5 mL of thioacetamide RS. Precipitate is not formed. Add 2 M sodium hydroxide, dropwise. A white gelatinous precipitate is formed which dissolves in excess of 2 M sodium hydroxide. Gradually add ammonium chloride RS. A white gelatinous precipitate is produced.

B. Weigh and pulverize the tablets. Dissolve the quantity of powder equivalent to 15 mg of aluminum hydroxide in 2 mL of water. It meets aluminum ion reactions (5.3.1.1).

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

ACIDITY NEUTRALIZATION CAPACITY

Transfer sample amount equivalent to one tablet into a 250 mL beaker. Add 70 mL of water and mix with a magnetic stirrer for approximately one minute. Transfer 25 mL of M hydrochloric acid VS and shake for 15 minutes. Titrate excess acid immediately and, for an additional period not exceeding five minutes, with 0.5 M sodium hydroxide VS to obtain a stable pH of 3.5 (for 10 to 15 seconds). Carry out the test at a temperature of $(37 \pm 3)^\circ\text{C}$. Not less than 5 mEq of acid is consumed and not less than 55.0% of the expected mEq value from the stated quantity of aluminum hydroxide is obtained. Each mg of $\text{Al}(\text{OH})_3$ has a neutralizing capacity of 0.0385 mEq.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh and pulverize 20 tablets. Weigh the quantity of powder equivalent to 1.2 g of $\text{Al}(\text{OH})_3$, add 15 mL of concentrated hydrochloric acid and heat until dissolved. Dilute with water to approximately 100 mL, shake, quantitatively filter into a 500 mL volumetric flask, washing the filter with water. Complete the volume with water and shake. Transfer 20 mL of this solution to a 250 mL erlenmeyer flask, add 25 mL of 0.05 M disodium edetate VS and 20 mL of acetic acid-ammonium acetate buffer and heat to boiling for five minutes. Cool, add 50 mL of ethyl alcohol and 2 mL of

0.025% (w/v) dithizone. Titrate the solution with 0.05 M zinc sulfate VS until pink color. Perform a blank test, using 20 mL of water in place of 20 mL of sample solution. Each mL of 0.05 M disodium edetate VS is equivalent to 3.900 mg of $\text{Al}(\text{OH})_3$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

ALUMINUM HYDROXIDE GEL

Aluminum hydroxide gel is an amorphous aluminum hydroxide suspension in which there is partial substitution of carbonate for hydroxide. It contains not less than 90.0% and not more than 110.0% the stated quantity of $\text{Al}(\text{OH})_3$.

IDENTIFICATION

A. To 1 g of the sample, add 4 mL of concentrated hydrochloric acid. Heat at 60 °C for one hour, cool, dilute to 50 mL with water and filter if necessary. To 10 mL of the obtained solution, add 0.5 mL of 2 M hydrochloric acid and 0.5 of thioacetamide RS. Precipitate is not formed. Slowly add 5 mL of 2 M sodium hydroxide and allow to stand for one hour. A white gelatinous precipitate is formed which dissolves by the addition of 5 mL of 2 M sodium hydroxide. Add, slowly, 5 mL of ammonium chloride RS and allow to stand for 30 minutes. A white gelatinous precipitate is produced again.

B. To 1 g of the sample, add 4 mL of concentrated hydrochloric acid. Heat at 60 °C for one hour, cool, dilute to 50 mL with water and filter if necessary. The solution obtained meets aluminum ion reactions **(5.3.1.1)**.

CHARACTERISTICS

pH (5.2.19). 5.5 to 8.0.

PURITY TESTS

Chloride limit. Transfer a sample amount, accurately weighed, equivalent to 0.6 g of $\text{Al}(\text{OH})_3$, for porcelain capsule. Add 0.1 mL of potassium chromate RS and 25 mL of water. Shake. Drop 0.1 M silver nitrate until pink color persists. Not more than 8 mL of 0.1 M silver nitrate is used (4.7%).

Arsenic (5.3.2.5). Dissolve a sample amount, accurately weighed, equivalent to 0.3 g of $\text{Al}(\text{OH})_3$, in 20 mL of 10 M sulfuric acid. Proceed as described in *Spectrophotometric Method, Method I*. Not more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Dissolve 2.3 g of the sample in 20 mL of M hydrochloric acid and 10 mL of water. Add 0.5 mL of nitric acid and allow to boil for 30 seconds. Cool, add 2 g of ammonium chloride and 2 g of ammonium thiocyanate and extract with two 10 mL portions of a mixture of isoamyl alcohol and ethyl ether (1:1). Add, to the aqueous phase, 2 g of citric acid and dilute to 40 mL with water. Use 18 mL of the solution obtained and proceed as described in *Method I*. Not more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Dissolve a sample amount, accurately weighed, equivalent to 0.15 g of $\text{Al}(\text{OH})_3$, in 5 mL of 3 M hydrochloric acid, heating if necessary. Filter and proceed as described in *Limit test for sulfates*. Not more than 0.8% (8000 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Transfer a sample amount, accurately weighed, equivalent to 1.5 g of $\text{Al}(\text{OH})_3$, to a beaker and add 15 mL of concentrated hydrochloric acid, heating for complete dissolution. Cool, transfer to a 500 mL volumetric flask and complete the volume with water. Transfer 20 mL of the solution to a 250 mL erlenmeyer flask and add, under constant agitation, 25 mL of 0.05 M disodium edetate VS and 20 mL of acetic acid-ammonium acetate buffer. Heat for five minutes. Cool, add 50 mL of ethyl alcohol and 2 mL of 0.025% (w/v) dithizone in ethyl alcohol. Titrate with 0.05 M zinc sulfate VS until color turns from greenish-violet to pink. Carry out blank test, using 20 mL of water, and make the necessary corrections. Each mL of 0.05 M disodium edetate VS is equivalent to 3.900 mg of $\text{Al}(\text{OH})_3$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

CALCIUM HYDROXIDE*Calcii hydroxidum*Ca(OH)₂; 74.09

calcium hydroxide; 04696

Calcium hydroxide

[1305-62-0]

It contains not less than 95.0% and not more than 100.5% of Ca(OH)₂.

DESCRIPTION

Physical characteristics. White or off-white fine powder.

Solubility. Practically insoluble in water, soluble in glycerol. Soluble in acids, with heat release.

IDENTIFICATION

A. Transfer 0.8 g of the sample to a glass mortar, add 10 mL of water, 0.5 mL of phenolphthalein TS and homogenize. Red color develops. Add 17.5 mL of *M* hydrochloric acid. The suspension becomes colorless, without effervescence. Pulverize the mixture for one minute. Red color develops again. Add 6 mL of *M* hydrochloric acid and pulverize. The solution turns colorless.

B. Dissolve 0.1 g of the sample in hydrochloric acid RS and dilute to 10 mL with water. The solution meets calcium ion reactions **(5.3.1.1)**.

PURITY TESTS

Alkalinity and alkaline earth metals. Dissolve 1 g of the sample in a mixture with 10 mL of hydrochloric acid and 40 mL of water. Heat to boiling and add 50 mL of oxalic acid RS. Neutralize with ammonia and complete the volume to 200 mL with water. Allow to stand for an hour and filter with a suitable filter. To 100 mL of the filtrate, carefully add 0.5 mL of sulfuric acid. Evaporate to dryness and incinerate. Not more than 20 mg of residues (4.0% of sulfates).

Carbonate limit. Add 5 mL of *M* hydrochloric acid VS to the solution obtained in Assay. Titrate with *M* sodium hydroxide VS using 0.5 mL of methyl orange TS as indicator. Each mL of *M* hydrochloric acid VS is equivalent to 50.05 mg of calcium carbonate. Not more than 5.0% of CaCO₃.

Limit for acid-insoluble substances. Dissolve 2 g of the sample in 30 mL of hydrochloric acid. Heat to boiling and filter. Wash residue with hot water and incinerate. The residue weight is not greater than 10 mg. Not more than 0.5%.

Arsenic (5.3.2.5). Dissolve 0,75 g of the sample in 15 mL of hydrochloric acid. Proceed as described in *Spectrophotometric Method, Method I*. The addition of 20 mL of 3.5 *M* sulfuric acid specified in the procedure can be omitted. Not more than 0.0004% (4 ppm).

Chlorides (5.3.2.1). Dissolve 1.07 g of the sample in 7 mL of nitric acid. Dilute to 40 mL with water and proceed as described in *Limit test for chlorides*. Not more than 0.033% (330 ppm).

Sulfates (5.3.2.2). Dissolve 0.3 g of the sample in 10 mL of hydrochloric acid RS and proceed as described in *Limit test for sulfates*. Not more than 0.4% (4 000 ppm).

Heavy metals (5.3.2.3). Dissolve 1 g of the sample in 10 mL of 3 M hydrochloric acid and evaporate in water bath to dryness. Dissolve the residue in 20 mL of water. Filter. Proceed as described in *Method I*. Not more than 0.002% (20 ppm).

Loss on ignition (5.2.9.2). Determine in 1 g of the sample. Incinerate in muffle furnace at 900 ± 25 °C until constant weight. Not more than 34.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 1.5 g of the sample and transfer to a glass mortar. Add 20 mL to 30 mL of water and 0.5 mL of phenolphthalein TS. Titrate with M hydrochloric acid VS, pulverizing the substance until red color disappears. Each mL of M hydrochloric acid VS is equivalent to 37.045 mg of $\text{Ca}(\text{OH})_2$.

PACKAGING AND STORAGE

In tightly closed, non-metallic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Astringent.

MAGNESIUM HYDROXIDE

Magnesii hydroxidum

Mg(OH)₂; 58.32
magnesium hydroxide; 04697
Magnesium hydroxide
[1309-42-8]

It contains not less than 95.0% and not more than 100.5% of Mg(OH)₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white fine powder, amorphous.

Solubility. Practically insoluble in water. Soluble in diluted acid solutions.

IDENTIFICATION

A. Prepare sample aqueous suspension and add phenolphthalein TS. Pink color develops.

B. Dissolve about 15 mg of the sample in 2 mL of nitric acid RS and neutralize with an 8.5% (w/v) sodium hydroxide solution. The solution meets magnesium ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 5 g of the sample in a mixture with 50 mL of acetic acid RS and 50 mL of water. A slight effervescence develops. Boil for two minutes and dilute to 100 mL with 2 M acetic acid. Filter in a porcelain or silica crucible filter, previously calcined and tared, with adequate porosity to obtain a clear filtrate. The preparation obtained is not more intensely stained than the *Color reference solution* **(5.2.12)** described below.

Color Reference Solution: prepare a mixture of three parts of *Cobalt Chloride Base Solution*, 2.4 parts of *Cupric Sulfate Base Solution*, three parts of *Ferric Chloride Base Solution* and 1.6 parts of 1% hydrochloric acid (w/v). At the time of use, dilute 3.75 volumes of this solution with 6.25 volumes of 1% hydrochloric acid (w/v).

Limit for acetic acid-insoluble substances. The residue weight obtained in *Preparation aspect*, after washing with 2 M acetic acid, desiccation and incineration at 600°C, is not more than 5 mg. Not more than 0.1%.

Soluble substances limit. Mix 2 g of the sample in 100 mL of water and boil for five minutes. Filter while still hot in a sintered glass funnel, cool and dilute to 100 mL with water. Evaporate 50 mL of the obtained solution to dryness and dry between 100 °C and 105 °C. The residue weight is not more than 20 mg. Not more than 2.0%.

Arsenic (5.3.2.5). Determine in 5 mL of the preparation obtained in *Preparation aspect*. Proceed as described in *Visual method*. Not more than 0.0004% (4 ppm).

Calcium (5.3.2.7). Dilute 1.3 mL of the preparation obtained in *Preparation aspect* to 150 mL with water. Determine in 15 mL of this solution. Not more than 1.5% (15 000 ppm).

Chlorides (5.3.2.1). Determine in 7 mL of the preparation obtained in *Preparation aspect*. For the *Standard Preparation*, use 1 mL of 0.01 M hydrochloric acid. Not more than 0.1% (1000 ppm).

Iron (5.3.2.4). Dissolve 0.143 g of the sample in 5 mL of 2 M hydrochloric acid and dilute to 10 mL with distilled water. Use 1 mL of the solution obtained and proceed as described in *Method I*. Use 10 mL of *Standard iron solution (1 ppm Fe)*. Not more than 0.07% (700 ppm).

Heavy metals (5.3.2.3). Dissolve 1 g of the sample in 15 mL of 3 M hydrochloric acid and evaporate in water bath to dryness. Towards the end of evaporation, shake the residue frequently to disintegrate it, so as to obtain a fine dry powder. Dissolve the residue in 20 mL of water and filter. Add 2 mL of M acetic acid to the filtrate and dilute with water to 25 mL. Not more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Determine in 2 mL of the preparation obtained in *Preparation aspect*. For the *Standard Preparation* use 1 mL of 0.005 M sulfuric acid. Not more than 0.5% (5000 ppm).

Loss on drying (5.2.9.1). Dry in oven at 105 °C for two hours. Not more than 2.0%.

Loss on ignition (5.2.9.2). Determine in 0.5 g of the sample. Gradually heat up to 900 °C ± 25 °C and calcine until constant weight. Not more than 41.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.1 g of the sample in 2 mL of 2 M hydrochloric acid and proceed as described in *Complexometric titrations (5.3.3.4)* for magnesium. Each mL of 0.1 M disodium edetate VS is equivalent to 5.832 mg of Mg(OH)₂.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antacid.

POTASSIUM HYDROXIDE

Kalii hydroxidum

KOH; 56.11

potassium hydroxide; 04698

Potassium hydroxide

[1310-58-3]

It contains not less than 85.0% and not more than 100.5% of KOH.

DESCRIPTION

Physicochemical characteristics. White or slightly yellowish, crystalline particles, supplied as spheres, zests, sticks or irregular pieces. Hygroscopic and deliquescent, quickly absorbs atmospheric carbon dioxide. Melting point (5.2.2): around 360 °C.

Solubility. Very soluble in water, freely soluble in ethyl alcohol.

IDENTIFICATION

A. Dissolve 0.1 g of the sample in 10 mL of water. Dilute 1 mL to 100 mL with the same solvent. The pH (5.2.19) of the resulting solution is not less than 10.5.

B. The 1% (w/v) aqueous solution meets potassium ion reactions (5.3.1.1).

PURITY TESTS

Preparation aspect. Dissolve 5 g of the sample in carbon dioxide-free water and dilute to 50 mL with the same solvent. The preparation obtained is clear (5.2.25) and colorless (5.2.12).

Carbonate limit. Proceed as described in Assay. Each mL of *M* hydrochloric acid required for the bromophenol blue TS solution changeover is equivalent to 69.103 mg of K₂CO₃. Not more than 2.0%.

Sodium limit. Proceed as described in Atomic spectrometry (5.2.13.1.1). Dissolve 1 g of the sample in 50 mL of water, add 5 mL of sulfuric acid and complete the volume to 100 mL with water. Dilute 1 mL of this solution to 10 mL with water. Prepare the reference solution by dissolving in water 0.5084 g of sodium chloride, previously dried at 105 °C for three hours, and dilute to 1000 mL with the same solvent (0.2 mg/mL of sodium). Dilute with water to prepare standard solutions as required. Read at 589 nm, using a hollow sodium cathode lamp and an air-acetylene flame as radiation source. Not more than 1.0% (10 000 ppm).

Aluminum (5.3.2.10). Required for potassium hydroxide intended for the preparation of hemodialysis solutions. Dissolve 20 g of the sample in 100 mL of water and add 10 mL of acetate buffer pH 6.0. Proceed as described in Limit test for aluminum. Use, as reference solution, a mixture of 2 mL of Standard aluminum solution (2 ppm Al), 10 mL of acetate buffer pH 6.0 and 98 mL of water. For blank use a mixture of 10 mL of acetate buffer solution pH 6.0 and 100 mL of water. Not more than 0.00002% (0.2 ppm).

Chlorides (5.3.2.1). Weigh, accurately, about 8.75 g of the sample and transfer to a 25 mL volumetric flask. Dissolve in 10 mL of water. Slowly add 2 mL of nitric acid, cool and complete the volume with

nitric acid RS. Use 20 mL of this solution and proceed as described in *Limit test for chlorides*. Not more than 0.005% (50 ppm).

Iron (5.3.2.4). Use *Method III*. Dissolve 10 g of the sample in 15 mL of water. Carefully add 12 mL of hydrochloric acid, cool, dilute with 7.3% (w/v) hydrochloric acid and complete to 50 mL with the same solvent. Use 5 mL of this solution. Not more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dilute 10 mL of the solution obtained in the test for *Iron* to 20 mL with water. Use, as reference, *Standard lead solution (1 ppm Pb)*. Not more than 0.001% (10 ppm).

Sulfates (5.3.2.2). Weigh, accurately, about 15 g of the sample and transfer to a 50 mL volumetric flask. Dissolve in 15 mL of water. Slowly add 12 mL of hydrochloric acid, cool and dilute to 50 mL with diluted hydrochloric acid. Use 30 mL of the solution obtained and 1 mL of 0.005 M sulfuric acid standard solution. Proceed as described in *Limit test for sulfates*. Not more than 0.005% (50 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 2 g of the sample and dissolve in 25 mL of carbon dioxide-free water. Add 25 mL of freshly prepared 0.025 M barium chloride and 0.3 mL of phenolphthalein TS. Slowly, under agitation, add 25 mL of M hydrochloric acid VS. Continue titration with M hydrochloric acid VS until the indicator changeover, from pink to colorless. Add 0.3 mL of bromophenol blue TS solution and continue titration with M hydrochloric acid VS until the indicator changeover, from bluish-violet to yellow. Each mL of M hydrochloric acid VS used in the second part of the titration is equivalent to 69.103 mg of K₂CO₃. Each mL of M hydrochloric acid VS used in the combination of titrations is equivalent to 56.110 mg of total alkalinity, calculated as KOH.

PACKAGING AND STORAGE

In tightly closed, non-metallic containers.

LABELING

Comply with current legislation.

SODIUM HYDROXIDE*Natrii hydroxidum*

NaOH; 40.00
sodium hydroxide; 04699
Sodium hydroxide
[1310-73-2]

It contains not less than 97.0% and not more than 100.5% of NaOH.

DESCRIPTION

Physical characteristics. White to off-white crystalline mass, supplied as spheres, scales or other shapes, deliquescent, promptly absorbs carbon dioxide.

Solubility. Very soluble in water, freely soluble in ethyl alcohol.

IDENTIFICATION

The 5% (w/v) sample solution meets sodium ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Transfer, accurately weighed, 5 g of the sample to a 50 mL volumetric flask. Dilute with water, complete the volume and homogenize. The 10% (w/v) aqueous preparation is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). Not less than 11.0%. Determine in aqueous solution at 0.01% (w/v).

Carbonate limit. Determine as described in *Assay*. Not more than 2.0% considering the calculation for Na_2CO_3 .

Potassium. Acidify 5 mL of a solution at 5% (w/v) with 6 M acetic acid and add five drops of sodium cobaltinitrite RS. No precipitate is formed.

Heavy metals (5.3.2.3). Weigh 1 g of the sample, dissolve in 20 mL of water and add 8 mL of 3 M hydrochloric acid. Use *Method I*. Not more than 0.002% (20 ppm).

ASSAY

Dissolve 2 g of the sample, accurately weighed, in 80 mL of carbon dioxide-free water. Add 0.3 mL of phenolphthalein TS and titrate with M hydrochloric acid VS. Add 0.3 mL of methyl orange TS solution and continue titration with M hydrochloric acid VS. Each mL of M hydrochloric acid VS used in the second part of the titration is equivalent to 0.1060 g of Na_2CO_3 , and each mL of M hydrochloric acid VS used in the total titration is equivalent to 40.000 mg of total alkali, calculated as NaOH.

PACKAGING AND STORAGE

In tightly closed, non-metallic containers.

LABELING

Comply with current legislation.

CATEGORY

Alkalinizing agent.

DILUTED SODIUM HYPOCHLORITE SOLUTION

Aqueous sodium hypochlorite solution. It contains not less than 2.0% (w/v) and not more than 3.0% (w/v) of NaClO, equivalent to not less than 1.9% (w/v) and not more than 2.9% (w/v) of active chlorine. Clear, pale greenish-yellow liquid with chlorine odor. It is susceptible to light and gradually deteriorates.

IDENTIFICATION

A. Mix 5 mL of the sample with 1 mL of 15% (w/v) potassium iodide. Orange color quickly develops, which soon disappears. Then add five drops of 2 M hydrochloric acid and drops of starch RS. The solution turns blue.

B. Mix 1 mL of the sample with 50 mg of phenolphthalein. The liquid turns reddish.

C. To 5 mL of the sample, add five drops of 2 M hydrochloric acid. There is an increase in the intensity of the greenish color and gaseous chlorine bubbles are formed.

D. Immerse, in 1 mL of the sample, red litmus paper. The paper color turns blue, then fades.

E. The acidified solution obtained in the *Identification* test **C.** meets the flame test for sodium ions (**5.3.1.1**).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). Not less than 11.0%.

PURITY TESTS

Calcium limit. Transfer 10 g of the sample to a 150 mL beaker, add 10 mL of water, 5 mL of hydrochloric acid and 1 g of potassium iodide. Heat for five minutes, cool and add 2 mL of 30% (v/v) hydrogen peroxide. Evaporate to dryness, cool, add 2 mL of hydrochloric acid and 2 mL of 30% (v/v) hydrogen peroxide. Wash the beaker inner walls with a few milliliters of water and filter if necessary. Add 3 mL of ammonium hydroxide and 5 mL of 3.5%(w/v) ammonium oxalate to the filtrate. Quantitatively transfer to a Nessler tube, complete the volume to 25 mL and homogenize. No turbidity produced within 15 minutes exceeds that of the standard preparation, obtained by submitting 10 mL of standard calcium solution (10 ppm Ca) to the same treatment given to the sample. Not more than 0.001% (10 ppm).

ASSAY

Quantitatively transfer 3 mL of the sample or volume containing between 60 mg and 90 mg of NaClO or between 57 mg and 87 mg of active chlorine into a 250 mL erlenmeyer with cap. Add approximately 50 mL of water, 1 g of potassium iodide and 10 mL of 6 M acetic acid. Put a stopper, shake and allow to stand for 15 minutes, protected from light. Wash the flask sides with a few milliliters of water and titrate the iodine formed with 0.1 M sodium thiosulfate VS. Add 1 mL of starch RS when the solution turns greenish-yellow. Continue the titration until the blue color

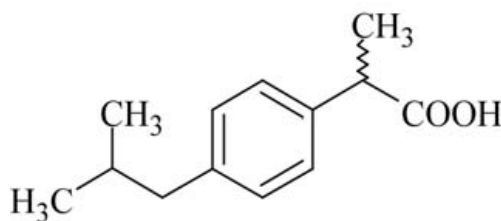
disappears. Each mL of 0.1 M sodium thiosulfate VS is equivalent to 3.723 mg of NaClO and to 3.545 mg of active chlorine

PACKAGING AND STORAGE

In tightly closed and opaque containers, preferably at a temperature below 25 °C.

LABELING

Comply with current legislation

IBUPROFEN*Ibuprofenum* $C_{13}H_{18}O_2$; 206.29

ibuprofen; 04766

 α -Methyl-4-(2-methylpropyl)benzeneacetic acid

[15687-27-1]

It contains not less than 97.0% and not more than 103.0% of $C_{13}H_{18}O_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, freely soluble in ethyl alcohol and in methyl alcohol. Soluble in aqueous solutions of alkali hydroxides.

Physicochemical constants.

Melting range (5.2.2): 75 °C to 78 °C.

Optical rotation (5.2.8). +0,05° to -0,05°. Determine in solution at 2,5% (w/v) in methyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ibuprofen CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 240 nm to 300 nm, of 0.025% (w/v) solution in 0.1 M sodium hydroxide, there are maximum and minimum points only at the same wavelengths as similar ibuprofen CRS solution. The respective absorbances, calculated in relation to the anhydrous substance, at the wavelengths of 264 nm and 273 nm, differ not more than 3.0%.

PURITY TESTS

Preparation aspect. The 10% (w/v) preparation in ethyl alcohol is clear **(5.2.25)**.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of *n*-hexane, ethyl acetate and glacial acetic acid (15:5:1) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 100 mg/mL sample solution in methylene chloride.

Solution (2): 1 mg/mL sample solution in methylene chloride.

Develop chromatogram. Remove plate and allow to air dry. Spray with 1% *p*-dimethylaminobenzaldehyde (w/v) in a mixture of ethyl alcohol and hydrochloric acid (10:1), heat in oven at 100 °C for five minutes and nebulize with 5% ferric chloride (w/v) . Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram with *Solution (2)* (1,0%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Water (5.2.20.1). Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the substance. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.5 g of the sample and dissolve in 100 mL of ethyl alcohol. Add 3 mL of phenolphthalein TS and titrate with 0.1 *M* sodium hydroxide VS until turning pink. Carry out the blank test and make the necessary corrections. Each mL of 0.1 *M* sodium hydroxide VS is equivalent to 20.629 mg of C₁₃H₁₈O₂.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Analgesic, anti-inflammatory.

IBUPROFEN TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{13}H_{18}O_2$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Mix a quantity of powder equivalent to 0.5 g of ibuprofen with 20 mL of acetone, shake, filter and evaporate the filtrate to dryness in air stream, without heating. In the infrared absorption spectrum **(5.2.14)** of the residue obtained, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ibuprofen CRS spectrum, prepared identically.

B. Recrystallize the residue obtained in test **A.** from *Identification* with petroleum ether. The residue melting temperature **(5.2.2)** is about 75°C.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 7.2, 900 mL.

Apparatus: baskets, 150 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium and dilute, if necessary, with phosphate buffer pH 7.2 to adequate concentration. Measure the absorbances of the solutions at 221 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the quantity of $C_{13}H_{18}O_2$ dissolved in the medium, comparing the readings obtained with that of the ibuprofen CRS solution at the concentration of 0.002% (w/v), prepared in the same solvent.

Tolerance: not less than 60% (Q) of the stated quantity of $C_{13}H_{18}O_2$ dissolve in 30 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel as support, and a mixture of *n*-hexane, ethyl acetate and glacial acetic acid (15:5:1) as mobile phase. Separately apply to the plate 5 µL of each of the solutions described below.

Solution (1): shake the quantity of powder equivalent to 0.2 g of ibuprofen with 10 mL of chloroform, filter and reserve the filtrate. Repeat the procedure with two more 10 mL portions of chloroform

and combine the chloroform extracts. Evaporate the filtrate to about 1 mL and add chloroform sufficient to 2 mL.

Solution (2): dilute one volume of *Solution (1)* to 100 volumes with chloroform.

Develop chromatogram. Remove the plate, allow to air dry, spray with *p*-dimethylaminobenzaldehyde at 1% (w/v) in a mixture of ethyl alcohol and hydrochloric acid (10:1), heat in oven at 100 °C for five minutes and nebulize with 5% (w/v) aqueous ferric chloride solution. No secondary stain obtained in the chromatogram with *Solution (1)* is more intense than the main stain obtained with *Solution (2)* (1.0%).

Water (5.2.20.1). Not more than 5.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh and pulverize the tablets. Shake the quantity of powder equivalent to 0.5 g of ibuprofen with 20 mL of chloroform. Filter through a sintered glass funnel and wash the residue obtained with 50 mL of ethyl alcohol, previously neutralized with 0.1 M sodium hydroxide VS, using phenolphthalein TS as indicator. Titrate with 0.1 M sodium hydroxide VS until pink changeover. Each mL of 0.1 M sodium hydroxide VS is equivalent to 20.629 mg of C₁₃H₁₈O₂.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 264 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow rate of 1.2 mL/minute.

Mobile phase: mixture of phosphoric acid, water and methyl alcohol (3:247:750).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 100 mg of ibuprofen to a 100 mL volumetric flask, add 70 mL of *Mobile Phase*, shake for 30 minutes, complete the volume with the *Mobile Phase* and homogenize. Centrifuge a portion of the suspension obtained and use the supernatant liquid.

Standard solution: dissolve an accurately weighed quantity of ibuprofen CRS in *Mobile phase* to obtain a 1 mg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The tailing factor is not more than 2.0 and the relative standard deviation of the replicate areas under the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₃H₁₈O₂ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

IBUPROFEN ORAL SUSPENSION

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{13}H_{18}O_2$.

IDENTIFICATION

A. Evaporate drops of *Solution (1)* and *Solution (2)* from the identification test **B.** in cold air stream. In the infrared absorption spectrum (**5.2.14**) of the *Solution (1)* residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the spectrum of the *Solution (2)* residue, prepared identically.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of *n*-hexane, butyl acetate and glacial acetic acid (17:3:1) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): transfer volume of the oral suspension equivalent to 200 mg of ibuprofen to separating funnel, add 10 mL of chloroform, mix for one minute. After phase separation, pass the lower layer through a filter containing 2 g of anhydrous sodium sulfate. Use the filtrate. Use a portion of this solution for the identification test **A**.

Solution (2): 20 mg/mL ibuprofen CRS solution in chloroform.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.6 to 4.6.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 7.2, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 60 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and proceed as described in *Assay*. Prepare solutions as described below.

Internal standard solution: 0.3 mg/mL benzophenone solution in acetonitrile.

Sample solution: remove aliquot from the dissolution medium and filter. Mix 5 mL of the filtrate with 5 mL of the *Internal Standard Solution*.

Standard solution: dissolve an accurately weighed quantity of ibuprofen CRS in the dissolution medium to obtain a solution at 0.011Q mg/mL; Q is the labeled quantity, in mg, of ibuprofen in each mL of oral suspension. Mix 5 mL of this solution with 5 mL of the *Internal Standard Solution*.

Inject, separately, 10 µL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₃H₁₈O₂ dissolved in the medium, using the formula:

$$90.000(C/L)(D/P_u)(R_u/R_s)$$

where

C = concentration, in mg/mL, of ibuprofen CRS in the *Standard Solution*;

L = labeled quantity, in mg/mL, of ibuprofen in the oral suspension;

D = density, in g/mL, of the oral suspension;

P_u = weight, in g, of the oral suspension added to the dissolution medium;

R_u = ratio between the areas under the peaks of ibuprofen and benzophenone obtained in the *Sample solution* and

R_s = ratio between the areas under the peaks of ibuprofen and benzophenone obtained in the *Standard solution*

Tolerance: not less than 80% (Q) of the stated quantity of C₁₃H₁₈O₂ dissolve in 60 minutes.

PURITY TESTS

Limit of Ibuprofen Related Compound C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 2 mL/minute.

Mobile phase: mixture of 0.01 M phosphoric acid and acetonitrile (63:37).

Diluent: mixture of acetonitrile and water (1:1).

Solution (1): shake the flask containing the sample and immediately transfer the equivalent of 60 mg of ibuprofen to a 50 mL volumetric flask and complete the volume with *Diluent*. Transfer 20 mL to a 50 mL volumetric flask and complete the volume with acetonitrile. The solution must be filtered through a 0.22 µm membrane.

Solution (2): dissolve an accurately weighed quantity of ibuprofen related compound C CRS in acetonitrile to obtain a 0.5 mg/mL solution. Transfer 3 mL to a 50 mL volumetric flask and complete the volume with *Diluent*. Transfer 2 mL of this solution to a 50 mL volumetric flask, add 18 mL of *Diluent* and complete the volume with acetonitrile. The solution must be filtered through a 0.22 µm membrane. This standard solution contains approximately 0.0012 mg/mL of *ibuprofen related compound C*.

Resolution solution: transfer 1.5 mL of *Solution (1)* and 9 mL of ibuprofen CRS solution at 1.2 mg/mL in *Diluent*, prepared in the *Assay*, to a 25 mL volumetric flask and complete the volume with acetonitrile. The solution must be filtered through a 0.22 µm membrane.

Procedure: inject, separately, 35 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The relative retention times are about 1.0 for ibuprofen

and 1.3 for *ibuprofen related compound C*. The resolution between *ibuprofen related compound C* and ibuprofen peaks is not less than 1.5. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%. Calculate the percentage of *ibuprofen related compound C* in the oral suspension using the formula:

$$(12.500C/VL)(A_u/A_s)$$

where

C = concentration, in mg/mL, of *ibuprofen related compound C* CRS in the *Solution (2)*;

V = quantity, in mL, of oral suspension used to prepare the *Sample Solution* from the *Assay*;

L = labeled quantity, in mg/mL, of ibuprofen in the oral suspension;

A_u and A_s = areas under the peaks of *ibuprofen related compound C* obtained from *Solution (1)* and *Solution (2)*.

Not more than 0.25%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 2 mL/minute.

Mobile phase: mixture of 0.01 M phosphoric acid and acetonitrile (63:37).

Diluent: mixture of acetonitrile and water (1:1).

Internal standard solution: 3.2 mg/mL benzophenone solution in acetonitrile.

Sample solution: shake the flask containing the sample and immediately transfer the equivalent to 60 mg of ibuprofen to a 50 mL volumetric flask and complete the volume with *Diluent*. Transfer 10 mL of this solution and 2.5 mL of the *Internal standard solution* to a 25 mL volumetric flask and complete the volume with acetonitrile.

Standard solution: 1.2 mg/mL ibuprofen CRS solution in *Diluent*. Transfer 10 mL of this solution and 2.5 mL of the *Internal standard solution* to a 25 mL volumetric flask and complete the volume with acetonitrile.

Inject 5 µL replicates of the *Standard solution*. The relative retention times are 0.9 for benzophenone and 1.0 for ibuprofen. The resolution between ibuprofen and benzophenone is not less than 1.5. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 5 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₃H₁₈O₂ in the oral suspension, using the formula:

$$125C(D/P_u)(R_u/R_s)$$

where

C = concentration, in mg/mL, of ibuprofen CRS in the *Standard Solution*;

D = density, in g/mL of the oral suspension;

P_u = mass, in g, of the oral suspension used to prepare the *Sample solution*;

R_u = ratio between the areas under the ibuprofen and benzophenone peaks obtained in the *Sample solution* and

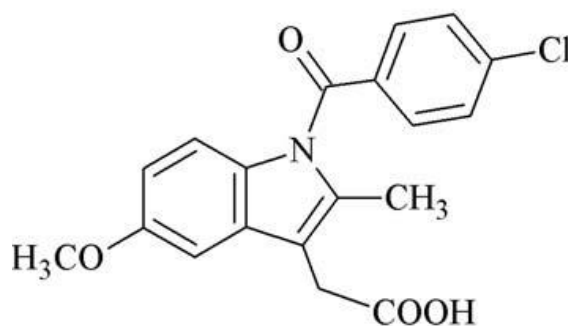
R_s = ratio between the areas under the ibuprofen and benzophenone peaks obtained in the *Standard solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

INDOMETHACIN*Indometacinum*C₁₉H₁₆ClNO₄; 357.79

indomethacin; 04889

1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid

[53-86-1]

It contains not less than 98.0% and not more than 101.0% of C₁₉H₁₆ClNO₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellow crystalline powder. Presents polymorphism.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 158 °C to 162 °C.

IDENTIFICATION

The tests B., C. and D. can be omitted if test A. is carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the indomethacin CRS spectrum, prepared identically. If the spectra obtained are not identical, dissolve the substances separately in methyl alcohol and evaporate to dryness. Obtain new spectra with the residues.

B. In the ultraviolet absorption spectrum (**5.2.14**), at the range of 300 nm to 400 nm, of 0.0025% (w/v) solution in mixture of methyl alcohol and hydrochloric acid (9:1), there is maximum peak at 318 nm. The absorbance at 318 nm is between 0.425 and 0.475.

C. Add, to 0.1 mL of 1% (w/v) sample solution in ethyl alcohol, 2 mL of freshly prepared mixture of 25% (w/v) hydroxylamine hydrochloride and sodium hydroxide RS (1:3). Add 2 mL of 20% (w/v) hydrochloric acid, 1 mL of 1.3% (w/v) ferric chloride and homogenize. Pink-violet color develops.

D. Add, to 0.5 mL of 1% (w/v) sample solution in ethyl alcohol, 0.5 mL of *p*-dimethylaminobenzaldehyde RS. Dissolve the formed precipitate, with agitation. Heat in water bath. Blueish-green color is produced. Heat for five minutes and cool in ice bath for two minutes. Precipitate is formed and the color turns to greyish-green. Add 3 mL of ethyl alcohol. The preparation becomes clear and pink-violet.

PURITY TESTS

Related substances. Proceed as described in *Thin Layer Chromatography (5.2.17.1)*, using suspension of silica gel HF₂₅₄ in monobasic sodium phosphate at 4.68% (w/v) to prepare the chromatoplate support, and a mixture of petroleum ether and ethyl ether (30:70) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): 20 mg/mL sample solution in methyl alcohol. Prepare immediately before use.

Solution (2): dilute 1 mL of *Solution (1)* to 200 mL with methyl alcohol to obtain a 0.1 mg/mL solution.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.5%).

Heavy metals (5.3.2.3). Use 2 g of the solution and proceed as described in *Method IV*. Prepare the standard solution using 4 mL of *Standard lead solution (10 ppm Pb)*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample, in oven at 105 °C, until constant weight. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Quantitatively dissolve about 0.3 g of the sample in 75 mL of acetone. Bubble carbon dioxide-free nitrogen for 15 minutes. Add 0.1 mL of phenolphthalein TS and titrate with 0.1 M sodium hydroxide VS, keeping the nitrogen flow constant, until pink color. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium hydroxide VS is equivalent to 35.779 mg of C₁₉H₁₆ClNO₄.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 300 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: 0.01 M sodium phosphate monobasic and 0.01 M sodium phosphate dibasic solution, prepared using a mixture of acetonitrile and water (1:1) as solvent.

Sample solution: quantitatively transfer about 0.1 g of the sample to a 100 mL volumetric flask. Dissolve in *Mobile Phase* and complete the volume with the same solvent. Homogenize. Transfer 10 mL of this solution to a 100 mL volumetric flask, complete the volume with *Mobile phase*. Homogenize.

Standard solution: 0.1 mg/mL indomethacin CRS solution in *Mobile phase*.

The column efficiency is not less than 500 theoretical plates/column. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₉H₁₆ClNO₄ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-inflammatory, analgesic.

INDOMETHACIN CAPSULES

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{19}H_{16}ClNO_4$.

IDENTIFICATION

A. Weigh the capsules, remove contents and weigh again. Homogenize the powder and mix quantity equivalent to 50 mg of indomethacin with 10 mL of acetone for two minutes and filter. Transfer 5 mL of the filtrate to a capped Erlenmeyer flask, add 20 mL of water and shake for two minutes until a crystalline precipitate is formed. Filter and collect the crystals. Dry the crystals at room temperature and Dry in oven at 100 °C, under reduced pressure, for two hours. In the infrared absorption spectrum **(5.2.14)** of the crystals, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the indomethacin CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 300 nm to 350 nm of the sample solution obtained in *Assay*, there is maximum point at 320 nm, identical to that observed in the spectrum of the standard solution.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel as support, and a mixture of chloroform and methyl alcohol (4:1) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described below.

Solution (1): weigh the capsules, remove contents and weigh again. Homogenize the content of the capsules. Mix quantity of powder equivalent to 25 mg of indomethacin with 25 mL of methyl alcohol, obtaining a 1 mg/mL solution. Filter.

Solution (2): 1 mg/mL indomethacin CRS solution in methyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position and intensity to the one obtained with *Solution (2)*.

D. Mix the quantity of the powder obtained in test **A** from *Identification*, equivalent to 25 mg of indomethacin, with 2 mL of water and add 2 mL of 2 M sodium hydroxide. Light yellow color develops, which quickly fades.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer the contents of each capsule to a 100 mL volumetric flask. Add 10 mL of water, allow to stand for 10 minutes, occasionally shaking. Add 75 mL of methyl alcohol, shake mechanically for 10 minutes, complete the volume with methyl alcohol, homogenize

and filter. Dilute successively with mixture of methyl alcohol and phosphate buffer pH 7.2 (1:1) to concentration of 0.0025% (w/v). Proceed as described in Assay.

DISSOLUTION TEST (5.1.5)

Dissolution medium: mixture of phosphate buffer pH 7.2 and water (1:4), 750 mL.

Apparatus: baskets, 100 rpm.

Time: 20 minutes).

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute, if necessary, in mixture of phosphate buffer pH 7.2 and water (1:4) to adequate concentration. Measure the absorbances of the solutions at 318 nm (5.2.14) using the same solvent for zero adjustment. Calculate the quantity of $C_{19}H_{16}ClNO_4$ dissolved in the medium, comparing the readings obtained with that of the indomethacin CRS solution at the concentration of 0.0025% (w/v), prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{19}H_{16}ClNO_4$ dissolve in 20 minutes.

PURITY TESTS

Related substances. Proceed as described in *Related substances* in the monograph of *Indomethacin*. Prepare *Solutions (1)* and *(2)* as described below.

Solution (1): weigh the capsules, remove contents and weigh again. Homogenize the content of the capsules. Mix quantity of powder equivalent to 0.1 g of indomethacin with 5 mL of chloroform and filter, obtaining a 20 mg/mL solution.

Solution (2): transfer 1 mL of *Solution (1)* to a 200 mL volumetric flask and complete the volume with chloroform, obtaining a 0.1 mg/mL solution..

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.5%).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh 20 capsules, remove contents and weigh again. Homogenize the content of the capsules. Accurately weigh quantity of powder equivalent to approximately 50 mg of indomethacin and transfer to a 100 mL volumetric flask, add 10 mL of water and allow to stand for 10 minutes, shaking occasionally. Add 75 mL of methyl alcohol, homogenize, complete the volume with methyl alcohol and filter. Transfer 5 mL of the filtrate to a 100 mL volumetric flask and complete the volume with mixture of phosphate buffer pH 7.2 and methyl alcohol (1:1) to obtain a 0,0025% (w/v) solution. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the

resulting solutions at 320 nm, using mixture of phosphate buffer pH 7.2 and methyl alcohol (1:1) for zero adjustment. Calculate the quantity of $C_{19}H_{16}ClNO_4$ in the capsules from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 193$, at 320 nm, in mixture of phosphate buffer pH 7.2 and methyl alcohol (1:1) .

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

INDOMETHACIN SUPPOSITORIES

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{19}H_{16}ClNO_4$.

IDENTIFICATION

A. Weigh the suppositories, pulverize or cut into small pieces and mix until obtaining a homogeneous mass. Dissolve quantity equivalent to 0.1 g of indomethacin in 50 mL of hot water and filter. Wash residue with hot water, allow to air dry. Dissolve the residue in 5 mL of chloroform and evaporate until dryness. In the infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the indomethacin CRS spectrum, prepared identically.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel as support, and a mixture of chloroform and glacial acetic acid (19:1) as mobile phase. Apply to the plate, separately, 10 μ L of the freshly prepared solutions described below.

Solution (1): weigh the suppositories, pulverize or cut into small pieces and mix until obtaining a homogeneous mass. Transfer quantity equivalent to 25 mg of indomethacin to a 125 mL separating funnel, add 15 mL of water and 50 mL of ethyl ether and shake until dissolved. Transfer the ether layer to a 200 mL volumetric flask and extract the aqueous layer with two more 50 mL portions of ethyl ether. Combine the ether extracts and complete the volume with ethyl ether.

Solution (2): 0.125 mg/mL indomethacin CRS solution in mixture of methyl alcohol and ethyl ether (1:100). Dissolve in methyl alcohol in advance.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position and intensity to the one obtained with *Solution (2)*.

C. Weigh the suppositories, pulverize or cut into small pieces and mix until obtaining a homogeneous mass. Shake quantity equivalent to 25 mg of indomethacin with 5 mL of water until a white suspension is produced. Add 2 mL of 2 M sodium hydroxide. Light yellow color develops, which quickly fades.

CHARACTERISTICS

Disintegration test (5.1.4.2). Carry out the test for 90 minutes in phosphate buffer pH 6.8 using three accurately weighed suppositories. After testing, remove each suppository, dry on filter paper and weigh. Not less than 75% of each suppository is dissolved.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Proceed as described in *Ultraviolet absorption spectrophotometry* (**5.2.14**). Transfer each suppository to a 100 mL volumetric flask containing 80 mL of a mixture of methyl alcohol and glacial acetic acid (199:1), mechanically shake until the suppository dissolves and complete the volume with the same solvent. Homogenize and filter. Dilute successively with mixture of methyl alcohol and glacial acetic acid (199:1) to concentration of 0.0025% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of

the resulting solutions at 320 nm, using methyl alcohol and glacial acetic acid (199:1) for zero adjustment. Calculate the quantity of $C_{19}H_{16}ClNO_4$ in the suppositories from the readings obtained.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh 10 suppositories, pulverize or cut into small pieces and mix until obtaining a homogeneous mass. Accurately weigh quantity equivalent to about 0.1 g of indomethacin, transfer to a 50 mL volumetric flask with the aid of 40 mL of methyl alcohol and shake until completely dispersed. Complete the volume with methyl alcohol and filter. Transfer 2 mL of the filtrate to a 100 mL volumetric flask and complete the volume with mixture of phosphate buffer pH 7.2 and methyl alcohol (1:1). Prepare a standard solution in the same concentration, using the same solvents. Measure the absorbances of the solutions at 318 nm, using mixture of phosphate buffer pH 7.2 and methyl alcohol (1:1) for zero adjustment. Calculate the quantity of $C_{19}H_{16}ClNO_4$ in the suppositories from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 193$, at 318 nm, in mixture of phosphate buffer pH 7.2 and methyl alcohol (1:1).

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

POTASSIUM IODIDE*Kalii iodidum*

KI; 166.00
potassium iodide; 04965
Potassium iodide
[7681-11-0]

It contains not less than 99.0% and not more than 100.5% of KI, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder or colorless crystals.

Solubility. Very soluble in water, freely soluble in glycerol and soluble in ethyl alcohol.

IDENTIFICATION

A. The 10% (w/v) solution in carbon dioxide-free water meets the iodide ion reactions **(5.3.1.1)**.

B. The 10% (w/v) solution in carbon dioxide-free water meets potassium ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. The preparation used in the test **A.** from *Identification* is clear **(5.2.25)** and colorless **(5.2.12)**.

Alkalinity. To 12.5 mL of the solution used in the test **A.** from *Identification*, add 0.1 mL of bromothymol blue TS and titrate with 0.01 M hydrochloric acid until yellow color. Not more than 0.5 mL of 0.01 M hydrochloric acid.

Iodates. To 10 mL of the solution used in the test **A.** from *Identification*, add 0.25 mL of iodide-free starch TS and 0.2 mL of M sulfuric acid. Allow to stand, protected from light, for two minutes. Blue color is not developed.

Thiosulfate. To 10 mL of the solution used in the test **A.** from *Identification*, add 0.1 mL of iodide-free starch TS and 0.1 mL of 0.005 M iodine. Blue color develops.

Iron (5.3.2.4). Dilute 5 mL of the solution obtained in the test **A.** from *Identification* to 10 mL with water. Proceed as described in *Limit test for iron, Method I*. Not more than 0.002% (20 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Use 20 mL of the solution obtained in test **A.** from *Identification*. Not more than 0.001% (10 ppm).

Sulfates (5.3.2.2). Dissolve 8 g of the sample in 15 mL of water. Proceed as described in *Limit test for sulfates*. Not more than 0.015% (150 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for three hours. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 1.5 g of the sample, dissolve in water and complete the volume to 100 mL with the same solvent. To 20 mL of this solution, add 40 mL of concentrated hydrochloric acid and titrate with 0.05 M potassium iodate VS until color turns from brown to yellow. Add 5 mL of chloroform. Continue the titration, shaking vigorously, until the chloroform layer discolors. Each mL of 0.05 M potassium iodate VS is equivalent to 16.600 mg of KI.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antithyroid drug.

SODIUM IODIDE***Natrii iodidum***

NaI; 149.89
sodium iodide; 04969
Sodium iodide
[7681-82-5]

It contains not less than 99.0% and not more than 101.5% of NaI, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or colorless crystals hygroscopic.

Solubility. Very soluble in water and freely soluble in ethyl alcohol.

IDENTIFICATION

A. Dissolve 10 g of the sample in carbon dioxide-free water and complete the volume to 100 mL with the same solvent. The resulting solution meets iodide ion reactions **(5.3.1.1)**.

B. It meets sodium ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 10 g of the sample in carbon dioxide-free water and complete the volume to 100 mL with the same solvent. The preparation is clear **(5.2.25)** and colorless **(5.2.12)**.

Alkalinity. To 12.5 mL of the preparation obtained in *Preparation aspect*, add 0.1 mL of bromothymol blue TS. Not more than 0.7 mL of 0.01 M hydrochloric acid is required for indicator changeover.

Barium. A 20% (w/v) sample solution, acidified with hydrochloric acid, should not become turbid with the addition of 1% (w/v) potassium sulfate.

Iodides. To 10 mL of the solution used in *Preparation aspect*, add 0.25 mL of iodide-free starch TS and 0.2 mL of M sulfuric acid. Allow to stand for two minutes, protected from light. Blue color is not developed.

Nitrate, nitrite and ammonia. Add 5 mL of M sodium hydroxide and approximately 0.2 g of metallic aluminum to a solution of 1 g of the sample in 5 mL of water, in a test tube with capacity of 40 mL. Insert a cotton swab into the top of the tube and place a piece of red litmus paper on the mouth of the test tube. Heat in water bath for 15 minutes. Blue coloration on the paper is not observed.

Potassium. A solution of 1 g of the sample in 2 mL of water must not precipitate with 1 mL of sodium bitartrate RS.

Thiosulfates. To 10 mL of the solution obtained in *Preparation aspect*, add 0.1 mL of iodized starch RS and 0.1 mL of 0,005 M iodine. Blue color develops.

Iron (5.3.2.4). Use *Method I*. Use 10 mL of the preparation obtained in *Preparation aspect* and proceed as described in *Limit test for iron*. Use 0.2 mL of *Standard iron solution (100 ppm Fe)*. Not more than 0.002% (20 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Weigh 2 g of the sample, dissolve in 2 mL of water and proceed as described in *Limit test for heavy metals*. Not more than 0.001% (10 ppm).

Sulfates (5.3.2.2). Use 20 mL of the preparation obtained in *Preparation aspect* and dilute to 15 mL with water. Proceed as described in *Limit test for sulfates*. Not more than 0.015% (150 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for three hours. Not more than 3.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.3 g of the sample previously desiccated and dissolve in 10 mL of water. Add 15 mL of hydrochloric acid and titrate with 0.1 M potassium iodate VS until color turns from red to yellow. Add 5 mL of chloroform and continue the titration, shaking vigorously, until the chloroform layer discolors. Each mL of 0.1 M potassium iodate VS is equivalent to 29.978 mg of NaI.

PACKAGING AND STORAGE

In tightly closed containers and protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Expectorant and antihyperthyroid drug.

IODINE

Iodum

I₂; 253.80

iodine; 04983

Iodine

[7553-56-2]

It contains not less than 99.5% and not more than 100.5% of I.

DESCRIPTION

Physical characteristics. Fine, violet crystals with metallic shine.

Solubility. Very slightly soluble in water, soluble in ethyl alcohol, slightly soluble in glycerol. Very soluble in concentrated iodide solutions.

IDENTIFICATION

A. In a test tube, heat a small portion of the sample. Violet vapors are released, which condense on the tube walls in the form of bluish crystals.

B. To a saturated sample solution, add starch RS solution. Blue color is produced. Heat until discolored. Under cooling, blue color reappears.

PURITY TESTS

Cyanide. Vigorously shake 1 g of the sample with 30 mL of water and filter. To 5 mL of the filtrate, add ten drops of 0.1 M sodium thiosulfate, one ferrous sulfate crystal, one drop of ferric chloride RS and boil. Allow to cool. Acidify with hydrochloric acid. Blue color is not developed.

Sulfate. Dilute 3 mL of the obtained filtrate in *Cyanide* to 5 mL with water, add one drop of hydrochloric acid and five drops of barium chloride RS. Turbidity is not observed.

Bromide and chloride limit. Pulverize 3 g of the sample and mix with 20 mL of water. Filter, wash the filter with water and dilute to 30 mL with the same solvent. Add 1 g of zinc powder. After solution discoloration, filter, wash the filter with water and complete the volume to 40 mL with the same solvent. To 10 mL of the solution, add 3 mL of ammonia and 6 mL of 0.1 M silver nitrate solution. Then filter again, wash the filter with water and complete the volume to 20 mL with the same solvent. Treat 10 mL of the solution with 1.5 mL of nitric acid. After one minute, the opalescence presented by the preparation should not be more intense than that of a standard preparation obtained simultaneously with a mixture of 10.75 mL of water, 0.25 mL of 0.01 M hydrochloric acid, 0.2 mL of 20% nitric acid (w/v) and 0.3 mL of 0.1 M silver nitrate solution. Not more than 0.025% (250 ppm).

Residue by evaporation. Quantitatively transfer about 5 g of the sample to a porcelain dish, heat in water bath until all the iodine is sublimed, then dry in oven at 105 °C for one hour. Not more than 0.05%.

ASSAY

Quantitatively transfer about 0.2 g of iodine to an Erlenmeyer flask containing 1 g of potassium iodide and 2 mL of water. Add 1 mL of diluted acetic acid and, after dissolution, add 50 mL of water. Titrate with 0.1 M sodium thiosulfate VS at a temperature below 15 °C until discoloration from dark yellow to pale yellow. Add some drops of starch TS and continue with the titration until the blue color disappears. Each mL of 0.1 M sodium thiosulfate VS is equivalent to 12.690 mg of I.

PACKAGING AND STORAGE

In hermetically closed containers, protected from light and heat.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial, antihyperthyroid.

IODINE, STRONG TINCTURE

The strong iodine tincture consists of 6.5 g of iodine, in the presence of sodium iodide and diluted ethyl alcohol. It contains not less than 5.85 g and not more than 7.15 g of iodine in 100 mL of solution. Sodium iodide can be replaced by potassium iodide containing not less than 2.25 g of iodide in 100 mL of solution.

IDENTIFICATION

- A.** Add one drop of sample to a 0.2% (w/v) starch solution. Blue color is produced.
- B.** Evaporate about 3 mL of the sample in water bath until dryness. The residue meets sodium ion reaction 1 (**5.3.1.1**).
- C.** The precipitate obtained in test **B.** from *Identification* meets iodide ion reactions (**5.3.1.1**).

PURITY TEST

Alcohol (5.3.3.8). Proceed as described in *Determination of alcohol*. Between 82 and 88.5% (v/v).

ASSAY

Iodine. Transfer 5 mL of the strong iodine tincture to an Erlenmeyer flask containing 20 mL of water. Add three drops of starch TS and titrate with 0.1 M sodium thiosulfate VS. Each mL of 0.1 M sodium thiosulfate VS is equivalent to 12.690 mg of iodine (I).

Sodium iodide or potassium iodide. Transfer 5 mL of the strong iodine tincture to an Erlenmeyer flask containing 30 mL of water and add 10 mL of hydrochloric acid. Titrate with 0.05 M potassium iodate VS until light brown color. Add 5 mL of chloroform and continue the titration, shaking vigorously, until the chloroform layer discolors. From the volume of 0.05 M potassium iodate VS used, subtract half the volume of 0.1 M sodium thiosulfate VS used in the iodine assay. Each mL of the remaining 0.05 M potassium iodate VS is equivalent to 14.989 mg of sodium iodide (NaI) or 16.600 mg of potassium iodide (KI).

PACKAGING AND STORAGE

In tightly closed containers, protected from heat.

LABELING

Comply with current legislation.

IODINE, WEAK TINCTURE

The weak iodine tincture consists of 2 g of iodine, in the presence of 2.4 g of sodium iodide in 100 mL of 50% (v/v) ethyl alcohol. It contains not less than 1.8 g and not more than 2.2 g of iodine in 100 mL of solution. Sodium iodide can be replaced by potassium iodide containing not less than 1.35 g of iodide in 100 mL of solution.

IDENTIFICATION

A. Add one drop of sample to a 0.2% (w/v) starch solution. Blue color is produced.

B. Evaporate about 3 mL of the sample in water bath until dryness. The residue meets sodium ion reaction 1 (5.3.1.1) and iodide reactions (5.3.1.1).

PURITY TEST

Alcohol (5.3.3.8). Proceed as described in *Determination of alcohol*. Between 44 and 50%.

ASSAY

Iodine. Transfer 5 mL of the weak iodine tincture to an Erlenmeyer flask containing 20 mL of water. Add three drops of starch TS and titrate with 0.1 M sodium thiosulfate VS. Each mL of 0.1 M sodium thiosulfate VS is equivalent to 12.690 mg of iodine (I).

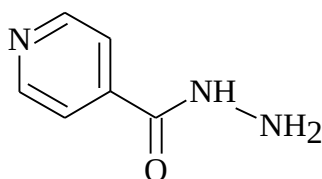
Sodium iodide or potassium iodide. Transfer 5 mL of the weak iodine tincture to an Erlenmeyer flask containing 30 mL of water and add 10 mL of hydrochloric acid. Titrate with 0.05 M potassium iodate VS until light brown color. Add 5 mL of chloroform and continue the titration, shaking vigorously, until the chloroform layer discolors. From the volume of 0.05 M potassium iodate VS used, subtract half the volume of 0.1 M sodium thiosulfate VS used in the iodine assay. Each mL of the remaining 0.05 M potassium iodate VS is equivalent to 14.989 mg of sodium iodide (NaI) or 16.600 mg of potassium iodide (KI).

PACKAGING AND STORAGE

In tightly closed containers, protected from heat.

LABELING

Comply with current legislation.

ISONIAZID*Isoniazidum*C₆H₇N₃O; 137.14

isoniazid; 05092

4-pyridinecarboxylic acid hydrazide

[54-85-3]

It contains not less than 98.0% and not more than 102.0% of C₆H₇N₃O, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or colorless crystalline powder.

Solubility. Freely soluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 170 °C to 174 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the isoniazid CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 350 nm of the sample solution obtained in method **B.** from *Assay*, there are maximum absorption points at 212 nm and 265 nm, identical to those observed in the spectrum of the standard solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **C.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

pH (5.2.19). 6.0% to 8.0%. Determine in aqueous solution at 5% (w/v).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and a mixture of water, acetone, methyl alcohol and ethyl acetate (5:20:10:75) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 1 g of the sample in mixture of water and acetone (1:1) and complete to 10 mL with the same solvent.

Solution (2): dissolve 50 mg of hydrazine sulfate in 50 mL of water and complete to 100 mL with acetone. Transfer 10 mL of this solution to a 100 mL volumetric flask, add 0.2 mL of *Solution (1)* and complete the volume with mixture of water and acetone (1:1).

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.2%). Spray plates with *p*-dimethylaminobenzaldehyde RS1. An additional stain, corresponding to hydrazine, appears on the chromatogram. Any secondary stain, corresponding to hydrazine, obtained in the chromatogram with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (2)* (0.05%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at $105\text{ }^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for four hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

ASSAY

Use one of the methods described below.

A. Accurately weigh about 250 g of the sample. Transfer to a 100 mL volumetric flask and complete the volume with water. Transfer volumetrically 20 mL of this solution to a 250 mL erlenmeyer. Add 100 mL of distilled water, 20 mL of hydrochloric acid RS, 0.2 g of potassium bromide and 0.05 mL of methyl red TS. Titrate with 0.0167 M potassium bromate VS until the indicator red color disappears. Each mL of 0.0167 M potassium bromate VS is equivalent to 3.429 mg of isoniazide ($\text{C}_6\text{H}_7\text{N}_3\text{O}$).

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 25 mg of the sample and dissolve in 0.01 M hydrochloric acid. Place in ultrasonic bath, if necessary, and complete the volume to 250 mL with the same solvent. Dilute with 0.01 M hydrochloric acid to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 265 nm, using hydrochloric acid for zero adjustment. Calculate the content of $\text{C}_6\text{H}_7\text{N}_3\text{O}$ in the sample from the readings obtained.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 μm), kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Phosphate buffer pH 6.9: prepare 0.1 M monobasic potassium phosphate solution and adjust the pH to 6.9 with concentrated sodium hydroxide solution RS. Add five drops of triethanolamine per liter of prepared buffer and homogenize.

Mobile phase: mixture of *Phosphate buffer pH 6.9* and methyl alcohol (95:5).

Sample solution: quantitatively transfer about 32 mg of the sample to a 100 mL volumetric flask with the aid of 40 mL of *Mobile phase* and place in ultrasonic bath for 10 minutes. Complete the volume with the same solvent to obtain a 0.32 mg/mL solution.

Standard solution: dissolve an accurately weighed quantity of isoniazid CRS in *Mobile phase* to obtain a 0.32 mg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is not less than 1800 theoretical plates. The retention factor is not less than 2.35. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₆H₇N₃O in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

ISONIAZID TABLETS

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_6H_7N_3O$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 350 nm of the sample solution obtained in method **B.** from Assay, there are maximum absorption points at 212 nm and 265 nm, identical to those observed in the spectrum of the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **C.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

C. Weigh and pulverize the tablets. Transfer quantity of powder equivalent to 1 mg of isoniazid to an Erlenmeyer flask, add 50 mL of ethyl alcohol and shake. To 5 mL of the resulting solution, add 0.1 g of sodium tetraborate and 5 mL of 5% (w/v) 1-chloro-2,4-dinitrobenzene in ethyl alcohol. Evaporate in water bath until dryness and heat for another 10 minutes. Add 10 mL of methyl alcohol to the residue and homogenize. Purple-reddish color develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.01 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in 0.01 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 265 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the quantity of $C_6H_7N_3O$ dissolved in the medium, comparing the readings obtained with that of the isoniazid CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_6H_7N_3O$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh and pulverize 20 tablets. Dissolve quantity of powder equivalent to 0.4 g of isoniazid in water, transfer to a 250 mL volumetric flask, complete the volume with water and shake. Filter. Transfer 50 mL of the obtained solution into an Erlenmeyer flask. Add 50 mL of water, 20 mL of hydrochloric acid RS and 0.2 g of potassium bromide and titrate with 0.0167 M potassium bromate VS solution, determining the endpoint potentiometrically. Each mL of 0.0167 M potassium bromate is equivalent to 3.429 mg of $C_6H_7N_3O$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.1 mg of isoniazid to a 100 mL volumetric flask and add 50 mL of 0,01 M hydrochloric acid. Place in ultrasonic bath for 15 minutes, shake mechanically for 15 minutes and complete the volume with the same solvent. Homogenize and filter. Carry out successive dilutions up to a concentration of 0.001% (w/v), using the same solvent. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 265 nm, using 0,01 M hydrochloric acid for zero adjustment. Calculate the quantity of $C_6H_7N_3O$ in the tablets from the readings obtained.

C. Proceed as described in method **C.** from Assay in the *Isoniazid* monograph. Prepare *Sample solution* as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 32 mg of isoniazid to a 100 mL volumetric flask, add 40 mL of *Mobile Phase* and place in ultrasonic bath for 10 minutes. Cool to room temperature, complete the volume with *Mobile Phase* and centrifuge for five minutes to obtain a 0.32 mg/mL solution.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of isoniazid ($C_6H_7N_3O$) in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

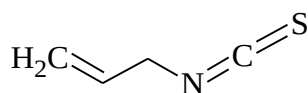
PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

ALLYL ISOTHIOCYANATE



C₄H₅NS; 99.15

allyl isothiocyanate; 09889

3-Isothiocyanate-1-propene

[57-06-7]

It contains not less than 93.0% and not more than 105.0% of C₄H₅NS.

DESCRIPTION

Physical characteristics. Viscous liquid, ranging from colorless to slightly yellow. It is a strong tear agent with an irritating odor and, during handling, eye protection should be used and inhalation should be avoided.

Physicochemical constants.

Relative density (5.2.5): 1.013 to 1.020.

Distillation range (5.2.3): 148 °C to 154 °C.

Refractive index (5.2.6): 1.527 to 1.531, determined at 20 °C.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in sodium chloride or potassium bromide, there are absorption bands at 700 cm⁻¹, 950 cm⁻¹, 980 cm⁻¹, 1300 cm⁻¹, 1340 cm⁻¹, 1350 cm⁻¹, 1410 cm⁻¹, 1420 cm⁻¹, 1650 cm⁻¹, 2100 cm⁻¹ and 2200 cm⁻¹.

PURITY TESTS

Phenols. Dilute 1 mL of sample in 5 mL of ethyl alcohol and add one drop of ferric chloride RS. Blue precipitate is not formed.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Quantitatively transfer about 4 mL of the sample to a 100 mL volumetric flask and complete the volume with ethyl alcohol. Transfer 5 mL of this solution to a distillation flask, together with 50 mL of 0.1 M silver nitrate VS and 5 mL of 10% (v/v) ammonia solution. Connect the flask to a reflux condenser, heat in water bath for one hour and cool at room temperature. Disconnect the flask

from the reflux condenser, transfer the content to a 100 mL volumetric flask and complete the volume with water. Filter the solution, discarding 10 mL of the initial filtrate volume. For each 50 mL of filtrate, add 5 mL of nitric acid, 2 mL of ferric ammonium sulfate RS and titrate excess silver nitrate with 0.1 M ammonium thiocyanate VS. Carry out a blank test using 5 mL of ethyl alcohol, in place of the sample solution. Each mL of 0.1 M silver nitrate VS is equivalent to 4.958 mg of C_4H_5NS .

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antisecretory.

ISOTRETINOIN CAPSULES

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{20}H_{28}O_2$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution* chromatogram.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 353 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (10 μ m), kept at room temperature; *Mobile phase* flow rate of 1.4 mL/minute.

Mobile phase: mixture of methyl alcohol and water (77:23) with 0.5% (v/v) of glacial acetic acid.

Sample solution: weigh 20 capsules, remove the content and weigh again. Homogenize the content of the capsules. Transfer quantity of powder equivalent to 20 mg of isotretinoin to a 100 mL amber volumetric flask. Dilute in 80 mL of methyl alcohol and shake mechanically for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of the filtrate to a 25 mL amber volumetric flask and complete the volume with methyl alcohol, obtaining a 40 μ g/mL solution.

Standard solution: transfer 20 mg of isotretinoin CRS, accurately weighed, to a 50 mL amber volumetric flask. Dissolve in methyl alcohol and complete the volume with the same solvent. Transfer 5 mL of the solution above to a 50 mL amber volumetric flask and complete the volume with methyl alcohol.

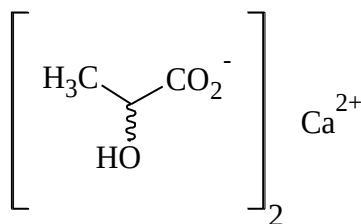
Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of isotretinoin ($C_{20}H_{28}O_2$) in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CALCIUM LACTATE*Calcii lactas*C₆H₁₀CaO₆; 218.22

calcium lactate; 00275

Calcium salt of 2-hydroxypropanoic acid (1:2)

[814-80-2]

C₆H₁₀CaO₆.xH₂O

calcium lactate hydrate; 11414

2(S)-hydroxypropanoic acid calcium salt hydrate (1:2:?)

[949014-28-2]

It contains not less than 98.0% and not more than 101.0% of C₆H₁₀CaO₆, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder or granules. Calcium lactate pentahydrate is efflorescence and becomes anhydrous at 120 °C.

Solubility. Calcium lactate pentahydrate is soluble in water and practically insoluble in ethyl alcohol.

IDENTIFICATION

A. It meets calcium ion reactions **(5.3.1.1)**.

B. It meets lactate ion reactions **(5.3.1.1)**.

PURITY TESTS

Acidity. Titrate 20 mL of sample solution (1:20) with 0.1 M sodium hydroxide VS, using phenolphthalein TS as indicator. Neutralization is achieved using not more than 0.5 mL of sodium hydroxide (0.45% as lactic acid).

Volatile fatty acids. Shake approximately 0.5 g with 1 mL of sulfuric acid and heat. Volatile fatty acid odor is not released.

Loss on drying (5.2.9.1). Distribute 1 g to 2 g of the sample evenly in a layer of not more than 3 mm in a suitable weighing bottle. Dry in oven at 120 °C for four hours. Water loss is: pentahydrate, 20.0% to 30.0%; trihydrate, 15.0% to 20.0%; monohydrate 5.0% to 8.0% and the anhydrous form, not more than 3.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh a sample amount that contains the equivalent to about 0.35 g of anhydrous lactate. Dissolve in 150 mL of acidified water with 2 mL of diluted hydrochloric acid. Under agitation (preferably with a magnetic stirrer), add approximately 30 mL of 0.05 M disodium edetate VS. Add 15 mL of sodium hydroxide RS, 0.3 g of hydroxynaphthol blue indicator and continue the titration until turning blue. Each mL of 0.05 M disodium edetate VS is equivalent to 10.911 mg of $C_6H_{10}CaO_6$.

PACKAGING AND STORAGE

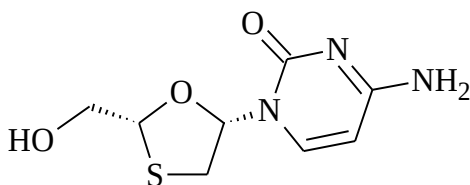
In hermetic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Calcium replenisher and electrolyte replenisher.

LAMIVUDINE**Lamivudinum**C₈H₁₁N₃O₃S; 229.26

lamivudine; 05152

4-Amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1*H*)-pyrimidone
[134678-17-4]

It contains not less than 98.0% and not more than 102.0% of C₈H₁₁N₃O₃S, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White to yellowish-white crystalline powder.

Solubility. Freely soluble in water, moderately soluble in methyl alcohol and ethyl alcohol. Freely soluble in 0.1 *M* hydrochloric acid and in 0.1 *M* sodium hydroxide.

Physicochemical constants.

Melting range (5.2.2): 176 °C to 178 °C.

Specific optical rotation (5.2.8): –135 to –146, in relation to the anhydrous substance. Determine in solution at 0.8% (w/v) in methyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the lamivudine CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm of the sample solution obtained in method **A. from Assay**, there is maximum point at 270 nm, identical to that observed in the standard solution spectrum.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B. from Assay**, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, using a chromatograph equipped with an ultraviolet detector at 270 nm; column with 250 mm in

length and 4.6 mm in internal diameter, packed with hydroxypropylbetacyclodextrin (5 µm); *Mobile Phase* flow of 1 mL/minute.

Mobile phase: mixture of 0.1 M ammonium acetate and methyl alcohol (95:5).

Solution (1): dissolve 15 mg of the sample in *Mobile phase* and complete to 10 mL with the same solvent.

Procedure: inject 20 µL of *Solution (1)*, record the chromatograms and measure the areas under the peaks. The sum of the areas under the secondary peaks, except the area under the main peak, is not greater than 1.0% of the total area under the peaks obtained. Do not consider peaks related to the solvent.

Heavy metals (5.3.2.3). Use *Method I*. Not more than 0.001% (10 ppm).

Water (5.2.20.1). Not more than 2.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 50 g of the sample and dissolve in water. Complete the volume to 100 mL with the same solvent. Dilute, successively, in water until concentration of 0.0015% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 270 nm, using water for zero adjustment. Calculate the content of C₈H₁₁N₃O₃S in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 277 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Acetate buffer pH 3.8: dissolve 1.9 g of ammonium acetate in 900 mL of water, adjust the pH to 3.8 ± 0.2 with glacial acetic acid and complete the volume to 1000 mL.

Mobile phase: mixture of *Acetate buffer pH 3.8* and methyl alcohol (95:5).

Sample solution: weigh, accurately, about 20 mg of the sample and transfer to a 50 mL volumetric flask. Dissolve with *Mobile Phase* and complete the volume with the same solvent.

Standard solution: weigh, accurately, about 20 mg of lamivudine CRS to a 50 mL volumetric flask. Dissolve with *Mobile Phase* and complete the volume with the same solvent.

Inject 10 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₈H₁₁N₃O₃S in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antirretroviral.

LAMIVUDINE TABLETS

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_8H_{11}N_3O_3S$. Tablets must be coated.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer quantity of powder equivalent to 0.15 g of lamivudine to a mortar, add 10 mL of methyl alcohol, mix and filter. Evaporate the filtrate to residue and dry in oven at 40 °C for two hours. The residue meets the test **A.** in *Identification* of the *Lamivudine* monograph.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from *Assay*, there is maximum point at 270 nm, identical to that observed in the spectrum of the standard solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Not more than 30 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 100 mL volumetric flask containing 70 mL of water and shake until complete tablet disintegration. Place in ultrasonic bath for 15 minutes, complete the volume with the same solvent, homogenize and filter. Proceed as described in method **A.** of *Assay* from "Dilute until concentration...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in water until adequate concentration. Measure the absorbances at 270 nm (**5.2.14**), using water for zero adjustment. Calculate the quantity of $C_8H_{11}N_3O_3S$ dissolved in the medium, comparing the readings obtained with that of the lamivudine CRS solution at the concentration of 0.0015% (w/v), prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_8H_{11}N_3O_3S$ dissolve in 30 minutes.

PURITY TESTS

Water (5.2.20.1). Not more than 3.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.15 g of lamivudine to a 100 mL volumetric flask, add 70 mL of water, place in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Homogenize and filter. Dilute successively to a concentration of 0.0015% (w/v) using water as solvent. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 270 nm, using water for zero adjustment. Calculate the amount of $C_8H_{11}N_3O_3S$ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Proceed as described in method **B.** from Assay in the *Lamivudine* monograph. Prepare *Sample solution* as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.2 mg of lamivudine to a 100 mL volumetric flask and add 50 mL of *Mobile phase*. Shake mechanically for 10 minutes and complete the volume with the *Mobile phase*. Homogenize and filter. Transfer 10 mL to a 50 mL volumetric flask, complete the volume with the *Mobile phase* and homogenize.

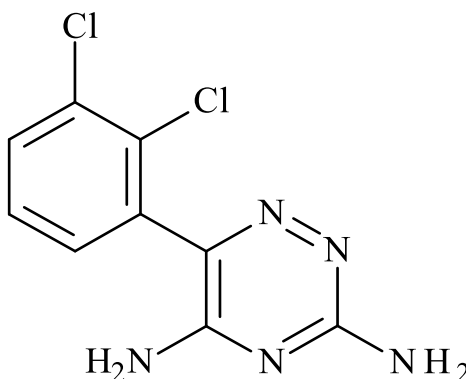
Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_8H_{11}N_3O_3S$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

LAMOTRIGINE*Lamotriginum* $C_9H_7Cl_2N_5$; 256.09

lamotrigine; 05153

6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine

[84057-84-1]

It contains not less than 98.0% and not more than 102.0% of $C_9H_7NCl_2N_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Moderately soluble in methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 216 °C to 218 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the lamotrigine CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 370 nm, of the 0.002% (w/v) solution in 0.01 M hydrochloric acid, there is a maximum point at 269 nm, identical to that observed in the spectrum of similar lamotrigine CRS solution.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel 60 F₂₅₄, as support, and a mixture of chloroform, methyl alcohol and dimethylformamide (16:3.5:0.5), as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): 1.0 mg/mL sample solution in methyl alcohol.

Solution (2): 1.0 mg/mL lamotrigine CRS solution in methyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm) or expose plate to iodine vapors. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for two hours. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 279 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of 0.3% (v/v) triethylamine, with pH adjusted to 4.0 with 10% (v/v) phosphoric acid, and methyl alcohol (62:38).

Sample solution: dissolve an accurately weighed quantity of the sample in methyl alcohol to obtain a 0.5 mg/mL solution. Transfer 4 mL of this solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 40 µg/mL solution.

Standard solution: dissolve an accurately weighed quantity of lamotrigine CRS in methyl alcohol to obtain a 0.5 mg/mL solution. Transfer 4 mL of this solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 40 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is not less than 5000 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₉H₇NCl₂N₅ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anticonvulsant.

LAMOTRIGINE TABLETS

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_9H_7Cl_2N_5$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Use quantity of powder equivalent to 10 mg of lamotrigine. Proceed as described in test **B.** from *Identification* in the *Lamotrigine* monograph.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in the *Assay* method in the *Lamotrigine* monograph. Prepare *Sample solution* as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 25 mg of lamotrigine to a 50 mL volumetric flask with the aid of 30 mL of methyl alcohol. Place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, filter and homogenize. Transfer 4 mL of this solution to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_9H_7Cl_2N_5$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

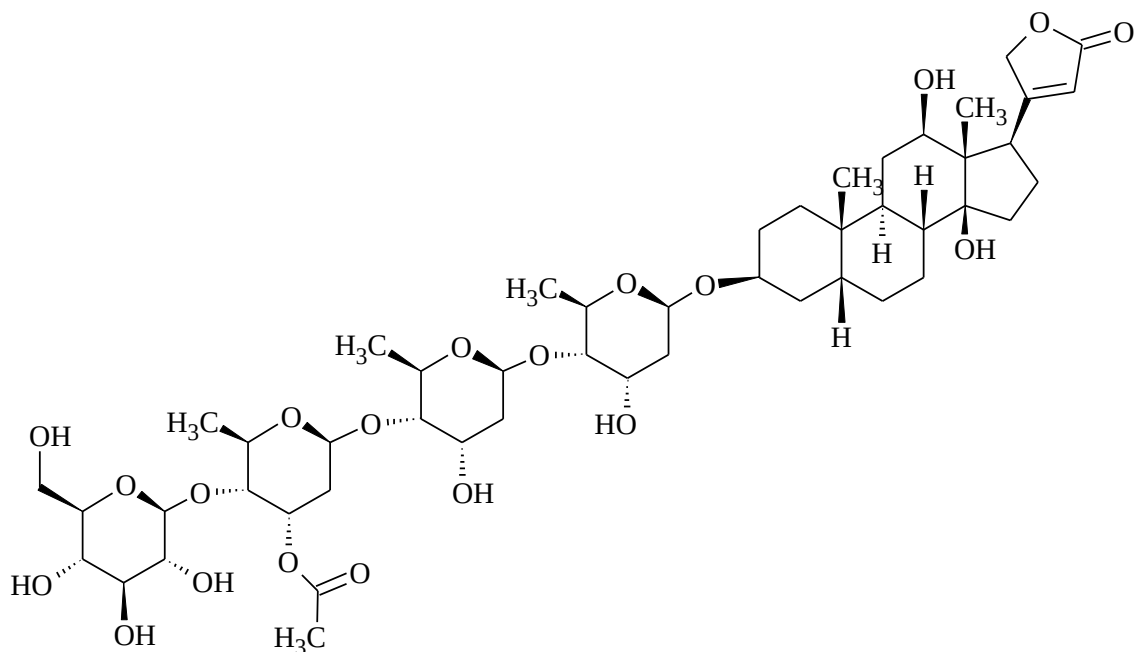
In tightly closed containers, at room temperature.

LABELING

Comply with current legislation.

LANATOSIDE C

Lanatosidum C



$C_{49}H_{76}O_{20}$; 985.13

lanatoside C; 05156

(3 β ,5 β ,12 β)-3-[(O- β -D-Glycopyranosyl-(1 $\rightarrow$ 4)-O-3-O-acetyl-2,6-dideoxy- β -D-ribo-hexopyranosyl-(1 $\rightarrow$ 4)-O-2,6-dideoxy- β -D-ribo-hexopyranosyl-(1 $\rightarrow$ 4)-2,6-dideoxy- β -D-ribo-hexopyranosyl)oxy]-12,14-dihydroxy-card-20(22)-enolide
[17575-22-3]

It contains not less than 97.0% and not more than 102.0% of $C_{49}H_{76}O_{20}$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or slightly yellow crystalline powder., hygroscopic.

Solubility. Practically insoluble in water, soluble in methyl alcohol, in dioxane and in pyridine.

Physicochemical constants.

Specific optical rotation (5.2.8): +32.0 to +35.5, in relation to the desiccated substance. Determine in solution at 2% (w/v) in methyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the lanatoside C CRS spectrum, prepared identically.

B. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

C. Dissolve 0.5 mg of the sample in 0.2 mL of ethyl alcohol at 60% (v/v). Add 0.1 mL of 2% (w/v) 3,5-dinitrobenzoic acid in ethyl alcohol and 0.1 mL of 2 M sodium hydroxide. Violet color develops.

D. Dissolve 5 mg of the sample in 5 mL of glacial acetic acid and add 0.05 mL of ferric chloride RS. Carefully add 2 mL of sulfuric acid, without shaking. Allow to stand. A non-reddish brown ring develops at the interface and a yellowish-green color, which turns greenish-blue, diffuses from the ring.

PURITY TESTS

Preparation aspect. The 2% (w/v) preparation in methyl alcohol is clear **(5.2.25)** and colorless **(5.2.12)**.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and a mixture of toluene, ethyl alcohol, methylene chloride and water (60:30:20:1) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 20 mg/mL sample solution in methyl alcohol.

Solution (2): 2 mg/mL sample solution in methyl alcohol.

Solution (3): 2 mg/mL lanatoside C CRS solution in methyl alcohol.

Solution (4): 0.3 mg/mL lanatoside C CRS solution in methyl alcohol.

Solution (5): 0.2 mg/mL lanatoside C CRS solution in methyl alcohol.

Solution (6): 0.1 mg/mL lanatoside C CRS solution in methyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Spray with 5% sulfuric acid (v/v) in ethyl alcohol. In the chromatogram obtained with *Solution (1)*, no secondary stain is more intense than the main stain obtained in the chromatogram with *Solution (4)* (1.5%). Not more than three secondary stains are more intense than the main stain obtained in the chromatogram with *Solution (6)* (0.5%) and not more than one of these stains is more intense than the main stain obtained with *Solution (5)* (1.0%).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample, in oven at 105 °C, under reduced pressure, over phosphorus pentoxide until constant weight. Not more than 7.5%.

Residue by incineration (5.2.10). Determine in 0,1 g of the sample. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Visible absorption spectrophotometry* (**5.2.14**). Accurately weigh about 50 mg of the sample and dissolve in ethyl alcohol. Dilute to 50 mL with the same solvent. Dilute with ethyl alcohol until concentration of 0.005% (w/v). Prepare a standard solution in the same concentration, using the same solvent. To 5 mL of each diluted solution, add 3 mL of alkaline sodium picrate RS and allow to stand in water bath at temperature between 19 °C and 21 °C for 40 minutes, protected from light. Measure the absorbances of the solutions at 484 nm, using mixture of 5 mL of ethyl alcohol and 3 mL of alkaline sodium picrate RS solution for zero adjustment. Calculate the content of $C_{49}H_{76}O_{20}$ in the sample from the readings obtained.

PACKAGING AND STORAGE

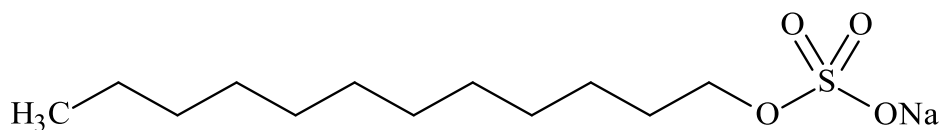
In tightly closed glass containers, protected from light, stored at temperature below 10 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Cardiotonic glycoside.

SODIUM LAURYL SULFATE*Natrii laurilsulfas* $C_{12}H_{25}NaO_4S$; 288.38

sodium lauryl sulfate; 05178

Sulfuric acid monododecyl ester sodium salt (1:1)

[151-21-3]

Sodium lauryl sulfate is a mixture of sodium alkyl sulfates consisting primarily of the sodium salt of sulfuric acid monododecyl ester (1:1). It contains not less than 85.0% of sodium alkyl sulfates, expressed as $C_{12}H_{25}NaO_4S$, in relation to the desiccated substance. The total content of sodium chloride and sodium sulfate is not more than 8.0%.

DESCRIPTION

Physical characteristics. White or slightly yellow powder or crystal. Slight characteristic odor.

Solubility. Freely soluble in water forming an opalescent solution or mixture, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. Dissolve 0.1 g of the sample in 10 mL of water and shake. Abundant foaming is formed.

B. Mix 0.1 mL of the solution obtained in the test **A.** from *Identification* with 0.1 mL of 0.1% (w/v) methylthioninium chloride and 2 mL of diluted sulfuric acid RS. Add 2 mL of methylene chloride and shake. Intense blue color develops in the methylene chloride layer.

C. Mix approximately 10 mg of the sample with 10 mL of ethyl alcohol. Heat to boiling in water bath, shaking frequently. Immediately filter and evaporate the ethyl alcohol. Dissolve the residue in 8 mL of water, add 3 mL of hydrochloric acid RS, evaporate the solution to half its volume and allow to cool. Separate by filtering the solidified fatty alcohols. Add 1 mL of 6.1% (w/v) barium chloride to the filtrate. A white crystalline precipitate is produced.

D. A 10% (w/v) sample solution meets sodium ion reactions **(5.3.1.1)**.

E. A 10% (w/v) sample solution acidified with hydrochloric acid and gently boiled for 20 minutes meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

Alkalinity. Accurately weigh about 1 g of the sample and dissolve in 100 mL of carbon dioxide-free water. Add 0.1 mL of phenol red TS and titrate with 0.1 M hydrochloric acid VS. Not more than 0.6 mL of 0.1 M hydrochloric acid VS should be used.

Non-esterified alcohol limit. Accurately weigh about 10 g of the sample and dissolve in 100 mL of water, add 100 mL of ethyl alcohol and extract the solution three times with 50 mL of pentane each. If necessary, add sodium chloride to facilitate separation of the two phases. Collect the organic phases and wash three times with 50 mL of water each. Eliminate water from the organic solution with anhydrous sodium sulfate, filter and evaporate in water bath until solvent is fully removed. Heat the residue at 105 °C for 15 minutes and cool. The residue mass must be not more than 4.0%.

Total alcohol limit. Accurately weigh approximately 5g of the sample into an 800 mL Kjeldahl flask. Add 150 mL of water, 50 mL of hydrochloric acid and some glass boiling beads. Attach the Kjeldahl flask in a reflux condenser. Heat carefully to avoid excessive foaming and boil for four hours. Cool, wash the condenser with ethyl ether, collecting the ethyl ether into the flask, and transfer the content to a separating funnel. Wash the flask twice with ethyl ether and add the washings to the separating funnel. Extract the solution with two 75 mL portions of ethyl ether. In a previously weighed beaker, collect the ether combined extracts, evaporate in water bath and dry the residue at 105 °C for 30 minutes. Cool and weigh. The residue represents the total of alcohols. The residue mass must be not less than 59.0% of the sample mass used.

Heavy metals (5.3.2.3). Use *Method III*. Accurately weigh about 1 g of the sample. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample in oven at 105 °C, for two hours. Not more than 3.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Sodium alkyl sulfates. Accurately weigh about 0.115 g of the sample, dissolve in 20 mL of water, heat if necessary. Transfer to a 100 mL volumetric flask and complete the volume with the same solvent. Remove a 20 mL aliquot from this solution and transfer to a 125 mL erlenmeyer flask, add 15 mL of chloroform and 10 mL of sulfan blue-dimidium bromide RS. Titrate with 0.004 M benzethonium chloride VS, with vigorous agitation, until the chloroform layer pink color turns grayish-blue. Before each titrant addition, check the complete separation of the layers. Each mL of 0.004 M benzethonium chloride VS is equivalent to 1.154 mg of sodium alkyl sulfates, calculated as $C_{12}H_{25}NaO_4S$.

Sodium chloride. Accurately weigh about 0.5 g of the sample and dissolve in 50 mL of water. Add diluted nitric acid (1:20), dropwise, until the solution is neutral to the litmus paper. Add 2 mL of potassium chromate RS and titrate with 0.1 M silver nitrate VS. Each mL of 0.1 M silver nitrate VS is equivalent to 5.844 mg of sodium chloride.

Sodium sulfate. Accurately weigh about 1 g of sample and transfer to a 250 mL beaker. Add 35 mL of water, heat to dissolve. Add 2 mL of M nitric acid to the heated solution, mix and add 50 mL of ethyl alcohol. Heat the solution to boiling. Slowly add, under agitation, 10 mL of 3.31% (w/v) lead nitrate solution. Cover the beaker with a watch glass, boil gently for five minutes and allow to stand.

If the supernatant is turbid, allow to stand for another 10 minutes, heat to boiling and allow to stand again. When the solution is nearly boiling, decant as much liquid as possible through a 9 cm diameter quantitative filter paper, black band, fast filtration, ash-free. Wash four times by decanting, using each time 50 mL of 50% ethyl alcohol (v/v) and bring the mixture to boiling. Transfer the filter paper to the original beaker and immediately add 30 mL of water, 20 mL of 0.05 *M* disodium edetate VS and 1 mL of ammonium chloride buffer pH 10.7. Heat until precipitate dissolves. Allow to cool. Add 0.2 mL of eriochrome black T TS and titrate with 0.05 *M* zinc sulfate VS. Each mL of 0.05 *M* disodium edetate is equivalent to 7.102 mg of sodium sulfate.

PACKAGING AND STORAGE

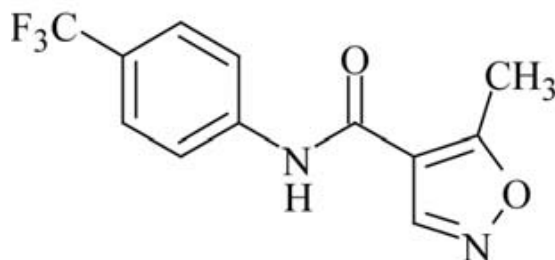
In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Anionic surfactant.

LEFLUNOMIDE*Leflunomidum*

C₁₂H₉F₃N₂O₂; 270.21

leflunomide; 05192

5-Methyl-*N*-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide
[75706-12-6]

It contains not less than 98.0% and not more than 102.0% of C₁₂H₉F₃N₂O₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder. Presents polymorphism.

Solubility. Practically insoluble in water, soluble in methyl alcohol, in ethyl alcohol and in isopropyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 165 °C to 167 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the leflunomide CRS spectrum.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 370 nm, of the 0.001% (w/v) sample solution in mixture of acetonitrile and water (50:50), there is a maximum point at 260 nm, identical to the one observed in the spectrum of similar leflunomide CRS solution.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel 60 F₂₅₄ as support, and mixture of methylene chloride and ethyl acetate (97:3) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): 0.1 mg/mL sample solution in ethyl acetate.

Solution (2): 0.1 mg/mL solution of leflunomide CRS in ethyl acetate.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm) or expose plate to iodine vapors. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

D. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for two hours. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at 25 °C; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water and acetonitrile (50:50).

Sample solution: transfer 20 mg of the sample, accurately weighed, to a 100 mL volumetric flask, with the aid of 60 mL of *Mobile phase*. Shake, if necessary, complete the volume with the same solvent and homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask and complete the volume with *Mobile Phase* (inject this solution immediately or within 24 hours after preparation, if stored under refrigeration).

Standard solution: dissolve an accurately weighed quantity of leflunomide CRS in *Mobile Phase* to obtain a solution at 40 µg/mL (inject this solution immediately or within 24 hours after preparation, if stored under refrigeration).

Inject 20 µL replicates of the *Standard solution*. The column efficiency must be not less than 3000 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₂H₉F₃N₂O₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In hermetic containers, protected from light and in refrigerator.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-rheumatic.

LEFLUNOMIDE TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{12}H_9F_3N_2O_2$. The tablets must be coated.

IDENTIFICATION

A. Weigh and pulverize the tablets. Use quantity of powder equivalent to 10 mg of leflunomide and transfer to a 100 mL volumetric flask, with the aid of 60 mL of mixture with acetonitrile and water (50:50). Shake for 10 minutes and complete the volume with the same solvent. Homogenize and filter. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with mixture of acetonitrile and water (50:50). In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 370 nm of this solution, there is maximum point at 260 nm, identical to that observed in the leflunomide CRS spectrum, prepared identically.

B. Weigh and pulverize the tablets. Use quantity of powder equivalent to 5 mg of leflunomide, dissolve in 50 mL of ethyl acetate, homogenize and filter. Proceed as described in test **C.** from *Identification* in the *Leflunomide* monograph.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: de-aerated water, 1000 mL, for tablets containing 10 or 20 mg.

Apparatus: paddles, 100 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium and immediately filter in a filter with a porosity of 0.45 μ m and dilute, if necessary, with the *Dissolution medium*, until appropriate concentration. Measure the solutions absorbances at 260 nm **(5.2.14)**, using water for zero adjustment. Calculate the quantity of $C_{12}H_9F_3N_2O_2$ dissolved in the medium, comparing the readings obtained with the leflunomide CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent. Acetonitrile can be used to dissolve leflunomide CRS in a volume not exceeding 2% in the final solution.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{12}H_9F_3N_2O_2$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in the *Assay* method in the *Leflunomide* monograph. Prepare *Sample solution* as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 10 mg of leflunomide and transfer to a 50 mL volumetric flask with the aid of 30 mL of *Mobile phase*. Shake, mechanically for 15 minutes, complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

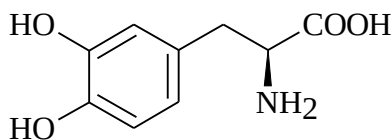
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{12}H_9F_3N_2O_2$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

LEVODOPA***Levodopum***

C₉H₁₁NO₄; 197.19
levodopa; 05249
3-Hydroxy-L-tyrosine
[59-92-7]

It contains not less than 98.0% and not more than 102.0% of C₉H₁₁NO₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water, practically insoluble in ethyl alcohol. Freely soluble in *M* hydrochloric acid and moderately soluble in 0.1 *M* hydrochloric acid.

Physicochemical constants.

Optical rotation (5.2.8): -1,27° to -1,34°, in relation to the desiccated substance. Dissolve 0.2 g of the sample and 5 g of methenamine in 10 mL of *M* hydrochloric acid. Dilute to 25 mL with the same acid and homogenize. Store the solution protected from light (25 °C) for three hours before determination.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the levodopa CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 230 nm to 350 nm, of the 0.003% (w/v) solution in 0.1 *M* hydrochloric acid, there is a maximum point at 280 nm, identical to that observed in the spectrum of similar levodopa CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

pH (5.2.19). 4.5 to 7.0. Determine in suspension at 1% (w/v) in carbon dioxide-free water, obtained after 15 minutes of shaking the sample with the solvent.

Light absorption. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 230 nm to 350 nm, of 0,003% (w/v) solution in 0.1 M hydrochloric acid, there is a maximum point at 280 nm. The specific absorptivity, A(1%, 1 cm), is 137 to 147, at 280 nm, in 0.1 M hydrochloric acid.

Related substances. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using cellulose plate as support, and mixture of glacial acetic acid, water and butyl alcohol (25:25:50) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): Dissolve 0.1 g of the sample in 5 mL of anhydrous formic acid and dilute to 10 mL with methyl alcohol. Prepare extemporaneously.

Solution (2): transfer 0.5 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with methyl alcohol.

Solution (3): dissolve 30 mg of tyrosine in 1 mL of anhydrous formic acid and dilute to 100 mL with methyl alcohol. Mix 1 mL of this solution with 1 mL of *Solution (1)*.

Develop chromatogram. Remove plate and allow to dry under hot air. Spray with a freshly prepared mixture of ferric chloride RS and potassium ferricyanide RS (1:1). Examine immediately. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.5%). The test will only be valid if the chromatogram obtained with *Solution (3)* presents, above the main stain, a distinct stain, more intense than the stain in the chromatogram obtained with *Solution (2)*.

Heavy metals (5.3.2.3). Use *Method III*. Prepare the standard using 2 mL of *Standard lead solution* (10 ppm Pb). Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105 °C for four hours. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium* (**5.3.3.5**). Accurately weigh about 0.18 mg of the sample and dissolve in 5 mL of anhydrous formic acid. Heat if necessary. Allow to cool and add 25 mL of glacial acetic acid and 25 mL of dioxane. Titrate with 0.1 M perchloric acid VS, using 0.1 mL of methylrosaniline chloride TS, until color turns green. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 19.719 mg of C₉H₁₁NO₄.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Carry out the procedure protected from light and keep the solutions at a temperature of 10 °C until injection. Use chromatograph equipped with ultraviolet detector at 280 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Diluent: mixture of trifluoroacetic acid and water (1:1.000).

Mobile phase: mixture of *Diluent* and tetrahydrofuran (97:3).

Sample solution: dissolve an accurately weighed quantity of the sample in *Diluent* to obtain a 0.4 mg/mL solution.

Standard solution: dissolve an accurately weighed quantity of levodopa CRS in *Diluent* to obtain a 0.4 mg/mL solution.

Resolution solution: dissolve accurately weighed quantity of levodopa SQR and levotyrosine CRS in *Diluent* to obtain a 10 µg/mL solution of each substance.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are approximately 1.0 for levodopa and 1.3 for levotyrosine. The resolution between the levotyrosine and levodopa peaks is not less than 3.0. The tailing factor for the levodopa peak is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₉H₁₁NO₄ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

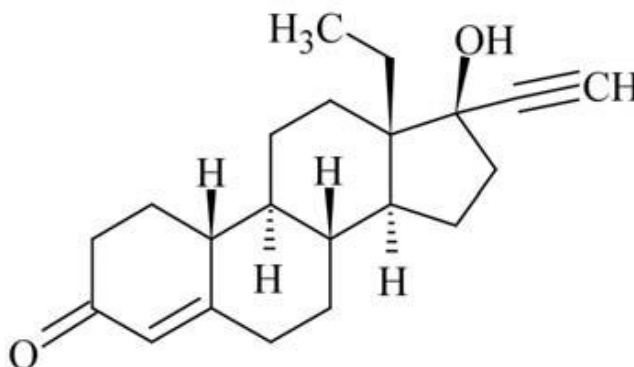
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiparkinsonian.

LEVONORGESTREL*Levonorgestrelum* $C_{21}H_{28}O_2$; 312.45

levonorgestrel; 05279

(17 α)-13-Ethyl-17-hydroxy-18,19-dinorpregn-4-en-20-in-3-one
[797-63-7]

It contains not less than 98.0% and not more than 102.0% of $C_{21}H_{28}O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 232 °C to 239 °C. The interval between the beginning and the end of the melting does not exceed 4 °C.

Specific optical rotation (5.2.8): -30 to -35. Determine in solution at 2% (w/v) in chloroform.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the levonorgestrel CRS spectrum, prepared identically.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of acetone and chloroform (20:80) as mobile phase. Apply to the plate, separately, 10 μ L of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.5 g of the sample in chloroform and dilute to 25 mL with the same solvent.

Solution (2): transfer 2.5 mL of *Solution (1)* to a 25 mL volumetric flask and complete the volume with chloroform. Transfer 2.5 mL of this solution to a 50 mL volumetric flask and complete the volume with the same solvent.

Solution (3): transfer 10 mL of *Solution (2)* to a 25 mL volumetric flask and complete the volume with chloroform.

Solution (4): dissolve 5 mg of levonorgestrel CRS and 5 mg of ethinylestradiol CRS in chloroform and dilute to 50 mL with the same solvent.

Develop chromatogram. Remove plate and allow to air dry. Spray with 10% (w/v) phosphomolybdic acid in *n*-propyl alcohol. Heat the plate at 100 °C and 105 °C for 15 minutes and examine immediately. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.5%), and not more than two of these stains are more intense than that obtained with *Solution (3)* (0.2%). The test is only valid if the chromatogram obtained with *Solution (4)* presents two clearly separated stains.

Ethinyl limit. Proceed as described in method **A.** from Assay. Each mL of 0.1 M sodium hydroxide VS is equivalent to 2.503 mg of ethinyl. Not less than 7.81% and not more than 8.18%.

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105 °C for five hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh about 0.2 g of the sample and dissolve in 45 mL of tetrahydrofuran. Add 10 mL of 10% (w/v) silver nitrate to water. After one minute, titrate with 0.1 M sodium hydroxide VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M sodium hydroxide VS is equivalent to 31.245 mg of C₂₁H₂₈O₂.

B. By *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 100 g of the sample and dissolve in ethyl alcohol. Dilute, successively, in ethyl alcohol until concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 241 nm, using ethyl alcohol for zero adjustment. Calculate the content of C₂₁H₂₈O₂ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Contraceptive.

LEVONORGESTREL AND ETHINYLESTRADIOL TABLETS

It contains not less than 90.0% and not more than 110.0% of the declared quantity of levonorgestrel ($C_{21}H_{28}O_2$) and ethinylestradiol ($C_{20}H_{24}O_2$).

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and mixture of methyl alcohol and chloroform (1:99) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize 15 tablets and extract with 30 mL of acetone. Filter and evaporate to dryness. Dissolve the residue obtained with 1 mL of chloroform.

Solution (2): prepare 0.75 mg/mL solution of levonorgestrel CRS in chloroform.

Solution (3): prepare 0.45 mg/mL solution of ethinylestradiol CRS in chloroform.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The stains related to levonorgestrel and ethinylestradiol obtained with *Solution (1)* correspond in position and color to the main ones obtained with *Solutions (2)* and *(3)*. Spray with 2% (w/v) *p*-toluenesulfonic acid in water. Heat at 110 °C for 10 minutes. Examine under ultraviolet light (365 nm). The stains related to levonorgestrel and ethinylestradiol are blue.

B. The retention times of the main peaks of the *Sample solution* chromatogram, obtained in the *Assay*, correspond to the ones from the main peaks of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.0005% (w/v) polysorbate 80 solution in water, 500 mL.

Apparatus: paddles, 75 rpm.

Time: 60 minutes.

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 247 nm (for determination of levonorgestrel) and with spectrofluorometric detector with excitation wavelengths at 285 nm and emission wavelengths at 310 nm (for determination of ethinylestradiol); column with 150 mm in length and 4.0 mm in

internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm to 10 µm), kept at room temperature; *Mobile Phase* flow rate of 1.0 mL/minute..

Mobile phase: mixture of acetonitrile and water (60:40).

Sample solution: after the test, remove aliquot from the dissolution medium, filter in polyvinylidene filter, discarding the first milliliters. For uncoated tablets, remove aliquots from the dissolution medium at periods of 30 minutes (taking care to replenish the volume of each tank) and 60 minutes. For dragees, carry out this procedure only at period of 60 minutes.

Standard solution: prepare a solution containing levonorgestrel CRS and ethinylestradiol CRS in *Dissolution medium*, to obtain concentrations close to those of levonorgestrel and ethinylestradiol, respectively, in the *Sample solution*.

Inject 100 µL replicates of the *Standard solution*. The relative retention times are approximately 0.7 for ethinylestradiol and 1.0 for levonorgestrel. The relative standard deviation of the replicate areas under the peaks recorded is not more than 3.0%.

Procedure: inject, separately, 100 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of levonorgestrel (C₂₁H₂₈O₂) and ethinylestradiol (C₂₀H₂₄O₂) dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: for uncoated tablets, not less than 80% (Q) of the stated quantity of levonorgestrel (C₂₁H₂₈O₂) and 75% (Q) of the stated quantity of ethinylestradiol (C₂₀H₂₄O₂) dissolve in 60 minutes. For dragees, not less than 60% (Q) of the stated quantity of levonorgestrel (C₂₁H₂₈O₂) and 60% (Q) of the stated quantity of ethinylestradiol (C₂₀H₂₄O₂) dissolve in 60 minutes. When tablet coating does not have the function of modifying the release of actives, the same recommended tolerance for uncoated tablets can be adopted.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 215 nm; 150 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of water and acetonitrile (51:49).

Diluent: mixture of water and acetonitrile (40:60).

Sample solution: weigh and pulverize 20 to 30 tablets. Transfer quantity of powder equivalent to 250 µg of levonorgestrel to a centrifuge tube and add 4 mL of *Diluent*. Heat at 60 °C for 25 minutes, shake and place in ultrasonic bath for another 25 minutes. Cool, centrifuge and use the clear

supernatant.

Standard solution: prepare a solution of levonorgestrel CRS and ethinylestradiol CRS in the *Diluent* containing, respectively, 0.625 mg and 0.125 mg/mL. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with the *Diluent*, obtaining a solution with 62.5 µg/mL of levonorgestrel and 12.5 µg/mL of ethinylestradiol.

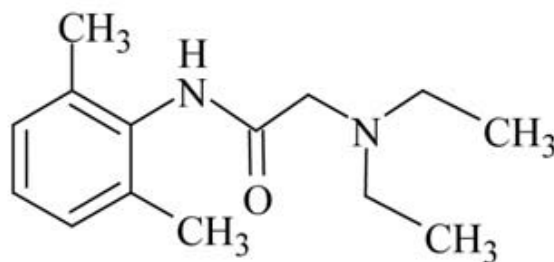
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of levonorgestrel ($C_{21}H_{28}O_2$) and ethinylestradiol ($C_{20}H_{24}O_2$) in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

LIDOCAINE*Lidocainum*C₁₄H₂₂N₂O; 234.34

lidocaine ;05313

2-(Diethylamino)-N-(2,6-dimethylphenyl)acetamide

[137-58-6]

It contains not less than 99.0% and not more than 101.0% of C₁₄H₂₂N₂O in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, very soluble in ethyl alcohol. Soluble in diluted hydrochloric acid.

Physicochemical constants.

Melting range (5.2.2): 66 °C to 70 °C.

IDENTIFICATION

The identification tests B., C. and D. can be omitted if test A. is carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the lidocaine CRS spectrum, prepared identically.

B. Dissolve, with heating, 0.2 g of the sample in mixture with 0.5 mL of diluted hydrochloric acid and 10 mL of water. Add 10 mL of 1% picric acid (w/v). The precipitate, washed with water and dried, has a melting temperature (5.2.2) around 230 °C, with decomposition.

C. To about 5 mg of the sample, add 0.5 mL of fuming nitric acid. Evaporate to dryness in water bath, cool and dissolve the residue in 5 mL of acetone. Add 0.2 mL of 0.5 M ethanolic potassium hydroxide. Green color develops.

D. Dissolve about 0.1 g of the sample in 1 mL of ethyl alcohol and add 0.5 mL of a 10% (w/v) cobalt nitrate solution. A blueish-green precipitate is produced.

PURITY TESTS

Preparation aspect. Dissolve 1 g of the sample in 3 mL of hydrochloric acid and dilute to 10 mL with water. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

Limit of 2,6-dimethylaniline. Dissolve 0.25 mg of the sample in methyl alcohol and dilute to 10 mL with the same solvent. To 2 mL of the above solution, add 1 mL of 1% (w/v) *p*-dimethylaminobenzaldehyde in methyl alcohol and 2 mL of glacial acetic acid. Allow to stand for 10 minutes. Any yellow coloration in the solution under test is not more intense than that of a reference solution, prepared simultaneously, using 2 mL of 2,6-dimethylaniline 0.00025% (w/v) in methyl alcohol. Not more than 0.002% (20 ppm).

Chlorides (5.3.2.1). Dissolve 1.4 g of the sample in mixture with 3 mL of nitric acid and 12 mL of water and proceed as described in *Limit test for chlorides*. Not more than 0.0035% (35 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Determine in 1 g of the sample. Not more than 0.002% (20 ppm).

Sulfate (5.3.2.2). Dissolve 0.5 g of the sample in 5 mL of ethyl alcohol and dilute to 25 mL with water. Not more than 0.1% (1000 ppm).

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

Water (5.2.20.1). Determine in 1 g of sample. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 0.2 g of the desiccated sample, under reduced pressure, on silica gel for 24 hours in 50 mL of glacial acetic acid and shake until complete dissolution. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid is equivalent to 23.434 mg of $C_{14}H_{22}N_2O$.

PACKAGING AND STORAGE

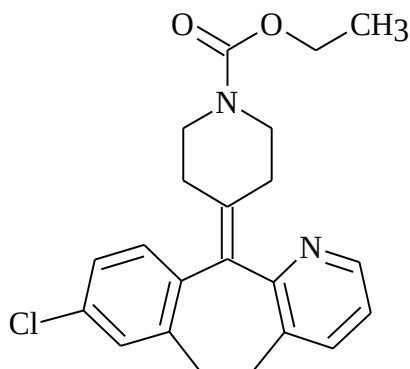
In hermetic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Local anesthetic.

LORATADINE**Loratadinum**C₂₂H₂₃ClN₂O₂; 382.89

loratadine; 05416

4-(8-Chloro-5,6-dihydro-11Hbenzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylic acid ethyl ester

[79794-75-5]

It contains not less than 98.5% and not more than 102.0% of C₂₂H₂₃ClN₂O₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder. Presents polymorphism.

Solubility. Insoluble in water, freely soluble in methyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 132 °C to 137 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the loratadine CRS spectrum, prepared identically. If the spectra obtained are not identical, dissolve the substances separately in acetone and evaporate to dryness. Obtain new spectra with the residues.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances.

Note: according to the synthesis route, carry out Test 1 or Test 2. Test 2 is recommended if 4,8-dichloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-one is a potential related substance.

Test 1. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm), kept between 25 °C and 35 °C; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of 0.01 M anhydrous dibasic potassium phosphate, methyl alcohol and acetonitrile (7:6:6). Adjust with phosphoric acid to pH 7.2.

Diluent: transfer 400 mL of 0.05 M hydrochloric acid and 80 mL of anhydrous 0.6 M potassium phosphate dibasic to a 1000 ml volumetric flask and complete the volume with mixture of methyl alcohol and acetonitrile (1:1). Homogenize.

Solution (1): 0.8 µg/mL solution of loratadine CRS in *Diluent*.

Solution (2): quantitatively transfer about 40 g of the sample to a 100 mL volumetric flask. Dissolve, place in ultrasonic bath for 10 minutes and complete the volume with *Diluent*.

The relative retention times are about 0.79 for 4-(8-chloro-11-fluoro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ethyl)-1-piperidinecarboxylate and 1.00 for loratadine. The relative standard deviation of replicate areas under the peaks recorded is not more than 4,0%.

Procedure: inject, separately, 50 µL of each solution, record the chromatograms and measure the areas under all the peaks of *Solution (2)* and that under the main peak of *Solution (1)*. Calculate the percentage of each impurity relative to the area under the main peak of the *Solution (1)* and the response factors for the impurities (the response factor for 4-(8-chloro-11-fluoro-6,11-di-ethyl hydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-yl)-1-piperidinecarboxylate is 0.25). Not more than 0.2% of 4-(8-chloro-11-fluoro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-yl)-Ethyl 1-piperidine carboxylate, 0.1% of individual impurities and 0.3% of total impurities.

Test 2. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow rate of 1.2 mL/minute.

Eluent A: dissolve 0.96 g of sodium 1-pentanesulfonate monohydrate in 900 mL of water. Adjust with 10% phosphoric acid (v/v) to pH 3.00 ± 0.05 and dilute with water to 1000 mL.

Eluent B: use acetonitrile.

Mobile Phase gradient: adopt the gradient system described in the following table.

<i>Time (minutes)</i>	<i>Eluent (A) (%)</i>	<i>Eluent (B) (%)</i>	<i>Elution</i>
0	75	25	isocratic
0 – 20	75 → 50	25 → 50	linear gradient
20 – 30	50 → 40	50 → 60	linear gradient

30 – 35	40 → 30	60 → 70	linear gradient
35 – 45	30	70	isocratic
45 – 50	75	25	isocratic

Solution (1): dissolve accurately weighed quantities of loratadine CRS, 8-chloro-6,11-dihydro-11-(4-piperidylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine CRS (loratadine compound related CRS) and 8-chloro-6,11-dihydro-11-(*N*-methyl-4-piperynylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine CRS (loratadine related compound B CRS) in methyl alcohol to obtain a 0.1 mg/mL solution of each compound. Transfer 1 mL of this solution to a 10 mL volumetric flask, add 2 mL of *Eluent A* and complete the volume with methyl alcohol.

Solution (2): weigh, accurately, about 0.1 g of the sample and transfer to a 10 mL volumetric flask. Add 2 mL of methyl alcohol and shake until dissolved. Add 2 mL of *Eluent A* and complete the volume with methyl alcohol.

Inject 20 µL replicates of the *Solution (1)*. The resolution between the peak of loratadine related compound A and loratadine related compound B is not less than 1.5. The relative standard deviation of the areas under the loratadine peak in the replicates is not more than 10,0%. The relative retention times and response factors are described in the table below. For unknown impurities, the response factor is 1.00.

<i>Related compound</i>	<i>Relative retention time</i>	<i>Response factor</i>
Loratadine related compound A	0.50	1.00
Loratadine related compound B	0.53	0.89
8-Chloro-6,11-dihydro-5 <i>H</i> -benzo[5,6]cyclohepta[1,2- <i>b</i>]pyridin-11-one	0.70	0.60
8-Chloro-6,11-dihydro-11-[<i>N</i> -methyl-4-piperidiny]11-hydroxy-5 <i>H</i> -benzo[5,6]cyclohepta[1,2- <i>b</i>]pyridine	0.75	0.46
4,8-Dichloro-6,11-dihydro-5 <i>H</i> -benzo[5,6]cyclohepta[1,2- <i>b</i>]pyridin-11-one	1.23	0.92
8-Chloro-6,11-dihydro-11-[<i>N</i> -ethoxycarbonyl-4-piperidiny]11-hydroxy-5 <i>H</i> -benzo[5,6]cyclohepta[1,2- <i>b</i>]pyridine	1.60	0.42
4,8-Dichloro-6,11-dihydro-11-[<i>N</i> -ethoxycarbonyl-4-piperidylidene]-5 <i>H</i> -benzo[5,6]cyclohepta[1,2- <i>b</i>]pyridine	1.83	1.08
Loratadine	1.00	1.00

Procedure: inject, separately, 20 µL of each solution, record the chromatograms and measure the areas under the peaks. Not more than 0.1% of loratadine related compound A, 0.1% of loratadine related compound B, 0.1% of each individual impurity and 0.3% of total impurities.

Heavy metals (5.3.2.3). Proceed as described in *Method III*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 100 °C, until constant weight. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 0.3 g of the sample in 50 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 38.289 mg of $C_{22}H_{23}ClN_2O_2$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 μ m), kept between 25 °C and 35 °C; *Mobile phase* flow rate of 1.0mL/minute.

Mobile phase: mixture of 0.01 M anhydrous potassium phosphate dibasic, methyl alcohol and acetonitrile (7:6:6). Adjust with phosphoric acid to pH 7.2.

Diluent: transfer 400 mL of 0.05 M hydrochloric acid and 80 mL of anhydrous 0.6 M potassium phosphate dibasic to a 1000 mL volumetric flask and complete the volume with mixture of methyl alcohol and acetonitrile (1:1). Homogenize.

Sample solution: quantitatively transfer about 40 mg of the sample to a 100 mL volumetric flask. Dissolve, place in ultrasonic bath for 10 minutes and complete the volume with *Diluent*. Homogenize.

Standard solution: 0.4 mg/mL loratadine CRS solution in *Diluent*.

Inject 15 μ L replicates of the *Standard solution*. The relative standard deviation of replicate areas under the peaks recorded is not more than 2,0%.

Procedure: inject, separately, 15 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{22}H_{23}ClN_2O_2$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antihistamine.

LORATADINE TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of ethyl ether and diethylamine (40:1), as mobile phase. Apply to the plate, separately, 5 μ L of each of the freshly prepared solutions described below.

Solution (1): transfer quantity of powder from the tablets equivalent to approximately 20 mg of loratadine to a centrifuge tube. Add 5 mL of mixture of chloroform and methyl alcohol (1:1), shake for 30 minutes and centrifuge.

Solution (2): 4 mg/mL solution of loratadine CRS in mixture of chloroform and methyl alcohol (1:1).

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 60 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 280 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the quantity of $C_{22}H_{23}ClN_2O_2$ dissolved in the medium, comparing the readings obtained with that of the loratadine CRS solution at a concentration of 0.001% (w/v) prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{22}H_{23}ClN_2O_2$ dissolves in 60 minutes.

PURITY TESTS

Related substances. Proceed as described in method **B.** of Assay in the *Loratadine* monograph. Prepare solutions as described below.

Solution (1): use the *Sample Solution* described in Assay of this monograph (tablets).

Solution (2): transfer 5 mL of the *Standard solution* to a 100 mL volumetric flask, complete the volume with *Diluent* and homogenize. Dilute this solution to obtain a concentration of 0.8 µg/mL of loratadine CRS.

Inject 50 µL replicates of the *Solution (1)*. The relative retention times are about 0.79 for 4-(8-chloro-11-fluoro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ethyl)-1-piperidinecarboxylate and 1.0 for loratadine. The relative standard deviation of the replicate areas under the loratadine peak is not more than 4.0%.

Procedure: inject, separately, 50 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Not more than 0.2% of 4-(8-chloro-11-fluoro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl)- Ethyl 1-piperidine carboxylate. Not more than 0.1% of any individual impurity. The sum of all impurities, except 4-(8-chloro-11-fluoro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-yl) Ethyl -1-piperidine carboxylate, is not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in method **B.** of Assay in the *Loratadine* monograph. Prepare *Sample solution* as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 100 g of loratadine to a 250 mL volumetric flask. Add 100 mL of 0.05 M hydrochloric acid and shake for 40 minutes. Add 75 mL of a mixture of methyl alcohol and acetonitrile (1:1) and homogenize. Add 20 mL of 0.6 M anhydrous potassium phosphate dibasic and homogenize for five minutes. Complete the volume with mixture of methyl alcohol and acetonitrile (1:1) and homogenize.

Inject 15 µL replicates of the *Standard solution*. The retention factor is not less than 3.5. The tailing factor is not more than 1.7. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 15 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₂H₂₃ClN₂O₂ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers at temperature of 2 °C to 30 °C.

LABELING

Comply with current legislation.

LORATADINE ORAL SOLUTION

It contains not less than 94.0% and not more than 105.0% of the stated quantity of loratadine ($C_{22}H_{23}ClN_2O_2$).

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of ethyl ether and diethylamine (40:1), as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): transfer volume of the oral solution equivalent to 10 mg of loratadine to a centrifuge tube. Add 10 mL of 0.2 M sodium hydroxide and 2 mL of methylene chloride. Shake for 10 minutes. Centrifuge. Use the organic phase.

Solution (2): 5 mg/mL loratadine CRS solution in methylene chloride.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 2.5 to 3.1.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm), kept between 30 °C and 40 °C; *Mobile phase* flow rate of 2.0 mL/minute.

Mobile phase: 0.015 M sodium lauryl sulfate solution in a mixture of water and acetonitrile (1:1). Adjust pH to 2.6± 0.1 with phosphoric acid.

Diluent: mixture of *Mobile phase* and water (2:1).

Solution (1): 0.002 mg/mL loratadine CRS solution in *Diluent*.

Solution (2): transfer 5 mL of *Solution (1)* to a 50 mL volumetric flask and complete the volume with *Diluent*.

Solution (3): transfer volume of the oral solution containing the equivalent to 20 mg of loratadine to a glass flask with cap. Add 1 mL of a 3% (w/v) hydrogen peroxide solution and homogenize. Cap

the flask and heat at 65°C for 18 to 24 hours. Cool to room temperature. Transfer 5 mL to a 25 mL volumetric flask and complete the volume with *Diluent*.

Solution (4): transfer volume of the oral solution containing the equivalent to 5 mg of loratadine to a 25 mL volumetric flask and complete the volume with *Diluent*. Homogenize.

Inject 50 µL replicates of the *Solution (3)*. The relative retention times are about 0.70 for 4-(8-chloro-5,6-dihydro-4-(hydroxymethyl)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ethyl-ylidene)-1-piperidinecarboxylate, 0.84 for 4-[8-chloro-5,6-dihydro-2-(hydroxymethyl)-11H-benzo[5,6]cyclohepta[1,2-b]ethyl]pyridine-11-ylidene]-1-piperidinecarboxylate and 1.0 for loratadine. The resolution between the peaks of loratadine and 4-[8-chloro-5,6-dihydro-2-(hydroxymethyl)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene Ethyl]-1-piperidinecarboxylate is not less than 3.0. Inject 50 µL replicates of the *Solution (1)*. The tailing factor for the loratadine peak is between 0.7 and 1.1. Inject 50 µL replicates of the *Solution (2)*. The relative standard deviation of the replicate areas under the loratadine peak is not more than 10.0%.

Procedure: inject 50 µL of *Solution (4)*, record the chromatograms and measure the areas under the peaks. Not more than 0.3% of 4-(8-chloro-5,6-dihydro-4-(hydroxymethyl)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene Ethyl)-1-piperidinecarboxylate. Not more than 0.3% of 4-(8-chloro-5,6-dihydro-2-(hydroxymethyl)-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene)-ethyl 1-piperidinecarboxylate. Not more than 0.2% of any other individual impurity and the sum of all impurities is not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use a chromatograph equipped with an ultraviolet detector at 254 nm; 300mm long, 4 mm internal diameter column, packed with silica chemically bonded to phenyl group (10 µm), kept between 20 °C and 30 °C; *Mobile phase* flow rate of 2.0 mL/minute.

0.05 M potassium phosphate monobasic: transfer approximately 6.8 g of potassium phosphate monobasic to a 1000 mL volumetric flask. Dissolve and complete the volume with water and homogenize. Adjust pH to 3.0± 0.1 with phosphoric acid.

Mobile phase: mixture of 0.05 M monobasic potassium phosphate and acetonitrile (7:3).

Internal standard solution: 0.3 mg/mL butylparaben solution in mixture of water and acetonitrile (7:3).

Sample solution: transfer volume of the oral solution containing the equivalent to 5 mg of loratadine to a 50 mL volumetric flask. Add 5 mL of the *Internal standard solution* and complete the volume with mixture of water and acetonitrile (7:3). Homogenize.

Standard solution: 1 mg/mL loratadine CRS solution in acetonitrile. Transfer 5 mL of this solution to a 50 mL volumetric flask and add 5 mL of the *Internal Standard Solution* and 12 mL of water. Complete the volume with mixture of water and acetonitrile (7:3). Homogenize.

Inject 10 µL replicates of the *Standard solution*. The relative retention times are about 0.78 for butylparaben and 1.0 for loratadine. The resolution between loratadine and butylparaben is not less than 1.9. The tailing factor is not more than 1.6. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject 10 µL of the *Standard Solution* and the *Sample Solution* separately, record the chromatograms and measure the areas under the peaks corresponding to loratadine and butylparaben. Calculate the quantity of C₂₂H₂₃ClN₂O₂ in the oral solution from the responses obtained for the loratadine/butylparaben ratio with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

LORATADINE AND PSEUDOEPHEDRIN SULFATE ORAL SOLUTION

It contains not less than 94.0% and not more than 105.0% of the declared quantities of loratadine ($C_{22}H_{23}ClN_2O_2$) and pseudoephedrine sulfate ($(C_{10}H_{15}NO)_2 \cdot H_2SO_4$).

IDENTIFICATION

The relation between the retention times of the main peaks and the peak of the internal standard in the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the relation between the retention times of the main peaks and the peak of the internal standard in the *Standard solution* chromatogram.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 247 nm; column with 300 mm in length and 3.9 mm in internal diameter, packed with silica chemically bonded to octadecylsilane group (10 μ m), kept at a temperature between 20 °C and 30 °C; *Mobile Phase* flow rate of 2 mL/minute.

Solution A: dissolve 3 g of monobasic ammonium phosphate in a mixture of water, methyl alcohol and phosphoric acid (150:110:1).

Mobile phase: mixture of *Solution A* and acetonitrile (60:40).

Internal standard solution: 0.1 mg/mL butylparaben solution in *Mobile Phase*.

Sample solution: transfer volume of the oral solution equivalent to 5 mg of loratadine to a 50 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 2 mL of the obtained solution to a 10 mL volumetric flask, add 1 mL of *Internal standard solution* and complete the volume with *Mobile Phase*. Homogenize.

Standard solution: accurately weigh approximately 24 mg of pseudoephedrine sulfate CRS and transfer to a 100 mL volumetric flask. Add 10 mL of loratadine CRS solution at 0.2 mg/mL in *Mobile Phase* and 10 mL of *Internal Standard Solution*. Complete the volume with the *Mobile phase* and homogenize.

Inject 20 μ L replicates of the *Standard solution*. The relative retention times are about 0.4 for pseudoephedrine sulfate, 0.6 for butylparaben, and 1.0 for loratadine. The resolution between the peaks of loratadine and butylparaben is not less than 2.0. The tailing factor is not more than 1.6.

The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

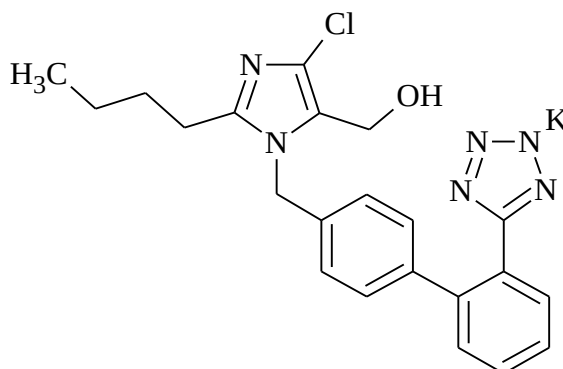
Procedure: inject 20 µL of the *Standard Solution* and the *Sample Solution* separately, record the chromatograms and measure the areas under the peaks corresponding to pseudoephedrine sulfate, butylparaben and loratadine. Calculate the quantities of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ and $C_{22}H_{23}ClN_2O_2$ in the oral solution from the responses obtained for the pseudoephedrine sulfate/butylparaben and loratadine/butylparaben ratios with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

LOSARTAN POTASSIUM*Losartanum kalicum*C₂₂H₂₂ClKN₆O; 461.01

losartan potassium; 05432

Potassium salt of 2-butyl-4-chloro-1-[[2'-(2*H*-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl]-1*H*-imidazol-5- methanol (1:1)
[124750-99-8]

It contains not less than 98.5% and not more than 101.0% of C₂₂H₂₂ClKN₆O, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Soluble in water and ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only at the same wavelengths and with the same relative intensities as those observed in the losartan potassium CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of a 0.001% (w/v) sample solution in methyl alcohol, there is a absorption maximum absorption point identical to that observed in the spectrum of similar losartan potassium CRS solution.

C. It meets potassium ion reactions (**5.3.1.1**).

PURITY TESTS

Limit for cyclohexane and isopropyl alcohol. Proceed as described in *Gas chromatography* (**5.2.17.5**). Use gas chromatograph equipped with flame ionization detector; capillary column with 30 m in length and 0.53 mm in internal diameter, packed with phenyl-methylpolysiloxane (5:95), with a film thickness of 1.5 µm; column temperature according to the following parameters: place at 50 °C for five minutes and increase to 200 °C at 30 °C per minute and keep for five minutes. Keep

the injector and detector temperatures at 220 °C. Use helium as carrier gas at a linear velocity of about 6 mL/minute.

Sample solution: dissolve an accurately weighed quantity of the sample in dimethylformamide to obtain a 50 mg/mL solution.

Standard solution: prepare a solution, in dimethylformamide, containing 0.05 mg/mL of cyclohexane and 0.05 mg/mL of isopropyl alcohol.

Inject 1 µL replicates of the *Standard Solution*. The retention times are approximately two minutes for isopropyl alcohol and four minutes for cyclohexane. The resolution between the cyclohexane and isopropyl alcohol peaks is not less than 4.0. The relative standard deviation of the replicate areas under the recorded peaks is not more than 8.0%.

Procedure: inject, separately, 1 µL of the *Standard Solution* and the *Sample Solution*. Record the chromatograms and measure the areas under the peaks. The areas under the peaks for cyclohexane and isopropyl alcohol obtained in the *Sample Solution* must not be greater than the areas under the peaks for cyclohexane and for isopropyl alcohol obtained in the *Standard Solution*. Not more than 0.1% of cyclohexane and 0.1% of isopropyl alcohol.

Chromatographic purity. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; chromatographic column of 250 mm in length and 4.0 mm in internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile Phase* flow rate of 1 mL/minute.

Eluent A: 0.1% (v/v) phosphoric acid solution in water.

Eluent (B): acetonitrile.

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent (A) (%)</i>	<i>Eluent (B) (%)</i>	<i>Elution</i>
0 – 25	75 → 10	25 → 90	linear gradient
25 – 35	10	90	isocratic
35 – 45	10 → 75	90 → 25	linear gradient
45 – 50	75	25	isocratic

Sample solution: dissolve 30 mg of the sample in methyl alcohol and dilute to 100 mL with the same solvent, obtaining a 300 µg/mL solution.

Resolution solution: dissolve accurately weighed quantity of losartan potassium CRS and triphenylmethanol in methyl alcohol and quantitatively dilute to obtain a solution at 0.3 mg/mL and 0.002 mg/mL, respectively.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are about 1.0 for losartan and 1.9 (about 20 minutes) for triphenylmethanol. The tailing factor for the losartan peak is not more than 1.6.

Procedure: Inject 10 µL of the *Sample Solution*, record the chromatograms and measure the areas of all the peaks obtained. The area of any secondary peak is not more than 0.2% of the total area under the peaks obtained. The sum of the areas under the secondary peaks, except that under the main peak, is not more than 0.5% of the total area under the peaks obtained. Do not include solvent-related peaks in the calculations.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20.1). Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 0.18 g of the sample and dissolve in 50 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS. Determine the endpoint potentiometrically or using 1-naphtholbenzene TS as indicator. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 23.051 mg of $C_{22}H_{22}ClKN_6O$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm, chromatographic column of 250 mm in length and 4.0 mm in internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), maintained at a temperature of 35 °C, *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of 0.1% (v/v) phosphoric acid solution in water and acetonitrile (60:40). Make the necessary adjustments.

Sample solution: dissolve an accurately weighed quantity of the sample in methyl alcohol to obtain a solution at 250 µg/mL.

Standard solution: dissolve an accurately weighed quantity of losartan potassium CRS in methyl alcohol and quantitatively dilute to obtain a solution at 250 µg/mL.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is not less than 4000 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{22}H_{22}ClKN_6O$ in the sample from the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

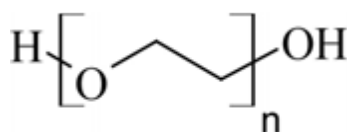
In tightly closed containers at room temperature.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antihypertensive.

MACROGOL***Macrogolum***H(OCH₂CH₂)_nOH

macrogol; 05474

 α -Hydro- ω -hydroxypoly(oxy-1,2-ethanediyl)

[25322-68-3]

Macrogol is an addition polymer of ethylene oxide and water, represented by the formula above, where n is the average number of ethylene oxide groups. The average molecular mass is not less than 95.0% and not more than 105.0% of the labeled nominal value, when this is less than 1000; not less than 90.0% and not more than 110.0% of the labeled nominal value, when it is between 1000 and 7000; and not less than 87.5% and not more than 112.5% of the labeled nominal value when greater than 7000.

DESCRIPTION

Physical characteristics. Clear or slightly turbid liquid, viscous, colorless, slightly hygroscopic and with a slight characteristic odor or an odorless white solid, creamy consistency, in the form of powder or flakes that dissolve in water.

Solubility. Soluble in water, acetone, ethyl alcohol, miscible with other glycols and aromatic hydrocarbons, insoluble in ethyl ether and aliphatic hydrocarbons.

Physicochemical constants.

Viscosity (5.2.7): determine in a capillary viscometer with flow time of not less than 200 seconds and temperature kept at $(98.9 \pm 0.3)^\circ\text{C}$. The viscosity must be within the limits established in **Table 1**, according to the average molecular mass of the sample. For samples whose average molecular mass is not listed in the table, calculate the limits by interpolation.

Table 1 – Viscosity limit for macrogol samples.

<i>Average molecular mass</i>	<i>Viscosity range in centistokes</i>	<i>Average molecular mass</i>	<i>Viscosity range in centistokes</i>
200	3.9 to 4.8	2200	43.0 to 56.0
300	5.4 to 6.4	2300	46.0 to 60.0
400	6.8 to 8.0	2400	49.0 to 65.0
500	8.3 to 9.6	2500	51.0 to 70.0
600	9.9 to 11.3	2600	54.0 to 74.0
700	11.5 to 13.0	2700	57.0 to 78.0
800	12.5 to 14.5	2800	60.0 to 89.0
900	15.0 to 17.0	2900	64.0 to 88.0
1000	16.0 to 19.0	3000	67.0 to 93.0
1100	18.0 to 22.1	3250	73.0 to 105.0

1200	20.0 to 24.5	3350	76.0 to 110.0
1300	22.0 to 27.5	3500	87.0 to 123.0
1400	24.0 to 30.0	3750	99.0 to 140.0
1450	25.0 to 32.0	4000	110.0 to 158.0
1500	26.0 to 33.0	4250	123.0 to 177.0
1600	28.0 to 36.0	4500	140.0 to 200.0
1700	31.0 to 39.0	4750	155.0 to 228.0
1800	33.0 to 42.0	5000	170.0 to 250.0
1900	35.0 to 45.0	5500	206.0 to 315.0
2000	38.0 to 49.0	6000	250.0 to 390.0
2100	40.0 to 53.0	6500	295.0 to 480.0
		7000	350.0 to 590.0
		7500	405.0 to 735.0
		8000	470.0 to 900.0

IDENTIFICATION

Determination of average molecular mass.

Phthalic anhydride solution: add 49 g of phthalic anhydride in an amber erlenmeyer flask and dissolve in 300 mL of freshly distilled pyridine, in the presence of phthalic anhydride. Shake the erlenmeyer flask vigorously until completely dissolved. Add 7 g of imidazole and mix carefully to fully dissolve. Allow the solution to stand for 16 hours before use.

Sample preparation for liquid macrogols: carefully introduce 25 mL of the *Phthalic anhydride solution* into a dry erlenmeyer flask, resistant to pressure and heat. Add to the erlenmeyer quantity of sample accurately weighed, equivalent to its nominal weight divided by 160. Cap the flask and wrap it with a jacket or safety mesh.

Sample preparation for solid macrogols: carefully introduce 25 mL of the *Phthalic anhydride solution* in a dry erlenmeyer flask, resistant to pressure and heat. Add to the flask a quantity of sample, accurately weighed, equivalent to its nominal weight divided by 160 (due to the solubility limit, do not use more than 25 g). Add 25 mL of freshly distilled pyridine in the presence of phthalic anhydride. Shake until effective solution. Cap the erlenmeyer and wrap with a safety jacket.

Procedure: transfer the erlenmeyer flask to water bath at a temperature between 96 °C and 100 °C, so that the height of the bath water corresponds to the height of the liquid inside the erlenmeyer. Remove the erlenmeyer flask from the bath after five minutes, without removing the safety jacket, shake for 30 seconds to ensure homogeneity. Heat for another 30 minutes (60 minutes for macrogol of molecular mass above 3000). Remove the flask from the bath and allow to cool to room temperature. Uncap the flask carefully to remove any pressure. Remove the safety jacket. Add 10 mL of water and shake. Wait two minutes, add 0.5 mL of a mixture of phenolphthalein TS and pyridine (1:99). Titrate with 0.5 M sodium hydroxide VS until pink color persists for 15 seconds. Carry out a blank test using mixture of 25 mL of *Phthalic anhydride solution* and quantity of pyridine equivalent to that added to the sample.

Calculate the average molecular mass according to the expression:

$$P = \frac{[2000 \times m]}{[B - S] \times (M)}$$

where

P = average molecular mass in g/mol;

m = sample mass in grams;

B = volume of 0.5 M sodium hydroxide VS consumed by the blank;

S = volume of 0.5 M sodium hydroxide VS consumed by the sample;

M = molarity of the sodium hydroxide solution.

PURITY TESTS

Preparation aspect. The 10% (w/v) aqueous preparation is clear **(5.2.25)** and colorless **(5.2.12)** for liquid samples and not more than slightly turbid for solid samples.

pH (5.2.19). 4.5 to 7.5. Determine in a solution prepared by dissolving 5 g of the sample in 100 mL of carbon dioxide-free water and adding 0.3 mL of saturated potassium chloride solution.

Arsenic (5.3.2.5). Proceed as described in *Spectrophotometric Method, Method II*. Not more than 0.0003% (3 ppm).

Heavy metals (5.3.2.3). Mix 4 g of the sample with 5 mL of 0.1 M hydrochloric acid and dilute with water to 25 mL. Proceed as described in *Method I*. Not more than 0.0005% (5 ppm).

Residue by incineration (5.2.10). Determine in 25 g of the sample in a platinum crucible. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

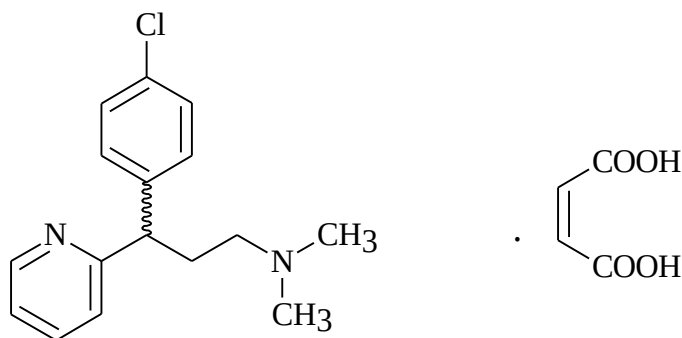
In tightly closed containers. Some plastics are softened by macrogol.

LABELING

Comply with current legislation.

CATEGORY

Pharmacotechnical adjuvant.

CHLORPHENIRAMINE MALEATE*Chlorphenamini maleas*

$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$; 390.86

chlorpheniramine maleate; 02442

γ -(4-Chlorophenyl)-*N,N*-dimethyl-2-pyridinepropamine (2Z)-2-butenedioate (1:1)

[113-92-8]

It contains not less than 98.0% and not more than 100.5% of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Freely soluble in water, soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 130 °C to 135 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the chlorpheniramine maleate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 230 nm to 350 nm, of 0.003% (w/v) sample solution in 0.1 M hydrochloric acid, there is a maximum point at 265 nm.

PURITY TESTS

pH (5.2.19). 4.0 to 5.0. Determine in aqueous solution at 2% (w/v).

Related substances. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel HF₂₅₄ as support, and mixture of ethyl acetate, methyl alcohol and M acetic acid (50:30:20) as mobile phase. Separately apply to the plate 2 µL of each solution, described below.

Solution (1): 5 % (w/v) sample solution in chloroform.

Solution (2): 0.01 % (w/v) sample solution in chloroform.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, except for the two main stains, corresponding to chlorpheniramine and maleic acid, is not more intense than that obtained in the chromatogram with *Solution (2)* (0.2%).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Quantitatively dissolve about 0.4 g of the sample, previously desiccated, in 20 mL of glacial acetic acid. Add two drops of methylrosanilinium chloride TS and titrate with 0.1 M perchloric acid VS until color turns greenish-blue. Carry out the blank test and make the necessary corrections. Alternatively, determine the endpoint potentiometrically. Each mL of 0.1 M perchloric acid is equivalent to 19.543 mg of $C_{16}H_{19}ClN_2.C_4H_4O_4$.

PACKAGING AND STORAGE

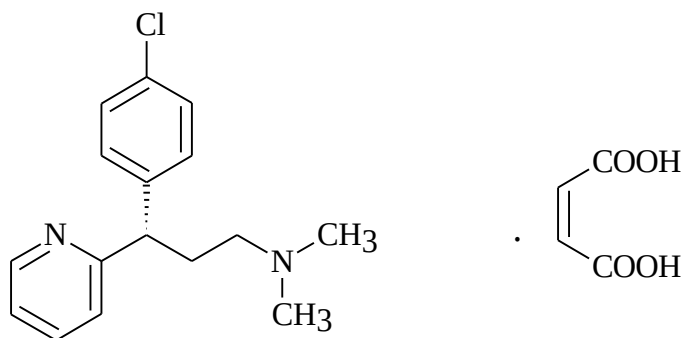
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antihistamine.

DEXCHLORPHENIRAMINE MALEATE*Dexchlorpheniramini maleas*

$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$; 390.86

dexchlorpheniramine maleate; 02839

(γ S)- γ -(4-chlorophenyl)-*N,N*-dimethyl-2-pyridinepropamine 2Z)-2-butenedioate (1:1)
[2438-32-6]

It contains not less than 98.0% and not more than 100.5% of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Freely soluble in water and in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 110 °C to 115 °C.

Specific optical rotation (5.2.8): +39.5 to +43.0, in relation to the desiccated substance. Determine in solution at 5% (w/v) in dimethylformamide.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the dexchlorpheniramine maleate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range from 200 nm to 400 nm, of solution at 0.002% (w/v) in water, there are maximum and minimum absorption points only on the same wavelengths as the ones observed in the spectrum of similar dexchlorpheniramine maleate CRS solution.

PURITY TESTS

pH (5.2.19). 4.0 to 5.0. Determine in 1% (w/v) aqueous solution in carbon dioxide-free water.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 65 °C for four hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh about 0.4 g of the sample previously desiccated and dissolve in 50 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS determining the endpoint potentiometrically or using 0.1 mL of methylrosaniline chloride TS until color turns green from blue. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 19.543 mg of $C_{16}H_{19}ClN_2.C_4H_4O_4$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiallergic.

DEXCHLORPHENIRAMINE MALEATE TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{16}H_{19}ClN_2.C_4H_4O_4$.

IDENTIFICATION

A. Pulverize to a fine powder, quantity of tablets equivalent to 150 mg of dexchlorpheniramine maleate. Add 100 mL of *M* acetic acid and shake mechanically for 10 minutes. Filter in a sintered glass funnel. Adjust the filtrate pH to 11.0 with 0.1% (w/v) sodium hydroxide. Transfer to a separating funnel and extract with six 100-mL portions of hexane. Filter each extract obtained using suitable medium to allow efficient separation between the organic phase and the aqueous phase. Combine the extracts and concentrate in heated bath to a reduced volume. Transfer to a smaller container and evaporate to the point where hexane vapors are no longer noticeable. Transfer the oily residue using four 3 mL portions of dimethylformamide to a 15 mL cylinder with cap, complete the volume with the same solvent and shake. Centrifuge if necessary. The optical rotation **(5.2.8)** is between $+0.24^\circ$ and $+0.35^\circ$.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Not more than 1.0%.

Disintegration test (5.1.4.1). Up to 15 minutes in water at 37°C .

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Pulverize each tablet to a fine powder, quantitatively transfer to a 10 mL volumetric flask and add 9 mL of mixture of water and trifluoroacetic acid (100:0.8). Proceed as described in *Assay*, starting from "Shake mechanically...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 500 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after testing, remove aliquot from the dissolution medium and filter. Proceed as described in *Assay*. Prepare *Standard solution* as described below.

Standard solution: dissolve an accurately weighed quantity of dexchlorpheniramine maleate CRS in water and dilute adequately to obtain a solution at $4\text{ }\mu\text{g/mL}$.

Inject 40 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 40 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{16}H_{19}ClN_2.C_4H_4O_4$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 75% (Q) of the stated quantity of $C_{16}H_{19}ClN_2.C_4H_4O_4$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 262 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 0.8 mL/minute.

Eluent (A): mixture of water and trifluoroacetic acid (100:0.05).

Eluent (B): mixture of acetonitrile and trifluoroacetic acid (100:0.05).

Mobile Phase gradient: adopt the gradient system described in the following table.

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>
0	100	0
10	50	50
11	0	100
16	0	100

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 10 mg of dexchlorpheniramine maleate to a 50 mL volumetric flask. Add 40 mL of mixture of water and trifluoroacetic acid (100:0.8). Shake mechanically for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Dilute with water to obtain a 40 µg/mL solution.

Standard solution: dissolve an accurately weighed quantity of dexchlorpheniramine maleate CRS in water and dilute adequately to obtain a solution at 40 µg/mL.

Inject 20 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{16}H_{19}ClN_2.C_4H_4O_4$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

DEXCHLORPHENIRAMINE MALEATE ORAL SOLUTION

It contains not less than 90.0% and not more than 110.0% of the stated quantity of dexchlorpheniramine maleate.

IDENTIFICATION

A. Dilute the equivalent to 20 mg of dexchlorpheniramine maleate to 50 mL with hydrochloric acid (1:120). Dilute 10 mL to 100 mL with the same solvent. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from Assay, there are maximum points identical to those observed in the spectrum of the standard solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Quantitatively transfer the volume of the oral solution equivalent to 8 mg of dexchlorpheniramine maleate to a 250 mL separating funnel and adjust the solution pH to 11.0 with *M* sodium hydroxide. Extract with two 75 mL portions of hexane and combine the extracts in a second separating funnel. Repeat the extraction with three 50 mL portions of hydrochloric acid (1:120), completing the volume to 200 mL with the same solvent. In another container, accurately weigh approximately 40 mg of dexchlorpheniramine maleate CRS, dissolve in water and complete to 100 mL with the same solvent. Transfer 10 mL of this solution to a separating funnel and adjust the pH to 11.0 with *M* sodium hydroxide. Extract with two 50 mL portions of hexane, shaking each portion for two minutes, before phase separation. Combine the extracts in a second separating funnel, extract with two 40 mL portions of hydrochloric acid (1:120). Combine the extracts in a volumetric flask and complete the volume to 100 mL with the same solvent. Filter the solution, discarding the first portions of the filtrate. Measure the absorbances **(5.2.14)** of the resulting solutions at 264 nm, using hydrochloric acid (1:120) for zero adjustment. Calculate the quantity of $C_{16}H_{19}ClN_2.C_4H_4O_4$ in the oral solution from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 262 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water, acetonitrile and trifluoroacetic acid (70:30:0.5).

Sample solution: transfer known volume of sample to volumetric flask. Add water and homogenize to obtain a 40 µg/mL solution. Filter.

Standard solution: dissolve an accurately weighed quantity of dexchlorpheniramine maleate CRS in water to obtain a solution at 40 µg/mL. Homogenize and filter.

Inject 20 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

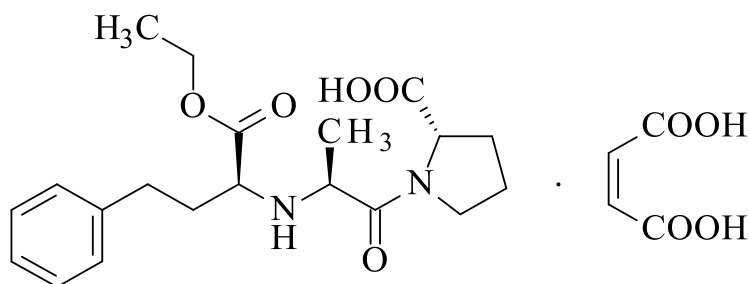
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{16}H_{19}ClN_2.C_4H_4O_4$ in the oral solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

ENALAPRIL MALEATE*Enalaprili maleas*C₂₀H₂₈N₂O₅·C₄H₄O₄; 492.53

enalapril maleate; 03370

N-[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl-L-proline (2*Z*)-2-butenedioate (1:1)

[76095-16-4]

It contains not less than 98.0% and not more than 102.0% of C₂₀H₂₈N₂O₅·C₄H₄O₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Moderately soluble in water, freely soluble in methyl alcohol. Soluble in diluted alkali hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 143 °C to 145 °C.

Specific optical rotation (5.2.8): -48 to -51, at 20 °C, in relation to the desiccated substance. Determine in 1% (w/v) solution in carbon dioxide-free water.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the enalapril maleate CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

pH (5.2.19). 2.4 to 2.9. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed as described in method **B.** from Assay. Inject 50 µL of the *Sample solution*. Calculate the percentage of each peak obtained in the *Sample Solution* chromatogram, excluding the peaks related to maleic acid and to enalapril. Do not include the solvent peaks in the calculations. Not more than 2.0% of total impurities.

Heavy metals (5.3.2.3). Use *Method III*. Determine in 2 g of sample. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, not greater than 5 mmHg, for two hours. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

A. Weigh, accurately, about 0.1 g of the sample, transfer to a 250 mL erlenmeyer flask and dissolve in 30 mL of carbon dioxide-free water. Titrate with 0.1 M sodium hydroxide VS and determine the endpoint potentiometrically, up to the second inflection point. Each mL of 0.1 M sodium hydroxide VS is equivalent to 16.417 mg of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 215 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm), kept at temperature of 50 °C ; *Mobile phase* flow rate of 2.0 mL/minute.

Mobile phase: mixture of *Phosphate buffer pH 2.2* and acetonitrile (75:25).

Enalaprilat solution: dissolve an accurately weighed quantity of enalaprilat CRS in water to obtain a 0.4 mg/mL solution.

Enalapril diketopiperazine solution: melt approximately 20 mg of enalapril maleate CRS in the center of a 100 mL beaker on a heating plate (approximately 5 to 10 minutes of heating). Immediately remove the beaker from the plate and allow to cool. Add 50 mL of acetonitrile to the residue and place in ultrasonic bath for a few minutes to dissolve. The solution generally contains between 0.2 and 0.4 mg/mL of enalapril diketopiperazine.

Sample solution: dissolve an accurately weighed quantity of the sample in phosphate buffer pH 2.2 to obtain a 0.3 mg/mL solution. Place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent. Homogenize.

Standard solution: dissolve an accurately weighed quantity of enalapril maleate CRS in phosphate buffer pH 2.2 to obtain a 0.3 mg/mL solution. Place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent. Homogenize.

Resolution solution: prepare 0.3 mg/mL enalapril maleate CRS solution in phosphate buffer pH 2.2 and add appropriate volume of *Enalaprilat solution* to obtain 0.003 mg/mL enalaprilat CRS solution. Transfer 0.75 mL of the *Enalapril diketopiperazine solution* to a 25 mL volumetric flask and complete the volume with the solution prepared in advance.

Inject 50 µL replicates of the *Resolution solution*. The column efficiency is not less than 1000 theoretical plates/meter for enalaprilat; not less than 300 theoretical plates/meter for enalapril and not less than 2500 theoretical plates/meter for enalapril diketopiperazine. The relative retention times are about 0.3 for maleic acid, 0.5 for enalaprilat, 1.0 for enalapril, and greater than 1.5 for enalapril diketopiperazine. The enalapril tailing factor is not more than 2.0. The resolution is not less than 2.0 between enalaprilat and maleic acid, between enalapril and enalaprilat, and between enalapril and enalapril diketopiperazine. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0% for enalapril and 5.0% for enalaprilat.

Procedure: inject, separately, 50 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

THERAPEUTIC CLASS

Antihypertensive.

ENALAPRIL MALEATE TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity: transfer each tablet to a corresponding volumetric flask to obtain a 0.1 mg/mL solution. Add phosphate buffer pH 2.2 and place in ultrasonic bath until the tablet completely disintegrates. Proceed as described in *Assay*, starting from "Shake mechanically for 30 minutes...". Prepare *Standard solution* in phosphate buffer pH 2.2 to obtain a 0.1 mg/mL enalapril maleate CRS solution.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 6.8, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium and dilute, if necessary, with phosphate buffer pH 6.8 to adequate concentration. Calculate the quantity of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ dissolved in the medium, proceeding as described in *Uniformity of unit doses*.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ dissolve in 30 minutes.

PURITY TESTS

Related substances. Proceed as described in *Assay*. Inject 50 µL of the *Sample solution*. Calculate the percentage of each peak obtained in the *Sample Solution* chromatogram, excluding the peak related to maleic acid and to enalapril. Do not include solvent-related peaks in the calculations. Not more than 5.0% of total impurities.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in method **B.** of Assay in the *Enalapril maleate* monograph. Prepare solutions as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 20 mg of enalapril maleate to a 100 mL volumetric flask, add phosphate buffer pH 2.2 and place in ultrasonic bath for 15 minutes. Shake mechanically for 30 minutes. Complete the volume with the same solvent to obtain a 0.2 mg/mL solution. Homogenize, and filter, discarding the first 5 mL of the filtrate.

Standard solution: dissolve an accurately weighed quantity of enalapril maleate CRS in phosphate buffer pH 2.2 to obtain a 0.2 mg/mL solution. Place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent. Homogenize.

Resolution solution: prepare 0.2 mg/mL enalapril maleate CRS solution in phosphate buffer pH 2.2 and add appropriate volume of *Enalaprilat solution* to obtain 0.002 mg/mL enalaprilat CRS solution. Transfer 0.5 mL of the *Enalapril diketopiperazine solution* to a 25 mL volumetric flask and complete the volume with the solution prepared in advance.

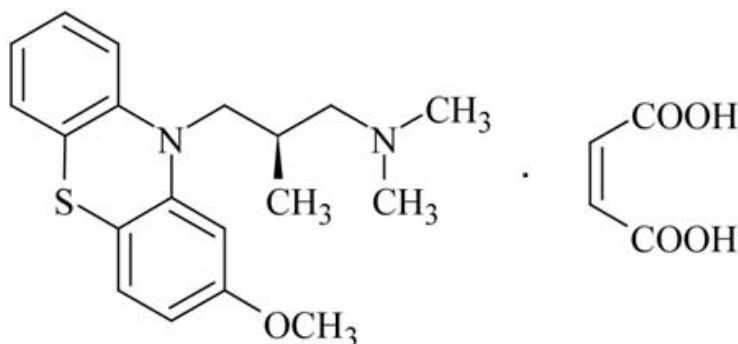
Procedure: inject, separately, 50 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{20}H_{28}N_2O_5 \cdot C_4H_4O_4$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

LEVOMEPROMAZINE MALEATE*Levomepromazini maleas*

$C_{19}H_{24}N_2OS \cdot C_4H_4O_4$; 444.55

levomepromazine maleate; 05265

(β R)-2-methoxy-N,N, β -trimethyl-10H-phenothiazine-10-propanamine (2Z)-2-butenedioate (1:1)
[7104-38-3]

It contains not less than 98.5% and not more than 101.0% of $C_{19}H_{24}N_2OS \cdot C_4H_4O_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, or slightly yellowish, crystalline powder. It darkens when exposed to air and light.

Solubility. Slightly soluble in water and in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): -7,0 to -8,5, in relation to the desiccated substance. Determine in solution at 5% (w/v) in dimethylformamide.

IDENTIFICATION

The identification tests B. and C. can be omitted if test A. is carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the levomepromazine maleate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 230 nm to 350 nm, of 0,001% (w/v) solution in methyl alcohol, there are maximum points at 254 nm and 308 nm. The absorbance values are approximately 0.6 and 0.1, respectively.

C. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel GF₂₅₄ as support, and mixture of water, anhydrous formic acid and isopropyl ether (3:7:90) as mobile phase. Apply

to the plate, separately, in band of 10mm X 2mm, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 20 mg/mL sample solution in mixture of water and acetone (1:9).

Solution (2): 5 mg/mL maleic acid CRS solution in mixture of water and acetone (1:9).

Develop chromatogram (12 cm). Remove the plate, dry at 120°C for 10 minutes. Examine under ultraviolet light (254 nm). *Solution (1)* presents a stain on the application point and another main stain which corresponds in position, color and intensity to that obtained with *Solution (2)*.

PURITY TESTS

pH (5.2.19). 3.5 to 5.5. Proceed protected from intense light. Weigh 0,5 g of the sample and add 25 mL of carbon dioxide-free water. Shake and allow to settle. Check the supernatant pH.

Related substances. Proceed protected from intense light. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of acetone, diethylamine and cyclohexane (10:10:80) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): 20 mg/mL sample solution in mixture of water and acetone (1:9).

Solution (2): dilute 0.5 mL of *Solution (1)* to 100 mL with mixture of water and acetone (1:9).

Develop chromatogram (15 cm). Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (2)* (0.5%).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 100 °C to 105 °C for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 0.35 g of the sample and dissolve in 50 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 44.455 mg of C₁₉H₂₄N₂OS·C₄H₄O₄.

PACKAGING AND STORAGE

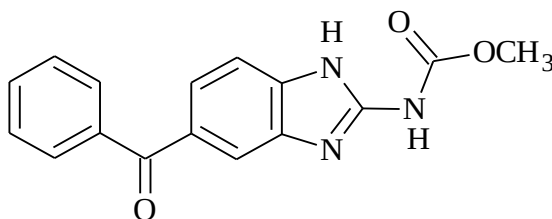
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antipsychotic; neuroleptic.

MEBENDAZOLE**Mebendazolum**C₁₆H₁₃N₃O₃; 295.30

mebendazole; 05515

N-(6-benzoyl-1H-benzimidazol-2-yl)carbamic acid methyl ester

[31431-39-7]

It contains not less than 98.0% and not more than 102.0% of C₁₆H₁₃N₃O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to slightly yellow fine powder.

Solubility. Practically insoluble in water and in ethyl alcohol. Soluble in formic acid and practically insoluble in mineral acids.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the mebendazole CRS spectrum, prepared identically.

B. Dissolve 30 mg of the sample in 2 mL of anhydrous formic acid and successively dilute in isopropyl alcohol to a concentration of 0.00075% (w/v). In the ultraviolet absorption spectrum (**5.2.14**) in the range of 230 nm to 320 nm, there are maximum points at 247 nm and 312 nm, identical to those observed in the spectrum of similar mebendazole CRS solution.

C. Dissolve 40 mg of the sample in 2 mL of anhydrous formic acid and add 5 mL of ethyl alcohol acidified with some drops of hydrochloric acid. Shake vigorously and filter. Add about 3 mg of p-phenylenediamine hydrochloride to the filtrate and shake. Add about 0.1 g of zinc powder and allow to stand for two minutes. Add 5 mL of acid ammoniacal ferric sulfate RS. Violet color develops.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ as support, and mixture of chloroform, methyl alcohol and anhydrous formic acid (90:5:5) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 50 mg of the sample in 1 mL of anhydrous formic acid and complete the volume to 10 mL with chloroform.

Solution (2): dissolve 50 mg of mebendazole CRS in 1 mL of anhydrous formic acid and complete the volume to 10 mL with chloroform.

Solution (3): transfer 1 mL of *Solution (2)* into a 200 mL volumetric flask and complete the volume with mixture of chloroform and anhydrous formic acid (9:1). Homogenize.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* chromatogram corresponds in position, color and intensity to the one obtained with *Solution (2)*. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (3)* (0.5%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for four hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 0.225 g of the sample and dissolve in 30 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 29.530 mg of C₁₆H₁₃N₃O₃.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anthelmintic.

MEBENDAZOLE TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{16}H_{13}N_3O_3$.

IDENTIFICATION

Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel G as support and mixture of chloroform, methyl alcohol and 96% (w/w) formic acid (90:5:5) as mobile phase. Apply to the plate, separately, 10 μ L of each of the freshly prepared solutions described below.

Solution (1): pulverize not less than 10 tablets to a fine powder, weigh the equivalent to 0.2 g of mebendazole, add 20 mL of mixture of chloroform and 96% formic acid (w/w) (19:1), place in water bath for one to two minutes, cool and filter.

Solution (2): prepare 10 mg/mL mebendazole CRS solution in mixture of chloroform and 96% formic acid (w/w) (19:1).

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity: transfer each tablet to a 100 mL volumetric flask, add 20 mL of formic acid and wait for the tablet to completely disintegrate. Heat in water bath for 15 minutes. Cool, complete the volume with isopropyl alcohol. Homogenize and filter. Dilute in isopropyl alcohol until concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 310 nm (5.2.14), using mixture of 96% formic acid (w/w) and isopropyl alcohol (1:500) for zero adjustment. Calculate the quantity of $C_{16}H_{13}N_3O_3$ in the tablets from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 1.0% (w/v) sodium lauryl sulfate in 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 120 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute with *Dissolution medium* to adequate concentration. Measure the absorbances of the solutions at 248 nm (5.2.14), using *Dissolution medium* for zero adjustment. Calculate the quantity of $C_{16}H_{13}N_3O_3$

dissolved in the medium, comparing the readings obtained with that of the mebendazole CRS solution at the concentration of 0.0005% (w/v), prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated quantity of $C_{16}H_{13}N_3O_3$ dissolve in 120 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 50 mg of mebendazole to a 100 mL volumetric flask, add 10 mL of formic acid and shake mechanically for 15 minutes. Complete the volume with isopropyl alcohol. Homogenize and filter. Transfer 1 mL of the filtrate to a 100 mL volumetric flask, add 5 mL of 0.1 M hydrochloric acid, complete the volume with isopropyl alcohol and homogenize. Prepare standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 290 nm, using mixture of 0.1 M hydrochloric acid and isopropyl alcohol (5:95) for zero adjustment. Calculate the quantity of $C_{16}H_{13}N_3O_3$ in the tablets from the readings obtained.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Prepare solutions as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.1 mg of mebendazole to a 100 mL volumetric flask, add 50 mL of formic acid and heat in water bath at 50 °C for 15 minutes. Cool and complete the volume with water. Homogenize and filter in a medium porosity glass filter. Transfer 10 mL of the filtrate to a separating funnel, add 50 mL of chloroform and 50 mL of water. Shake for two minutes, allow the phases to separate and transfer the chloroform layer to a second separating funnel. Wash the aqueous layer with two 10 mL portions of chloroform, combining the chloroform extracts in the second separating funnel. Discard the aqueous layer. Wash the combined chloroform extracts with mixture of 0.1 M hydrochloric acid and 10% formic acid (v/v) (4:50). Transfer the chloroform layer to a 100 mL volumetric flask. Wash the aqueous layer with two 10 mL portions of chloroform, combining the chloroform extracts in the same volumetric flask. Complete the volume with isopropyl alcohol and homogenize. Transfer 5 mL of the solution to a 100 mL volumetric flask, complete the volume with isopropyl alcohol and homogenize.

Standard solution: transfer 20 mg of mebendazole CRS to a 100 mL volumetric flask, add 90 mL of chloroform, 7 mL of isopropyl alcohol and 2 mL of 10% (v/v) formic acid. Shake until complete dissolution and complete the volume with isopropyl alcohol. Transfer 5 mL of this solution to a 200 mL volumetric flask. Complete the volume with isopropyl alcohol and homogenize.

Blank solution: transfer 45 mL of chloroform to a 100 mL volumetric flask, add 1 mL of 10% (v/v) formic acid, complete with isopropyl alcohol and homogenize. Transfer 5 mL of the solution to a 100 mL volumetric flask, complete with isopropyl alcohol and homogenize.

Measure the absorbances of the resulting solutions at 274 nm, using the *Blank solution* for zero adjustment. Calculate the quantity of $C_{16}H_{13}N_3O_3$ in the tablets from the readings obtained.

C. By High performance liquid chromatography (5.2.17.4) Use chromatograph equipped with ultraviolet detector at 247 nm; 300mm long, 3.9 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (3 μ m to 10 μ m), kept at 30 °C; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.05 M potassium phosphate monobasic (60:40). Adjust pH to 5.5 with 0.1 M phosphoric acid or with M sodium hydroxide.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.5 g of mebendazole to a 100 mL volumetric flask, add 50 mL of formic acid and heat in water bath at 50 °C for 15 minutes. Shake mechanically for one hour, complete the volume with water, homogenize and filter. Transfer 5 mL of the filtrate to a 100 mL volumetric flask, complete the volume with mixture of formic acid and methyl alcohol (1:9) and homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase*, homogenize and filter.

Standard solution: transfer 25 mg of mebendazole CRS, accurately weighed, to a 100 mL volumetric flask. Add 10 mL of formic acid and heat in water bath at 50 °C for 15 minutes. Shake mechanically for five minutes, add 80 mL of methyl alcohol and cool. Complete the volume with the same solvent and homogenize. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with *Mobile phase*, homogenize and filter.

Inject 15 μ L replicates of the *Standard solution*. The column efficiency is not less than 2500 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 15 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{16}H_{13}N_3O_3$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

MEBENDAZOLE ORAL SUSPENSION

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{16}H_{13}N_3O_3$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the *Sample solution* obtained in method **A.** from *Assay*, there are maximum and minimum points identical to those observed in the spectrum of the standard solution.

B. The main stain of the chromatogram from *Solution (1)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Aspect. Completely empty the content of 10 previously shaken flasks into corresponding clean and dry cylinders, fitted with a cap, and observe immediately under suitable visibility conditions. The content flows smoothly, the suspension is homogeneous, viscous, free of lumps and foreign particles. After 24 hours standing, it may show slight sedimentation, which resuspends under agitation.

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.0 to 7.5.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of chloroform, methyl alcohol and formic acid (90:5:5) as mobile phase. Apply to the plate, separately, 50 µL of each of the freshly prepared solutions described below.

Solution (1): to an aliquot equivalent to 10 mg of mebendazole, add 1 mL of formic acid, shake until dissolved, complete the volume to 10 mL with chloroform, homogenize and filter.

Solution (2): weigh 10 mg of mebendazole CRS, add 1 mL of formic acid, shake until dissolved, complete the volume to 10 mL with chloroform and homogenize.

Solution (3): transfer 1 mL of *Solution (2)* into a 200 mL volumetric flask, complete the volume with mixture of chloroform and formic acid (9:1) and homogenize.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not greater or more intense than that obtained with *Solution (3)* (0.5%).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer volume of the oral suspension equivalent to 100 mg of mebendazole to a 100 mL volumetric flask. Add 30 mL of formic acid and shake until complete dissolution. Complete the volume with formic acid and mix. Transfer 5 mL of this solution to a 50 mL volumetric flask, add 5 mL of 0.1 M hydrochloric acid, shake and complete the volume with isopropyl alcohol. Heat to slight boiling and filter. Cool and dilute in isopropyl alcohol until concentration of 0.001% (w/v). Prepare standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 310 nm, using 0.1 M hydrochloric acid and isopropyl alcohol (1:9) for zero adjustment. Calculate the quantity of $C_{16}H_{13}N_3O_3$ in the oral suspension from the readings obtained.

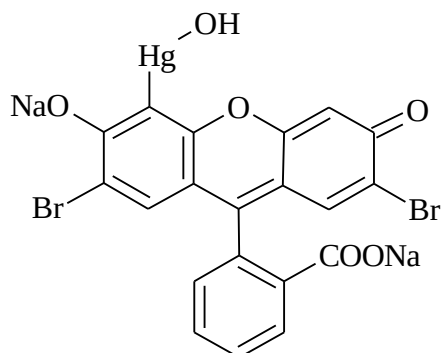
B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer volume of the oral suspension equivalent to 100 mg of mebendazole to a 100 mL volumetric flask, add 50 mL of formic acid and place in water bath at 50 °C for 15 minutes. Cool, complete the volume with water, homogenize and filter through a medium porosity glass filter. Transfer 10 mL of the filtrate to a separating funnel, add 50 mL of chloroform, 50 mL of water and shake for two minutes. Allow the phases to separate and transfer the chloroform layer to a second separating funnel. Wash the aqueous layer with two 10 mL portions of chloroform and add the washed chloroform extracts to the second separating funnel. Wash the combined chloroform extracts with mixture of M hydrochloric acid and 10% (v/v) formic acid (4:50). Transfer the chloroform layer to a 100 mL volumetric flask. Wash the aqueous layer with two 10 mL portions of chloroform, add the extract to the volumetric flask, complete with isopropyl alcohol and mix. Dilute in isopropyl alcohol until concentration of 0.0005% (w/v). Prepare standard solution in the same concentration, using the same solvents. Prepare blank as described below. Transfer 45 mL of chloroform to a 100 mL volumetric flask, add 1 mL of 10% formic acid (v/v), complete with isopropyl alcohol, homogenize, transfer 5 mL of this solution to a 100 mL volumetric flask, complete with isopropyl alcohol and homogenize. Measure the absorbances of the resulting solutions at 274 nm, using blank for zero adjustment. Calculate the quantity of $C_{16}H_{13}N_3O_3$ in the oral suspension from the readings obtained.

PACKAGING AND STORAGE

In tightly closed amber glass containers.

LABELING

Comply with current legislation.

MERBROMIN***Merbrominum***

$C_{20}H_8Br_2HgNa_2O_6$; 750.66

merbromin; 05676

(2'7'-Dibromo-3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-4'-yl)

hydroxymercury sodium salt (2:1)

[129-16-8]

It contains not less than 22.4% and not more than 26.7% of mercury (Hg = 200.59) and not less than 18.0% and not more than 22.4% of bromine (Br = 79.90), in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Metallic green to reddish brown scale or granule.

Solubility. Freely soluble in water, however, it sometimes leaves a small quantity of insoluble material, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. The 0.05% (w/v) solution has a red color and yellowish green fluorescence.

B. To 5 mL of 0.4% (w/v) solution add three drops of sulfuric acid RS. A reddish-orange precipitate is formed.

C. Heat 0.1 g of the sample with small iodine crystals in a test tube. Red crystals are sublimated at the top of the tube. If yellow crystals are produced, rub with a glass rod. The color of the crystals turns red.

D. Weigh 0.1 g of the sample and add 12 mL of 16.67% (w/v) sodium hydroxide solution. Evaporate to dryness with agitation and incinerate at 600 °C for one hour. Dissolve the residue in 5 mL of water and acidify with hydrochloric acid. Add three drops of chlorine RS, 2 mL of chloroform and shake; in the chloroform layer, a yellowish-brown color is produced.

PURITY TESTS

Solution aspect. Dissolve 0.4 g of the sample in 20 mL of water, add 3 mL of sulfuric acid RS and filter. The filtrate color is not more intense than that of the *SC C Color Standard Solution (5.2.12)*.

Mercury insoluble compounds. Dissolve 2.5 g of the sample in 50 mL of water and allow to stand for 24 hours, protected from light. Centrifuge and wash the precipitate with small portions of water until the last wash is colorless. Transfer the precipitate to a flask with a ground stopper, add exactly 5 mL of 0.05 M iodine VS and allow to stand for one hour, shaking frequently. Add, dropwise, 4.3 mL of 0.1 M sodium thiosulfate VS, under agitation. Add 1 mL of starch TS. Blue color develops.

Soluble halogens.

Sample preparation: dissolve 5 g of the sample in 80 mL of water, add 10 mL of 10% (w/v) nitric acid and dilute to 100 mL with water. Homogenize and filter. Transfer 40 mL of the filtrate to a Nessler tube, add 6 mL of 10% (w/v) nitric acid and dilute to 50 mL with water.

Standard preparation: in a Nessler tube add 0.25 mL of 0.01 M hydrochloric acid, 6 mL of 10% (w/v) nitric acid and dilute to 50 mL with water.

Procedure: add 1 mL of 0.1 M silver nitrate to the tubes, mix thoroughly and allow to stand for five minutes protected from light. Any turbidity produced in the *Sample preparation* must not be more intense than that obtained in the *Standard preparation*.

Mercury soluble salts.

Sample preparation: transfer 5 mL of the filtrate obtained in *Solution Aspect* to a test tube and add 5 mL of water.

Standard preparation: dissolve 40 mg of mercury (II) chloride, accurately weighed, in water and dilute to 1000 mL with the same solvent. To 20 mL of this solution, add 3 mL of sulfuric acid RS. Transfer 5 mL of the previous solution to a test tube and add 5 mL of water.

Procedure: add a drop of sodium sulfide RS to the tubes. Any turbidity produced in the *Sample preparation* must not be more intense than that obtained with the *Standard preparation*.

Loss on drying (5.2.9.1). Determine in 2 g of the sample. Dry in oven at 105 °C for five hours. Not more than 5.0%.

ASSAY

Mercury.

Accurately weigh about 0.6 g of the previously powdered and dried sample, transfer to a flask with a stopper and dissolve with 50 mL of water. Add 8 mL of glacial acetic acid, 20 mL of chloroform and exactly 30 mL of 0.05 M iodine VS. Cap hermetically and allow to stand for an hour, shaking frequently and vigorously. Titrate excess iodine with 0.1 M sodium thiosulfate VS, under vigorous agitation, using 1 mL of starch TS. Carry out the blank test and make the necessary corrections. Each mL of 0.05 M iodine VS is equivalent to 10.030 mg of Hg.

Bromine.

Accurately weigh, in a porcelain crucible, approximately 0.5 g of the sample previously powdered and dried, add 2 g of potassium nitrate, 3 g of anhydrous potassium carbonate, 3 g of anhydrous sodium carbonate and homogenize. Cover the surface of the mixture with 3 g of equal parts of anhydrous potassium carbonate and anhydrous sodium carbonate and calcine at 400°C to 500°C for one hour. Cool, dissolve and quantitatively transfer the calcined mixture to an erlenmeyer flask with the aid of 80 mL of hot water and acidify with nitric acid. Add 25 mL of 0.1 M silver nitrate VS and shake. Titrate excess silver nitrate with 0.1 M ammonium thiocyanate VS using 2 mL of ammonium ferric sulfate RS as indicator. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M silver nitrate VS is equivalent to 7.990 mg of Br.

PACKAGING AND STORAGE

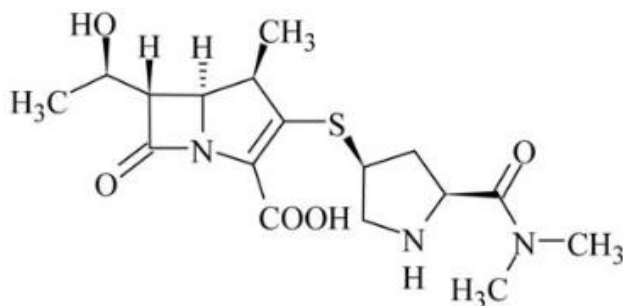
In tightly closed and opaque containers.

LABELING

Comply with current legislation.

CATEGORY

Preservative.

MEROPENEM***Meropenemum***

$C_{17}H_{25}N_3O_5S$; 383.46

meropenem; 05688

(4*R*,5*S*,6*S*)-3-[[[(3*S*,5*S*)-5-[(dimethylamino)carbonyl]-3-pyrrolidinyl]thio]-6-[(1*R*)-1-hydroxyethyl]-4-methyl acid -7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid
[96036-03-2]

$C_{17}H_{25}N_3O_5S \cdot 3H_2O$; 437.51

meropenem trihydrate; 09494

(4*R*,5*S*,6*S*)-3-[[[(3*S*,5*S*)-5-[(dimethylamino)carbonyl]-3-pyrrolidinyl]thio]-6-[(1*R*)-1-hydroxyethyl]-4-methyl acid -7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid hydrate (1:3)
[119478-56-7]

It contains not less than 98.0% and not more than 101.0% of $C_{17}H_{25}N_3O_5S$ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or pale yellow crystalline powder.

Solubility. Slightly soluble in water, very slightly soluble in ethyl alcohol. Soluble in 5% (w/v) monobasic potassium phosphate.

Physicochemical constants.

Melting range (5.2.2): 230 °C to 240 °C, with decomposition.

Specific optical rotation (5.2.8): -17 to -21. Determine in aqueous solution at 0,5% (w/v) at 20 °C..

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the meropenem CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.003% (w/v) solution in water, there are maximum and minimum points only at the same wavelengths as similar meropenem CRS solution.

PURITY TESTS

pH (5.2.19). 4.0 to 6.0. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at temperature of 40°C; *Mobile phase* flow rate of 1.6 mL/minute. If necessary, adjust the *Mobile Phase* flow so that the meropenem retention time is between five to seven minutes.

Mobile phase: mixture of *Diluent* and acetonitrile (1000:70).

Diluent: add 1 mL of triethylamine to 900 mL of water, adjust pH to 5.0 ± 0.1 with 10% (v/v) phosphoric acid and dilute to 1000 mL with water.

Solution (1): dissolve quantity, accurately weighed, of the sample in *Diluent* and quantitatively dilute to obtain a solution at 5 mg/mL. Inject immediately after preparation.

Solution (2): dissolve quantity, accurately weighed, of meropenem CRS in *Diluent* and quantitatively dilute to obtain a solution at 25 µg/mL. Inject immediately after preparation or store in refrigerator for not more than 24 hours.

Inject 10 µL replicates of the *Solution (2)*. The column efficiency is not less than 2500 theoretical plates. The tailing factor is not more than 1.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject 10 µL of *Solution (1)* and *Solution (2)* separately and record the chromatograms for not less than three times the retention time related to the meropenem peak. The main impurities are observed at the retention times of 0.45 and 1.9 related to the meropenem peak. Calculate the percentage of each impurity present in the sample from the following formula:

$$\left(\frac{C_p}{C_a}\right) \times P \times \left(\frac{A_i}{A_p}\right)$$

where

C_p = concentration, in mg/mL, of meropenem CRS in the standard solution;

C_a = concentration, in mg/mL, of meropenem CRS in the sample solution;

P = declared potency, in relation to the anhydrous substance, of meropenem in the chemical reference substance;

A_i = area under the peak corresponding to any individual impurity obtained in the sample solution;

A_p = area under the peak corresponding to the meropenem obtained in the standard solution.

Not more than 0.3% of any of the two main impurities, in relation to the anhydrous substance. Not more than 0.1% of any other individual impurity, in relation to the anhydrous substance. Calculated in relation to the anhydrous substance, the sum of all other impurities, with the exception of the main impurities, must be not more than 0.3%.

Water (5.2.20.1). 11.4 to 13.4.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Incinerate at $(500 \pm 50)^\circ\text{C}$. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

When indicated on the label that the substance is sterile, the sample complies with the Sterility and with the Bacterial endotoxin tests. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test. Use the *Membrane Filtration Method*.

Bacterial endotoxins (5.5.2.2). Not more than 0.125 EU/mg of meropenem.

ASSAY

Use one of the methods described below.

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 298 nm; 250 mm long, 4 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group ($3\ \mu\text{m}$ to $10\ \mu\text{m}$), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Buffer pH 3.0: prepare 0.03 M monobasic potassium phosphate solution. Adjust pH to 3.0 ± 0.1 with phosphoric acid.

Mobile phase: mixture of *Buffer pH 3.0* and acetonitrile (90:10).

Note: inject the solutions, described below, immediately after preparation or keep refrigerated for not more than 24 hours.

Sample solution: dissolve an accurately weighed quantity of the sample in water and dilute to obtain a 0.2 mg/mL meropenem ($\text{C}_{17}\text{H}_{25}\text{N}_3\text{O}_5\text{S}$) solution. Transfer 5 mL to a 25 mL volumetric flask and complete the volume with water, obtaining solution at 40 $\mu\text{g/mL}$.

Standard solution: dissolve an accurately weighed quantity of meropenem CRS in water and dilute to obtain a 0.2 mg/mL meropenem ($\text{C}_{17}\text{H}_{25}\text{N}_3\text{O}_5\text{S}$) solution. Transfer 5 mL to a 25 mL volumetric flask and complete the volume with water, obtaining solution at 40 $\mu\text{g/mL}$.

Inject 20 μL replicates of the *Standard solution*. The column efficiency must be not less than 4000 theoretical plates for the meropenem peak. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $\text{C}_{17}\text{H}_{25}\text{N}_3\text{O}_5\text{S}$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

B. Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)* by agar diffusion using cylinders.

Microorganism: Kocuria rhizophila ATCC 9341.

Culture media: culture medium number 1 for microorganism maintenance, sterile saline solution for inoculum standardization, culture medium number 11 for base layer and for inoculum preparation.

Sample solution: weigh quantity of sample equivalent to 30 mg of meropenem, transfer to a 100 mL volumetric flask and complete with sterile water. Dilute to obtain concentrations of 1.5 µg/mL, 3.0 µg/mL and 6.0 µg/mL, using sterile water as diluent.

Standard solution: weigh quantity of meropenem CRS equivalent to 30 mg of meropenem, transfer to a 100 mL volumetric flask and complete with sterile water. Dilute to obtain concentrations of 1.5 µg/mL, 3.0 µg/mL and 6.0 µg/mL, using sterile water as diluent.

Procedure: add 20 mL of culture medium number 11 to each plate, wait to solidify, add 5 mL of 0,5% inoculum and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*, adding 0.2 mL of the freshly prepared solutions to the cylinders. Calculate the sample potency, in µg of meropenem per milligram, from the standard potency and the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at room temperature.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

MEROPENEM TRIHYDRATE POWDER FOR INJECTION SOLUTION

It contains not less than 90.0% and not more than 120.0% of the stated quantity of meropenem ($C_{17}H_{25}N_3O_5S$). Meropenem powder for injection solution is a sterile dry mixture of meropenem trihydrate and sodium carbonate.

IDENTIFICATION

A. Weigh three units individually, remove the content, wash the respective flasks, dry and weigh again. Homogenize the content of the flasks. Shake quantity of powder with water and quantitatively dilute with the same solvent to concentration of 0.002% (w/v). Filter, if necessary. In the ultraviolet absorption spectrum (**5.2.14**) of the sample solution, in the range of 200 nm to 400 nm, there is maximum point at 298 nm, identical to that observed in the spectrum of similar meropenem CRS solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

pH (5.2.19). 7.3 to 8.3. Determine in aqueous solution at 5% (w/v).

Determination of weight (5.1.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), kept at 40 °C; *Mobile phase* flow rate of 1.6 mL/minute. If necessary, adjust the *Mobile Phase* flow so that the meropenem retention time is between five to seven minutes.

Mobile phase: mixture of *Diluent* and acetonitrile (1000:70).

Diluent: add 1 mL of triethylamine to 900 mL of water, adjust pH to 5.0 ± 0.1 with 10% (v/v) phosphoric acid and dilute to 1000 mL with water.

Solution(1): weigh three units individually, remove the content, wash the respective flasks, dry and weigh again. Homogenize the content of the flasks. Shake quantity, accurately weighed, of powder with *Diluent* and quantitatively dilute with the same solvent to obtain a solution at 5 mg/mL. Inject immediately after preparation.

Solution (2): dissolve quantity, accurately weighed, of meropenem CRS in *Diluent* and quantitatively dilute with the same solvent to obtain a solution at 25 μ g/mL. Inject immediately after preparation or store in refrigerator for not more than 24 hours.

Inject 10 µL replicates of the *Solution (2)*. The column efficiency is not less than 2500 theoretical plates. The tailing factor is not more than 1.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject 10 µL of *Solution (1)* and *Solution (2)* separately and record the chromatograms for not less than three times the retention time related to the meropenem peak. The main impurities are observed at the retention times of 0.45 and 1.9 related to the meropenem peak. Calculate the percentage of each impurity present in the sample according to the following formula:

$$10 \times \left(\frac{C \times P}{m} \right) \times \left(\frac{A_i}{A_p} \right)$$

where

C = concentration, in mg/mL, of meropenem CRS in *Solution (2)*;

P = declared potency, in relation to the anhydrous substance, of meropenem CRS;

m = quantity of meropenem, in mg, in the mass of powder weighed for the preparation of *Solution (1)*;

A_i = area under the peak corresponding to any individual impurity obtained in *Solution (1)*;

A_p = area under the peak corresponding to the meropenem obtained in *Solution (2)*.

Not more than 0.8% of impurity with a relative retention time of 0.45, in relation to the meropenem peak. Not more than 0.6% of impurity with a relative retention time of 1.9, in relation to the meropenem peak.

Sodium. Proceed as described in *Flame atomic absorption spectrometry (5.2.13.1.1)*. Use a spectrometer equipped with air and acetylene flame and a hollow sodium cathode lamp with light emission at 589.6 nm.

Potassium chloride solution: dissolve 38.1 g of potassium chloride in water and dilute to 1000 mL with the same solvent.

Sample solution: weigh three units individually, remove the content, wash the respective flasks, dry and weigh again. Homogenize the content of the flasks. Transfer quantity of powder equivalent to 25 mg of meropenem to a 200 mL volumetric flask and complete the volume with water. Transfer 5 mL to a 50 mL volumetric flask, add 5 mL of the *Potassium chloride solution* and complete the volume with water.

Standard solution: dissolve 28.67 mg of sodium chloride in water, previously dried at 105 °C for two hours, to obtain a sodium chloride solution at 28.67 µg/mL. Transfer 5 mL to a 50 mL volumetric flask, add 5 mL of the *Potassium chloride solution* and complete the volume with water.

Blank: transfer 5 mL of *Potassium chloride solution* to a 50 mL volumetric flask and complete the volume with water.

Procedure: measure the absorbances of the *Standard solution* and the *Sample solution*, using *Blank* for zero adjustment. Calculate the quantity of sodium, in mg, in the powder for meropenem

injection solution, from the readings obtained. It contains between 80% and 120% of the declared quantity of sodium.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 65 °C, under reduced pressure, for six hours. Between 9.0 and 12.0%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Use the *Membrane Filtration Method*.

Bacterial endotoxins (5.5.2.2). Not more than 0.125 EU/mg of meropenem.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 298 nm; 250mm long, 4 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (3 µm to 10 µm), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Buffer pH 3.0: prepare 0,03 M monobasic potassium phosphate solution. Adjust pH to 3.0± 0.1 with phosphoric acid.

Mobile phase: mixture of *Buffer pH 3.0* and acetonitrile (90:10).

Note: inject the solutions, described below, immediately after preparation or keep refrigerated for not more than 24 hours.

Sample solution: weigh 20 units individually, remove the content, wash the respective flasks, dry and weigh again. Homogenize the content of the flasks. Dissolve an accurately weighed quantity of powder in water to obtain a 0.2 mg/mL meropenem (C₁₇H₂₅N₃O₅S) solution. Transfer 5 mL to a 25 mL volumetric flask and complete the volume with water, obtaining solution at 40 µg/mL.

Standard solution: dissolve an accurately weighed quantity of meropenem CRS in water to obtain a 0.2 mg/mL meropenem (C₁₇H₂₅N₃O₅S) solution. Transfer 5 mL to a 25 mL volumetric flask and complete the volume with water, obtaining solution at 40 µg/mL.

Inject 20 µL replicates of the *Standard solution*. The column efficiency must be not less than 4000 theoretical plates for the meropenem peak. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₇H₂₅N₃O₅S in the powder for injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at room temperature.

LABELING

Comply with current legislation.

GEMIFLOXACIN MESYLATE TABLETS

It contains gemifloxacin mesylate equivalent to not less than 90.0% and not more than 110.0% of the declared quantity of gemifloxacin ($C_{18}H_{20}FN_5O_4$).

IDENTIFICATION

The identification test A. can be omitted if test B. is carried out. The identification test B. can be omitted if test A. is carried out.

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 220 nm to 400 nm of the sample solution obtained in method **A.** from Assay, there are maximum and minimum points only at the same wavelengths observed in the standard solution spectrum.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel F₂₅₄ as support, and mixture of butyl alcohol, water, ammonium hydroxide and acetone (10:15:15:60), as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below. Proceed protected from direct light.

Solution (1): weigh and pulverize the tablets. Place in ultrasonic bath, for 15 minutes, quantity of powder equivalent to 10 mg of gemifloxacin with 100 mL of methyl alcohol. Filter the solution.

Solution (2): dissolve an accurately weighed quantity of gemifloxacin mesylate CRS in methyl alcohol to obtain 0.1 mg/mL gemifloxacin solution.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 6.0, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 60 minutes.

Procedure: proceed protected from direct light. After the test, remove aliquot from the dissolution medium, filter and proceed as described in method **B.** from Assay. Prepare the *Sample solution* and the *Standard solution* as described below.

Sample solution: transfer 5 mL of the sample to a 25 mL volumetric flask and complete the volume with *Mobile phase*.

Standard solution: dissolve an accurately weighed quantity of gemifloxacin mesylate CRS in phosphate buffer pH 6.0 to obtain a 350 µg/mL gemifloxacin solution. Dilute successively with *Mobile Phase* to obtain a concentration of 70 µg/mL of gemifloxacin.

Tolerance: not less than 70% (Q) of the stated quantity of C₁₈H₂₀FN₅O₄ dissolve in 60 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed protected from direct light, as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 60 mg of gemifloxacin to a 200 mL volumetric flask and add 150 mL of methyl alcohol. Place in ultrasonic bath at room temperature for 30 minutes, complete the volume with methyl alcohol and filter. Dilute successively, with the same solvent, to a concentration of 0.0006% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 272 nm, using methyl alcohol for zero adjustment. Calculate the quantity of C₁₈H₂₀FN₅O₄ in the tablets from the readings obtained.

B. Proceed protected from direct light, as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 272 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at temperature of 25 °C; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of aqueous solution of 0.3% (v/v) triethylamine pH 3.0, previously adjusted with 10% (v/v) phosphoric acid and acetonitrile (80:20).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 50 mg of gemifloxacin to a 200 mL volumetric flask and add 150 mL of methyl alcohol. Place in ultrasonic bath at room temperature for 30 minutes, complete the volume with methyl alcohol. Transfer 4 mL of the this solution to a 50 mL volumetric flask, complete the volume with methyl alcohol to obtain concentration of 20 µg/mL of gemifloxacin. Homogenize and filter.

Standard solution: dissolve an accurately weighed quantity of gemifloxacin mesylate CRS in methyl alcohol and dilute adequately to obtain a 20 µg/mL gemifloxacin solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency for the gemifloxacin peak is not less than 2000 theoretical plates/meter. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.5%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity in mg of $C_{18}H_{20}FN_5O_4$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

C. Proceed protected from direct light, as described in *Microbiological Assay of Antibiotics (5.5.3.3)* by agar diffusion using cylinders.

Microorganism: *Staphylococcus epidermidis* ATCC 12228.

Culture media: culture medium number 1 for microorganism maintenance, sterile saline solution for inoculum standardization, culture medium number 1 for base layer and for inoculum preparation.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 50 mg of gemifloxacin to a 200 mL volumetric flask and add 150 mL of methyl alcohol. Place in ultrasonic bath at room temperature for 30 minutes, complete the volume with methyl alcohol and homogenize. Dilute, successively, to concentrations of 0.5 µg/mL, 1.5 µg/mL and 4.5 µg/mL, using 1% Potassium phosphate buffer, sterile, pH 6.0 (*Solution 1*) as diluent.

Standard solution: dissolve an accurately weighed quantity of gemifloxacin mesylate CRS in methyl alcohol and dilute adequately to obtain a 0.1 mg/mL gemifloxacin solution. Dilute, successively, to concentrations of 0.5 µg/mL, 1.5 µg/mL and 4.5 µg/mL, using 1% Potassium phosphate buffer, sterile, pH 6.0 (*Solution 1*) as diluent.

Procedure: add 20 mL of medium number 1 to each plate, wait to solidify, add 5 mL of 2% inoculum and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*, using cylinders, adding 0.2 mL of the freshly prepared solutions to the cylinders. Calculate the quantity in mg of gemifloxacin ($C_{18}H_{20}FN_5O_4$) in the tablets from the standard potency and from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

SODIUM METABISULFITE***Natrii metabisulfis***Na₂S₂O₅; 190.11SO₂; 64.06

sodium metabisulfite; 05711

Sodium salt of disulfuric acid (2:1)

[7681-57-4]

Sodium metabisulfite contains quantity of Na₂S₂O₅ equivalent to not less than 65.0% and not more than 67.4% of SO₂.

DESCRIPTION

Physical characteristics. White, off-white or colorless crystals. Efflorescent.

Solubility. Freely soluble in water and slightly soluble in ethyl alcohol.

IDENTIFICATION

The 5% (w/v) solution meets sodium ion and sulfite ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 3.5 to 5.0. Determine in 5% (w/v) solution in carbon dioxide-free water.

Limit for Thiosulfates. Mix 2.2 g of the sample with 10 mL of *M* hydrochloric acid. Heat gently for five minutes, cool and transfer to a small test tube. If there is turbidity, it is not greater than that produced by 0.1 mL of 0.1 *M* sodium thiosulfate, treated under the same conditions (0.05%).

Arsenic (5.3.2.5). Mix 0.6 g of the sample with 2 mL of water in a beaker. Add, dropwise, 1.5 mL of nitric acid. Evaporate in water bath until dryness. Heat over the flame until no more vapors are produced. Transfer the residue and complete to 25 mL. Proceed as described in *Method I*. Not more than 0.0005% (5 ppm).

Iron (5.3.2.4). Dissolve 0.5 g of the sample in 14 mL of diluted hydrochloric acid (2 in 7) and evaporate in water bath to dryness. Dissolve the residue in 7 mL of diluted hydrochloric acid (2 in 7) and, again evaporate to dryness. Dissolve the residue in a mixture of 2 mL of hydrochloric acid and 20 mL of water, add three drops of bromine water RS. Heat to boiling to release bromine. Cool. Dilute with water to 40 mL. Not more than 0.002% (20 ppm).

Heavy metals (5.3.2.3). Dissolve 1 g of the sample in 10 mL of water, add 5 mL of hydrochloric acid and evaporate in water bath to dryness. Dissolve the residue in 25 mL of water. Not more than 0.002% (20 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.2 g of the sample in 50 mL of 0.05 M iodine VS. Allow to stand for five minutes protected from light. Add 1 mL of hydrochloric acid and titrate excess iodine with 0.1 M VS sodium thiosulfate using, as indicator, 3 mL of starch TS solution, which should be added towards the end of the titration. Each mL of 0.05 M iodine VS is equivalent to 3.203 mg of SO₂.

PACKAGING AND STORAGE.

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Antioxidant.

POTASSIUM METAPHOSPHATE*Kalii metaphosphas* $(\text{KPO}_3)_x$

potassium metaphosphate; 05721

Potassium salt of metaphosphoric acid (1:1)

[7790-53-6]

Potassium metaphosphate is a linear chain polymer with a high degree of polymerization. It contains the equivalent to not less than 59.0% and not more than 61.0% of P_2O_5 .

DESCRIPTION

Physical characteristics. White powder.

Solubility. Insoluble in water. Soluble in diluted aqueous solutions of alkali metal salts (except potassium).

Physicochemical constants.

Viscosity (5.2.7): mix 0.3 g of the sample with 200 mL of 0.35% sodium pyrophosphate (w/v) using a magnetic stirrer. Determine the viscosity of the clear preparation obtained or of the liquid phase of the mixture obtained, after 30 minutes of continuous agitation. Between 6.5 cP and 15 cP.

IDENTIFICATION

A. Add 1 g of the finely powdered sample, slowly and with vigorous agitation, to 100 mL of 2% sodium chloride (w/v). A gelatinous mass is formed.

B. Heat to boiling, for 30 minutes, mixture of 0.5 g of the sample, 10 mL of nitric acid and 50 mL of water, and cool. The resulting solution meets phosphate ion reactions (5.3.1.1) and potassium ion reactions (5.3.1.1).

PURITY TESTS

Limit for fluorides. Transfer 5 g of the sample, 25 mL of water, 50 mL of perchloric acid, five drops of 50% silver nitrate (w/v) and some glass beads to a 250 mL distillation flask, connected to a condenser containing a thermometer and capillary tube, both in contact with the liquid. Connect a small, water-filled addition funnel or steam generator to the capillary tube. Adapt the flask to a distillation system with 1/3 of the bottom of the flask in the flame. Distill into a 250 mL volumetric flask until the temperature reaches 135 °C. Add water through the addition funnel or introduce steam through a capillary to keep the temperature between 135 °C and 140 °C. Continue the distillation until collecting 225 mL to 240 mL, then complete the volume with water and homogenize. Transfer 50 mL of this solution to a Nessler tube and, to the standard Nessler tube, transfer 50 mL of water. Add to each tube 0.1 mL of filtered alizarin TS solution and 1 mL of freshly prepared 0.025% (w/v) hydroxylamine hydrochloride solution and homogenize. Add, dropwise, and under agitation, 0.05 M sodium hydroxide to the tube containing the sample, until the color matches that of the standard tube (slightly pink). Next, add 1 mL of 0.1 M hydrochloric acid to each tube and homogenize. Using a burette with a graduation of 0.05 mL, slowly add to the tube containing the sample, a sufficient quantity of thorium nitrate at 0.025% (w/v), so that, after homogenization, the

color of the liquid turns pink. Record the volume of added solution, add the same accurately weighed volume to the standard tube and homogenize. Then, with the aid of the burette, add sodium fluoride RS (10 µg of F/mL), to turn the coloration of the two tubes similar, after dilution to the same volume. Homogenize and allow air bubbles to escape before proceeding to final color comparison. Check the endpoint by adding one or two drops of sodium fluoride RS to the standard tube. Distinct coloration is observed. The volume of sodium fluoride required for the reference solution should not exceed 1 mL (0.001%).

Arsenic (5.3.2.5). Dissolve 1 g of the sample in 15 mL of 3 M hydrochloric acid and proceed as described in *Spectrophotometric method, Method I*. Not more than 0.0003% (3 ppm).

Lead (5.3.2.12). Dissolve 1 g of the sample in 10 mL of 3 M hydrochloric acid and proceed as described in *Limit test for lead*. Not more than 0.0005% (5 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Add 10 mL of 3 M hydrochloric acid to 1 g of the sample and heat until it no longer dissolves. Add 15 mL of water, homogenize and filter. Not more than 0.002% (20 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Mix 0.2 g of the sample with 15 mL of nitric acid and 30 mL of water, boil for 30 minutes, cool and dilute to approximately 100 mL with water. Heat to 60 °C, add excess ammonium molybdate RS1 and heat at 50 °C for 30 minutes. Filter and wash the precipitate, first with 0.5 M nitric acid and then with 1% (w/v) potassium nitrate, until no acid residue is detected in the filtrate (use litmus paper). Add 25 mL of water to the precipitate, dissolve with 50 mL of M sodium hydroxide VS, add phenolphthalein TS and titrate excess M sodium hydroxide VS with 0.5 M sulfuric acid VS. Each mL of M sodium hydroxide VS is equivalent to 3.086 mg of P₂O₅.

PACKAGING AND STORAGE

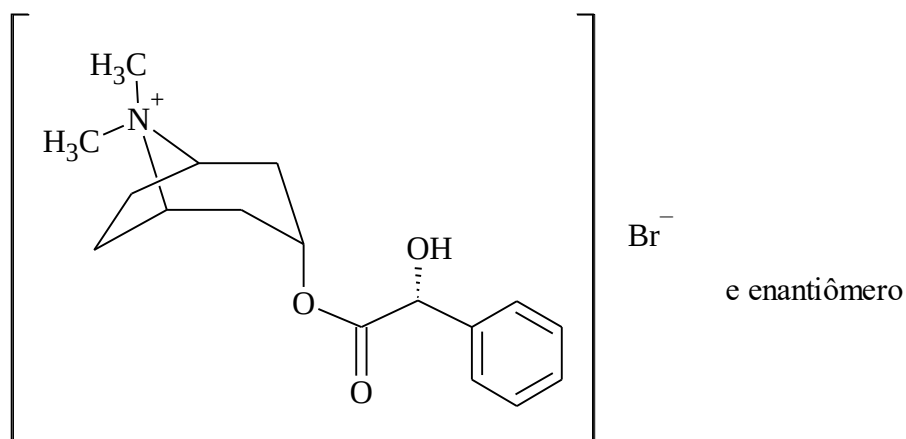
In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Buffering agent.

HOMATROPINE METHYLBROMIDE*Homatropini methylbromidum*C₁₇H₂₄BrNO₃; 370.29

homatropine methylbromide; 04747

(3-*endo*)-3-[(2-hydroxy-2-phenylacetyl)oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane (1:1)
bromide
[80-49-9]

It contains not less than 98.5% and not more than 100.5% of C₁₇H₂₄BrNO₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or colorless crystals. **Melting point (5.2.2):** around 190 °C.

Solubility. Freely soluble in water, soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the homatropine methylbromide CRS spectrum, prepared identically. If differences in spectra are observed, dissolve sample and standard separately in methyl alcohol and recrystallize by adding dioxane to each solution.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.1% (w/v) sample solution in ethyl alcohol, there is a maximum point at 258 nm, identical to the one observed in the spectrum of a similar homatropine methylbromide CRS solution.

C. Dissolve 0.1 g of the sample in 5 mL of water and add mercuric potassium iodide RS. A white or slightly yellowish precipitate is produced. Precipitate is not produced by the addition of solutions of alkali hydroxides or carbonates, even in sample concentrated solutions (distinction from most alkaloids).

D. Dissolve 0.1 g of the sample in 5 mL of water and add ammonium reineckate RS. A red precipitate is produced.

E. The 5% (w/v) aqueous solution meets bromide ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. The sample preparation at 5% (w/v) in carbon dioxide-free water is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). 4.5 to 6.5. Determine in 1% (w/v) solution in carbon dioxide-free water.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of anhydrous formic acid, water and ethyl acetate (16.5:16.5:67) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.2 g of the sample in mixture of water and methyl alcohol (1:9) and dilute to 5 mL with the same solvent.

Solution (2): dilute 0.5 mL of *Solution (1)* to 100 mL with mixture of water and methyl alcohol (1:9).

Develop chromatogram. Remove the plate, dry in oven between 100 °C and 105 °C, until the solvent odor is not perceptible. Allow to cool. Spray with diluted potassium iodobismutate RS and then with 3% (w/v) hydrogen peroxide RS. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.5%).

Volatile organic impurities. Proceed as described in *Gas chromatography (5.2.17.5)*. Use gas chromatograph equipped with flame ionization detector; chromatographic column of fused silica (30 m long and 0.53 mm internal diameter), packed with silica chemically bonded to phenylmethylpolysiloxane (5:95) (5 µm); pre-column of 5 m long and 0.53 mm internal diameter with phenylmethylsiloxane deactivated silica. Program the column temperature according to the following parameters: place at 35 °C for five minutes and increase to 175 °C at the rate of 8 °C per minute; increase to 260 °C at 35 °C ratio per minute and hold for not less than 16 minutes. Keep the injector and detector temperatures at 70°C and 260°C, respectively. Use helium as carrier gas at a linear velocity of about 35 cm/second.

Sample solution: dissolve, in organic compounds-free water, an accurately weighed quantity of the sample to obtain a 20 mg/mL solution.

Standard solution: prepare solution, in organic compounds-free water, containing 0.04 µg/mL of benzene, 12.0 µg/mL of methylene chloride, 1.2 µg/mL of chloroform, 7.6 µg/mL of dioxane and 1.6 µg/mL of trichlorethylene.

Inject 1 µL replicates of the *Standard Solution*. The resolution among any of the components is not less than 1.0. The relative standard deviation of the replicate areas under the recorded peaks is not more than 15.0%.

Procedure: inject, separately, 1 µL of the *Standard Solution* and the *Sample Solution*. Record the chromatograms and measure the areas under the peaks. The areas under the peaks obtained with

the *Sample solution*, related to benzene, methylene chloride, chloroform, dioxane and trichlorethylene must not be greater than the areas under the peaks related to benzene, methylene chloride, chloroform, dioxane and trichlorethylene obtained with the *Standard solution*, corresponding to not more than 2 ppm, 600 ppm, 60 ppm, 380 ppm and 80 ppm, respectively.

Loss on drying (5.2.9.1). Dry in oven at 105 °C for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Dissolve 0.3 g of the sample in 10 mL of water. Titrate with 0.1 M silver nitrate VS. Determine endpoint potentiometrically, using silver indicator electrode and silver/silver chloride reference electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 37.029 mg of $C_{17}H_{24}BrNO_3$.

B. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh about 0.7 g of the sample and dissolve in mixture of 50 mL of glacial acetic acid and 10 mL of mercury acetate RS. Add one drop of methylrosanilinium chloride TS and titrate with 0.1 M perchloric acid VS until blue color turns bluish-green. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 37.029 mg of $C_{17}H_{24}BrNO_3$.

PACKAGING AND STORAGE

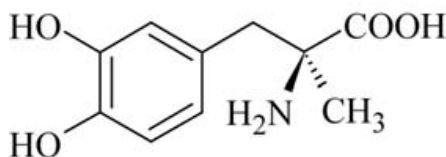
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anticholinergic.

METHYLDOPA***Methyldopum*** $C_{10}H_{13}NO_4$; 211.22

methyldopa; 05799

3-Hydroxy- α -methyl-L-tyrosine

[555-30-6]

 $C_{10}H_{13}NO_4 \cdot 1\frac{1}{2}H_2O$; 238.24

methyldopa sesquihydrate; 09496

3-Hydroxy- α -methyl-L-tyrosine hydrate (2:3)

[41372-08-1]

It contains not less than 98.0% and not more than 101.0% of $C_{10}H_{13}NO_4$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or yellowish-white crystalline powder, or colorless or nearly colorless crystals.

Solubility. Slightly soluble in water, in glacial acetic acid and in methyl alcohol, very slightly soluble in ethyl alcohol. Soluble in diluted mineral acid solutions.

Physicochemical constants.

Specific optical rotation (5.2.8): -25 to -28, in relation to the anhydrous substance. Determine in solution at 4,4% (w/v) in aluminum chloride RS.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the methyldopa CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0,004% (w/v) solution in 0.1 M hydrochloric acid, there is a maximum point at 280 nm, calculated in relation to the anhydrous substance. The absorbance differs by not more than 3% compared to that of a methyldopa CRS solution prepared identically.

C. Add three drops of 0.4% (w/v) ninhydrin in sulfuric acid to 10 mg of the sample. After 10 to 15 minutes, a dark violet color develops. Add three drops of water. The color turns pale yellowish brown.

PURITY TESTS

Acidity. Dissolve 1 g of the sample, under heating, in carbon dioxide-free water. Add one drop of methyl red TS and titrate with 0.1 M sodium hydroxide until a yellow color develops. Not more than 0.5 mL of the titrant is used for the indicator changeover.

Limit for 3-*O*-methyldopa. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using chromatographic cellulose, with 0.25 mm thick, as support, and mixture of butyl alcohol, glacial acetic acid and water (65:15:25) as mobile phase. Apply to the plate, separately, 20 µL of the *Solution (1)* and 10 µL of the *Solution (2)* freshly prepared, described below.

Solution (1): dissolve 0.1 g of the sample in methyl alcohol and dilute to 10 mL with the same solvent, obtaining a 10 mg/mL solution.

Solution (2): dissolve 5 mg of 3-*O*-methyldopa CRS in methyl alcohol and dilute to 50 mL with the same solvent, obtaining a 0.1 mg/mL solution

Develop chromatogram. Remove plate, dry under heated air. Spray with *p*-nitroaniline and sodium nitrite RS and dry under heated air. Spray with 20% (w/v) decahydrate sodium carbonate (w/v). Any secondary stain, corresponding to 3-*O*-methyldopa obtained in the chromatogram with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (2)* (1.0%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20.1). Determine in 0.2 g of sample. Between 10.0 and 13.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 0.2 g of the sample and dissolve in 25 mL of glacial acetic acid, heating if necessary. Add 50 mL of acetonitrile, 0.1 mL of methylrosanilinium chloride TS and titrate with 0.1 M perchloric acid VS until indicator turns blue. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 21.122 mg of C₁₀H₁₃NO₄.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antihypertensive.

METHYLDOPA TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{10}H_{13}NO_4$. The tablets must be coated.

IDENTIFICATION

A. Weigh and pulverize the tablets. Use quantity of powder equivalent to 10 mg of methyldopa. Add three drops of 0.4% (w/v) ninhydrin in sulfuric acid. After 10 to 15 minutes, a dark violet color develops. Add three drops of water. The color turns pale yellowish brown.

B. Weigh and pulverize the tablets. Use quantity of powder equivalent to 10 mg of methyldopa, add 2 mL of 0.05 M sulfuric acid, 2 mL of ferrous tartrate RS and 0.25 mL of 6 M ammonium hydroxide. A dark violet color develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 20 minutes).

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 280 nm **(5.2.14)**, using the same solvent for zero adjustment. Calculate the quantity of $C_{10}H_{13}NO_4$ dissolved in the medium, comparing the readings obtained with that of the methyldopa CRS solution at the concentration of 0.005% (w/v), prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{10}H_{13}NO_4$ dissolve in 20 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.1 g of methyldopa to a 100 mL volumetric flask, add 50 mL of 0.05 M sulfuric acid and shake mechanically for 15 minutes. Complete the volume with the same solvent. Homogenize and filter. Accurately weigh about 50 mg of methyldopa CRS, transfer to a 50 mL volumetric flask, dissolve and complete the volume with 0.05 M sulfuric acid and

homogenize. Separately transfer 5 mL of the standard and sample solutions to 100 ml volumetric flasks. Prepare blank in parallel using 5 mL of 0.05 M sulfuric acid. Add, to each flask, 5 mL of ferrous tartrate RS and complete the volume with ammonium acetate buffer pH 8.5. Measure the absorbances of the resulting solutions at 520 nm, using blank for zero adjustment. Calculate the quantity of $C_{10}H_{13}NO_4$ in the tablets from the readings obtained.

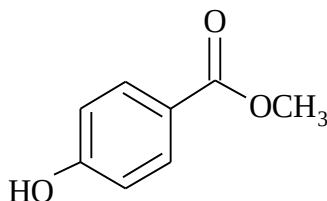
PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

METHYLPARABEN
Methylis parahydroxybenzoas



$C_8H_8O_3$; 152.15
methylparaben; 05809
4-hydroxybenzoic acid methyl ester.
[99-76-3]

It contains not less than 98.0% and not more than 102.0% of $C_8H_8O_3$.

DESCRIPTION

Physical characteristics. White or colorless crystalline powder.

Solubility. Slightly soluble in water, freely soluble in acetone, ethyl alcohol and ethyl ether.

Physicochemical constants.

Melting range (5.2.2): 125 °C to 128 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the methylparaben CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 230 nm to 280 nm, of 0.0005% (w/v) solution in ethyl alcohol, there is a maximum point at 258 nm. The absorbance at 258 nm is of 0.52 to 0.56.

PURITY TESTS

Preparation aspect. Dissolve 1 g of the sample in ethyl alcohol and dilute to 10 mL with the same solvent. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

Acidity. To 2 mL of the preparation obtained in *Preparation aspect*, add 3 mL of ethyl alcohol, 5 mL of carbon dioxide-free water and 0.1 mL of bromocresol green TS. Not more than 0.1 mL of 0.1 M sodium hydroxide is used to promote the indicator changeover.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of anhydrous formic acid, ethyl acetate and methylene chloride (2:10:88), as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.1 g of the sample in methyl alcohol and dilute to 5 mL with the same solvent.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with methyl alcohol.

Develop chromatogram. Remove plate and allow to dry under hot air current. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (1.0%).

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Accurately weigh about 1 g of the sample, transfer to an erlenmeyer flask with ground stopper and add 20 mL of *M* sodium hydroxide VS. Adapt reflux condenser and heat at 70 °C for one hour. Cool to room temperature. Titrate the excess *M* hydrochloric acid VS with 0.5 *M* sulfuric acid VS. Determine the endpoint potentiometrically, proceeding with titration until the second inflection point. Carry out the blank test and make the necessary corrections. Each mL of *M* sodium hydroxide VS is equivalent to 152.150 mg of $C_8H_8O_3$.

PACKAGING AND STORAGE

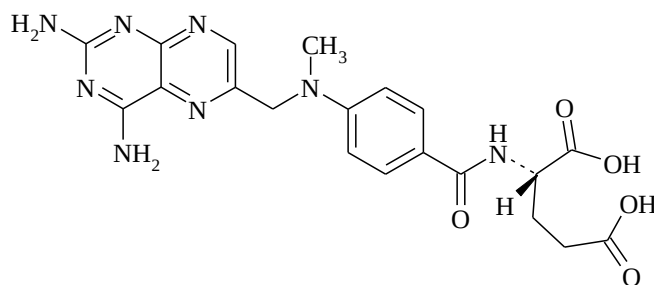
In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Preservative.

METHOTREXATE***Methotrexatum***

$C_{20}H_{22}N_8O_5$; 454.45

methotrexate; 05884

N-[4-[[2,4-diamino-6-pteridiny]methyl]methylamino]benzoyl]-L-glutamic acid
[59-05-2]

It contains not less than 98.0% and not more than 102.0% of $C_{20}H_{22}N_8O_5$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Orangish or yellow crystalline powder, hygroscopic.

Solubility. Practically insoluble in water and in ethyl alcohol. Freely soluble in alkali and carbonate solutions, slightly soluble in 6 M hydrochloric acid solution.

Physicochemical constants.

Specific optical rotation (5.2.8): +19,0 to +24,0, in relation to the anhydrous substance. Determine in 10 mg/mL solution in 0.05 M sodium carbonate using 2.0 dm tube.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the methotrexate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in 0.1 M hydrochloric acid, there are maximum and minimum absorption points identical to the ones observed in the methotrexate CRS spectrum, prepared identically.

PURITY TESTS

Related substances. Proceed as described in Assay. Prepare solutions as described below.

Solution (1): transfer 0.1 g of the sample, accurately weighed, to a 100 mL volumetric flask and dilute with *Mobile phase* to complete the volume and homogenize.

Solution (2): dissolve an accurately weighed quantity of methotrexate CRS in *Mobile phase* to obtain a 5.0 µg/mL solution.

Procedure: inject, separately, 10 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Record the *Solution (1)* chromatogram for not more than three times the methotrexate retention time. The sum of the areas under the peaks, except that under the methotrexate obtained in *Solution (1)*, is less than four times the area under the main peak of *Solution (2)* (2.0%) and the area under no peak is greater than that under the peak obtained with *Solution (2)* (0.5%).

Enantiomeric purity. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 302 nm; 150mm long, 4.0 mm internal diameter column, packed with silica gel chemically bonded to bovine albumin (thickness of 7 µm and pore diameter of 30 nm), kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mix 500 mL of anhydrous dibasic sodium phosphate solution at 0.71% (w/v) and 600 mL of monobasic sodium phosphate solution at 0.69% (w/v). Adjust pH to 6.9 with 2 M sodium hydroxide. Mix 920 mL of this solution with 80 mL of isopropyl alcohol.

Solution (1): quantitatively dissolve about 20 mg of the sample in *Mobile phase* and complete to 100 mL with the same solvent.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL using *Mobile phase*.

Resolution solution: quantitatively dissolve about 4 mg of methotrexate RS in *Mobile phase* and complete to 100 mL with the same solvent.

Inject 20 µL replicates of the *Resolution solution*. The resolution between (S)-methotrexate and (R)-methotrexate peaks is not less than 3.0.

Procedure: inject, separately, 20 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The content of (R)-methotrexate in *Solution (1)* compared to methotrexate contained in *Solution (2)* is not more than 3.0%.

Heavy metals (5.3.2.3). Determine in 1.0 g of the sample. Proceed as described below.

Sample preparation: transfer sample to silica crucible and mix with 4 mL of 25% (w/v) magnesium sulfate solution in 5.5% (v/v) sulfuric acid. Carefully heat. If the mixture is liquid, evaporate to dryness in water bath. Gradually heat to incinerate at maximum temperature of 800 °C until white or gray residue is obtained. Cool and moisten the residue with some drops of 5.5% sulfuric acid (v/v). Evaporate, incinerate and cool. The total incineration period must be not more than two hours. Dissolve the residue obtained with two 5 mL portions of 0.2 M hydrochloric acid and add 0.1 mL of phenolphthalein TS. Add concentrated ammonia solution until a pink color is developed. Allow to cool and discolor the solution with glacial acetic acid. Add 0.5 mL of excess acid, filter if necessary, and dilute the solution with water to a volume of 20 mL.

Standard Preparation: prepare as described for *Sample preparation*, using 5 mL of *Standard Lead Solution (10 ppm Pb)* in place of the sample. To 10 mL of the obtained solution, add 2 mL of the *Sample Preparation*.

Monitoring preparation: prepare as described for *Sample preparation*, adding to the substance under test 5 mL of *Standard Lead Solution (10 ppm Pb)*. To 10 mL of the obtained solution, add 2 mL of the *Sample Preparation*.

Blank solution: mixture of 10 mL of water and 2 mL of the *Sample Preparation*.

Procedure: transfer 12 mL of each solution obtained to a Nessler tube, add 2 mL of *Acetate Buffer pH 3.5* and homogenize immediately. In each tube, add 1.2 mL of *Thioacetamide reagent* and homogenize. The brown color in the *Sample preparation* should not be more intense than in the *Standard preparation*. In the *Standard Preparation* there must be a light brown color when compared to the *Blank Solution*. The *Monitoring preparation* stains at least as intensely as the *Standard Preparation*. Not more than 0.005% (50 ppm).

Water (5.2.20.1). Determine according to *Direct method*. Use 0.1 g of sample. Not more than 12.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 302 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group; *Mobile phase* flow of 1.2 mL/minute.

Phosphate buffer pH 6.0: prepare 0.2 M dibasic sodium phosphate solution and 0.1 M anhydrous citric acid (63:37). Adjust to pH 6.0, if necessary, with 0.1 M anhydrous citric acid or 0.2 M sodium phosphate dibasic.

Mobile phase: mixture of *Phosphate buffer pH 6.0* and acetonitrile (90:10). Degas and filter. Make adjustments if necessary.

Sample solution: transfer, accurately weighed, 25 g of the sample to a 250 mL volumetric flask. Dilute with *Mobile phase* to complete the volume and homogenize.

Standard solution : dissolve an accurately weighed quantity of methotrexate CRS in *Mobile phase* to obtain a 0.1 mg/mL solution.

Resolution solution: prepare a solution at 0.1 mg/mL of methotrexate CRS and at 0.1 mg/mL of folic acid in *Mobile Phase*.

Inject 10 µL replicates of the *Resolution solution*. The relative retention times are 0.35 for folic acid and 1.0 for methotrexate. The resolution between methotrexate and folic acid peaks is not more than 8.0. Inject 10 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the recorded peaks is not more than 2.5% for methotrexate.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₀H₂₂N₈O₅ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

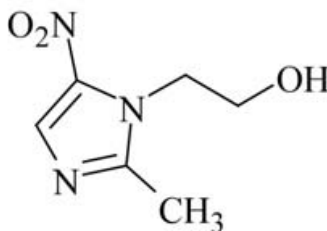
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antineoplastic.

METRONIDAZOLE***Metronidazolum*** $C_6H_9N_3O_3$; 171.16

metronidazole; 05902

2-Methyl-5-nitro-1*H*-imidazol-1-ethanol

[443-48-1]

It contains not less than 99.0% and not more than 101.0% of $C_6H_9N_3O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or slightly yellowish crystalline powder.

Solubility. Moderately soluble in water and in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 159 °C to 163 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the metronidazole CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Preparation aspect. Dissolve 1 g of the substance in 10 mL of 50% (v/v). hydrochloric acid. The preparation is clear **(5.2.25)**.

Related substances. Proceed as described in method **B.** from *Assay*. Prepare the solutions for the test as described below.

Solution (1): 1 mg/mL metronidazole solution in *Mobile phase*.

Solution (2): 1 µg/mL solution of metronidazole CRS and 2 µg/mL of 2-methyl-5-nitroimidazole in *Mobile phase*.

Inject replicates (not less than six) of 30 μ L of the *Standard solution*. The relative retention times are approximately 0.75 for 2-methyl-5-nitroimidazole and 1.0 for metronidazole. The resolution between the peak of metronidazole and 2-methyl-5-nitroimidazole is not less than 2.0. The tailing factor for the metronidazole peak is not more than 2.0. The relative standard deviation of the replicate areas under the recorded peaks is not more than 6.0%.

Procedure: inject separately 30 μ L of *Solution (1)* and of *Solution (2)*. Record the chromatograms and measure the areas under the peaks. Determine the percentage of each impurity found in *Solution (1)*, in which the individual content of 2-methyl-5-nitroimidazole is not more than 0.1% in relation to metronidazole. The percentage for any other individual impurity is not more than 0.1% and not more than 0.2% for the sum of all impurities present.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Quantitatively dissolve about 0.3 g of the sample, previously desiccated, in 20 mL of acetic anhydride and heat slowly if necessary. Cool, add one drop of malachite green TS and titrate with 0.1 M perchloric acid VS, using microburette, until color turns greenish-yellow. Carry out the blank test and make the necessary corrections. Alternatively, determine the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 17.116 mg of C₆H₉N₃O₃.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 319 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μ m); kept at 30 °C; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of methyl alcohol and water (1:4).

Sample solution: weigh, accurately, about 100 mg of the sample, transfer to a volumetric flask and complete the volume to 100 mL with *Mobile phase*. Dilute 3 mL of the resulting solution to 100 mL with *Mobile phase*.

Standard solution: dissolve an accurately weighed amount of metronidazole CRS in the *Mobile phase* and dilute adequately to obtain a solution at 30 μ g/mL.

Inject 30 μ L replicates of the *Standard solution*. The tailing factor in relation to the metronidazole peak is not more than 2.0 and the relative standard deviation of the replicate areas under the recorded peaks is not more than 2.0%.

Procedure: Inject 30 μ L of *Sample solution* and *Standard solution* separately and record the chromatograms for not less than twice the retention time of the main peak and measure the areas

under the peaks. Calculate the content of $C_6H_9N_3O_3$ in the sample, from the responses obtained with the metronidazole, the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

METRONIDAZOLE TABLETS

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_6H_9N_3O_3$. The tablets must be coated.

IDENTIFICATION

The identification test B. can be omitted if tests A., C. and D. are carried out. The identification tests C. and D. can be omitted if tests A. and B. are carried out.

A. Weigh and pulverize the tablets. Shake the quantity of powder equivalent to 0.1 g of metronidazole with 40 mL of chloroform for 15 minutes. Filter and evaporate the filtrate to dryness. Proceed as described in test **A.** from *Identification* in the *Metronidazole* monograph.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. Weigh and pulverize the tablets. Use quantity of powder equivalent to 0.1 g of metronidazole, heat in water bath with 10 mg of zinc powder, 1 mL of water and 0.25 mL of hydrochloric acid for five minutes. Cool in ice bath and add 0.5 mL of sodium nitrite RS. Remove excess nitrite with sulfamic acid. Add 0.5 mL of 2-naphthol RS1 and 2 mL of 0.5 M sodium hydroxide. An orangish-red color develops.

D. Weigh and pulverize the tablets. Shake quantity of powder equivalent to 0.2 g of metronidazole with 4 mL of 0.5 M sulfuric acid. Filter. To the filtrate, add 10 mL of picric acid RS and allow to stand. The melting point of the precipitate, after being washed with water and dried at 105 °C, is approximately 150 °C.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 250 mL volumetric flask. Add 100 mL of 1% (v/v) hydrochloric acid and shake for 30 minutes. Complete the volume with the same solvent and homogenize. Filter, discarding the first 15 mL of the filtrate. Proceed as described in method **A.** of *Assay* from "Carry out successive dilutions...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 1000 mL.

Apparatus: baskets, 100 rpm.

Time: 60 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 274 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the quantity of $C_6H_9N_3O_3$ dissolved in the medium, comparing the readings obtained with that of the metronidazole CRS solution at the concentration of 0.002% (w/v), prepared in the same solvent.

Tolerance: not less than 85% (Q) of the stated quantity of $C_6H_9N_3O_3$ dissolve in 60 minutes.

PURITY TESTS

Limit for 2-Methyl-5-nitroimidazole. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel F₂₅₄, as support, and mixture of chloroform, dimethylformamide and 90%(v/v) formic acid (80:25:5), as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize 20 tablets. Shake the quantity of powder equivalent to 0.2 g of metronidazole with 5 mL of mixture of chloroform and methyl alcohol (1:1) for five minutes. Filter.

Solution (2): 0.2 mg/mL 2-methyl-5-nitroimidazole CRS solution in mixture of chloroform and methyl alcohol (1:1).

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any stain corresponding to 2-methyl-5-nitroimidazole obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (2)* (0.5%).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.2 g of metronidazole to a 100 mL volumetric flask, add 70 mL of 1% (v/v) hydrochloric acid. Shake mechanically for 15 minutes and complete the volume with the same solvent. Filter. Carry out successive dilutions up to a concentration of 0.002% (w/v), using 1% (v/v) hydrochloric acid as solvent. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 278 nm, using 1% (v/v) hydrochloric acid for zero adjustment. Calculate the quantity of $C_6H_9N_3O_3$ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (3 µm to 10 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water and methyl alcohol (80:20).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.5 g of metronidazole to a 50 mL volumetric flask, add 30 mL of methyl alcohol and shake mechanically for 30 minutes. Complete the volume with methyl alcohol and allow to decant. Transfer 5 mL of the supernatant to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Standard solution: 0.5 mg/mL metronidazole CRS solution in *Mobile phase*.

Inject 10 µL replicates of the *Standard solution*. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₆H₉N₃O₃ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

METRONIDAZOLE INJECTION SOLUTION

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_6H_9N_3O_3$.

IDENTIFICATION

A. Transfer volume of the injection solution equivalent to about 0.1 g of metronidazole into a separating funnel and shake with 9 g of sodium chloride for five minutes. Extract with 20 mL of acetone. Separate the top layer and evaporate to dryness. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the metronidazole CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.5 to 7.0.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 0.35 EU/mg of metronidazole.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer volume of the injection solution equivalent to 50 g of metronidazole to a 100 mL volumetric flask and complete the volume with 0.1 M hydrochloric acid. Dilute, successively, in 0.1 M hydrochloric acid, to a concentration of 0.002% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 277 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the quantity of $C_6H_9N_3O_3$ in the injection solution from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph with ultraviolet detector at 320 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 μ m to 10 μ m); *Mobile phase* flow rate of 2.0 mL/minute.

Mobile phase: mixture of 0.073% (w/v) potassium phosphate monobasic and methyl alcohol (93:7). Adjust pH to 4.0 ± 0.5 with 0.1 M phosphoric acid.

Sample solution: transfer volume of the injection solution equivalent to 25 mg of metronidazole to a 25 mL volumetric flask and complete the volume with water. Transfer 2 mL of the solution

obtained to a 10 mL volumetric flask containing 2 mL of methyl alcohol and complete the volume with *Mobile phase*.

Standard solution: quantitatively transfer about 25 mg of metronidazole CRS to a 25 mL volumetric flask, dissolve in methyl alcohol and complete the volume with the same solvent. Transfer 2 mL of the solution obtained to a 10 mL volumetric flask containing 2 mL of water and complete the volume with *Mobile phase*.

Inject 20 µL replicates of the *Standard solution*. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

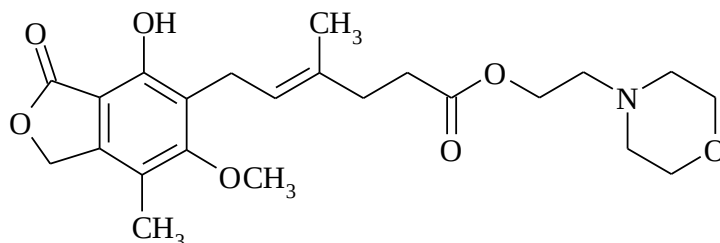
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₆H₉N₃O₃ in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

MYCOPHENOLATE MOFETIL*Mycophenolas mofetil*C₂₃H₃₁NO₇; 433.50

mycophenolate mofetil; 00290

2-(morpholin-4-yl)ethyl (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate.

[128794-94-5]

It contains not less than 98.0% and not more than 102.0% of C₂₃H₃₁NO₇, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water and moderately soluble in anhydrous ethyl alcohol.

Physicochemical constants.

Melting temperature (5.2.2): between 93 °C and 94 °C.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the mycophenolate mofetil CRS spectrum, prepared identically.

PURITY TESTS

Preparation aspect. Dissolve 0,10 g of the sample in ethyl alcohol and dilute to 10 mL with the same solvent. The preparation is clear **(5.2.25)** and colorless **(5.2.12)**.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 250 nm; 250mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm); kept at 45 °C, *Mobile phase* flow rate of 1.5 mL/minute. *Prepare the solutions immediately before use or store between 4 °C and 8 °C.* Protect the solutions from light. Keep the chromatograph sampler at a temperature of 10°C and store the injection solutions at that temperature for 15 minutes before injecting.

Triethylamine solution: transfer 3 mL of triethylamine to a 1000 mL volumetric flask, complete the volume with water and homogenize. Adjust pH to 5.3 with phosphoric acid.

Mobile phase: mixture of *Triethylamine solution* and acetonitrile (65:35).

Solution (1): dissolve 20 mg of the sample in acetonitrile, dilute to 10 mL with the same solvent and homogenize.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with acetonitrile. Dilute 1 mL of the resulting solution to 10 mL with acetonitrile and homogenize.

Solution (3): dissolve 5 mg of mycophenolate mofetil to identify the CRS peaks containing impurities A (2-(morpholin-4-yl)ethyl(4E)-6-(4,6-dihydroxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate), B (2-(morpholin-4-yl)ethyl(4E)-6-[(1R)-4-hydroxy-6-methoxy-7-methyl-1-[2-(morpholin-4-yl)ethoxy]-3-oxo-1,3-dihydroisobenzofuran-5-yl]-4-methylhex-4-enoate), D (2-(morpholin-4-yl)ethyl(4E)-6-(4,6-dimethoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate), E (methyl(4E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate), F (acid(4E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoic or mycophenolic acid), G (2-(morpholin-4-yl)ethyl(4E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate N-oxide) and H (7-hydroxy-5-methoxy-4-methyl-6-[2-[(2R)-2-methyl-5-oxotetrahydrofuran-2-yl]ethyl]isobenzofuran-1(3H)-one) in acetonitrile and dilute to 2.5 mL with the same solvent.

Procedure: inject 10 µL of *Solution (1)*, *Solution (2)* and *Solution (3)* separately and record the chromatograms for not less than three times the main peak retention time. The relative retention times are about 0.3 for impurity F, 0.4 for impurity A, 0.5 for impurity H, 0.6 for impurity G, 0.8 for impurity B, 1.0 for mycophenolate mofetil, 1.2 for impurity D and 1.6 for impurity E. The test will only be valid if the chromatogram obtained with *Solution (3)* presents a resolution of not less than 2.0 between the peaks of impurity H and impurity A. To calculate the impurity B content, multiply the area under the peak by 2.1. The area under the peak of impurity F obtained with *Solution (1)* is not more than five times the area under the main peak obtained with *Solution (2)* (0.5%); the area under the peak of impurity B obtained with *Solution (1)* is not more than twice the area under the main peak obtained with *Solution (2)* (0.2%) and the areas under the peaks of impurities A, D, E, G and H obtained with *Solution (1)* are not more than equal to the area under the main peak obtained with *Solution (2)* (0.1%). For any other impurity, the area under the peak of the impurity obtained with *Solution (1)* is not more than equal to the area under the main peak of the chromatogram obtained with *Solution (2)* (0.1%). The total of impurities is not more than seven times the area under the main peak of the chromatogram obtained with *Solution (2)* (0.7%). The exclusion limit is 0.5 times the area under the main peak of the chromatogram obtained with *Solution (2)* (0.05%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.400 g of the sample in 50 mL of anhydrous acetic acid and titrate with 0.1 M perchloric acid VS, determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 43.350 mg of $C_{23}H_{31}NO_7$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Immunosuppressant.

MYCOPHENOLATE MOFETIL TABLETS

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_{23}H_{31}NO_7$. The tablets must be coated.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer each tablet to a 200 mL volumetric flask and add 150 mL of 0.1 M hydrochloric acid. Shake mechanically, until disintegration, and then place in ultrasonic bath for 15 minutes. Complete the volume with 0.1 M hydrochloric acid, homogenize and filter. Carry out successive dilutions up to a concentration of 0.0025% (w/v), using 0.1 M hydrochloric acid as solvent. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 250 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the quantity of $C_{23}H_{31}NO_7$ in the tablets from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 15 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute, if necessary, with 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 250 nm **(5.2.14)**, using *Dissolution medium* for zero adjustment. Calculate the quantity of $C_{23}H_{31}NO_7$ dissolved in the medium, comparing the readings obtained with that of the mycophenolate mofetil CRS solution at the concentration of 0.0022% (w/v), prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{23}H_{31}NO_7$ dissolve in 15 minutes.

PURITY TESTS

Related substances. Proceed as described in *Assay*. Prepare the *Standard solution* and the *Test solution* as described below.

Solution (1): weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 25 mg of mycophenolate mofetil to a 50 mL volumetric flask, add 30 mL of *Mobile Phase* and place in ultrasonic bath for 10 minutes. Complete the volume with the *Mobile phase*, homogenize and filter.

Solution (2): dissolve an accurately weighed quantity of mycophenolate mofetil CRS in the *Mobile phase* and dilute adequately to obtain a solution at 0.0025 mg/mL.

Procedure: inject, separately, 25 µL of *Solution (1)* and of *Solution (2)*, record the chromatograms for not less than three times the main peak retention time and measure the areas under the peaks. The relative retention times are approximately 1.0 for mycophenolate mofetil and 2.4 for mycophenolic acid. Calculate, individually, the percentages of impurities according to the expression:

$$\left(\frac{C_p}{C_t}\right) \times \left(\frac{r_t}{r_p}\right) \times \left(\frac{1}{F}\right) \times 100$$

where

C_p = concentration, in mg/mL, of mycophenolate mofetil in *Solution (2)*;

C_t = concentration, in mg/mL, of mycophenolate mofetil in *Solution (1)*;

r_p = response under the peak of mycophenolate mofetil in *Solution (2)*;

r_t = area under the peak of mycophenolic acid or of other impurities in *Solution (1)*;

F = correction factor.

Use 1.4 as correction factor to calculate the percentage of mycophenolic acid and 1.0 for other impurities. Not more than 0.5% of mycophenolic acid and 0.1% for other individual impurities and 0.7% of total impurities.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 250 nm; 250mm long, 4.0 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm); kept at 45 °C, *Mobile phase* flow rate of 1.5 mL/minute. *Prepare the solutions immediately before use or store between 4 °C and 8 °C*. Protect the solutions from light.

Triethylamine solution: transfer 10 mL of triethylamine to a 1000 mL volumetric flask, complete the volume with water. Adjust pH to 3.0 with phosphoric acid.

Mobile phase: mixture of *Triethylamine solution* and acetonitrile (70:30) (v/v).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 25 mg of mycophenolate mofetil to a 50 mL volumetric flask, add 30 mL of *Mobile Phase* and place in

ultrasonic bath for 10 minutes. Complete the volume with the *Mobile phase*, homogenize and filter. Quantitatively dilute to obtain a 50 µg/ml solution.

Standard solution: dissolve an accurately weighed quantity of mycophenolate mofetil CRS in the *Mobile phase* and dilute adequately to obtain a solution at 50 µg/mL.

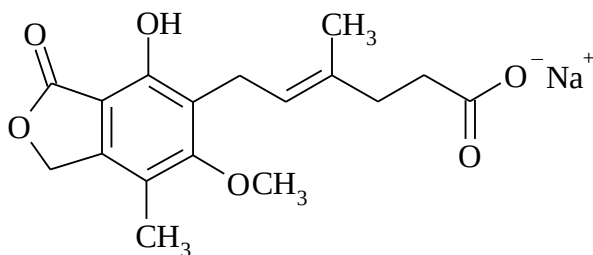
Procedure: inject, separately, 25 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₂₃H₃₁NO₇ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

MYCOPHENOLATE SODIUM***Mycophenolas natrium***C₁₇H₁₉NaO₆; 342.32

sodium mycophenolate; 00291

6-(4-Hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexanoic acid sodium salt
[37415-62-6]

It contains not less than 98.0% and not more than 102.0% of (C₁₇H₁₉NaO₆), in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water and in ethyl alcohol, soluble in methyl alcohol. Practically insoluble in 0.1 M hydrochloric acid and freely soluble in 0.1 M sodium hydroxide.

Physicochemical constants.

Melting temperature (5.2.2): between 189 °C and 191 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the sodium mycophenolate CRS spectrum, prepared identically.

B. The 1.0% (w/v) sample solution meets sodium ion reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use chromatograph equipped with ultraviolet detector at 216 nm; 250mm long, 3.0 mm internal diameter column, packed with silica gel chemically bonded to octadecylsilane group (5 µm); kept at 50 °C°, *Mobile phase* flow rate of 0.8 mL/minute.

Eluent A: mixture of acetonitrile, phosphoric acid and water (100:0.2:900). Degas and filter.

Eluent B: mixture of acetonitrile, phosphoric acid and water (800:0.2:200). Degas and filter.

Diluent: mixture of water and methyl alcohol (90:10).

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 35	90 → 10	10 → 90	linear gradient
35 – 40	10	90	isocratic
40 – 45	10 → 90	90 → 10	linear gradient

Solution (1): sample solution containing sodium mycophenolate at 0.08 mg/mL in *Diluent*. Protect against the action of light.

Solution (2): solution of sodium mycophenolate CRS at 0.08 mg/mL in *Diluent*. Protect against the action of light.

Solution (3): quantitatively transfer about 1 mg of mycophenolate mofetil related-substance B ((RS)-7-hydroxy-5-methoxy-4-methyl-6-[2-(5-methyl-2-oxo-tetrahydrofuran-5-yl)ethyl]-3H-isobenzofuranyl-1-one) into a 50 mL volumetric flask, dissolve in 5 mL of methyl alcohol and complete the volume with water. Transfer 4 mL of this solution to a 100 mL volumetric flask and complete the volume with *Diluent*.

Solution (4): solution of sodium mycophenolate CRS at 0.08 mg/mL in *Solution (3)*.

Solution (5): solution of sodium mycophenolate CRS at 0.024 µg/mL diluted in *Diluent*.

Inject 10 µL replicates of the *Solution (4)*. The relative retention times are 0.9 for mycophenolate mofetil related-substance B and 1.0 for mycophenolate sodium. The resolution between the peaks of mycophenolate sodium and mycophenolate mofetil related-substance B is not less than 1.5. The relative standard deviation of the replicate areas under the recorded peaks is not more than 2%. Inject 10 µL replicates of the *Solution (5)*. The signal/noise ratio is greater than 10.

Procedure: inject, separately, 10 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of each related substance using the following formula:

$$(A_i/A_p) \times (C_p/C_a) \times 100$$

where

A_i = area under the peak of each related substance in the chromatogram obtained with *Solution (1)*;
 A_p = area under the peak relative to mycophenolate sodium in the chromatogram obtained with *Solution (2)*;

C_p = concentration, in mg/mL, of mycophenolate sodium, in *Solution (2)*;

C_a = concentration, in mg/mL, of mycophenolate sodium, in *Solution (1)*;

Not more than 0.1% of mycophenolate mofetil related substance B, not more than 0.07% for another individual impurity and not more than 0.4% for the sum of all impurities. Do not consider peaks related to the solvent or with an area less than 0.05%.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for two hours. Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 250 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm); kept at 30 °C, *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.05%(v/v) phosphoric acid (55:45).

Diluent: mixture of methyl alcohol and water (55:45).

Sample solution: quantitatively transfer about 22.5 mg of the sample to a 50 mL volumetric flask. Add 30 mL of *Diluent*, place in ultrasonic bath for 10 minutes and complete the volume with *Diluent*. Transfer 2 mL of this solution to a 25 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Standard solution: dissolve an accurately weighed quantity of mycophenolic acid CRS in methyl alcohol to obtain a solution equivalent to 0.45 mg/mL of sodium mycophenolate. Transfer 2 mL to a 25 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Procedure: inject, separately, 25 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₇H₁₉NaO₆ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Immunosuppressant.

MYCOPHENOLATE SODIUM TABLETS

Enteric coated tablets must contain not less than 90.0% and not more than 110.0% of the stated quantity of mycophenolic acid ($C_{17}H_{20}O_6$).

IDENTIFICATION

A. The retention time of the main peak from the *Sample solution* chromatogram, obtained in Assay, corresponds to the one from the main peak of the *Standard solution*.

B. Weigh and pulverize 20 tablets. Shake quantity of powder equivalent to 100 mg of sodium mycophenolate with 10 mL of water. Filter. It meets test 1 of sodium ion (**5.3.1.1**).

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Acid stage:

Dissolution medium: 0.1 M hydrochloric acid and 0.1 M sodium hydroxide.

Apparatus: paddles, 50 rpm.

Time: 120 minutes.

Procedure: after testing, remove aliquot from the dissolution medium and filter. Measure the absorbances of the solutions at 250 nm (**5.2.14**), using 0.1 M hydrochloric acid for zero adjustment. Calculate the quantity of $C_{17}H_{20}O_6$ dissolved in the medium, comparing the readings obtained with that of the *Standard mycophenolic acid solution (1)*, prepared as described below.

Standard mycophenolic acid solution (1): accurately weigh approximately 11.25 mg of mycophenolic acid CRS, transfer to a 50 mL volumetric flask. Dissolve and complete the volume with methyl alcohol. Dilute with 0.1 M hydrochloric acid to a concentration of 0.0018% (w/v).

Tolerance: not more than 2.0% (Q) of the stated quantity of $C_{17}H_{20}O_6$ dissolve in 120 minutes.

Buffer stage:

Dissolution medium: After removing the aliquot from the *Acid Stage*, add 250 mL of 0.2 M *Tribasic Sodium Phosphate Buffer*, prepared as described below, and adjust the pH in each tank to 6.8 using 0.1 M sodium hydroxide or 0.1 M hydrochloric acid.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

0.2 M Tribasic Sodium Phosphate Buffer: dissolve 76.02 g of tribasic sodium phosphate dodecahydrate in 1000 mL of water.

Procedure: after testing, remove aliquot from the dissolution medium and filter. Measure the absorbances of the solutions at 250 nm (**5.2.14**), using mixture of 0.1 M hydrochloric acid and 0.2 M tribasic sodium phosphate buffer (3:1) for zero adjustment. Calculate the quantity of $C_{17}H_{20}O_6$ dissolved in the medium, comparing the readings obtained with that of the *Standard mycophenolic acid solution* (2), prepared as described below.

Standard mycophenolic acid solution (2): accurately weigh approximately 11.25 mg of mycophenolic acid CRS, transfer to a 50 mL volumetric flask. Dissolve and complete the volume with methyl alcohol. Dilute, successively, with a mixture of 0.1 M hydrochloric acid and 0.2 M tribasic sodium phosphate buffer (3:1) to a concentration of 0.0018% (w/v).

Tolerance: not less than 75% (Q) of the stated quantity of $C_{17}H_{20}O_6$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography* (**4.2.17.4**). Use chromatograph equipped with ultraviolet detector at 250 nm; 150mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 μ m); kept at 30 °C, *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of methyl alcohol and 0.05%(v/v) phosphoric acid (55:45).

Diluent: mixture of methyl alcohol and water (55:45).

Sample solution: transfer five tablets to a 500 mL volumetric flask, add 275 mL of methyl alcohol and place in ultrasonic bath for 15 minutes. Complete the volume with water and filter. Carry out successive dilutions with *Diluent* until obtaining a solution at 0.036 mg/mL of mycophenolic acid.

Standard solution: accurately weigh approximately 11.25 mg of mycophenolic acid CRS, transfer to a 50 mL volumetric flask. Dissolve and complete the volume with methyl alcohol. Dilute successively with *Diluent* until obtaining a solution at 0.036 mg/mL.

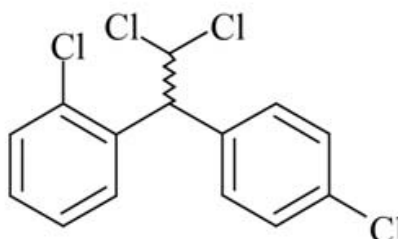
Procedure: inject, separately, 25 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{17}H_{20}O_6$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

MITOTANE**Mitotanium**C₁₄H₁₀Cl₄; 320.03

mitotane; 06020

1-Chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene

[53-19-0]

It contains not less than 97.0% and not more than 103.0% of C₁₄H₁₀Cl₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White granular powder, composed of colorless crystals with pleasant odor.

Solubility. Practically insoluble in water, soluble in ethyl alcohol, in methyl alcohol and in fixed and fatty oils.

Physicochemical constants.

Melting range (5.2.2): 75 °C to 81 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the mitotane CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.02% (w/v) solution in methyl alcohol, there are maximum and minimum points only at the same wavelengths of similar mitotane CRS solution.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for two hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 0.1 mg of the sample and dissolve in methyl alcohol. Dilute successively, with the same solvent, to a concentration of 0.02% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 268 nm, using methyl alcohol for zero adjustment. Calculate the content of $C_{14}H_{10}Cl_4$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation. Handle with exceptional care.

THERAPEUTIC CLASS

Antineoplastic.

MITOTANE TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{14}H_{10}Cl_4$.

IDENTIFICATION

Weigh and pulverize the tablets. Shake quantity of powder equivalent to 0.5 g of mitotane in 10 mL of water. Filter in a sintered glass funnel and wash the residue with two 5 mL portions of water. Transfer the residue to a small beaker, add 4 mL of ethyl alcohol, heat to boiling and filter immediately. Cool, filter the mitotane crystals, wash once with 2 mL of ethyl alcohol, and dry at 60 °C under reduced pressure for two hours. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the mitotane CRS spectrum, prepared identically.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Not more than 15 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

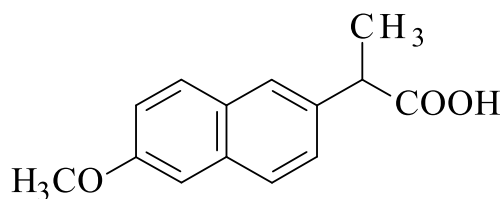
Weigh and pulverize 20 tablets. Accurately weigh the quantity of powder equivalent to approximately 0.1 g of mitotane and transfer to a 250 mL volumetric flask, add 100 mL of methyl alcohol, shake for five minutes, complete the volume with methyl alcohol, homogenize and filter. Transfer 25 mL of the filtrate to a 50 mL volumetric flask, complete the volume with methyl alcohol and homogenize to obtain a 0.02 % (w/v) solution. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 268 nm, using methyl alcohol for zero adjustment. Calculate the quantity of $C_{14}H_{10}Cl_4$ in the tablets from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

NAPROXEN***Naproxenum*** $C_{14}H_{14}O_3$; 230.26

naproxen; 06233

(α S)-6-Methoxy- α -methyl-2-naphthaleneacetic acid

[22204-53-1]

It contains not less than 99.0% and not more than 101.0% of $C_{14}H_{14}O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water. Soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 154 °C to 158 °C.

Specific optical rotation (5.2.8): +59,0 to +62,0. Determine in solution at 2% (w/v) in ethyl alcohol.

IDENTIFICATION

The identification tests B. and C. can be omitted if test A. is carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the naproxen CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of a 0.0025% (w/v) sample solution in methyl alcohol, there are maximum absorption points only at the same wavelengths and with the same relative intensities as those observed in the naproxen CRS spectrum, prepared identically. The absorptivity at 271 nm, calculated from the spectrum obtained with the desiccated sample, varies not more than 3% in relation to the spectrum obtained with the naproxen CRS sample.

C. Dissolve about 10 mg of the sample in 5 mL of methyl alcohol, add 5 mL of water, 2 mL of potassium iodide RS, 5 mL of a potassium iodate solution (1:100) and homogenize. A brownish-yellow color develops. Add 5 mL of chloroform and homogenize. A slight red or purple color develops in the chloroform layer.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄, with thickness of 250 µm, as support, and mixture of hexane, methylene chloride, tetrahydrofuran and acetic acid (50:30:17:3), as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve about 100 mg of the sample, accurately weighed, in 10 mL of a mixture of chloroform and ethyl alcohol (1:1).

Solution (2): transfer 2 mL of *Solution (1)* to a 100 mL volumetric flask, complete the volume with mixture of chloroform and ethyl alcohol (1:1) and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete with the same solvent and homogenize.

Develop the chromatogram until the front of the mobile phase ascends 12 cm above the application line. Remove plate and allow to air dry. Examine under ultraviolet light. The main stain obtained in the chromatogram with *Solution (1)* corresponds in position to the one obtained with *Solution (2)*. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, can have maximum intensity equal to that obtained with *Solution (2)* (0.2%).

Enantiomeric purity. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 263 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica gel π-acceptor/π-donor (1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene) for chiral separation (*S,S*) (5 µm), kept at 25 °C; *Mobile Phase* flow of 2 mL/minute.

Mobile phase: mixture of glacial acetic acid, acetonitrile, isopropyl alcohol and hexane (0.5:5:10:84.5).

Solution (1): dissolve 25 mg of the sample in tetrahydrofuran, dilute to 50 mL with the same solvent and homogenize. Transfer 2 mL of the solution to a 20 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Solution (2): transfer 2.5 mL of the *Solution (1)* to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Solution (3): dissolve 5 mg of racemic naproxen CRS (2R)-2-(6-methoxynaphthalen-2-yl)propanoic acid - *R* enantiomer) in 10 mL of tetrahydrofuran and dilute to 100 mL with *Mobile Phase*.

Inject, separately, 20 µL replicates of the *Solution (2)* and of *Solution (3)*, record the chromatograms and measure the areas under the peaks. The resolution between the peaks of naproxen and (2R)-2-(6-methoxynaphthalen-2-yl)propanoic acid (impurity G) is not less than 3.0.

Procedure: inject, separately, 20 µL of *Solution (1)* and of *Solution (2)*, record the chromatograms for not less than one and a half time the naproxen retention time (retention time around five minutes) and measure the areas under the peaks. The area under the peak related to (2R)-2-(6-methoxynaphthalen-2-yl)propanoic acid (impurity G) obtained with *Solution (1)* is not more than equal to the area under the main peak obtained with *Solution (2)* (2.5%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 200 mg of the sample and dissolve in a mixture of 75 mL of methyl alcohol and 25 mL of water. Titrate with 0.1 M sodium hydroxide VS using phenolphthalein TS until violet color is formed. Each mL of 0.1 M sodium hydroxide VS is equivalent to 23.026 mg of C₁₄H₁₄O₃. Carry out the blank test and make the necessary corrections.

PACKAGING AND STORAGE

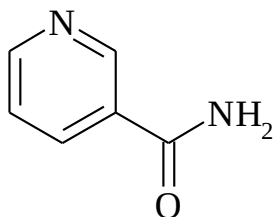
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Analgesic and anti-inflammatory.

NICOTINAMIDE*Nicotinamidum*

C₆H₆N₂O; 122.13

nicotinamide; 06346

Pyridinecarboxamide

[98-92-0]

It contains not less than 99.0% and not more than 101.0% of C₆H₆N₂O, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or colorless crystals.

Solubility. Freely soluble in water, soluble in glycerol, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 128 °C to 131 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the nicotinamide CRS spectrum, prepared identically.

B. The ratio between the absorbance **(5.2.14)** measured at 245 nm and 262 nm of a 0.0002% (w/v) sample solution in water ranges between 0.63 and 0.67.

PURITY TESTS

pH (5.2.19). 6.0% to 8.0%. Determine in aqueous solution at 5% (w/v).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and mixture of water, ethyl alcohol and chloroform (4:45:48) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 80 mg/mL sample solution in mixture of ethyl alcohol and water (50:50).

Solution (2): dilute 0.5 mL of *Solution (1)* to 200 mL in mixture of ethyl alcohol and water (50:50).

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Examine the plate. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.25%).

Heavy metals (5.3.2.3). Proceed as described in *Thioacetamide reaction methods, Method I*. Not more than 0.003% (30 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample, in desiccator under reduced pressure, for four hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.4.5)*. Dissolve about 0.25 g of the sample previously desiccated in 20 mL of glacial acetic acid, heating if necessary. Add 5 mL of acetic anhydride, titrate with 0.1 M perchloric acid VS, using methylrosanilinium chloride TS as indicator, until greenish-blue color. Carry out the blank test and proceed with the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 12.213 mg of C₆H₆N₂O.

PACKAGING AND STORAGE

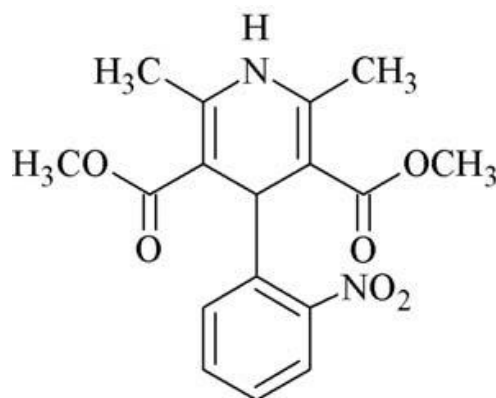
In hermetic containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vitamin B component.

NIFEDIPINE***Nifedipinum***C₁₇H₁₈N₂O₆; 346.34

nifedipine; 06352

1,4-Dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid 3,5-dimethyl ester

[21829-25-4]

It contains not less than 98.0% and not more than 102.0% of C₁₇H₁₈N₂O₆, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Yellow and tasteless crystals.

Solubility. Practically insoluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 171 °C to 175 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the nifedipine CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described in method **B.** from *Assay*. Prepare solutions as described below. Perform the tests immediately after preparing the solutions.

Solution (1): 1 mg/mL nifedipine CRS solution in methyl alcohol.

Solution (2): dilute 3 mL of *Solution (1)* to 10 mL using *Mobile phase*.

Solution (3): 1 mg/mL solution of nifedipine nitrophenylpyridine analog CRS (dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate) in methyl alcohol.

Solution (4): from *Solution (3)* to prepare 0.6 µg/mL solution of nifedipine nitrophenylpyridine analog CRS in *Mobile Phase*.

Solution (5): 1 mg/mL solution of nifedipine nitrosophenylpyridine analog CRS (dimethyl 4-(2-nitrosophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate) in methyl alcohol.

Solution (6): from *Solution (5)* to prepare 0.6 µg/mL solution of nifedipine nitrosophenylpyridine analog CRS in *Mobile Phase*.

Solution (7): mixture of *Mobile Phase*, *Solution (4)* and *Solution (6)* (1:1:1).

Solution (8): mixture of *Solution (2)*, *Solution (4)* and *Solution (6)* (1:1:1).

Inject replicates of *Solution (8)*. The relative retention times are about 0.8 for nifedipine nitrophenylpyridine analog, 0.9 for nifedipine nitrosophenylpyridine analog, and 1.0 for nifedipine. The resolution between nifedipine and nifedipine nitrophenylpyridine analog is not less than 1.5. The resolution between nifedipine and nifedipine nitrosophenylpyridine analog is not less than 1.0. The relative standard deviation of the replicate areas under the recorded peaks of nifedipine nitrophenylpyridine analog and nifedipine nitrosophenylpyridine analogue is not more than 10.0%.

Procedure: separately inject 25 µL of the *Sample Solution* of Assay **B.(1)** and of the *Solution (7)*. Record the chromatograms and measure the areas under the peaks. The area under the peak corresponding to the nifedipine nitrophenylpyridine analog obtained with *the Sample Solution* must not be greater than the area under the main peak obtained with the *Solution (7)* (0.2%). The area under the peak corresponding to the nifedipine nitrosophenylpyridine analog obtained with *the Sample Solution* must not be greater than the area under the main peak obtained with the *Solution (7)* (0.2%).

Chlorides (5.3.2.1). Not more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Sulfates (5.3.2.2). Not more than 0.05% (500 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for two hours. Not more than 0.5%.

Residue by incineration (5.2.10). Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14, protected from direct light using the freshly prepared solutions. Accurately weigh about 100 mg of the sample and dissolve in 70 mL of ethyl alcohol. Complete the volume to 100 mL with the same solvent. Dilute, successively, in ethyl alcohol until concentration of 10 µg/mL. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 236 nm, using ethyl alcohol for zero adjustment. Calculate the content of C₁₇H₁₈N₂O₆ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography* (5.2.17.4), protected from direct light, using chromatograph equipped with ultraviolet detector at 235 nm; column with 250 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); kept at room temperature, *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water, acetonitrile and methyl alcohol (50:25:25).

Sample solution: quantitatively transfer about 25 mg of the sample to a 250 mL volumetric flask. Dissolve in 25 mL of methyl alcohol, complete with *Mobile phase* and mix to obtain a 0.1 mg/mL solution. Prepare just before use.

Standard solution: dissolve an accurately weighed quantity of nifedipine CRS in *Mobile phase* to obtain a 0.1 mg/mL solution. Prepare just before use.

Inject 25 µL replicates of the *Standard solution*. The column efficiency is not less than 16 000 theoretical plates/meter; the tailing factor is not more than 1.5 and the relative standard deviation of the replicate areas under recorded peaks is not more than 1.0%.

Procedure: inject, separately, 25 µL of the *Standard Solution* and the *Sample Solution*. Record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₇H₁₈N₂O₆ in the sample from the responses with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vasodilator.

NIFEDIPINE CAPSULES

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{17}H_{18}N_2O_6$.

IDENTIFICATION

Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel G, with thickness of 0.5 mm, as support, and mixture of ethyl acetate and cyclohexane(1:1) as mobile phase. Apply to the plate, separately, 0,5 mL of each of the freshly prepared solutions described below.

Solution (1): transfer the content of three capsules to a centrifuge tube and wash the inside of the capsules with 20 mL of 0.1 M sodium hydroxide. Volumetrically add 20 mL of methylene chloride to the tube and cap. Shake gently and release the pressure in the tube. Cap hermetically and shake for one hour. Centrifuge for 10 minutes between 2000 and 2500 rpm Remove the supernatant aqueous phase with a syringe and transfer 5 mL of the lower transparent layer to a beaker.

Solution (2): 1.2 mg/mL nifedipine CRS solution in methylene chloride.

Develop the chromatogram protected from direct light. Remove plate and allow to air dry. Examine immediately under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in color, intensity and position to the one obtained with *Solution (2)*. Spray plate with *TLC visualization reagent solution* Each chromatogram presents a light orangish band on a yellow background.

TLC visualization reagent solution: dissolve 3 g of bismuth subnitrate and 30 g of potassium iodide in 10 mL of 3 M hydrochloric acid and transfer to a 100 mL volumetric flask. Complete the volume with water and homogenize. Transfer 10 mL to a 100 mL volumetric flask. Add 10 mL of 3 M hydrochloric acid and complete the volume with water. Homogenize.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer the content of each capsule to a 200 mL volumetric flask. Wash the inside of the capsules with small quantities of methyl alcohol, collecting the washing liquids in the flask. Complete the volume with methyl alcohol and homogenize. Transfer 5 mL to a 100 mL volumetric flask and complete the volume with methyl alcohol. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 350 nm (5.2.14), using methyl alcohol for zero adjustment. Calculate the quantity of $C_{17}H_{18}N_2O_6$ in the capsules from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: simulated gastric fluid (without pepsin), 900 mL.

Apparatus: baskets, 50 rpm.

Time: 20 minutes).

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute with *Dissolution medium* to adequate concentration. Measure the absorbances of the solutions at 340 nm (**5.2.14**), using *Dissolution medium* for zero adjustment. Calculate the quantity of $C_{17}H_{18}N_2O_6$ dissolved in the medium, comparing the readings obtained with that of the nifedipine CRS solution at the concentration of 0.005% (w/v), prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{17}H_{18}N_2O_6$ dissolve in 20 minutes.

PURITY TESTS

Related substances. Proceed as described in method **B**. of *Assay* in the *Nifedipine* monograph. Prepare solutions as described below.

Note: proceed with the test immediately after the preparation of *Solution (1)* and *Solution (5)*, protecting them from direct light.

Solution (1): dissolve an accurately weighed quantity of nifedipine CRS in methyl alcohol to obtain a 1 mg/mL solution. Dilute with *Mobile phase* to obtain a 0.3 mg/mL solution.

Solution (2): dissolve an accurately weighed quantity of nitrophenylpyridine CRS in methyl alcohol to obtain a 1 mg/mL solution. Dilute with *Mobile phase* to obtain a 6 µg/mL solution.

Solution (3): dissolve an accurately weighed quantity of nitrosophenylpyridine CRS in methyl alcohol to obtain a 1 mg/mL solution. Dilute with *Mobile phase* to obtain a 1.5 µg/mL solution.

Solution (4): mix 5 mL of *Solution (2)*, 5 mL of *Solution (3)* and 5 mL of *Mobile Phase*. Homogenize.

Solution (5): proceed as described for *Sample solution* in method **B**. from *Assay*.

Solution (6): mix equal volumes of *Solution (1)*, *Solution (2)* and *Solution (3)*.

Inject 25 µL replicates of the *Solution (6)*. The relative retention times are about 0.8 for nitrophenylpyridine, 0.9 for nitrosophenylpyridine and 1.0 for nifedipine. The resolution between nitrosophenylpyridine and nitrophenylpyridine is not less than 1.5. The resolution between nifedipine and nitrosophenylpyridine is not less than 1.0. The relative standard deviation of the replicate areas under the recorded peaks corresponding to nitrophenylpyridine and to nitrosophenylpyridine is not more than 10.0%.

Procedure: inject, separately, 25 µL of the *Solution (4)* and of *Solution (5)*, record the chromatograms and measure the areas under the peaks. Calculate the quantity, in mg, of the nitrophenylpyridine and nitrosophenylpyridine impurities in the capsules according to the formula:

$$\frac{V}{5} \times C \times \left(\frac{r_5}{r_4} \right)$$

where

V = total volume, in mL, of *Solution (5)*;

C = concentration of nitrophenylpyridine or nitrosophenylpyridine, in mg/mL, in *Solution (4)*;

r_5 = area under the peak corresponding to nitrophenylpyridine or to nitrosophenylpyridine in the chromatogram obtained with *Solution (5)*;

r_4 = area under the peak corresponding to nitrophenylpyridine or to nitrosophenylpyridine in the chromatogram obtained with *Solution (4)*;

Not more than 2.0% of nitrophenylpyridine and 0.5% of nitrosophenylpyridine, in relation to the nifedipine content.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*, protected from direct light. Transfer the content of 10 capsules to a 100 mL volumetric flask. Wash the inside of the capsules with small quantities of methyl alcohol, collecting the washing liquids in the flask. Complete the volume with the same solvent and homogenize. Dilute with methyl alcohol until concentration of 0.005% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 350 nm, using methyl alcohol for zero adjustment. Calculate the quantity of $C_{17}H_{18}N_2O_6$ in the capsules from the readings obtained.

B. Proceed as described in method **B.** of Assay in the *Nifedipine* monograph. Prepare *Sample solution* as described below.

Sample solution: transfer the content of five capsules to a 100 mL volumetric flask, with the aid of small portions of methyl alcohol. Complete the volume with the same solvent and homogenize. Dilute, successively, in *Mobile phase*, to obtain a 0.1 mg/mL nifedipine solution.

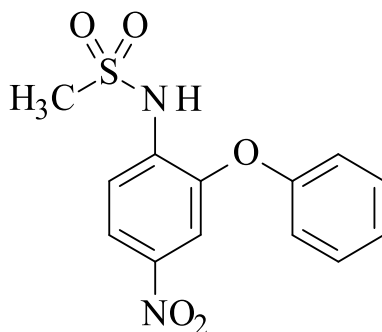
Procedure: inject, separately, 25 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{17}H_{18}N_2O_6$ in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In hermetically closed containers, protected from light, at temperature between 15 °C and 25 °C.

LABELING

Comply with current legislation.

NIMESULIDE*Nimesulidum* $C_{13}H_{12}N_2O_5S$; 308.31

nimesulide; 06391

N-(4-Nitro-2-phenoxyphenyl)methanesulfonamide

[51803-78-2]

It contains not less than 98.5% and not more than 101.5% of $C_{13}H_{12}N_2O_5S$, in relation to the desiccated substance.

DESCRIPTION

Physicochemical characteristics. Pale yellow crystalline powder, slightly oily to the touch. Non-hygroscopic. Presents polymorphism.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol and in methyl alcohol, very soluble in acetonitrile. Soluble in alkali hydroxide solutions. Insoluble in acid solutions.

IDENTIFICATION

The identification test C. can be omitted if tests A. and B. are carried out. The identification test B. can be omitted if tests A. and C. are carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample desiccated at 105 °C, until constant weight, and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the nimesulide CRS spectrum, prepared identically.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ activated in oven for 30 minutes at 105 °C, as support, and mixture of methyl alcohol and acetonitrile (80:20), as mobile phase. Apply to the plate, separately, 4 µL of each of the freshly prepared solutions described below.

Solution (1): weigh, accurately, about 75 mg of the sample, transfer to a 100 mL volumetric flask and complete the volume with acetone. Transfer 1 mL of this solution to a 10 mL volumetric flask and complete the volume with the same solvent.

Solution (2): weigh, accurately, about 75 mg of nimesulide CRS, transfer to a 100 mL volumetric flask and complete the volume with acetone. Transfer 1 mL of this solution to a 10 mL volumetric flask and complete the volume with the same solvent.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm and 365 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **C.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Heavy metals (5.3.2.3). Use *Method IV*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for four hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh, accurately, about 0.24 g of the sample, dissolve in 30 mL of acetone neutralized in advance and add 20 mL of water. Titrate with 0.1 M sodium hydroxide VS, determine the endpoint potentiometrically. Each mL of 0.1 M sodium hydroxide VS is equivalent to 30.831 mg of $C_{13}H_{12}N_2O_5S$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Quantitatively transfer about 0.1 g of the sample to a 100 mL volumetric flask, dissolve and complete the volume with 0.01 M sodium hydroxide. Dilute successively, with the same solvent, to a concentration of 0.00015% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 392 nm, using 0.01 M sodium hydroxide for zero adjustment. Calculate the content of $C_{13}H_{12}N_2O_5S$ in the sample from the readings obtained.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 150 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.8 mL/minute.

Mobile phase: mixture of water and acetonitrile (50:50).

Sample solution: quantitatively transfer about 50 mg of the sample to a 100 mL volumetric flask, dissolve in *Mobile Phase* and complete the volume with the same solvent. Dilute, successively, in *Mobile phase*, to obtain a 20 µg/mL solution.

Standard solution: dissolve an accurately weighed quantity of nimesulide CRS in *Mobile phase* to obtain a 20 µg/mL solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₃H₁₂N₂O₅S in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-inflammatory.

NIMESULIDE TABLETS

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $C_{13}H_{12}N_2O_5S$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Prepare 0.01% (w/v) nimesulide solution in chloroform. Filter. Dry the filtrate in water bath until dryness. Dry the residue at 105°C until constant weight. Proceed as described in test **A.** from *Identification* in the *Nimesulide* monograph.

B. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** from *Assay*, there are maximum absorption points at 212 nm and 392 nm, identical to those observed in the spectrum of the standard solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described in method **A.** from *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: potassium phosphate buffer pH 7.4 with 2% polysorbate 80 (v/v), 900 mL.

Apparatus: paddles, 75 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in water until adequate concentration. Measure the absorbances of the solutions at 392 nm **(5.2.14)**, using the same solvent for zero adjustment. Calculate the quantity of $C_{13}H_{12}N_2O_5S$ dissolved in the medium, comparing the readings obtained with the nimesulide CRS solution at the concentration of 0.0015% (w/v) prepared with the same solvent as the sample.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{13}H_{12}N_2O_5S$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.1 g of nimesulide to a 100 mL volumetric flask, add 60 mL of 0.01 M sodium hydroxide and shake for 40 minutes. Complete the volume with the same solvent and filter. Dilute successively to a concentration of 0.002% (w/v) using 0.01 M sodium hydroxide. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 392 nm, using 0.01 M sodium hydroxide for zero adjustment. Calculate the quantity of $C_{13}H_{12}N_2O_5S$ in the tablets from the readings obtained.

B. Proceed as described in method **C.** of Assay in the *Nimesulide* monograph. Prepare *Sample solution* as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 50 mg of nimesulide to a 100 mL volumetric flask, add 60 mL of *Mobile phase* and shake mechanically for 40 minutes. Complete the volume with *Mobile phase* and filter. Dilute, successively, in *Mobile phase*, to obtain a 20 µg/mL solution.

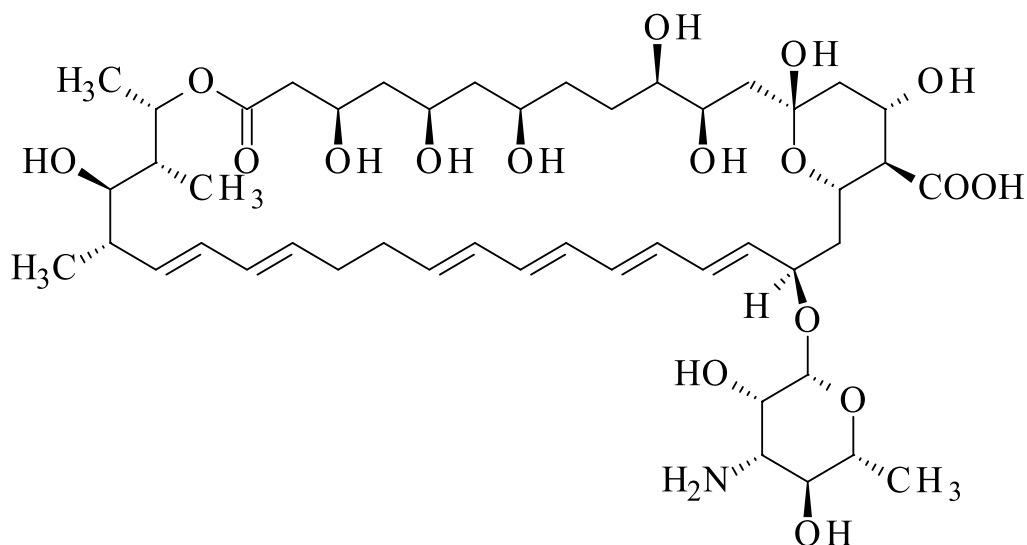
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{13}H_{12}N_2O_5S$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

NYSTATIN***Nystatinum***C₄₇H₇₅NO₁₇; 926.11

nystatin; 06410

Nystatin

[1400-61-9]

Nystatin is a substance or a mixture of two or more substances produced by *Streptomyces noursei* Brown et al. (*Streptomycetaceae*). It has a potency of not less than 4400 IU of nystatin per milligram or 5000 IU of nystatin per milligram if intended for the production of powder for oral suspension.

DESCRIPTION

Physical characteristics. Hygroscopic powder, fine, yellow or brown.

Solubility. Practically insoluble in water, slightly soluble in methyl alcohol and practically insoluble in ethyl alcohol.

IDENTIFICATION

A. Accurately weigh about 100 000 IU of the sample and dissolve in mixture of 5 mL of glacial acetic acid and 50 mL of methyl alcohol. Complete the volume to 100 mL with methyl alcohol. Dilute in methyl alcohol until concentration of 40 IU/mL. In the ultraviolet absorption spectrum **(5.2.14)**, at the range of 220 nm to 350 nm, in the solution obtained, there are maximum points at 230 nm, 291 nm, 305 nm and 319 nm. The ratio between the absorbance values measured at 291 nm and 305 nm ranges between 0.61 and 0.73. The ratio between the absorbance values measured at 319 nm and 305 nm ranges between 0.83 and 0.96. The ratio between the absorbance values measured at 230 nm and 280 nm ranges between 0.83 and 1.25.

B. Add 0.1 mL of hydrochloric acid to 2 mg of the sample. Brown color is produced.

C. Add 0.1 mL of sulfuric acid to 2 mg of the sample. It produces a brown color, which turns violet under standing.

PURITY TESTS

pH (5.2.19). 6.0% to 8.0%. Determine in aqueous solution at 3% (w/v).

Crystallinity. Suspend some particles of the sample in mineral oil, transfer to a glass slide and examine on a microscope equipped with polarized light. The particles display birefringence, which is extinguished when moving the sample through micrometric adjustment.

Nystatin A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Protect from direct light. Use chromatograph equipped with ultraviolet detector at 304 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at temperature of 30 °C; *Mobile phase* flow of 1.0 mL/minute.

Eluent A: 0.05 M ammonium acetate and acetonitrile (71:29).

Eluent B: 0.05 M ammonium acetate and acetonitrile (40:60).

Mobile Phase gradient: adopt the gradient system described in the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 25	100	0	isocratic
25 – 35	100 → 0	0 → 100	linear gradient
35 – 40	0	100	isocratic
40 – 45	0 → 100	100 → 0	linear gradient
45 – 60	100	0	equilibrium

Solution (1): dissolve quantity, accurately weighed, of the sample in dimethylsulfoxide, to obtain a solution at 0.4 mg/mL. Use this solution within not more than 24 hours after preparation, kept under refrigeration.

Solution (2): dissolve quantity, accurately weighed, of nystatin CRS in dimethylsulfoxide, to obtain a solution at 0.4 mg/mL. Use this solution within not more than 24 hours after preparation, kept under refrigeration.

Solution (3): dissolve 20 mg of nystatin CRS in 25 mL of methyl alcohol, dilute to 50 mL with water and homogenize. Add 2 mL of hydrochloric acid diluted in 10 mL of the previously prepared solution and wait for one hour at room temperature before using.

Inject 20 µL replicates of the *Solution (3)*. The average retention time for nystatin A is 14 minutes. The resolution between the two main peaks is not less than 3.5.

Procedure: inject, separately, 20 µL of the *Solution (1)* and *Solution (2)*, record the chromatograms and measure the areas under the peaks. Disregard peaks obtained before two minutes of running. Not less than 85% of nystatin A. Not more than 4.0% of any other component.

Heavy metals (5.3.2.3). Prepare the standard solution using 2 mL of *Standard lead solution (10 ppm Pb)*. Proceed as described in *Method IV*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 0,1 g of the sample. Dry in oven at 60 °C, under reduced pressure, not greater than 5 mmHg, for three hours. Not more than 5.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 3.5%.

Nystatin intended for the production of powder for extemporaneous oral suspension complies with the following additional test.

Dispersibility. Quantitatively transfer about 0.2 g of the sample to a 200 mL beaker with water and slowly disperse with a glass rod. Allow to stand for two minutes. The material should be suspended and there will be not more than a small sediment. If there is sedimentation, determine the potency of the suspended part, as described in *Microbiological test of antibiotics (5.5.3.3)*. Volumetrically transfer the suspended sample to the high-speed blender, add dimethylformamide so as to obtain a concentration of 400 IU/mL and shake for three to five minutes. Dilute this solution with sterile 1% *Potassium Phosphate Buffer, pH 6.0 (Solution 1)* to obtain a solution with concentration equivalent to that of the standard. Not less than 90.0% of the expected nystatin potency.

ASSAY

Proceed as described in *Microbiological assay of antibiotics (5.5.3.3)*, by the agar diffusion method **(5.5.3.3.1)**.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at temperature between 2 °C and 8 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antifungal.

NYSTATIN VAGINAL TABLETS

It contains not less than 90.0% and not more than 140.0% of the stated quantity of nystatin. Vaginal tablets contain binding, diluent and lubricant agents.

IDENTIFICATION

Weigh and pulverize the vaginal tablets. Transfer quantity of powder equivalent to 100 000 IU of nystatin to a 100 mL volumetric flask. Add 5 mL of glacial acetic acid and 50 mL of methyl alcohol. Homogenize. Add methyl alcohol sufficient to produce 100 mL. Filter. Transfer 1 mL of this solution to a 25 mL volumetric flask and complete the volume with methyl alcohol. Prepare blank assay using the same solvents and omitting the addition of the sample. In the ultraviolet absorption spectrum **(5.2.14)**, at the range of 250 nm to 350 nm, there are maximum peaks of the solution obtained at 291 nm, 305 nm and 319 nm. The ratio between the absorbance values measured at 291 nm and 305 nm ranges between 0.61 and 0.73. The ratio between the absorbance values measured at 319 nm and 305 nm ranges between 0.83 and 0.96.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.2). Not more than 60 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Loss on drying (5.2.9.1). Weigh and pulverize the vaginal tablets. Determine in 0.1 g of the sample, in oven at 60 °C, under reduced pressure, for three hours. Not more than 5.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed protected from direct light. Weigh and pulverize 20 vaginal tablets. Weigh quantity of powder equivalent to 200 000 IU, add 50 mL of dimethylformamide and shake for one hour. Centrifuge. Transfer 10 mL of the supernatant to a 200 mL volumetric flask and complete the volume with solution 4 described in *Microbiological assay of antibiotics* **(5.5.3.3)**. Proceed as described in *Microbiological assay by agar diffusion* **(5.5.3.3.1)**.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at temperature below 25 °C.

LABELING

Comply with current legislation.

NYSTATIN VAGINAL CREAM

It contains not less than 90.0% and not more than 130.0% of the stated quantity of $C_{47}H_{75}NO_{17}$.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Assay* in the *Nystatin* monograph. Prepare *Sample solution* as described below.

Sample solution: mix, accurately, in a high-speed blender, quantity of vaginal cream with dimethylformamide, to obtain a concentration of approximately 400 IU/mL of nystatin. Dilute successively this solution in *sterile 10% potassium phosphate buffer, pH 6.0 (Solution 4)* to obtain solutions in the concentration range suitable to the standard curve.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at temperature below 25 °C.

LABELING

Comply with current legislation.

NYSTATIN ORAL SUSPENSION

It contains not less than 90.0% and not more than 130.0% of the stated quantity of nystatin. The oral suspension contains flavoring, preservative and dispersing agents.

IDENTIFICATION

Transfer quantity of the oral suspension containing 100,000 IU of nystatin to a 100 mL volumetric flask and add mixture of glacial acetic acid and methyl alcohol (5:50). Homogenize. Complete volume with methyl alcohol and filter. Transfer 1 mL of this solution to a 25 mL volumetric flask and complete the volume with methyl alcohol. Prepare blank assay using the same solvents and omitting the addition of the sample. In the ultraviolet absorption spectrum **(5.2.14)**, at the range of 250 nm to 350 nm, there are maximum peaks of the solution obtained at 291 nm, 305 nm and 319 nm. The ratio between the absorbance values measured at 291 nm and 305 nm ranges between 0.61 and 0.73. The ratio between the absorbance values measured at 319 nm and 305 nm ranges between 0.83 and 0.96.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.5 to 6.0. If the product contains glycerin, the pH should be between 6.0 and 7.5.

Uniformity of unit doses (5.1.6). Complies with the test. It must be carried out if the drug is packaged in unit doses.

Procedure for content uniformity. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer the content of an oral suspension flask to a 100 mL volumetric flask, complete the volume with methyl alcohol and homogenize. Quantitatively dilute this solution with methyl alcohol to obtain a 25 IU/mL nystatin solution. At the same time, prepare a solution of nystatin CRS in methyl alcohol at 25 IU/mL. Measure the absorbances of the solutions at 304 nm, using methyl alcohol for zero adjustment. Calculate the quantity of nystatin in the oral suspension from the readings obtained.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

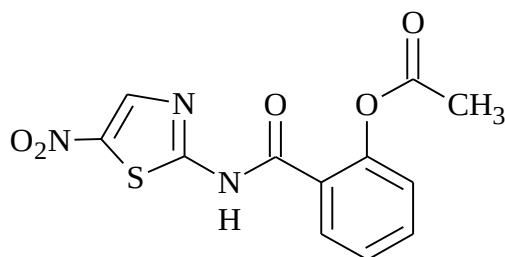
Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)* and *Microbiological Assay by agar diffusion (5.5.3.3.1)*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at temperature below 25 °C.

LABELING

Comply with current legislation.

NITAZOXANIDE***Nitazoxanidum***

$C_{12}H_9N_3O_5S$; 307.28

nitazoxanide; 06413

2-Acetyloxy-*N*-(5-nitro-2-thiazolyl)benzamide

[55981-09-4]

It contains not less than 98.0% and not more than 102.0% of $C_{12}H_9N_3O_5S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to slightly yellow crystalline powder.

Solubility. Practically insoluble in water, soluble in acetonitrile, very slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting point (5.2.2): 202 °C

IDENTIFICATION

The identification test B. can be omitted if test C. is carried out. The identification test C. can be omitted if test B. is carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the nitazoxanide CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 220 nm to 400 nm of the sample solution obtained in method A. from Assay, there are maximum and minimum points only at the same wavelengths observed in the standard solution spectrum.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method B. from Assay, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, protected from direct light,. Use chromatograph equipped with ultraviolet detector at 210 nm;

250mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm); kept at 30 °C, *Mobile phase* flow rate of 1.0 mL/minute.

Diluent: mixture of *Eluent A* and *Eluent B* (70:30).

Eluent (A): acetonitrile.

Eluent B: water previously adjusted to pH 2.5 with phosphoric acid.

Mobile Phase gradient: adopt the gradient system described in the following table.

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>
0	30	70
25.0	70	30
30.0	70	30
30.1	30	70
40.0	30	70

Solution (1): dissolve quantity accurately weighed of the sample in *Diluent* to obtain a solution at 2.5 mg/mL of nitazoxanide.

Solution (2): dissolve accurately weighed quantity of nitazoxanide CRS, nitazoxanide related compound A and acetylsalicylic acid in the *Diluent* to obtain a 2.5 µg/ml solution of each of the three substances.

Inject 35 µL replicates of the *Solution (2)*. The resolution between nitazoxanide related compound A and acetylsalicylic acid is not less than 2.0. The tailing factor for the nitazoxanide peak is not more than 2.0. The relative standard deviation of the replicate areas under the nitazoxanide peaks recorded is not more than 10.0%.

Procedure: inject, separately, 35 µL of the *Solution (1)* and *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of each impurity present in the sample according to the formula:

$$100 (1/F) (A_i/A_p) (C_p/C_a)$$

where

F = relative response factor for each impurity;

A_i = area under the peak corresponding to any individual impurity obtained in *Solution (1)*;

A_p = area under the peak corresponding to the nitazoxanide obtained in *Solution (2)*.

C_p = concentration, in mg/mL, of nitazoxanide in *Solution (2)*;

C_a = concentration, in mg/mL, of nitazoxanide in *Solution (1)*;

The limits of impurities are presented below:

<i>Impurities</i>	<i>Relative retention time</i>	<i>Relative response factor</i>	<i>Limit (%)</i>

Nitazoxanide related compound A (2-Amino-5-nitrothiazole)	0.35	0.46	0.1
Acetylsalicylic acid (2-(acetyloxy)benzoic acid)	0.48	0.91	0.1
Salicylic acid (2-hydroxybenzoic acid)	0.61	3.7	0.1
Nitazoxanide	1.0	-	-
Other individual impurities	-	1.0	0.1

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 60 °C for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*, protected from direct light. Accurately weigh about 120 mg of the sample and add 50 mL of acetonitrile. Complete the volume to 100 mL with the same solvent. Dilute successively with water at pH 4.5 previously adjusted with phosphoric acid 10% (v/v) to a concentration of 0.0012% (w/v). Prepare standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 345 nm, using water pH 4.5 for zero adjustment. Calculate the content of $C_{12}H_9N_3O_5S$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, protected from direct light,. Use chromatograph equipped with ultraviolet detector at 240 nm; 250mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (4 µm to 5 µm), kept at 25 °C; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of 0.1% (v/v) phosphoric acid, pH 5.3 previously adjusted with triethylamine, and acetonitrile (45:55).

Sample solution: quantitatively transfer about 100 mg of the sample to a 100 mL volumetric flask, complete the volume with acetonitrile and homogenize. Transfer 3 mL of this solution to a 100 mL volumetric flask, complete the volume with acetonitrile and homogenize, obtaining a solution at 30 µg/mL.

Standard solution: quantitatively transfer about 10 mg of nitazoxanide CRS to a 100 mL volumetric flask, complete the volume with acetonitrile and homogenize. Transfer 3 mL of this solution to a 10 mL volumetric flask, complete the volume with acetonitrile and homogenize, obtaining a solution at 30 µg/mL.

Inject 20 µL replicates of the *Standard solution*. The column efficiency for the nitazoxanide peak is not less than 2000 theoretical plates/meter. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₂H₉N₃O₅S in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiparasitic.

NITAZOXANIDE TABLETS

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_{12}H_9N_3O_5S$. Tablets must be coated.

IDENTIFICATION

The identification test B. can be omitted if test C. is carried out. The identification test C. can be omitted if test B. is carried out.

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, protected from direct light, using silica gel 60 F₂₅₄ as support, and mixture of hexane and ethyl alcohol (60:40) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize 10 tablets. Transfer quantity of powder equivalent to 50 mg of nitazoxanide to a 50 mL volumetric flask and add 40 mL of acetonitrile. Place in ultrasonic bath at room temperature for 10 minutes, complete the volume with acetonitrile, homogenize and filter.

Solution (2): 1 mg/mL nitazoxanide CRS solution in acetonitrile.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 220 nm to 400 nm of the sample solution obtained in method **A.** from Assay, there are maximum and minimum points only at the same wavelengths observed in the standard solution spectrum.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: pH 7.5 phosphate buffer containing 6% of cetrimonium bromide, kept at 25 °C, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 45 minutes.

Procedure: proceed protected from direct light. After the test, remove aliquot from the dissolution medium, prepare *Sample solution* and proceed as described in method **B.** from Assay. Inject, separately, 20 µL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{12}H_9N_3O_5S$ dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*, prepared as described below.

Sample solution: after testing, remove aliquot from the dissolution medium, filter and dilute, if required, in acetonitrile to obtain a 27.77 µg/mL nitazoxanide solution.

Standard solution: quantitatively transfer about 13.88 mg of nitazoxanide CRS to a 100 mL volumetric flask. Add 5 mL of acetonitrile, place in ultrasonic bath for 30 minutes and complete the volume with Phosphate buffer pH 7.5 containing 6% of cetrimonium bromide. Dilute successively with acetonitrile to obtain a 27.77 µg/mL nitazoxanide solution.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{12}H_9N_3O_5S$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed protected from direct light, as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 15 mg of nitazoxanide to a 100 mL volumetric flask and add 50 mL of acetonitrile. Place in ultrasonic bath at room temperature for 10 minutes, complete the volume with acetonitrile, homogenize and filter. Transfer 2 mL of the filtrate to a 25 mL volumetric flask and complete the volume with water with pH previously adjusted to 4.5 with 10% (v/v) phosphoric acid, obtaining a solution at 12 µg/mL. Prepare standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 345 nm, using water with a pH previously adjusted to 4.5 with 10% (v/v) phosphoric acid for zero adjustment. Calculate the quantity of $C_{12}H_9N_3O_5S$ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, protected from direct light,. Use chromatograph with ultraviolet detector at 240 nm; pre-column (optional) with 4,0 mm of length and 3.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (4 µm); column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (4 µm to 5 µm); kept at 25 °C; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of 0.1% (v/v) phosphoric acid, pH 5.3 previously adjusted with triethylamine, and acetonitrile (45:55).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 50 mg of nitazoxanide to a 250 mL volumetric flask, add 150 mL of acetonitrile and place in ultrasonic bath

for 10 minutes. Complete the volume with acetonitrile, homogenize and filter. Transfer 3 mL of the filtrate to a 20 mL volumetric flask and complete the volume with acetonitrile, obtaining a 30 µg/mL solution.

Standard solution: quantitatively transfer about 10 mg of nitazoxanide CRS to a 100 mL volumetric flask, complete the volume with acetonitrile and homogenize. Transfer 3 mL of this solution to a 10 mL volumetric flask and complete the volume with acetonitrile, obtaining a 30 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency for the nitazoxanide peak is not less than 2000 theoretical plates/meter. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₂H₉N₃O₅S in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

NITAZOXANIDE POWDER FOR ORAL SUSPENSION

The powder for oral suspension is a mixture of nitazoxanide with one or more coloring, flavoring, buffering, sweetening and preservative agents. It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{12}H_9N_3O_5S$.

IDENTIFICATION

The identification test B. can be omitted if test C. is carried out. The identification test C. can be omitted if test B. is carried out.

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, protected from direct light, using silica gel 60 F₂₅₄ as support, and mixture of hexane and ethyl alcohol (60:40) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): reconstitute the content of three flasks as indicated on the label and homogenize. Transfer quantity of oral suspension equivalent to 50 mg of nitazoxanide to a 50 mL volumetric flask and add 40 mL of acetonitrile. Place in ultrasonic bath at room temperature for 10 minutes, complete the volume with acetonitrile, homogenize and filter.

Solution (2): 1 mg/mL nitazoxanide CRS solution in acetonitrile.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 220 nm to 400 nm of the sample solution obtained in method **A.** from *Assay*, there are maximum and minimum points only at the same wavelengths observed in the standard solution spectrum.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test. Determine in powder before reconstituting.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 7.5 containing 6% of cetrimonium bromide, kept at 25 °C, 900 mL.

Apparatus: paddles, 100 rpm.

Time: 45 minutes.

Procedure: proceed protected from direct light. After the test, remove aliquot from the dissolution medium, prepare *Sample solution* and proceed as described in method **B.** from *Assay*. Inject, separately, 20 µL of the *Standard solution* and *Sample solution*, record the chromatograms and

measure the areas under the peaks. Calculate the quantity of $C_{12}H_9N_3O_5S$ dissolved in the medium from the responses obtained with the *Standard Solution* and the *Sample Solution*, prepared as described below.

Sample solution: after testing, remove aliquot from the dissolution medium, filter and dilute, if required, in acetonitrile to obtain a 27.77 µg/mL nitazoxanide solution.

Standard solution: quantitatively transfer about 13.88 mg of nitazoxanide CRS to a 100 mL volumetric flask. Add 5 mL of acetonitrile, place in ultrasonic bath for 30 minutes and complete the volume with phosphate buffer pH 7.5 containing 6% of cetrimonium bromide. Dilute successively with acetonitrile to obtain a 27.77 µg/mL nitazoxanide solution.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{12}H_9N_3O_5S$ dissolve in 45 minutes.

PURITY TESTS

Water (5.2.20.1). Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed protected from direct light, as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Reconstitute the content of three flasks as indicated on the label and homogenize. Transfer volume of oral suspension equivalent to 15 mg of nitazoxanide to a 100 mL volumetric flask and add 50 mL of acetonitrile. Place in ultrasonic bath at room temperature for 10 minutes, complete the volume with acetonitrile, homogenize and filter. Transfer 2 mL of the filtrate to a 25 mL volumetric flask and complete the volume with water with pH previously adjusted to 4.5 with 10% (v/v) phosphoric acid, obtaining a 12 µg/mL solution. Prepare standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 345 nm, using water with a pH previously adjusted to 4.5 with 10% (v/v) phosphoric acid for zero adjustment. Calculate the quantity of $C_{12}H_9N_3O_5S$ in the oral suspension from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, protected from direct light,. Use chromatograph with ultraviolet detector at 240 nm; pre-column (optional) with 4,0 mm of length and 3.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (4 µm); column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (4 µm to 5 µm); kept at 25 °C; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of 0.1% (v/v) phosphoric acid with pH previously adjusted to 5.3 with triethylamine, and acetonitrile (45:55).

Sample solution: reconstitute the content of three flasks as indicated on the label and homogenize. Transfer volume of oral suspension equivalent to 50 mg of nitazoxanide to a 250 mL volumetric flask, add 150 mL of acetonitrile and place in ultrasonic bath for 10 minutes. Complete the volume with acetonitrile, homogenize and filter. Transfer 3 mL of the filtrate to a 20 mL volumetric flask and complete the volume with acetonitrile, obtaining a 30 µg/mL solution.

Standard solution: quantitatively transfer about 10 mg of nitazoxanide CRS to a 100 mL volumetric flask, complete the volume with acetonitrile and homogenize. Transfer 3 mL of this solution to a 10 mL volumetric flask and complete the volume with acetonitrile, obtaining a 30 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency for the nitazoxanide peak is not less than 2000 theoretical plates/meter. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

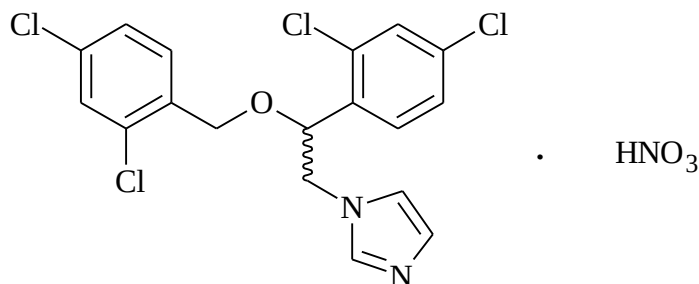
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₂H₉N₃O₅S in the oral suspension from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

MICONAZOLE NITRATE***Miconazoli nitrato***C₁₈H₁₄Cl₄N₂O.HNO₃; 479.14

miconazole nitrate; 05929

1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-1*H*-imidazole nitrate (1:1)
[22832-87-7]

It contains not less than 98.0% and not more than 101.0% of C₁₈H₁₄Cl₄N₂O.HNO₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Very slightly soluble in water, moderately soluble in methyl alcohol and slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 178 °C to 184 °C.

Specific optical rotation (5.2.8): -0,10 to +0,10, in relation to the desiccated substance. Determine in solution at 1% (w/v).

IDENTIFICATION

Test A. can be omitted if tests **B.**, **C.** and **D.** are carried out. **Test B** can be omitted if tests **A.**, **C.** and **D.** are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the miconazole nitrate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.04% (w/v) solution in mixture of 0.1 *M* hydrochloric acid and isopropyl alcohol (1:10), there are maximum and minimum points only at the same wavelengths of similar miconazole nitrate CRS solution.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using octadecylsilane silica gel as support, and mixture of ammonium acetate RS, dioxane and methyl alcohol (20:40:40), as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 6 mg/mL sample solution in mobile phase.

Solution (2): 6 mg/mL miconazole nitrate CRS solution in mobile phase.

Solution (3): dissolve 30 mg of miconazole nitrate CRS and 30 mg of econazole nitrate CRS in 5 mL of mobile phase.

Develop chromatogram. Remove the plate, allow to air dry and nebulize with iodine. Examine under visible light. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. The test is only valid if, in the chromatogram obtained with *Solution (3)*, there are two clearly separated stains.

D. It meets nitrate ion reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 235 nm; column with 100 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 µm), kept at room temperature; *Mobile phase* flow rate of 2.0 mL/minute.

Mobile phase: weigh 6 g of ammonium acetate and transfer to a 1000 mL volumetric flask, add 300 mL of acetonitrile, 320 mL of methyl alcohol and complete the volume with water.

Solution (1): quantitatively transfer about 0.1 g of the sample to a 10 mL volumetric flask and complete the volume with *Mobile Phase*. Homogenize.

Solution (2): quantitatively transfer approximately 25 mg of miconazole nitrate CRS and 25 mg of econazole nitrate CRS to a 25 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize. Dilute 2.5 mL to a 100 mL volumetric flask and complete the volume with the same solvent

Solution (3): transfer 1 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with *Mobile phase*. Dilute 5 mL of this solution to 20 mL, completing the volume with *Mobile phase*.

Equilibrate the chromatographic system for 30 minutes. Inject 10 µL of *Solution (3)*. Adjust the chromatographic system so that the height of the main peak obtained in the chromatogram with *Solution (3)* is less than 50% of the full scale. Inject 10 µL of *Solution (2)*. The retention time for miconazole nitrate is approximately 20 minutes and for econazole nitrate is 10 minutes. The resolution between the peaks obtained with *Solution (2)* is not less than 10, if necessary, adjust the composition of the *Mobile Phase*.

Procedure: inject separately 10 µL of *Solution (1)* and of *Solution (3)*. Record the chromatograms and measure the areas under the peaks. In the chromatogram obtained with *Solution (1)*, the individual area under the peaks, except the main peak, is not greater than the area under the main peak obtained with *Solution (3)* (0.25%). The sum of the areas under all peaks recorded is not more than

twice the area under the main peak obtained with *Solution (3)* (0.5%). Disregard peaks with areas below 0.2 times the area under the main peak, obtained with *Solution (3)* (0.05%) and the peaks related to the nitrate ion.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven between 100 °C and 105 °C for two hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 0.35 g of the previously desiccated sample, dissolve in 50 mL of glacial acetic acid, heating slightly if necessary, and titrate potentiometrically with 0.1 M perchloric acid VS. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS used is equivalent to 47.914 mg of $C_{18}H_{14}Cl_4N_2O.HNO_3$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antifungal.

SILVER NITRATE

Argenti nitras

AgNO₃; 169.87
silver nitrate; 06427
Silver salt(1+) of nitric acid (1:1)
[7761-88-8]

It contains not less than 99.0% and not more than 100.5% of AgNO₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Large colorless crystals, transparent or small white crystals.

Solubility. Very soluble in water, soluble in ethyl alcohol, moderately soluble in ammonia water.

IDENTIFICATION

A. To 10 ml of 10% (w/v) solution, add one drop of diphenylamine RS and homogenize. Carefully pour the solution into a test tube containing 2 mL of sulfuric acid. A blue color develops on the interface.

B. The 2% (w/v) solution meets silver ion reactions (5.3.1.1).

C. The 2% (w/v) solution meets nitrate ion reactions (5.3.1.1).

PURITY TESTS

Preparation aspect. The 10% (w/v) aqueous preparation is clear (5.2.25) and colorless (5.2.12).

Acidity and alkalinity. To 2 mL of 4% (w/v) solution, add 0.1 mL of bromocresol green TS. Blue color develops. To 2 mL of 10% (w/v) solution, add 0.1 mL of phenol red TS. A yellow color develops.

Aluminum, copper, lead and bismuth. Dissolve 1 g of the sample in mixture with 4 mL of 13.5 M ammonia and 6 mL of water. The preparation is clear (5.2.25) and colorless (5.2.12).

Residue by evaporation. To 30 mL of 4% solution (w/v), add 7.5 mL of diluted hydrochloric acid, shake vigorously, heat for five minutes in water bath and filter. Evaporate 20 mL of the filtrate in water bath and dry the residue in oven, between 100 °C and 105 °C. Not more than 2 mg (0,3%).

ASSAY

Dry the sample in advance, on silica, for four hours, protected from light. Accurately weigh about 0.3 g of the desiccated sample and dissolve in 50 mL of water. Add 2 mL of nitric acid and 2 mL of ammoniacal ferric sulfate RS and homogenize. Titrate with 0.1 M ammonium thiocyanate VS until reddish-yellow coloration. Each mL of 0.1 M ammonium thiocyanate VS is equivalent to 16.987 mg of AgNO₃.

PACKAGING AND STORAGE

In tightly closed, non-metallic containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

SILVER NITRATE OPHTHALMIC DROPS

It contains not less than 95.0% and not more than 105.0% of AgNO_3 . The ophthalmic drops is buffered by the addition of sodium acetate.

IDENTIFICATION

A. The ophthalmic drops meets silver ion reactions **(5.3.1.1)**.

B. The ophthalmic drops meets nitrate ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Aspect. The ophthalmic drops is clear **(5.2.25)** and colorless **(5.2.12)**.

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.5 to 6.0.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

ASSAY

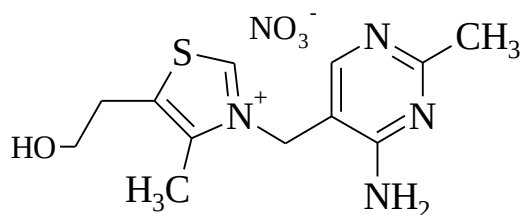
Transfer volume of the ophthalmic drops equivalent to 50 mg of silver nitrate to an erlenmeyer flask, dilute with 20 mL of water, add 1 mL of nitric acid, 1 mL of ammoniacal ferric sulfate RS and homogenize. Titrate with 0.02 M ammonium thiocyanate VS until reddish-yellow coloration. Each mL of 0.02 M ammonium thiocyanate VS is equivalent to 3.397 mg of AgNO_3 .

PACKAGING AND STORAGE

In tightly closed inert containers, protected from light.

LABELING

Comply with current legislation.

THIAMINE NITRATE*Thiamini nitras*C₁₂H₁₇N₅O₄S; 327.36

thiamine nitrate; 08514

3[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium nitrate
[532-43-4]

It contains not less than 98.0% and not more than 101.0% of C₁₂H₁₇N₅O₄S, in relation to the desiccated substance.

DESCRIPTION

Physicochemical characteristics. White or off-white crystalline powder.

Solubility. Moderately soluble in water and slightly soluble in ethyl alcohol.

IDENTIFICATION

The identification tests C, D, and E, can be omitted if tests A, and B, are carried out. The identification test A, can be omitted if tests B., C., D, and E, are carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the thiamine nitrate CRS spectrum, prepared identically.

B. Prepare a 20 mg/mL sample solution. To 2 mL of this solution, add 2 mL of sulfuric acid. Cool and add 2 mL of ferrous sulfate RS. A brown ring appears at the junction of the two liquids.

C. Dissolve 5 mg of the sample in mixture of 1 mL of lead acetate RS and 1 mL of 2.5 M sodium hydroxide. Heat in water bath for some minutes. Upon sample dissolution, a yellow color is produced. Heat the solution, the color turns brown and after standing, a precipitate is formed.

D. A 20 mg/mL sample solution produces a white precipitate when a solution containing 6.5 g of mercury chloride in 100 mL of water is added; and a reddish-brown precipitate when adding 0.1 M iodine VS. A precipitate is also produced when adding trinitrophenol RS.

E. Prepare solution by dissolving approximately 5 mg of the sample in 5 mL of 0.5 M sodium hydroxide. To this solution, add 0.5 mL of potassium ferrocyanide RS and 5 mL of isobutyl alcohol. Shake vigorously for two minutes and allow the liquid layers to separate. When illuminated from

above by a beam of vertical ultraviolet light and viewed at a right angle, the air-liquid meniscus shows a vivid blue fluorescence, which disappears when it is made alkaline again.

PURITY TESTS

pH (5.2.19). 6.0 to 7.5. Determine in sample solution at 2% (w/v).

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; chromatographic column of 150 mm in length and 4 mm in internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile Phase* flow of 0.75 mL/minute.

Eluent A: 0.005 M sodium 1-octane sulfonate in diluted glacial acetic acid (1:100).

Eluent B: mixture of methyl alcohol and acetonitrile (3:2).

Mobile phase: mixture of *Eluent A* and *Eluent B* (60:40).

Sample solution: 1 mg/mL sample solution in *Mobile phase*.

Procedure: inject 10 µL of *Sample solution* and record the chromatograms for not less than the triple of the main peak retention time. Calculate the total percentage of secondary peaks in the portion of thiamine nitrate used according to the following formula:

$$\text{Porcentagem de impurezas} = \left(\frac{r_U}{r_T} \right) \times 100$$

where

r_U = sum of the areas under all peaks, except for that under the thiamine nitrate peak;

r_T = sum of the areas under all peaks.

Not more than 1.0% of total impurities.

Chlorides (5.3.2.1). Not more than 0.06% (600 ppm).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105 °C for two hours. Not more than 1.0%.

Residue by incineration (5.2.10). Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 140 mg of thiamine nitrate, dissolve in 5 mL of formic acid and add 50 mL of acetic anhydride. Titrate immediately with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 16.368 mg of $C_{12}H_{17}N_5O_4S$.

PACKAGING AND STORAGE

In hermetically closed containers and protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vitamin.

SODIUM NITRITE

Natrii nitris

NaNO₂; 69.00

sodium nitrite; 06433

Nitrous acid sodium salt (1:1)

[7632-00-0]

It contains not less than 97.0% and not more than 101.0% of NaNO₂, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Granular powder or transparent, colorless hexagonal crystals, or white, opaque and deliquescent mass.

Solubility. Freely soluble in water, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. The 10% (w/v) solution meets nitrite ion reactions (5.3.1.1).

B. The 10% (w/v) solution meets sodium ion reactions (5.3.1.1).

PURITY TEST

Heavy metals (5.3.2.3). Use *Method III*. Determine in 1 g of the sample. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in oven at 105 °C for four hours. Not more than 0.25%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 1 g of sample, transfer to a 100 mL volumetric flask, dissolve in water and complete the volume with the same solvent. Transfer 15 mL of this solution to a flask containing mixture of 50 mL of 0.02 M potassium permanganate VS, 100 mL of water and 5 mL of sulfuric acid. When adding the sample solution, immerse the pipette tip under the surface of the permanganate mixture. Heat the mixture to 40 °C, allow to stand for five minutes and add 25 mL of 0.05 M oxalic acid VS. Heat the mixture to 80°C and heat titrate with 0.02 M potassium permanganate VS. Each mL of 0.02 M potassium permanganate VS is equivalent to 3.450 mg of NaNO₂.

PACKAGING AND STORAGE

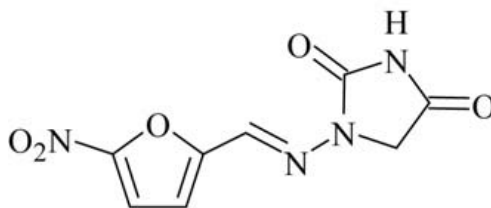
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Vasodilator; antidote for cyanide poisoning.

NITROFURANTOIN***Nitrofurantoinum***

$C_8H_6N_4O_5$; 238.16

nitrofurantoin; 06438

1-[[[5-Nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione
[67-20-9]

$C_8H_6N_4O_5 \cdot H_2O$; 256.17

nitrofurantoin monohydrate; 11389

1-[[[5-Nitro-2-furanyl)methylene]amino]-2,4-imidazolidinedione hydrate (1:1)
[17140-81-7]

It contains not less than 98.0% and not more than 102.0% of $C_8H_6N_4O_5$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Yellow crystalline powder or yellow crystals. In solid form or in solution, it undergoes discoloration by alkalis and exposure to light, and decomposition by contact with metals, except stainless steel and aluminum.

Solubility. Very slightly soluble in water and in ethyl alcohol.

IDENTIFICATION

The identification tests B., C. and E. can be omitted if tests A. and D. are carried out. The identification tests A. and D. can be omitted if tests B., C. and E. are carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample, desiccated at 140 °C for 30 minutes and dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the nitrofurantoin CRS spectrum, prepared identically.

B. Proceed protected from intense light. In the ultraviolet absorption spectrum (**5.2.14**), at the range of 220 nm to 400 nm, of the sample solution obtained in method **A.** from Assay, there are maximum absorption points at 266 nm and at 367 nm. The ratio between the absorbance values measured at 367 nm and 266 nm ranges between 1.36 and 1.42.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

D. Dissolve 10 mg of the sample in 10 mL of dimethylformamide. Transfer 1 mL of the solution to a test tube and add 0.1 mL of 0.5 M ethanolic potassium hydroxide. A brown color develops.

E. Dissolve 5 mg of the sample in 5 mL of 0.1 M sodium hydroxide. An intense yellow colored solution is produced, which then turns reddish-orange.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of nitromethane and methyl alcohol (9:1) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.25 g of the sample in dimethylformamide and complete the volume to 10 mL with acetone.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with acetone.

Develop chromatogram. Remove the plate, allow to air dry and heat between 100 °C and 105 °C for five minutes. Examine under ultraviolet light at 254 nm. Spray with phenylhydrazine hydrochloride RS. Heat the plate again between 100 °C and 105 °C for 10 minutes. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram with *Solution (2)* (1,0%).

Water (5.2.20). Dry the sample at 140 °C for 30 minutes. Not more than 1.0% for the anhydrous form and between 6.5% and 7.5% for the monohydrate form.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*, protected from intense light. Accurately weigh about 0.12 g of the sample and dissolve in 25 mL of dimethylformamide. Complete the volume to 500 mL with water. Transfer 2.5 mL of the solution to a 100 mL volumetric flask and complete the volume with a solution containing 1.8% (w/v) sodium acetate and 0.14% (v/v) glacial acetic acid. Prepare standard solution in the same concentration, using the same solvents. Measure the absorbance of the resulting solution at 367 nm using the sodium acetate and acetic acid solution described above for the zero adjustment. Calculate the content of C₈H₆N₄O₅ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane (5 µm), kept at room temperature; adjust the chromatographic conditions so that the retention time of the nitrofurantoin peak is eight minutes.

Phosphate buffer pH 7.0: dissolve 6.8 g of monobasic potassium phosphate in 500 mL of water and adjust to pH 7.0 with M ammonium hydroxide. Complete the volume to 1000 mL with water and homogenize.

Mobile phase: mixture of *Phosphate buffer pH 7.0* and tetrahydrofuran (9:1). Filter and degas.

Internal standard solution: dissolve an accurately weighed quantity of acetanilide in water and dilute adequately to obtain a solution at 1 mg/mL.

Sample solution: quantitatively dissolve about 25 mg of the sample in 20 mL of dimethylformamide. Add 25 mL of the *Internal Standard Solution*, complete the volume to 50 mL with dimethylformamide and homogenize.

Standard solution: quantitatively dissolve about 25 mg of nitrofurantoin CRS in 20 mL of dimethylformamide. Add 25 mL of the *Internal Standard Solution*, complete the volume to 50 mL with dimethylformamide and homogenize.

Inject 5 µL or 10 µL replicates of the *Standard solution*. The resolution between acetanilide and nitrofurantoin is not less than 3.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 5 µL or 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₈H₆N₄O₅ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at temperature below 25 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

NITROFURANTOIN TABLETS

It contains not less than 90.0% and not more than 110.0% the stated quantity of $C_8H_6N_4O_5$. The tablets must be coated.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out. The identification tests B. and C. can be omitted if test A. is carried out.

A. Weigh and pulverize the coated tablets. Shake quantity of powder equivalent to 0.1 g of nitrofurantoin with 10 mL of 6 M acetic acid. Heat for some minutes and carry out hot filtration. Cool to room temperature, collect the precipitate and dry at 105 °C for one hour until constant weight. The residue meets the test **A.** in *Identification* of the *Nitrofurantoin* monograph.

B. In the ultraviolet absorption spectrum (**5.2.14**), at the range of 220 nm to 400 nm, of the sample solution obtained in method **A.** from *Assay*, there are maximum absorption points at 266 nm and at 367 nm. The ratio between the absorbance values measured at 367 nm and 266 nm ranges between 1.36 and 1.42.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 7.2, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 60 minutes and 120 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute with dissolution medium to adequate concentration. Measure the absorbances of the solutions at 375 nm (**5.2.14**) using the same solvent for zero adjustment. Calculate the quantity of $C_8H_6N_4O_5$ dissolved in the medium, comparing the readings obtained with that of the nitrofurantoin CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: not less than 25% (Q) of the stated quantity of $C_8H_6N_4O_5$ dissolve in 60 minutes and not less than 85% (Q) of the stated quantity of $C_8H_6N_4O_5$ dissolve in 120 minutes.

PURITY TESTS

Related substances. Proceed as described in *Related substances* in the *Nitrofurantoin* monograph. Prepare *Solution (1)* as described below.

Solution (1): weigh and pulverize the coated tablets. Dissolve quantity of powder equivalent to 0.1 g of nitrofurantoin in 10 mL of a filtered mixture of dimethylformamide and acetone (1:9).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

A. Weigh and pulverize 20 coated tablets. Proceed as described in method **A.** of Assay in the *Nitrofurantoin* monograph, using quantity of powder equivalent to 0.12 g of nitrofurantoin. Calculate the quantity of $C_8H_6N_4O_5$ in the coated tablets from the readings obtained.

B. Proceed as described in method **B.** of Assay in the *Nitrofurantoin* monograph. Prepare *Sample solution* as described below.

Sample solution: weigh and pulverize 20 coated tablets. Transfer quantity of powder equivalent to 50 mg of nitrofurantoin to a 100 mL volumetric flask, add 40 mL of dimethylformamide and shake mechanically for 15 minutes. Add 50 mL of *Internal standard solution*, homogenize and allow to cool to room temperature. Complete the volume with dimethylformamide and homogenize. Filter through a 0.45 μ m nylon filter.

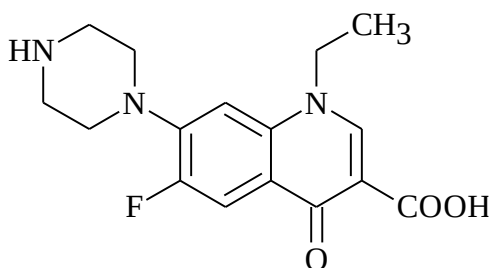
Procedure: inject 5 μ L or 10 μ L of the *Standard Solution* and the *Sample Solution* separately, record the chromatograms and measure the areas under the peaks corresponding to nitrofurantoin and acetanilide. Calculate the content of $C_8H_6N_4O_5$ in the coated tablets from the responses obtained for the nitrofurantoin and acetanilide ratio, in the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at temperature below 25 °C.

LABELING

Comply with current legislation.

NORFLOXACIN***Norfloxacinum***C₁₆H₁₈FN₃O₃; 319.34

norfloxacin; 06497

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinicocarboxylic acid
[70458-96-7]

It contains not less than 99.0% and not more than 101.0% of C₁₆H₁₈FN₃O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to pale yellow crystalline powder. Sensitive to light and moisture.

Solubility. Slightly soluble in water, freely soluble in acetic acid, slightly soluble in ethyl alcohol and very slightly soluble in methyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample, previously desiccated, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the norfloxacin CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of 0.0005% (w/v) solution in 0.1 M sodium hydroxide, there is a maximum point at 273 nm. The absorbance differs by not more than 3.0% from that of a similar norfloxacin CRS solution, prepared in the same solvent. Use amber glassware.

PURITY TESTS

Chromatographic purity. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel washed in advance with methyl alcohol and air dried as support, and mixture of chloroform, methyl alcohol, toluene, diethylamine and water (40:40:20:14:8) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 8.0 mg/mL sample solution in mixture of methyl alcohol and methylene chloride (1:1).

Solution (2): accurately weigh about 4.0 mg of norfloxacin CRS and dissolve with 1 mL of glacial acetic acid, add 4 mL of methyl alcohol and mix. Dilute 1 mL of this solution in 19 mL of mixture of methyl alcohol and methylene chloride (1:1).

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm and 365 nm). The main stain obtained in the chromatogram with *Solution (2)* is equivalent to 0.5% of impurity. Compare the intensities of any secondary stains obtained in the chromatogram of *Solution (1)* with the main stain obtained in the chromatogram of *Solution (2)*. The sum of the intensities of all the secondary stains obtained in the chromatogram of *Solution (1)* is not more than equal to the main one obtained with *Solution (2)* (0.5%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.0015% (15 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry at 105 °C, under reduced pressure, for two hours. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Accurately weigh approximately 240 mg of the sample and dissolve in 80 mL of glacial acetic acid. Titrate immediately with 0.1 M perchloric acid VS and determine the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 31.934 mg of $C_{16}H_{18}FN_3O_3$.

PACKAGING AND STORAGE

In tightly closed containers and protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

ALUMINUM ZIRCONIUM OCTACHLOROHYDRATE

$\text{Al}_8\text{ZrCl}_8(\text{OH})_{20}$; 930.84

aluminum zirconium octachlorohydrate; 09831

Aluminum zirconium chloride hydroxide

[98106-55-9]

Aluminum zirconium octachlorohydrate is a hydrated polymeric complex of basic aluminum and zirconium chloride. It contains not less than 90.0% and not more than 110.0% of aluminum zirconium octachlorohydrate in relation to the anhydrous substance.

IDENTIFICATION

The 10% (w/v) aqueous solution meets chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 3.0 to 5.0. Determine in aqueous solution at 15% (w/v).

Aluminum content. Weigh 0.15 g of the sample, transfer to a 150 mL beaker, add 5 mL of water and 15 mL of hydrochloric acid. Keep in ebullition for five minutes. Then add 40 mL of water and 30 mL of 0.05 M disodium edetate VS. Boil again for five minutes. Allow to cool, add 15 mL of acetic acid-ammonium acetate buffer and adjust with concentrated ammonia solution to pH 4.5. Add 20 mL of ethyl alcohol and adjust the pH to 4.6 with concentrated ammonia solution. Add 10 drops of 0.025% (w/v) dithizone in ethyl alcohol. Titrate with 0.1 M zinc sulfate VS until a pink-purple color appears. Carry out the blank test and make the necessary corrections. Calculate the percentage of aluminum with the formula:

$$\frac{2,698[15 M_e - (zM_z + Z_e)]}{W}$$

where

M_e = molarity of 0.05 M disodium edetate VS;

z = volume of 0.1 M zinc sulfate VS used;

M_z = molarity of 0.1 M zinc sulfate VS;

W = quantity in grams of the sample and

Z_e = equivalent volume of 0.05 M disodium edetate VS used by the quantity of zirconium, calculated according to the formula:

$$\left(\frac{Zr}{M_e}\right) \times \left(\frac{W}{92,97}\right)$$

where

Zr = percentage of zirconium determined in the *Zirconium content* test and

92.97 = zirconium atomic mass corrected for the 2% hafnium content.

Use the obtained result to calculate the atomic ratio of aluminum/zirconium and the atomic ratio of (aluminum + zirconium)/chloride.

Zirconium content. Weigh 250 g of the sample, transfer to a 150 mL beaker, add 5 mL of water and 15 mL of hydrochloric acid. Keep in ebullition for six to eight minutes. Then add from 30 mL to 40 mL of water and 5 mL of hydrochloric acid and heat to boiling. Add one drop of 0.1% (w/v) xylenol orange solution and titrate the solution, while still hot, with 0.05 M disodium edetate VS until color turns yellow from pink. Carry out the blank test and make the necessary corrections. Each mL of 0.05 M disodium edetate VS is equivalent to 46.485 mg of zirconium. Not less than 12.8% and not more than 15.4% of zirconium. Use the obtained result to calculate the atomic ratio of aluminum/zirconium and the atomic ratio of (aluminum + zirconium)/chloride.

Aluminum/zirconium atomic ratio. Divide the percentage of aluminum found in the test for *Aluminum content* by the percentage of zirconium found in the test for *Zirconium content* and multiply by 92.97/26.98, where 92.97 is the zirconium atomic mass corrected to 2% of hafnium and 26.98 is the aluminum atomic mass. Not less than 6.0% and not more than 10.0%.

Chloride content. Weigh 250 g of the sample, transfer to a 250 mL beaker, add 120 mL of water and 20 mL of M nitric acid. Shake until dissolution and titrate with 0.1 M silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 M silver nitrate VS is equivalent to 3.545 mg of chloride. Not less than 16.5% and not more than 19.0% of chloride. Use the obtained result to calculate the aluminum/zirconium atomic ratio and the (aluminum + zirconium)/chloride atomic ratio.

(Aluminum + zirconium)/chloride atomic ratio. Calculate the (aluminum + zirconium)/chloride atomic ratio according to the formula:

$$\frac{Al/26,98 + Zr/92,97}{Cl/35,45}$$

where

Al, *Zr* and *Cl* = percentages of aluminum, zirconium and chloride, determined in the tests for *Aluminum content*, *Zirconium content* and *Chloride content*, respectively;

26.98 = aluminum atomic mass;

92.97 = zirconium atomic mass corrected for 2% of hafnium and

35.45 = chloride atomic mass;

The ratio is between 0.9 and 1.5.

Arsenic (5.3.2.5). Determine in 1.5 g of sample. Proceed as described in *Limit test for arsenic*. Not more than 0.0002% (2 ppm).

Iron (5.3.2.4). Use *Method I*. Weigh 0.667 g of the sample and add 40 mL of water. Proceed as described in *Limit test for iron*. Not more than 0.015% (150 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Weigh 1 g of the sample and add 40 mL of water. If the preparation is not clear, heat to 80 °C for some minutes, then allow to cool. If the preparation still remains turbid, repeat the process adding 3 mL of hydrochloric acid. Adjust pH between 3 and 4

with 6 M ammonium hydroxide. Proceed as described in *Limit test for heavy metals* using 2 mL of the standard 10 ppm lead solution. Not more than 0.002% (20 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Calculate the percentage of aluminum zirconium octachlorohydrate, according to the formula:

$$Al \times \left\{ \frac{26,98y + 92,97 + 17,01 \left[3y + 4 - \left(\frac{y+1}{z} \right) \right] + 35,45 \left(\frac{y+1}{z} \right)}{26,98y} \right\}$$

where

Al = percentage of aluminum found in the test for *Aluminum Content*;

y = aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*;

z = (aluminum + zirconium)/chloride atomic ratio found in the test for *(Aluminum + zirconium)/chloride atomic ratio*;

26.98 = aluminum atomic mass;

92.97 = zirconium atomic mass corrected for 2% of hafnium;

17.01 = molecular mass of the hydroxide anion (OH) and

35.45 = chloride (Cl) atomic mass;

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiperspirant.

ALUMINUM ZIRCONIUM OCTACHLOROHYDRATE SOLUTION

It contains not less than 90.0% and not more than 110.0% of the stated quantity of anhydrous aluminum octachlorohydrate. Aluminum zirconium octachlorohydrate is a polymeric complex of basic aluminum chloride. It has an atomic ratio of aluminum/zirconium between 6.0 and 10.0, and of (aluminum+zirconium)/chloride between 0.9 and 1.5. The following solvents can be used: water, propylene glycol or dipropylene glycol.

IDENTIFICATION

A. When the solution is prepared in propylene glycol, add about 10 mL of isopropyl alcohol to 2 g of the solution. Mix and filter. Evaporate the filtrate in water bath until reducing the volume to 1 mL. In the infrared absorption spectrum **(5.2.14)** of a film from this solution, dispersed in silver chloride, there are maximum absorption points only on the same wavelengths as the ones observed in the spectrum of a propylene glycol film, prepared identically. When the solution is prepared in dipropylene glycol, add about 10 mL of isopropyl alcohol to 2 g of the solution. Mix and filter. Evaporate the filtrate in water bath until reducing the volume to 1 mL. In the infrared absorption spectrum **(5.2.14)** of a film from this solution, dispersed in silver chloride, there are maximum absorption points only on the same wavelengths as the ones observed in the spectrum of a dipropylene glycol film, prepared identically.

B. The solution containing the equivalent to about 100 mg of aluminum zirconium octachlorohydrate per mL meets chloride ion reactions **(5.3.1.1)**.

CHARACTERISTICS

pH (5.2.19). 3.0 to 5.0. Determine in a solution prepared by diluting 3 g of the solution with water to complete the volume to 10 mL.

PURITY TESTS

Aluminum content. Weigh 0.15 g of the sample, transfer to a 150 mL beaker, add 5 mL of water and 15 mL of hydrochloric acid. Keep in ebullition for five minutes. Then add 40 mL of water and 30 mL of 0.05 M disodium edetate VS. Keep in ebullition for five minutes again. Allow to cool, add 15 mL of acetic acid-ammonium acetate buffer and adjust with concentrated ammonia solution to pH 4.5. Add 20 mL of ethyl alcohol and adjust the pH to 4.6 with concentrated ammonia solution. Add 10 drops of 0.025% (w/v) dithizone in ethyl alcohol. Titrate with 0.1 M zinc sulfate VS until a pink-purple color appears. Carry out blank test. Calculate the percentage of aluminum with the formula:

$$\frac{2,698[15 M_e - (zM_z + Z_e)]}{W}$$

where

M_e = molarity of 0.05 M disodium edetate VS;

z = volume of 0.1 M zinc sulfate VS used, in mL;

M_z = molarity of 0.1 M zinc sulfate VS;

W = quantity in grams of the sample and

Z_e = equivalent volume of 0.05 M disodium edetate VS used by the quantity of zirconium, calculated according to the formula:

$$\left(\frac{Zr}{M_e}\right) \times \left(\frac{W}{92,97}\right)$$

where

Zr = percentage of zirconium determined in the *Zirconium content* test;

92.97 = zirconium atomic mass corrected for the 2% hafnium content.

Use the obtained result to calculate the aluminum/zirconium atomic ratio and the (aluminum + zirconium)/chloride atomic ratio.

Zirconium content. Weigh 500 g of the sample, transfer to a 150 mL beaker, add 5 mL of water and 15 mL of hydrochloric acid. Keep in ebullition for six to eight minutes. Then add from 30 mL to 40 mL of water and 5 mL of hydrochloric acid and heat to boiling. Add one drop of xylenol orange TS and titrate the solution, while still hot, with 0.05 M disodium edetate VS until color turns yellow from pink. Carry out blank test. Each mL of 0.05 M disodium edetate VS is equivalent to 46.485 mg of zirconium. Not less than 12.8% and not more than 15.4% of zirconium. Use the obtained result to calculate the aluminum/zirconium atomic ratio and the (aluminum + zirconium)/chloride atomic ratio.

Aluminum/zirconium atomic ratio. Divide the percentage of aluminum found in the test for *Aluminum content* by the percentage of zirconium found in the test for *Zirconium content* and multiply by 92.97/26.98, where 92.97 is the zirconium atomic mass corrected to 2% of hafnium and 26.98 is the aluminum atomic mass. Not less than 6.0% and not more than 10.0%.

Chloride content. Weigh 500 g of the sample, transfer to a 250 mL beaker, add 120 mL of water and 20 mL of M nitric acid. Shake until dissolution and titrate with 0.1 M silver nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 M silver nitrate VS is equivalent to 3.545 mg of chloride. It contains not less than 16.5% and not more than 19.0% of chloride. Use the obtained result to calculate the aluminum/zirconium atomic ratio and the (aluminum + zirconium)/chloride atomic ratio.

(Aluminum + zirconium)/chloride atomic ratio. Calculate the (aluminum + zirconium)/chloride atomic ratio according to the formula:

$$\frac{Al/26,98 + Zr/92,97}{Cl/35,45}$$

where

Al , Zr and Cl = percentages of aluminum, zirconium and chloride, determined in the tests for *Aluminum content*, *Zirconium content* and *Chloride content*, respectively;

26.98 = aluminum atomic mass;

92.97 = zirconium atomic mass corrected for 2% of hafnium and

35.45 = chloride atomic mass;

The ratio is between 0.9 and 1.5.

Arsenic (5.3.2.5). Use *Method I*. Determine in 1.5 g of sample. Not more than 0.0002% (2 ppm).

Iron (5.3.2.4). Use *Method III*. Transfer 5.3 g of aluminum zirconium octachlorohydrate solution to a 100 mL volumetric flask and complete the volume with water. Transfer 5 mL of the sample solution and 2 mL of the standard solution to separate beakers and, to each beaker, add 5 mL of 6 M nitric acid. Cover with watch glass and boil the solutions for three to five minutes. Cool. Not more than 0.0075% (75 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Weigh 2 g of aluminum zirconium octachlorohydrate solution and add 40 mL of water. If the preparation is not clear, heat to 80 °C for some minutes, then allow to cool. If the preparation still remains turbid, repeat the process adding 3 mL of hydrochloric acid. Adjust pH between 3 and 4 with 6 M ammonium hydroxide. Use 2 mL of *Standard lead solution* (10 ppm). Not more than 0.001% (10 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Calculate the percentage of aluminum zirconium octachlorohydrate in the solution, according to the following formula:

$$Al \times \left\{ \frac{26,98y + 92,97 + 17,01 \left[3y + 4 - \left(\frac{y+1}{z} \right) \right] + 35,45 \left(\frac{y+1}{z} \right)}{26,98y} \right\}$$

where

Al = percentage of aluminum found in the test for *Aluminum Content*;

y = aluminum/zirconium atomic ratio found in the *Aluminum/zirconium atomic ratio* test;

z = (aluminum + zirconium)/chloride atomic ratio found in the *(Aluminum + zirconium)/chloride atomic ratio* test;

26.98 = aluminum atomic mass;

92.97 = zirconium atomic mass corrected for 2% of hafnium;

17.01 = molecular mass of the hydroxide anion (OH) and

35.45 = chloride (Cl) atomic mass;

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

OFLOXACIN
Ofloxacinum



$C_{18}H_{20}FN_3O_4$; 361.37

ofloxacin; 06574

9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic
[83380-47-6]

It contains not less than 98.5% and not more than 101.5% of $C_{18}H_{20}FN_3O_4$, in relation to the desiccated substance.

DESCRIPTION

Physicochemical characteristics. Colorless needle-shaped crystals. Melting temperature **(5.2.2)**: 250 °C, with decomposition.

Physicochemical constants.

Optical rotation (5.2.8): $-1,0^\circ$ to $+1,0^\circ$, in relation to the desiccated substance. Determine in solution at 1% (w/v) in chloroform.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the desiccated sample dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the ofloxacin CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.00067% (w/v) solution in 0.1 M hydrochloric acid, there are maximum points identical to those observed in the spectrum of similar ofloxacin CRS solution.

PURITY TESTS

Arsenic (5.3.2.5). Use *Method II*. Not more than 0.0001% (1 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 2 g of the sample. Dry in oven at 105 °C for four hours. Not more than 0.2%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Transfer approximately 0.1 g of the sample dried in advance to a 400 mL beaker, add 275 mL of acetic anhydride and shake until dissolved. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Use the first of the two inflection points. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 36.137 mg of $C_{18}H_{20}FN_3O_4$.

B. Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)*, by agar diffusion using cylinders.

Microorganisms *Kocuria rhizophila* ATCC 9341.

Culture media: medium number 1, for microorganism maintenance, sterile saline solution for inoculum standardization, and medium number 11, for base layer and inoculum layer on the plate.

Sample solution: accurately weigh approximately 0.25 g of sample, transfer to a 250 mL volumetric flask with the aid of 100 mL of *sterile 0.1 M Potassium Phosphate Buffer, pH 8.0 (Solution 2)*, shake for 30 minutes and complete the volume with the same diluent. Dilute, successively, to concentrations of 20 µg/mL, 30 µg/mL and 45 µg/mL, using *0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2)* as diluent.

Standard solution: accurately weigh approximately 50 mg of ofloxacin CRS, transfer to a 50 mL volumetric flask and complete the volume with *0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2)*. Dilute, successively, to concentrations of 20 µg/mL, 30 µg/mL and 45 µg/mL, using *0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2)* as diluent.

Procedure: add 20 mL of culture medium number 11 to each plate, wait to solidify, add 5 mL of 0.5% inoculum and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*, adding 0.2 mL of the freshly prepared solutions to the cylinders. Calculate the sample potency, in µg of ofloxacin per milligram, from the standard potency and the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimicrobial.

OFLOXACIN TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{18}H_{20}FN_3O_4$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Prepare solution containing the equivalent to 0.00067% (w/v) of ofloxacin in 0.1 M hydrochloric acid, shake mechanically for 20 minutes and filter. In the ultraviolet absorption spectrum **(5.2.14)** in the range of 200 nm to 400 nm, there are maximum points identical to those observed in the spectrum of similar ofloxacin CRS solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **A.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 294 nm; column with 100 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group, kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Buffer solution: dissolve 2.72 g of monobasic potassium phosphate in 1000 mL of water and adjust the pH to 3.3 ± 0.1 with phosphoric acid RS.

Eluent A: mixture of *Buffer solution* and acetonitrile (88:12). Degas and filter.

Eluent B: mixture of acetonitrile and *Buffer solution* (60:40). Degas and filter.

Mobile Phase gradient: adopt the gradient system described in the following table.

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 8	100	0	isocratic
8 – 25	100 → 40	0 → 60	linear gradient
25 – 26	40 → 100	60 → 0	linear gradient
26 – 40	100	0	isocratic

Solution (1): weigh and pulverize not less than 20 tablets. Transfer quantity of powder equivalent to 100 mg of ofloxacin to a 100 mL volumetric flask, add approximately 70 mL of methyl alcohol and place the flask in ultrasonic bath for 20 minutes. Complete the volume with methyl alcohol and filter in 0,45 µm filter, discarding the first 5 mL of the filtrate.

Solution (2): dissolve an accurately weighed quantity of ofloxacin CRS in methyl alcohol and dilute to obtain a 4 µg/mL solution.

Inject 10 µL replicates of the *Solution (2)*. The tailing factor for the ofloxacin peak is not more than 2.0. The relative standard deviation of replicate areas under the peaks recorded is not more than 5,0%.

Procedure: inject, separately, 10 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of each impurity present in the sample according to the formula:

$$100 (1/F) (A_i/A_p) (C_p/C_a)$$

where

F = relative response factor for each impurity;

A_i = area under the peak corresponding to any individual impurity obtained in *Solution (1)*;

A_p = area under the peak corresponding to the ofloxacin obtained in *Solution (2)*;

C_p = concentration, in mg/mL, of ofloxacin in *Solution (2)*;

C_a = concentration, in mg/mL, of ofloxacin in *Solution (1)*, based on the declared value.

The limits of impurities are presented below:

<i>Impurities</i>	<i>Relative retention time</i>	<i>Relative response factor</i>	<i>Limit (%)</i>
Impurity A (2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6 acid -carboxylic)	0.5	1	0.3
Impurity B (9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid)	3.6	0.22	0.3
Other individual impurities	-	1	0.2
Total impurities	-	-	1.0

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 294 nm; 100 mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group, kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Buffer solution: dissolve 2.72 g of monobasic potassium phosphate in 1000 mL of water and adjust the pH to 3.3 ± 0.1 with phosphoric acid RS.

Mobile phase: mixture of *buffer solution* and acetonitrile (88:12). Degas and filter.

Diluent 1: mixture of methyl alcohol and glacial acetic acid (75:25).

Diluent 2: mixture of water and acetonitrile (90:10).

Sample solution: weigh and pulverize not less than 20 tablets. Transfer quantity of powder equivalent to 100 mg of ofloxacin to a 100 mL volumetric flask, add approximately 70 mL of *Diluent 1* and place the flask in ultrasonic bath for 20 minutes. Complete the volume with *Diluent 1* and filter this solution in a 0.45 μm filter. Transfer 2 mL of the solution to a 100 mL volumetric flask, complete the volume with *Diluent 2* and shake.

Standard solution: transfer approximately 25 mg of ofloxacin CRS to a 25 mL volumetric flask and dissolve in *Diluent 1* (1 mg/mL). Transfer 2 mL of this solution to a 100 mL volumetric flask and complete the volume with *Diluent 2* (20 $\mu\text{g/mL}$).

Inject 20 μL replicates of the *Standard solution*. The tailing factor for the ofloxacin peak is not more than 2.0. The relative standard deviation of replicate areas under the peaks recorded is not more than 2,0%.

Procedure: inject, separately, 20 μL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the amount of $\text{C}_{18}\text{H}_{20}\text{FN}_3\text{O}_4$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

B. Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)*, by agar diffusion using cylinders.

Microorganism: *Kocuria rhizophila* ATCC 9341.

Culture media: medium number 1, for microorganism maintenance, sterile saline solution for inoculum standardization, and medium number 11, for base layer and inoculum layer on the plate.

Sample solution: weigh and pulverize 20 tablets. Use quantity of powder equivalent to 0.25 g of ofloxacin, quantitatively transfer to a 250 mL volumetric flask with the aid of 100 mL of sterile 0.1 M potassium phosphate buffer, pH 8.0 (*Solution 2*). Shake for 30 minutes, complete the volume with the same diluent and filter. Dilute to obtain concentrations of 0.05 $\mu\text{g/mL}$, 0.10 $\mu\text{g/mL}$ and 0.20 $\mu\text{g/mL}$, using potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) as diluent.

Standard solution: accurately weigh approximately 25 mg of ofloxacin CRS, transfer to a 25 mL volumetric flask and complete the volume with potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) and shake. Dilute to obtain concentrations of 0.05 $\mu\text{g/mL}$, 0.10 $\mu\text{g/mL}$ and 0.20 $\mu\text{g/mL}$, using potassium phosphate buffer, sterile, pH 8.0 (*Solution 2*) as diluent.

Procedure: add 20 mL of culture medium number 11 to each plate, wait to solidify, add 5 mL of 0.5% inoculum and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*, adding 0.2 mL of the freshly prepared solutions to the cylinders. Calculate the quantity in mg of ofloxacin in the tablets from the standard potency and from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

OFLOXACIN OPHTHALMIC DROPS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{18}H_{20}FN_3O_4$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of chloroform, methyl alcohol and ammonium hydroxide solution (1 in 30) (150:75:15) as mobile phase. Separately apply to the plate 2 µL of each solution, described below.

Solution (1): dilute accurately measured quantity of the ophthalmic drops in mixture of chloroform and methyl alcohol (1:1), so as to obtain a solution at 0.3 mg/mL.

Solution (2): dissolve quantity of ofloxacin CRS in mixture of chloroform and methyl alcohol (1:1) to obtain a 0.3 mg/mL solution.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **A.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 6.0 to 6.8.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 294 nm; 250mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to an octadecylsilane group (5 µm); kept at 35 °C, *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of 0.24% (w/v) sodium lauryl sulfate, acetonitrile and glacial acetic acid (580:400:20). Degas and filter.

Sample solution: transfer quantity of ophthalmic drops equivalent to 3 mg ofloxacin to a 50 mL volumetric flask, dilute with 0.05 M hydrochloric acid and shake.

Standard solution: dissolve accurately weighed quantity of ofloxacin CRS and dilute with 0.05 M hydrochloric acid to obtain a 60 µg/mL solution.

Resolution Solution: prepare a solution containing about 0.1 mg/mL of ofloxacin CRS and about 2.4 mg/mL in acetonitrile.

Inject 20 µL replicates of the *Resolution solution*. The resolution between propylparaben and ofloxacin is not less than 2.0. Inject 20 µL replicates of the *Standard solution*. The tailing factor for the ofloxacin peak is not more than 3.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₈H₂₀FN₃O₄ in the ophthalmic drops from the responses obtained with the *Standard solution* and the *Sample solution*.

B. Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3.1)* by agar diffusion using cylinders.

Microorganism: *Kocuria rhizophila* ATCC 9341.

Culture media: medium number 1, for microorganism maintenance, sterile saline solution for inoculum standardization, and medium number 11, for base layer and inoculum layer on the plate.

Sample solution: dilute the ophthalmic drops to obtain concentrations of 0.05 µg/mL, 0.10 µg/mL and 0.20 µg/mL, using *Potassium phosphate buffer solution, sterile, pH 8.0 (Solution 2)* as diluent.

Standard solution: accurately weigh approximately 25 mg of ofloxacin CRS, transfer to a 25 mL volumetric flask and complete the volume with *Potassium phosphate buffer solution, sterile, pH 8.0 (Solution 2)* and shake. Dilute to obtain concentrations of 0.05 µg/mL, 0.10 µg/mL and 0.20 µg/mL, using *Potassium phosphate buffer solution, sterile, pH 8.0 (Solution 2)* as diluent.

Procedure: add 20 mL of culture medium number 11 to each plate, wait to solidify, add 5 mL of 0.5% inoculum and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*, adding 0.2 mL of the freshly prepared solutions to the cylinders. Calculate the quantity of ofloxacin in the ophthalmic drops from the standard potency and from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

PEANUT OIL

Arachidis oleum

peanut oil; 09887
[8002-03-7]

Refined fixed oil obtained from the seeds of one or more cultivated varieties of *Arachis hypogaea* L.
– LEGUMINOSAE.

DESCRIPTION

Physical characteristics. Almost colorless or slightly yellowish oil, viscous, odorless or with a slight pleasant odor.

Solubility. Insoluble in water, soluble in mineral oils, slightly soluble in ethyl alcohol, miscible with ethyl ether, petroleum ether, chloroform and carbon disulfide.

Physicochemical constants.

Relative density (5.2.5): 0.912 to 0.920.

Refractive index (5.2.6): 1.462 to 1.464. Determine at 40 °C.

Solidification temperature (5.2.29.3): 26 °C to 33 °C. Determine in dry fatty acid mixture.

IDENTIFICATION

Saponify 5 g of the sample with 2.5 mL of 7.5 M sodium hydroxide and 12.5 mL of ethyl alcohol, by heating, until boiling. Evaporate the ethyl alcohol, dissolve the soap in 50 mL of hot water and add hydrochloric acid until the free fatty acids separate as an oily layer. Cool the mixture, remove the separated fatty acids and dissolve in 75 mL of ethyl ether. To the ether solution, add heated 10% (w/v) lead acetate solution in ethyl alcohol. Allow to stand for 18 hours. Filter the supernatant liquid and transfer the precipitate to the filter with the aid of ethyl ether. Place the precipitate in mixture of 40 mL of 3 M hydrochloric acid and 20 mL of water. Heat until the oil layer becomes completely clear. Cool, decant the aqueous solution and boil the fatty acids with water acidified with hydrochloric acid, until the lead is eliminated. [Fatty acids are lead free when 100 mg, dissolved in 10 mL of ethyl alcohol, does not darken by adding two drops of 10% sodium sulfate (w/v)]. Allow the fatty acids to solidify and press them between filter papers on a cool surface to dry. Dissolve the solid fatty acids in 25 mL of 90% (v/v) ethyl alcohol, heat moderately, cool to 15 °C and keep at this temperature until the fatty acids crystallize. Filter the fatty acids obtained. Recrystallize them with hot 90% (v/v) ethyl alcohol and dry in a desiccator, under reduced pressure, for four hours. The arachidonic acid obtained melts (5.2.2) at a temperature between 73 °C and 76 °C.

PURITY TESTS

Acidity value (5.2.29.7). Not more than of 2 mL of 0.02 M sodium hydroxide VS is required to neutralize the free fatty acids present in 10 g of the sample.

Iodine value (5.2.29.10). 84 to 100.

Saponification value (5.2.29.8). 185 to 195.

Unsaponifiable matter (5.2.29.14). Not more than 1.5%.

Cotton oil. In a test tube, shake 5 mL of the sample with 5 mL of mixture of *n*-amyl alcohol and 1% sulfur (w/v) in carbon disulfide (1:1). Carefully heat until carbon disulphide evaporates. Submerge the tube to a third of its depth in boiling saturated sodium chloride solution. No red color develops for 15 minutes.

Sesame oil. Shake the sample with an equal volume of mixture of 90% ethyl alcohol (v/v) and ammonium hydroxide (9:1). Heat in water bath until the ethyl alcohol and ammonia are eliminated. Mix 2 mL of the residue with 1 mL of 1% sucrose (w/v) in hydrochloric acid and allow to stand for five minutes. The acid layer must not turn pink or, if the color pink appears, it must be not more than equal to that obtained by repeating the test with sucrose.

Other vegetable oils. In a flask with reflux condenser, saponify 1 mL of the sample with 5 mL of 2 *M* ethanolic potassium hydroxide for five minutes. Add 1.5 mL of glacial acetic acid and 50 mL of 70% (v/v) ethyl alcohol. Heat until the solution becomes clear. Cool slowly and measure the temperature of the liquid. Turbidity occurs at a temperature of not less than 39 °C.

Rancid. Mix 1 mL of the 10% (v/v) sample in ethyl ether with 1 mL of hydrochloric acid and add 1 mL of 0.1% (w/v) phloroglucinol in ethyl ether. Red or pink color is not developed.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

In hermetic, light-resistant containers, protected from exposure to excessive heat.

LABELING

Comply with current legislation.

CATEGORY

Pharmacotechnical adjuvant.

SESAME OIL***Sesami oleum***

sesame oil; 09888

Sesame glyceride and fatty oils

[8008-74-0]

Refined fixed oil obtained from the seeds of one or more cultivated varieties of *Sesamum indicum* L. – PEDALIACEAE.

DESCRIPTION

Physical characteristics. Pale yellow oil, almost odorless, with a mild flavor. Composed mainly of linoleic and oleic acids.

Solubility. Insoluble in water, soluble in chloroform, in ethyl ether and in petroleum ether, slightly soluble in ethyl alcohol.

Physicochemical constants.

Relative density (5.2.5): 0.916 to 0.921.

Solidification temperature (5.2.29.3): 20 and 25 °C. Use the dry fatty acid mixture.

IDENTIFICATION

Shake 1 mL of the sample and 10 mL of 1% sucrose (w/v) in hydrochloric acid for 30 seconds. The acidic layer turns pink and turns red when standing (distinction from most other fixed oils).

PURITY TESTS

Acidity value (5.2.29.7). Not more than of 2 mL of 0.02 M sodium hydroxide VS is required to neutralize the free fatty acids present in 10 g of the sample.

Iodine value (5.2.29.10). 103 to 116.

Saponification value (5.2.29.8). 188 to 195.

Unsaponifiable matter (5.2.29.14). Not more than 1.5%.

Triglyceride composition. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with refractive index detector; two columns in series of 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm); kept at 30 °C, *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of acetonitrile and methylene chloride (60:40).

Sample solution: quantitatively transfer about 0.2 g of the sample to a 10 mL volumetric flask and complete the volume with *Mobile Phase*. The fatty acids present in the sample are: linoleic acid (L),

oleic acid (O), palmitic acid (P) and stearic acid (E). The triglycerides present in the sample are: trilinolein (LLL), 1,2-dilinoleoyl-3-oleyl-rac-glycerol (OLL), 1,2-dilinoleoyl-3-palmitoylarac-glycerol (PLL), 1,2-dioleoyl-3-linoleoyl-rac-glycerol (OOL), 1-palmitoyl-2-oleyl-3-linoleoyl-rac-glycerol (POL), triolein (OOO), 1-linoleoyl-2-oleoyl-3-stearoyl-rac-glycerol (EOL) and 1,2-dioleoyl-3-palmitoylarac-glycerol (POO).

Resolution solution: transfer 30 mg of OLL and 30 mg of PLL, accurately weighed, to a 10 mL volumetric flask and complete the volume with *Mobile phase*.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.93 for OLL and 1.0 for PLL. The resolution between the PLL and OLL peaks is not less than 1.8. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.5%.

Procedure: Inject 20 µL of the *Sample Solution*, record the chromatograms and measure the areas of all the peaks obtained. Calculate the percentage of each triglyceride in the sesame oil sample, in relation to the sum of the observed peaks. Do not consider peaks related to the solvents. The table below indicates the relative retention times and percent composition of triglycerides.

<i>Triglyceride</i>	<i>Relative retention time</i>	<i>Composition (%)</i>
LLL	0.55	7.0 to 19.0.
OLL	0.65	13.0 to 30.0.
PLL	0.69	5.0 to 9.0.
OOL	0.77	14.0 to 25.0.
POL	0.82	8.0 to 16.0.
OOO	0.93	5.0 to 14.0.
EOL	0.97	2.0 to 8.0.
POO	1.0	2.0 to 8.0.

Cotton oil. In a test tube, shake 5 mL of the sample and 5 mL of mixture of *n*-amyl alcohol and 1% sulfur (w/v) in carbon disulfide (1:1). Carefully heat until carbon disulphide evaporates. Submerge the tube to a third of its depth in boiling saturated sodium chloride solution. No red color develops for 15 minutes.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

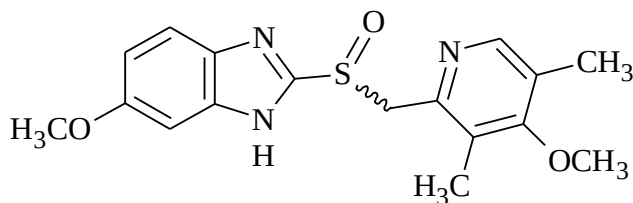
In hermetic, light-resistant containers, protected from excessive heat.

LABELING

Comply with current legislation.

CATEGORY

Pharmacotechnical excipient.

OMEPRAZOLE***Omeprazolum***C₁₇H₁₉N₃O₃S; 345.42

omeprazole; 06602

6-Methoxy-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole
[73590-58-6]

It contains not less than 98.0% and not more than 102.0% of C₁₇H₁₉N₃O₃S, in relation to the substance.

DESCRIPTION

Physical characteristics. White or off-white powder. Presents polymorphism.

Solubility. Very slightly soluble in water, moderately soluble in ethyl alcohol and methyl alcohol. Soluble in diluted alkali hydroxide solutions.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the omeprazole CRS spectrum. If there are differences between the spectra, dissolve sample and omeprazole CRS separately in methyl alcohol and evaporate to dryness. Obtain new spectra with the residues.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 230 nm to 350 nm, of 0.002% (w/v) solution in 0.1 *M* sodium hydroxide, there are maximum points at 276 nm and 305 nm. The ratio between the absorbance values measured at 305 nm and 276 nm ranges between 1.6 and 1.8.

C. The main stain of the chromatogram from *Solution (2)*, obtained in *Related compounds 1*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

PURITY TESTS

Preparation aspect. The 2% (w/v) preparation in methylene chlorid is clear (**5.2.25**) and colorless (**5.2.12**).

Light absorption. The absorbance of the 2% (w/v) solution in methylene chloride, measured at 440 nm, is not more than 0.10.

Related compounds 1. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel F₂₅₄ as support, and mixture of isopropyl alcohol, methylene chloride and methylene chloride

saturated with ammonia (1:2:2) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 100 mg of the sample in 2 mL of mixture of methyl alcohol and methylene chloride (1:1).

Solution (2): dilute 1 mL of *Solution (1)* to 10 mL with methyl alcohol.

Solution (3): dissolve 10 mg of omeprazole CRS in 2 mL of methyl alcohol.

Solution (4): dilute 1 mL of *Solution (1)* to 10 mL with mixture of methyl alcohol and methylene chloride (1:1). Dilute 1 mL of the resulting solution to 100 mL with the same diluent.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (4)* (0.1%).

Related compounds 2. Proceed as described in method **B**. from Assay. Prepare *Solution (1)* as described below:

Solution (1): dissolve 16 mg of the sample in *Mobile phase* and dilute to 10 mL with the same solvent. Dilute 1 mL of the resulting solution to 10 mL with *Mobile phase*.

Procedure: inject 40 µL of *Solution (1)* and record the chromatograms for not less than twice the main peak retention time. Measure the areas under all the peaks obtained. The area under any secondary peaks, except that under the main peak, is not more than 0.3% of the total area under the peaks obtained with *Solution (1)*. The sum of the areas under the secondary peaks, except that under the main peak, is not more than 1.0% of the total area under the peaks obtained with *Solution (1)*. Do not include solvent-related peaks in the calculations.

Volatile organic impurities. Proceed as described in *Gas chromatography (5.2.17.5)*. Use chromatograph equipped with flame ionization detector, using helium as carrier gas; capillary column of fused silica with 30 m in length and 0.53 mm in internal diameter, packed with (poly (cyanopropyl) (phenyl) dimethyl siloxane), with film thickness of 1.8 µm; column temperature at 40 °C for 20 minutes, soon increased to 240 °C and kept for 20 minutes; injector temperature at 140 °C and detector temperature at 260 °C.

Sample solution: transfer 100 mg of the sample to a 10 mL flask, add 5 mL of dimethylacetamide and 1 g of anhydrous sodium sulfate, cap and heat at 80°C for one hour.

Standard solution: prepare solution in dimethylacetamide, containing 12.0 µg/mL of methylene chloride, 1.2 µg/mL of chloroform, 7.6 µg/mL of dioxane and 1.6 µg/mL of trichlorethylene. Transfer 5 mL of the resulting solution to a 10 mL volumetric flask, add 1 g of anhydrous sodium sulfate, cap and heat at 80 °C for one hour.

Inject replicas of the *Standard Solution* through appropriate headspace. The resolution among any of the components is not less than 3.0. The relative standard deviation of the replicate areas under the recorded peaks is not more than 15.0%.

Procedure: inject, separately, through the appropriate headspace, the *Standard Solution* and the *Sample Solution*. Record the chromatograms and measure the areas under the peaks. The areas under the peaks related to methylene chloride, chloroform, dioxane and trichlorethylene obtained with the *Sample solution* must not be greater than the areas under the peaks related to methylene chloride, chloroform, dioxane and trichlorethylene obtained with the *Standard solution*, corresponding to not more than 600 ppm, 60 ppm, 380 ppm and 80 ppm, respectively.

Heavy metals (5.3.2.3). Use *Method I*. Not more than 0.5%.

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 60 °C, under reduced pressure, for four hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh about 1.1 g of the sample and dissolve in 50 mL of mixture of water and ethyl alcohol (1:4). Titrate with 0.5 M sodium hydroxide VS. Determine the endpoint potentiometrically. Each mL of 0.5 M sodium hydroxide VS is equivalent to 172.710 mg of $C_{17}H_{19}N_3O_3S$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 280 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow rate of 0.8 mL/minute.

Phosphate buffer pH 7.6: dissolve 0.725 g of monobasic sodium phosphate and 4.472 g of anhydrous dibasic sodium phosphate in 300 mL of water, dilute to 1000 mL with the same solvent and homogenize. Transfer 250 mL of the resulting solution to a 1000 mL volumetric flask, add approximately 980 mL of water, adjust the pH to 7.6 with phosphoric acid and complete the volume with water.

Mobile phase: mixture of *Phosphate buffer pH 7.6* and acetonitrile (3:1).

Sample solution: dissolve an accurately weighed quantity of the sample in a mixture of 0.01 M sodium borate and acetonitrile (3:1) and dilute with the same solvent to obtain a 200 μ g/mL solution.

Standard solution: dissolve an accurately weighed quantity of omeprazole CRS in mixture of 0.01 M sodium borate and acetonitrile (3:1) and dilute with the same solvent to obtain a 200 μ g/mL solution.

Diluted standard solution: transfer 5 mL of the *Standard solution* to a 10 mL volumetric flask, complete the volume with the same mixture of 0.01 M sodium borate and acetonitrile (3:1), obtaining a solution at 100 µg/mL.

Inject 20 µL replicates of the *Diluted standard solution*. The column efficiency must be not less than 3000 theoretical plates. The capacity factor is not less than 6.0. The tailing factor is not more than 1.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₇H₁₉N₃O₃S in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In hermetic containers, protected from light and moisture, between 2 °C and 8 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antisecretory.

MAGNESIUM OXIDE***Magnesii oxidum***

MgO; 40.30

magnesium oxide; 06728

Magnesium oxide

[1309-48-4]

It contains not less than 96.0% and not more than 100.5% of MgO, in relation to the incinerated substance.

DESCRIPTION

Physical characteristics. Amorphous, fine, white powder.

Solubility. Practically insoluble in water. Soluble in diluted acids, producing slight effervescence.

IDENTIFICATION

Dissolve about 15 mg of the sample in 2 mL of 20% (w/v) nitric acid and neutralize with 8.5% (w/v) sodium hydroxide. The solution meets magnesium ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 5 g of the sample in a mixture with 70 mL of acetic acid RS and 30 mL of water. Heat to boiling for two minutes, cool and complete the volume to 100 mL with 0.045 M acetic acid. Filter, if necessary, on a porcelain or silica filter, previously calcined and tared, whose porosity allows for a clear filtrate. Store any residue that may be present. The reference solution is a mixture (1:1) of the solution described below and 1% (w/v) hydrochloric acid: mix 3 mL of the *Cobalt II chloride base solution*, 3 mL of the *Ferric chloride base solution*, 2.4 mL of *Cupric sulfate base solution*, prepared as described in *Color of liquids (5.2.12)*, and 1.6 mL of 1% hydrochloric acid (w/v). 10 mL of the sample solution is not more stained than 10 mL of the reference solution.

Limit for acetic acid-insoluble substances. Wash, dry and calcine at 600 °C any residue collected during the preparation of the sample solution in *Preparation aspect*. The residue mass is not more than 5 mg, which represents not more than 0.1%.

Limit for soluble substances and free alkalis. To 2 g of the sample add 100 mL of water and heat to boiling for five minutes. Carry out hot filtration through a porous glass filter. Add two drops of methyl red TS to 50 mL of the filtrate and titrate with 0.05 M sulfuric acid VS. Not more than 2 mL of 0.05 M sulfuric acid VS are used. Evaporate 25 mL of the filtrate to dryness and dry between 100 °C and 105 °C. The residue mass is not greater than 10 mg. Not more than 2.0%.

Arsenic (5.3.2.5). Determine in 15 mL of the sample solution obtained in *Preparation aspect*. Proceed as described in *Visual method*. Not more than 0.0004% (4 ppm).

Calcium (5.3.2.7). Dilute 1.3 mL of the sample solution obtained in *Preparation aspect* to 150 mL with water. Proceed as described in *Limit test for calcium*, using 15 mL of this solution. Not more than 1.5%.

Iron (5.3.2.4). Dissolve 40 mg of the sample in 5 mL of 2 M nitric acid, heat to boiling for one minute and dilute to 50 mL with water. Dilute 25 mL of the obtained solution to 45 mL with water, add 2 mL of hydrochloric acid and proceed as described in *Method I*. Use 1 mL of *Standard iron solution* (10 ppm Fe). Not more than 0.05% (500 ppm).

Heavy metals (5.3.2.3). Dissolve 2 g of the sample in 35 mL of 3 M hydrochloric acid and evaporate in water bath to dryness. Towards the end of evaporation, shake the residue frequently to disintegrate it, so as to obtain a fine dry powder. Dissolve the residue in 20 mL of water and evaporate until dryness. Dissolve the residue again in 20 mL of water, filter if necessary and dilute with water to 40 mL. Dilute 20 mL of the obtained solution to 25 mL with water and proceed as described in *Method I*. Not more than 0.002% (20 ppm).

Sulfates (5.3.2.2). To 2 g of the sample, add 30 mL of hydrochloric acid RS. Filter, if necessary, add 1 mL of barium chloride RS, complete the volume to 50 mL with water and heat in water bath for 10 minutes. Any opalescence obtained is not more intense than that presented by 0.2 mg of sulfate ion, in an equal volume of liquid, containing the same quantities of reagents. Not more than 0.01% (100 ppm).

Loss on ignition (5.2.9.2). Determine in 1 g of the sample. Incinerate in muffle furnace at 900 ± 25 °C until constant weight. Not more than 10.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Complexometric titrations for magnesium (5.3.3.4)*. Incinerate the sample at 800 °C for one hour. Weigh, accurately, about 0.32 g of the residue, dissolve in 7 mL of 3 M hydrochloric acid and dilute with 100 mL of water. Titrate 20 mL of the solution obtained using 0.1 M disodium edetate VS. Each mL of 0.1 M disodium edetate VS is equivalent to 4.030 mg of MgO.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation. It must be informed whether light or heavy magnesium oxide.

CATEGORY

Pharmacotechnical adjuvant, antacid, saline hyperosmotic laxative.

ZINC OXIDE*Zinci oxidum*

ZnO; 81.38
zinc oxide; 06730
Zinc oxide
[1314-13-2]

It contains not less than 99.0% and not more than 100.5% of ZnO, in relation to the incinerated substance.

DESCRIPTION

Physical characteristics. White or slightly yellowish fine, amorphous powder.

Solubility. Insoluble in water and ethyl alcohol (at 96%). Soluble in diluted mineral acids.

IDENTIFICATION

A. It turns yellow when subjected to strong heating, which disappears after cooling.

B. Dissolve 0.1 g of the sample in 1.5 mL of hydrochloric acid RS and dilute to 5 mL with water. The solution meets zinc ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 1 g of the sample in 15 mL of hydrochloric acid RS. Effervescence is not observed during dissolution. The preparation obtained is colorless **(5.2.12)** and is not more opalescent than the *Reference suspension II* **(5.2.25)**.

Alkalinity. Shake 1 g of the sample with 10 mL of boiling water. Add two drops of phenolphthalein TS and filter. If the filtrate is red, not more than 0.3 mL of 0.1 M hydrochloric acid is required to promote the indicator changeover.

Cadmium limit. Proceed as described in *Atomic absorption spectrophotometry* **(5.2.13.1)**. Prepare the *Standard* and *Sample solutions* for the test as described below.

Sample solution: dissolve 2 g of the sample in 14 mL of mixture of water and lead and cadmium-free nitric acid (1:1). Boil for one minute, cool and dilute to 100 mL with water.

Standard solutions: prepare standard solutions using cadmium standard solution (0.1% Cd) and diluting with nitric acid at 3.5% (v/v) free of cadmium and lead.

Procedure: Measure the absorbances of the resulting solutions at 228.8 nm, using a cadmium hollow cathode lamp as source of radiation and acetylene flame. Not more than 0.001% (10 ppm).

Lead. Dissolve 2 g of the sample in 20 mL of water and add 5 mL of glacial acetic acid in water bath until it dissolves completely. Add five drops of potassium chromate RS. The solution obtained does not produce any turbidity or precipitate.

Arsenic (5.3.2.5). Determine in 0.6 g of the sample. Proceed as described in *Spectrophotometric Method, Method I*. Not more than 0.0005% (5 ppm).

Iron (5.3.2.4). Dissolve 0.5 g of the sample in 1 mL of hydrochloric acid RS and dilute to 10 mL with water. Proceed as described in *Method I*. Use *Standard iron solution (100 ppm Fe)*. Not more than 0.02% (200 ppm).

Loss on ignition (5.2.9.2). Determine in 1 g of sample. Incinerate in muffle furnace at 850 ± 25 °C until constant weight. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Quantitatively dissolve about 0.8 g of the sample previously calcined at 850 °C for one hour in 2 mL of water and 3 mL of hydrochloric acid, transfer to a 100 mL volumetric flask and complete the volume with water. Pipet 10 mL of this solution, add 80 mL of water and then add a solution of sodium hydroxide (1 in 50) until solid particles appear in suspension. Add approximately 5 mL of ammonium chloride buffer pH 10.7 and two drops of eriochrome black T TS and titrate with 0.05 M disodium edetate VS. Each mL of 0.05 M disodium edetate VS is equivalent to 4.069 mg of ZnO.

PACKAGING AND STORAGE

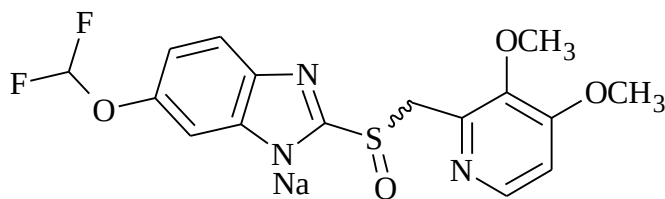
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Skin protectors.

PANTOPRAZOLE SODIUM*Pantoprazoli natricum*C₁₆H₁₄F₂N₃NaO₄S; 405.35

pantoprazole sodium; 06819

6-(Difluoromethoxy)-2-[[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole, sodium salt (1:1)

[138786-67-1]

C₁₆H₁₄F₂N₃NaO₄S.1,5H₂O; 432.37

pantoprazole sodium sesquihydrate; 09514

6-(Difluoromethoxy)-2-[[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole hydrated sodium salt (2:2:3)

[164579-32-2]

It contains not less than 98.0% and not more than 102.0% of C₁₆H₁₄F₂N₃NaO₄S in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder, hygroscopic.

Solubility. Freely soluble in water, soluble in methyl alcohol and ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 139 °C to 160 °C, with decomposition.

Specific optical rotation (5.2.8): -1.0 to +1.0, in relation to the anhydrous substance. Determine in aqueous solution at 5% (w/v).

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the pantoprazole sodium CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 210 nm to 360 nm, of 0.001% (w/v) solution in methyl alcohol, there is a maximum point at 289 nm.

C. Dissolve 20 mg of the sample in 1 mL of water. The solution meets sodium ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. The 10% (w/v) aqueous preparation is clear **(5.2.25)** and slightly yellowish **(5.2.12)**.

pH (5.2.19). 9.0 to 11.5. Determine in aqueous solution at 2% (w/v).

Light absorption. The maximum absorbance of the 2% aqueous solution (w/v), measured at 440 nm, is 0.025 and the minimum transmittance, measured at 650 nm, is 97%. The maximum absorbance of the 10% aqueous solution (w/v), measured at 440 nm, is 0.1 and the minimum transmittance, measured at 650 nm, is 95%.

Heavy metals (5.3.2.3). Use *Method III*. Determine in 1 g of sample desiccated in oven at 60 °C, under reduced pressure, for four hours. Not more than 0.002% (20 ppm).

Water (5.2.20.1). Between 4.5 and 8.0%.

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 60 °C, under reduced pressure, for four hours. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Quantitatively dissolve about 0.35 g of the sample in 50 mL of ethyl alcohol. Titrate with 0.1 M hydrochloric acid VS. Determine the endpoint potentiometrically or use bromophenol blue TS as indicator, with color turning green. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M hydrochloric acid VS is equivalent to 40.535 mg of $C_{16}H_{14}F_2N_3NaO_4S$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 290 nm; column with 150 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of acetonitrile and water (75:25).

Sample solution: dissolve an accurately weighed quantity of the sample in 0.1 M sodium hydroxide to obtain a 0.1 mg/mL solution. Dilute in phosphate-sodium lauryl sulfate buffer pH 6.8 to a concentration of 0.1 mg/mL. Dilute the obtained solution in mixture of acetonitrile and water (50:50) to a concentration of 10 µg/mL.

Sample solution: dissolve an accurately weighed quantity of pantoprazole sodium CRS in 0.1 M sodium hydroxide to obtain a 1 mg/mL solution. Dilute in phosphate-sodium lauryl sulfate buffer pH 6.8 to a concentration of 0.1 mg/mL. Dilute the obtained solution in mixture of acetonitrile and water (50:50) to a concentration of 10 µg/mL.

Inject 20 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{16}H_{14}F_2N_3NaO_4S$ in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and under refrigeration.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antisecretory.

PANTOPRAZOLE SODIUM CAPSULES

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_{16}H_{15}F_2N_3O_4S$. Capsules must be gastro-resistant.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Quantitatively transfer the content of each capsule, quantitatively, to a 50 mL volumetric flask and add 25 mL of 0.1 M sodium hydroxide, place in ultrasonic bath for five minutes and under mechanical agitation for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Dilute in phosphate buffer pH 6.8 to obtain a solution at 80 µg/mL. Dilute in mixture of acetonitrile and water (50:50) to obtain a 8,0 µg/mL solution. Prepare the *Standard Solution* as described in *Assay*, to obtain an 8.0 µg/mL pantoprazole solution. Proceed as described in *Assay*.

DISSOLUTION TEST (5.1.5)

Acid stage:

Dissolution medium: 0.1 M hydrochloric acid, 500 mL.

Apparatus: baskets, 100 rpm.

Time: 60 minutes.

Procedure: after 60 minutes, lift the baskets and remove 25 mL of dissolution medium from each tank. Filter and dilute, if necessary, with mixture of acetonitrile and water (50:50) to a theoretical concentration of 8.0 µg/mL, assuming 10% dissolution of pantoprazole. Prepare *Standard solution* as described below.

Standard solution: dissolve an accurately weighed quantity of pantoprazole sodium CRS to obtain a 80 mg/mL pantoprazole base solution. Dilute with phosphate buffer pH 6.80 to concentration of 0.8 mg/mL. Then dilute with mixture of acetonitrile and water (50:50) to a concentration of 8.0 µg/mL.

Proceed as described in *Assay*. Calculate the quantity of pantoprazole dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Buffer stage:

Dissolution medium: add 425 mL of phosphate buffer pH 11.0 to the 475 mL of 0.1 M hydrochloric acid remaining in the tanks. If necessary, adjust the pH to 6.80 with 20% (v/v) phosphoric acid or 40% (w/v) sodium hydroxide.

Apparatus: baskets, 100 rpm.

Time: 60 minutes.

Procedure: use the same capsules submitted to the *Acid stage*. After 60 minutes, lift the baskets and remove aliquot from the dissolution medium. Filter, if necessary. Dilute with mixture of acetonitrile and water (50:50) to a concentration of 8.8 µg/mL. Prepare *Standard solution* as described below.

Standard solution: 8.0 µg/mL pantoprazole solution.

Proceed as described in *Assay*. Calculate the quantity of pantoprazole dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 80% (Q) of the stated quantity of C₁₆H₁₅F₂N₃O₄S dissolve in 60 minutes in the *Buffer Stage*.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in method **B.** of *Assay* in the *Sodium pantoprazole* monograph. Prepare the *Sample solution* and the *Standard solution* as described below.

Sample solution: Weigh not less than 10 capsules, remove the content and weigh again. Homogenize the content of the capsules. Quantitatively transfer quantity of powder equivalent to 50 mg of pantoprazole base to a 50 mL volumetric flask. Add about 25 mL of 0.1 M sodium hydroxide, submit to ultrasonic bath for five minutes and mechanical agitation for 15 minutes. Complete the volume with the same solvent. Homogenize and filter in adequate filter paper. Dilute the filtrate with phosphate buffer pH 6.80 to concentration of 0.1 mg/mL. Then dilute with mixture of acetonitrile and water (50:50) to a concentration of 10 µg/mL.

Standard solution: dissolve an accurately weighed quantity of pantoprazole sodium CRS in 0.1 M sodium hydroxide to obtain a 1.0 mg/mL pantoprazole base solution. Dilute with phosphate buffer pH 6.80 to concentration of 0.1 mg/mL. Then dilute with mixture of acetonitrile and water (50:50) to a concentration of 10 µg/mL.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₆H₁₅F₂N₃O₄S in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

PANTOPRAZOLE SODIUM GASTRO-RESISTANT GRANULES

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $C_{16}H_{15}F_2N_3O_4S$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in Assay, corresponds to the one from the main peak of the *Standard solution*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: mixture of 475 mL of 0.1 M hydrochloric acid and 425 mL of phosphate buffer pH 11.0, with pH adjusted to 6.8 with phosphoric acid 20% (v/v) or sodium hydroxide 40% (w/v).

Apparatus: baskets, 100 rpm. **Note**: it must be ensured that the baskets have a mesh opening smaller than the size of the granules.

Time: 60 minutes.

Procedure: carry out the test with a mass of granules equivalent to 40 mg of pantoprazole in each basket. After the test, remove aliquot from the dissolution medium, filter and dilute, if necessary, with mixture of acetonitrile and water (50:50) to concentration close to 8.8 µg/mL. Prepare the *Standard Solution* as described in Assay, to obtain an 8.0 µg/mL pantoprazole base solution. Proceed as described in Assay. Calculate the quantity of $C_{16}H_{15}F_2N_3O_4S$ dissolved in the medium, from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{16}H_{15}F_2N_3O_4S$ dissolve in 60 minutes.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 60 °C, under reduced pressure, for four hours. Not more than 6.0%.

GASTRO-RESISTANCE

Use apparatus for *Dissolution Test (5.1.5)*.

Medium: 0.1 M hydrochloric acid, 500mL

Apparatus: baskets, 100 rpm. **Note**: it must be ensured that the baskets have a mesh opening smaller than the size of the granules.

Time: 120 minutes.

Procedure: carry out the test with a mass of granules equivalent to 20 mg of pantoprazole in each basket. After two hours, lift the platform. Remove the baskets, quantitatively transfer the granules from each basket to 25 mL volumetric flasks by dissolution with 13 mL of 0.1 M sodium hydroxide. Submit to ultrasonic bath for five minutes and mechanical agitation for 15 minutes. Complete the volume with 0.1 M sodium hydroxide. Filter on adequate filter paper. Dilute the filtrate with phosphate buffer pH 6.8 to concentration of 80 µg/mL. Dilute the obtained solution in mixture of acetonitrile and water (50:50) to a concentration of 8.0 µg/mL. Prepare the *Standard Solution* as described in Assay, to obtain an 8.0 µg/mL pantoprazole base solution. Proceed as described in Assay. Calculate the quantity of $C_{16}H_{15}F_2N_3O_4S$ dissolved in 0.1 M sodium hydroxide from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: after resisting for two hours in 0.1 M hydrochloric acid, the individual values of the quantities dissolved in 0.1 M sodium hydroxide are not less than 80% and the average value is not less than 85% of the declared quantity of $C_{16}H_{15}F_2N_3O_4S$.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in method **B.** of Assay in the *Sodium pantoprazole* monograph. Prepare the *Sample solution* and the *Standard solution* as described below.

Sample solution: pulverize sufficient quantity of the sample. Quantitatively transfer quantity of powder equivalent to 25 mg of pantoprazole base to a 25 mL volumetric flask. Add about 13 mL of 0.1 M sodium hydroxide, submit to ultrasonic bath for five minutes and mechanical agitation for 15 minutes. Complete the volume with the same solvent. Homogenize and filter in adequate filter paper. Dilute the filtrate with phosphate buffer pH 6.8 to concentration of 0.1 mg/mL. Then dilute with mixture of acetonitrile and water (50:50) to a concentration of 10 µg/mL.

Standard solution: dissolve an accurately weighed quantity of pantoprazole sodium CRS in 0.1 M sodium hydroxide to obtain a 1.0 mg/mL pantoprazole base solution. Dilute with phosphate buffer pH 6.80 to concentration of 0.1 mg/mL. Then dilute with mixture of acetonitrile and water (50:50) to a concentration of 10 µg/mL.

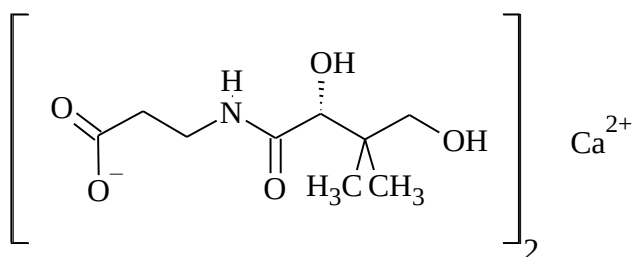
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{16}H_{15}F_2N_3O_4S$ in the granules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CALCIUM PANTOTHENATE*Calcii pantothenas*C₁₈H₃₂CaN₂O₁₀; 476.54

calcium pantothenate; 00317

N-[(2*R*)-2,4-dihydroxy-3,3-dimethyl-1-oxobutyl]-β-alanine calcium salt (1:2)

[137-08-6]

It contains not less than 98.0% and not more than 101.0% of C₁₈H₃₂CaN₂O₁₀, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White powder, slightly hygroscopic.

Solubility. Freely soluble in water, soluble in glycerol, slightly soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +25,0 to +27,5, in relation to the desiccated substance.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the calcium pantothenate CRS spectrum, prepared identically.

B.

The main stain of the chromatogram from *Solution (2)*, obtained in *Limit for 3-aminopropionic acid*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

C. Dissolve 2.5 g of the sample in 50 mL of water. Transfer 1 mL of the solution to a test tube and add 1 mL of sodium hydroxide RS and 0.1 mL of cupric sulfate RS. Blue color will be formed.

D. It meets calcium ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. The 5% (w/v) aqueous preparation is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). 6.8 to 8.0. Determine in solution at 5% (w/v).

Limit for 3-aminopropionic acid. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of water, glacial acetic acid and ethyl alcohol (20:30:50) as mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.2 g of the sample in water and complete the volume to 5 mL with the same solvent.

Solution (2): dilute 1 mL of *Solution (1)* to 10 mL with water.

Solution (3): dissolve 20 mg of calcium pantothenate CRS in water and dilute to 5 mL with the same solvent.

Solution (4): dissolve 10 mg of 3-aminopropionic acid CRS in water and dilute to 50 mL with the same solvent.

Develop chromatogram. Remove the plate, allow to air dry and spray the plate with ninhydrin RS. Heat at 110 °C for 10 minutes. The stain corresponding to 3-aminopropionic acid in the chromatogram obtained with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (4)*. Not more than 0.5%.

Volatile organic impurities. Proceed as described in *Gas chromatography (5.2.17.5)*. Use chromatograph equipped with flame ionization detector, using mixture of nitrogen, synthetic air and hydrogen (1:1:10) as auxiliary gases to the detector flame; capillary column of 30 m in length and 0.53 mm in internal diameter, packed with stationary phase bonded to 5% of phenylpolysiloxane and 95% to methylpolysiloxane, with film thickness of 5 µm; column temperature from 35 °C to 260 °C (35 °C held for five minutes, increased to 175 °C at 8 °C per minute, increased to 260 °C at 35 °C and held at this temperature for not less than 16 minutes), injector temperature at 70 °C and detector temperature at 260 °C; use helium as carrier gas; carrier gas flow rate of 1.0 mL/minute.

Sample solution: dissolve in 50 mL of organic compound-free water, about 1 g of sample, accurately weighed.

Standard solution: prepare a solution, in organic compound-free water, containing in each mL, 10 µg of methylene chloride, 1 µg of chloroform, 2 µg of benzene, 2 µg of dioxane and 2 µg of trichlorethylene.

Procedure: inject, separately, 1 µL of the *Sample solution* and the *Standard solution* in the gas chromatograph. Record the chromatograms and measure the area under the peaks. Identify, based on the retention time, any peaks present in the *Sample Solution* chromatogram. The presence and identification of the peaks in the chromatogram must be established by comparing the chromatograms of the *Sample Solution* and *Standard Solution*. Limits: benzene 2 ppm, chloroform 50 ppm, dioxane 100 ppm, methylene chloride 500 ppm and trichlorethylene 80 ppm.

Chlorides (5.3.2.1). Dissolve 1.8 g of the sample in 40 mL of water and proceed as described in *Limit test for chlorides*. Not more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Dissolve 1.0 g of sample in 10 mL of water and use 2 mL of *Standard Lead Solution (10 ppm Pb)*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 2 g of sample. Dry in oven at 105 °C for three hours. Not more than 3.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 180 g of the sample in 50 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS. Determine the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 23.827 mg of $C_{18}H_{32}CaN_2O_{10}$.

PACKAGING AND STORAGE

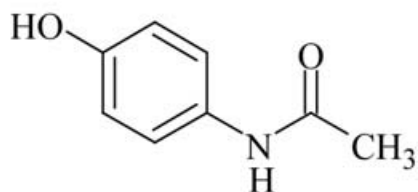
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Food supplement.

PARACETAMOL*Paracetamolum* $C_8H_9NO_2$; 151.17

paracetamol; 06827

N-(4-Hydroxyphenyl)acetamide

[103-90-2]

It contains not less than 98.0% and not more than 101.0% of $C_8H_9NO_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Moderately soluble in water, soluble in boiling water, freely soluble in ethyl alcohol. Soluble in *M* sodium hydroxide.

Physicochemical constants.

Melting range (5.2.2): 168 °C to 172 °C.

IDENTIFICATION

The identification test B. can be omitted if tests A. and C. are carried out. The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the paracetamol CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.0005% (w/v) solution in mixture of 0.1 *M* hydrochloric acid and methyl alcohol (1:100), there are maximum and minimum points only at the same wavelengths of similar paracetamol CRS solution.

C. To 10 mL of a 1% (w/v) sample solution, add one drop of ferric chloride RS. A blue-violet color develops.

PURITY TESTS

pH (5.2.19). 5.3 to 6.5. Determine in the saturated solution.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 245 nm; 250mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm), kept at 35 °C; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of 1.79% (w/v) dibasic sodium phosphate solution, 0.78% (w/v) monobasic sodium phosphate solution and methyl alcohol containing 0.46% (w/v) of hydroxide tetrabutylammonium at 40% (w/v) (375:375:250).

Solution (1): dissolve 0.2 g of the sample in 2.5 mL of methyl alcohol containing 4.6 g/L of a 40% (w/v) tetrabutylammonium hydroxide solution and dilute to 10 mL with a mixture of dibasic sodium phosphate 1.79% (w/v) and monobasic sodium phosphate 0.78% (w/v) (1:1).

Solution (2): dilute 1 mL of *Solution (1)* to 50 mL with *Mobile phase*. Dilute 5 mL of this solution to 100 mL with the same solvent.

Solution (3): dilute 1 mL of *Solution (2)* to 10 mL using *Mobile phase*.

Solution (4): dissolve 5 mg of 4-aminophenol, 5 mg of paracetamol CRS and 5 mg of chloroacetanilide in methyl alcohol, and dilute to 20 ml with the same solvent. Dilute 1 mL to 250 mL with *Mobile phase*.

Solution (5): dissolve 20 mg of 4-nitrophenol in methyl alcohol and dilute to 50 mL with the same solvent. Dilute 1 mL to 20 mL with *Mobile phase*.

The relative retention times for paracetamol (retention time = about four minutes) are about 0.8 for 4-aminophenol, 3.0 for 4-nitrophenol and 7.0 for chloroacetanilide. The resolution between paracetamol and 4-aminophenol is not less than 4.0. The signal-noise ratio is not less than 50 for chloroacetanilide.

Procedure: inject, separately, 20 µL of each freshly prepared solution and record the chromatograms for not less than six times the paracetamol peak retention time and measure the areas under the peaks. The chloroacetanilide area obtained with *Solution (1)* is not greater than 0.2 times the area under the peak obtained with *Solution (4)* (0.001%). The area under the 4-aminophenol peak, obtained with *Solution (1)*, is not greater than the area under the peak obtained with *Solution (4)* (0.005%). The area under the 4-nitrophenol peak, obtained with *Solution (1)*, is not greater than the area under the peak obtained with *Solution (5)* (0.05%). The sum of the areas under all the peaks obtained with *Solution (1)*, except that under the paracetamol peak, is not greater than the area under the main peak obtained with *Solution (2)* (0.1%). To calculate the total impurities disregard peaks with areas below the one under the main peak in the chromatogram obtained with *Solution (3)* (0.01%).

Readily carbonizable substances. Dissolve 0.5 g of sample in 5 mL of sulfuric acid. The solution color is not more intense than that of the *SC A Color Standard Solution (5.2.12)*.

Sulfides. Weigh approximately 2.5 g of the sample in a 50 mL beaker. Add 5 mL of ethyl alcohol and 1 mL of *M* hydrochloric acid. Moisten a filter paper impregnated with lead acetate with water and place on a watch glass. Cover the beaker with the watch glass in such a way that one end of the paper is in the opening of the flask. Heat on an electric plate until boiling. No stains or colors develops on the lead acetate paper.

Chlorides (5.3.2.1). Shake 1 g of the sample in 25 mL of water, filter and add 1 mL of 2 M nitric acid. Not more than 0.014% (140 ppm).

Sulfates (5.3.2.2). Shake 1 g of the sample with 25 mL of water, quantitatively filter into a Nessler tube. Not more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Dissolve 1 g of the sample in a mixture of acetone and water (85:15) and dilute to 20 mL with the same solvent mixture. Transfer the solution obtained to a Nessler tube and proceed as described in *Method II*. Not more than 0.002% (20 ppm).

Water (5.2.20.1). Not more than 0.5%.

Residue by incineration (5.2.10). Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh approximately 0.15 g of sample, dissolve in 50 mL of 0.1 M sodium hydroxide, add 100 mL of water, shake mechanically for 15 minutes and add water sufficient for 200 mL. Homogenize and filter. Dilute 10 mL of the filtrate to 100 mL with water. Transfer 10 mL of the resulting solution to a 100 mL volumetric flask, add 10 mL of 0.1 M sodium hydroxide and complete the volume with water. Prepare the standard solution in the same concentration, using 0.01 M sodium hydroxide as solvent. Measure the absorbances of the resulting solutions at 257 nm, using 0.01 M sodium hydroxide for zero adjustment. Calculate the content of $C_8H_9NO_2$ in the sample from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 715$, at 257 nm.

PACKAGING AND STORAGE

In tightly closed and opaque containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Analgesic and antipyretic.

PARACETAMOL TABLETS

It contains not less than 95.0% and not more than 105.0% the stated quantity of $C_8H_9NO_2$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Extract the quantity of powder equivalent to 0.5 g of paracetamol with 20 mL of acetone. Filter, evaporate the filtrate and dry at 105 °C. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the paracetamol CRS spectrum, prepared identically.

B. Heat to boiling 0.1 g of the residue obtained in the test **A.** from *Identification* with 1 mL of hydrochloric acid for three minutes, add 10 mL of water and cool. No precipitate is formed. Add 0.05 mL of 0.0167 M potassium dichromate. Violet color develops, which does not turn red.

C. The melting temperature **(5.2.2)** of the residue obtained in the test **A.** from *Identification* is approximately 169 °C.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 5.8, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in phosphate buffer pH 5.8 to adequate concentration. Measure the absorbances of the solutions at 243 nm **(5.2.14)**, using the same solvent for zero adjustment. Calculate the quantity of $C_8H_9NO_2$ dissolved in the medium, comparing the readings obtained with that of the paracetamol CRS solution at 0.00075% (w/v), prepared in the same solvent.

Tolerance: not less than 80% (Q) of the stated quantity of $C_8H_9NO_2$ dissolve in 30 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography*, using silica gel GF₂₅₄ as support, and mixture of chloroform, acetone and toluene (65:25:10) as mobile phase. Apply to the

plate, separately, 200 µL of *Solution (1)* and 40 µL of each of *Solutions (2), (3) and (4)*, described below.

Solution (1): weigh and pulverize the tablets. Transfer quantity of powder equivalent to 1 g of paracetamol to a 15 mL centrifuge tube with a ground glass cap. Add 5 mL of ethyl ether and shake mechanically for 30 minutes. Centrifuge at 1000 revolutions per minute for 15 minutes or until supernatant is clear. Use the supernatant.

Solution (2): dilute 1 mL of *Solution (1)* to 10 mL with ethyl alcohol.

Solution (3): prepare 0.005% (w/v) solution of *p*-chloroacetanilide in ethyl alcohol.

Solution (4): dissolve 0.25 g of *p*-chloroacetanilide and 0.1 g of paracetamol in ethyl alcohol and complete the volume to 100 mL.

Develop the chromatogram in an unsaturated chamber, until the mobile phase reaches 14 cm from the origin. Remove the plate, dry with a stream of hot air and examine under ultraviolet light (254 nm). Any stain corresponding to *p*-chloroacetanilide, obtained in the chromatogram with *Solution (1)* is not more intense than that obtained in the chromatogram with *Solution (3)* (0.005%). Any secondary stain obtained in the chromatogram with *Solution (2)*, with *R_f* value lower than that of *p*-chloroacetanilide, is not more intense than that obtained in the chromatogram with *Solution (3)* (0.25%). The test is only valid if the chromatogram obtained with *Solution (4)* shows two clearly separated main stains, with the stain corresponding to *p*-chloroacetanilide having the highest *R_f* value.

4-Aminophenol limit. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 272 nm; column with 200 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (10 µm); *Mobile phase* flow rate of 2 mL/minute.

Mobile phase: prepare 0.01 M sodium butanesulfonate solution using a mixture of water, methyl alcohol and formic acid (85:15:0.4) as solvent.

Solution (1): weigh and pulverize the tablets. Transfer quantity of powder equivalent to 1 g of paracetamol to a 100 mL volumetric flask, add 15 mL of methyl alcohol and shake. Complete the volume with water, homogenize and filter.

Solution (2): prepare 0.001% (w/v) solution of 4-aminophenol in 15% (v/v) methyl alcohol.

Procedure: inject, separately, 20 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The area under the peak corresponding to 4-aminophenol obtained in the chromatogram with *Solution (1)* is not greater than the area under the main peak obtained in the chromatogram with *Solution (2)* (0.1%).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.15 g of paracetamol to a 200 mL volumetric flask. Add 50 mL of 0.1 M sodium hydroxide, 100 mL of water, shake mechanically for 15 minutes and complete the volume with water. Homogenize, filter and dilute 10 mL of the filtrate to 100 mL with water. Transfer 10 mL of the resulting solution to a 100 mL volumetric flask, add 10 mL of 0.1 M sodium hydroxide and complete the volume with water. Prepare standard solution in the same concentration in 0.01 M sodium hydroxide. Measure the absorbances of the resulting solutions at 257 nm (**5.2.14**), using 0.01 M sodium hydroxide for zero adjustment. Calculate the quantity of $C_8H_9NO_2$ in the tablets from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{cm}) = 715$, at 257 nm, in 0.01 M sodium hydroxide.

B. Proceed as described in *High performance liquid chromatography* (**5.2.17.4**). Use chromatograph equipped with ultraviolet detector at 243 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group, kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of water and methyl alcohol (75:25).

Sample Solution: weigh and pulverize 20 tablets. Dissolve accurately weighed quantity of sample in *Mobile Phase*. Shake mechanically for 10 minutes and place in ultrasonic bath for five minutes. Dilute with the same solvent to a concentration of 10 µg/mL of paracetamol.

Standard solution: dissolve an accurately weighed quantity of paracetamol CRS in *Mobile phase* to obtain a 10 µg/mL solution.

Inject 10 µL replicates of the *Standard solution*. The column efficiency is not less than 1000 theoretical plates/meter. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_8H_9NO_2$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

PARACETAMOL ORAL SOLUTION

It contains not less than 90% and not more than 110% of the stated quantity of $C_8H_9NO_2$.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out. The identification test C. can be omitted if tests A. and B. are carried out.

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the sample solution obtained in method **A.** of Assay, there is maximum absorption at 249 nm, identical to that observed in the spectrum of the standard solution.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel GF₂₅₄ as support, and mixture of methylene chloride and methyl alcohol (4:1) as mobile phase. Apply to the plate, separately, 20 µL of each one of the freshly prepared solutions described below.

Solution (1): dilute the oral solution in methyl alcohol until concentration of 1 mg/mL.

Solution (2): dissolve 10 mg of paracetamol CRS in methyl alcohol and complete the volume to 10 mL with the same solvent.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light. The main stain obtained with *Solution (1)* corresponds in position to the one obtained with *Solution (2)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

Drip test (5.1.8). Complies with the test.

pH (5.2.19). 3.8 to 6.5.

PURITY TESTS

4-Aminophenol limit. Proceed as described in *High performance liquid chromatography* (**5.2.17.4**). Use chromatograph equipped with ultraviolet detector at 272 nm; 200mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (10 µm), kept at 25 °C; *Mobile phase* flow rate of 2.0 mL/minute.

Mobile phase: prepare 0.01 M sodium butanesulfonate solution using mixture of water, methyl alcohol and formic acid (85:15:0.4) as solvent.

Solution (1): 4.8 mg/mL sample solution in *Mobile phase*. Filter if necessary.

Solution (2): prepare a 24 µg/mL 4-aminophenol CRS solution in *Mobile phase*.

Procedure: inject, separately, 20 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The area under the peak corresponding to 4-aminophenol obtained in the chromatogram with *Solution (1)* is not greater than the area under the main peak obtained in the chromatogram with *Solution (2)* (0.5%). In the chromatogram obtained with *Solution (1)*, peaks with a long retention time may occur due to the presence of preservatives in the formulation.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Transfer volume of the oral solution to a volumetric flask and dilute with methyl alcohol to obtain a 1 mg/mL solution. Transfer 1 mL of the resulting solution to a 100 mL volumetric flask, add 1 mL of 0.1 M hydrochloric acid, complete the volume with methyl alcohol and homogenize. Prepare standard solution in the same concentration, using the same solvents. Measure the absorbances of the resulting solutions at 249 nm, using 0.1 M hydrochloric acid methanol solution for zero adjustment. Calculate the quantity of $C_8H_9NO_2$ in the oral solution from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 880$, at 249 nm

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 243 nm; 250mm long, 4.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at 25 °C; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water and methyl alcohol (75:25).

Sample solution: transfer volume accurately weighed of the oral solution equivalent to 200 mg of paracetamol to a 200 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 1 mL of this solution to a 100 mL volumetric flask, complete the volume with *Mobile phase*, homogenize and filter.

Standard solution: dissolve an accurately weighed quantity of paracetamol CRS in *Mobile phase* to obtain a final concentration of 0.01 mg/mL.

Inject 10 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

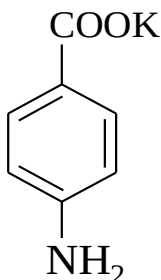
Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_8H_9NO_2$ in the oral solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

Store protected from light.

LABELING

Comply with current legislation.

POTASSIUM PARAMINOBENZOATE***Kalii 4-aminobenzoas***

C₇H₆KNO₂; 175.23

potassium paraminobenzoate; 09818

4-aminobenzoic acid potassium salt (1:1)

[138-84-1]

It contains not less than 98.5% and not more than 101.0% of C₇H₆KNO₂ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White, crystalline powder.

Solubility. Freely soluble in water, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in 0.001 M sodium hydroxide, there are maximum and minimum points only at the same wavelengths as similar potassium paraminobenzoate CRS solution.

B. Dissolve about 400 mg of the sample in 10 mL of water. Add 1 mL of 3 M hydrochloric acid, filter and wash the precipitate with two 5 mL aliquots of cold water. Wash with ethyl alcohol and allow to recrystallize. Dry the precipitate obtained at 110 °C for one hour. The melting point **(5.2.2)** of the paraminobenzoic acid thus obtained is between 186.0°C and 189.5°C.

C. The 1% (w/v) sample solution meets potassium ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 8.0 to 9.0. Determine in solution at 5% (w/v).

Diazotized volatile substances limit. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Prepare the solutions as described below.

Solution (1): transfer 5 g of potassium aminobenzoate into a 50 mL volumetric flask. Add quantity of M sodium hydroxide sufficient to dissolve the sample and make the medium alkaline to

phenolphthalein. Complete the volume to 50 mL. Distill in distillation system with steam carrier and collect about 95 mL of distillate in a 100 mL flask. Complete the volume with water and mix.

Solution (2): dissolve 10 mg of p-toluidine in 5 mL of methyl alcohol in a 100 mL volumetric flask. Complete the volume with water and homogenize. Transfer 1 mL of this solution to a 100 mL volumetric flask, complete the volume with the same solvent and homogenize.

Procedure: transfer 20 mL of *Solution (1)* and 20 mL of *Solution (2)*, separately, to 100 mL volumetric flasks. Carry out blank test, transferring 20 mL of water to a third 100 mL volumetric flask. Add 5 mL of *M* hydrochloric acid to each volumetric flask and cool in ice bath. Add, dropwise, under agitation, 2 mL of 0.1 *M* sodium nitrite VS. Allow to stand for five minutes for the diazotization reaction to complete. Quickly add 10 mL of freshly prepared cooled guaiacol solution by dissolving 0.2 g of guaiacol in 100 mL of *M* sodium hydroxide. Homogenize and allow to stand for 30 minutes. Determine the absorbance (**5.2.14**) of the solutions at 405 nm, using the blank solution for zero adjustment. The absorbance of *Solution (1)* must not be greater than that presented by *Solution (2)* (0.002%).

Chlorides (5.3.2.1). Dissolve 1.8 g of the sample in 40 mL of water and proceed as described in *Limit test for chlorides*. Not more than 0.02% (200 ppm).

Sulfates (5.3.2.2). Dissolve 6 g of the sample in 40 mL of water and proceed as described in *Limit test for sulfates*. Not more than 0.02% (200 ppm).

Heavy metals (5.3.2.3). Use *Method IV*. Use 1 g of the sample in a silica crucible. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Accurately weigh approximately 1 g to 2 g of sample and dry in oven at 105 °C, under reduced pressure, for two hours. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 500 g of sample and transfer to a beaker. Add 25 mL of water and 25 mL of 3 *M* hydrochloric acid. Mix and cool in ice bath. Titrate with 0.1 *M* sodium nitrite VS. Determine the endpoint potentiometrically using a platinum-calomel electrode. Each mL of 0.1 *M* sodium nitrite VS is equivalent to 17.523 mg of C₇H₆KNO₂.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Analgesic.

POTASSIUM PERCHLORATE*Kalii perchloras*KClO₄; 138.55

potassium perchlorate; 09834

Potassium salt of perchloric acid (1:1)

[7778-74-7]

It contains not less than 99.0% and not more than 100.5% of KClO₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or colorless crystals.

Solubility. Moderately soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. Prepare a 10% (w/v) solution of the sample in water and add some drops of methylthioninium chloride RS. A violet precipitate is produced.

B. Dissolve 0.1 g of the sample in 5 mL of water. Add 5 mL of indigo carmine RS and heat until ebullition. The solution color does not fade.

C. It meets potassium ion reactions (5.3.1.1).

D. It meets chloride ion reactions (5.3.1.1).

E. It meets chlorate ion reactions (5.3.1.1).

PURITY TESTS

Preparation aspect. The 1% (w/v) aqueous preparation is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 5.0 to 6.5. Determine in aqueous solution at 1.4% (w/v).

Acidity or alkalinity. Accurately weigh about 5 g of the sample and add 90 mL of water. Heat to boiling. Allow to cool and filter. Dilute the filtrate to 100 mL with carbon dioxide-free water. To 5 mL of this solution, add 5 mL of water and 0.1 mL of phenolphthalein TS. Not more than 0.25 mL of 0.01 M sodium hydroxide is required to change the indicator color. To another 5 mL of this solution, add 5 mL of water and 0.1 mL of bromocresol green TS solution. Not more than 0.25 mL of 0.01 M hydrochloric acid is required to change the indicator color.

Volatile organic impurities. Proceed as described in *Gas chromatography* (5.2.17.5). Use chromatograph equipped with flame ionization detector, using a mixture of nitrogen, synthetic air and hydrogen (1:1:10) as auxiliary gases to the detector flame; capillary column of 30 m in length and 0.53 mm in internal diameter, packed with stationary phase bonded to 5% of phenylpolysiloxane and 95% to methylpolysiloxane, with film thickness of 5 µm; column temperature from 35 °C to 260 °C (35 °C held for five minutes, increased to 175 °C to 8 °C per minute,

increased to 260 °C to 35 °C per minute and held at this temperature for not less than 16 minutes), injector temperature of 70 °C and detector temperature of 260 °C; use helium as carrier gas; carrier gas flow rate of 1 mL/minute.

Sample solution: dissolve in 50 mL of organic compound-free water, about 1 g of sample, accurately weighed.

Standard solution: prepare a solution, in organic compound-free water, containing in each mL, 10 µg of methylene chloride, 1 µg of chloroform, 2 µg of benzene, 2 µg of dioxane and 2 µg of trichlorethylene.

Procedure: inject, separately, 1 µL of the *Sample solution* and the *Standard solution* in the gas chromatograph. Record the chromatograms and measure the area under the peaks. Identify, based on the retention time, any peaks present in the *Sample Solution* chromatogram. The presence and identification of the peaks in the chromatogram must be established by comparing the chromatograms from the *Sample Solution* and the *Standard Solution*. Limits: benzene 2 ppm, chloroform 50 ppm, dioxane 100 ppm, methylene chloride 500 ppm and trichlorethylene 80 ppm.

Insoluble substances limit. Dissolve 20 g of the sample in 150 mL of warm water. Filter through a pre-weighed medium porosity filter. Wash with three 50 mL portions of warm water. Dry the residue at 105 °C for three hours. The residue weight does not exceed 0.005% (50 ppm).

Calcium limit. The opalescence developed in the *Sample Preparation* after 15 minutes is not more intense than in the *Standard Preparation*, prepared similarly. Not more than 0.01% (100 ppm).

Sample preparation: weigh, accurately, about 5 g of the sample, transfer to a beaker and add 90 mL of water. Heat to boiling. Allow to cool, filter to a 100 mL volumetric flask and complete the volume with carbon dioxide-free water. In a Nessler tube, mix 0.2 mL of ethanolic calcium standard solution (100 ppm Ca) and 1 mL of ammonium oxalate RS. After one minute, add 1 mL of 2 M acetic acid and 15 mL of the solution containing the previously prepared sample.

Sample preparation: transfer to a Nessler tube (50 mL capacity and 22 mm internal diameter) 0.2 mL of ethanolic calcium standard solution (100 ppm Ca) and mix with 1 mL of ammonium oxalate RS. Wait one minute, add a mixture of 7.5 mL of the calcium standard solution (10 ppm Ca), 1 mL of diluted acetic acid and 7.5 mL of distilled water.

Sodium. Prepare a 10% (w/v) solution of the sample. Immerse a platinum loop in this solution and place on the flame. Intense yellow color is not produced on the flame.

Chlorides (5.3.2.1). Weigh 10 g of sample and proceed as described in *Limit test for chlorides*. Not more than 0.003% (30 ppm).

Chlorides and chlorates (5.3.2.1). Accurately weigh about 3.5 g of the sample, add 30 mL of water, 1 mL of nitric acid and 0.1 g of sodium nitrite. Allow to cool and proceed as described in *Limit test for chlorides*. Not more than 0.01% (100 ppm) (calculated as chlorides).

Sulfates (5.3.2.2). Accurately weigh about 2 g of the sample and dissolve in 40 mL of water. Proceed according to the *Limit Test for sulfates*. Use 0.5 mL of the standard solution. Not more than 0.012% (120 ppm).

Heavy metals (5.3.2.3). Dissolve 2 g of the sample in 25 mL of water, adjust the pH to the range between 3.0 and 4.0 with *M* acetic acid or 6 *M* ammonium hydroxide. Dilute with water and homogenize. Proceed according to the *Limit Test for Heavy Metals, Method I*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample, on silica gel. Dry for 12 hours. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ion Exchange Chromatography (5.2.17.4)*. Use chromatograph equipped with conductivity detector, chromatographic column with 7.5 cm in length and 4.6 mm in internal diameter, packed with a porous polyacrylate or polymethacrylate gel with quaternary ammonia groups of about 10 µm; *Mobile Phase* flow rate of 1.2 mL/minute.

Mobile phase: dissolve 1.66 g of phthalic acid in 1000 mL of water. Adjust the pH to 4.5 with approximately 0.45 g of lithium hydroxide. Filter.

Sample solution: dissolve an accurately weighed quantity of the sample in water to obtain a 0.5 mg/mL solution. Transfer 20 mL of this solution to a 100 mL volumetric flask and complete the volume with water, obtaining a 0.1 mg/mL solution.

Standard solution: dissolve an accurately weighed quantity of potassium perchlorate CRS in water to obtain a 0.5 mg/mL solution. Transfer 20 mL of this solution to a 100 mL volumetric flask and complete the volume with water, obtaining a 0.1 mg/mL solution.

Procedure: inject, separately, 50 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of KClO₄ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antithyroid drug.

POTASSIUM PERMANGANATE*Kalii permanganas*KMnO₄; 158.03

Potassium permanganate; 07000

Permanganic acid potassium salt (1:1)

[7722-64-7]

It contains not less than 99.0% and not more than 100.5% of KMnO₄, in relation to the desiccated substance.

Note: *Caution! Dangerous explosions can occur if placed in contact with organic or freely oxidizable substances, either in solution or in dry state.*

DESCRIPTION

Physical characteristics. Dark violet crystals, with metallic shine, unalterable in air.

Solubility. Freely soluble in boiling water and soluble in cold water.

IDENTIFICATION

A. Dissolve 50 mg of the sample in 5 mL of water. Add 1 mL of ethyl alcohol and 0.3 mL of sodium hydroxide RS. Green color develops. Heat to boiling. Dark brown precipitate is formed.

B. It meets permanganate ion reactions (5.3.1.1).

C. Filter the mixture obtained in test **A.** from *Identification*. The filtrate meets potassium ion reactions (5.3.1.1).

PURITY TESTS

Preparation aspect. Dissolve 0,75 g of the sample in 25 mL of water and add 3 mL of ethyl alcohol. Heat to boiling for two minutes. Cool and complete the volume to 30 mL with water. Filter. The preparation obtained is colorless (5.2.12).

Water-insoluble substances limit. Dissolve, by heating, 0.5 g of the sample in 50 mL of water. Filter with a medium porosity glass filter, previously tared and washed with water, until obtaining a colorless filtrate. Collect the residue and dry in oven at (102.5 ± 2.5) °C. The residue mass is not more than 5 mg (1.0%).

Chlorides (5.3.2.1). To 10 mL of the preparation obtained in *Preparation aspect*, complete the volume to 15 mL with water. Proceed as described in *Limit test for chlorides*. Not more than 0.02% (200 ppm).

Sulfates (5.3.2.2). To 12 mL of the preparation obtained in *Preparation aspect*, complete the volume to 15 mL with water. Proceed as described in *Limit test for sulfates*. Not more than 0.05% (500 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry under reduced pressure over silica gel for 18 hours. Not more than 0.5%.

ASSAY

Accurately weigh about 0.125 g of the sample and dissolve in 25 mL of water. Add a previously prepared solution of 2 mL of sulfuric acid in 5 mL of water, and 50 mL of 0.05 M oxalic acid VS. Heat the solution to about 80°C. Titrate excess oxalic acid with 0.02 M potassium permanganate VS until a pale pink color is produced, persistent for 15 seconds. Each mL of 0.05 M oxalic acid VS is equivalent to 3.161 mg of KMnO_4 .

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation

THERAPEUTIC CLASS

Topical antiseptic.

WHITE PETROLATUM

white petrolatum; 09104
White paraffin oil; white vaseline
[308069-07-0]

Purified mixture of semi-solid hydrocarbons obtained from petroleum. May contain adequate stabilizer.

DESCRIPTION

Physical characteristics. Unctuous, semi-solid, white or slightly yellowish mass, practically odorless and tasteless.

Solubility. Practically insoluble in water, soluble in chloroform, in ethyl ether, in petroleum ether, in carbon disulfide and in oils, practically insoluble in glycerol and in ethyl alcohol.

Physicochemical constants.

Relative density (5.2.5): 0.815 to 0.880. Determine at 60 °C.

Melting range (5.2.2): 38 °C to 60 °C.

PURITY TESTS

Color of liquids (5.2.12). Melt approximately 10 g of the sample in water bath and transfer 5 mL of the liquid to a clear glass test tube approximately with 16 mm in diameter and 150 mm in length. The hot, melted liquid should not be darker than a solution prepared by mixing 1.6 mL of *Ferric chloride base solution* and 3.4 mL of water in a similar tube. Carry out the comparison against a white background, with the tubes being held directly against the background at such an angle that there is no fluorescence.

Acidity or alkalinity. Weigh 35 g of the sample in a beaker and add 100 mL of boiling water. Cover, place on a heating plate and keep the water boiling temperature for five minutes. Then allow to stand until the phases separate. Remove the aqueous layer and transfer to an erlenmeyer flask. Wash the sample with two additional 50 mL portions of boiling water. Combine the three aqueous layers (the first one plus the washing waters), add 0.1 mL of phenolphthalein TS and heat to boiling. The solution should not turn pink. If the solution remains colorless, add 0.1 mL of methyl orange TS. The solution should not turn red or pink.

Consistency.

Apparatus. Determine consistency using a penetrometer equipped with a 150 g, cone-shaped polished metallic piston and a detachable steel tip. The tip of the cone must have an angle of 30° and the truncated end a diameter of (0.381 ± 0.025) mm. The diameter of the tip base must be (8.38 ± 0.05) mm and the tip length must be (14.94 ± 0.05) mm. The remaining portion of the cone should have an angle of 90°, height of about 28 mm and maximum diameter at base of about 65 mm. The test containers must be flat-bottomed metal cylinders with diameter of (100 ± 6) mm and height of not less than 65 mm. They must be produced with 1.6 mm metal and have well-sealed, impermeable lids.

Procedure: heat a sufficient quantity of the sample in oven to $(82 \pm 2.5)^\circ\text{C}$ and transfer to the cylinders previously heated to the same temperature, filling up to 6 mm from the edge. Cool to $(25 \pm 2.5)^\circ\text{C}$ for a period of not less than 16 hours, protecting from room exposure. Two hours before testing, place the cylinders in water bath at $(25 \pm 0.5)^\circ\text{C}$. If room temperature is below 23.5°C or above 26.5°C , adjust the cone temperature to $(25 \pm 0.5)^\circ\text{C}$ by placing in water bath. Without disturbing the sample surface, place the cylinder on the penetrometer table and lower the cone until the tip touches the sample surface at a point 25 mm to 38 mm below the edge of the cylinder. Reset the instrument to zero and quickly release the piston, allowing it to be free for five seconds. Fix the piston and carry out the reading. Carry out three or more readings, each spaced apart so that the penetration areas do not overlap. When penetration exceeds 20 mm, use a separate sample container for each test. Read penetration up to tenths of a millimeter. Calculate the average of three or more readings and run more tests, up to a total of 10, if individual results differ from the average by more than 3%. The average of all tests must be not less than 10 mm and not more than 30 mm, indicating a consistency value between 100 and 300.

Organic acids. Accurately weigh 20 g of the sample and add 100 mL of mixture with ethyl alcohol neutralized in advance with 0.1 M sodium hydroxide and water (1:2). Shake the solution and heat to boiling. Add 1 mL of phenolphthalein TS and titrate quickly with 0.1 M sodium hydroxide VS, under vigorous agitation, until a pink color is observed in the hydroalcoholic layer. Not more than 0.4 mL of 0.1 M sodium hydroxide VS is required to promote the indicator changeover.

Fixed oils, fats and resin. Heat 10 g of the sample with 50 mL of 5 M sodium hydroxide at 100°C for 30 minutes. Separate the aqueous layer and acidify with 2.5 M sulfuric acid. No separation of any oily or solid material should occur.

Residue by incineration (5.2.10). Determine in 2 g of the sample. No release of irritating odor should occur during heating. Not more than 0.05%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Excipient.

LIQUID PETROLATUM

Paraffinum liquidum

liquid petrolatum; 09388
Paraffin oils
[8012-95-1]

Mixture of liquid hydrocarbons obtained from petroleum.

DESCRIPTION

Physical characteristics. Oily, clear, colorless liquid and non-fluorescent in daylight.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol and miscible with hydrocarbons.

Physicochemical constants.

Relative density (5.2.5): 0.827 to 0.890.

Viscosity (5.2.7): 110 mPa.s to 230 mPa.s.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the liquid petrolatum CRS spectrum, prepared identically.

B. In a test tube, carefully boil 1 mL of the sample with 1 mL of 0.1 M sodium hydroxide, with continuous agitation for about 30 seconds. Allow to cool to room temperature, forming two phases. Add 0.1 mL of phenolphthalein TS to the aqueous phase. Pink color is produced.

PURITY TESTS

Acidity or alkalinity. Vigorously shake 10 mL of sample with 20 mL of boiling water for one minute. Separate the aqueous phase and filter. To 10 mL of the filtrate, add 0.1 mL of phenolphthalein TS. The solution is colorless. Not more than 0.1 mL of 0.1 M sodium hydroxide is required to turn the indicator color to pink.

Polycyclic aromatic hydrocarbons. Use reagents for spectrophotometry. Place 25 ml into a 125 mL separating funnel with a ground stopper. Add 25 mL of hexane, previously shaken twice with 5 mL of dimethylsulfoxide. Mix and add 5 mL of dimethylsulfoxide. Shake vigorously for one minute and allow to stand to form two phases. Transfer the bottom layer to a second separating funnel and add an additional 2 mL of hexane and shake vigorously. Allow to stand to form two phases. Separate the bottom layer and measure the absorbance (5.2.14) between 260 nm to 420 nm. Prepare blank in parallel, using the bottom layer, obtained by vigorously shaking 5 mL of dimethylsulfoxide with 25.0 mL of hexane in a separating funnel. Prepare a 7 mg/L naphthalene standard solution in isooctane and measure the absorbance at 275 nm using isooctane as blank. At no wavelength between 260 nm and 420 nm does the absorbance of the sample solution exceed one third of the absorbance of the standard solution at 275 nm.

Carbonizable substances. Use a ground-stoppered tube approximately 125 mm in length and 18 mm in internal diameter, graduated into 5 mL and 10 mL; wash with chromic acid cleaning solution, rinse with water and dry. Introduce 5 mL of the sample and 5 mL of nitrogen-free sulfuric acid. Insert the stopper and shake as vigorously as possible in the tube longitudinal direction for five seconds. After removing the stopper, immediately place the tube in water bath, avoiding contact of the tube with the bottom or side of the bath, and heat. After two minutes, four minutes, six minutes and eight minutes, remove the tube from the bath and shake as vigorously as possible in the tube longitudinal direction for five seconds. At the end of 10 minutes of heating, remove the tube from the water bath and allow to stand for 10 minutes. Centrifuge at 2000 g for five minutes. Transfer 4 mL of the top layer to a clean test tube. The color is not more intense **(5.2.12)** than 4 mL of a mixture of 0.6 mL of a brown standard solution and 9.4 mL of a 1% (w/v) hydrochloric acid solution. The bottom layer is not more intensely colored than a mixture of 0.5 mL of *Cupric sulfate base solution*, 1.5 mL of *Cobalt chloride base solution*, 3 mL of *Ferric chloride base solution* and 2 mL of 1% (w/v) hydrochloric acid.

Solid paraffins. Dry sufficient quantity of sample by heating at 100 °C for two hours and cool in a desiccator with sulfuric acid. Store in a glass tube with an internal diameter of 25 mm, close the tube and place in ice-water bath. After four hours, the liquid is translucent enough to easily see a black line, 0.5 mm wide, on a white background, when placed vertically behind the tube.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

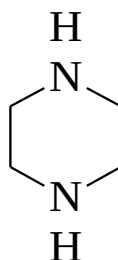
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Pharmacotechnical adjuvant.

PIPERAZINE*Piperazinum* $C_4H_{10}N_2$; 86.14

piperazine; 07099

Piperazine

[110-85-0]

 $C_4H_{10}N_2 \cdot 6H_2O$; 194.23

piperazine hexahydrate; 09518

Piperazine hexahydrate

[142-63-2]

It contains not less than 98.0% and not more than 101.0% of $C_4H_{10}N_2$, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or whitish lumps or flakes.

Solubility. Soluble in water and ethyl alcohol.

Physicochemical constants.

Melting temperature (5.2.2): between 109 °C and 113 °C.

IDENTIFICATION

A. Dissolve approximately 0.2 g of the sample in 5 mL of diluted hydrochloric acid and add, with agitation, 1 mL of 50% (w/v) sodium nitrite solution. Cool in ice bath for 15 minutes, shake if necessary to induce crystallization. Filter the precipitate on a fritted funnel, wash the precipitate with 10 mL of cold water and dry at 105 °C: the *N,N'*-dinitrosopiperazine thus obtained melts **(5.2.2)** between 156 °C and 160 °C.

B. It meets citrate ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 10 g of the sample in water and dilute to 50 mL with the same solvent. The obtained preparation is not more colored **(5.2.12)** than the reference solution, prepared by adding 2 mL of *Ferric chloride base solution* in water and diluted to 50 mL with the same solvent, when compared in Nessler tubes.

Primary amines and ammonia. Dissolve 0.2 g of the sample in 10 mL of water, add 1 mL of acetone and 0.5 mL of freshly prepared 10% (w/v) sodium nitroprusside solution. Mix and allow to stand for 10 minutes. Measure the absorbance **(5.2.14)** of this solution at 520 nm and 600 nm, preparing the blank in the same form as the solution described above, except for the sample. The ratio of the absorbance at 600 nm to the absorbance at 520 nm is not more than 0.5 (equivalent to about 0.7% primary amines and ammonia).

Water (5.2.20.1). Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 0.15 g of the sample and dissolve in 75 mL of glacial acetic acid. Titrate potentiometrically with 0.1 M perchloric acid VS, using the silver-glass electrode system. When approaching the changeover point, heat the solution between 68 °C and 70 °C and then complete the titration. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid is equivalent to 4.307 mg of C₄H₁₀N₂.

PACKAGING AND STORAGE

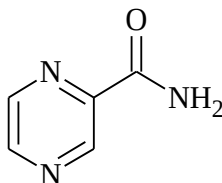
In hermetic containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anthelmintic.

PYRAZINAMIDE*Pyrazinamidum*

C₅H₅N₃O; 123.12
pyrazinamide; 07141
2-Pyrazinecarboxamide
[98-96-4]

It contains not less than 99.0% and not more than 101.0% of C₅H₅N₃O in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Moderately soluble in water, slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 188 °C to 191 °C.

IDENTIFICATION

Test A. can be omitted if tests B., C and D. are carried out. Tests B. and C. can be omitted if tests A. and D. are carried out.

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the pyrazinamide CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of the 0.001% (w/v) solution in water, there are maximum and minimum points identical to those observed in the spectrum of similar pyrazinamide CRS solution.

C. Dissolve 0.1 g of the sample in 5 mL of water. Add 1 mL of acidified ferrous sulfate RS. Orangish color develops. Add 1 mL of sodium hydroxide RS. The solution turns dark blue.

D. Heat to boiling 20 mg of the sample with 5 mL of 5 M sodium hydroxide. Characteristic ammonia odor is released.

PURITY TESTS

Preparation aspect. Dissolve 0.5 g of the sample in carbon dioxide-free water and dilute to 50 mL with the same solvent. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

Acidity or alkalinity. To 25 mL of the solution obtained in *Preparation aspect*, add 0.5 mL of phenolphthalein TS and 0.2 mL of 0.01 M sodium hydroxide. The preparation turns pink. Add 1.0 mL of 0.01 M hydrochloric acid. The preparation turns colorless. Add 0.12 mL of methyl red TS. The preparation turns red.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of glacial acetic acid, water and butyl alcohol (20:20:60) as mobile phase. Apply to the plate, separately, 50 µL of each of the freshly prepared solutions described below.

Solution (1): transfer 0.1 g of the sample to a 10 mL volumetric flask, dilute in mixture of methyl alcohol and methylene chloride (1:9) and complete the volume with the same solvent.

Solution (2): transfer 1 mL of *Solution (1)* to a 50 mL volumetric flask and complete the volume with mixture of methyl alcohol and methylene chloride (1:9). Transfer 1 mL of this solution to a 10 mL volumetric flask and complete the volume with the same solvent.

Solution (3): transfer 10 mg of nicotinic acid CRS to a 10 mL volumetric flask, dissolve in mixture of methyl alcohol and methylene chloride (1:9), add 1 mL of *Solution (1)* and complete the volume with the same solvent.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.2%). The test is only valid if, in the chromatogram obtained with *Solution (3)*, there are two clearly separate main stains.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20.1). Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Quantitatively dissolve about 0.1 g of the sample in 50 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 12.312 mg of C₅H₅N₃O.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Tuberculostatic.

PYRAZINAMIDE TABLETS

It contains not less than 93.0% and not more than 107.0% of the stated quantity of $C_5H_5N_3O$.

IDENTIFICATION

*The Identification tests **B**, **C** and **D** can be omitted if test **A** is carried out. The Identification test **B** can be omitted if tests **A**, **C** and **D** are carried out. The identification test **C** can be omitted if tests **A**, **B** and **D** are carried out.*

A. Weigh and pulverize the tablets. Shake the quantity of powder equivalent to 50 mg of pyrazinamide with 50 mL of absolute ethyl alcohol. Filter and evaporate the filtrate to dryness. Dry the residue in oven at 105 °C for 30 minutes. The residue complies with the test **A** in *Identification* of the *Pyrazinamide* monograph.

B. In the ultraviolet absorption spectrum (**5.2.14**) in the range of 200 nm to 400 nm of the sample solution obtained in method **A** from *Assay*, there are maximum absorption points identical to those observed in the spectrum of the standard solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. Weigh and pulverize the tablets. Boil quantity of powder equivalent to 20 mg of pyrazinamide in 5 mL of 5 M sodium hydroxide. Characteristic ammonia odor is released.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer each tablet to a 500 mL volumetric flask containing 350 mL of water and wait for the tablet to completely disintegrate. Proceed as described in method **A** of *Assay* from "Place in ultrasonic for 15 minutes...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL

Apparatus: paddles, 50 rpm.

Time: 45 minutes

Procedure: after the test, remove aliquot from the dissolution medium and dilute, if necessary, in water, until adequate concentration. Measure the absorbances at 268 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the quantity of $C_5H_5N_3O$ dissolved in the medium, comparing the readings obtained with that from the pyrazinamide CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent. Alternatively, calculate using $A (1\%, 1\text{ cm}) = 650$, at 268 nm, in water.

Tolerance: not less than 75% (Q) of the stated quantity of $C_5H_5N_3O$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Related substances* in the *Pyrazinamide* monograph. Prepare solutions as described below.

Solution (1): weigh and pulverize the tablets. Dissolve quantity of powder equivalent to 0.1 g of pyrazinamide with 50 mL of mixture of methyl alcohol and chloroform (1:9), shake for 15 minutes. Filter, evaporate the filtrate to dryness and dissolve the residue with the same solvent to complete 10 mL.

Solution (2): transfer 1 mL of *Solution (1)* to a 50 mL volumetric flask and complete the volume with mixture of methyl alcohol and chloroform (1:9). Transfer 1 mL of this solution to a 10 mL volumetric flask and complete the volume with the same solvent.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). Any stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.2%).

Water (5.2.20.1). Not more than 3.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.1 g of pyrazinamide to a 500 mL volumetric flask containing 350 mL of water. Place in ultrasonic bath for 15 minutes and shake mechanically for 15 minutes. Complete the volume with the same solvent. Homogenize and filter. Dilute, in water, until concentration of 0.001% (w/v). Prepare standard solution in the same concentration, using the

same solvent. Measure the absorbances of the resulting solutions at 268 nm wavelength, using water for zero adjustment. Calculate the quantity of $C_5H_5N_3O$ in the tablets from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 650$, at 268 nm, in water.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 270 nm; 150 mm long and 3.6 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 μm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: transfer 0.6805 g of monobasic potassium phosphate for a 250 mL volumetric flask, dissolve in water and complete the volume with the same solvent. Transfer the solution obtained to a 1000 mL volumetric flask. Add 18 mL of 0.2 M sodium hydroxide and complete the volume with water. Adjust pH to 3.0 ± 0.2 with phosphoric acid. Mix 10 mL of acetonitrile with 1000 mL of this solution, filter and degas.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.1 g of pyrazinamide to a 100 mL volumetric flask, add 70 mL of water. Place in ultrasonic bath for 15 minutes and shake mechanically for 15 minutes. Complete the volume with the same solvent. Homogenize and filter. Transfer 1 mL of the filtrate to a 25 mL volumetric flask and complete the volume with water.

Standard solution: transfer 50 mg of pyrazinamide CRS to a 50 mL volumetric flask, dissolve in water and complete the volume with the same solvent. Transfer 1 mL of this solution to a 25 mL volumetric flask and complete the volume with water, obtaining a 40 $\mu\text{g/mL}$ solution.

Resolution solution: transfer 1 mL of hydrochloric acid to a 5 mL volumetric flask and complete the volume with the *Standard solution*. Place this solution in boiling water bath for five minutes to form pyrazinoic acid. Cool.

Inject 20 μL replicates of the *Standard solution*. The column efficiency must be not less than 2500 theoretical plates. The tailing factor is not more than 1.3. Inject 20 μL replicates of the *Resolution solution*. The relative retention times are approximately 0.45 for pyrazinoic acid and 1.0 for pyrazinamide. The resolution between pyrazinoic acid and pyrazinamide should be not less than 6.0.

Procedure: inject, separately, 20 μL of each of the *Standard* and *sample solutions*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_5H_5N_3O$ in the tablets from the responses obtained for the *Standard* and *sample solutions*.

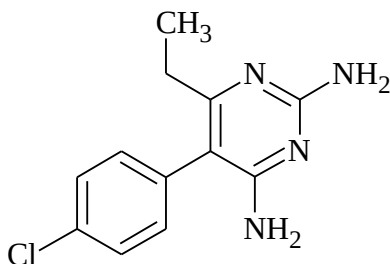
PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

PYRIMETHAMINE
Pyrimethaminum



C₁₂H₁₃ClN₄; 248.71

pyrimethamine; 07170

5-(4-Chlorophenyl)-6-ethyl-2,4-pyrimidinediamine

[58-14-0]

It contains not less than 99.0% and not more than 101.0% of C₁₂H₁₃ClN₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Off-white crystalline powder or colorless crystals.

Solubility. Practically insoluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 239 °C to 243 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the pyrimethamine CRS spectrum.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in 0.1 M hydrochloric acid, prepared with heating, if necessary, there are maximum and minimum points only at the same wavelengths of similar pyrimethamine CRS solution.

C. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position and intensity to the one obtained with *Solution (3)*.

D. Transfer to crucible approximately 1 g of sample and 5 g of anhydrous sodium carbonate. Mix and heat until ignition. Cool, add 5 mL of hot water, place in ultrasonic bath for five minutes, filter and neutralize the filtrate with nitric acid. The resulting solution meets chloride ion reactions **(5.3.1.1)**.

PURITY TESTS

Acidity or alkalinity. Transfer 1 g of the sample to a 50 mL volumetric flask, dissolve in 30 mL of water, shake for two minutes, complete the volume with the same solvent and filter. To 10 mL of the solution, add 0.5 mL of phenolphthalein TS. Not more than 0.2 mL of 0.01 M sodium hydroxide is required to promote the indicator changeover. Add 0.05 mL of methyl red TS and 0.4 mL of 0.01 M hydrochloric acid. An orangish-red color develops.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of chloroform, *n*-propyl alcohol, glacial acetic acid and toluene (4:8:12:76) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in mixture of methyl alcohol and chloroform (1:9).

Solution (2): transfer 1 mL of *Solution (1)* to a 10 mL volumetric flask and complete the volume with mixture of methyl alcohol and chloroform (1:9).

Solution (3): 1 mg/mL of pyrimethamine CRS solution in mixture of methyl alcohol and chloroform (1:9).

Solution (4): transfer 2.5 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with mixture of methyl alcohol and chloroform (1:9). Transfer 1 mL of this solution to a 10 mL volumetric flask and complete the volume with mixture of solvents.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (4)* (0.25%).

Sulfates (5.3.2.2). Transfer 1 g of the sample to a 50 mL volumetric flask, dissolve in 30 mL of water, shake for two minutes, complete the volume with the same solvent and filter. Use 15 mL of the filtrate. Prepare standard sulfate solution at a concentration of 0,001% (10 ppm). Not more than 0.008% (80 ppm).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven between 100 °C and 105 °C for four hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 0.2 g of the sample and dissolve in 25 mL of glacial acetic acid, heating gently. Cool and titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 24.871 mg of C₁₂H₁₃ClN₄.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antimalarial and antitoxoplasmosis.

PYRIMETHAMINE TABLETS

It contains not less than 93.0% and not more than 107.0% of the stated quantity of $C_{12}H_{13}ClN_4$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer quantity of powder equivalent to 0.2 g of pyrimethamine to a beaker and add 25 mL of acetone, heat to boiling for 2 minutes and filter through a sintered glass crucible. Repeat this treatment three times with 25 mL portions of acetone. Evaporate the combined filtrates in water bath to dryness, using an air stream. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the pyrimethamine CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 250 nm to 300 nm, of the sample solution obtained in *Assay*, there are maximum absorption points only at the same wavelengths and with the same relative intensities as those observed in the standard solution spectrum, prepared identically.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute with 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 272 nm **(5.2.14)** using the same solvent for zero adjustment. Calculate the quantity of $C_{12}H_{13}ClN_4$ dissolved in the medium, comparing the readings obtained with that of the pyrimethamine CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated quantity of $C_{12}H_{13}ClN_4$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

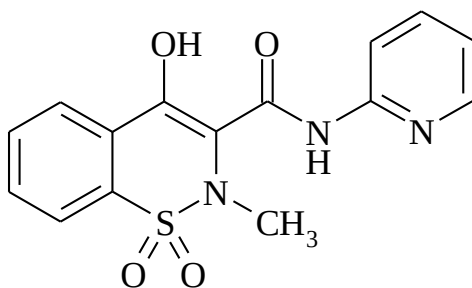
Proceed as described in *Ultraviolet absorption spectrophotometry* (**5.2.14**). Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 25 mg of pyrimethamine to a 100 mL volumetric flask and add 50 mL of 0.1 M hydrochloric acid. Heat in water bath for 10 minutes and place in ultrasonic bath for 30 minutes. Cool, complete the volume with the same solvent. Filter. Transfer 5 mL of the filtrate to a 100 mL volumetric flask and complete the volume with 0.1 M hydrochloric acid, obtaining a 12.5 µg/mL solution. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 272 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the quantity of C₁₂H₁₃ClN₄ in the tablets from the readings obtained. Alternatively, calculate considering A (1%, 1 cm) = 316, at 272 nm, in 0.1 M hydrochloric acid.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

PIROXICAM*Piroxicamum*C₁₅H₁₃N₃O₄S; 331.35

piroxicam; 07211

1,1-dioxide 4-hydroxy-2-methyl-*N*-2-pyridinyl-2*H*-1,2-benzothiazine-3-carboxamide
[36322-90-4]It contains not less than 97.0% and not more than 103.0% of C₁₅H₁₃N₃O₄S.

DESCRIPTION

Physical characteristics. White, or slightly yellowish, crystalline powder or solid. Presents polymorphism.**Solubility.** Very slightly soluble in water and slightly soluble in ethyl alcohol. Slightly soluble in alkaline aqueous solutions and very slightly soluble in diluted acid solutions.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the non-desiccated sample, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the piroxicam CRS spectrum, prepared identically.**B.** In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in 0.01 *M* methanolic hydrochloric acid, there are maximum and minimum points only at the same wavelengths of similar piroxicam CRS solution.**C.** Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support, and mixture of toluene and glacial acetic acid (95:5) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.*Solution (1):* 1 mg/mL sample solution in mixture of chloroform and methyl alcohol (1:1).*Solution (2):* 1 mg/mL solution of piroxicam CRS in mixture of chloroform and methyl alcohol (1:1).Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position to the one obtained with *Solution (2)*.

PURITY TESTS

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.005% (50 ppm).

Water (5.2.20.1). Not more than 0.5%.

Residue by incineration (5.2.10). Not more than 0.3%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow rate of 1.2 mL/minute.

Phosphate buffer: dissolve approximately 7.72 g of anhydrous citric acid in 400 mL of water and, separately, dissolve approximately 5.35 g of anhydrous dibasic sodium phosphate in 100 mL of water. Add the phosphate solution to the citric acid solution, dilute with water to a 1000 mL volume and homogenize.

Mobile phase: mixture of *Phosphate buffer* and methyl alcohol (55:45).

Diluent: mixture of 0.01 M methanolic hydrochloric acid and water (4:1).

Sample solution: quantitatively transfer about 25 mg of the sample to a 50 mL volumetric flask, add 25 mL of 0.01 M methanolic hydrochloric acid and place in ultrasonic bath for 15 minutes. Complete the volume with 0.01 M methanolic hydrochloric acid and homogenize. Transfer 5 mL of this preparation to a 50 mL volumetric flask, complete the volume with *Diluent* and homogenize, obtaining a solution at 50 µg/mL.

Standard solution: dissolve an accurately weighed quantity of piroxicam CRS in 0.01 M hydrochloric acid to obtain a 0.25 mg/mL solution. Transfer 5 mL of this solution to a 25 mL volumetric flask, complete the volume with *Diluent* and homogenize, obtaining a solution at 50 µg/mL.

Inject 25 µL replicates of the *Standard solution*. The column efficiency is not less than 500 theoretical plates. The tailing factor is not more than 1.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 25 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₅H₁₃N₃O₄S in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-inflammatory.

PIROXICAM CAPSULES

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $C_{15}H_{13}N_3O_4S$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the *Sample solution* obtained in method **A.** from *Assay*, there is maximum absorption at 354 nm, identical to that observed in the spectrum of the *Standard solution*.

B. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described in method **A.** from *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, remove 10 mL aliquot from the dissolution medium, filter and dilute in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 242 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the quantity of $C_{15}H_{13}N_3O_4S$ dissolved in the medium using the value of $A(1\%, 1\text{ cm}) = 352$, at 242 nm, in 0.1 M hydrochloric acid.

Tolerance: not less than 70% (Q) of the stated quantity of $C_{15}H_{13}N_3O_4S$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of toluene and glacial acetic acid (90:10) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): mix quantity of powder containing 80 mg of piroxicam with 25 mL of methylene chloride, filter and place the filtrate to dryness using a rotary evaporator. Dissolve the residue in 2 mL of methylene chloride.

Solution (2): dilute 1 mL of *Solution (1)* to 20 mL with methylene chloride.

Solution (3): prepare 2 mg/mL piroxicam CRS solution in methylene chloride.

Solution (4): dilute 2 mL of *Solution (2)* to 50 mL with methylene chloride.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)* is not more intense than that obtained with *Solution (4)* (0.2%). Disregard any stain remaining in the application line.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh 20 capsules, remove the content and weigh again. Homogenize the content of the capsules. Transfer quantity of powder equivalent to 25 mg of piroxicam to a 250 mL volumetric flask and complete the volume with 0.1 M sodium hydroxide. Dilute successively, with the same solvent, to a final concentration of 0.001% (w/v). Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 354 nm, using 0.1 M sodium hydroxide for zero adjustment. Calculate the quantity of $C_{15}H_{13}N_3O_4S$ in the capsules from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 248 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (10 μ m), kept at room temperature; *Mobile phase* flow rate of 2 mL/minute.

Sodium phosphate dibasic buffer: dissolve 5.35 g of sodium phosphate dibasic dodecahydrate in 100 mL of water. Dissolve 7.72 g of citric acid in 400 mL of water. Transfer the two solutions to a 1000 mL volumetric flask and complete the volume with water.

Mobile phase: mixture of methyl alcohol and *Sodium phosphate dibasic buffer* (60:40).

Sample solution: weigh 20 capsules, remove the content and weigh again. Homogenize the content of the capsules. Transfer quantity of powder equivalent to 10 mg of piroxicam to a 200 mL volumetric flask, add 150 mL of 0.01 M methanolic hydrochloric acid, shake and place in ultrasonic bath at room temperature for 30 minutes. Cool and complete the volume with the same solvent. Filter the solution with quantitative filter.

Standard solution: prepare a 50 μ g/mL piroxicam CRS solution in 0.01 M methanolic hydrochloric acid. If necessary, submit the solution to ultrasonic bath at room temperature.

Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{15}H_{13}N_3O_4S$ in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

PIROXICAM GEL

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $C_{15}H_{13}N_3O_4S$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of ethyl acetate, methyl alcohol and glacial acetic acid (80: 10: 1), as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): mix quantity of gel containing 10 mg of piroxicam with 0.1 mL of saturated hydrochloric acid solution until the solution becomes turbid. Dilute to 5 mL with 0.01 M methanolic hydrochloric acid. Shake thoroughly, centrifuge and use the clear supernatant solution. Filter, if necessary.

Solution (2): prepare a 0.2% (w/v) piroxicam CRS solution in 0.01 M methanolic hydrochloric acid.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* chromatogram corresponds in position and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

pH (5.2.19). 7.2 to 8.2. Determine in gel solution at 10% (w/v).

Determination of weight (5.1.1). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 248 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (3 µm to 10 µm), and pre-column with 100 mm of length and 4.6 mm of internal diameter chemically bonded to octylsilane group (5 µm), kept at temperature of 40°C, *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of 0.05 M sodium phosphate dibasic dihydrate, with pH adjusted to 3.5 with phosphoric acid, acetonitrile and methyl alcohol (55:30:15).

Sample solution: transfer quantity of gel equivalent to 5 mg of piroxicam to a 100 mL volumetric flask, add 5 mL of 0.01 M methanolic hydrochloric acid and shake for 30 minutes. Add 50 mL of *Mobile Phase* and shake vigorously for 30 minutes. Complete the volume with the *Mobile phase* and shake. Filter the solution with a glass microfiber filter of 1.0 µm pore diameter.

Standard solution: prepare a 1 µg/mL piroxicam CRS solution in 0.01 M methanolic hydrochloric acid. If necessary, submit the solution to ultrasonic bath at room temperature. Take a 5 mL aliquot of this solution, transfer to a 100 mL volumetric flask and complete the volume with *Mobile phase*.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{15}H_{13}N_3O_4S$ in the gel from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

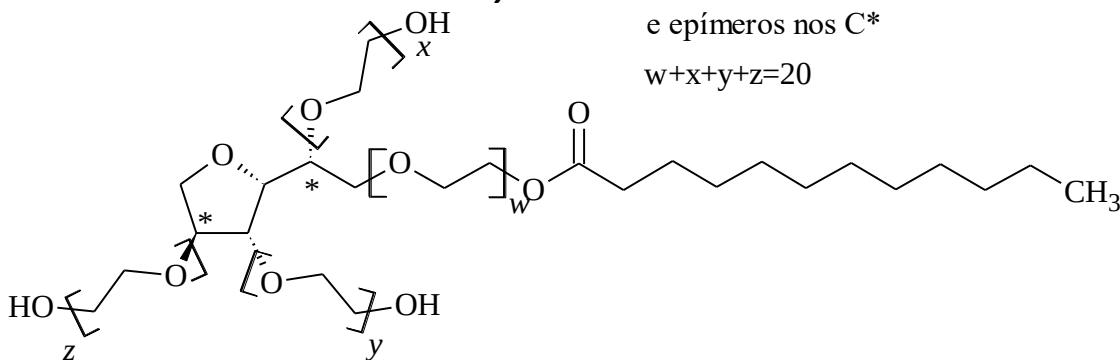
LABELING

Comply with current legislation.

POLYSORBATE 20***Polysorbatum 20***

e epímeros nos C*

$$w+x+y+z=20$$



polysorbate 20; 07272

Poly(oxy-1,2-ethanediyl) derivatives of sorbitan monododecanoate
[9005-64-5]

Mixture of sorbitol partial lauric esters and their copolymerized anhydrides, with approximately 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides. Lauric acid, used in esterification, can contain varying quantities of other fatty acids.

DESCRIPTION

Physicochemical characteristics. Oily, clear or slightly opalescent liquid, yellowish or amber.
Relative density (5.2.5): about 1.1.

Solubility. Miscible with water, absolute ethyl alcohol, ethyl acetate and methyl alcohol. Practically insoluble in fixed oils and in liquid paraffin.

IDENTIFICATION

A. Dissolve 0.5 g of the sample in water, at about 50 °C and dilute to 10 mL with the same solvent. The solution produces abundant foaming after shaking. Add 0.5 g of sodium chloride and heat until ebullition. The turbidity formed disappears with cooling to about 50 °C.

B. Heat, under reflux, 4 g of the sample in water bath for 30 minutes with 40 mL of 5% potassium hydroxide (w/v). Cool to about 80 °C, acidify with 20 mL of 2 M nitric acid and heat under reflux for about 10 minutes to separate the emulsion. Fatty acids remain on the surface as an oily liquid. Cool to room temperature and extract with 50 mL of petroleum ether (distillation range between 40 °C and 60 °C), avoiding vigorous agitation. Wash the organic phase with three 5 mL portions of water and evaporate in water bath until dryness. The acidity value **(5.2.29.7)**, determined in 0.3 g of the residue with 50 mL of the solvent ranges from 245 to 300.

C. Dissolve 0.1 g of the sample in 5 mL of chloroform, add 0.1 g of potassium thiocyanate and 0.1 g of cobalt(II) nitrate and mix with a glass rod. Blue color develops.

PURITY TESTS

Acidity value (5.2.29.7). Determine in 5 g of the sample. Not more than 2.0.

Hydroxyl value (5.2.29.12). 96 to 108. Determine in 2 g of the sample.

Iodine value (5.2.29.10). Not more than 5.0.

Saponification value (5.2.29.8). 40 to 50. Use 15 mL of 0.5 M alcoholic potassium hydroxide VS and dilute with 50 mL of water before titration.

Reducing impurities. Dissolve 2 g of the sample in 25 mL of hot water, add 25 mL of M sulfuric acid and 0.1 mL of ferroin TS. Titrate with 0.01 M ceric ammonium nitrate VS, shaking continuously until color changes from red to persistent greenish-blue for 30 seconds. Carry out the blank test and make the necessary corrections. Not more than 2 mL of 0.01 M ceric ammonium nitrate VS are required.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20). Determine in 1 g. Not more than 3.0.

Residue by incineration (5.2.10). Transfer 2 g of the sample to a platinum or silica crucible, add 0.5 mL of sulfuric acid and heat in water bath for two hours. Carefully heat over a gas burner until complete carbonization. Add 2 mL of nitric acid and 0.25 mL of sulfuric acid to the carbonized mass, heat carefully until white smoke develops and incinerate at 600 °C until constant weight. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

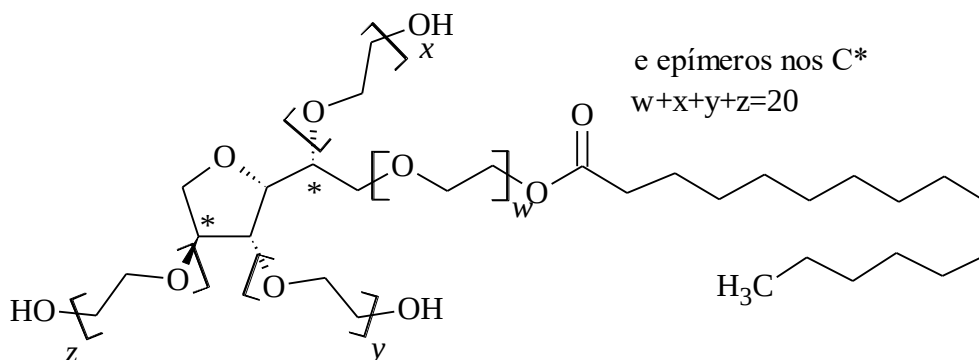
In tightly closed containers and protected from light.

LABELING

Comply with current legislation.

CATEGORY

Non-ionic surfactant. Emulsifier, solubilizer and humectant.

POLYSORBATE 40***Polysorbatum 40***

polysorbate 40; 07273

Poly(oxy-1,2-ethanediyl) derivatives of sorbitan monohexadecanoate
[9005-66-7]

Sorbitol palmitic ester and its anhydrides copolymerized, with approximately 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides.

DESCRIPTION

Physical characteristics. Yellow liquid with a strong characteristic odor.

Solubility. Soluble in water and ethyl alcohol. Insoluble in mineral oil and vegetable oils.

IDENTIFICATION

A. To 5 mL of sample solution (1:20), add 5 mL of *M* sodium hydroxide. Heat to boiling for some minutes, cool and acidify with 3 *M* hydrochloric acid. The preparation becomes strongly opalescent.

B. To 2 mL of sample solution (1:20), add, dropwise, 0.5 mL of bromine water RS. Bromine does not discolor (unlike polysorbate 80).

PURITY TESTS

Hydroxyl value (5.2.29.12). 89 to 105. Determine in 2 g of the sample.

Saponification value (5.2.29.8). 41 to 52. Use 15 mL of 0.5 *M* ethanolic potassium hydroxide VS and dilute with 50 mL of water before titration.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20). Determine in 1 g. Not more than 3.0.

Residue by incineration (5.2.10). Transfer 2 g of the sample to a platinum or silica crucible, add 0.5 mL of sulfuric acid and heat in water bath for two hours. Carefully heat over a gas burner until complete carbonization. Add 2 mL of nitric acid and 0.25 mL of sulfuric acid to the carbonized mass,

heat carefully until white smoke develops and incinerate at 600 °C until constant weight. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

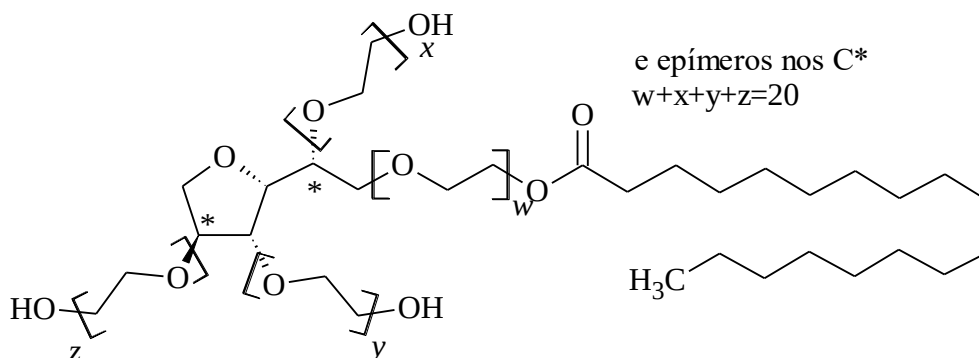
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

CATEGORY

Non-ionic surfactant. Emulsifier, solubilizer and humectant.

POLYSORBATE 60***Polysorbatum 60***

polysorbate 60; 07274

Poly(oxy-1,2-ethanediyl) derivatives of sorbitan mono-octadecanoate
[9005-67-8]

Mixture of sorbitol partial stearic esters and their copolymerized anhydrides, with approximately 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides. The stearic acid used for esterification may contain other fatty acids, especially palmitic acid.

DESCRIPTION

Physicochemical characteristics. Yellowish-brown gelatinous mass. It presents itself as a clear liquid at temperature above 25°C. *Relative density (5.2.5):* about 1.1.

Solubility. Miscible with water, absolute ethyl alcohol, ethyl acetate and methyl alcohol. Practically insoluble in fixed oils and in liquid paraffin.

IDENTIFICATION

A. Dissolve 0.5 g of the sample in water, at about 50 °C and complete the volume to 10 mL with the same solvent. The solution produces abundant foaming after shaking. Add 0.5 g of sodium chloride and heat until boiling. The turbidity formed disappears with cooling to about 50 °C.

B. Heat, under reflux, 4 g of the sample in water bath for 30 minutes with 40 mL of 5% potassium hydroxide (w/v). Cool to about 80 °C, acidify with 20 mL of 2 M nitric acid and boil under reflux for about 10 minutes to separate the emulsion. Fatty acids remain on the surface as an oily liquid. Cool to room temperature and extract with 50 mL of petroleum ether (distillation range between 40 °C and 60 °C), avoiding vigorous agitation. Wash the organic phase with three 5 mL portions of water and evaporate in water bath until dryness. The acidity value **(5.2.29.7)**, determined in 0.5 g of the residue with 50 mL of the solvent ranges from 190 to 220.

C. Dissolve 0.1 g of the sample in 5 mL of chloroform, add 0.1 g of potassium thiocyanate and 0.1 g of cobalt(II) nitrate and mix with a glass rod. Blue color develops.

D. To 5 mL of sample solution (1:20), add 5 mL of M sodium hydroxide. Heat for some minutes, cool and acidify with 3 M hydrochloric acid. The preparation becomes strongly opalescent.

PURITY TESTS

Acidity value (5.2.29.7). Determine in 5 g of the sample. Not more than 2.0.

Hydroxyl value (5.2.29.12). 81 to 96. Determine in 2 g of the sample.

Iodine value (5.2.29.10). Not more than 5.0.

Saponification value (5.2.29.8). 45 to 55. Use 15 mL of 0.5 M ethanolic potassium hydroxide VS and dilute with 50 mL of water before titration.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20). Determine in 1 g of the sample. Not more than 3.0.

Residue by incineration (5.2.10). Transfer 2 g of the sample to a platinum or silica crucible, add 0.5 mL of sulfuric acid and heat in water bath for two hours. Carefully heat over a gas burner until complete carbonization. Add 2 mL of nitric acid and 0.25 mL of sulfuric acid to the carbonized mass, heat carefully until white smoke develops and incinerate at 600 °C until constant weight. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

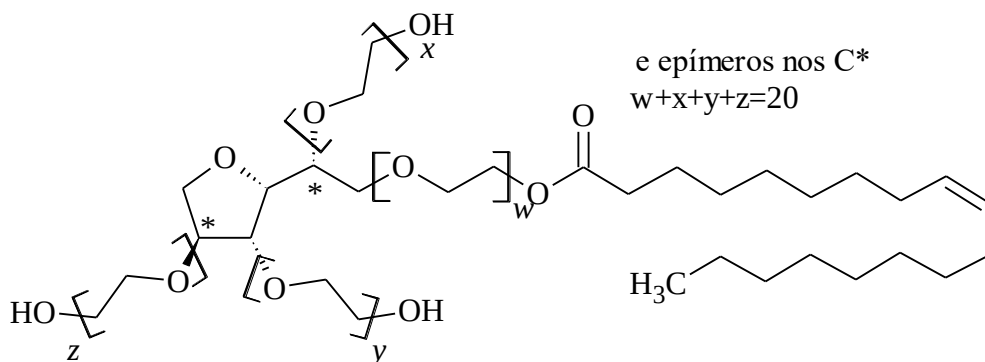
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

CATEGORY

Non-ionic surfactant. Emulsifier, solubilizer and humectant.

POLYSORBATE 80***Polysorbatum 80***

polysorbate 80; 07275

Sorbitan mono-(9Z)-9-octadecenoate poly(oxy-1,2-ethanediyl) derivatives
[9005-65-6]

Mixture of sorbitol partial oleic esters and their copolymerized anhydrides, with approximately 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides.

DESCRIPTION

Physicochemical characteristics. Clear, oily liquid, yellow or light brown, with a characteristic odor and slightly bitter taste. *Relative density (5.2.5):* about 1.1. *Viscosity (5.2.7):* about 400 mPa.

Solubility. Miscible with water, absolute ethyl alcohol, ethyl acetate and methyl alcohol. Practically insoluble in fixed oils and in liquid paraffin.

IDENTIFICATION

A. Dissolve 0.5 g of the sample in water, at about 50 °C and complete the volume to 10 mL with the same solvent. The solution produces abundant foaming after shaking. Add 0.5 g of sodium chloride and heat until boiling. The turbidity formed disappears with cooling to about 50 °C.

B. Heat, under reflux, 4 g of the sample in water bath for 30 minutes with 40 mL of 5% potassium hydroxide (w/v). Cool to about 80 °C, acidify with 20 mL of 2 M nitric acid and heat to ebullition for about 10 minutes under reflux to separate the emulsion. Fatty acids remain on the surface as an oily liquid. Cool to room temperature and extract with 50 mL of petroleum ether (distillation range between 40 °C and 60 °C), avoiding vigorous agitation. Wash the organic phase with three 5 mL portions of water and evaporate in water bath until dryness. Resuspend the residue obtained with a mixture of 2 mL of nitric acid and 3 mL of water. Carefully add, in small portions, 0.5 g of sodium nitrite and allow to stand at room temperature. The fatty acid layer solidifies in four hours.

C. Dissolve 0.1 g of the sample in 5 mL of chloroform, add 0.1 g of potassium thiocyanate and 0.1 g of cobalt(II) nitrate and mix with a glass rod. Blue color develops.

D. To 5 mL of sample solution (1:20), add 5 mL of M sodium hydroxide. Heat to boiling for some minutes, cool and acidify with 3 M hydrochloric acid. The preparation becomes strongly opalescent.

E. To 2 mL of sample solution (1:20), add, dropwise, 0.5 mL of bromine water RS. Bromine does not discolor (unlike polysorbate 40).

PURITY TESTS

Acidity value (5.2.29.7). Not more than 2.0.

Hydroxyl value (5.2.29.12). 65 to 80. Determine in 2 g of the sample.

Iodine value (5.2.29.10). 18 to 24.

Saponification value (5.2.29.8). 45 to 55. Use 15 mL of 0.5 M ethanolic potassium hydroxide VS and dilute with 50 mL of water before titration.

Reducing impurities. Dissolve 2 g of the sample in 25 mL of hot water, add 25 mL of M sulfuric acid and 0.1 mL of ferroin TS. Titrate with 0.01 M ceric ammonium nitrate VS, shaking continuously until color changes from red to greenish-blue, persistent for 30 seconds. Carry out the blank test and make the necessary corrections. Not more than 5 mL of 0.01 M ceric ammonium nitrate VS are required.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20). Determine in 1 g of the sample. Not more than 3.0.

Residue by incineration (5.2.10). Transfer 2 g of the sample to a platinum or silica crucible, add 0.5 mL of sulfuric acid and heat in water bath for two hours. Carefully heat over a gas burner until complete carbonization. Add 2 mL of nitric acid and 0.25 mL of sulfuric acid to the carbonized mass, heat carefully until white smoke develops and incinerate at 600 °C until constant weight. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

In tightly closed containers and protected from light.

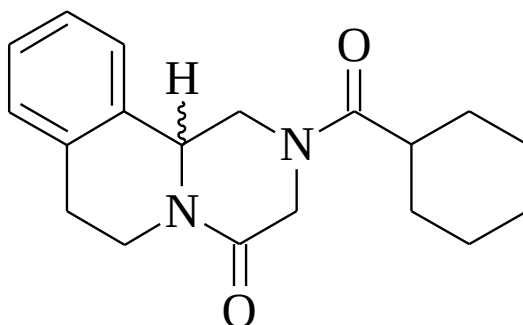
LABELING

Comply with current legislation.

CATEGORY

Non-ionic surfactant. Emulsifier, solubilizer and humectant.

PRAZIQUANTEL
Praziquantelum



$C_{19}H_{24}N_2O_2$; 312.41

praziquantel; 07321

2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*pyrazino[2,1-*a*]isoquinolin-4-one
 [55268-74-1]

It contains not less than 98.5% and not more than 101.0% of $C_{19}H_{24}N_2O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder. Presents polymorphism.

Solubility. Very slightly soluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 136 °C to 142 °C.

IDENTIFICATION

In the infrared absorption spectrum **(5.2.14)** of the sample, previously desiccated and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the praziquantel CRS spectrum, prepared identically.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 210 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water and acetonitrile (55:45).

Solution (1): dissolve 40 mg of the sample in *Mobile phase* and dilute to 10 mL with the same solvent, obtaining a 4 mg/mL solution.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with *Mobile phase*. Dilute 5 mL of this solution to 10 mL with *Mobile Phase*, obtaining a 20 µg/mL solution.

Solution (3): 0.2 mg/mL praziquantel CRS solution in *Mobile phase*.

Solution (4): dissolve 5 mg of 2-benzoyl-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (*impurity A*) CRS in *Solution (3)* and dilute to 5 mL with the same solvent. Dilute 1 mL of this solution to 10 mL with *Mobile Phase*, obtaining a 20 µg/mL *impurity A* and praziquantel solution.

Inject 20 µL replicates of the *Solution (4)*. The resolution between *impurity A* and praziquantel is not less than 3.0.

Procedure: inject, separately, 20 µL of *Solution (1)* and of *Solution (2)*, record the chromatograms for not less than five times the praziquantel retention time and measure the areas under the peaks. The area under any peak obtained in the chromatogram with *Solution (1)*, except under the main peak, is not greater than the area under the main peak obtained with *Solution (2)* (0.5%). The area under not more than one of the secondary peaks obtained in the chromatogram with *Solution (1)*, except under the main peak, is greater than 0.4 times the area under the main peak obtained with *Solution (2)* (0.2 %). The sum of the areas under all the peaks obtained with *Solution (1)*, except under the main peak, is not greater than the area under the main peak obtained with *Solution (2)* (0.5%). Disregard peaks with areas below 0.1 times the area under the main peak obtained with *Solution (2)* (0.05%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 50 °C, under reduced pressure, for two hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 210 nm; column with 250 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm to 10 µm); *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of water and acetonitrile (40:60).

Sample solution: quantitatively transfer about 45 mg of the sample to a 25 mL volumetric flask. Complete the volume with the *Mobile phase* and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*.

Standard solution: dissolve an accurately weighed quantity of praziquantel CRS in the *Mobile phase* and dilute adequately with the same solvent to obtain a solution at 0.18 mg/mL.

Inject 10 µL replicates of the *Standard solution*. The tailing factor is not more than 1.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₉H₂₄N₂O₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anthelminthic.

PRAZIQUANTEL TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{19}H_{24}N_2O_2$.

IDENTIFICATION

Proceed as described in *Thin Layer Chromatography* (5.2.17.1), using silica gel G as support and ethyl acetate as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Transfer quantity of powder equivalent to 30 mg of praziquantel to a centrifuge tube and add 5 mL of methyl alcohol. Shake for five minutes and centrifuge. Use the clear supernatant.

Solution (2): 6 mg/mL praziquantel CRS solution in methyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test. Not more than 15 minutes.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.2% (w/v) sodium lauryl sulfate in 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 60 minutes.

Procedure: after testing, remove aliquot from the dissolution medium and filter. Measure the absorbances of the solutions at 263 nm (5.2.14), using *Dissolution medium* for zero adjustment. Calculate the quantity of $C_{19}H_{24}N_2O_2$ dissolved in the medium, comparing the readings obtained with that of the *Standard solution*, prepared as described below.

Standard solution: dissolve an accurately weighed quantity of praziquantel CRS to obtain a solution whose concentration is $L/90$ mg/mL, where L is the stated quantity, in milligrams, of praziquantel per tablet. Transfer 5 mL of the solution obtained to a 50 mL volumetric flask, dilute and complete the volume with *Dissolution medium*.

Tolerance: not less than 75% (Q) of the stated quantity of $C_{19}H_{24}N_2O_2$ dissolve in 60 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in Assay in the *Praziquantel* monograph. Prepare *Sample solution* as described below.

Sample Solution: weigh and pulverize the tablets. Transfer quantity of powder equivalent to 150 mg of praziquantel to a 100 mL volumetric flask. Add 70 mL of *Mobile phase* and place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 3 mL to a 25 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

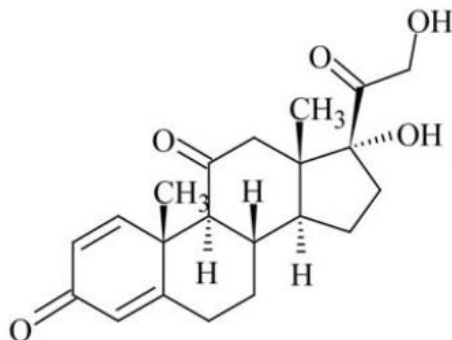
Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₉H₂₄N₂O₂ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

PREDNISONE*Prednisonum*C₂₁H₂₆O₅; 358.43

prednisone; 07341

17,21-Dihydroxypregna-1,4-diene-3,11,20-trione

[53-03-2]

It contains not less than 97.0% and not more than 103.0% of C₂₁H₂₆O₅, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder. Presents polymorphism.

Solubility. Very slightly soluble in water, slightly soluble in ethyl alcohol, in dioxane and in methyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +167 to +175. Determine in solution at 0,5% (w/v) in dioxane.

IDENTIFICATION

The identification tests B. and D. can be omitted if tests A. and C. are carried out. The identification test A. can be omitted if tests B., C. and D. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the prednisone CRS spectrum, prepared identically. If the spectra obtained are not identical, dissolve, separately, prednisone CRS and sample in acetone and evaporate to dryness. Obtain new spectra with the residues.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of the 0.001% (w/v) sample solution in ethyl alcohol, there is a maximum point at 239 nm, identical to the one observed in the spectrum of a similar prednisone CRS solution. The absorbance at 239 nm is between 0.405 and 0.435.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ plate as support, and mixture of methylene chloride, ethyl ether, methyl alcohol and water (77:15:8:1.2) as

mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 1 mg/mL sample solution in mixture of chloroform and methyl alcohol (9:1).

Solution (2): 1 mg/mL solution of prednisone CRS in mixture of chloroform and methyl alcohol (9:1).

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). Spray with 20% (v/v) sulfuric acid in ethyl alcohol. Heat the plate at 120 °C for 10 minutes. Cool. Examine under ultraviolet light (365 nm). The stain obtained with *Solution (1)* chromatogram corresponds in position, color and intensity to the one obtained with *Solution (2)*.

D. Dissolve 6 mg of the sample in 2 mL of concentrated sulfuric acid. Allow to stand for five minutes. Orangish color develops. Pour the solution, dropwise and under agitation, into 10 mL of water. The color changes to yellow and gradually to bluish-green.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250mm long, 4.0 mm internal diameter column, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at 45 °C; *Mobile phase* flow rate of 2.5 mL/minute.

Solution (1): dissolve 25 mg of the sample in methyl alcohol and dilute to 10 mL with the same solvent.

Solution (2): dissolve 2 mg of prednisone CRS and 2 mg of prednisolone CRS in methyl alcohol and dilute to 100 mL with the same solvent.

Solution (3): dilute 1 mL of *Solution (1)* to 100 mL with methyl alcohol.

Eluent A: in a 1000 mL volumetric flask, add 100 mL of acetonitrile with 200 mL of methyl alcohol and 650 mL of water. Homogenize. Adjust volume to 1000 mL with water and mix again.

Eluent (B): acetonitrile.

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0	100	0	stabilize
0 – 25	100	0	isocratic
25 – 40	100 → 40	0 → 60	linear gradient
40 – 41	40 → 0	60 → 100	linear gradient
41 – 46	0	100	isocratic
46 – 47	0 → 100	100 → 0	linear gradient
47 – 52	100	0	stabilize

Equilibrate the column for 30 minutes with *Eluent B* at a flow rate of 2.5 mL/minute and then with *Eluent A* for five minutes. Proceed under the described elution conditions between 40 and 52 minutes. Adjust the system sensitivity so that the height of the main peak of the chromatogram obtained with 20 µL of *Solution (3)* is not less than 50% of the total of the full scale. Inject 20 µL replicates of the *Solution (2)*. The retention times are about 19 minutes for prednisone and 23 minutes for prednisolone. The resolution between prednisolone and prednisone is not less than 2.7; if necessary, adjust the acetonitrile concentration in *Eluent A*.

Procedure: separately inject 20 µL of methyl alcohol as blank, 20 µL of *Solution (1)* and 20 µL of *Solution (3)*. In the chromatogram obtained with *Solution (1)*, the area under no peaks, except the area under the main peak, is not more than 0.25 times the area under the main peak of the chromatogram obtained with *Solution (3)* (0.25%). The sum of the areas under all the peaks, except that under the main peak, is not more than 0.75 times the area under the main peak obtained with *Solution (3)* (0.75%). Discard any peaks obtained in the blank run and any peaks with an area less than 0.05 times the area under the main peak of the chromatogram obtained with *Solution (3)* (0.05%).

Water (5.2.20.1). Not more than 1.0% for the anhydrous substance and not more than 5.0% for the monohydrate substance.

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105°C. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 0.1 g of sample. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 240 nm; column with 250 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water, tetrahydrofuran and methyl alcohol (69:25:6.2).

Internal Standard Solution: accurately weigh approximately 11 mg of acetanilide and dissolve in 33 mL of methyl alcohol. Complete the volume to 100 mL with purified water to obtain a solution at 110 µg/mL.

Sample solution: accurately weigh about 20 mg of the sample and dissolve in 33 mL of methyl alcohol. Complete the volume to 100 mL with purified water to obtain a solution at 0.2 mg/mL. Transfer 5 mL of this solution and 5 mL of the *Internal Standard Solution* to a 50 mL volumetric flask. Complete the volume with mixture of methyl alcohol and water (1:2) and homogenize, obtaining a 20 µg/mL prednisone solution.

Standard Solution: accurately weigh approximately 20 mg of prednisone CRS and dissolve in 33 mL of methyl alcohol. Complete the volume to 100 mL with purified water to obtain a solution at 0.2 mg/mL. Transfer 5 mL of this solution and 5 mL of the *Internal standard solution* to a 50 mL volumetric flask. Complete the volume with mixture of methyl alcohol and water (1:2) and homogenize, obtaining a 20 µg/mL prednisone solution.

Inject 20 µL replicates of the *Internal Standard Solution*. The resolution between prednisone and acetanilide is not less than 3.0. The relative standard deviation of the replicate areas under the recorded peaks is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₁H₂₆O₅ in the sample from the responses obtained for the prednisone/acetanilide ratio with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Steroidal anti-inflammatory.

PREDNISONE TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{21}H_{26}O_5$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Shake quantity of powder equivalent to 20 mg of prednisone with about 100 mL of chloroform. Filter and evaporate to dryness. Dry the residue in oven at 105 °C for three hours. In the infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the prednisone CRS spectrum, prepared identically.

B. To about 6 mg of the residue obtained in method **A.** from *Identification*, add 2 mL of sulfuric acid. Allow to stand for five minutes. Orangish color develops. Pour the solution, dropwise and under agitation, into 10 mL of water. The orange color changes first to yellow and then gradually to bluish-green.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described in method **B.** from *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 500 mL (for tablets containing 10 mg or less of prednisone) or 900 mL (for tablets containing more than 10 mg of prednisone).

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in water until adequate concentration. Measure the absorbances of the solutions at 242 nm, using the same solvent for zero adjustment. Calculate the quantity of $C_{21}H_{26}O_5$ dissolved in the medium, comparing the readings obtained with the prednisone CRS solution at the concentration of 0.001% (w/v), prepared in the same solvent, but with prior addition of ethyl alcohol to ensure solubilization. The quantity of ethyl alcohol must not exceed 5% of the total volume.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{21}H_{26}O_5$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 5 mg of prednisone to a 50 mL volumetric flask and add 30 mL of ethyl alcohol. Shake mechanically for 15 minutes and complete the volume with the same solvent. Homogenize and filter. Dilute, successively, in ethyl alcohol to concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 239 nm, using ethyl alcohol for zero adjustment. Calculate the quantity of $C_{21}H_{26}O_5$ in the tablets from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 420$, at 239 nm, in ethyl alcohol.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Proceed as described in Assay in the *Prednisone* monograph. Prepare the *Sample solution* as described below.

Sample Solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 20 mg of prednisone to a 100 mL volumetric flask and add 5 mL of water. Place in ultrasonic bath for one minute. Add 50 mL of methyl alcohol and place in ultrasonic bath for one more minute. Complete the volume with purified water and homogenize. Transfer 5 mL of this solution and 5 mL of the *Internal standard solution* to a 50 mL volumetric flask and complete the volume with mixture of methyl alcohol and water (1:2) to obtain a 20 µg/mL prednisone solution. Homogenize and filter.

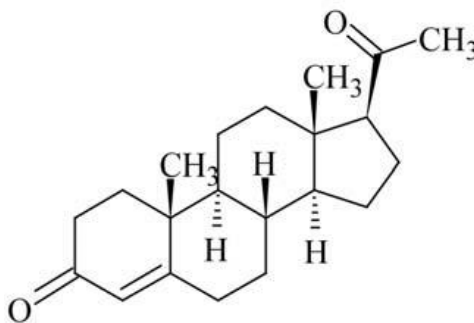
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{21}H_{26}O_5$ in the tablets from the responses obtained for the prednisone/acetanilide ratio with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

PROGESTERONE***Progesteronum***

$C_{21}H_{30}O_2$; 314.47
 progesterone; 07413
 Pregn-4-ene-3,20-dione
 [57-83-0]

It contains not less than 97.0% and not more than 103.0% of $C_{21}H_{30}O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, or slightly yellowish, crystalline powder. Stable on air.

Solubility. Practically insoluble in water, soluble in ethyl alcohol and in dioxane, slightly soluble in vegetable oils.

Physicochemical constants.

Melting range (5.2.2): 126 °C to 131 °C. It has a polymorph with melting point around 121 °C.

Specific optical rotation (5.2.8): +175 to +183, in relation to the desiccated substance. Determine in solution at 2% (w/v) in dioxane.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the progesterone CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in ethyl alcohol, there are maximum and minimum points only at the same wavelengths of similar progesterone CRS solution.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of chloroform and ethyl acetate (2:1) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.1 g of the sample in ethyl alcohol and complete to 10 mL with the same solvent.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL of ethyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram with *Solution (2)* (1,0%).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105 °C for two hours. Not more than 0.5%.

Residue by incineration (5.2.10). Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Quantitatively transfer about 20 mg of the sample to a 100 mL volumetric flask, dissolve and complete the volume with ethyl alcohol. Dilute successively, with the same solvent, to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 240 nm, using ethyl alcohol for zero adjustment. Calculate the content of C₂₁H₃₀O₂ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

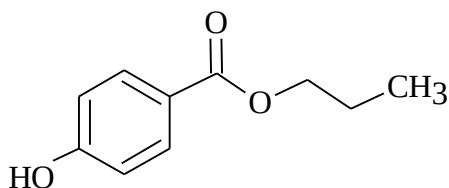
LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Progestin.

PROPYLPARABEN
Propylis parahydroxybenzoas



$C_{10}H_{12}O_3$; 180.20

propylparaben; 07461

4-hydroxybenzoic acid propyl ester.

[94-13-3]

It contains not less than 98.0% and not more than 102.0% of $C_{10}H_{12}O_3$.

DESCRIPTION

Physical characteristics. White, crystalline powder.

Solubility. Very slightly soluble in water, freely soluble in methyl alcohol, in ethyl alcohol and in ethyl ether.

Physicochemical constants.

Melting range (5.2.2): 96.0 °C to 99.0 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the propylparaben CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 230 nm to 280 nm, of 0.0005% (w/v) solution in ethyl alcohol, there is a maximum point at 258 nm. The absorbance at 258 nm is between 0.440 and 0.475.

PURITY TESTS

Preparation aspect. The 10% (w/v) preparation in ethyl alcohol is clear **(5.2.25)** and colorless **(5.2.12)**.

Acidity. To 2 mL of the solution described in *Preparation aspect*, add 3 mL of ethyl alcohol, 5 mL of carbon dioxide-free water and 0.1 mL of bromocresol green TS. Not more than 0.1 mL of 0.1 M sodium hydroxide is required to promote the indicator changeover.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and anhydrous formic acid, ethyl acetate and methylene chloride (2:10:88), as

mobile phase. Apply to the plate, separately, 5 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.1 g of the sample in methyl alcohol and dilute to 5 mL with the same solvent.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with methyl alcohol.

Develop chromatogram. Remove the plate, allow to dry under a stream of hot air and examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (1.0%).

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Accurately weigh about 1 g of the sample, transfer to an erlenmeyer flask with ground stopper and add 20 mL of *M* sodium hydroxide VS. Adapt reflux condenser and heat at 70 °C for one hour. Cool to room temperature. Titrate the excess *M* hydrochloric acid VS with 0.5 *M* sulfuric acid VS. Determine the endpoint potentiometrically, proceeding with titration until the second inflection point. Carry out the blank test and make the necessary corrections. Each mL of *M* sodium hydroxide VS is equivalent to 180.200 mg of C₁₀H₁₂O₃.

PACKAGING AND STORAGE

In tightly closed containers.

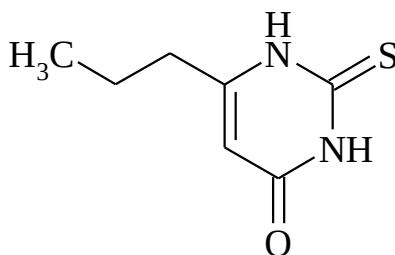
LABELING

Comply with current legislation.

CATEGORY

Preservative.

PROPYLTHIOURACIL
Propylthiouracilum



C₇H₁₀N₂OS; 170.23

propylthiouracil; 07462

3-dihydro-6-propyl-2-thioxopyrimidine-4(1*H*)-one
[51-52-5]

It contains not less than 98.0% and not more than 100.5% of C₇H₁₀N₂OS, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water and moderately soluble in ethyl alcohol. Soluble in alkaline solutions.

Physicochemical constants.

Melting range (5.2.2): 217 °C to 221 °C.

IDENTIFICATION

The Identification tests B. and C. can be omitted if test A. is carried out. The Identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the propylthiouracil CRS spectrum, prepared identically.

B. Dissolve about 20 mg of the sample in 8 mL of bromine water RS and shake for some minutes. Heat until mixture loses color, cool and filter. Add 2 mL of barium chloride at 61 g/L to the filtrate. A white precipitate is formed whose color does not turn violet after the addition of 5 mL of 8.5% (w/v) sodium hydroxide.

C. Examine the chromatograms obtained from the *Related substances* test under ultraviolet light at 254 nm before exposing the plate to iodine vapor. The main stain obtained with *Solution (2)* corresponds in position and intensity to the one obtained with *Solution (3)*.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄, with thickness of 250 µm as support, and mixture of chloroform, isopropyl alcohol and glacial acetic acid (50:6:0.1) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve about 100 mg of accurately weighed propylthiouracil in methyl alcohol and complete to 10 mL with the same solvent.

Solution (2): transfer 1 mL of *Solution (1)* to a 10 mL volumetric flask and complete the volume with methyl alcohol.

Solution (3): dissolve 10 mg of propylthiouracil CRS in methyl alcohol and complete to 10 mL with the same solvent.

Solution (4): dissolve 50 mg of thiourea in methyl alcohol and dilute to 100 mL with the same solvent. Transfer 1 mL to a 100 mL volumetric flask and complete the volume with methyl alcohol.

Solution (5): transfer 1 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with methyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light at 254 nm. Expose the plate to iodine vapors for 10 minutes. The stain obtained with *Solution (1)* chromatogram is not greater in intensity than the one corresponding in position obtained with *Solution (4)* (0.05%). Any other stain obtained from the chromatogram of *Solution (1)* chromatogram, apart from the one described above and the main one, does not have greater intensity than the stain obtained from the chromatogram of *Solution (5)* (1.0%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for two hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh approximately 300 mg of the sample and dissolve in mixture of 30 mL of 0.1 M sodium hydroxide VS and 30 mL of water, heat to boiling and shake the mixture until completely dissolved. Add 50 mL of 0.1 M silver nitrate with agitation and heat gently for five minutes. Allow to cool. Titrate with 0.1 M sodium hydroxide VS, determine the endpoint potentiometrically. The volume of 0.1 M sodium hydroxide VS used is equal to the sum of the volume initially added and

the volume used in the final titration. Each mL of 0.1 *M* sodium hydroxide VS is equivalent to 8.512 mg of C₇H₁₀N₂OS.

PACKAGING AND STORAGE

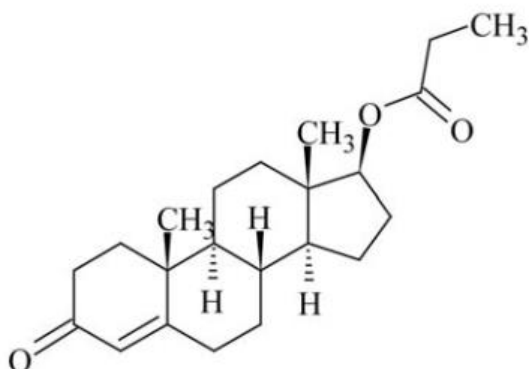
In tightly closed containers and protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antithyroid agent.

TESTOSTERONE PROPIONATE*Testosteroni propinas* $C_{22}H_{32}O_3$; 344.49

testosterone propionate; 08458

(17 β)-17-(1-Oxopropoxy)androst-4-en-3-one

[57-85-2]

It contains not less than 97.0% and not more than 103.0% of $C_{22}H_{32}O_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. white or off-white powder, or colorless crystals.

Solubility. Practically insoluble in water, freely soluble in ethyl alcohol, soluble in vegetable oils.

Physicochemical constants.

Melting range (5.2.2): 118 °C to 123 °C.

Specific optical rotation (5.2.8): +83 to +90, in relation to the desiccated substance. Determine in 1% (w/v) solution in absolute ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the testosterone propionate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in ethyl alcohol, there are maximum and minimum points only at the same wavelengths of similar testosterone propionate CRS solution. Absorbance values measured at 241 nm differ by not more than 3.0%.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel HF₂₅₄ as support and mixture of chloroform and diethylamine (19:1), as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 40 mg of the sample in 2 mL of ethyl alcohol.

Solution (2): dilute 1 mL of *Solution (1)* to 100 mL with ethyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram with *Solution (2)* (1,0%).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 105 °C for two hours. Not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 25 mg of the sample and dissolve in absolute ethyl alcohol. Complete the volume to 250 mL with the same solvent. Dilute 10 mL of the solution to 100 mL with absolute ethyl alcohol to obtain a 0.001% (w/v) solution. Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 240 nm, using absolute ethyl alcohol for zero adjustment. Calculate the content of C₂₂H₃₂O₃ in the sample from the readings obtained. Alternatively, calculate considering A (1%, 1 cm) = 490, at 240 nm, in absolute ethyl alcohol.

PACKAGING AND STORAGE

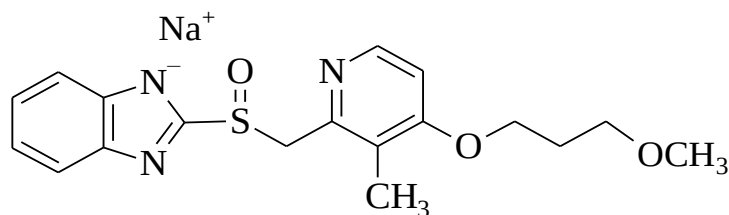
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Androgenic hormone.

RABEPRAZOLE SODIUM*Rabeprazoli natricum*C₁₈H₂₀N₃NaO₃S; 381.43

rabeprazole sodium; 07595

2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl]-1H-benzimidazole sodium salt
[117976-90-6]

It contains not less than 98.0% and not more than 102.0% of C₁₈H₂₀N₃NaO₃S in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or slightly yellow crystalline powder, hygroscopic.

Solubility. Very soluble in water and methyl alcohol, freely soluble in ethyl alcohol.

IDENTIFICATION

The Identification test B. can be omitted if test C. is carried out. The Identification test C. can be omitted if test B. is carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated at 120 °C, under reduced pressure for three hours, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the rabeprazole sodium CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 220 nm to 400 nm of the sample solution obtained in method A. from Assay, there are maximum and minimum points only at the same wavelengths observed in the standard solution spectrum.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method B. from Assay, corresponds to the one from the main peak of the *Standard solution*.

D. Dissolve 20 mg of the sample in 2 mL of water. The solution meets sodium ion reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described in method B. from Assay. Prepare *Solution (1)* as described below.

Solution (1): quantitatively transfer about 40 mg of the sample to a 100 mL volumetric flask and complete the volume with acetonitrile. Transfer 5 mL of the this solution to a 50 mL volumetric flask, complete the volume with methyl alcohol to obtain a 40 µg/mL rabeprazole sodium solution.

Procedure: inject 20 µL of *Solution (1)*, record the chromatograms and measure the areas under all the peaks obtained. The sum of the areas under the secondary peaks, except that under the solvent peak, is not more than 1.5% of the area under the main peak. No area is greater than 0.5% of the area under the main peak. Disregard peaks with areas below 0.05 times the area under the main peak. Do not include solvent-related peaks in the calculations.

Heavy metals (5.3.2.3). Use *Method I*. Not more than 0.001% (10 ppm).

Water (5.2.20.1). Determine in 0.3 g of sample. Not more than 7.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 100 mg of the sample and dissolve in water with pH previously adjusted to 10.0 with ammonium hydroxide RS. Complete the volume to 100 mL with the same solvent. Dilute successively, with the same solvent, to a concentration of 0.0012% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 291 nm, using water pH 10.0 for zero adjustment. Calculate the content of $C_{18}H_{20}N_3NaO_3S$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 282 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm) with inactivated base, kept at 30 °C; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of water and acetonitrile (65:35).

Sample solution: dissolve an accurately weighed quantity of acetonitrile to obtain a 0.4 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with acetonitrile, obtaining a 40 µg/mL solution.

Standard solution: dissolve an accurately weighed quantity of rabeprazole sodium CRS in acetonitrile, to obtain a 40 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with acetonitrile, obtaining a 40 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is not less than 2000 theoretical plates/meter. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.5%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{18}H_{20}N_3NaO_3S$ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antisecretory.

RABEPRAZOLE SODIUM TABLETS

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_{18}H_{20}N_3NaO_3S$. Tablets must be coated.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel 60 F₂₅₄, as support, and mixture of methyl alcohol and ethyl acetate (10:90) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize 10 tablets. Transfer quantity of powder equivalent to 50 mg of rabeprazole sodium to a 50 mL volumetric flask and add 40 mL of methyl alcohol. Place in ultrasonic bath at room temperature for 20 minutes, complete the volume with methyl alcohol, homogenize and filter.

Solution (2): 1 mg/mL rabeprazole sodium CRS solution in methyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. Expose to iodine vapors until stains appear. The stains are yellowish-brown.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test. Proceed as described in *Enteric coated tablets (gastro-resistant)*

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Acid stage:

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 120 minutes.

Buffer stage pH 9.0:

Dissolution medium: borate buffer pH 9.0, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 30 minutes.

Sample solution: after testing, remove aliquot from the dissolution medium, filter and dilute, if required, in acetonitrile to obtain a 11 µg/mL rabeprazole sodium solution.

Standard solution: quantitatively transfer about 27.5 mg of rabeprazole sodium CRS to a 50 mL volumetric flask, complete the volume with mixture of acetonitrile and borate buffer pH 9.0 (50:50) and homogenize. Transfer 1 mL of the this solution to a 50 mL volumetric flask, complete the volume with mixture of acetonitrile and borate buffer pH 9.0 (50:50) and homogenize to obtain a 11 µg/mL rabeprazole sodium solution.

Procedure: after the *Acid stage* test, transfer the tablets to tanks containing 900 mL of borate buffer pH 9.0 and carry out the *Buffer stage pH 9.0* test. After the test, remove aliquot from the dissolution medium, prepare *Sample solution* and proceed as described in *Assay*. Inject, separately, 20 µL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{18}H_{20}N_3NaO_3S$ dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 85% (Q) of the stated quantity of $C_{18}H_{20}N_3NaO_3S$ dissolve in 30 minutes.

PURITY TESTS

Related substances. Proceed as described in *Assay*. Prepare *Sample solution* as described below.

Sample solution: transfer 10 tablets to a 200 mL volumetric flask, add 20 mL of water pH 10.0, previously adjusted with ammonium hydroxide, and place in ultrasonic bath at room temperature for 20 minutes, complete the volume with acetonitrile, homogenize and filter on paper. Transfer 2 mL of this solution to a 25 mL volumetric flask, complete the volume with acetonitrile and homogenize, obtaining a solution at 40 µg/mL.

Procedure: Inject 20 µL of the *Sample Solution*, record the chromatograms and measure the areas under all the peaks obtained. The sum of the areas under the secondary peaks, except that under the solvent peak, is not more than 3.0% of the area under the main peak. No area is greater than 1.0% of the area under the main peak. Disregard peaks with areas below 0.05 times the area under the main peak. Do not include solvent-related peaks in the calculations.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 282 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm) with inactivated base, kept at 30 °C; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of water and acetonitrile (65:35).

Sample solution: transfer 10 tablets to a 200 mL volumetric flask, add 20 mL of water pH 10.0, previously adjusted with ammonium hydroxide, and place in ultrasonic bath at room temperature for 20 minutes. Complete the volume with acetonitrile, homogenize and filter on paper. Dilute an

aliquot of the resulting clear solution with acetonitrile to obtain a 40 µg/mL rabeprazole sodium solution.

Standard solution: dissolve an accurately weighed quantity of rabeprazole sodium CRS in acetonitrile, to obtain a 0.4 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with acetonitrile, obtaining a 40 µg/mL solution.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is not less than 2000 theoretical plates/meter. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.5%.

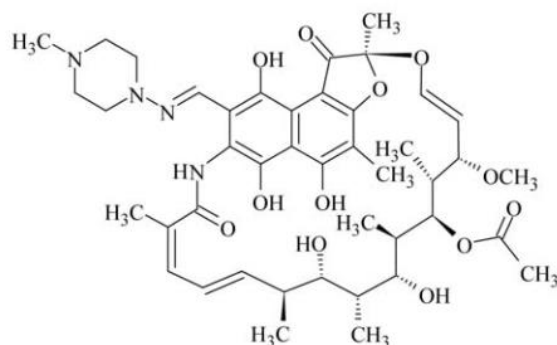
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{18}H_{20}N_3NaO_3S$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at room temperature.

LABELING

Comply with current legislation.

RIFAMPICIN***Rifampicinum***

$C_{43}H_{58}N_4O_{12}$; 822.95

rifampicin; 07719

3-[[[4-Methyl-1-piperazinyl]imino]methyl]rifamycin
[13292-46-1]

It contains not less than 97% and not more than 102% of $C_{43}H_{58}N_4O_{12}$.

DESCRIPTION

Physical characteristics. Reddish-brown to brownish-red crystalline powder.

Solubility. Slightly soluble in water, soluble in methyl alcohol, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated, dispersed in liquid paraffin-based paste, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the rifampicin CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), at the range of 220 nm to 500 nm, of the sample solution obtained in Assay, there are maximum absorption points at 237 nm, 254 nm, 334 nm and 475 nm. The ratio between the absorbance values measured at 334 nm and 475 nm is about 1.75.

C. Suspend about 25 mg of the sample in 25 mL of purified water. Shake for five minutes and filter. To 5 mL of the filtrate, add 1 mL of 10% (w/v) ammonium persulfate in a phosphate buffer solution pH 7.4 and shake for some minutes. The solution changes from orangish-yellow to red-violet without precipitate formation.

PURITY TESTS

pH (5.2.19). 4.5 to 6.5. Determine in 0.1% (w/v) of the sample suspension in carbon dioxide-free water.

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 100 mm of length

and 4.6 mm of internal diameter, packed with silica chemically bonded to octylsilane group (5 µm); *Mobile phase* flow rate of 1.5 mL/minute.

Phosphate buffer: dissolve 136.1 g of monobasic potassium phosphate in about 500 mL of water, add 6.3 mL of phosphoric acid, dilute with water to 1000 mL and homogenize.

Mobile phase: prepare mixture of water, acetonitrile, *Phosphate buffer*, *M* citric acid and 0.5 *M* sodium perchlorate (510:350:100:20:20), filter through a membrane filter with pores of 0.7 µm or less and degas. Make adjustments if necessary.

Diluent: prepare mixture of water, acetonitrile, *M* dibasic potassium phosphate, *M* monobasic potassium phosphate and *M* citric acid (640:250:77:23:10).

Solution (1): transfer about 0.2 g of the sample to a 100 mL volumetric flask, dissolve with acetonitrile, dilute and complete the volume. Place in ultrasonic bath for about 30 seconds, if necessary, to ensure complete dissolution. Use this solution within a maximum period of two hours.

Solution (2): transfer 5 mL of *Solution (1)* to a 50 mL volumetric flask, dilute with *Diluent*, complete the volume and homogenize. Prepare this solution immediately before use.

Solution (3): transfer 10 mL of *Solution (1)* to a 100 mL volumetric flask, dilute with acetonitrile, complete the volume and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, dilute with *Diluent*, complete the volume and homogenize. Prepare this final dilution immediately before use.

Resolution solution: dissolve previously weighed quantity of rifampicin CRS and rifampicin quinone CRS in acetonitrile to obtain a solution at 0.1 mg/mL of each. Transfer 1 mL of this solution to a 10 mL volumetric flask, dilute with *Diluent*, complete the volume and homogenize.

Inject 50 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.6 for rifampicin quinone and 1.0 for rifampicin. The resolution between the rifampicin and rifampicin quinone peaks is not less than 4.0.

Procedure: inject about 50 µL of the *Solution (2)* and of *Solution (3)*, record the chromatograms and measure the areas under the peaks. Calculate the percentage of each related substance by the formula:

$$r_{Ti}/\left(r_D + 0,01 \sum r_{Ti}\right)$$

where r_{Ti} is the area under the peak of each related substance in the chromatogram obtained with *Solution (2)*, r_D is the area under the rifampicin peak in the chromatogram obtained with *Solution (3)*, and $\sum r_{Ti}$ is the sum of the areas under all peaks of the related substances obtained from the chromatogram of *Solution (2)*: not more than 1.5% of rifampicin quinone is found; not more than 1.0% of any other related substance is found and a total of not more than 3.5% of the total individual related substances, apart from rifampicin quinone, having a retention time above 3.0 in relation to the rifampicin retention time, is found.

Heavy metals (5.3.2.3). Use *Method III*. Determine in 1 g of the sample. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 80 °C, at pressure of not more than 670 Pa, for four hours. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 2 g of the sample. Not more than 0.1%.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Dissolve 0.1 g of the sample in methyl alcohol and complete the volume to 100 mL with the same solvent. Dilute 2 mL of the solution to 100 mL with phosphate buffer pH 7.4. Measure the absorbance of the resulting solution at 475 nm, using phosphate buffer pH 7.4 for zero adjustment. Calculate the content of $C_{43}H_{58}N_4O_{12}$ in the sample considering $A(1\%, 1\text{ cm}) = 187$, at 475 nm, in phosphate buffer pH 7.4.

PACKAGING AND STORAGE

In tightly closed containers, protected from light at temperature not exceeding 25 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial (tuberculostatic).

RIFAMPICIN CAPSULES

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{43}H_{58}N_4O_{12}$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of chloroform and methyl alcohol (90:10) as mobile phase. Apply, separately, to the plate 3 μ L of each solution described below.

Solution (1): dissolve quantity of powder, equivalent to about 50 mg of rifampicin, with 5 mL of chloroform and filter.

Solution (2): 10 mg/mL sample solution in chloroform.

Develop chromatogram. Remove plate, allow to air dry. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Transfer the content of each capsule to a 100 mL volumetric flask. Wash the capsule body and cap with mixture of acetonitrile and methyl alcohol (1:1) and transfer to the volumetric flask. Dilute to obtain a 1.5 mg/mL solution. Place in ultrasonic bath for about five minutes and cool to room temperature. Transfer 10 mL of this solution to a 50 mL volumetric flask, complete the volume with *Diluent* and shake. Proceed as described in *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: baskets, 100 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in 0.1 M hydrochloric acid until adequate concentration. Measure the absorbances of the solutions at 475 nm (**5.2.14**), using the same solvent for zero adjustment. Calculate the quantity of $C_{43}H_{58}N_4O_{12}$ dissolved in the medium, comparing the readings obtained with that of the rifampicin CRS solution at a concentration of 0.0032% (w/v) prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated quantity of $C_{43}H_{58}N_4O_{12}$ dissolve in 45 minutes.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 100 mg of sample. Dry in oven at 60 °C, under reduced pressure, for three hours. Not more than 3.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; 250 mm long, 4.0 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Phosphate buffer: dissolve 136.1 g of monobasic potassium phosphate in about 500 mL of water, add 6.3 mL of phosphoric acid, dilute with water to 1000 mL and shake. Adjust the pH to 3.1 ± 0.1 .

Mobile phase: mixture of water, acetonitrile, *Phosphate buffer*, *M* citric acid and 0.5 *M* sodium perchlorate (510:350:100:20:20). Degas and filter.

Diluent: mixture of water, acetonitrile, *M* dibasic potassium phosphate, monobasic potassium phosphate and *M* citric acid (640:250:77:23:10).

Sample solution: weigh 20 capsules, remove the content and weigh again. Homogenize the content of the capsules. Quantitatively transfer quantity of powder equivalent to about 300 mg of rifampicin to a 200 mL volumetric flask, add about 180 mL of mixture of acetonitrile and methyl alcohol (1:1) and place in ultrasonic bath for five minutes. Transfer 10 mL of this solution to a 50 mL volumetric flask, complete the volume with acetonitrile and shake. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with *Diluent* and shake.

Standard solution: accurately weigh approximately 37.5 mg of rifampicin CRS, transfer to a 25 mL volumetric flask and complete the volume with mixture of acetonitrile and methyl alcohol (1:1). Transfer 10 mL of this solution to a 50 mL volumetric flask, complete the volume with acetonitrile and shake. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with *Diluent* and shake. Each mL of the *Standard Solution* contains approximately 0.03 mg of rifampicin CRS.

Resolution solution: dissolve an accurately weighed quantity of rifampicin quinone CRS in mixture of acetonitrile and methyl alcohol (1:1) to obtain a solution with a concentration of 0.1 mg/mL. Transfer 1.5 mL of this solution and 5 mL of rifampicin CRS solution at 0.3 mg/mL to a 50 mL volumetric flask, complete the volume with *Diluent* and shake.

Inject 50 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.6 for rifampicin quinone and 1.0 for rifampicin. The resolution between the rifampicin quinone and rifampicin peaks is not less than 4.0. Inject 50 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 50 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{21}H_{23}FCINO_2$ in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

RIFAMPICIN ORAL SUSPENSION

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{43}H_{58}N_4O_{12}$.

IDENTIFICATION

A. To 0.1 g of rifampicin, add 30 mL of water and shake with two portions of 50 mL of chloroform. Dry the combined extracts with anhydrous sodium sulfate, filter and evaporate the dry filtrate at a temperature not exceeding 70 °C. In the infrared absorption spectrum (**5.2.14**) of the residue, after washing with 1 mL of ethyl ether and drying at 70 °C, there are maximum absorption points at the same wavelengths and with the same relative intensities as those observed in the rifampicin CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**) in the range of 220 nm to 500 nm of the *Sample solution* obtained in method **B.** from *Assay*, there are maximum absorption points at 237 nm, 254 nm, 334 nm and 475 nm, identical to those observed in the spectrum of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.2 to 4.8. Determine in the reconstituted suspension as indicated on the label.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 120 mm of length and 4.6 mm of internal diameter, packed with silica gel chemically bonded to octylsilane group (5 µm); *Mobile phase* flow of 1.5 mL/minute.

Mobile phase: mixture of 35 volumes of acetonitrile and 65 volumes of a solution containing 0.1% (v/v) phosphoric acid, sodium perchlorate at 1.9 mg/mL, citric acid at 5.9 mg/mL and monobasic potassium phosphate at 20.9 mg/mL.

Diluent: mixture of citric acid solution at 210.1 mg/mL, monobasic potassium phosphate solution at 136.1 mg/mL, dibasic potassium phosphate solution at 174.2 mg/mL, acetonitrile and water (10:23:77:250:640).

Note: Prepare *Solutions (1), (2), (3), (4), (5) and (6)* immediately before use.

Solution (1): add 5 mL of water to quantity of oral suspension containing 20 mg of rifampicin and extract with four successive 10 mL portions of methylene chloride. Filter the combined extracts and evaporate to dryness at a temperature not exceeding 40 °C. Dissolve the residue in 10 mL of acetonitrile and dilute 5 mL of this solution to 50 mL with the *Diluent*. Homogenize.

Solution (2): transfer 1 mL of *Solution (1)* to a 100 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Solution (3): dissolve quantity, accurately weighed, of rifampicin quinone CRS in *Diluent* to obtain a solution at 0.3 mg/mL. Transfer 1 mL of this solution to a 100 mL volumetric flask and complete the volume with *Diluent*, obtaining a 3 µg/mL solution.

Solution (4): dissolve quantity, accurately weighed, of rifampicin N-oxide CRS in *Diluent* to obtain a 0.2 mg/mL solution. Transfer 1 mL of this solution to a 100 mL volumetric flask and complete the volume with *Diluent*, obtaining a 2 µg/mL solution.

Solution (5): dissolve quantity, accurately weighed, of 3-formylrifamycin CRS in *Diluent* to obtain a 0.1 mg/mL solution. Transfer 10 mL of this solution to a 100 mL volumetric flask and complete the volume with *Diluent*, obtaining a 10 µg/mL solution.

Solution (6): transfer 10 mL of *Solution (3)* to an erlenmeyer flask, add 5 mL of *Diluent* and homogenize. Transfer 5 mL of this solution to an erlenmeyer flask, add 5 mL of *Solution (2)* and homogenize.

Inject 20 µL replicates of the *Solution (6)*. Adjust the detector's sensitivity so that the height of the two main peaks is not less than half the scale. The resolution between the rifampicin and rifampicin quinone peaks is not less than 4.0. If necessary, adjust the acetonitrile concentration in the *Mobile Phase*.

Procedure: inject, separately, 20 µL of the *Solution (2)*, *Solution (3)*, *Solution (4)* and *Solution (5)*, record the chromatograms and measure the areas under the peaks. Inject 20 µL of *Solution (1)* and develop the chromatogram for not less than three times the rifampicin peak retention time. The area under the peak corresponding to rifampicin quinone is not greater than the area under the peak of the chromatogram obtained with *Solution (3)* (1.5%). The area under the peak corresponding to rifampicin N-oxide is not greater than the area under the peak of the chromatogram obtained with *Solution (4)* (1.0%); the area under the peak corresponding to 3-formylrifamycin is not greater than the area under the peak of the chromatogram obtained with *Solution (5)* (5.0%) and the area under any secondary peak is not greater than the area under the peak of the chromatogram obtained with *Solution (2)* (1.0%). Disregard any peak with retention time shorter than the rifampicin quinone peak.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Dilute volume of the oral suspension equivalent to 0.4 g of rifampicin in 500 mL of methyl alcohol and homogenize. Dilute 2 mL of this solution in 100 mL of phosphate buffer pH 7.0 and measure the absorbance at 475 nm. Calculate the content of C₄₃H₅₈N₄O₁₂ in the sample considering A(1%, 1 cm) = 187, at 475 nm, in phosphate buffer pH 7.0. Calculate the quantity of C₄₃H₅₈N₄O₁₂ in the oral suspension from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 100 mm of length and 4.6 mm of internal diameter packed with silica chemically bonded to octadecylsilane group (5 µm).

Phosphate buffer: dissolve 136.1 g of monobasic potassium phosphate in 500 mL of water, add 6.3 mL of phosphoric acid and homogenize. Complete the volume to 1000 mL with water and homogenize.

Mobile phase: mixture of water, acetonitrile, *Phosphate buffer*, *M* citric acid and 0.5 *M* sodium perchlorate (500:360:100:20:20). Filter through membrane with pores of 0.7 µm or smaller and degas.

Diluent: prepare mixture of acetonitrile and water (1:1).

Sample solution: shake the flask containing the sample and immediately transfer 5 mL of the bubble-free oral suspension to a 100 mL volumetric flask. Dissolve, complete the volume with *Diluent* and homogenize. Transfer 5 mL of the resulting solution to a 50 mL volumetric flask, complete the volume with *Diluent* and homogenize.

Standard solution: dissolve an accurately weighed quantity of rifampicin CRS in *Diluent* to obtain a 0.5 mg/mL solution. Place in ultrasonic bath for about 30 seconds, if necessary, to dissolve. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with *Diluent* and homogenize. Use this preparation within not more than one hour.

Resolution solution: dissolve adequate quantities of rifampicin CRS and rifampicin quinone CRS in acetonitrile to obtain a solution at 0.1 mg/mL of each. Transfer 1 mL of this solution to a 10 mL volumetric flask, dilute and complete the volume with mixture of water, acetonitrile, *M* dibasic potassium phosphate, *M* monobasic potassium phosphate and *M* citric acid (640:250:77:23:10). Homogenize.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.6 for rifampicin quinone and 1.0 for rifampicin. The resolution between the rifampicin and rifampicin quinone peaks is not less than 4.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₄₃H₅₈N₄O₁₂ in the oral suspension from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation

RITONAVIR CAPSULES

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{37}H_{48}N_6O_5S_2$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.7% (w/v) sodium lauryl sulfate, 900 mL.

Apparatus: paddles, 25 rpm Use paddles coated with inert material.

Times: 60 and 120 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute until adequate concentration. Proceed as described in *Assay*. Inject, separately, 20 μ L of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{37}H_{48}N_6O_5S_2$ dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 40% (Q) of the stated quantity of $C_{37}H_{48}N_6O_5S_2$ dissolve in 60 minutes and not less than 75% (Q) of the stated quantity of $C_{37}H_{48}N_6O_5S_2$ dissolve in 120 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 210 nm; column with 125 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of methyl alcohol and water (67:23).

Sample solution: weigh 20 capsules, remove the content and weigh again. Quantitatively transfer the equivalent to 20 mg of ritonavir to a 100 mL volumetric flask, add 70 mL of *Mobile phase*, shake

and complete the volume with the same solvent. Homogenize and filter, obtaining a 0.2 mg/mL solution.

Standard solution: dissolve an accurately weighed quantity of ritonavir CRS in *Mobile phase* to obtain a 0.2 mg/mL solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₃₇H₄₈N₆O₅S₂ in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

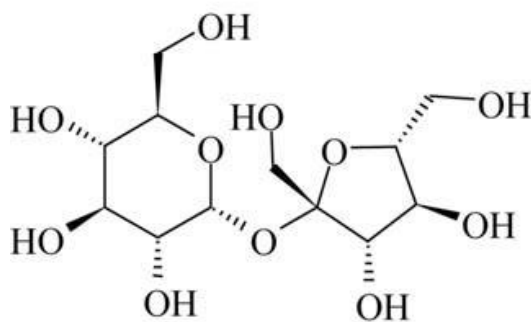
In tightly closed containers.

LABELING

Comply with current legislation.

SUCROSE

Saccharum



$C_{12}H_{22}O_{11}$; 342.30

sucrose; 07854

β -D-Fructofuranosyl- α -D-glycopyranoside

[57-50-1]

Sugar obtained from *Saccharum officinarum* L. (POACEAE), *Beta vulgaris* L. (CHENOPODIACEAE) and other sources.

DESCRIPTION

Physical characteristics. White or colorless crystalline powder or crystalline mass, odorless and sweet.

Solubility. Very soluble in water, freely soluble in 70% (v/v) ethyl alcohol, insoluble in chloroform and ethyl ether.

Physicochemical constants.

Specific optical rotation (5.2.8): not less than +65.9 in relation to the desiccated substance at 105 °C for two hours. Determine in aqueous solution at 26% (w/v).

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of water, methyl alcohol, glacial acetic acid and ethylene chloride (10:15:25:50) as mobile phase. Apply, separately, to the plate 2 μ L of each solution described below, drying each application point.

Solution (1): dissolve 10 mg of the sample in mixture of water and methyl alcohol. (2:3). Complete the volume to 20 mL with the same mixture of solvents.

Solution (2): dissolve 10 mg of sucrose CRS in mixture of water and methyl alcohol. (2:3). Complete the volume to 20 mL with the same mixture of solvents.

Solution (3): dissolve 10 mg of glucose CRS, lactose CRS, fructose CRS and sucrose CRS in mixture of water and methyl alcohol (2:3) and complete to 20 mL with the same solvent mixture.

Develop the chromatogram until the front of the mobile phase ascends 17 cm above the application line. Remove the plate and dry in hot air. Spray with a 0.5% (w/v) thymol solution in mixture of ethyl alcohol and sulfuric acid (95:5). Heat the plate at 130 °C for 10 minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. The *Solution (3)* chromatogram must present four clearly separated stains.

B. Dilute 1 mL of the preparation obtained in *Preparation aspect* to 100 mL with water. To 5 mL of this solution, add 2 mL of freshly prepared 2 M sodium hydroxide solution and 0.15 mL of 10% (w/v) cupric sulfate solution in water. The resulting preparation is blue and clear, remaining unchanged under heating. To the hot preparation, add 4 mL of hydrochloric acid RS solution, heat to boiling and add 4 mL of 2 M sodium hydroxide solution. An orange precipitate forms immediately.

PURITY TESTS

Preparation aspect. Dissolve 150 g of the sample in carbon dioxide-free distilled water and complete the volume to 300 mL. The preparation obtained is clear (**5.2.25**), odorless and does not stain more than the *SC F Color Standard Solution* diluted in water at a 1:4 ratio (**5.2.12**).

Alkalinity. Add 0.3 mL of phenolphthalein TS to 10 mL of the preparation obtained in *Preparation aspect*. The preparation is colorless. Not more than 0.3 mL of 0.01 M sodium hydroxide is required to turn the indicator to pink.

Dextrins. To 2 mL of the preparation obtained in *Preparation aspect*, add 8 mL of water, 0.05 mL of 2 M hydrochloric acid and 0.05 mL of 0.1 M iodine solution. The preparation remains yellow.

Glucose and invert sugar. Dissolve 20 g of the sample in water, complete the volume to 100 mL and filter, if necessary. Transfer 50 mL of the clear liquid to a 250 mL beaker, add 50 mL of alkaline cupric tartrate RS, cover the beaker with watch glass and heat the mixture to boiling in approximately four minutes. Continue to boil for exactly two minutes. Add, immediately, 100 mL of freshly boiled cold water and collect the cuprous oxide precipitate in a tared funnel with a medium pore filter plate. Wash the filter residue with hot water, followed by 10 mL of ethyl alcohol and finally by 10 mL of ethyl ether. Dry at 105°C for one hour. The cuprous oxide weight is not more than 0.112 g.

Stained substances. Filter 100 mL of the preparation obtained in *Preparation aspect*, using a glass plate with average pore of 1 µm and diameter of 24 mm. The filter does not stain blue. To 100 mL of the preparation obtained in *Preparation aspect*, stored in test tube, add 1 mL of diluted hypophosphorous acid. Cap the tube. No unpleasant odor is noticed within an hour. When examined under ultraviolet light (365 nm), the preparation obtained in *Preparation aspect* does not show more intense fluorescence than the reference solution, containing 0.4 ppm of quinine sulfate in 0.005 M sulfuric acid.

Barium. To 10 mL of the preparation obtained in *Preparation aspect*, add 1 mL of 4 M sulfuric acid. After one hour, any opalescence developed in the preparation is not more intense than that of the preparation obtained in *Preparation aspect*, diluted in distilled water at a 10:1 ratio.

Sulfites limit. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Dissolve 5 g of the sample in 40 mL of water, add 2 mL of M sodium hydroxide and complete to 50 mL with water - use as *Sample solution*. Separately, dissolve 76 mg of sodium metabisulfite and complete to 50 mL with water; pipet 5 mL of this solution and complete with water to 100 mL. Pipet 3 mL of this solution and complete with water to 100 mL - use this solution as standard. Separately, pipet 10 mL of the

Sample solution and the *Standard solution*, add 1 mL of 3 M hydrochloric acid, 2 mL of discolored fuchsin RS and 2 mL of formaldehyde solution, allowing to stand for 30 minutes. Prepare blank in parallel, using 10 mL of water and the same reagents, in the same quantities. Measure the absorbances of the solutions at 583 nm. The absorbance of the *Sample solution* is not greater than the absorbance of the *Standard solution*. Not more than 0.0015% (15 ppm) of SO₂. If the *Standard Solution* does not show a red-purple to blue-purple color, the test result is invalid.

Heavy metals (5.3.2.3). Not more than 0.0005% (5 ppm).

Residue by incineration (5.2.10). Dissolve 5 g of the sample in 5 mL of water, add 2 mL of sulfuric acid, evaporate to dryness and incinerate to constant weight. Not more than 0.02%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

PACKAGING AND STORAGE

In hermetic containers.

LABELING

Comply with current legislation

CATEGORY

Coating agent; diluent for capsules and tablets; excipient for syrups.

ORAL REHYDRATION SALTS

Salts for oral rehydration consist of a dry mixture of sodium chloride, sodium bicarbonate and glucose. Alternatively, sodium bicarbonate can be replaced by sodium citrate (anhydrous or dihydrate). It may contain glucose monohydrate in place of the anhydrous form, as long as the sodium bicarbonate or sodium citrate are packaged separately, following the main content. It presents characteristic flavor.

It contains not less than 90.0% and not more than 110.0% of sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) or citrate ($\text{C}_6\text{H}_5\text{O}_7^{3-}$), in relation to indicated quantity of sodium chloride, potassium chloride and sodium bicarbonate [or sodium citrate (anhydrous or dihydrate)].

It contains not less than 90.0% and not more than 110.0% of the declared quantity of glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) or glucose monohydrate ($\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$).

IDENTIFICATION

- A.** It meets sodium ion reactions (5.3.1.1).
- B.** It meets potassium ion reactions (5.3.1.1).
- C.** It meets chloride ion reactions (5.3.1.1).
- D.** It meets bicarbonate ion reactions, if present (5.3.1.1).
- E.** It meets citrate ion reactions, if present (5.3.1.1). Use three to five drops of the reconstituted solution, as indicated on the label, and 20 mL of acetic anhydride-pyridine RS.
- F.** Add some drops of the reconstituted solution, as indicated on the label, to 5 mL of hot alkaline cupric tartrate RS. A large quantity of red precipitate with cuprous oxide is produced.
- G.** When heated, the mixture melts, expands, and chars, producing odor of burned sugar.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 50 °C, until constant weight. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Glucose.

Weigh, accurately, portion of the sample equivalent to about 20 g of glucose, transfer to a 100 mL volumetric flask and complete the volume with water. Transfer 50 mL of this solution to a 100 mL

volumetric flask. Add 0.2 mL of 6 M ammonium hydroxide and complete the volume with water. If the preparation is turbid, filter on filter paper. If this is not enough, add activated charcoal mesh ASTM (20-35) from 0.5 mm to 0.75 mm, filter again through filter paper and finally filter through a 0.45 µm membrane. Determine the specific optical rotation **(5.2.8)** at 25 °C. Calculate the quantity, in grams, of glucose ($C_6H_{12}O_6$) in the salt for oral rehydration, considering 52.7 as the specific optical rotation at 25 °C. When the label indicates glucose monohydrate ($C_6H_{12}O_6 \cdot H_2O$), consider 47.9 as the specific optical rotation at 25 °C.

Sodium.

Proceed as described in *Atomic absorption spectrometry (5.2.13.1.1)*, use *Method I*. Use the following conditions: air-acetylene flame and 589.6 nm wavelength. Dissolve an accurately weighed quantity of the sample, equivalent to about 25 mg of sodium, in 50 mL of water. Then dilute by a factor of 500 times with water. Prepare 1 g/L sodium standard stock solution in water using analytical grade sodium chloride (NaCl). Plot the analytical curve with the sodium standard solutions at the following concentrations: 0.25 mg/L; 0.5 mg/L; 1.0 mg/L; 2.0 mg/L and 2.5 mg/L. Prepare the standard solutions by sequential dilution in water. Add to the standard solution and to the sample solution quantity equivalent to 0.5% (v/v) of a 10 g/L cesium chloride solution (CsCl).

Potassium.

Proceed as described in *Atomic absorption spectrometry (5.2.13.1.1)*, use *Method I*. Use the following conditions: air-acetylene flame and 769.9 nm wavelength. Dissolve an accurately weighed quantity of the sample, equivalent to about 25 mg of potassium, in 50 mL of water. Then dilute by a factor of 500 times with water. Prepare 1 g/L potassium standard stock solution in water, using analytical grade potassium chloride (KCl). Plot the analytical curve with the potassium standard solutions at the following concentrations: 0.25 mg/L; 0.5 mg/L; 1.0 mg/L; 2.0 mg/L and 2.5 mg/L. Prepare the standard solutions by sequential dilution in water. Add to the standard solution and to the sample solution quantity equivalent to 0.5% (v/v) of a 10 g/L cesium chloride solution (CsCl).

Bicarbonate (if present).

Dissolve an accurately weighed quantity of the sample, equivalent to about 0.1 g of bicarbonate (HCO_3^-) (consider that each milligram of sodium bicarbonate is equivalent to 0.726 mg of HCO_3^-) in 100 mL of water. Add three drops of methyl orange TS and titrate with 0.1 M hydrochloric acid VS. Each mL of 0.1 M hydrochloric acid VS is equivalent to 6.101 mg of bicarbonate (HCO_3^-).

Chloride.

Dissolve an accurately weighed quantity of the sample, equivalent to approximately 55 mg of chloride (Cl^-) (consider that each milligram of potassium chloride and sodium chloride is equivalent, respectively, to 0.476 mg and 0.607 mg of Cl^-), transfer to a 250 mL beaker, add 100 mL of water and 10 mL of M nitric acid. Shake until dissolution and titrate with 0.1 M silver nitrate VS, determining the endpoint potentiometrically, using silver-silver chloride electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 3.545 mg of chloride.

Citrate (if present).

Dissolve an accurately weighed quantity of the sample, equivalent to 0.1 g of citrate ($C_6H_5O_7^{3-}$), in 80 mL of glacial acetic acid. Heat to about 50 °C, cool, dilute to 100 mL with glacial acetic acid and

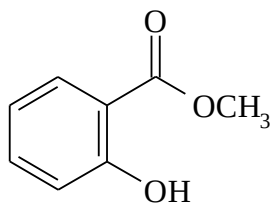
then allow to stand for 10 minutes. Potentiometrically titrate 20 mL of this solution with 0.1 *M* perchloric acid VS. Each mL of 0.1 *M* perchloric acid VS is equivalent to 6.303 mg of citrate ($\text{C}_6\text{H}_5\text{O}_7^{3-}$). Each mg of sodium citrate is equivalent to 0.643 mg of citrate ($\text{C}_6\text{H}_5\text{O}_7^{3-}$).

PACKAGING AND STORAGE

In tightly closed containers and at temperature below 30 °C. Sodium bicarbonate and sodium citrate components can be omitted from the mixture and packaged separately, following the main content.

LABELING

Comply with current legislation.

METHYL SALICYLATE*Methylis salicylas* $C_8H_8O_3$; 152.15

methyl salicylate; 00344

Methyl 2-hydroxybenzoate

[119-36-8]

It contains not less than 98.0% and not more than 100.5% of $C_8H_8O_3$.

DESCRIPTION

Physical characteristics. Colorless liquid, slightly yellowish, with a characteristic odor.

Solubility. Slightly soluble in water, miscible with ethyl alcohol, chloroform, glacial acetic acid, fatty acids and volatile oils.

Physicochemical constants.

Relative density (5.2.5): 1.180 to 1.186.

Refractive index (5.2.6): 1.535 to 1.538.

Boiling range (5.2.3): 221 °C to 225 °C.

IDENTIFICATION

A. Heat for five minutes, in water bath, mixture of 0.25 mL of the sample and 2 mL of sodium hydroxide RS. Add 3 mL of 5.5% (v/v) sulfuric acid. A white crystalline precipitate is produced. Filter, wash the residue with water and dry at 105 °C. The residue obtained melts **(5.2.2)** between 156°C and 161 °C.

B. Mix one drop of the sample with 5 mL of water. Add one or two drop of ferric chloride RS. Violet color develops.

C. Heat some drops of the sample with 5 mL of cupric sulfate RS. Green color develops.

PURITY TESTS

Preparation aspect. Add 10 mL of ethyl alcohol to 2 mL of the sample. The preparation is clear **(5.2.25)** and not more stained than the *Reference solution (5.2.12)* described below.

Reference solution: mix 2.5 mL of *Ferric chloride base solution*, 0.6 mL of *Cobalt II chloride base solution* and 7 mL of 1% (v/v) hydrochloric acid. Dilute 2.5 mL of this solution to 100 mL using 1% (v/v) hydrochloric acid.

Acidity. Dissolve 5 g of the sample in 50 mL of ethyl alcohol, previously neutralized with 0.1 M sodium hydroxide VS, using bromocresol green TS until blue color. Not more than 0.4 mL of 0.1 M sodium hydroxide VS is required to restore the blue color.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

BIOLOGICAL SAFETY TESTS.

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.5 g of the sample and dissolve in 25 mL of ethyl alcohol. Add 0.05 mL of phenol red TS and neutralize with 0.1 M sodium hydroxide VS. Add 50 mL of 0.1 M sodium hydroxide VS to the neutralized solution. Heat in water bath, under reflux, for 30 minutes and cool. Titrate with 0.1 M hydrochloric acid VS. Calculate the volume of 0.1 M sodium hydroxide VS required in the saponification. Carry out the blank test and make the necessary corrections. The difference between the titrations represents the quantity of sodium hydroxide required in the saponification. Each mL of 0.1 M sodium hydroxide VS is equivalent to 15.215 mg of $C_8H_8O_3$.

PACKAGING AND STORAGE

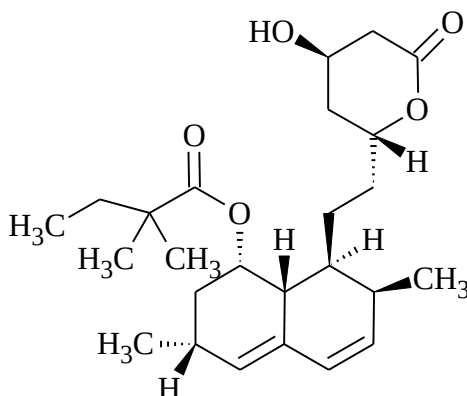
In hermetically closed containers, protected from light and heat.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Analgesic and antirheumatic.

SIMVASTATIN***Simvastatinum***

$C_{25}H_{38}O_5$; 418.57

simvastatin; 08016

(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl-2,2-dimethylbutanoate 3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalene-1-yl
[79902-63-9]

It contains not less than 98.0% and not more than 102.0% of $C_{25}H_{38}O_5$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water and freely soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): between +285 to +298. Determine in solution at 0.5% (w/v) in acetonitrile.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the non-desiccated sample dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the simvastatin CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related substances. Proceed as described in *Assay*. *Procedure:* inject, separately, 5 μ L of the *Sample solution*, record the chromatograms and measure the areas under the peaks. Identify the impurities specified in the table below. Measure the area under all the peaks and calculate the percentage of

each impurity and the total simvastatin impurities. Disregard peaks with areas with less than 0.05% of the area under the main peak.

<i>Related compound</i>	<i>Relative retention time</i>	<i>Limit (%)</i>
simvastatin hydroxy acid	0.45	0.4
epilovastatin and lovastatin	0.60	1.0
methylenesimvastatin	0.80	0.4
simvastatin	1.00	-
acetylsimvastatin	2.38	0.4
anhydrosimvastatin	2.42	0.4
simvastatin dimer	3.80	0.4
other individual impurities	-	0.1
total impurities except lovastatin and epilovastatin	-	1.0

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample, in oven at 60 °C, under reduced pressure, for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 238 nm; column with 33 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 3 mL/minute.

Note: *Simvastatin solutions are stable for three days when stored at up to 4°C. Without refrigeration, they must be injected immediately after preparation.*

Buffer solution: dissolve 1.4 g of monobasic potassium phosphate in 1000 mL of water and adjust the pH to 4.0 ± 0.1 with phosphoric acid R.

Diluent: mixture of acetonitrile and *Buffer solution* (3:2).

Diluted phosphoric acid: transfer 1 mL of concentrated phosphoric acid to a 1000 mL volumetric flask; complete the volume with water and homogenize.

Eluent A: mixture of acetonitrile and *Diluted phosphoric acid* (50:50).

Eluent B: transfer 1 mL of concentrated phosphoric acid to a 1000 mL volumetric flask; complete the volume with acetonitrile and homogenize.

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent (A) (%)</i>	<i>Eluent (B) (%)</i>	<i>Elution</i>
0 – 4.5	100	0	isocratic
4.5 – 4.6	100 → 95	0 → 5	linear gradient
4.6 – 8.0	95 → 25	5 → 75	linear gradient
8.0 – 11.5	25	75	linear gradient
11.5 – 11.6	25 → 100	75 → 0	isocratic
11.6 – 13.0	100	0	isocratic

Sample solution: quantitatively transfer about 75 mg of simvastatin to a 50 mL volumetric flask, dissolve in about 30 mL of *Diluent*, complete the volume with the same solvent and homogenize.

Standard solution: dissolve an accurately weighed quantity of simvastatin CRS in *Diluent* to obtain a 1.5 mg/mL solution.

Resolution solution: obtain a 1.5 mg/mL simvastatin CRS solution and a 0.015 mg/mL lovastatin CRS solution in *Diluent*.

Inject 5 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.6 for lovastatin and 1.0 for simvastatin. The resolution between the simvastatin and lovastatin peaks is not less than 2.0. Inject 5 µL replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 5 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₅H₃₈O₅ in the sample, from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In opaque, tightly closed containers, under nitrogen atmosphere or under refrigeration, and protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Lipid-lowering agent.

SIMVASTATIN CAPSULES

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{25}H_{38}O_5$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Weigh each capsule individually, transfer the content to a 50 mL volumetric flask and weigh again. Add 25 mL of methyl alcohol. Proceed as described in *Assay* from "Place in ultrasonic bath for 10 minutes...".

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.5% (w/v) sodium lauryl sulfate in 0.01 M aqueous monobasic sodium phosphate solution, 900 mL, pH 7.0.

Apparatus: paddles, 75 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute, if necessary, in dissolution medium until adequate concentration and proceed as described in *Assay*. Calculate the quantity of $C_{25}H_{38}O_5$ dissolved in the medium, comparing the areas obtained with the simvastatin solution CRS at 22.2 µg/mL, prepared with the same diluent.

Tolerance: not less than 75% (Q) of the stated quantity of $C_{25}H_{38}O_5$ dissolve in 45 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 238 nm; 250 mm long and 4.0 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm); kept at 30 °C, *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: methyl alcohol and 0.1% (v/v) phosphoric acid (80:20).

Sample solution: weigh 20 capsules, remove the content and weigh again. Transfer accurately weighed quantity of powder from the capsules content to a volumetric flask of adequate capacity, add methyl alcohol up to approximately 70% of the total capacity of the volumetric flask and place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, homogenize and centrifuge for four minutes at 4000 rpm. Dilute the appropriate volume of the supernatant with acetonitrile to obtain a 40 µg/mL solution.

Standard solution: dissolve an accurately weighed quantity of simvastatin CRS in methyl alcohol to obtain a 0.4 mg/mL solution. Dilute with the same solvent to obtain a 40 µg/mL solution.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₂₅H₃₈O₅ in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

SIMVASTATIN TABLETS

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $C_{25}H_{38}O_5$. The tablets must be coated.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Weigh individually and transfer each tablet to a 50 mL volumetric flask, add not more than 2 mL of water (not more than 4% of the volumetric flask capacity). Shake until the tablet disintegrates. Add 35 mL of methyl alcohol, place in ultrasonic bath for 15 minutes and complete the volume with the same solvent. Centrifuge for four minutes at 4000 rpm. Dilute adequate volume of the supernatant to a concentration of 0.0008% (w/v), using methyl alcohol as solvent. Prepare a standard solution in the same concentration, using the same solvent. Obtain the second derivative spectrum and measure the maximum amplitude of the solutions at 247 nm, using methyl alcohol for zero adjustment. Alternatively, obtain the spectrum in the range of 200 nm to 300 nm, record the absorbances in 1 nm increments. Calculate the second-order derivative and measure the absorbance at 247 nm. Calculate the quantity of $C_{25}H_{38}O_5$ in the tablets from the readings obtained.

DISSOLUTION TEST (5.1.5)

Dissolution medium: solution containing 0.5% (w/v) sodium lauryl sulfate and 0.01 M monobasic sodium phosphate, prepared as follows: dissolve 30 g of sodium lauryl sulfate and 8.28 g of monobasic sodium phosphate in 6000 mL of water and adjust the pH to 7.0 with 50% (w/v) sodium hydroxide solution, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute, if necessary, with dissolution medium until adequate concentration. Obtain the second derivative spectrum and measure the maximum amplitude of the solutions at 248 nm, using dissolution medium for zero adjustment. Alternatively, obtain the spectrum in the range of 200 nm to 300 nm, record the absorbances in 1 nm increments. Calculate the second-order derivative and measure the absorbance at 248 nm, using the same solvent for zero adjustment. Calculate the quantity of

C₂₅H₃₈O₅ dissolved in the medium, comparing the readings obtained with that of the simvastatin CRS solution at the concentration of 0.0008% (w/v), prepared in the same solvent.

Tolerance: not less than 75% (Q) of the stated quantity of C₂₅H₃₈O₅ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder to a volumetric flask of adequate capacity, add methyl alcohol up to approximately 70% of the total capacity of the volumetric flask and place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent. Centrifuge for four minutes at 4000 rpm. Dilute adequate volume of the supernatant to concentration of 0.0008% (w/v), using methyl alcohol as solvent. Prepare a standard solution in the same concentration, using the same solvent. Obtain the second derivative spectrum and measure the maximum amplitude of the solutions at 247 nm, using methyl alcohol for zero adjustment. Alternatively, obtain the spectrum in the range of 200 nm to 300 nm, record the absorbances in 1 nm increments. Calculate the second-order derivative and measure the absorbance at 247 nm. Calculate the quantity of C₂₅H₃₈O₅ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 238 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm); kept at 30 °C, *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: mixture of acetonitrile and 0.1% (v/v) phosphoric acid (65:35).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder to a volumetric flask of adequate capacity, add acetonitrile up to approximately 70% of the total capacity of the volumetric flask and place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent, homogenize and centrifuge for four minutes at 4000 rpm. Dilute adequate volume of the supernatant to concentration of 40 µg/mL, using acetonitrile as solvent.

Standard solution: dissolve an accurately weighed quantity of simvastatin CRS in acetonitrile to obtain a 0.4 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with acetonitrile, obtaining a 40 µg/mL solution.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₂₅H₃₈O₅ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

ORGAN PRESERVATION SOLUTIONS

Solutiones ad conservationem partium corporis

Organ preservation solutions are aqueous and sterile preparations used for the preservation, protection and/or perfusion of mammalian organs intended for transplantation.

They contain electrolytes, usually in a concentration close to the intracellular electrolyte composition.

They may contain carbohydrates (such as glucose or mannitol); amino acids; calcium complexing agents (such as citrates or phosphates); hydrocolloids (such as starch or gelatin derivatives); as well as other auxiliary substances aimed, for example, to make the preparation isotonic with blood serum; to adjust or stabilize the pH, or to prevent component degradation; these auxiliary substances do not impair the desired action for the preparation and, at chosen concentrations, do not have a toxic effect or cause abnormal local irritation. Organ conservation solutions can also contain active ingredients or active ingredients can be added immediately before use.

Examined under appropriate visibility conditions, organ preservation solutions are practically particle-free.

Organ preservation solutions may also be in the form of concentrated solutions to be diluted, immediately before use, with an appropriate liquid to a prescribed volume. Once diluted, these solutions meet the requirements established for organ preservation solutions.

They are prepared from products and by methods that ensure sterility; prevent the introduction of contaminants and the growth of microorganisms; related recommendations are given in the text *Sterile products* (8.1).

Unless for justified and authorized exceptions, they are prepared with *Water for injections* and do not contain antimicrobial preservatives.

CHARACTERISTICS

pH (5.2.19). 5.0 to 8.0. Carry out the test at room temperature.

Osmolality (5.2.28). Between 250 and 380 mosmole/kg.

PURITY TESTS

Hydroxymethylfurfural. If the sample contains glucose, it meets the hydroxymethylfurfural test. Proceed as described in *Ultraviolet absorption spectrophotometry* (5.2.14). Transfer solution volume containing equivalent to 25 mg of glucose to a suitable container. Add 5.0 mL of 100 g/L *p*-toluidine reagent solution in isopropyl alcohol containing 10% v/v glacial acetic acid and then 1.0 mL of 5 g/L barbituric acid reagent solution. Allow the mixture to stand for two to three minutes. The absorbance at 550 nm is not greater than that of a standard, prepared simultaneously and under the same conditions, replacing the sample with the same volume of a reagent solution containing 10 µg of hydroxymethylfurfural.

Contamination by particles (5.1.7.1). Carry out the test for sub-visible particles in 50 mL of the sample. The solution contains not more than, per milliliter, 50 particles greater than 10 µm in

diameter and five particles greater than 25 µm in diameter. Labeled products stating they are used with a terminal filter are exempt from these requirements.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 0.5 EU/mL of sample.

Note: *for solutions to which no validated bacterial endotoxin detection method can be applied, the sample complies with the additional test below.*

Pyrogens (5.5.2.1). Complies with the test. Unless justified and authorized exception, inject 10 mL/kg of rabbit mass.

PACKAGING AND STORAGE

The solutions for organ preservation are stored in *Containers for medicines and related (6)*. Sealing of these containers is ensured by appropriate means. Lids ensure sealing; they prevent the penetration of microorganisms and any other contaminating agent and usually enable, without being displaced, to fully or partly collect the package content. In the plastic material or elastomer that constitutes this closure, there is a strength and an elasticity adapted to the penetration of a needle, not releasing, as much as possible, fragments.

Organ preservation solutions must be cooled before use to a temperature below room temperature (usually between 2°C and 6°C) to lower the organ temperature and reduce its metabolism.

LABELING

Comply with current legislation. The label must state that the solution should not be used by injection; the formula of the organ preservation solution must be expressed in grams per liter and in millimoles per liter; the nominal volume of the organ preservation solution contained in the container; osmolality, expressed in milliosmoles per kilogram; that unused quantities of the ready-to-use solution, concentrated solution or diluted solution should be discarded; the storage conditions and, when suitable, that the solution should be used with a terminal filter. In the case of a concentrated solution, indicate on the label that the solution must be diluted immediately before use, using an appropriate liquid.

SOLUTIONS FOR PERITONEAL DIALYSIS

Soluciones ad peritonealem dialysim

Peritoneal dialysis solutions are preparations containing electrolytes with a concentration close to plasma electrolyte composition. They contain glucose in varying concentrations or other suitable osmotic agents.

They must be supplied in plastic, rigid or semi-rigid containers; flexible plastic containers equipped with special connection device (containing a volume lower than their nominal capacity and in closed system) or in glass containers.

Containers made of plastic material and their caps meet the requirements for *Plastic Containers (6.2)* and *Elastomer Caps (6.2.2)*.

Various formulations can be used. The concentrations of the components per liter of solution are generally within the limits listed on **Table 1**. This table also describes the specification limits for each of the components, based on the labeled values.

Table 1 – Concentration of components per liter of solution and specification limits.

<i>Solution components</i>	<i>Concentration in mmole/L</i>	<i>Concentration in mEq/L</i>	<i>Specification limit (%)</i>
Sodium	125-150	125-150	97.5 to 102.5.
Potassium	0-4.5	0-4.5	95.0 to 105.0.
Calcium	0-2.5	0-5.0	95.0 to 105.0.
Magnesium	0.25-1.5	0.50-3.0	95.0 to 105.0.
Acetate, lactate or bicarbonate	30-60	30-60	95.0 to 105.0.
Chloride	90-120	90-120	95.0 to 105.0.
Glucose	25-250		95.0 to 105.0.

When bicarbonate is present, the sodium bicarbonate solution is provided in a separate compartment or container and is added to the electrolyte solution just before use.

Unless justified and authorized, antioxidants such as metabisulfite salts are not added to the solutions.

IDENTIFICATION

Depending on its composition, the sample meets the following identification reactions:

A. Potassium: it meets the reaction 2 **(5.3.1.1)**.

B. Calcium: to 0.2 mL of the prescribed solution, add 0.5 mL of a 2 g/L solution of glyoxal-hydroxanil in ethyl alcohol at 96% RS, 0.2 mL of diluted sodium hydroxide RS and 0.2 mL of sodium carbonate RS solution. Shake with 1 to 2 mL of chloroform and add 1 to 2 mL of water. The chloroform phase turns reddish.

C. Sodium: use 0.5 mL of the solution. Add 1.5 mL of methoxyphenylacetic reagent and cool in iced water for 30 minutes. A large white crystalline precipitate is formed. Place in water at 20 °C and shake for five minutes. The precipitate does not disappear. Add 1 mL of 6 M ammonia RS. The precipitate dissolves completely. Add 1 mL of ammonium carbonate RS solution. No precipitate is formed.

D. Chloride: it meets the reaction 1 (5.3.1.1).

E. Acetate: in a test tube with a stopper and a curved tube, place 5 mL of the sample and 1 mL of hydrochloric acid; heat and collect some milliliters of the distillate. To 3 mL of the distillate, successively add 0.25 mL of lanthanum nitrate solution; 0.1 mL of 0.05 M iodine and 0.05 mL of ammonia RS. Carefully heat to boiling. In some minutes, a blue precipitate or a dark blue color develops.

F. Lactate and bicarbonate: identification is carried out in conjunction with the Assay.

G. Magnesium: to 0.1 mL of titan yellow TS add 10 mL of water, 2 mL of the sample and 1 mL of M sodium hydroxide. Pink color develops.

H. Glucose: To 5 mL of the sample, add 2 mL of diluted sodium hydroxide solution and 0.05 mL of copper sulfate solution. The preparation turns blue and clear. Heat to boiling; a large red precipitate forms.

CHARACTERISTICS

Aspect. The preparation obtained is clear (5.2.25) and not more intensely stained than the reference solution A₄, prepared as described below.

Reference solution A₄: prepare *Standard Solution A* (yellow) as indicated on **Table 2** from the base solutions of cobalt chloride (red) and ferric chloride (yellow) (5.2.12). From *Standard Solution A*, prepare *Reference Solution A₄* according to **Table 3**.

Procedure: in identical test tubes of neutral, colorless and transparent glass, with an external diameter of 12 mm, compare 2.0 mL of the sample with 2.0 mL of the *Reference Solution A₄*. Observe shades in diffused natural light. Observe horizontally on white background.

Table 2 – Volumes in milliliters of solutions

<i>Standard solution</i>	<i>Yellow solution</i>	<i>Red solution</i>	<i>Hydrochloric acid at 10 g/L of HCl</i>
A (yellow)	2.4	0.6	7.0

Table 3 – Volumes in milliliters of reference solutions A.

<i>Reference solution</i>	<i>Standard solution A</i>	<i>Hydrochloric acid at 10 g/L of HCl</i>
A ₁	100.0	0.0
A ₂	75.0	25.0
A ₃	50.0	50.0
A ₄	25.0	75.0

A ₅	12.5	87.5
A ₆	5.0	95.0
A ₇	2.5	97.5

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.0 to 6.5. If the solution contains bicarbonate, the pH is between 6.5 and 8.0.

PURITY TESTS

Aluminum. Proceed as described in *Limit test for aluminum (5.3.2.10)*. Use *Method I*. Not more than 10 ppm. Measure 200 mL of the sample, adjust to pH 6.0 using 0.1 molar hydrochloric acid solution or 0.1 molar sodium hydroxide solution and add 10 mL of acetate buffer solution pH 6.0. The solution meets the limit test for aluminum (10 µg/L). Use as standard a mixture of 1 mL of 2 ppm aluminum (Al) solution, 10 mL of acetate buffer solution pH 6.0 and 9 mL of water and as blank a mixture of 10 mL of acetate buffer solution pH 6.0 pH and 10 mL of water.

Hydroxymethylfurfural. Measure a sample volume equivalent to 25 mg of glucose, add 5 mL of *p*-toluidine solution at 100 g/L in isopropyl alcohol containing 10% v/v glacial acetic acid and then 1 mL of barbituric acid solution at 5 g/L. The absorbance **(5.2.14)** of the solution, determined at 550 nm after standing for two to three minutes, is not greater than the absorbance of a standard prepared simultaneously and under the same conditions, with a solution containing 10 µg of hydroxymethylfurfural in the same volume as the problem solution. If the solution contains bicarbonate, use as a standard a solution containing 20 µg of hydroxymethylfurfural.

Contamination by particles (5.1.7.1). Use *Method I*. Carry out the test for non-visible particles in 50 mL of the sample.

Table 4 - Limit number of particles per milliliter.

<i>Particles greater than 10 µm</i>	<i>Particles greater than 25 µm</i>
25	3

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 0.05 EU/mL of sample.

Note: for solutions to which no validated bacterial endotoxin detection method can be applied, the sample complies with the additional test below.

Pyrogens (5.5.2.1). Complies with the test. Inject each rabbit, per kilogram of body weight, with volume corresponding to 10 mL of sample.

ASSAY

Sodium.

Proceed as described in *Atomic emission spectrometry (5.2.13.2)*. Use *Method II*. Prepare the solutions described below.

Sample solution: if necessary, dilute the sample with water to obtain an appropriate dilution for the instrument used.

Standard solutions: prepare standard solutions from the 200 ppm sodium (Na) solution.

Procedure: determine the absorbance at 589.0 nm using a sodium hollow cathode lamp as radiation source and an air-propane or air-acetylene flame.

Potassium.

Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*. Use *Method I*. Prepare the solutions described below.

Sample solution: if necessary, dilute the sample with water to obtain an appropriate dilution for the instrument used. To 100 mL of solution add 10 mL of 22 g/L sodium chloride solution.

Standard solutions: prepare standard solutions from the 100 ppm potassium (K) solution. To 100 mL of each standard solution add 10 mL of 22 g/L sodium chloride solution.

Procedure: determine the absorbance at 766.5 nm using a sodium hollow cathode lamp as radiation source and air-propane or air-acetylene flame.

Calcium.

Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*. Use *Method I*. Prepare the solutions described below.

Sample solution: if necessary, dilute the sample with water to obtain an appropriate dilution for the instrument used.

Standard solutions: prepare standard solutions from the 400 ppm calcium (Ca) solution.

Procedure: determine the absorbance at 422.7 nm using a calcium hollow cathode lamp as radiation source and an air-propane or air-acetylene flame.

Magnesium.

Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*. Use *Method I*. Prepare the solutions described below.

Sample solution: if necessary, dilute the sample with water to obtain an appropriate dilution for the instrument used.

Standard solutions: prepare standard solutions from the 100 ppm magnesium (Mg) solution.

Procedure: determine the absorbance at 285.2 nm using a magnesium hollow cathode lamp as radiation source and an air-propane or air-acetylene flame.

Total chloride

Use accurately weighed volume of sample corresponding to about 60 mg of chloride and complete to 50 mL with water. Add 5 mL of diluted nitric acid, 25 mL of 0.1 M silver nitrate VS and 2 mL of dibutyl phthalate and shake. Titrate with 0.1 M ammonium thiocyanate VS in the presence of 2 mL of ammonium ferric sulfate RS solution, until reddish-yellow color. Each mL of 0.1 M silver nitrate is equivalent to 3.545 mg of Cl.

Acetate.

Use a sample volume corresponding to about 0.7 mmole of acetate and add 10.0 mL of 0.1 M hydrochloric acid VS. Titrate with 0.1 M sodium hydroxide VS and plot the potentiometric curve. Determine the volume of 0.1 M sodium hydroxide VS used between the two inflection points. Each mL of 0.1 M sodium hydroxide VS is equivalent to 0.1 mmole of acetate.

Lactate.

Use a sample volume corresponding to about 0.7 mmole of lactate and add 10.0 mL of 0.1 M hydrochloric acid VS. Add 50 mL of acetonitrile. Titrate with 0.1 M sodium hydroxide VS and plot the potentiometric curve. Determine the volume of 0.1 M sodium hydroxide VS used between the two inflection points. Each mL of 0.1 M sodium hydroxide VS is equivalent to 0.1 mmole of lactate.

Sodium bicarbonate.

Use a sample volume corresponding to about 0.1 g of sodium bicarbonate. Titrate with 0.1 M hydrochloric acid VS. Determine the equivalence point potentiometrically. Each mL of 0.1 M hydrochloric acid VS is equivalent to 8.401 mg of NaHCO_3 .

Lactate and bicarbonate.

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with differential refractometer detector; column with 300 mm length and 7.8 mm of inner diameter, packed with cation-exchange resin (9 μm), kept at 60 °C; *Mobile phase* flow rate of 0.6 mL/minute.

Mobile phase: 0.005 M sulfuric acid previously degassed with helium;

Sample solution: use the sample.

Standard solution: dissolve an accurately weighed quantity of lactate and bicarbonate in water and complete the volume to 100 mL to obtain concentrations close to 90%, 100% and 110% of the contents indicated on the label.

Procedure: inject, in duplicate, 20 μL of the *Sample solution* and 20 μL of each *Standard solution*. When chromatograms are recorded under prescribed conditions, peaks are eluted in the following order: lactate and then bicarbonate. Determine the lactate and bicarbonate content in the sample by interpolating the area under the peak for lactate and of the peak height for bicarbonate from the linear regression line obtained with the *Standard solutions*.

Reducing sugars (expressed as anhydrous glucose).

Transfer a sample volume corresponding to 25 mg of glucose to a 250 mL polished-necked erlenmeyer flask. Add 25 mL of cupric citrate RS and some pieces of pumice stone. Attach a reflux still and boil for exactly 10 minutes. Cool and add 3 g of potassium iodide dissolved in 3 mL of water. Carefully add, in small portions at a time, 25 mL of 25% w/w sulfuric acid solution. Titrate with 0.1 M sodium thiosulfate VS in the presence of starch TS solution added near the end of the titration. Carry out a blank test under the same conditions, using 25,0 mL of water. Calculate the content of reducing sugars expressed as anhydrous glucose ($C_6H_{12}O_6$) using the values recorded on **Table 5**.

Table 5 – Correspondence between volumes of 0.1 M sodium thiosulfate and anhydrous glucose.

<i>Volume of 0.1 M sodium thiosulfate (mL)</i>	<i>Anhydrous glucose (mg)</i>
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3.

STORAGE

Do not store at temperature below 4°C.

LABELING

Comply with current legislation. The label must indicate: the preparation formula, expressed in grams per liter and in millimoles per liter; the calculated osmolality, expressed in milliosmoles per liter; the nominal volume of the solution in the container; that the solution is free of bacterial and pyrogenic endotoxins; storage conditions and that unused quantities are disregarded.

SOLUTIONS FOR HEMOFILTRATION AND HEMODIAFILTRATION

Soluciones ad haemocolaturam haemodiacolaturamque

Hemofiltration and hemodiafiltration solutions are sterile, non-pyrogenic preparations for parenteral use, containing electrolytes in concentrations close to those of human plasma. Glucose can be part of the formulation.

They must be supplied in plastic, rigid or semi-rigid containers; containers of flexible plastic material, stored in closed protective covers, or glass containers.

Containers made of plastic material and their caps meet the requirements for *Plastic Containers (6.2)* and *Elastomer Caps (6.2.2)*.

The formulations listed in **Table 1** are used for hemofiltration and hemodiafiltration, usually with the following limits of concentrations per liter of solution. In the table there are also the specification limits, in relation to the labeled value, for each component.

Table 1 – Concentration of components per liter of solution and specification limits.

<i>Solution components</i>	<i>Concentration in mmole/L</i>	<i>Concentration in mEq/L</i>	<i>Specification limit (%)</i>
Sodium	125-150	125-150	97.5 to 102.5.
Potassium	0-4.5	0-4.5	95.0 to 105.0.
Calcium	1.0-2.5	2.0-5.0	95.0 to 105.0.
Magnesium	0.25-1.5	0.50-3.0	95.0 to 105.0.
Acetate, lactate or bicarbonate	30-60	30-60	95.0 to 105.0.
Chloride	90-120	90-120	95.0 to 105.0.
Glucose	0-25		95.0 to 105.0.

When bicarbonate is present, the sodium bicarbonate solution is provided in a separate compartment or container and is added to the electrolyte solution just before use.

The formulations listed in **Table 2** can also be used for hemofiltration and hemodiafiltration, usually with the following limits of concentrations per liter of solution. In the table there are also the specification limits, in relation to the labeled value, for each component.

Table 2 – Concentration of components per liter of solution and specification limits.

<i>Solution components</i>	<i>Concentration in mmole/L</i>	<i>Concentration in mEq/L</i>	<i>Specification limit (%)</i>
Sodium	130-167	130-167	97.5 to 102.5.
Potassium	0-4.0	0-4.0	95.0 to 105.0.
Bicarbonate	20-167	20-167	95.0 to 105.0.
Chloride	0-147	0-147	95.0 to 105.0.

Antioxidants, such as metabisulfite salts, are not added to the solutions.

IDENTIFICATION

Depending on its composition, the sample meets the following identification reactions:

A. Potassium: it meets the reaction 2 (5.3.1.1).

B. Calcium: to 0.2 mL of the prescribed solution, add 0.5 mL of a 2 g/L solution of glyoxal-hydroxanil in ethyl alcohol at 96% RS, 0.2 mL of diluted sodium hydroxide RS and 0.2 mL of sodium carbonate RS solution. Shake with 1 mL to 2 mL of chloroform and add 1 mL to 2 mL of water. The chloroform phase turns reddish.

C. Sodium: use 0.5 mL of the solution. Add 1.5 mL of methoxyphenylacetic reagent and cool in iced water for 30 minutes. A large white crystalline precipitate is formed. Place in water at 20 °C and shake for five minutes. The precipitate does not disappear. Add 1 mL of 6 M ammonia RS. The precipitate dissolves completely. Add 1 mL of ammonium carbonate RS solution. No precipitate is formed.

D. Chloride: it meets the reaction 1 (5.3.1.1).

E. If the solution does not contain glucose, it responds to the following acetate reaction: use 3 mL of the prescribed solution. Successively add 0.25 mL of lanthanum nitrate solution; 0.1 mL of 0.05 M iodine and 0.05 mL of ammonia RS. Carefully heat to boiling. In some minutes, a blue precipitate or a dark blue color develops. If the solution contains glucose, it responds to the following reaction: in a test tube with a stopper and a curved tube, place 5 mL of the sample and 1 mL of hydrochloric acid; heat and collect some milliliters of the distillate. In this case, carry out the reaction described above for the solution that does not contain glucose.

F. Lactate: it meets lactate reaction (5.3.1.1).

G. Carbonate and bicarbonate: meet both carbonate and bicarbonate reactions (5.3.1.1).

H. Magnesium: to 0.1 mL of titan yellow RS, add 10 mL of water, 2 mL of the sample and 1 mL of M sodium hydroxide. Pink color develops.

I. Glucose: To 5 mL of the sample, add 2 mL of diluted sodium hydroxide solution and 0.05 mL of copper sulfate solution. The solution turns blue. Heat to boiling; a large red precipitate forms.

CHARACTERISTICS

Aspect. The sample is clear (5.2.25). If it does not contain glucose, it is colorless (5.2.12); if it contains glucose it is not more colored than the reference solution A₇, prepared as described below.

Reference solution A₇: prepare standard solution A (yellow) as indicated in **Table 3** from the base solutions of cobalt chloride (red) and ferric chloride (yellow) (5.2.12). From standard solution A, prepare reference solution A₇ according to **Table 4**.

Procedure: in identical test tubes of neutral, colorless and transparent glass, with external diameter of 12 mm, compare 2.0 mL of the sample with 2.0 mL of the reference solution A₇. Observe shades in diffused natural light. Observe horizontally on white background.

Table 3 – Volumes in milliliters of solutions

<i>Standard solution</i>	<i>Yellow solution</i>	<i>Red solution</i>	<i>Hydrochloric acid at 10 g/L of HCl</i>
A (yellow)	2.4	0.6	7.0

Table 4 – Volumes in milliliters of reference solutions A.

<i>Reference solution</i>	<i>Standard solution A</i>	<i>Hydrochloric acid at 10 g/L of HCl</i>
A ₁	100.0	0.0
A ₂	75.0	25.0
A ₃	50.0	50.0
A ₄	25.0	75.0
A ₅	12.5	87.5
A ₆	5.0	95.0
A ₇	2.5	97.5

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). If the sample contains glucose, the pH is between 4.5 and 6.5. If the solution contains bicarbonate, the pH is between 7.0 and 8.5. If the solution contains glucose and bicarbonate, the pH is between 5.0 and 7.5.

PURITY TESTS

Aluminum. Proceed as described in *Limit test for aluminum (5.3.2.10)*. Use *Method I*. Not more than 10 ppm. Use 200 mL of the sample, adjust to pH 6.0 using 0.1 M hydrochloric acid solution or 0.1 M sodium hydroxide solution and add 10 mL of acetate buffer solution pH 6.0. The solution meets the limit test for aluminum (10 µg/L). Use as standard a mixture of 1 mL of 2 ppm aluminum (Al) solution, 10 mL of acetate buffer solution pH 6.0 and 9 mL of water and as blank a mixture of 10 mL of acetate buffer solution pH 6.0 pH and 10 mL of water.

Hydroxymethylfurfural. Use a sample volume equivalent to 25 mg of glucose, add 5 mL of *p*-toluidine solution at 100 g/L in isopropyl alcohol containing 10% v/v glacial acetic acid and then 1 mL of barbituric acid solution at 5 g/L. The absorbance **(5.2.14)** of the solution, determined at 550 nm after standing for two to three minutes, is not greater than the absorbance of a standard prepared simultaneously and under the same conditions, with a solution containing 10 µg of hydroxymethylfurfural in the same volume as the problem solution. If the solution contains bicarbonate, use as a standard a solution containing 20 µg of hydroxymethylfurfural.

Contamination by particles (5.1.7.1). Use *Method I*. Carry out the test for non-visible particles in 50 mL of the sample.

Table 5 - Limit number of particles per milliliter.

<i>Particles greater than 10 µm</i>	<i>Particles greater than 25 µm</i>
25	3

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 0.05 EU/mL of sample.

Note: for solutions to which no validated bacterial endotoxin detection method can be applied, the sample complies with the additional test below.

Pyrogens (5.5.2.1). Complies with the test. Inject each rabbit, per kilogram of body weight, with volume corresponding to 10 mL of sample.

ASSAY

Sodium.

Proceed as described in *Atomic emission spectrometry (5.2.13.2)*. Use *Method II*. Prepare the solutions described below.

Sample solution: if necessary, dilute the sample with water to obtain an appropriate dilution for the instrument used.

Standard solutions: prepare standard solutions from the 200 ppm sodium (Na) solution.

Procedure: determine the absorbance at 589.0 nm using a sodium hollow cathode lamp as radiation source and an air-propane or air-acetylene flame.

Potassium.

Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*. Use *Method I*. Prepare the solutions described below.

Sample solution: if necessary, dilute the sample with water to obtain an appropriate dilution for the instrument used. To 100 mL of solution add 10 mL of 22 g/L sodium chloride solution.

Standard solutions: prepare standard solutions from the 100 ppm potassium (K) solution. To 100 mL of each standard solution add 10 mL of 22 g/L sodium chloride solution.

Procedure: determine the absorbance at 766.5 nm using a sodium hollow cathode lamp as radiation source and air-propane or air-acetylene flame.

Calcium.

Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*. Use *Method I*. Prepare the solutions described below.

Sample solution: if necessary, dilute the sample with water to obtain an appropriate dilution for the instrument used.

Standard solutions: prepare standard solutions from the 400 ppm calcium (Ca) solution.

Procedure: determine the absorbance at 422.7 nm using a calcium hollow cathode lamp as radiation source and an air-propane or air-acetylene flame.

Magnesium.

Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*. Use *Method I*. Prepare the solutions described below.

Sample solution: if necessary, dilute the sample with water to obtain an appropriate dilution for the instrument used.

Standard solutions: prepare standard solutions from the 100 ppm magnesium (Mg) solution.

Procedure: determine the absorbance at 285.2 nm using a magnesium hollow cathode lamp as radiation source and an air-propane or air-acetylene flame.

Total chloride

Use accurately weighed volume of sample corresponding to about 60 mg of chloride and complete to 50 mL with water. Add 5 mL of diluted nitric acid, 25 mL of 0.1 M silver nitrate VS and 2 mL of dibutyl phthalate and shake. Titrate with 0.1 M ammonium thiocyanate VS in the presence of 2 mL of ammonium ferric sulfate RS solution until reddish-yellow color. Each mL of 0.1 M silver nitrate VS is equivalent to 3.545 mg of Cl^- .

Acetate.

Use a sample volume corresponding to about 0.7 mmole of acetate and add 10.0 mL of 0.1 M hydrochloric acid VS. Titrate with 0.1 M sodium hydroxide VS and plot the potentiometric curve. Determine the volume of 0.1 M sodium hydroxide VS used between the two inflection points. Each mL of 0.1 M sodium hydroxide is equivalent to 0.1 mmole of acetate.

Lactate.

Use a sample volume corresponding to about 0.7 mmole of lactate and add 10.0 mL of 0.1 M hydrochloric acid VS. Add 50 mL of acetonitrile. Titrate with 0.1 M sodium hydroxide VS and plot the potentiometric curve. Determine the volume of 0.1 M sodium hydroxide VS used between the two inflection points. Each mL of 0.1 M sodium hydroxide VS is equivalent to 0.1 mmole of lactate.

Sodium bicarbonate.

Use a sample volume corresponding to about 0.1 g of sodium bicarbonate. Titrate with 0.1 M hydrochloric acid VS. Determine the equivalence point potentiometrically. Each mL of 0.1 M hydrochloric acid VS corresponds to 8.401 mg of NaHCO_3 .

Lactate and bicarbonate.

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with differential refractometer detector; column with 300 mm length and 7.8 mm of inner diameter, packed with cation-exchange resin (9 μm), kept at 60 °C; *Mobile phase* flow rate of 0.6 mL/minute.

Mobile phase: 0.005 M sulfuric acid previously degassed with helium;

Sample solution: use the sample.

Standard solution: dissolve an accurately weighed quantity of lactate and bicarbonate in 100 mL of water to obtain concentrations close to 90%, 100% and 110% of the contents indicated on the label.

Procedure: inject, in duplicate, 20 µL of the *Sample solution* and 20 µL of each *Standard solution*. When chromatograms are recorded under prescribed conditions, peaks are eluted in the following order: lactate and then bicarbonate. Determine the lactate and bicarbonate content in the sample by interpolating the area under the peak for lactate and of the peak height for bicarbonate from the linear regression line obtained with the *Standard solutions*.

Reducing sugars (expressed as anhydrous glucose).

Transfer a sample volume corresponding to 25 mg of glucose to a 250 mL polished-necked erlenmeyer flask. Add 25 mL of cupric citrate RS and some pieces of pumice stone. Attach a reflux still and boil for exactly 10 minutes. Cool and add 3 g of potassium iodide dissolved in 3 mL of water. Carefully add, in small portions at a time, 25 mL of 25% w/w sulfuric acid solution. Titrate with 0.1 M sodium thiosulfate VS in the presence of starch TS solution added near the end of the titration. Carry out a blank test under the same conditions, using 25,0 mL of water. Calculate the content of reducing sugars expressed as anhydrous glucose (C₆H₁₂O₆) using **Table 6**.

Table 6 – Correspondence between 0.1 M sodium thiosulfate and anhydrous glucose.

<i>Volume of 0.1 M sodium thiosulfate (mL)</i>	<i>Anhydrous glucose (mg)</i>
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3.

STORAGE

Do not store at temperature below 4°C.

LABELING

Comply with current legislation. The label must indicate the preparation formula, expressed in grams per liter and in millimoles per liter; the calculated osmolality, expressed in milliosmoles per liter; the nominal volume of the solution in the container; that the solution is free of bacterial and pyrogenic endotoxins; storage conditions and that unused quantities are disregarded.

SOLUTIONS FOR IRRIGATION

Solutiones ad irrigationem

Irrigation solutions are sterile, large-volume aqueous solutions to be used for irrigation of body cavities, wounds and surfaces, for example, during surgical interventions.

Irrigation solutions can be solutions prepared by dissolving one or more active principles, electrolytes or osmotically active substances in water which meets the requirements of the monograph *Water for Injections*, or they can be composed of this water only. In the latter case, the solution can be labeled *Irrigation Water*. Irrigation solutions are usually adjusted to be isotonic with blood.

When examined under suitable visual conditions, irrigation solutions are practically free of particles.

Irrigation solutions are presented in single dose container. Containers and their lids must meet the requirements of the monograph *Containers for preparations for parenteral use* in accordance with recognized pharmacopoeias.

The container administration orifice is incompatible with intravenous administration equipment and prevents irrigation solutions from being administered using such equipment.

PRODUCTION

Irrigation solutions are produced using products and methods that guarantee sterility and prevent the introduction of contaminants and the growth of microorganisms; recommendations on this can be found in the text *Sterile products (8.1)*. During production it must be demonstrated that the nominal content can be removed from the container.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

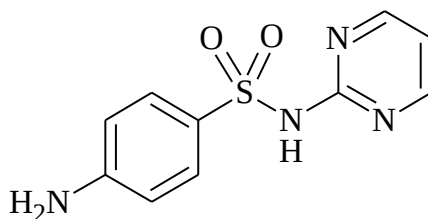
Bacterial endotoxins (5.5.2.2). Not more than 0.5 EU/mL of sample.

Note: for solutions to which no validated bacterial endotoxin detection method can be applied, the sample complies with the additional test below.

Pyrogens (5.5.2.1). Complies with the test. Inject each rabbit, per kilogram of body weight, with volume corresponding to 10 mL of sample.

LABELING

Comply with current legislation. The label must state that the preparation must not be used for parenteral use, that the preparation must be used only once, and that any unused fraction must be discarded.

SULFADIAZINE*Sulfadiazinum* $C_{10}H_{10}N_4O_2S$; 250.28

sulfadiazine; 08116

4-Amino-*N*-2-pyrimidinyl-benzenesulfonamide

[68-35-9]

It contains not less than 98.0% and not more than 102.0% of $C_{10}H_{10}N_4O_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish-white powder. Turns dark slowly by exposure to light.

Solubility. Practically insoluble in water, very slightly soluble in ethyl alcohol. Freely soluble in diluted alkali hydroxide solutions and soluble in 3 *M* hydrochloric acid.

Physicochemical constants.

Melting range (5.2.2): 252 °C to 256 °C, with decomposition.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the sulfadiazine CRS spectrum, prepared identically.

B. The main stain of the chromatogram from *Solution (1)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

C. Dissolve 50 mg of the sample in 2 mL of hydrochloric acid RS with heating. Cool in ice bath and add 2 mL of sodium nitrite RS. Dilute with 2 mL of iced water and add 1 mL of 2-naphthol SR. An orangish precipitate is produced.

D. Carefully heat 50 mg of the sample in a dry test tube with direct flame or sand bath. A reddish-brown color develops and the vapors released do not modify the moistened lead acetate paper.

E. Heat 1 g of the sample, gently, in a test tube, over low flame, until sublimation. With a glass rod, collect some milligrams of the sublimate and mix in a test tube with 1 mL of 5%(w/v) resorcinol in 90% (v/v) ethyl alcohol. Add 1 mL of sulfuric acid and shake. Dark red color is immediately produced. Dilute the mixture carefully with 25 mL of iced water and add excess of 6 M ammonia. Blue or reddish-blue color develops.

PURITY TESTS

Preparation aspect. Dissolve 1 g of the sample in mixture of 5 mL of sodium hydroxide RS and 20 mL of water. The preparation obtained is clear **(5.2.25)**.

Acidity. Heat 1 g of the sample with 50 mL of carbon dioxide-free water at 70 °C for five minutes. Quickly cool to 20°C and filter. Not more than 0.2 mL of 0.1 M sodium hydroxide is required to neutralize 25 mL of the filtrate, using phenolphthalein TS as indicator.

Related substances. Proceed as described in *Thin layer chromatography* **(5.2.17.1)**, using silica gel GF₂₅₄ as support, and mixture of chloroform, methyl alcohol and ammonium hydroxide (30:12:1) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): 100 µg/mL sample solution in mixture of toluene and dimethylformamide (2:1).

Solution (2): 100 µg/mL sulfadiazine CRS solution in mixture of toluene and dimethylformamide (2:1).

Solution (3): 2 µg/mL sulfanilamide solution in mixture of toluene and dimethylformamide (2:1).

Develop chromatogram. Remove plate and allow to air dry. Spray with 1 M hydrochloric acid in methyl alcohol and then with 1% (w/v) sodium nitrite in 90% (v/v) ethyl alcohol. Wait for three to five minutes and spray with 0.5% (w/v) *N*-(1-naphthyl)ethylenediamine dihydrochloride in 90% (v/v) ethyl alcohol. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (3)* (2.0%).

Arsenic (5.3.2.5). Proceed as described in *Visual method*. Not more than 0.0002% (2 ppm).

Chlorides (5.3.2.1). Boil 1 g in mixture of 10 mL of nitric acid RS and 5 mL of distilled water. Proceed as described in *Limit test for chlorides*. Not more than 0.035% (350 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Sulfates (5.3.2.2). Dissolve 1 g of the sample, with heating, in mixture with 10 mL of hydrochloric acid RS and 5 mL of water. Proceed as described in *Limit test for sulfates*. Not more than 0.12% (1200 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for two hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Diazotization titrations (5.3.3.1), Method 2*. Each mL of 0.1 M sodium nitrite VS is equivalent to 25.028 mg of $C_{10}H_{10}N_4O_2S$.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Dissolve an accurately weighed quantity of the sample in 0.1 M sodium hydroxide and dilute with the same solvent to a concentration of 0.001% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 254 nm, using 0.1 M sodium hydroxide for zero adjustment. Calculate the content of $C_{10}H_{10}N_4O_2S$ in the sample from the readings obtained.

C. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Weigh, accurately, about 0.5 g of the sample and transfer to a 200 mL volumetric flask using 30 mL of 0.1 M sodium hydroxide. Shake until dissolution, complete the volume with water and homogenize. Carry out successive dilutions in water up to concentration of 0.005% (w/v). Prepare a 0.25% (w/v) standard solution in mixture of water and 0.1 M sodium hydroxide (85:15). Carry out successive dilutions in water up to concentration of 0.005% (w/v). Transfer 2 mL of the *Standard Solution* and *Sample Solution* to 25 mL volumetric flasks. Add, to each flask, 1 mL of 2 M hydrochloric acid and 1 mL of 0.1% (w/v) sodium nitrite. Shake and allow to stand for two minutes. Add 1 mL of 0.5% ammonium sulfamate (w/v), shake and allow to stand for two minutes. Add 1 mL of *N*-(1-naphthyl) ethylenediamine RS dihydrochloride, shake and allow to stand for 10 minutes. Complete the volumes with water. Prepare blank in parallel. Measure the absorbances of the solutions at 540 nm, using blank for zero adjustment. Calculate the content of $C_{10}H_{10}N_4O_2S$ in the sample from the readings obtained.

PACKAGING AND STORAGE

In tightly closed opaque containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiseptic.

SULFADIAZINE TABLETS

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $C_{10}H_{10}N_4O_2S$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Use quantity of powder equivalent to 0.5 g of sulfadiazine and pulverize in a mortar with 5 mL of chloroform. Filter and discard the filtrate. Pulverize the residue with 10 mL of 6 M ammonium hydroxide for five minutes, add 10 mL of water and filter. Heat the filtrate to eliminate all the ammonia and cool. Add 6 M acetic acid until distinctly acidic reaction. Sulfadiazine precipitate is formed. Filter and wash the precipitate with cold water. Dry the residue at 105 °C for one hour. In the infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed with sulfadiazine CRS.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of 0.001% (w/v) solution of the residue obtained in the test **A.** from *Identification* in ethyl alcohol, there are maximum and minimum points only at the same wavelengths of similar sulfadiazine CRS solution.

C. The main stain obtained with *Solution (1)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (2)*.

D. Dissolve 50 mg of the residue obtained in test **A.** from *Identification* in 2 mL of 10% (w/v) hydrochloric acid, with heating. Cool in ice bath and add 2 mL of 10% (w/v) sodium nitrite. Dilute with 10 mL of iced water and add 2 mL of 2-naphthol RS. An orangish precipitate is produced.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described in method **A.** from *Assay*.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 90 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in 0.1 M sodium hydroxide until adequate concentration. Measure the absorbances of the solutions at 254 nm (**5.2.14**), using 0.1 M sodium hydroxide for zero adjustment. Calculate the quantity of $C_{10}H_{10}N_4O_2S$ dissolved in the medium, comparing the readings obtained with that of the sulfadiazine CRS solution at the concentration of 0.0005% (w/v), prepared in the same solvent.

Tolerance: not less than 70% (Q) of the stated quantity of $C_{10}H_{10}N_4O_2S$ dissolve in 90 minutes.

PURITY TESTS

Related substances. Proceed as described in *Related substances* in the *Sulfadiazine* monograph. Prepare *Solution (1)* using the residue obtained in test **A.** from *Identification*.

Water (5.2.20.1). Not more than 3.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 257 nm; column with 300 mm of length and 4.0 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group, kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: mixture of water, acetonitrile and glacial acetic acid (87:12:1).

Sample solution: weigh and pulverize not less than 20 tablets. Transfer quantity of powder, accurately weighed, equivalent to 0.1 g of sulfadiazine to a 100 mL volumetric flask, add 75 mL of 0.025 M sodium hydroxide, place in ultrasonic bath for 10 minutes, complete the volume with the same solvent, mix and filter.

Standard solution: dissolve accurately weighed quantity of sulfadiazine CRS in 0.025 M sodium hydroxide to obtain a 1 mg/mL solution.

Inject 10 μ L replicates of the *Standard solution*. The tailing factor is not more than 1.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

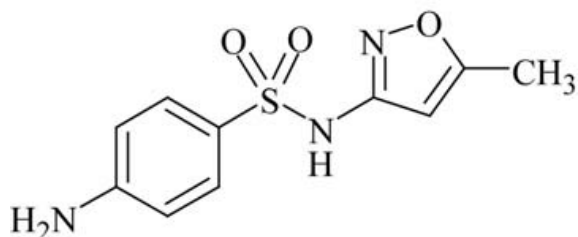
Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{10}H_{10}N_4O_2S$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

SULFAMETHOXAZOLE***Sulfamethoxazolum***

C₁₀H₁₁N₃O₃S; 253.28

sulfamethoxazole; 08134

4-Amino-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide
[723-46-6]

It contains not less than 99.0% and not more than 101.0% of C₁₀H₁₁N₃O₃S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, moderately soluble in ethyl alcohol. Freely soluble in diluted sodium hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 168 °C to 172 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the sulfamethoxazole CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0.001% (w/v) sample solution in 0.1 M sodium hydroxide, there are maximum and minimum points identical to those observed in the spectrum of similar sulfamethoxazole CRS solution. The absorbances of the solutions at 257 nm differ by not more than 2.0%.

C. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

D. Dissolve 0.1 g of the sample in 2 mL of 2 M hydrochloric acid. The solution obtained meets the primary aromatic amine reaction. **(5.3.1.1).**

PURITY TESTS

Alkalinity. Add 25 mL of water to 1.25 g of finely powdered sample. Heat at 70°C for five minutes. Cool in iced water for about 15 minutes and filter. Add 0.1 mL of bromothymol blue TS to 20 mL of the filtrate. Not more than 0.3 mL of 0.1 M sodium hydroxide is required to turn the indicator color.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of ammonia, water, nitromethane and dioxane (3:5:40:50) as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): transfer 0.1 g of the sample to a 5 mL volumetric flask. Dissolve in mixture of ammonia and methyl alcohol (2:48) and complete the volume with the same solvent.

Solution (2): dilute 1 mL of *Solution (1)* to 5 mL with mixture of ammonia and methyl alcohol (2:48).

Solution (3): transfer 20 mg of sulfamethoxazole CRS to a 5 mL volumetric flask, dissolve in 3 mL of mixture of ammonia and methyl alcohol (2:48) and complete the volume with the same solvent.

Solution (4): dilute 1.25 mL of *Solution (2)* to 50 mL with mixture of ammonia and methyl alcohol (2:48).

Develop chromatogram. Remove the plate, dry at 105 °C. Examine under ultraviolet light (254 nm). Any stain obtained in the chromatogram of *Solution (1)*, different from the main stain, is not more intense than that obtained in the chromatogram of *Solution (4)* (0,5%).

Sulfanilamide and sulfanilic acid. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of ethyl alcohol, *n*-heptane, chloroform and glacial acetic acid (25:25:25:7) as mobile phase. Apply to the plate, separately, 10 µL of the *Solution (1)* and of *Solution (3)* and 25 µL of the *Solution (2)* freshly prepared, described below.

Solution (1): transfer 0.1 g of the sample to a 10 mL volumetric flask. Dissolve in 0.1 mL of ammonium hydroxide and complete the volume with methyl alcohol.

Solution (2): dissolve 20 mg of sulfanilamide CRS and 20 mg of sulphanilic acid in 10 mL of ammonium hydroxide and complete the volume to a 100 mL volumetric flask with methyl alcohol. Transfer 2 mL of the solution to a 50 mL volumetric flask, add 10 mL of ammonium hydroxide and complete the volume with methyl alcohol.

Solution (3): transfer 0.1 g of sulfamethoxazole CRS to a 10 mL volumetric flask. Dissolve in 0.1 mL of ammonium hydroxide and complete the volume with methyl alcohol.

Develop chromatogram. Remove the plate, air dry. Pulverize the plate with modified Ehrlich's reagent. The retention factors (R_f) are: 0.7 for sulfamethoxazole, 0.5 for sulfanilamide and 0.1 for sulphanilic acid. No stain of sulphanilic acid and sulfanilamide in the chromatogram of *Solution (1)* is more intense than the corresponding one in the chromatogram of *Solution (2)* (0.2%).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for four hours, until constant weight. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Proceed as described in *Diazotization titrations* **(5.3.3.1)**, *Method 2*. Accurately weigh about 0.5 g of the sample and dissolve in mixture of 20 mL of glacial acetic acid and 40 mL of water and add 15 mL of hydrochloric acid. Cool to 15°C. Titrate immediately with 0.1 M sodium nitrite VS Determine the endpoint potentiometrically. Each mL of 0.1 M sodium nitrite VS is equivalent to 25.328 mg of $C_{10}H_{11}N_3O_3S$.

PACKAGING AND STORAGE

In hermetic containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

SULFAMETHOXAZOLE AND TRIMETHOPRIM TABLETS

It contains not less than 93.0% and not more than 107.0% of the declared quantity of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$).

IDENTIFICATION

A. Filter the aqueous layer reserved in method **A.** from Assay. Add, dropwise, sufficient quantity of 2 M hydrochloric acid to acidify and extract with 50 mL of ethyl ether. Wash the ethereal layer with 10 mL of water, mix with 5 g of anhydrous sodium sulfate, filter and evaporate the filtrate to dryness. Dissolve the residue in a minimum volume of 5% (w/v) anhydrous sodium carbonate, add dropwise M hydrochloric acid until precipitation and filter. Wash the precipitate with water and dry at 105 °C to constant weight. In the infrared absorption spectrum (**5.2.14**) of the residue, previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the sulfamethoxazole CRS spectrum.

B. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 50 mg of trimethoprim to a separating funnel, add 30 mL of 0.1 M sodium hydroxide and shake. Extract with four 50 mL portions of chloroform, washing each extract with two 10 mL portions of 0.1 M sodium hydroxide and with 10 mL of water. Shake with 5 mg of anhydrous sodium sulfate, filter and dry at 105 °C to constant weight. In the infrared absorption spectrum (**5.2.14**) of the residue, previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the trimethoprim CRS spectrum.

C. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support and mixture of chloroform, isopropyl alcohol and diethylamine (60:50:10), as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Transfer quantity of powder equivalent to 4 mg of trimethoprim to a 10 mL volumetric flask, add 8 mL of methyl alcohol. Place in ultrasonic bath for 15 minutes and shake mechanically for 15 minutes. Complete the volume with methyl alcohol. Homogenize and filter.

Solution (2): prepare 0.4 mg/mL trimethoprim CRS solution in methyl alcohol.

Solution (3): prepare 2 mg/mL sulfamethoxazole CRS solution in methyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The stains related to sulfamethoxazole and to trimethoprim, obtained with *Solution (1)*, correspond in position, color and intensity to the ones obtained with *Solution (2)* and *Solution (3)*, respectively.

D. The retention times of the main peaks of the *Sample solution* chromatogram, obtained in method **C.** from Assay, correspond to the ones from the main peaks of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described in method **C.** from Assay.

DISSOLUTION TEST (5.1.5)

Dissolution medium: 0.1 M hydrochloric acid, 900 mL.

Apparatus: paddles, 75 rpm.

Time: 60 minutes.

Procedure: after the test, use aliquot from the dissolution medium as *Sample Solution* and proceed as described in Method **C.** from Assay, making dilutions, if necessary.

Tolerance: not less than 70% (Q) of the declared quantity of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$) dissolve in 60 minutes.

PURITY TESTS

Water (5.2.20.1). Not more than 3.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use methods A. and B. or, solely, method C., described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 50 mg of trimethoprim to a 25 mL volumetric flask, dissolve in 0.1 M sodium hydroxide and complete the volume with the same solvent. Quantitatively transfer the solution to a separating funnel, extract with four 50 mL portions of chloroform, washing each extract with 20 mL of 0.1 M sodium hydroxide. Reserve the aqueous layer for test **A.** from *Identification*. Combine the chloroform extracts and extract with four 60 mL portions of *M* acetic acid. Combine the acid extracts, wash them with 5 mL of chloroform and dilute the aqueous extract to 250 mL with *M* acetic acid. Transfer 10 mL of this solution to a 100 mL volumetric flask, add 10 mL of *M* acetic acid and complete the volume with water. Prepare 0.002% (w/v) trimethoprim CRS standard solution using 0.1 M acetic acid as solvent. Measure the absorbances of the resulting solutions at 271 nm, using 0.1 M acetic acid for zero adjustment. Calculate the quantity of trimethoprim ($C_{14}H_{18}N_4O_3$) in the tablets from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 204$, at 271 nm.

B. Weigh and pulverize 20 tablets. Weigh quantity of powder equivalent to 0.5 g of sulfamethoxazole and proceed as described in *Assay* in the *Sulfamethoxazole* monograph.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow rate of 2.0 mL/minute.

Mobile phase: mix 1400 mL of water, 400 mL of acetonitrile and 2 mL of triethylamine in a 2000 mL volumetric flask. Adjust pH to 5.9 ± 0.1 with 0.2 M sodium hydroxide or glacial acetic acid. Complete the volume with water.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.16 g of sulfamethoxazole and 32 mg of trimethoprim to 100 mL volumetric flask. Add 70 mL of methyl alcohol. Place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent and filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Standard solution: prepare a solution to obtain a concentration of sulfamethoxazole CRS at 1.6 mg/mL and trimethoprim CRS at 0.32 mg/mL in methyl alcohol. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a solution containing sulfamethoxazole at 160 μ g/mL and trimethoprim at 32 μ g/mL.

Inject 20 μ L replicates of the *Standard solution*. The relative retention times are of 1.0 for trimethoprim and 1.8 for sulfamethoxazole. The resolution between sulfamethoxazole and trimethoprim peaks is not less than 5.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and of trimethoprim ($C_{14}H_{18}N_4O_3$) in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

SULFAMETHOXAZOLE AND TRIMETHOPRIM ORAL SUSPENSION

It contains not less than 90.0% and not more than 110.0% of the declared quantity of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) and trimethoprim ($C_{14}H_{18}N_4O_3$).

IDENTIFICATION

Test A. refers to trimethoprim identification and test B. refers to sulfamethoxazole identification. The identification test D. can be omitted if test C. is carried out.

A. To a volume of oral suspension equivalent to 40 mg of trimethoprim, add 30 mL of 0.1 M sodium hydroxide and shake. Proceed as described in test **B.** from *Identification* in the *Sulfamethoxazole and trimethoprim tablets* monograph.

B. Use the aqueous layer obtained in test **A.** from *Identification* and proceed as described in test **A.** from *Identification* in the *Sulfamethoxazole and trimethoprim tablets* monograph.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support and mixture of chloroform, methyl alcohol and dimethylformamide (100:10:5), as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): add 20 mL of methyl alcohol to quantity of oral suspension equivalent to 0.4 g of sulfamethoxazole and 80 mg of trimethoprim. Add 10 g of anhydrous sodium sulfate and homogenize. Centrifuge and use the supernatant.

Solution (2): 20 mg/mL sulfamethoxazole CRS solution in methyl alcohol.

Solution (3): 4 mg/mL trimethoprim CRS solution in methyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Spray with diluted potassium iodobismuthate. The stains related to sulfamethoxazole and to trimethoprim, obtained with *Solution (1)*, correspond in position, color and intensity to the ones obtained with *Solution (2)* and *Solution (3)*.

D. The retention times of the main peaks of the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, correspond to the ones from the main peaks of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 5.0 to 6.5.

PURITY TESTS

Trimethoprim degradation product limit. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and mixture of chloroform, methyl alcohol and ammonium hydroxide (80:20:2) as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): transfer volume of oral suspension equivalent to 40 mg of trimethoprim to separating funnel. Extract with three portions of 25 mL of mixture with chloroform and methyl alcohol (8:2). Combine the chloroform extracts in a beaker and evaporate in water bath until dryness. Dissolve the residue in 2 mL of the same solvent.

Solution (2): prepare 20 mg/mL trimethoprim CRS solution in mixture of chloroform and methyl alcohol (8:2).

Solution (3): dissolve volume of *Solution (2)* in mixture of chloroform and methyl alcohol (8:2) to obtain a 0.1 mg/mL solution.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). Trimethoprim produces a stain with R_f of approximately 0.7 and its degradation product produces a stain with R_f between 0.3 and 0.5. Any stain obtained in the chromatogram with *Solution (1)*, with R_f of approximately 0.3 and 0.5, is not more than equal in size and intensity to that obtained with *Solution (3)* (0.5%).

Limit of sulfanilamide, sulfanilic acid and sulfamethoxazole N₄-glucoside. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of ethyl alcohol and methyl alcohol (95:5), heptane, chloroform and glacial acetic acid (25:25:25:7) as mobile phase. Apply to the plate, separately, 50 µL of each of the freshly prepared solutions described below.

Solution (1): transfer volume of oral suspension equivalent to 0.2 g of sulfamethoxazole to a 100 mL volumetric flask, containing 10 mL of ammonium hydroxide. Add 50 mL of methyl alcohol. Shake for three minutes and complete the volume with the same solvent. Filter.

Solution (2): transfer 20 mg of sulfamethoxazole CRS to a 10 mL volumetric flask. Dissolve in 1 mL of ammonium hydroxide and complete the volume with methyl alcohol.

Solution (3): transfer 10 mg of sulfanilamide CRS to a 50 mL volumetric flask. Dissolve in 5 mL of ammonium hydroxide and complete the volume with methyl alcohol. Transfer 5 mL of this solution to a 100 mL volumetric flask. Add 10 mL of ammonium hydroxide and complete the volume with methyl alcohol.

Solution (4): transfer 10 mg of sulfanilic acid CRS to a 50 mL volumetric flask, dissolve in 5 mL of ammonium hydroxide and complete the volume with methyl alcohol. Transfer 3 mL of this solution to a 100 mL volumetric flask. Add 10 mL of ammonium hydroxide and complete the volume with methyl alcohol.

Solution (5): transfer 3 mg of sulfamethoxazole N₄-glucoside CRS to a 50 mL volumetric flask. Dissolve in 5 mL of ammonium hydroxide and complete the volume with methyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Spray with modified Ehrlich's reagent and allow to stand for 15 minutes. Sulfamethoxazole presents stains with R_f of about 0.7. Any stains obtained in the chromatogram with *Solution (1)*, with R_f of approximately 0.5, 0.1 and 0.3, are not more than equal in size and intensity to the stains obtained in the chromatograms with *Solutions (3)*, (4) and (5), respectively, corresponding to not more than 0.5% of sulfanilamide, 0.3% of sulfanilic acid and 3.0% of sulfamethoxazole N₄-glucoside.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet and visible absorption spectrophotometry (5.2.14)*. Protect the solutions from light.

Procedure for sulfamethoxazole: transfer to a separating funnel volume of the oral suspension equivalent to 0.2 g of sulfamethoxazole. Add 10 mL of 0.1 M sodium hydroxide. Extract with six portions of 25 mL of chloroform. Combine the chloroform extracts and reserve for *Procedure for Trimethoprim*. Transfer the aqueous solution to a 200 mL volumetric flask and complete the volume with water. Transfer 5 mL of the resulting solution to a 100 mL volumetric flask and complete the volume with water. Transfer 2 mL of this solution to a 50 mL volumetric flask, add 4 mL of 0.5 M hydrochloric acid and 1 mL of 0.1% (w/v) sodium nitrite. Allow to stand in ice bath for 10 minutes. Add 2 mL of 0.5% ammonium sulfamate (w/v) and allow to stand for 10 minutes. Add 1 mL of 0.1% (w/v) *N*-(1-naphthyl)ethylene-diamine dihydrochloride, allow to stand for 10 minutes and complete the volume with water. For the preparation of the standard solution, transfer 50 mg of sulfamethoxazole CRS to a 50 mL volumetric flask containing 2.5 mL of 0.1 M sodium hydroxide and complete the volume with water. Transfer 5 mL of this solution to a 100 mL volumetric flask and complete the volume with water. Repeat the procedure from "Transfer 2 mL of this solution to a 50 mL volumetric flask...". Measure the absorbances of the resulting solutions at 538 nm, using water for zero adjustment. Calculate the quantity of sulfamethoxazole ($C_{10}H_{11}N_3O_3S$) in the oral suspension from the readings obtained.

Procedure for Trimethoprim: extract the chloroform solution reserved in the *Procedure for Sulfamethoxazole* with four 20 mL portions of 1 M acetic acid. Combine the aqueous extracts, wash with 5 mL of chloroform and dilute to 100 mL with 1 M acetic acid. Transfer 2 mL of the solution to a 50 mL volumetric flask, add 5 mL of 1 M acetic acid and complete the volume with water to obtain a 0.0016% (w/v) solution. Prepare a trimethoprim CRS solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 271 nm, using 1 M acetic acid for zero adjustment. Calculate the quantity of trimethoprim ($C_{14}H_{18}N_4O_3$) in the oral suspension from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 204$, at 271 nm, in 1 M acetic acid.

B. Proceed as described in method **C.** from *Assay in the Sulfamethoxazole and trimethoprim tablets monograph*. Prepare the *Sample solution* as described below.

Sample solution: transfer volume of the oral suspension equivalent to 0.16 g of sulfamethoxazole and 32 mg of trimethoprim to a 100 mL volumetric flask. Add 70 mL of methyl alcohol. Place in ultrasonic bath for 10 minutes, occasionally shaking. Complete the volume with the same solvent, homogenize and filter. Transfer 5 mL of the filtrate to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of sulfamethoxazole

(C₁₀H₁₁N₃O₃S) and of trimethoprim (C₁₄H₁₈N₄O₃) in the oral suspension from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

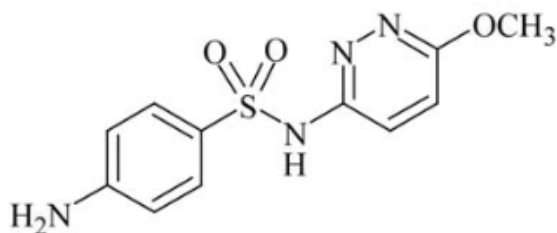
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

SULFAMETHOXYPYRIDAZINE

Sulfamethoxypyridazinum



$C_{11}H_{12}N_4O_3S$; 280.30

sulfamethoxypyridazine; 08136

4-Amino-*N*-(6-methoxy-3-pyridazinyl)-benzenesulfonamide
[80-35-3]

It contains not less than 99.0% and not more than 101.0% of $C_{11}H_{12}N_4O_3S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White to yellowish-white crystalline powder.

Solubility. Very soluble in water, slightly soluble in ethyl alcohol. Freely soluble in diluted solutions of mineral acids and alkali hydroxides.

Physicochemical constants.

Melting range (5.2.2): 182 °C to 183 °C.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the sulfamethoxypyridazine CRS spectrum, prepared identically.

B. It meets the primary aromatic amine reaction (5.3.1.1).

PURITY TESTS

Acidity. Heat 1 g of the sample in 50 mL of carbon dioxide-free water at around 70 °C for five minutes, cool to 20 °C and filter. The 25 mL filtrate aliquot requires not more than 0.35 mL of 0.1 M sodium hydroxide for neutralization.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C until constant weight. Not more than 0.5%.

ASSAY

Proceed as described in *Diazotization titrations* **(5.3.3.1)**, *Method 2*. Each mL of 0.1 M sodium nitrite VS is equivalent to 28.030 mg of $C_{11}H_{12}N_4O_3S$.

PACKAGING AND STORAGE

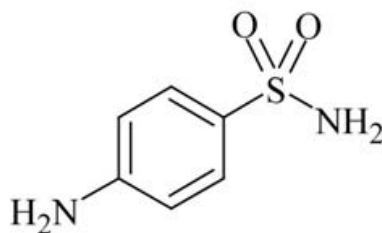
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

SULFANILAMIDE*Sulfanilamidum* $C_6H_8N_2O_2S$; 172.20

sulfanilamide; 08141

4-Aminobenzenesulfonamide

[63-74-1]

it contains not less than 99.0% and not more than 101.0% of $C_6H_8N_2O_2S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish-white crystalline powder.

Solubility. Slightly soluble in water, moderately soluble in ethyl alcohol. Soluble in alkali hydroxide solutions.

Physicochemical constants.

Melting range (5.2.2): 164.5 °C to 166 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the sulfanilamide CRS spectrum, prepared identically.

B. Dissolve 5 mg of the sample in 10 mL of 0.1 M hydrochloric acid. The solution, without acidification, meets the primary aromatic amine reactions **(5.3.1.1)**.

PURITY TESTS

Acidity. Heat at about 70 °C, for five minutes, 1 g of the sample in 50 mL of freshly boiled distilled water. Cool in ice bath for 15 minutes and filter. Add 0.1 mL of bromothymol blue TS to 20 mL of the filtrate. Not more than 0.1 mL of 0.02 M sodium hydroxide is required to neutralize the sample.

Heavy metals (5.3.2.3). Use *Method I*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C until constant weight. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Proceed as described in *Diazotization titrations (5.3.3.1), Method 2*. Each mL of 0.1 M sodium nitrite VS is equivalent to 17.220 mg of $C_6H_8N_2O_2S$.

PACKAGING AND STORAGE

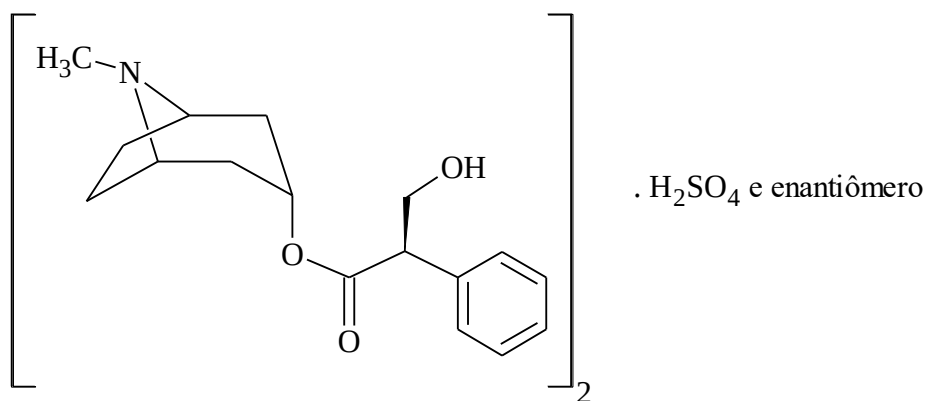
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

ATROPINE SULFATE*Atropini sulfas* $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$; 676.82

atropine sulfate; 00935

 α -(hydroxymethyl)benzeneacetic acid (3-*endo*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester sulfate (1:2)

[55-48-1]

 $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$; 694.84

atropine sulfate monohydrate; 11393

 α -(hydroxymethyl)benzeneacetic acid (3-*endo*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester sulfate hydrate (1:2:1)

[5908-99-6]

It contains not less than 98.5% and not more than 101.0% of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$ in relation to the anhydrous substance.

DESCRIPTION

Physicochemical characteristics. Colorless crystals or white crystalline powder, efflorescence in dry air, slowly altered by light. It melts at temperature not below 187 °C (**5.2.2**), determined immediately after desiccation of the sample at 120 °C for four hours.

Solubility. Very soluble in water, freely soluble in ethyl alcohol and in glycerin.

IDENTIFICATION

The identification tests C., D. and E. can be omitted if tests A., B. and F. are carried out.

A. Dissolve 50 mg of the sample in 25 mL of 0.01 M hydrochloric acid, add 2 mL of M sodium hydroxide and extract with two 10 mL portions of ethyl ether. Dry the ether extract with anhydrous sodium sulfate, filter, wash with 5 mL of ethyl ether and evaporate the filtrate at room temperature. Dry the residue over silica gel, under reduced pressure. Carry out, in parallel, the same procedure using 50 mg of atropine sulfate CRS. In the infrared absorption spectrum (**5.2.14**) of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same

wavelengths and with the same relative intensities as the ones observed in the atropine sulfate CRS spectrum.

B. Proceed as described in *Related substances*. The main stain obtained with *Solution (1)* corresponds in color, size and intensity to the one obtained with *Solution (4)*.

C. To 1 mg of the sample, add 0.2 mL of fuming nitric acid and evaporate in water bath to dryness. Dissolve the residue in 2 mL of acetone and add 0.1 mL of 3% (w/v) potassium hydroxide solution in methyl alcohol. Violet color is produced.

D. Dissolve approximately 25 mg of sample in 5 mL of water, acidify with 2 M hydrochloric acid and add 1 mL of aqueous-acetic potassium iodobismuthate. An orangish or orangish-red precipitate forms immediately.

E. To 1 mL of 5% aqueous solution (w/v) of the sample, add 1 mL of water and 0.5 mL of 0.1 M iodine solution. A dark precipitate is produced.

F. The 5% (w/v) aqueous solution meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 4.5 to 6.2. Determine in solution at 2% (w/v).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of acetone, water and concentrated ammonium solution (90:7:3) as mobile phase. Apply, separately, to the plate 10 µL of each solution described below.

Solution (1): 2% (w/v) sample solution in methyl alcohol.

Solution (2): 0.02% (w/v) sample solution in methyl alcohol.

Solution (3): 0.01% (w/v) sample solution in methyl alcohol.

Solution (4): 2% (w/v) atropine sulfate CRS solution in methyl alcohol.

Develop chromatogram. Remove the plate and dry at 100 °C to 105 °C for 15 minutes. Allow to cool and spray with diluted potassium iodobismuthate RS until stains are formed. No secondary stain obtained in the chromatogram with *Solution (1)*, is more intense than that obtained with *Solution (2)* (1.0%), and not more than one stain is more intense than the stain obtained with *Solution (3)* (0.5%).

Apoatropine limit. Prepare 0.1% (w/v) solution in 0.01 M hydrochloric acid. Measure the absorbances at 245 nm **(5.2.14)** using the same solvent for zero adjustment. The absorbance value is not more than 0.4 (0.5%).

Hyoscyamine. Dissolve 1.25 g, accurately weighed, in water for a final volume of 25 mL. Determine the solution optical rotation **(5.2.8)** at 25 °C. The observed rotation, in degrees, multiplied by 200 and divided by the length of the polarimetric tube used, in mm, is between -0.60° and +0.05°.

Water (5.2.20.1). Not more than 4.0%.

Residue by incineration (5.2.10). Determine in 1 g of the substance. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve about 1 g of the desiccated sample, accurately weighed, in 50 mL of glacial acetic acid and titrate with 0.1 M perchloric acid VS and determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 67.682 mg of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Mydriatic and adjuvant to general anesthetics.

ATROPINE SULFATE MONOHYDRATE INJECTION SOLUTION

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$.

IDENTIFICATION

A. Use sample volume equivalent to 10 mg of atropine sulfate, add 4 mL of *M* sodium hydroxide and extract with two 10 mL portions of ethyl ether. Dry the ether extract with anhydrous sodium sulfate, filter, wash with 5 mL of ethyl ether and evaporate the filtrate at room temperature. Dry the residue over silica gel, under reduced pressure. Carry out, in parallel, the same procedure using 50 mg of atropine sulfate CRS. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the atropine sulfate CRS spectrum.

B. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support and mixture of chloroform, acetone and diethylamine (50:40:10), as mobile phase. Apply, separately, to the plate 5 µL of each solution described as follows.

Solution (1): evaporate volume of the injection solution containing the equivalent to 5 mg of atropine sulfate, to dryness, in water bath. Pulverize the residue with 1 mL of ethyl alcohol, allow to stand and use the supernatant.

Solution (2): 0.5% (w/v) atropine sulfate CRS solution in ethyl alcohol.

Develop chromatogram. Remove the plate, dry at 105°C for 20 minutes. Allow to cool and spray with diluted potassium iodobismuthate RS. The stain obtained in the chromatogram with *Solution (1)* corresponds in size, color and position to that obtained in the chromatogram with *Solution (2)*.

C. Evaporate to dryness volume of the injection solution equivalent to 1 mg of atropine sulfate. Add to the residue 0.2 mL of fuming nitric acid and evaporate in water bath to dryness. Yellow residue is formed. After cooling, add 2 mL of acetone and 0.2 mL of 3% (w/v) potassium hydroxide solution in methyl alcohol. Violet color develops.

D. It meets sulfate ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.0 to 6.5.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 55.6 EU/mg of atropine sulfate.

ASSAY

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use chromatograph equipped with ultraviolet detector at 254 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm or 10 µm), kept at room temperature; *Mobile phase* flow rate of 2 mL/minute.

Acetate buffer: dissolve the equivalent to 6.8 g of sodium acetate in water, add 2.9 mL of glacial acetic acid and complete the volume to 1000 mL with water.

Mobile phase: transfer 5.1 g of tetrabutylammonium hydrogen sulfate to a 1000 mL volumetric flask, add 50 mL of acetonitrile and complete the volume with *Acetate buffer*. Adjust pH to 5.5 ± 0.1 with 5 M sodium hydroxide.

Sample solution: transfer sample volume equivalent to about 2 mg of atropine sulfate to a 25 mL volumetric flask, complete the volume with water and homogenize.

Standard solution: dissolve accurately weighed quantity of atropine sulfate CRS and dilute with water to obtain a concentration equivalent to 80 µg/mL of atropine sulfate.

Resolution Solution: dilute one volume of 2.5 µg/mL *p*-hydroxybenzoic acid aqueous solution with four volumes of *Standard Solution*.

Inject 100 µL replicates of the *Resolution solution*. The relative retention times are 1.0 for atropine sulfate and 1.6 for *p*-hydroxybenzoic acid. The resolution between the *p*-hydroxybenzoic acid and atropine sulfate peaks is not less than 2.2. Inject 100 µL replicates of the *Sample solution*. The relative standard deviation of the replicate areas under the obtained peaks is not more than 1.5%.

Procedure: inject, separately, 100 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

BARIUM SULFATE***Barii sulfas***

BaSO₄; 233.39

barium sulfate; 08162

Barium salt of sulfuric acid (1:1)

[7727-43-7]

It contains not less than 97.5% and not more than 100.5% of BaSO₄.

DESCRIPTION

Physical characteristics. Fine, white, dense or crystalline powder. Presents polymorphism.

Solubility. Practically insoluble in water and in organic solvents. Sparingly soluble in acids and alkaline solutions.

IDENTIFICATION

A. Mix 0.5 g of the sample, 2 g of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate. Heat the mixture in a crucible until completely melted. Add hot water and filter. Acidify the filtrate with hydrochloric acid. It meets sulfate ion reactions **(5.3.1.1)**.

B. Wash the residue obtained in test **A.** from *Identification* with water. Dissolve in 5 M acetic acid. It meets barium ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 3.5 to 10.0. Determine in sample aqueous suspension at 10% (w/w).

Sulfide limit. In a 500 mL erlenmeyer flask add 10 g of the sample and 100 mL of 0.5 M hydrochloric acid. Attach a filter paper to the top of the erlenmeyer flask. Moisten the central area of the filter paper with 0.15 mL of lead acetate RS. Boil the mixture gently for 10 minutes. Any darkening produced on the filter paper is not more intense than that produced by the standard, containing 5 µg of sulfide in 100 mL of 0.5 M hydrochloric acid and treated similarly. Not more than 0.00005% (0.5 ppm).

Acid-soluble substances limit. Cool the mixture obtained in *Sulfides* and add water to restore the initial volume. Filter on previously washed filter paper with mixture of 10 mL of 0.5 M hydrochloric acid and 90 mL of water. If necessary, filter the first portions again until a clear filtrate is obtained. Evaporate 50 mL of the filtrate to dryness in water bath and add two drops of hydrochloric acid and 10 mL of hot water. Filter again on filter paper, prepared as described above and wash the filter paper with 10 mL of hot water, collecting the filtrate in a tared container. Evaporate the filtrate, together with the washings, until dryness in water bath. Dry the residue in oven at 105 °C, for one hour. Not more than 0.3% (15 mg).

Soluble barium salt limit. Add 10 mL of water to the residue obtained in *Acid-Soluble Substances*. Filter on previously washed filter paper with mixture of 100 mL of 0.5 M hydrochloric acid and add 0.5 mL of M sulfuric acid. Any turbidity developed within 30 minutes is not more intense than that

produced by the standard, containing 50 µg of barium in 10 mL of water and 0.5 mL of *M* sulfuric acid, treated similarly. Not more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Heat 8.33 g of the sample to boiling with 50 mL of 4% acetic acid (v/v) for 10 minutes. Dilute to 50 mL with water and filter. Use 12 mL of the filtrate. Prepare the standard solution using the lead solution (10 ppm Pb). Not more than 0.001% (10 ppm).

Loss on ignition (5.2.9.2). Determine in 1 g of the sample. Incinerate in muffle furnace at 600 ± 25 °C until constant weight. Not more than 2.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.6 g of the sample and transfer to a previously tared platinum crucible. Add 10 g of anhydrous sodium carbonate and homogenize. Melt until a clear viscous liquid is obtained and heat for another 30 minutes. Cool, place the crucible in a 500 mL beaker, add 250 mL of water, shake and heat the set to remove the melted solid. Collect the crucible and wash with water, collecting the washing water in the beaker. Wash the inside of the crucible with 2 mL of 5 *M* acetic acid, then wash with water, collecting the portions again in the beaker. Continue to heat the beaker, under agitation, until the melted solid disintegrates. Cool in ice bath. Allow to stand until the solid decants. Filter the supernatant on filter paper (Whatman nr 40 or equivalent), preventing the precipitate from passing onto the filter paper. Wash the precipitate with two 10 mL portions of cooled 2% (w/v) anhydrous sodium carbonate solution, shake and wait for the solid to decant. Filter the supernatant, using the same filter paper, without transferring the precipitate. Wash the filter paper with five 1 mL portions of hydrochloric acid RS, then wash with water, collecting the filtrate in the beaker containing the barium carbonate precipitate. Add to the beaker 100 mL of water, 5 mL of hydrochloric acid, 10 mL of 40% (w/v) ammonium acetate, 25 mL of 10% (w/v) potassium dichromate and 10 g of urea. Cover the beaker with watch glass and heat to 85°C for not less than 16 hours. Filter while still hot, using a sintered glass funnel with fine porosity and previously tared. Transfer all the precipitate, with the aid of a glass rod, with rubberized tip. Wash the precipitate with 0.5% potassium dichromate (w/v) and then wash with 20 mL of water. Dry at 105 °C for two hours, cool and weigh. The barium chromate mass obtained, multiplied by 0.9213, is equivalent to the BaSO₄ mass.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Diagnostic agent for contrast medium.

CALCIUM SULFATE***Calcii sulfas***

CaSO₄; 136.14
calcium sulfate; 08164
Sulfuric acid calcium salt (1:1)
[7778-18-9]

CaSO₄.2H₂O; 172.17
calcium sulfate dihydrate; 08165
Sulfuric acid calcium salt hydrate (1:1:2)
[10101-41-4]

Calcium sulfate can be anhydrous or dihydrate. It contains not less than 98.0% and not more than 101.0% of CaSO₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white fine powder.

Solubility. Very slightly soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. It meets sulfate ion reactions **(5.3.1.1)**.

B. It meets calcium ion reactions **(5.3.1.1)**.

PURITY TESTS

Acidity or alkalinity. Shake, for five minutes, 1.5 g of the sample with 15 mL of carbon dioxide-free water. Allow to stand for five minutes and filter. To 10 mL of the filtrate add 0.1 mL of phenolphthalein TS and 0.25 mL of 0.01 M sodium hydroxide. Red color develops. Add 0.30 mL of 0.01 M hydrochloric acid. The solution turns colorless. Add 0.2 mL of methyl red TS. Reddish-orange color develops.

Arsenic (5.3.2.5). Dissolve, heating at 50 °C for five minutes, 1 g of the sample in 50 mL of 10% (v/v) hydrochloric acid. Cool and proceed as described in *Visual method*, using 15 mL of this solution. Not more than 0.001% (10 ppm).

Iron (5.3.2.4). Use *Method I*. Dissolve 0.1 g of the sample in 8 mL of 3 M hydrochloric acid. Use 1 mL of *Standard iron solution (10 ppm Fe)*. Not more than 0.01% (100 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Mix 2 g of the sample with 20 mL of water, add 25 mL of 3 M hydrochloric acid and heat to boiling for complete dissolution of the sample. Cool and add ammonium hydroxide to pH 7.0. Filter, reduce filtrate volume to 25 mL and filter again, if necessary, to obtain solution. Not more than 0.001% (10 ppm).

Loss on ignition (5.2.9.2). Determine at a minimum temperature of 250 °C, until constant weight. For the dihydrate form, the loss ranges between 19.0% and 23.0%. For the anhydrous form, not more than 1.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.15 g of the sample and dissolve in 120 mL of water. Proceed as described in *Complexometric titrations (5.3.3.4) for Calcium*, using 0.1 M disodium edetate VS. Each mL of 0.1 M disodium edetate VS is equivalent to 13.614 mg of CaSO₄.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

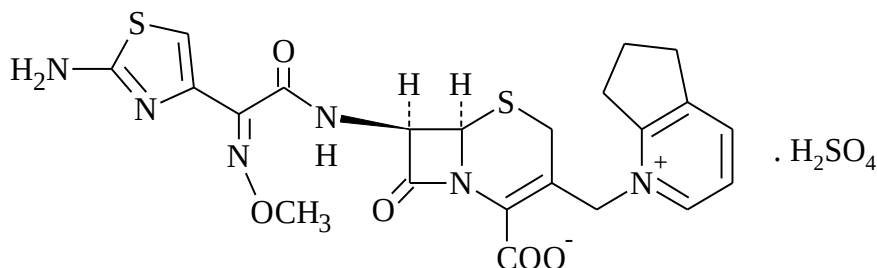
Comply with current legislation.

CATEGORY

Adjuvant.

CEFPIROME SULFATE

Cefpiromum sulfas



$C_{22}H_{22}N_6O_5S_2 \cdot H_2SO_4$; 612.65

cefpirome sulfate; 01887

[6*R*-[6 α ,7 β (*Z*)]]-1-[[7-[[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-sulfate 5-thia-1-azabicyclo[4.2.0]octo-2-en-3-yl]methyl]-6,7-dihydro-5*H*-cyclopenta[*b*]pyridinium.
[98753-19-6]

It contains not less than 98.0% and not more than 101.0% of $C_{22}H_{22}N_6O_5S_2 \cdot H_2SO_4$ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or pale yellow crystalline powder.

Solubility. Soluble in water, in ethyl alcohol and in methyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): -27 to -32, in relation to the anhydrous substance. Determine in aqueous solution at 5% (w/v).

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out. The identification tests B. and C. can be omitted if test A. is carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample previously desiccated at 60 °C, under reduced pressure, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the cefpirome sulfate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.0015% (w/v) solution in 0.1 *M* hydrochloric acid, there are maximum and minimum points only at the same wavelengths of similar cefpirome sulfate CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method B. from Assay, corresponds to the one from the main peak of the *Standard solution*.

D. It meets sulfate ion reactions (5.3.1.1).

PURITY TESTS

pH (5.2.19). 1.6 to 2.6. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed as described in method **B.** from Assay. Use the *Sample solution*.

Procedure: Inject 20 µL of the *Sample Solution*, record the chromatograms and measure the areas under all the peaks obtained. The sum of the areas under the secondary peaks, except that under the solvent peak, is not more than 1.0% of the area under the main peak. No area is greater than 0.3% of the area under the main peak. Do not include solvent-related peaks in the calculations.

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20.1). Not more than 2.0%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.2%.

BIOLOGICAL SAFETY TESTS

When indicated on the label that the substance is sterile, the sample complies with the Sterility and with the Bacterial endotoxin tests. When indicated that the substance must be sterilized during the production of sterile preparations, the sample complies with the Bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test. Proceed as described in *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). Not more than 0.1 EU/mg of cefpirome sulfate.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh about 30 mg of the sample and dissolve in 0.1 M hydrochloric acid. Complete the volume to 100 mL with the same solvent and homogenize. Dilute, successively, in 0.1 M hydrochloric acid, to a concentration of 0.0015% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 271 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the content of $C_{22}H_{22}N_6O_5S_2 \cdot H_2SO_4$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 265 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at temperature of 25°C; *Mobile phase* flow rate of 0.8 mL/minute.

Mobile phase: mixture of water and methyl alcohol (70:30).

Sample solution: quantitatively transfer about 30 mg of the sample to a 100 mL volumetric flask and complete the volume with water. Transfer 4 mL of this solution to a 100 mL volumetric flask, complete the volume with the same solvent and homogenize.

Standard solution: prepare a 12 µg/mL solution of cefpirome sulfate CRS in water.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{22}H_{22}N_6O_5S_2 \cdot H_2SO_4$ in the sample from the responses obtained with the *Standard Solution* and the *Sample Solution*.

C. Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)*, by agar diffusion using cylinders.

Microorganism: *Kocuria rhizophila* ATCC 9341.

Culture media: culture medium number 1 for microorganism maintenance, sterile saline solution for inoculum standardization, culture medium number 11 for base layer and for inoculum preparation.

Sample solution: weigh, accurately, about 24 mg of the sample and transfer to a 100 mL volumetric flask with water. Complete the volume with the same solvent and homogenize. Dilute, successively, to concentrations of 0.36 µg/mL, 0.72 µg/mL and 1.44 µg/mL, using 0,1 M sodium phosphate buffer, sterile, pH 8.0 as diluent.

Standard solution: weigh, accurately, the equivalent to 24 mg of cefpirome sulfate CRS and transfer to a 100 mL volumetric flask using water. Complete the volume with the same solvent and homogenize. Dilute to obtain concentrations of 0.36 µg/mL, 0.72 µg/mL and 1.44 µg/mL, using 0,1 M sodium phosphate buffer, sterile, pH 8.0 as diluent.

Procedure: add 20 mL of medium number 11 to each plate, wait to solidify, add 5 mL of 2% inoculum and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*, using cylinders, adding 0.2 mL of the freshly prepared solutions to the cylinders. Calculate the sample potency, in µg of cefpirome sulfate per milligram, from the standard potency and the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at temperature between 2 °C and 8 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

CEFPIROME SULFATE POWDER FOR INJECTION SOLUTION

It contains cefpirome sulfate equivalent to not less than 95.0% and not more than 110.0% of the stated quantity of cefpirome ($C_{22}H_{22}N_6O_5S_2$).

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out. The identification tests B. and C. can be omitted if test A. is carried out.

A. In the infrared absorption spectrum (**5.2.14**) of the sample previously desiccated at 60 °C, under reduced pressure, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the cefpirome sulfate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm, of 0.0012% (w/v) solution in 0.1 M hydrochloric acid, there are maximum and minimum points only at the same wavelengths of similar cefpirome sulfate CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. It meets sulfate ion reactions (**5.3.1.1**).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test. Determine in the diluent flask.

pH (5.2.19). 5.5 to 7.5. Determine after reconstitution of the sample with the diluent.

Determination of weight (5.1.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Related substances. Proceed as described in method **C.** from *Assay*. Use the *Sample solution*.

Procedure: Inject 20 µL of the *Sample Solution*, record the chromatograms and measure the areas under all the peaks obtained. The sum of the areas under the secondary peaks, except that under the solvent peak, is not more than 1.5% of the area under the main peak. No area is greater than 0.5% of the area under the main peak. Do not include solvent-related peaks in the calculations.

Water (5.2.20.1). Not more than 2.5%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Proceed as described in *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). Not more than 0.1 EU/mg of cefpirome.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Accurately weigh the equivalent to 24 mg of cefpirome and dissolve in 0.1 M hydrochloric acid. Complete the volume to 100 mL with the same solvent. Dilute, successively, in 0.1 M hydrochloric acid, to a concentration of 0.0012% (w/v). Prepare a standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 271 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the quantity of $C_{22}H_{22}N_6O_5S_2$ in the powder for injection solution from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 265 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), kept at temperature of 25°C; *Mobile phase* flow rate of 0.8 mL/minute.

Mobile phase: mixture of water and methyl alcohol (70:30).

Sample solution: quantitatively transfer quantity of sample equivalent to 25 mg of cefpirome to a 100 mL volumetric flask, complete the volume with water and homogenize. Transfer 4 mL of this solution to a 100 mL volumetric flask, complete the volume with the same solvent and homogenize.

Standard solution: prepare a 10 μ g/mL solution of cefpirome CRS in water.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of cefpirome ($C_{22}H_{22}N_6O_5S_2$) in the powder for injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

C. Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)*, by agar diffusion using cylinders.

Microorganism: *Kocuria rhizophila* ATCC 9341.

Culture media: culture medium number 1 for microorganism maintenance, sterile saline solution for inoculum standardization, culture medium number 11 for base layer and for inoculum preparation.

Sample solution: mix the contents of 10 units. Transfer quantity of powder equivalent to 20 mg of cefpirome to a 100 mL volumetric flask with water. Complete the volume with the same solvent and homogenize. Dilute to obtain concentrations of 0.3 μ g/mL, 0.6 μ g/mL and 1.2 μ g/mL, using 0.1 M sodium phosphate buffer, sterile, pH 8.0 as diluent.

Standard solution: weigh, accurately, the equivalent to 20 mg of cefpirome CRS and transfer to a 100 mL volumetric flask using water. Complete the volume with the same solvent and homogenize. Dilute to obtain concentrations of 0.3 μ g/mL, 0.6 μ g/mL and 1.2 μ g/mL, using 0.1 M sodium phosphate buffer, sterile, pH 8.0 as diluent.

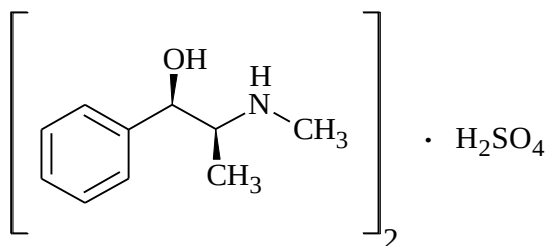
Procedure: add 20 mL of medium number 11 to each plate, wait to solidify, add 5 mL of 2% inoculum and proceed as described in *Microbiological test by agar diffusion (5.5.3.3.1)*, using cylinders, adding 0.2 mL of the freshly prepared solutions to the cylinders. Calculate the sample potency, in µg of cefpirome per milligram, from the standard potency and the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light and at temperature below 25 °C.

LABELING

Comply with current legislation.

EPHEDRINE SULFATE*Ephedrini sulfas* $(\text{C}_{10}\text{H}_{15}\text{NO})_2 \cdot \text{H}_2\text{SO}_4$; 428.54

ephedrine sulfate; 03311

 (αR) - α -[(1*S*)-1-(methylamino)ethyl]benzenemethanol sulfate (1:2)

[134-72-5]

It contains not less than 98.0% and not more than 101.0% of $(\text{C}_{10}\text{H}_{15}\text{NO})_2 \cdot \text{H}_2\text{SO}_4$, in relation to the desiccated substance.

DESCRIPTION

Physicochemical characteristics. Fine powder or white crystals, it darkens when exposed to light. Melting temperature (**5.2.2**) is about 245°C, with decomposition.

Solubility. Very soluble in water and slightly soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): -32 to -30, in relation to the desiccated substance. Determine in solution at 5% (w/v) in water.

IDENTIFICATION

A. In the infrared absorption spectrum (**5.2.14**) of the sample, dispersed in potassium bromide, there are maximum absorption points on the same wavelengths and with the same relative intensities observed in the ephedrine sulfate CRS spectrum, prepared identically.

B. It meets sulfate ion reactions (**5.3.1.1**).

PURITY TESTS

Acidity or alkalinity. Dissolve 1 g in 20 mL of distilled water and add one drop of methyl red TS. If the solution turns red or pink, it should turn yellow, by adding not more than 0.2 mL of 0.02 *M* sodium hydroxide. If it turns yellow, it should turn red by adding not more than 0.1 mL of 0.04 *M* sulfuric acid.

Chlorides (5.3.2.1). Not more than 0.15% (1500 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105 °C for three hours. Not more than 2.0%.

Residue by incineration (5.2.10). Determine in 1 g of the substance. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titration in non-aqueous medium (5.3.3.5)*. Transfer approximately 0.3 g of the sample, accurately weighed, to a separating funnel and dissolve in 10 mL of water, add 3 g of sodium chloride and 5 mL of *M* sodium hydroxide. Extract with four portions of 25 mL of chloroform. Shake the combined chloroform extracts with 10 mL of saturated sodium chloride solution and filter through cotton soaked with chloroform. Extract the aqueous layer with 10 mL of chloroform and add to the chloroform extract. Add methyl red TS and titrate with 0.1 *M* perchloric acid VS. Carry out the blank test and make the necessary corrections. Each mL of 0.1 *M* perchloric acid VS is equivalent to 21.427 mg of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Adrenergic (bronchodilator).

EPHEDRINE SULFATE INJECTION SOLUTION

It contains not less than 95.0% and not more than 105.0% of the stated quantity of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

IDENTIFICATION

A. Mix 1 mL of the sample with 5 mL of ethyl alcohol and evaporate in water bath. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points on the same wavelengths and with the same relative intensities observed in the ephedrine sulfate CRS spectrum, prepared identically.

B. It meets sulfate ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 4.5 to 7.0.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 1.7 EU/mg of ephedrine sulfate.

ASSAY

Quantitatively transfer the equivalent to 250 mg of ephedrine sulfate to a separating funnel. Add 10 mL of water, 3 g of sodium chloride, 5 mL of *M* sodium hydroxide and extract with four 25 mL portions of chloroform. Combine the chloroform extracts, shake with 10 mL of saturated sodium chloride solution and filter through cotton soaked with chloroform. Separate the phases and add 10 mL of chloroform to the aqueous phase. Combine the chloroform extracts, add methyl red TS and titrate with 0.1 *M* perchloric acid VS in dioxane. Carry out the blank test and make the necessary corrections. Each mL of 0.1 *M* perchloric acid VS is equivalent to 21.427 mg of $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

STREPTOMYCIN SULFATE POWDER FOR INJECTION SOLUTION

Streptomycin sulfate powder for injection solution is sterile streptomycin sulfate powder to be reconstituted in water for injection. It contains not less than 90.0% and not more than 115.0% of the stated quantity of $C_{12}H_{39}N_7O_{12}$.

IDENTIFICATION

A. Dissolve 10 mg of the powder in 5 mL of water, add 1 mL of *M* sodium hydroxide and heat in water bath for five minutes. Cool and add 2 mL of a 2% (w/v) ferric ammonium sulfate solution in 0.5 *M* sulfuric acid. Violet color is produced.

B. Dissolve 0.1 g of the sample powder in 2 mL of water, add 1 mL of a 10% (w/v) solution of 1-naphthol in ethyl alcohol and 2 mL of a 2% aqueous sodium hypochlorite solution (w/v). Reddish color is produced.

C. It meets sulfate ion reactions (5.3.1.1).

CHARACTERISTICS

pH (5.2.19). 5.0 to 8.0. Determine after reconstitution with diluent.

Determination of weight (5.1.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

PURITY TESTS

Loss on drying (5.2.9.1). Determine in 100 mg of the sample. Dry in oven at 60 °C, under reduced pressure (not greater than 5 mmHg) for three hours. Not more than 5.0%.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test. Use the *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). Not more than 0.25 EU/mg of streptomycin.

ASSAY

Use one of the methods described below.

Note: to carry out the following tests, use a minimum sampling of 10 flasks.

A. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Prepare sample solution and 0.2% (w/v) standard solution in water. Transfer 5 mL of each solution, separately, to 25 mL volumetric flasks. Add 1 mL of *M* sodium hydroxide to each flask and heat for four minutes in boiling water bath. Cool in iced water to room temperature. Add, to each flask, 2 mL of a 2% (w/v) ferric ammonium sulfate solution in 0.5 *M* sulfuric acid. Complete the volume with water, homogenize and allow to stand for 10 minutes. Prepare the blank in parallel, adding 5 mL of water in a 25 mL volumetric flask and proceed as described above, from "Add to each flask 1 mL...". Measure the

absorbances of the solutions at 520 nm **(5.2.14)**, using blank for zero adjustment. Calculate the potency in µg/mL of streptomycin $C_{12}H_{39}N_7O_{12}$ in the sample from the readings obtained and the potency of the standard.

B. Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)*, after reconstituting the content of the flasks, as indicated by the producer.

Microorganism: Bacillus subtilis ATCC 6633.

Sample solution: weigh equivalent quantity of the sample and dilute with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2), to obtain a solution containing 1 mg/mL of base. Dilute with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain solutions in the concentration ranges of 2.0 µg/mL, 1.0 µg/mL and 0.5 µg/mL.

Standard solution: dissolve accurately weighed quantity of streptomycin sulfate CRS in 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain a 1 mg/mL solution. Dilute successively with 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (Solution 2) to obtain solutions in the concentration ranges of 2.0 µg/mL, 1.0 µg/mL and 0.5 µg/mL.

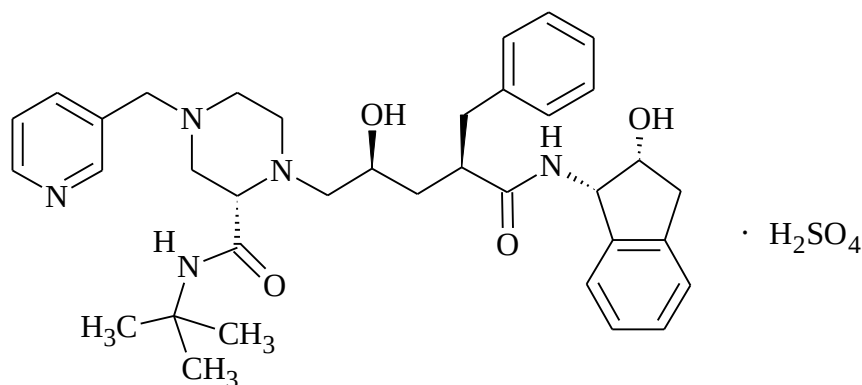
Procedure: add 20 mL of medium base number 5 to each plate, wait to solidify, add 5 mL of inoculum number 5 and proceed as described in *Microbiological Assay by agar diffusion (5.5.3.3.1)*. Calculate the quantity, in mg, of streptomycin ($C_{12}H_{39}N_7O_{12}$) in the powder for injection solution, from the standard potency and from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In hermetically closed containers, protected from humidity and at room temperature.

LABELING

Comply with current legislation.

INDINAVIR SULFATE*Indinaviri sulfas*C₃₆H₄₇N₅O₄·H₂SO₄; 711.88

indinavir sulfate; 04883

2,3,5-Trideoxy-*N*-[(1*S*,2*R*)-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl]-5-[(2*S*)-2-[(1,1-dimethylethyl)amino]carbonyl]-4-(3-pyridinylmethyl)-1-piperazinyl]-2-(phenylmethyl)-*D*-erythro-pentonamide (1:1)
[157810-81-6]

It contains not less than 98.5% and not more than 101.5% of C₃₆H₄₇N₅O₄·H₂SO₄ and not less than 13.2% and not more than 14.4% of sulfate, in relation to the anhydrous and ethyl alcohol-free substance.

DESCRIPTION

Physical characteristics. White or off-white amorphous powder.

Solubility. Freely soluble in water, soluble in methyl alcohol, very slightly soluble in acetonitrile.

Physicochemical constants.

Melting range (5.2.2): 150 °C to 154 °C, with decomposition.

Specific optical rotation (5.2.8): +122 to +129, in 1% (w/v) aqueous solution, in relation to the anhydrous and ethyl alcohol-free substance. Read at 25 °C, at a wavelength of 365 nm.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the indinavir sulfate CRS spectrum.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.004% (w/v) sample solution in 0.1 *M* hydrochloric acid, there are maximum points at 210 nm and 260 nm, identical to those observed in the spectrum of similar indinavir sulfate CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

D. The 0.5% (w/v) sample solution meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

Ethyl alcohol content. Proceed as described in *Gas chromatography (5.2.17.5)*. Use gas chromatograph equipped with flame ionization detector; chromatographic column with 30 m in length and 0.25 mm in internal diameter, packed with polyethylene glycol, with film thickness of 0.25 µm; column temperature at 45 °C; injector temperature at 260 °C and detector temperature at 280 °C; use nitrogen as carrier gas; carrier gas flow rate of 10 mL/minute. At the end of each run, the column temperature is increased to 230°C and kept for 18 minutes.

Sample solution: quantitatively transfer about 0.2 g of the sample to a 10 mL volumetric flask, dissolve in dimethylsulfoxide and complete the volume with the same solvent. Homogenize.

Standard solution: quantitatively transfer about 6.5 mL of absolute ethyl alcohol to a 100 mL volumetric flask, complete the volume with dimethylsulfoxide and homogenize. Transfer 5 mL of the resulting solution to a 25 mL volumetric flask and complete the volume with dimethylsulfoxide. Homogenize.

Procedure: inject, separately, 1 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of ethyl alcohol in the sample from the responses obtained with the *Standard solution* and the *Sample solution*. Between 5.0% and 8.0%.

Chromatographic purity. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, using chromatograph equipped with ultraviolet detector at 220 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); kept at room temperature, *Mobile phase* flow rate of 1 mL/minute.

Eluent A: dissolve 0.54 g of potassium phosphate monobasic and 2.79 g of potassium phosphate dibasic in 2000 mL of water.

Eluent B: acetonitrile.

Diluent: mixture of *Eluent A* and *Eluent B* (50:50).

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 40	80 → 30	20 → 70	linear gradient
40 – 45	30	70	isocratic
45 – 47	30 → 80	70 → 20	linear gradient
47 – 52	80	20	isocratic

Solution (1): quantitatively transfer about 50 mg of the sample to a 100 mL volumetric flask and dissolve in 70 mL of *Diluent*. Complete the volume with the same solvent. Homogenize, obtaining a 500 µg/mL solution.

Solution (2): prepare 500 µg/mL indinavir sulfate CRS solution in *Diluent*.

Inject 20 µL replicates of the *Solution (2)*. The column efficiency is not less than 4000 theoretical plates/meter. The retention factor for the indinavir peak is between 3.0 and 6.0. The tailing factor is not more than 2.0.

Procedure: inject 20 µL of *Solution (1)*, record the chromatograms and measure the areas under the peaks. Do not consider peaks related to the solvents. The area under any individual peak, except that under the main peak, is not more than 0.1% of the total area under the peaks obtained. The sum of the areas under all peaks, except that under the main peak, is not more than 0.5% of the total area under the peaks obtained.

Heavy metals (5.3.2.3). Use *Method I*. Not more than 0.001% (10 ppm).

Water (5.2.20.1). Not more than 1.5%.

Residue by incineration (5.2.10). Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Sulfate.

Weigh, accurately, about 0.5 g of the sample, transfer to a beaker and dissolve in 60 mL of mixture of methyl alcohol and water (50:50). Use specific electrode for lead and suitable reference electrode. Titrate with 0.1 M lead nitrate VS, determining the endpoint potentiometrically. Each mL of 0.1 M lead nitrate VS is equivalent to 9.604 mg of sulfate.

Indinavir sulfate.

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 260 nm; 250 mm long and 4.6 mm internal diameter column, packed with silica chemically bonded to octylsilane group (5 µm); kept at 40 °C, *Mobile phase* flow rate of 1 mL/minute.

Dibutylammonium phosphate buffer: dissolve 3 g of phosphoric acid and 1.7 mL of dibutylamine in 900 mL of water. Adjust pH to 6.5 ± 0.5 with M sodium hydroxide.

Mobile phase: mixture of *Dibutylammonium phosphate buffer* and acetonitrile (55:45).

Sample solution: quantitatively transfer about 58 mg of the sample to a 100 mL volumetric flask, add 70 mL of *Mobile Phase*. Shake mechanically for 15 minutes and place in ultrasonic bath for 15 minutes. Complete the volume with *Mobile phase*. Homogenize.

Standard Solution: prepare 0.58 mg/mL indinavir sulfate CRS solution in *Mobile Phase*, equivalent to 0.5 mg of indinavir per milliliter.

Inject 10 µL replicates of the *Standard solution*. The column efficiency is not less than 4000 theoretical plates/meter. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 1.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{36}H_{47}N_5O_4 \cdot H_2SO_4$ in the sample from the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In hermetic containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antirretroviral.

MAGNESIUM SULFATE***Magnesii sulfas***

MgSO₄; 120.36

magnesium sulfate; 08167

Magnesium salt of sulfuric acid (1:1)

[7487-88-9]

MgSO₄.7H₂O; 246.47

magnesium sulfate heptahydrate; 08168

sulfuric acid hydrate magnesium salt (1:1:7)

[10034-99-8]

It contains not less than 99.0% and not more than 100.5% of MgSO₄, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or bright colorless crystals.

Solubility. Freely soluble in water, very soluble in hot water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. It meets magnesium ion reactions **(5.3.1.1)**.

B. It meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 5 g of the sample in water and dilute to 50 mL with the same solvent. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

Acidity or alkalinity. Add one drop of phenol red TS to 10 mL of the preparation obtained in *Preparation aspect*. Not more than 0.2 mL of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is required to change the indicator color.

Arsenic (5.3.2.5). Determine in 15 mL of the preparation obtained in *Preparation aspect*. Proceed as described in *Spectrophotometric Method, Method I*. Not more than 0.0002% (2 ppm).

Chlorides (5.3.2.1). Determine in 1.2 g of the sample. Not more than 0.03% (300 ppm).

Iron (5.3.2.4). Determine in 5 mL of the preparation obtained in *Preparation aspect*. Proceed as described in *Method I*, using 10 mL of *Iron Standard Solution (1 ppm Fe)*. Not more than 0.002% (20 ppm).

Heavy metals (5.3.2.3). Not more than 0.001% (10 ppm).

Residue by incineration (5.2.10). Determine in 1 g of the sample. Dry in oven at 105 °C, for two hours, and then incinerate in a muffle at 450 °C ± 25 °C, until constant weight. Between 45.0% and 52.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Complexometric titrations for magnesium (5.3.3.4)*. Accurately weigh about 0.45 g of the sample. Each mL of 0.1 M disodium edetate VS is equivalent to 12.036 mg of MgSO₄.

PACKAGING AND STORAGE

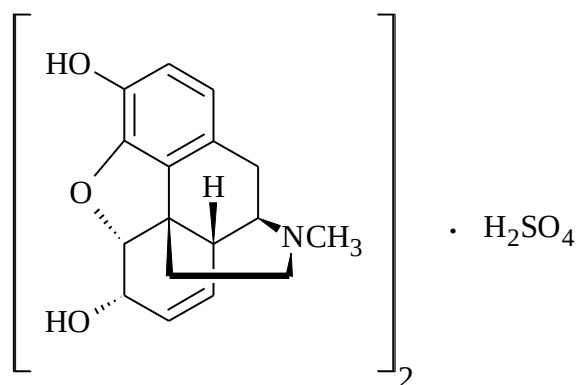
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Osmotic laxative; used in electrolyte therapy

MORPHINE SULFATE*Morphini sulfas* $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$; 668.76

morphine sulfate; 06114

 $(5\alpha,6\alpha)$ -7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol sulfate (1:2)
[64-31-3] $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$; 758.83

morphine sulfate pentahydrate; 09532

 $(5\alpha,6\alpha)$ -7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol sulfate hydrate (1:2:5)
[6211-15-0]

It contains not less than 98.0% and not more than 102.0% of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Freely soluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): -107 to -110, in relation to the anhydrous substance. Determine in solution at 2% (w/v) in water.

IDENTIFICATION

The Identification tests B, C., D. and E. can be omitted if test A. is carried out. The Identification tests B., C. and D can be omitted if tests A. and E. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample desiccated at 145 °C for one hour and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the morphine sulfate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 250 nm to 300 nm of the 0.01% (w/v) sample solution prepared in water, there is maximum absorption at 285 nm, identical to the spectrum of similar morphine sulfate CRS solution.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. In a porcelain capsule, add 0.5 mL of formaldehyde solution to 1 mg of the sample. Purple color develops, which turns violet.

E. It meets sulfate ion reactions **(5.3.1.1)**.

F. It meets alkaloid reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. The 2% (w/v) sample preparation in water is clear.

Acidity. Add 0,05 mL of methyl red TS to 10 mL of the preparation described in *Preparation aspect*. Not more than 0.2 mL of 0.02 M sodium hydroxide is required to develop yellow color.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of ammonia, acetone, ethyl alcohol, water and toluene (2.5:32.5:24.5:10.5:35) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): 20 mg/mL sample solution in mixture of water and ethyl alcohol (1:1).

Solution (2): dissolve 25 mg of codeine phosphate in 5 mL of *Solution (1)*, dilute 0.2 mL of this solution to 10 mL in mixture of water and ethyl alcohol (1:1).

Solution (3): dilute 0.1 mL of *Solution (1)* to 20 mL with mixture of water and ethyl alcohol (1:1).

Solution (4): dilute 2 mL of *Solution (3)* to 5 mL with mixture of water and ethyl alcohol (1:1).

Solution (5): dilute 2 mL of *Solution (3)* to 10 mL with mixture of water and ethyl alcohol (1:1).

Develop chromatogram. Remove plate and allow to air dry. Spray with potassium iodobismuthate RS and allow to air dry for 15 minutes. Spray with 3% (w/v) hydrogen peroxide RS. No secondary stain obtained in the chromatogram with *Solution (1)* is more intense than that obtained with *Solution (2)* (0.5%). No secondary stain obtained with *Solution (1)* can be more intense than the one obtained with *Solution (3)* (0.5%). Not more than two stains obtained with *Solution (1)* can be more intense than the one obtained with *Solution (4)* (0.2%). The test is only valid if in the chromatogram obtained with *Solution (2)* there are two clearly separated stains and if the stain obtained in the chromatogram obtained with *Solution (5)* is clearly visible.

Water (5.2.20.1). Determine in 0.2 g of sample. Between 10.4% and 13.4%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 0.5 g of the sample and dissolve in 120 mL of acetic anhydride. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 66.875 mg of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, using chromatograph equipped with ultraviolet detector at 284 nm; column with 300 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group; *Mobile phase* flow rate of 1.5 mL/minute.

Mobile phase: quantitatively dissolve about 0.73 g of sodium heptanesulfonate in 720 mL of water. Add 280 mL of methyl alcohol and 10 mL of glacial acetic acid.

Sample solution: dissolve an accurately weighed quantity of the sample in *Mobile phase* to obtain a 0.24 mg/mL solution.

Standard solution: dissolve an accurately weighed quantity of morphine sulfate CRS in *Mobile phase* to obtain a 0.24 mg/mL solution.

Inject 25 µL replicates of the *Standard solution*. The retention time is about 6.0 minutes for morphine sulfate. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 25 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$ in the sample from the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Opioid analgesic.

MORPHINE SULFATE TABLETS

It contains not less than 92.5% and not more than 107.5% of the stated quantity of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Weigh the equivalent to 20 mg of morphine sulfate, add 5 mL of water and shake. Filter and add 0.05 mL of ferric chloride RS to the filtrate. Blue color develops.

B. Weigh and pulverize the tablets. Weigh the equivalent to 10 mg of morphine sulfate and add 10 mL of water. Filter and add, to 5 mL of the filtrate, 0.15 mL of 1% potassium ferricyanide (w/v) and 0.05 mL of ferric chloride RS. Green color develops immediately, which quickly turns blue.

C. The pulverized powder from the tablets must meet sulfate ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described in method **B.** from Assay.

DISSOLUTION TEST (5.1.5)

Dissolution medium: phosphate buffer pH 6.5, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium and filter. Proceed as described in method **B.** from Assay except that the injection volume will be 200 µL. Calculate the quantity of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ dissolved in the medium, comparing the readings obtained with that of the morphine sulfate CRS solution at the concentration of 0.0033% (w/v), prepared in the same solvent.

Tolerance: not less than 70% (Q) of the stated quantity of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ dissolve in 45 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of 70% (v/v) ethyl alcohol, toluene, acetone and 13.5 M ammonia (35:35:32.5:2.5) as mobile phase. Apply to the plate, separately, 50 µL of each one of the freshly prepared solutions described below.

Solution (1): weigh the equivalent to 25 mg of morphine sulfate to obtain a 1 mg/mL sample solution in ethyl alcohol.

Solution (2): dissolve sufficient quantity of codeine sulfate CRS in *Solution (1)* to obtain a 1 mg/mL codeine sulfate solution. Remove an aliquot from this solution and dissolve in ethyl alcohol to obtain a 0.005 mg/mL codeine sulfate solution.

Solution (3): transfer a 2 mL aliquot from *Solution (2)* and dilute in 5 mL of ethyl alcohol.

Develop chromatogram. Remove plate and allow to air dry. Spray with potassium iodide and bismuth subnitrate RS and allow to air dry for 15 minutes. Spray with 3% (w/v) hydrogen peroxide. Codeine and morphine stains are bluish-gray and pink, respectively. No secondary stain obtained in the chromatogram with *Solution (1)* is more intense than that of codeine, obtained with *Solution (2)* (0.5%). No other secondary stain obtained is more intense than that obtained with *Solution (2)* (0.5%) and not more than two stains are more intense than that obtained with *Solution (3)* (0.2%). The test is only valid if in the chromatogram obtained with *Solution (2)* there are two clearly separated stains.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.1 g of morphine sulfate to 25 mL of water and 5 mL of *M* sodium hydroxide. Add 1 g of ammonium sulfate and shake mechanically until complete dissolution. Place in ultrasonic bath for 10 minutes if necessary. Add 20 mL of ethyl alcohol and extract with successive quantities of 40 mL, 20 mL, 20 mL and 20 mL of a mixture of chloroform and ethyl alcohol (3:1). Wash each extract with the same 5 mL of water, filter and evaporate the filtrate solvent. Dissolve the residue obtained in 10 mL of 0.05 *M* hydrochloric acid VS, boil, cool and add 15 mL of water. Titrate the excess of hydrochloric acid with 0.05 *M* sodium hydroxide SV, using methyl red TS as indicator. Each mL of 0.05 *M* SV hydrochloric acid is equivalent to 18.971 mg of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$ or to 16.719 mg of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$.

B. Proceed as described in method **B.** of Assay in the *Morphine sulfate* monograph. Prepare solutions as described below.

Sample Solution: weigh and pulverize 20 tablets. Dissolve accurately weighed quantity of morphine sulfate in *Mobile phase* to obtain a 0.3 mg/mL solution.

Standard solution: dissolve accurately weighed quantity of morphine sulfate CRS in *Mobile phase* to obtain a 0.3 mg/mL solution.

Inject 25 µL replicates of the *Standard solution*. The retention time is about six minutes for morphine sulfate. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 25 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In containers protected from light.

LABELING

Comply with current legislation.

MORPHINE SULFATE INJECTION SOLUTION

It contains not less than 90.0% and not more than 110.0% of the stated quantity of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$.

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and mixture of acetone, methyl alcohol and ammonium hydroxide (50:50:1) as mobile phase. Apply to the plate, separately, 20 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve accurately weighed quantity of the sample in mixture of methyl alcohol and water (1:1) to obtain a 0.05% (w/v) solution.

Solution (2): dissolve accurately weighed quantity of morphine sulfate CRS in mixture of methyl alcohol and water (1:1) to obtain a 0.05% (w/v) solution.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

C. Evaporate to dryness, in water bath, a volume equivalent to 5 mg of morphine sulfate. Dissolve the residue obtained in 5 mL of water, and add 0.15 mL of 1% potassium ferricyanide (w/v) and 0.05 mL of ferric chloride RS. Bluish-gray color develops, which quickly turns blue.

D. It meets sulfate ion reactions (5.3.1.1).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 2.5 to 6.5.

Related substances. Proceed as described in the *Related substances* test in the *Morphine sulfate tablets* monograph. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dilute the volume of the injection solution in mixture of ethyl alcohol and water (1:1), to obtain a 1% (w/v) morphine sulfate solution.

Solution (2): dissolve sufficient quantity of codeine sulfate CRS in *Solution (1)*, obtaining a 10 mg/mL codeine sulfate solution. Remove an aliquot from this solution and dissolve in mixture of ethyl alcohol and water (1:1), obtaining a 0.05 mg/mL codeine sulfate solution.

Solution (3): transfer a 2 mL aliquot from *Solution (2)* and dilute in 5 mL of mixture with ethyl alcohol and water (1:1).

The test is only valid if the chromatogram obtained with *Solution (2)* presents two clearly separated stains. Disregard any stain that has a retention factor (R_f) lower than 0.1.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Complies with the test. Not more than 14.29 EU/mg of morphine sulfate.

ASSAY

Proceed as described in method **B.** from *Assay* in the *Morphine sulfate* monograph. Prepare solutions as described below.

Sample solution: transfer volume of the injection solution equivalent to 25 mg of morphine sulfate to a 100 mL volumetric flask, using *Mobile phase* as solvent, to obtain a 0.25 mg/mL solution.

Standard solution: dissolve accurately weighed quantity of morphine sulfate CRS in *Mobile phase* to obtain a solution with final concentration of 0.25 mg/mL.

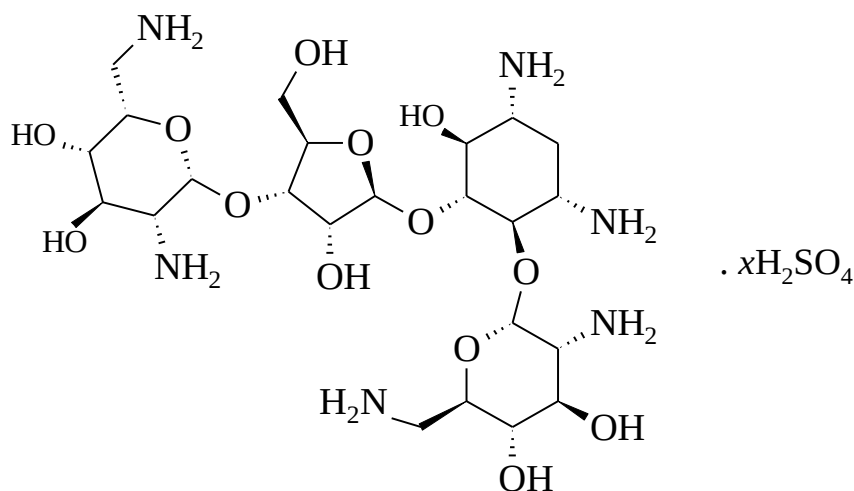
Procedure: inject, separately, 25 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $(C_{17}H_{19}NO_3)_2 \cdot H_2SO_4$ in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In type I glass containers, in cool place protected from light. Avoid freezing.

LABELING

Comply with current legislation.

NEOMYCIN SULFATE*Neomycini sulfas*

$C_{23}H_{46}N_6O_{13} \cdot xH_2SO_4$; 614.65 (base)

neomycin sulfate; 06284

Neomycin sulfate; 06284

[1405-10-3]

Neomycin sulfate is a mixture of sulfates of substances produced by *Streptomyces fradiae*, with its main component being 2-deoxy-4-O-(2,6-diamino-2,6-dideoxy- α -D-glycopyranosyl) sulfate. -5-O-[3-O-(2,6-diamino-2,6-dideoxy- β -L-idopyranosyl)- β -D-ribofuranosyl]-D-streptamine (neomycin B). It has a potency of not less than 600 μ g/mg of neomycin, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish-white crystalline powder, hygroscopic.

Solubility. Very soluble in water, very slightly soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +53.5 to +59.0, in relation to the desiccated substance. Determine in aqueous solution at 10% (w/v).

IDENTIFICATION

A. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel H as support and mixture of methyl alcohol, ammonium hydroxide, chloroform and water (6:3:2:1) as mobile phase. Apply to the plate, separately, 5 μ L of each one of the freshly prepared solutions described below.

Solution (1): prepare a 20 mg/mL sample solution in water.

Solution (2): prepare a 20 mg/mL neomycin sulfate CRS solution in water.

Develop chromatogram. Remove the plate and dry in hot air. Spray the plate with 1% (w/v) ninhydrin in butyl alcohol and heat at 105 °C for two minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

B. *Solution (1)* obtained in method **A.** of *Identification*, diluted to 5% (w/v) in water, meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 5.0 to 7.5. Determine in aqueous solution at 1% (w/v).

Related compounds 1. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support, and mixture of methylene chloride, ammonium hydroxide and methyl alcohol (10:20:20), as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): prepare a 25 mg/mL sample solution in water.

Solution (2): prepare a 0.5 mg/mL neamine CRS solution in water.

Solution (3): mix 0.5 mL of *Solution (1)* with 0.5 mL of *Solution (2)*.

Develop chromatogram. Remove the plate, allow to dry at between 100 °C and 105 °C for 10 minutes. Spray with stannous chloride and ninhydrin solution and heat at 100 °C for 15 minutes. Spray again with the same solution and heat at 110 °C for 15 minutes. Any stain obtained with *Solution (1)* chromatogram, corresponding to neamine, is not more intense than that obtained with *Solution (2)* (2.0%). The test is only valid if the chromatogram obtained with *Solution (3)* presents two clearly separated main stains.

Related compounds 2. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel G as support and mixture of methyl alcohol and 20% (w/v) sodium chloride (20:80) as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): prepare a 8 mg/mL sample solution in water.

Solution (2): prepare a 1.2 mg/mL framycetin sulfate CRS solution in water.

Solution (3): dilute 5 mL of *Solution (2)* to 25 mL with water.

Solution (4): prepare a 8 mg/mL neomycin sulfate CRS solution in water.

Develop chromatogram. Remove the plate, allow to dry between 100 °C and 105 °C for 10 minutes and spray with ethanolic acetic ninhydrin RS. Heat between 100 °C and 105 °C for 15 minutes. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (4)*. The stain with a lower R_f (neomycin C impurity) than the main stain is not more intense than that obtained with *Solution (2)* (15.0%), but it is more intense than the main stain obtained with *Solution (3)* (3%). The test is only valid if the chromatogram obtained with *Solution (4)* presents stain with R_f lower than that of the main stain.

Sulfate content. Quantitatively dissolve about 0.25 g of sample in 100 mL of water and adjust to pH 11.0 with concentrated ammonia solution. Add 10 mL of 0.1 M barium chloride VS and approximately 0.5 mg of phthalein purple. Titrate with 0.1 M disodium edetate VS. Add 50 mL of ethyl alcohol, when the color starts to change, and continue the titration until the blueish-violet color disappears. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M barium chloride VS is equivalent to 9.606 mg of sulfate (SO_4). It contains not less than 27.0% and not more than 31.0% of sulfate (SO_4), in relation to the desiccated substance.

Loss on drying (5.2.9.1). Determine in 0.1 g of the sample. Dry in oven at 60 °C, under pressure not exceeding 5 mm of mercury, for three hours. Not more than 8.0%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS

If using sterile neomycin sulfate, the sample meets the Sterility and Bacterial Endotoxin tests. If using neomycin sulfate, to be sterilized during the process of preparing parenteral pharmaceutical dosage forms, the sample meets the bacterial endotoxin test.

Sterility (5.5.3.2.1). Complies with the test. Use the *Membrane filtration method*.

Bacterial endotoxins (5.5.2.2). Not more than 1.30 EU/mg of neomycin.

ASSAY

Proceed as described in *Microbiological Assay of Antibiotics (5.5.3.3)*, by agar diffusion using cylinders.

Microorganism: Staphylococcus epidermidis ATCC 12228 or *Staphylococcus aureus* ATCC 6538P.

Culture media: sterile saline solution for inoculum standardization and culture medium number 11 for base layer and inoculum preparation.

Sample solution: weigh, accurately, the equivalent to 25 mg of the neomycin and transfer to a 25 mL volumetric flask, using 0.1 M Potassium phosphate buffer, sterile, pH 8.0 (solution 2). Complete the volume with the same solvent and homogenize. Dilute to obtain concentrations of 0.5 µg/mL, 1 µg/mL and 2 µg/mL, using Solution 2 as diluent, when using the microorganism *Staphylococcus epidermidis* ATCC 12228, or up to concentrations of 5 µg/mL, 10 µg/mL and 20 µg/mL, using Solution 2 as diluent, when using the microorganism *Staphylococcus aureus* ATCC 6538P.

Standard solution: weigh, accurately, the equivalent to 25 mg of neomycin CRS and transfer to a 25 mL volumetric flask using Solution 2. Complete the volume with the same solvent and homogenize. Dilute to obtain concentrations of 0.5 µg/mL, 1 µg/mL and 2 µg/mL, using Solution 2 as diluent, when using the microorganism *Staphylococcus epidermidis* ATCC 12228, or up to concentrations of 5 µg/mL, 10 µg/mL and 20 µg/mL, using Solution 2 as diluent, when using the microorganism *Staphylococcus aureus* ATCC 6538P.

Procedure: add 20 mL of medium culture number 11 to each plate, wait to solidify, add 5 mL of inoculum at 1% and proceed as described in *Microbiological Assay by agar diffusion (5.5.3.3.1)*.

Calculate the neomycin content in μg in the sample from the standard potency and the responses obtained with the *Standard Solution* and the *Sample Solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light. When the substance is intended for the production of parenteral preparations, the label must indicate whether the product is sterile or whether it must be sterilized during the process.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibiotic.

POTASSIUM SULFATE

Kalii sulfas

K_2SO_4 ; 174.26

potassium sulfate; 08171

Potassium salt of sulfuric acid (2:1)

[7778-80-5]

It contains not less than 99.0% of K_2SO_4 in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Crystalline powder or colorless or white crystals, with slightly saline taste.

Solubility. Soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

The preparation obtained in *Preparation aspect* meets potassium and sulfate ion reactions (5.3.1.1).

PURITY TESTS

Preparation aspect. Dissolve 1 g of the sample in 20 mL of water. The preparation obtained is clear (5.2.25) and colorless (5.2.12).

pH (5.2.19). 5.5 to 8.5. Determine in solution at 5% (w/v).

Water-insoluble substances limit. Dissolve 10 g of the sample in 100 mL of water in a beaker. Heat the covered beaker to ebullition in water bath for one hour. Filter the solution in a tared funnel with medium porosity (10 μ m to 15 μ m) and wash with hot water. Dry at 105 °C, cool in desiccator and weigh. Not more than 0.01% (100 ppm).

Chlorides (5.3.2.1). Determine in 1 g of the sample. Use 1 mL of solution containing 0.01 mg of chloride (Cl), equivalent to 0.028 mL of standard hydrochloric acid (0.01 M HCl VS). Not more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 110 °C for four hours. Not more than 1.0%.

BIOLOGICAL SAFETY TESTS.

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.5 g of the sample, previously desiccated, and dissolve in 200 mL of water and 1 mL of hydrochloric acid. Gradually add 8 mL of barium chloride RS heated to boiling. Heat the mixture in water bath for one hour. Collect the precipitate and wash with water until the last washing is not turbid by the addition of silver nitrate RS. Heat the precipitate between 500 °C and 600 °C, increasing the temperature slowly until obtaining barium sulfate. Dry to constant weight. Each g of the residue is equivalent to 0.7466 g of K₂SO₄.

PACKAGING AND STORAGE

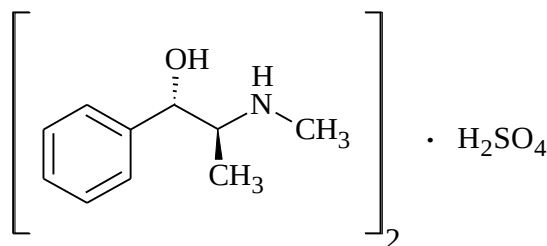
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Cathartic.

PSEUDOEPHEDRINE SULFATE*Pseudoephedrini sulfas* $(\text{C}_{10}\text{H}_{15}\text{NO})_2 \cdot \text{H}_2\text{SO}_4$; 428.54

pseudoephedrine sulfate; 07522

((α S)- α -[(1S)-1-(methylamino)ethyl]-benzenemethanol sulfate (1:2)

[7460-12-0]

It contains not less than 98.0% and not more than 100.5% of $(\text{C}_{10}\text{H}_{15}\text{NO})_2 \cdot \text{H}_2\text{SO}_4$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder.

Solubility. Moderately soluble in water, freely soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2). 174 °C to 179 °C.

Specific optical rotation (5.2.8): +56.0 to +59.0, in relation to the desiccated substance. Determine in solution at 5% (w/v) in water.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, previously desiccated, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the pseudoephedrine sulfate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm, of 0,05% (w/v) solution in water, there is a maximum point at 257 nm, calculated in relation to the anhydrous substance, differing by not more than 3%.

C. It meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

pH (5.2.19). 5.0 to 6.5. Determine in aqueous solution at 5% (w/v).

Heavy metals (5.3.2.3). Treat a solution of 2 g of the sample in 20 mL of 50% (v/v) ethyl alcohol with 5 mL of 5% (w/v) sodium hydroxide solution and five drops of sodium sulfide RS. Use *Standard lead solution (10 ppm Pb)* in the standard preparation. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 2 g of the sample, in oven at 105 °C, under reduced pressure, for two hours. Not more than 2.0%.

Residue by incineration (5.2.10). Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Dissolve 0.15 g of the sample in 50 mL of glacial acetic acid and titrate with 0.1 M perchloric acid VS. Determine the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 42.854 mg of pseudoephedrine sulfate ($C_{10}H_{15}NO)_2 \cdot H_2SO_4$.

PACKAGING AND STORAGE

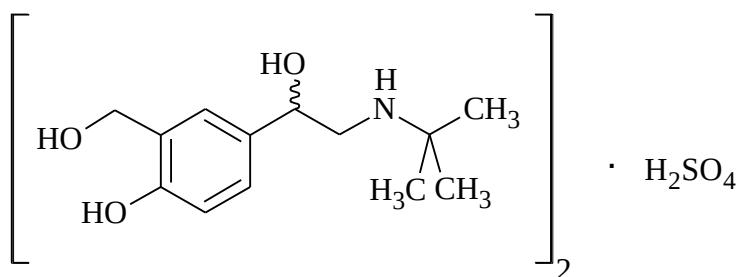
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Decongestant.

SALBUTAMOL SULFATE*Salbutamoli sulfas* $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$; 576.70

salbutamol sulfate; 07867

α 1-[[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol sulfate (1:2)
[51022-70-9]

It contains not less than 98.0% and not more than 101.0% of $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Freely soluble in water, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the salbutamol sulfate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 230 nm to 400 nm, of 0.008% (w/v) sample solution in 0.1 M hydrochloric acid, there is a maximum point at about 276 nm, whose absorbance ranges from 0.44 to 0.51.

C. Dissolve 10 mg of the sample in 50 mL of 2% (w/v) sodium tetraborate. Add 1 mL of 3% (w/v) 4-aminoantipyrine, 10 mL of 2% (w/v) potassium ferricyanide (w/v) and 10 mL of chloroform. Shake and allow the phases to separate. The chloroform layer develops orangish-red color.

D. Dissolve quantity of sample equivalent to 4 mg of salbutamol in 10 mL of water and filter. The filtrate meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. The preparation at 1% (w/v) carbon dioxide-free water is clear. **(5.2.25)**.

Acidity or alkalinity. Transfer 0,25 g of the sample to a 25 mL volumetric flask and complete the volume with carbon dioxide-free water. Add to 10 mL of this solution, 0.15 mL of methyl red TS and

0.2 mL of 0.01 M sodium hydroxide. The solution turns yellow. Not more than 0.4 mL of 0.01 M hydrochloric acid is required to turn color to red.

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, water and ammonium hydroxide (50:45:35:18:3), as mobile phase. Apply to the plate, separately, 50 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve accurately weighed quantity of the sample in water and dilute with methyl alcohol to obtain a 20 mg/mL solution.

Solution (2): dissolve accurately weighed quantity of salbutamol sulfate CRS in water and dilute with methyl alcohol to obtain a 0.1 mg/mL solution.

Develop chromatogram. Remove plate and allow to air dry. Expose to iodine vapors. Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (2)* (0.5%), and the sum of the intensities of all the secondary stains present is not more than 2.0%.

Water (5.2.20.1). Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Weigh, accurately, about 0.9 g of sample, transfer to a 250 mL erlenmeyer flask and dissolve in 50 mL of glacial acetic acid, with heating. Add two drops of oracet blue B TS and titrate with 0.1 M perchloric acid VS. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 57.670 mg of (C₁₃H₂₁NO₃)₂.H₂SO₄.

PACKAGING AND STORAGE

In tightly closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-asthmatic.

SALBUTAMOL SULFATE TABLETS

It contains salbutamol sulfate equivalent to not less than 90.0% and not more than 110.0% of the stated quantity of salbutamol ($C_{13}H_{21}NO_3$).

IDENTIFICATION

A. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the *Assay*, corresponds to the one from the main peak of the *Standard solution*.

B. Weigh and pulverize the tablets. Shake quantity of powder equivalent to 2.5 mg of salbutamol with 50 mL of 2% (w/v) sodium tetraborate. Add 1 mL of 3% (w/v) 4-aminoantipyrine, 10 mL of 2% (w/v) potassium ferricyanide (w/v) and 10 mL of chloroform. Shake and allow the phases to separate. The chloroform layer develops orangish-red color.

C. Weigh and pulverize the tablets. Use quantity of powder equivalent to 4 mg of salbutamol. Dissolve in 10 mL of water and filter. The filtrate meets sulfate ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 500 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, acidify with some drops of 1% (v/v) acetic acid and proceed as described in *Assay*. Prepare *Standard solution* as described below.

Standard solution: transfer quantity of salbutamol sulfate CRS, equivalent to 20 mg of salbutamol, to a 250 mL volumetric flask, add 150 mL of 1% (v/v) acetic acid. Place in ultrasonic bath for 15 minutes and complete the volume with water. Transfer 5 mL of this solution to a 100 mL volumetric flask and complete the volume with water, obtaining a 4 µg salbutamol solution per milliliter.

Inject, separately, 100 µL of the *Standard solution* and *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of salbutamol ($C_{13}H_{21}NO_3$) dissolved in the medium from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 80% (Q) of the declared quantity of salbutamol ($C_{13}H_{21}NO_3$) dissolve in 30 minutes.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, water and ammonium hydroxide (50:45:35:18:3), as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): weigh and pulverize the tablets. Transfer quantity of powder equivalent to 48 mg of salbutamol to an appropriate container. Add 60 mL of mixture of ethyl alcohol and water (1:2) and shake mechanically for 30 minutes. Filter. Evaporate the filtrate to dryness under reduced pressure in temperature below 40 °C. Dissolve the residue completely in 2 mL of water.

Solution (2): dissolve quantity of salbutamol sulfate CRS in water to obtain a 0.580 mg/mL solution, equivalent to 0.483 mg of salbutamol per milliliter.

Solution (3): dissolve quantity of salbutamol sulfate CRS in water to obtain a 0.218 mg/mL solution, equivalent to 0.183 mg of salbutamol per milliliter.

Solution (4): dissolve quantity of salbutamol sulfate CRS in water to obtain a 73 µg/mL solution, equivalent to 61 µg of salbutamol per milliliter.

Develop chromatogram. Remove plate, allow to air dry. Spray with 0.1% (w/v) solution of 3-methyl-2-benzothiazolinone hydrazone hydrochloride in mixture of methyl alcohol and water (9:1). Then spray with ammoniacal potassium ferricyanide and again with the 3-methyl-2-benzothiazolinone hydrazone hydrochloride solution. Any secondary stain obtained with *Solution (1)* is not more than equal in size or intensity to the stain obtained with *Solution (2)* (2%). Any other secondary stain obtained with *Solution (1)* is not more than equal in size and intensity to the main stain obtained with *Solution (3)* (0,75%). Not more than two secondary stains are equal in size and intensity as the stains obtained with *Solution (4)* (0.5%) The sum of the intensities of all secondary stains obtained in *Solution (1)* must be not more than 3.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*, using chromatograph equipped with ultraviolet detector at 276 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); kept at room temperature, *Mobile phase* flow rate of 1.5 mL/minute.

Sodium hexane sulfonate solution: dissolve 0.95 g of sodium 1-hexane sulfonate in 1000 mL of water. Add 10 mL of acetic acid and homogenize.

Mobile phase: mixture of *Sodium hexanesulfonate solution* and methyl alcohol (60:40).

Diluent: mixture of 1% (v/v) acetic acid and methyl alcohol (60:40).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 6 mg of salbutamol to a 200 mL volumetric flask. Add 150 mL of *Diluent*. Place in ultrasonic bath for 15 minutes. Shake mechanically for 45 minutes. Complete the volume with the same solvent to obtain a 30 µg/mL solution. Homogenize and filter.

Standard solution: transfer quantity of salbutamol sulfate CRS, equivalent to 12 mg of salbutamol, to a 100 mL volumetric flask, add 60 mL of *Diluent* and place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent. Transfer 25 mL of this solution to a 100 mL volumetric flask and complete the volume with the same solvent, obtaining a 30 µg salbutamol solution per milliliter. Homogenize.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is not less than 800 theoretical plates. The tailing factor is not more than 2.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of salbutamol (C₁₃H₂₁NO₃) dissolved in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers protected from light.

LABELING

Comply with current legislation.

SALBUTAMOL SULFATE ORAL SOLUTION

It contains salbutamol sulfate equivalent to not less than 90.0% and not more than 110.0% of the stated quantity of salbutamol ($C_{13}H_{21}NO_3$). It contains preservatives and sweetening agents. The oral solution may contain sugar.

IDENTIFICATION

A. In the visible absorption spectrum (**5.2.14**), in the range of 400 nm to 800 nm of the sample solution obtained in method **A.** from *Assay*, there is maximum point at 605 nm, identical to that observed in the standard solution spectrum.

B. To a volume of oral solution equivalent to 10 mg of salbutamol, add 50 mL of 2% (w/v) sodium tetraborate in water. Proceed as described in test **C.** from *Identification* in the *Salbutamol sulfate* monograph.

C. Use a volume of the oral solution equivalent to 4 mg of salbutamol. Dissolve in 10 mL of water and filter. The filtrate meets sulfate ion reactions (**5.3.1.1**).

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.3 to 5.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Protect the solutions from light. Transfer volume of the oral solution equivalent to 8 mg of salbutamol to a 100 mL volumetric flask. Add 70 mL of water. Place in ultrasonic bath for five minutes and complete the volume with the same solvent. Homogenize. Transfer 2 mL of this solution to a 250 mL separating funnel containing 80 mL of water. Add 4 mL of 5% (w/v) sodium bicarbonate, 4 mL of 0.1% (w/v) *N,N*-dimethyl-*p*-phenylenediamine sulfate and 4 mL of 8% (w/v) potassium ferricyanide. Shake and allow to stand for 20 minutes, protected from light. Extract with two 10 mL portions of chloroform, collect the extracts in a 25 mL volumetric flask and complete the volume with the same solvent. Homogenize. Prepare standard solution in the same concentration, using the same procedure. Measure the absorbances of the resulting solutions at 605 nm, using chloroform for zero adjustment. Calculate the quantity of salbutamol ($C_{13}H_{21}NO_3$) in the oral solution from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, using chromatograph equipped with ultraviolet detector at 276 nm; column with 150 mm of length and

4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); kept at room temperature, *Mobile phase* flow rate of 1.5 mL/minute.

Sodium hexane sulfonate solution: dissolve 0.95 g of sodium 1-hexane sulfonate in 1000 mL of water. Add 10 mL of acetic acid and homogenize.

Mobile phase: mixture of *Sodium hexanesulfonate solution* and methyl alcohol (60:40).

Diluent: mixture of 1% (v/v) acetic acid and methyl alcohol (60:40).

Sample solution: transfer volume of the oral solution equivalent to 6 mg of salbutamol to a 200 mL volumetric flask. Add 150 mL of *Diluent*. Shake. Complete the volume with the same solvent to obtain a 30 µg salbutamol solution per milliliter. Homogenize and filter.

Standard solution: transfer quantity of salbutamol sulfate CRS, equivalent to 12 mg of salbutamol, to a 100 mL volumetric flask, add 60 mL of *Diluent* and place in ultrasonic bath for 15 minutes. Complete the volume with the same solvent. Transfer 25 mL of this solution to a 100 mL volumetric flask and complete the volume with the same solvent, obtaining a 30 µg salbutamol solution per milliliter. Homogenize.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is not less than 800 theoretical plates. The tailing factor is not more than 2.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of salbutamol ($C_{13}H_{21}NO_3$) dissolved in the oral solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers protected from light.

LABELING

Comply with current legislation.

SODIUM SULFATE***Natrii sulfas***

Na_2SO_4 ; 142.04
sodium sulfate; 08173
Sulfuric acid sodium salt (2:1)
[7757-82-6]

$\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$; 322.19
sodium sulfate decahydrate; 09841
Sulfuric acid sodium salt decahydrate (2:1:10)
[7727-73-3]

It contains not less than 98.5% and not more than 101.0% of Na_2SO_4 , in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White crystalline powder or colorless clear crystals. Hygroscopic.

Solubility. Freely soluble in water and practically insoluble in ethyl alcohol.

IDENTIFICATION

A. The 5% (w/v) sample solution in water meets sulfate ion reactions **(5.3.1.1)**.

B. The 5% (w/v) sample solution in water meets sodium ion reactions **(5.3.1.1)**.

PURITY TESTS

Acidity or alkalinity. To 10 mL of a solution containing the equivalent to 1 g of Na_2SO_4 in 20 mL of water, add one drop of bromothymol blue TS. Not more than 0.5 mL of 0.01 M hydrochloric acid or 0.5 mL of 0.01 M sodium hydroxide is required to change the solution color.

Chloride (5.3.2.1). Not more than 0.02% (200 ppm) for sodium sulfate decahydrate and not more than 0.045% (450 ppm) for sodium sulfate.

Heavy metals (5.3.2.3). Use *Method I*. Not more than 0.001% (10 ppm) for sodium sulfate decahydrate and not more than 0.00225% (22.5 ppm) for sodium sulfate.

Loss on drying (5.2.9.1). Dry at 105 °C for four hours. For sodium sulfate decahydrate, the loss is between 51.0% and 57.0%. For sodium sulfate, not more than 0.5%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh the equivalent to 0.4 g of the anhydrous or desiccated sample, dissolve in 200 mL of water and add 1 mL of hydrochloric acid. Heat to boiling and gradually add small portions, with constant agitation, of an excess 12% (w/v) barium chloride solution (about 8 mL). Heat the mixture in water bath for one hour. Allow to decant, filter the precipitate and wash with water, until the washing waters are chloride-free. Dry, calcine and weigh. The barium sulfate mass obtained, multiplied by 0.6086, represents the equivalent to Na_2SO_4 .

PACKAGING AND STORAGE

In hermetic containers, with temperature not exceeding 30 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Laxative.

ZINC SULFATE***Zinci sulfas***

ZnSO₄; 161.44

zinc sulfate; 08174

Zinc sulfate

[7733-02-0]

ZnSO₄.H₂O; 179.45

zinc sulfate monohydrate; 08175

Zinc sulfate monohydrate

[7446-19-7]

ZnSO₄.7H₂O; 287.54

zinc sulfate heptahydrate; 09533

Zinc sulfate heptahydrate

[7446-20-0]

Zinc sulfate contains one, six or seven molecules of water of hydration. The monohydrate form contains not less than 89.0% and not more than 90.4% of ZnSO₄, corresponding to not less than 99.0% and not more than 101.0% of ZnSO₄.H₂O. The heptahydrate form contains not less than 55.6% and not more than 61.0% of ZnSO₄, corresponding to not less than 99.0% and not more than 108.7% of ZnSO₄.7H₂O.

DESCRIPTION

Physical characteristics. White to off-white crystalline powder or clear crystalline powder, efflorescent.

Solubility. Very soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. The preparation obtained in *Preparation aspect* at 5% (w/v) meets sulfate ion reactions **(5.3.1.1)**.

B. The preparation obtained in *Preparation aspect* at 5% (w/v) meets zinc ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. The 5% (w/v) aqueous preparation is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.9.1). 4.4 to 5.6. Determine in aqueous solution at 5% (w/v).

Acidity. A solution containing the equivalent to 28 mg of ZnSO₄ per mL does not develop a pinkish color in the presence of methyl orange TS.

Arsenic (5.3.2.5). Use *Spectrophotometric method, Method I*. Dissolve the equivalent quantity of the sample to 215 mg of ZnSO₄ in 35 mL of water. Not more than 0.0014% (14 ppm).

Chlorides (5.3.2.1). Determine in 5.5 mL of the preparation obtained in *Preparation aspect*, diluted in a 25 mL flask. Not more than 0.03% (300 ppm).

Iron (5.3.2.4). Determine in 2,0 mL of the preparation obtained in *Preparation aspect*. Not more than 0.01% (100 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Complexometric titrations (5.3.3.4)* for zinc. Determine in 200 mg of the sample. Carry out the blank test and make the necessary corrections.

PACKAGING AND STORAGE

In hermetically closed non-metallic containers.

LABELING

Comply with current legislation.

CATEGORY

Raw material for astringent preparations, zinc supplementation and for analytical testing applications.

FERROUS SULFATE***Ferrosi sulfas***FeSO₄; 151.91

ferrous sulfate; 08176

iron salt (2⁺) of sulfuric acid (1:1)

[7720-78-7]

It contains not less than 86.0% and not more than 90.0% of FeSO₄.

DESCRIPTION

Physical characteristics. White to grayish-yellow powder.

Solubility. Soluble in water, insoluble in ethyl alcohol.

IDENTIFICATION

A. It meets ferrous ion reactions **(5.3.1.1)**.

B. It meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

Manganese. Dissolve 1 g of the sample in 40 mL of water, add 10 mL of nitric acid and boil until red smoke release ceases. Add 0.5 g of ammonium peroxydisulfate and boil for 10 minutes. Eliminate any pink color that may have formed, adding, dropwise, 5% (w/v) sodium sulfite. Heat to boiling until sulfur dioxide odor disappears. Add 10 mL of water, 5 mL of phosphoric acid and 0.5 g of sodium periodate, heat to boiling for one minute and cool to room temperature. The obtained solution is not more intensely colored than the standard solution prepared under the same conditions, using 1 mL of 0.02 M potassium permanganate VS and the same quantities of reagents.

Basic sulfate. Dissolve 2 g of the sample in mixture of 7.5 mL of carbon dioxide-free water and 0.5 mL of 0.5 M sulfuric acid. The preparation is slightly turbid.

Insoluble substances limit. Dissolve 2 g of the sample in 20 mL of 1% (v/v) sulfuric acid, heat to boiling and place in water bath, in a covered beaker, for one hour. Filter and wash with 1% sulfuric acid (v/v). Dry the filter at 105 °C. Not more than 1 mg of residues (0.05%).

Ferric ion limit. Dissolve, in a capped erlenmeyer flask, 5 g of the sample in mixture of hydrochloric acid and carbon dioxide-free water (1:10) and add 3 g of potassium iodide. Cap the flask and allow to stand, protected from light, for five minutes. Titrate the released iodine with 0.1 M sodium thiosulfate VS, using 0.5 mL of starch TS as indicator, added close to the titration end. Prepare blank, omitting sample addition. The difference between the titrations represents the quantity of iodine released by the ferric ion. Not more than 4.5 mL of 0.1 M sodium thiosulfate VS is used (0.5%).

Copper limit. Proceed as described in *Atomic absorption spectrometry (5.2.13.1)*, use *Method II*. Transfer 2 g of the sample to a 100 mL volumetric flask, dissolve in 5% (v/v) nitric acid and complete the volume with the same solvent. In parallel, transfer 0.393 g of cupric sulfate pentahydrate for a 100 mL volumetric flask, dissolve in water and complete the volume with the same solvent, obtaining

standard copper solution (1000 ppm Cu). Dilute this solution in 5% (v/v) nitric acid to obtain the standard solutions. Measure the absorbances of the solutions at 324.7 nm. Not more than 0.005% (50 ppm).

Zinc limit. To 5 mL of the test solution obtained in *Heavy Metals*, add 1 mL of potassium ferrocyanide RS, dilute to 13 mL with water and allow to stand for five minutes. Any turbidity produced is not more intense than that obtained by mixing 10 mL of standard zinc solution (10 ppm Zn), 2 mL of 7 M hydrochloric acid and 1 mL of potassium ferrocyanide RS. Not more than 0.05% (500 ppm).

Arsenic (5.3.2.5). Dissolve 1 g of the sample in 10 mL of water and 15 mL of tin hydrochloric acid RS. Distill 20 mL of this solution. To the distillate, add 0.15 mL of bromine water RS, remove excess bromine with 0.15 mL of tin(II) chloride RS and dilute to 75 mL with water. Use 25 mL of the solution obtained and proceed as described in *Visual Method*. Not more than 0.0003% (3 ppm).

Heavy metals (5.3.2.3). Dissolve 1 g of the sample in 10 mL of 7 M hydrochloric acid, add 2 mL of concentrated hydrogen peroxide and boil until the volume is reduced to 5 mL. Allow to cool, dilute with 7 M hydrochloric acid to 20 mL and extract with three 20 mL portions of freshly distilled mixture of methyl isobutyl ketone and 7 M hydrochloric acid (100:1). Allow to stand, separate the aqueous phase and evaporate to half volume. Allow to cool and dilute to 25 mL with water, obtaining the test solution. Neutralize 7.5 mL of the test solution with ammonia RS and dilute to 15 mL with water. Use 12 mL of this solution and proceed as described in *Method I*. Not more than 0.005% (50 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Quantitatively dissolve about 0.5 g of the sample in mixture of water and M sulfuric acid (30:20). Titrate with 0.1 M ceric ammonium sulfate VS, using ferroin TS as indicator. Each mL of 0.1 M ceric ammonium sulfate VS is equivalent to 15.191 mg of FeSO₄.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antianemic.

FERROUS SULFATE TABLETS

It contains not less than 95.0% and not more than 110.0% the declared quantity of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The tablets must be coated.

IDENTIFICATION

A. Weigh and pulverize the tablets. Shake quantity of powder equivalent to 0.1 g of elemental iron with 20 mL of water and filter. It meets ferrous ion reactions **(5.3.1.1)**.

B. Weigh and pulverize the tablets. Shake quantity of powder equivalent to 0.1 g of elemental iron with 20 mL of hydrochloric acid RS and filter. It meets sulfate ion reactions. **(5.3.1.1)**.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.5 g of ferrous sulfate to a beaker containing a mixture of 20 mL of *M* sulfuric acid and 80 mL of freshly boiled and cooled water. Immediately filter the solution. Wash the filter and the precipitate with small portions of the mixture. Collect the filtrate and the washing waters, add orthophenanthroline TS and titrate immediately with 0.1 *M* ceric sulfate VS. Each mL of 0.1 *M* ceric sulfate is equivalent to 27.801 mg of ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$).

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation. The label must specify the quantities of ferrous sulfate (FeSO_4) and elemental iron (Fe) per tablet.

FERROUS SULFATE HEPTAHYDRATE***Ferrosi sulfas heptahydricus***FeSO₄·7H₂O; 278.01

Fe; 55.85

ferrous sulfate heptahydrate; 08177

Iron salt (2⁺) of sulfuric acid heptahydrate(1:1:7)

[7782-63-0]

It contains not less than 98.0% and not more than 105.0% of FeSO₄·7H₂O.

DESCRIPTION

Physical characteristics. Light green crystalline powder or blueish-green crystals, efflorescent in dry air. It oxidizes quickly in contact with moist air, forming brownish-yellow basic ferric sulfate.

Solubility. Freely soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. The preparation obtained in *Preparation aspect* meets ferrous ion reactions **(5.3.1.1)**.

B. The preparation obtained in *Preparation aspect* meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 2.5 g of the sample in carbon dioxide-free water, add 0.5 mL of *M* sulfuric acid and dilute to 50 mL with water. The preparation obtained is not more opalescent than the *Reference suspension II* **(5.2.25)**.

pH (5.2.19). 3.0 to 4.0. Determine in 5% (w/v) sample solution in carbon dioxide-free water.

Ferric ion limit. Transfer 5 g of the sample to a capped erlenmeyer flask and dissolve in mixture with 10 mL of hydrochloric acid and 100 mL of carbon dioxide-free water. Add 3 g of potassium iodide, cap and allow to stand for five minutes protected from light. Titrate the released iodine with 0.1 *M* sodium thiosulfate VS, using 0.5 mL of starch TS as indicator, added close to the endpoint. Carry out the blank test and make the necessary corrections. Not more than 4.5 mL of 0.1 *M* sodium thiosulfate VS are used in the titration (0.5%).

Manganese limit. Dissolve 1 g of the sample in 40 mL of water, add 10 mL of nitric acid and heat to boiling until red vapors are released. Add 0.5 g of ammonium peroxydisulfate and heat to boiling for 10 minutes. Eliminate any pink color that may have formed, adding, dropwise, 5% (w/v) sodium sulfite solution. Heat to boiling until sulfur dioxide odor disappears. Add 10 mL of water, 5 mL of phosphoric acid and 0.5 g of sodium periodate, heat to boiling for one minute and cool to room temperature. The obtained solution is not more intensely colored than the standard, prepared under the same conditions, using 1 mL of 0.02 *M* potassium permanganate VS and the same quantities of reagents (0.1%).

Zinc limit. Dissolve 1 g of the sample in 10 mL of hydrochloric acid RS, add 2 mL of concentrated hydrogen peroxide and heat to boiling until the volume is reduced to 5 mL. Cool, dilute to 20 mL

with hydrochloric acid RS, transfer to separating funnel and shake for three minutes with three 20 mL portions of methyl isobutyl ketone saturated with hydrochloric acid (prepared by shaking 100 mL of freshly distilled methyl isobutyl ketone with 1 mL of hydrochloric acid RS). Allow to stand, separate the aqueous layer and, in water bath, reduce volume to half. Cool, quantitatively transfer to a 25 mL volumetric flask and complete the volume with water. To 5 mL of this solution, add 1 mL of potassium ferrocyanide RS and dilute to 13 mL with water. After five minutes, any turbidity developed is not more intense than that produced by mixing 10 mL of standard zinc solution (10 ppm Zn), 2 mL of hydrochloric acid RS and 1 mL of potassium ferrocyanide RS. Not more than 0.05% (500 ppm).

Arsenic (5.3.2.5). Transfer 1 g of the sample to a 100 mL round-bottomed flask, equipped with distillation system. Add 40 mL of 4.5 M sulfuric acid, 2 mL of 30% (w/v) potassium bromide and immediately connect the flask to the distillation system. Add glass beads, heat the flask over low flame until the sample dissolves and distil until obtaining 25 mL of distillate. Transfer the distillate to the arsine generator flask and wash the condenser and other parts of the distillation system with small amounts of water, adding the washing waters to the arsine generator flask. Shake the flask in circular motion, add SR bromine water RS until obtaining slightly yellowish color and dilute with water to 35 mL. Proceed as described in *Spectrophotometric Method, Method I*. Not more than 0.0003% (3 ppm).

Chlorides (5.3.2.1). Determine in 1.2 g of the sample, using 1 mL of standard hydrochloric acid 0.01 M HCl SV), to prepare the standard. Not more than 0.03% (300 ppm).

Heavy metals (5.3.2.3). Dissolve 2 g of the sample in mixture with 1 mL of sulfuric acid RS and 40 mL of water. Add 0.05 g of hydroxylamine hydrochloride and heat to boiling for one minute. Cool to room temperature, quantitatively transfer to a 50 mL volumetric flask with water and complete the volume with the same solvent (*Solution A*). Transfer 15 mL of *Solution A* to a 50 mL Nessler tube and adjust the pH between 3.0 and 4.0 with 6 M ammonium hydroxide or M acetic acid. Add 2 mL of acetate buffer pH 3.5, dilute with water to 40 mL and homogenize. To prepare the standard, transfer 15 mL of *Solution A* to a 50 mL Nessler tube, dilute to 25 mL with water, adjust the pH between 3.0 and 4.0 with 6 M ammonium hydroxide or M acetic acid, add 2 mL of acetate buffer pH 3.5, 3 mL of *Standard lead solution (10 ppm Pb)*, dilute to 40 mL with water and homogenize. Add 1.2 mL of thioacetamide to the standard and sample, complete the volumes with water and homogenize. Allow to stand for two minutes. Examine the tubes from top to bottom on a white background. Any brown color developed in the sample preparation is not more intense than that developed in the standard preparation. Not more than 0.005% (50 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Quantitatively dissolve 0.5 g of the sample in mixture of 25 mL of M sulfuric acid and 25 mL of carbon dioxide-free water. Add two drops of ferroin TS and titrate immediately with 0.1 M ceric ammonium sulfate VS until reddish-orange color turns pale green. Each mL of 0.1 M ceric ammonium sulfate VS is equivalent to 27.801 mg of ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and to 5.585 mg of elemental iron (Fe).

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antianemic.

FERROUS SULFATE ORAL SOLUTION

It contains ferrous sulfate heptahydrate equivalent to not less than 94.0% and not more than 106.0% of the stated quantity of elemental iron (Fe).

IDENTIFICATION

A. It meets ferrous ion reactions **(5.3.1.1)**.

B. It meets sulfate ion reactions **(5.3.1.1)**.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 1.8 to 5.3.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Transfer accurately measured volume of oral solution equivalent to about 0.125 g of elemental iron (Fe) to erlenmeyer flask, add 80 mL of carbon dioxide-free water and 20 mL of *M* sulfuric acid. Add two drops of ferroin TS and titrate immediately with 0.1 *M* ceric ammonium sulfate VS until reddish-orange color turns pale green. Each mL of 0.1 *M* ceric ammonium sulfate VS is equivalent to 5.585 mg of elemental iron (Fe).

PACKAGING AND STORAGE

In tightly closed containers. Protect from light .

LABELING

Comply with current legislation. The label must specify the quantities of ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and of elemental iron (Fe) per milliliter of the oral solution.

SELENIUM SULFIDE

Selenii disulfidum

SeS₂; 143.09
Se; 78.97
selenium sulfide; 08182
Selenium sulfide
[7488-56-4]

It contains not less than 52.0% and not more than 55.5% of selenium (Se).

DESCRIPTION

Physical characteristics. Reddish-brown or orange powder.

Solubility. Practically insoluble in water and practically insoluble in organic solvents.

IDENTIFICATION

A. Boil 50 mg of sample with 5 mL of nitric acid for 30 minutes. Dilute to 50 mL with water and filter. For each 5 mL of the filtrate, add 10 mL of water and 5 g of urea. Heat to boiling, allow to cool and add 2 mL of 0.8%(w/v) potassium iodide solution. Yellow color is produced and quickly darkens (presence of selenium). This solution is used for test **B.** from *Identification*.

B. Allow the solution obtained in test **A.** from *Identification* to stand for 10 minutes and filter. The filtrate meets sulfate ion reactions (**5.3.1.1**).

PURITY TESTS

Soluble-selenium compounds limit. Proceed as described in *Visible absorption spectrophotometry* (**5.2.14**).

Sample solution: weigh 10 g of sample, add 100 mL of water and homogenize. Leave under constant agitation for an hour and filter. For every 10 mL of the filtrate add 2 mL of formic acid, complete to 50 mL with water and adjust the pH to 2.5 ± 0.5 with formic acid. Add 2 mL of 3,3'-diaminobenzidine tetrahydrochloride RS. Allow to stand for 45 minutes and adjust the pH between 6.5 ± 0.5 with 6 M ammonia. Shake the solution for one minute with 10 mL of toluene and allow the phases to separate. Discard the aqueous phase.

Standard solution: use 10 mL of a selenious acid solution containing 0.5 µg/mL of selenium. Proceed according to the preparation of the sample solution, starting with the addition of 2 mL of formic acid.

Determine the absorbances of the *Standard Solution* and the *Sample Solution* at 420 nm. Use blank with the same composition as the sample solution. The absorbance of the *Sample solution* is not greater than that of the *Standard solution*. Not more than 0.0005% (5 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh approximately 100 mg of the sample, add 25 mL of fuming nitric acid and heat in water bath for one hour. Allow to cool, transfer to a 250 mL volumetric flask containing 100 mL of water and complete for the volume of 250 mL with water. Transfer 50 mL of the solution, add 25 mL of water, 10 g of urea and heat to boiling. Allow to cool, add 3 mL of starch TS, 10 mL of 10% (w/v) potassium iodide and titrate immediately with 0.1 M sodium thiosulfate volumetric solution VS. Carry out blank test. Each mL of 0.1 M sodium thiosulfate is equivalent to 1.974 mg of Se.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antifungal.

SODIUM SULFITE***Natrii sulfis***

Na_2SO_3 ; 126.04

sodium sulfite; 08187

Sodium salt of sulfurous acid (2:1)

[7757-83-7]

It contains not less than 95.0% and not more than 100.5% of Na_2SO_3 .

DESCRIPTION

Physical characteristics. White powder.

Solubility. Freely soluble in water and very slightly soluble in ethyl alcohol.

IDENTIFICATION

A. The 5% (w/v) solution meets sodium ion reactions **(5.3.1.1)**.

B. The 0.9% (w/v) solution meets sulfite ion reactions **(5.3.1.1)**.

C. Dissolve 5 g of the sample in water and complete the volume to 100 mL with the same solvent. To a 5 mL aliquot add 0.5 mL of 0.05 M iodine. The resulting solution is colorless and meets sulfate ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 10 g of the sample in 25 mL of water and slowly add 15 mL of hydrochloric acid. Heat to boiling. Cool and complete the volume to 100 mL with water. The preparation obtained is clear **(5.2.25)** and colorless **(5.2.12)**.

Selenium limit. To 3 g of sample, add 10 mL of formaldehyde solution and carefully 2 mL of hydrochloric acid. Heat in water bath for 20 minutes. If pink color develops, it should not be more intense than that of a standard solution prepared, simultaneously and under the same conditions, with 1 g of the sample added with 0.2 mL of standard selenium solution (100 ppm Se). Not more than 0.001% (10 ppm).

Thiosulfates. Dissolve 2 g of the sample in 100 mL of water. Add 10 mL of formaldehyde solution and 10 mL of acetic acid. Wait five minutes. Add 0.5 mL of starch TS and titrate with 0.05 M iodine VS. Carry out blank test. The difference between the volumes used in the titrations is not more than 0.15 mL.

Zinc. Proceed as described in *Atomic absorption spectrometry* **(5.2.13.1)**, use *Method I*. Not more than 0.0025% (25 ppm).

Sample solution: dilute 2 mL of the preparation obtained in *Preparation aspect* to 10 mL with water.

Reference solutions: prepare reference solutions using standard zinc solution (100 ppm Zn), diluting with water when necessary.

Measure the absorbances at 213.9 nm, using a hollow cathode lamp as radiation source and air-acetylene flame.

Iron (5.3.2.4). Use *Method I*. Determine in 10 mL of the preparation obtained in *Preparation aspect*. Proceed as described in *Limit test for iron* using 0.1 mL of *Standard iron solution (100 ppm of Fe)*. Not more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Transfer 20 mL of the preparation obtained in *Preparation aspect* to a 50 mL Nessler tube. Complete the volume to 25 mL with water and proceed as described in *Limit test for heavy metals*. Not more than 0.001% (10 ppm).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.25 g of the sample and transfer to an erlenmeyer containing 50 mL of 0.05 M iodine VS. Shake until complete dissolution. Titrate excess of iodine with 0.1 M sodium thiosulfate VS, using 1 mL of starch TS as indicator. Carry out blank test. Each mL of 0.05 M iodine VS is equivalent to 6.302 mg of Na₂SO₃.

PACKAGING AND STORAGE

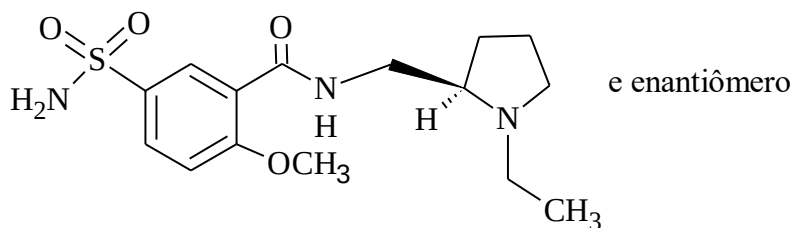
In tightly closed containers.

LABELING

Comply with current legislation.

CATEGORY

Antioxidant.

SULPIRIDE*Sulpiridum* $C_{15}H_{23}N_3O_4S$; 341.43

sulpiride; 08210

(RS)-5-(Aminosulfonyl)-N-[(1-ethyl-2-pyrrolidiny)methyl]-2-methoxybenzamide

[15676-16-1]

It contains not less than 98.5% and not more than 101.0% of $C_{15}H_{23}N_3O_4S$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, moderately soluble in methyl alcohol, slightly soluble in ethyl alcohol. Soluble in solutions of mineral acids and alkali hydroxides.

Physicochemical constants.

Melting range (5.2.2): 177 °C to 181 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the sulpiride CRS spectrum, prepared identically.

B. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, when observed under UV lamp at 254 nm, corresponds in position and intensity to the main stain obtained with *Solution (3)*.

C. Dissolve 1 mg of the sample in 0.5 mL of sulfuric acid and 0.05 mL of formaldehyde. It presents intense blue fluorescence when observed under ultraviolet light at 365 nm.

PURITY TESTS

Preparation aspect. Dissolve 1.0 g of the sample in 10 mL of glacial acetic acid. The preparation is clear **(5.2.25)** and its color has a maximum intensity equal to that of the *Color F Standard Solution (5.2.12)*.

Related compounds 1. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel F₂₅₄ plate as support, and mixture of ammonia, dioxane, methyl alcohol and methylene chloride (2:10:14:90) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.2 g of the sample in methyl alcohol and dilute to 10 mL with the same solvent. Place in ultrasonic bath until complete dissolution and homogenize.

Solution (2): dilute 1 mL of *Solution (1)* to 10 mL with methyl alcohol and homogenize.

Solution (3): dissolve 20 mg of sulpiride CRS in methyl alcohol, dilute to 10 mL with the same solvent and homogenize.

Solution (4): dissolve 5 mg of sulpiride impurity A in methyl alcohol, dilute to 25 mL with the same solvent and homogenize.

Solution (5): dilute 1 mL of *Solution (4)* to 10 mL with methyl alcohol and homogenize.

Develop the chromatogram in 10 cm of plate. Dry the plate at room temperature. Observe under UV lamp at 254 nm for test **B**. from *Identification*. Spray with ninhydrin RS solution, heat at 100 °C to 105 °C for 15 minutes and examine. In the chromatogram obtained with *Solution (1)*, any stain corresponding to impurity A is not more intense than the stain obtained with *Solution (5)* (0.1%).

Related compounds 2. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 240 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica gel chemically bonded to octylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1.5 mL/minute.

Monobasic potassium phosphate buffer pH 3.3: accurately weigh about 6.8 g of monobasic potassium phosphate and 1 g of sodium octane sulfonate, dilute in 1000 mL of water and adjust the pH to 3.3 with phosphoric acid R.

Mobile phase: mixture of potassium phosphate monobasic pH 3.3, acetonitrile and methyl alcohol (80:10:10).

Solution (1): quantitatively transfer about 0.1 g of the sample to a 100 mL volumetric flask. Complete the volume with *Mobile phase* and homogenize.

Solution (2): transfer 3 mL of the *Sample solution* to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize. Transfer 1.0 mL of this solution to a 10 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Solution (3): quantitatively transfer about 10 mg of sulpiride CRS and 10 mg of sulpiride impurity B to a 100 mL volumetric flask, complete the volume with *Mobile phase* and homogenize.

Inject 10 µL replicates of the *Solution (3)*. The resolution between impurity B and sulpiride peaks in *Solution (3)* is greater than 2.5.

Procedure: inject, separately, 10 µL of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The sum of the areas under the peaks related to the

impurities obtained in the chromatogram of *Solution (1)* is not more than equal to the area under the main peak obtained with *Solution (2)* (0.3%).

Chlorides (5.3.2.1). Dissolve 1.0 g of the sample in 20 mL of water. Filter through glass filter with a porosity between 16 and 40 µm. Pipet 10 mL of the filtrate and dilute to 15 mL with water. The solution meets the *Limit test for chlorides*. Not more than 0.01% (100 ppm).

Iron (5.3.2.4). Incinerate 1.0 g of sample. Add 1 mL of *M* hydrochloric acid, 3 mL of water and 0.1 mL of nitric acid. Heat in water bath for some minutes. Transfer the solution to a test tube. Wash the container with 4 mL of water and transfer to the test tube, dilute to 10 mL with water. Proceed as described in *Limit test for iron, Method III*. Not more than 0.001% (10 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 105°C. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 0.25 g of the sample in 80 mL of glacial acetic acid and titrate with 0.1 *M* perchloric acid VS, determining the endpoint potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 *M* perchloric acid VS is equivalent to 34.143 mg of C₁₅H₂₃N₃O₄S.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

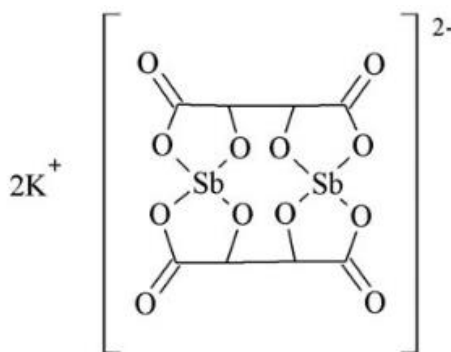
LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Dopamine receptor antagonist; neuroleptic.

ANTIMONY POTASSIUM TARTRATE



$C_8H_4K_2O_{12}Sb_2$; 613.82

antimony potassium tartrate; 00352

bis[μ -[(2*R*,3*R*)-2,3-Di(hydroxy- κ O)butanedioate(4-)- κ O1: κ O4]]di-antimonate(2-) of potassium
(2:2:2)
[11071-15-1]

$C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$; 667.87

antimony potassium tartrate sesquihydrate; 11395

bis[μ -[(2*R*,3*R*)-2,3-Di(hydroxy- κ O)butanedioate(4-)- κ O1: κ O4]]di-antimonate(2-) potassium
trihydrate (2:2:2 :3)
[28300-74-5]

It contains not less than 99.0% and not more than 103.0% of $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$.

DESCRIPTION

Physical characteristics. White or colorless crystalline powder.

Solubility. Freely soluble in water.

IDENTIFICATION

A. Dissolve a small amount of the sample in two drops of 5% (w/v) sodium periodate. Add one drop of 0.5 *M* sulfuric acid and, after five minutes, add some drops of sulfurous acid, followed by some drops of bleached fuchsin RS. Pink color develops in 15 minutes.

B. When heated to incandescence, burning occurs, with the release of burnt sugar odor, leading to a dark residue. When this residue is brought to the flame, violet color is developed.

C. Dissolve 1 g of sample in 20 mL of water. Acidify the solution with hydrochloric acid and add hydrogen sulfide RS. An orangish precipitate is formed, soluble in 0.05 *M* sodium hydroxide.

PURITY TESTS

Acidity or alkalinity. Dissolve 1 g of sample in 50 mL of water free from organic compounds and titrate with 0.01 *M* hydrochloric acid or 0.01 *M* sodium hydroxide at pH 4.5. Not more than 2 mL are required.

Arsenic (5.3.2.5). Use *Method II*. Dissolve 0.1 g of sample in 5 mL of hydrochloric acid. Add 10 mL of a freshly prepared solution of 20 g of stannous chloride in 30 mL of hydrochloric acid. Transfer to a staining comparison tube and allow to stand for 30 minutes. The white surface formed is not more intense than that produced when an equivalent solution containing 15 µg of arsenic is used. Not more than 0.015% (150 ppm).

Lead (5.3.2.12). Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 2 g of sample. Dry in oven at 105 °C until constant weight. Not more than 2.7%

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.5 g of the sample and dissolve in 50 mL of water. Add 5 g of potassium sodium tartrate, 2 g of sodium borate, 3 mL of iodized starch TS and titrate immediately with 0.1 M iodine VS until a persistent blue color develops. Each mL of 0.1 M iodine VS is equivalent to 16.697 mg of $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$

PACKAGING AND STORAGE

In tightly closed containers.

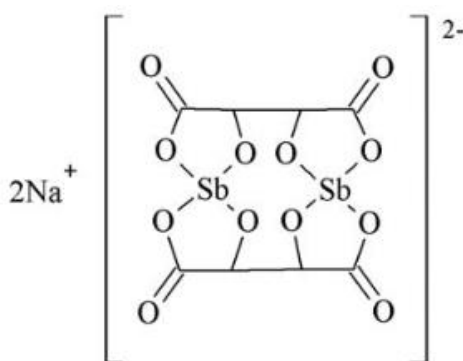
LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiparasitic.

ANTIMONY SODIUM TARTRATE



$C_8H_4Na_2O_{12}Sb_2$; 581.61

antimony sodium tartrate; 09845

bis[μ -[(2*R*,3*R*)-2,3-Di(hydroxy- κ O)butanedioate(4-)- κ O1: κ O4]]di-antimonate(2-) sodium (2:2:2)
[34521-09-0]

It contains not less than 98.0% and not more than 101.0% of $C_8H_4Na_2O_{12}Sb_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or colorless crystalline powder.

Solubility. Freely soluble in water.

IDENTIFICATION

A. Dissolve a small amount of the sample in two drops of 5% (w/v) sodium periodate. Add one drop of 0.5 *M* sulfuric acid and, after five minutes, add some drops of sulfurous acid, followed by some drops of bleached fuchsin RS. Pink color develops in 15 minutes.

B. It meets antimony ion reactions (5.3.1.1).

C. It meets sodium ion reactions (5.3.1.1).

PURITY TESTS

Acidity or alkalinity. Dissolve 1 g of sample in 50 mL of carbon dioxide-free water and titrate with 0.01 *M* hydrochloric acid or 0.01 *M* sodium hydroxide at pH 4.5. Not more than 2 mL are required.

Arsenic (5.3.2.5). Weigh 0.375 g of sample and proceed as described in *Limit test for arsenic, Method II*. Not more than 0.0008% (8 ppm).

Lead (5.3.2.12). Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 2 g of sample. Dry in oven at 105 °C until constant weight. Not more than 6.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.5 g of the sample and dissolve in 50 mL of water. Add 5 g of potassium sodium tartrate, 2 g of sodium borate, 3 mL of iodized starch TS and titrate immediately with 0.1 *M* iodine VS until a persistent blue color develops. Each mL of 0.1 *M* iodine VS is equivalent to 14.540 mg of $C_8H_4Na_2O_{12}Sb_2$.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antiparasitic.

METOPROLOL TARTRATE TABLETS

It contains not less than 95.0% and not more than 105.0% of the declared quantity of $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Transfer quantity of powder equivalent to 40 mg of metoprolol tartrate to a separating funnel. Add 25 mL of water and 4 mL of diluted ammonium hydroxide (1:3). Extract with 20 mL of chloroform, filtering the chloroform extract obtained through anhydrous sodium sulfate previously moistened with chloroform. Evaporate the chloroform to dryness, freeze the residue at $-18^{\circ}C$ for 30 minutes and allow to reach room temperature. In the infrared absorption spectrum **(5.2.14)** of the residue, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the metoprolol tartrate CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)** of the sample solution obtained in method **A.** from *Assay*, there are maximum and minimum points identical to those observed in the spectrum of the metoprolol tartrate CRS solution.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: simulated gastric fluid (without enzyme), 900 mL.

Apparatus: baskets, 100 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute in *Dissolution medium* to adequate concentration. Measure the absorbances of the solutions at 275 nm **(5.2.14)**, using *Dissolution medium* for zero adjustment. Calculate the quantity of $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$ dissolved in the medium, comparing the readings obtained with that of the metoprolol tartrate CRS solution at the concentration of 0.01% (w/v), prepared in the *Dissolution medium*.

Tolerance: not less than 75% (Q) of the declared quantity of $(C_{15}H_{25}NO_3)_2.C_4H_6O_6$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 75 mg of metoprolol tartrate to a 200 mL volumetric flask and add 150 mL of absolute ethyl alcohol. Homogenize and place in ultrasonic bath for 15 minutes. Complete the volume with absolute ethyl alcohol, homogenize and filter. Transfer 20 mL of the filtrate to a 50 mL volumetric flask, complete the volume with absolute ethyl alcohol and homogenize. Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the solutions at 274 nm, using absolute ethyl alcohol for zero adjustment. Calculate the quantity of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ in the tablets from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (3 μ m to 10 μ m), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute.

Mobile phase: dissolve 961 mg of sodium 1-pentanesulfonate monohydrate and 82 mg of anhydrous sodium acetate in a mixture of 550 mL of methyl alcohol and 470 mL of water, add 0.57 mL of glacial acetic acid and homogenize.

Diluent: prepare a mixture of methyl alcohol and 0.1 M hydrochloric acid (1:1).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 50 mg of metoprolol tartrate to a 50 mL volumetric flask, add 30 mL of *Diluent* and place in ultrasonic bath for 30 minutes. Complete the volume with *Diluent*, homogenize and filter. Dilute until concentration of 0.5 mg/mL, using *Mobile phase* as solvent.

Standard solution: dissolve accurately weighed quantity of metoprolol tartrate CRS in *Diluent* to obtain a 1 mg/mL solution. Dilute until concentration of 0.5 mg/mL, using *Mobile phase* as solvent.

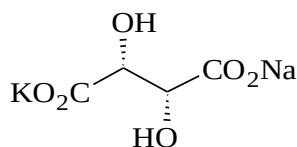
Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

POTASSIUM SODIUM TARTRATE*Kalii natrii tartras*C₄H₄KNaO₆; 210.16

potassium sodium tartrate; 09846

Sodium and potassium salt of (2*R*,3*R*)-2,3-dihydroxybutanedioic acid (1:1:1)

[304-59-6]

C₄H₄KNaO₆.4H₂O; 282.22

potassium sodium tartrate tetrahydrate; 11396

Sodium and potassium salt of (2*R*,3*R*)-2,3-dihydroxybutanedioic acid tetrahydrate (1:1:1:4)

[6381-59-5]

It contains not less than 99.0% and not more than 102.0% of C₄H₄KNaO₆, calculated in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline or colorless powder, clear crystals.

Solubility. Very soluble in water, practically insoluble in ethyl alcohol.

IDENTIFICATION

A. In 10 mL of a 5% (w/v) solution, add 10 mL of 6 *M* acetic acid. A white crystalline precipitate forms within 15 minutes.

B. It meets tartrate ion reactions (5.3.1.1).

C. It meets potassium ion reactions (5.3.1.1).

D. It meets sodium ion reactions (5.3.1.1).

PURITY TESTS

Acidity or alkalinity. Dissolve 5 g of the sample in 100 mL of water. To 5 mL of this solution, add 0.1 mL of phenolphthalein TS. Not more than 0.5 mL of 0.01 *M* hydrochloric acid or 0.01 *M* sodium hydroxide is required to turn the indicator color.

Barium and oxalates. To 5 mL of the solution obtained in the *Acidity or alkalinity* test, add 3 mL of calcium sulfate RS. Allow to stand for five minutes. Any opalescence in the preparation is not more intense than that obtained by mixing 3 mL of calcium sulfate RS and 5 mL of distilled water.

Ammonia (5.3.2.6). In 5 mL of the solution obtained in the *Acidity or alkalinity test*, carry out the *Limit test for ammonia*. Not more than 0.004% (40 ppm).

Calcium (5.3.2.7). Determine in 0.5 g of the sample. Not more than 0.02% (200 ppm).

Chlorides (5.3.2.1). Not more than 0.01% (100 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Sulfates (5.3.2.2). Determine in 4.8 g of the sample. Use 0.5 mL of standard sulfuric acid. Not more than 0.005% (50 ppm).

Water (5.2.20.1). Between 21.0 and 27.0.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 2 g of the sample in a tared porcelain crucible and ignite, slowly at first, until the salt is charred, protecting the charred salt from the flame during the whole process. Cool the crucible, place in a glass beaker and break the charred mass with a glass rod. Without removing the glass rod or crucible, add 50 mL of water and 50 mL of 0.25 *M* sulfuric acid VS, cover the beaker and boil the solution for 30 minutes. Filter and wash with hot water until the last washing is neutral to the litmus paper. Cool the filtrate and washings. Titrate excess acid with 0.5 *M* sodium hydroxide VS using, as indicator, a mixture of 10 mL of methyl red TS and 10 mL of methylthioninium chloride RS1. Carry out blank test. Each mL of 0.25 *M* sulfuric acid VS is equivalent to 52.540 mg of $C_4H_4KNaO_6$.

PACKAGING AND STORAGE

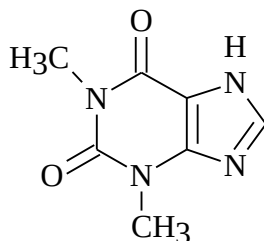
In closed containers.

LABELING

Comply with current legislation

CATEGORY

Cathartic.

THEOPHYLLINE***Theophyllinum***

$C_7H_8N_4O_2$; 180.17

theophylline; 08397

1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione

[58-55-9]

It contains not less than 99.0% and not more than 101.0% of $C_7H_8N_4O_2$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Slightly soluble in water, moderately soluble in ethyl alcohol. Soluble in solutions of alkali metal hydroxides, in ammonia and in mineral acids.

Physicochemical constants.

Melting range (5.2.2): 270 °C to 274 °C with the sample previously desiccated.

IDENTIFICATION

The Identification tests B., C., D. and E. can be omitted if test A. is carried out. The Identification test A. can be omitted if tests B., C., D. and E. are carried out.

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the theophylline CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of a 0.0005% (w/v) sample solution in 0.1 M hydrochloric acid, there are maximum absorption points only at the same wavelengths and with the same relative intensities as those observed in the theophylline CRS spectrum, prepared identically.

C. Heat about 10 mg of the sample in water bath, dissolved in 1 mL of 360 g/L potassium hydroxide, for three minutes. Add 1 mL of diazotized sulfanilic acid RS. A reddish color slowly forms.

D. Dissolve approximately 10 mg of the sample in 10 mL of water and add 0.5 mL of 5% (w/v) mercury(II) acetate. A white crystalline precipitate is produced after some minutes.

E. It meets xanthine reactions (5.3.1.1).

PURITY TESTS

Acidity. Dissolve about 0.5 g of the sample, accurately weighed, in 75 mL of water. Add 2 mL of 0.01 M sodium hydroxide and a drop of methyl red TS, a yellowish color forms.

Related substances. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel GF₂₅₄, with thickness of 250 µm as support, and mixture of acetone, chloroform, methyl alcohol, butyl alcohol and ammonia (3:3:2:2:1), as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve approximately 100 mg of theophylline, accurately weighed, in 3 mL of *N,N*-dimethylformamide and add 10 mL of methyl alcohol.

Solution (2): transfer 1 mL of *Solution (1)* to a 200 mL volumetric flask, complete the volume with methyl alcohol and homogenize.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light at 254 nm. The main stain obtained in the chromatogram with *Solution (1)* corresponds in position to the one obtained with *Solution (2)*. And no secondary stain obtained with *Solution (1)* is more intense than that obtained with *Solution (2)* (0,5%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1.0 g of the sample. Dry in oven at 105 °C for three hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1.0 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 150 mg of the sample and dissolve in 100 mL of water, add 20 mL of silver nitrate RS. Titrate with 0.1 M sodium hydroxide VS using bromothymol blue TS until color changeover. Each mL of 0.1 M sodium hydroxide VS is equivalent to 18.017 mg of C₇H₈N₄O₂.

PACKAGING AND STORAGE

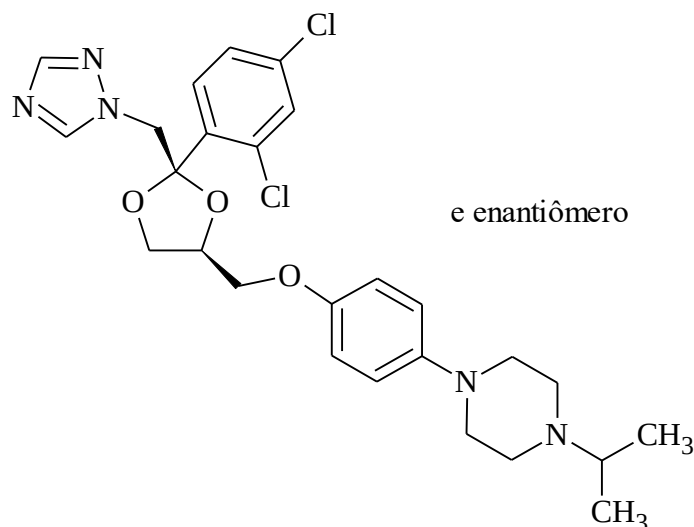
In tightly closed containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Bronchodilator.

TERCONAZOLE***Terconazolum***C₂₆H₃₁Cl₂N₅O₃; 532.47

terconazole; 08417

rel-1-[4-[[[(2*R*,4*S*)-2-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-4-(1-methylethyl)-piperazine
[67915-31-5]

It contains not less than 99.0% and not more than 101.0% of C₂₆H₃₁Cl₂N₅O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or off-white powder. Presents polymorphism.

Solubility. Practically insoluble in water, moderately soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): -0,10 to +0,10, in relation to the desiccated substance. Determine in solution at 10% (w/v) in methylene chloride.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the terconazole CRS spectrum, prepared identically. If the spectrum obtained shows differences, dissolve the sample and the standard separately in a minimum volume of acetone. Allow to evaporate until dryness and carry out a new spectrum with the residues.

B. Proceed as described in *Thin layer chromatography* (5.2.17.1), using silica gel G as support, and mixture of ammonium acetate RS, dioxane and methyl alcohol (20:40:40), as mobile phase. Apply to the plate, separately, 5 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 30 mg of the sample in methyl alcohol and dilute to 5 mL with the same solvent.

Solution (2): dissolve 30 mg of terconazole CRS in methyl alcohol and dilute to 5 mL with the same solvent.

Solution (3): dissolve 30 mg of terconazole CRS and 30 mg of ketoconazole CRS in methyl alcohol and dilute to 5 mL with the same solvent.

Develop chromatogram. Remove the plate, allow to air dry and heat for 15 minutes. Expose to iodine vapor until stains are formed. The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. The test is only valid if the chromatogram obtained with *Solution (3)* presents two clearly separated stains.

C. Add 0.3 mg of sodium carbonate anhydrous to 30 mg of the sample in a porcelain crucible. Heat to red for 10 minutes. Allow to cool. Extract the residue with 5 mL of nitric acid RS and filter. Add 1 mL of water to 1 mL of the filtrate. It meets chloride ion reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described in method **B.** from Assay. Prepare solutions as described below.

Solution (1): quantitatively dissolve about 0.1 g of the sample in methyl alcohol and dilute to 10 mL with the same solvent.

Solution (2): transfer 1 mL of *Solution (1)* to a 100 mL volumetric flask and complete the volume with methyl alcohol. Transfer 2.5 mL of this solution to a 10 mL volumetric flask and complete the volume with methyl alcohol.

Solution (3): dissolve 2.5 mg of terconazole CRS and 2 mg of ketoconazole CRS in methyl alcohol and dilute to 100 mL with the same solvent.

Inject 20 µL replicates of the *Solution (3)*. The relative retention times are approximately 0.8 for ketoconazole and 1.0 for terconazole. The resolution between terconazole and ketoconazole peaks is not less than 10.0. Make adjustments if necessary.

Procedure: separately inject 20 µL of methyl alcohol as blank, 20 µL of *Solution (1)* and 20 µL of *Solution (2)*. The area under any peak obtained in the chromatogram with *Solution (1)*, except under the main peak, is not greater than the area under the main peak obtained in the chromatogram with *Solution (2)* (0.25%). The sum of the areas under all the peaks, except that under the main peak, obtained with *Solution (1)*, is not greater than twice the area under the main peak, obtained in the chromatogram with *Solution (2)* (0.5%). Disregard peaks obtained with the blank or with area smaller than 0.2 times the area under the main peak obtained in the chromatogram with *Solution (2)* (0.05%).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 100°C to 105 °C until constant weight. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Quantitatively dissolve about 0.15 g of the sample in 70 mL of mixture of glacial acetic acid and methyl ethyl ketone (9:1). Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically, at the second inflection point. Each mL of 0.1 M perchloric acid VS is equivalent to 17.749 mg of C₂₆H₃₁Cl₂N₅O₃.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 220 nm; column with 125 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to inactivated octadecylsilane group (5 µm , kept at room temperature; *Mobile phase* flow rate of 2 mL/minute.

Eluent A: 3.4 mg/mL tetrabutylammonium hydrogen sulfate solution.

Eluent B: acetonitrile.

Mobile phase gradient: adopt the gradient system described on the following table:

<i>Time (minutes)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>	<i>Elution</i>
0 – 10	95 → 50	5 → 50	linear gradient
10 – 15	50	50	isocratic
15 – 20	95	5	stabilization

Sample solution: quantitatively dissolve an accurately weighed quantity of the sample in methyl alcohol to obtain a 0.5 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with methyl alcohol, obtaining a 50 µg/mL solution.

Standard solution: quantitatively dissolve an accurately weighed quantity of terconazole CRS in methyl alcohol to obtain a 0.5 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with methyl alcohol, obtaining a 50 µg/mL solution.

Resolution solution: dissolve 2.5 mg of terconazole CRS and 2 mg of ketoconazole CRS in methyl alcohol and dilute to 100 mL with the same solvent.

Inject 20 µL replicates of the *Resolution solution*. The relative retention times are approximately 0.8 for ketoconazole and 1.0 for terconazole. The relative standard deviation of the replicate areas

under the peaks recorded is not more than 2.0%. The resolution between terconazole and ketoconazole peaks should be not less than 10.0. Make adjustments if necessary.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₆H₃₁Cl₂N₅O₃ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antifungal.

TERCONAZOLE CREAM

It contains not less than 90.0% and not more than 120.0% of the declared quantity of $C_{26}H_{31}Cl_2N_5O_3$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the *Sample solution* obtained in method **A.** of Assay, there is maximum absorption at 226.6 nm, identical to that observed in the spectrum of the *Standard solution*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Quantitatively transfer quantity of cream equivalent to 14 mg of terconazole to a 100 mL volumetric flask and add 60 mL of 0.1 M hydrochloric acid. Shake for 30 minutes to disperse the cream and complete the volume with the same solvent. Filter. Dilute successively, with the same solvent to a concentration of 0.0014% (w/v). Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 226.6 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the quantity of $C_{26}H_{31}Cl_2N_5O_3$ in the cream from the readings obtained.

B. By *High performance liquid chromatography (5.2.17.4)* Proceed as described in method **B.** of Assay in the *Terconazole* monograph. Prepare *Sample solution* as described below.

Sample solution: quantitatively transfer quantity of cream equivalent to 40 mg of terconazole to a 100 mL volumetric flask and add 60 mL of 0.1 M hydrochloric acid. Shake for 30 minutes to disperse the cream and complete the volume with methyl alcohol and homogenize. Filter, discarding the first 5 mL of the filtrate. Transfer 25 mL of the filtrate to a 50 mL volumetric flask and complete the volume with methyl alcohol, obtaining a 200 µg/mL solution. Transfer 15 mL of this solution to a 50 mL volumetric flask and complete the volume with methyl alcohol, obtaining a 60 µg/mL solution.

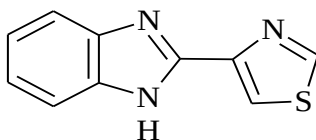
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{26}H_{31}Cl_2N_5O_3$ in the cream from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THIABENDAZOLE*Tiabendazolum* $C_{10}H_7N_3S$; 201.25

thiabendazole; 08493

2-(4-thiazolyl)-1*H*-benzimidazole

[148-79-8]

It contains not less than 98.0% and not more than 101.0% of $C_{10}H_7N_3S$ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White or off-white crystalline powder.

Solubility. Practically insoluble in water, slightly soluble in ethyl alcohol. Soluble in diluted mineral acids.

Physicochemical constants.

Melting range (5.2.2): 296 °C to 303 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample desiccated at 105 °C, until constant weight, and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the thiabendazole CRS spectrum, prepared identically.

B. Weigh 25 mg of the sample, dissolve in 0.1 *M* hydrochloric acid and dilute, in the same solvent, to concentration of 0.0005% (w/v). In the ultraviolet absorption spectrum **(5.2.14)** of the sample solution, in the range of 200 nm to 400 nm, there is maximum point at 302 nm, identical to that observed in the spectrum of similar thiabendazole CRS solution.

C. The main stain of the chromatogram from *Solution (2)*, obtained in *Related substances*, corresponds in position, color and intensity to the one obtained with *Solution (3)*.

D. Dissolve 10 mg of the sample in 5 mL of *M* hydrochloric acid, add 5 mg of *p*-phenylenediamine hydrochloride and shake until dissolved. Add about 0.1 g of zinc powder, shake and allow to stand for two minutes. Add 5 mL of freshly-prepared ferric ammonium sulfate RS. Blue or Violet-blue color develops.

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel HF₂₅₄ as support and mixture of water, acetone, glacial acetic acid and toluene (2.5:10:25:62.5), as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): transfer 0.1 g of the sample to a 10 mL volumetric flask. Dissolve in methyl alcohol and complete the volume with the same solvent.

Solution (2): transfer 1 mL of *Solution (1)* to a 10 mL volumetric flask and complete the volume with methyl alcohol.

Solution (3): transfer 25 mg of thiabendazole CRS to a 25 mL volumetric flask. Dissolve in methyl alcohol and complete the volume with the same solvent.

Solution (4): transfer 1 mL of *Solution (2)* to a 10 mL volumetric flask and complete the volume with methyl alcohol.

Solution (5): transfer 1 mL of *Solution (2)* to a 25 mL volumetric flask and complete the volume with methyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (4)* (1.0%), and only one stain is more intense than that obtained in the chromatogram with *Solution (5)* (0.4%).

Heavy metals (5.3.2.3). Use *Method III*. Not more than 0.001% (10 ppm).

Water (5.2.20.1). Not more than 0.5%.

Residue by incineration (5.2.10). Not more than 0.1%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Quantitatively dissolve about 0.15 g of the sample in 30 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically or using methylrosanilinium chloride TS until the color changes from blue to greenish-blue. Carry out the blank test and make the necessary corrections. Each mL of 0.1 M perchloric acid VS is equivalent to 20.125 mg of C₁₀H₇N₃S.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anthelmintic.

THIABENDAZOLE TABLETS

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_{10}H_7N_3S$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the sample solution obtained in method **B.** from Assay, there is maximum point at 302 nm, identical to that observed in the standard solution spectrum. **B.** The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **C.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

C. Weigh and pulverize the tablets. Use quantity of powder equivalent to 20 mg of thiabendazole. Add 5 mL of *M* hydrochloric acid, 5 mg of dimethyl-*p*-phenylenediamine hydrochloride and shake. Add 0.1 g of zinc powder, mix, wait for two minutes and add 10 mL of freshly-prepared ferric ammonium sulfate RS. Intense blue or violet color is produced.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test. Proceed as described in method **B.** from Assay.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Weigh and pulverize 20 tablets. Weigh quantity of powder equivalent to 0.15 g of thiabendazole and proceed as described in Assay in the *Thiabendazole* monograph.

B. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh and pulverize 20 tablets. Transfer a quantity of powder equivalent to 0.1 g of thiabendazole to a 100 mL volumetric flask. Add 75 mL of 0.1 *M* hydrochloric acid, heat in water bath for 15 minutes, shaking occasionally, cool, complete the volume to 100 mL with 0.1 *M* hydrochloric acid and filter. Transfer 10 mL of the filtrate to a 100 mL volumetric flask and complete the volume with 0.1 *M* hydrochloric acid. From this solution, pipet 5 mL, transfer to a 100 mL volumetric flask and complete the volume with the

same solvent, obtaining a 0.0005% (w/v) solution. Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 302 nm, using 0.1 M hydrochloric acid for zero adjustment. Calculate the quantity of $C_{10}H_7N_3S$ in the tablets, from the readings obtained.

C. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 300 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow rate of 2 mL/minute.

Phosphate buffer pH 3.5: dissolve 13.8 g of sodium phosphate monobasic in 2000 mL of water. Adjust the solution pH, with phosphoric acid, to 3.5 ± 0.05 .

Mobile phase: mixture of *Phosphate buffer pH 3.5* and methyl alcohol (54:46).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 0.2 g of thiabendazole to a 1000 mL volumetric flask, add 100 mL of 0.1 M hydrochloric acid, homogenize and heat in water bath at for 30 minutes. Allow to cool to room temperature and complete the volume with water. Homogenize and filter, discarding the first 20 mL of the filtrate.

Standard solution: dissolve an accurately weighed quantity of thiabendazole CRS in 0.1 M hydrochloric acid and make quantitative dilutions, if necessary, until obtaining a 2 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with water, obtaining a 0.2 mg/mL solution. Homogenize.

Inject 20 μ L replicates of the *Standard solution*. The column efficiency is not less than 960 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{10}H_7N_3S$ in the tablets from the responses obtained for the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THIABENDAZOLE OINTMENT

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_{10}H_7N_3S$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), at the range of 200 nm to 400 nm, of the sample solution obtained in *Assay*, there is maximum absorption at 302 nm, identical to that observed at the standard solution spectrum.

B. Place quantity of ointment equivalent to 10 mg of thiabendazole in 5 mL of *M* hydrochloric acid, add 5 mg of dimethyl-*p*-phenylenediamine hydrochloride and homogenize. Add about 0.1 g of zinc powder, shake and allow to stand for two minutes. Add 5 mL of ferric ammonium sulfate RS. Intense blue or Violet-blue color develops.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Weigh quantity of ointment equivalent to 50 mg of thiabendazole and quantitatively transfer to a separating funnel with a capacity of 250 mL, with the aid of 50 mL of ethyl ether. Shake to dissolve the ointment and extract with four 40 mL portions of 0.1 *M* hydrochloric acid. Combine the aqueous extract in a 250 mL volumetric flask and heat slightly to eliminate residues of ethyl ether. Cool and complete the volume with 0.1 *M* hydrochloric acid. Transfer 5 mL of this solution to a 200 mL volumetric flask and complete the volume with 0.1 *M* hydrochloric acid to obtain a 0.0005% solution (w/v). Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 302 nm, using 0.1 *M* hydrochloric acid for zero adjustment. Calculate the quantity of $C_{10}H_7N_3S$ in the ointment from the readings obtained.

PACKAGING AND STORAGE

In hermetically closed containers and protected from excessive heat.

LABELING

Comply with current legislation.

THIABENDAZOLE ORAL SUSPENSION

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_{10}H_7N_3S$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 400 nm of the *Sample solution* obtained in method **A.** of Assay, there is maximum absorption at 302 nm, identical to that observed in the thiabendazole CRS spectrum.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from Assay, corresponds to the one from the main peak of the *Standard solution*.

C. Transfer to a test tube a volume of oral suspension equivalent to 50 mg of thiabendazole, add 10 mL of *M* hydrochloric acid and shake vigorously. Add 5 mL to a test tube, add 5 mg of dimethyl-*p*-phenylenediamine hydrochloride and shake. Add 0.1 g of zinc powder and shake. Allow to stand for two minutes. Add 5 mL of ferric ammonium sulfate RS. Intense blue or violet-blue color is produced.

CHARACTERISTICS

Aspect. Completely empty the content of the number of flasks determined in **Table 1** in *Determination of volume* (**5.1.2**), previously shaken, in corresponding, clean and dry cylinders, with cap. Observe immediately under suitable visibility conditions. The content must flow smoothly, the suspension must be homogeneous, viscous, free of lumps and foreign particles. After 24 hours standing, it may show slight sedimentation, which resuspends under agitation.

Determination of volume (**5.1.2**). Complies with the test.

pH (**5.2.19**). 3.4 to 7.0. Determine in the reconstituted oral suspension as indicated on the label.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (**5.5.3.1.2**). Complies with the test.

Study of pathogenic microorganisms (**5.5.3.1.3**). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry* (**5.2.14**). Transfer volume of oral suspension equivalent to 0.25 g of thiabendazole to a 100 mL volumetric flask and add 75 mL of 0.1 *M* hydrochloric acid. Heat in water bath for 15 minutes, shaking occasionally. Cool to room temperature. Complete the volume with 0.1 *M* hydrochloric acid. Dilute, successively, in 0.1 *M* hydrochloric acid, to a concentration of 0.0005% (w/v). Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 302 nm, using 0.1 *M* hydrochloric acid for zero adjustment. Calculate the quantity of $C_{10}H_7N_3S$ in the oral suspension from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 254 nm; column with 300 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 2.0 mL/minute.

Phosphate buffer pH 3.1: dissolve 13.8 g of sodium phosphate monobasic monohydrate in 2000 mL of water. Adjust the solution pH with phosphoric acid to 3.10 ± 0.05 .

Mobile phase: mixture of *Phosphate buffer pH 3.1* and methyl alcohol (65:35).

Sample solution: transfer volume of the oral suspension, equivalent to 500 mg of thiabendazole, to a 250 mL volumetric flask, complete the volume with 0.1 M hydrochloric acid and homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask, complete the volume with water, obtaining a 0.2 mg/mL solution. Homogenize.

Standard solution: dissolve an accurately weighed quantity of thiabendazole CRS in 0.1 M hydrochloric acid to obtain a 2 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with water, obtaining a 0.2 mg/mL solution. Homogenize.

Inject 20 µL replicates of the *Standard solution*. The column efficiency is not less than 960 theoretical plates. The tailing factor is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

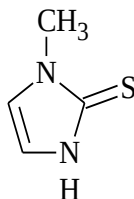
Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₀H₇N₃S in the oral suspension from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In hermetically closed containers and protected from excessive heat.

LABELING

Comply with current legislation.

THIAMAZOLE***Thiamazolum***

C₄H₆N₂S; 114.17

thiamazole; 08504

1,3-Dihydro-1-methyl-2*H*-imidazol-2-thione

[60-56-0]

It contains not less than 98.0% and not more than 101.0% of C₄H₆N₂S, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or slightly yellowish crystalline powder.

Solubility. Freely soluble in water, in methylene chloride and in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 143 °C to 146 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample desiccated at 105 °C for two hours and dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the thiamazole CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 300 nm, of 0.0005% (w/v) solution in 0.28% (v/v) sulfuric acid, there is maximum absorption at 211 nm and at 251 nm. The ratio between the absorbance values measured at 251 nm and 211 nm is comprised between 2.5 and 2.7.

C. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support, and mixture of ammonium hydroxide, isopropyl alcohol and toluene (1:24:75) as mobile phase. Apply to the plate, separately, 10 µL of each of the freshly prepared solutions described below.

Solution (1): 1 mg/mL sample solution in methyl alcohol.

Solution (2): 1 mg/mL thiamazole CRS solution in methyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). The stain obtained with *Solution (1)* chromatogram corresponds in position, color and intensity to

the one obtained with *Solution (2)*. Expose the plate to iodine vapors for 30 minutes, the chromatogram presents two clearly separated dots.

The version of the monograph evaluated in July 2014 does not have this final sentence. After exposure to iodine vapors for 30 minutes, the stain obtained with *Solution (1)* corresponds in position, color and intensity to that of the chromatogram obtained with *Solution (2)*.

PURITY TESTS

Related substances. Proceed as described in *Gas chromatography (5.2.17.5)*. Use gas chromatograph equipped with flame ionization detector, capillary column of 30 m in length and 0.25 mm in internal diameter, packed with polydiphenyldimethylsiloxane with special deactivation for basic compounds, with film thickness of 0.5 mm; column temperature from 100 °C to 250 °C (100 °C held for two minutes after injection, increased to 250 °C from two to seven minutes and held at 250 °C for seven to 22 minutes), temperature injector at 150 °C and detector temperature at 250 °C; using helium as carrier gas and auxiliary to the detector flame; 1.5 mL/minute flow rate.

Solution (1): 10 mg/mL sample solution in chloroform.

Solution (2): 0.01 mg/mL sample solution in chloroform.

Solution (3): dissolve 5 mg of 2,2-dimethoxy-N-methylethanamine CRS (Impurity A), 5 mg of 1-methyl-1H-imidazole CRS (Impurity B) and 5 mg of 1-methyl-2-(methylsulfanyl)-1H-imidazole CRS (Impurity C) in chloroform to complete 50 mL. Transfer 1 mL of this solution to a 10 mL volumetric flask and complete the volume with chloroform.

Inject, separately, 1 µL replicates of *Solution (2)* and *Solution (3)*. The thiamazole retention time is about 6.5 minutes. The relative retention times are about 0.3 for *Impurity A*, 0.4 for *Impurity B*, 0.7 for *Impurity C* and 1.0 for thiamazole. The resolution between the *Impurity B* and *Impurity A* peaks is not less than 1.5.

Procedure: inject, separately, 1 µL of the *Solution (1)*, *Solution (2)* and *Solution (3)*, using a 3:20 flow division. Record the chromatograms and measure the areas under the peaks corresponding to thiamazole. The areas obtained with *Impurities A, B* and *C* in the chromatogram of *Solution (1)* are not more than equal to the corresponding areas obtained with *Solution (3)* (0.1%). No area of any other impurity is greater than the area under the main peak obtained with *Solution (2)* (0.1%). The sum of the areas under all the peaks, except that under the main peak, obtained with the chromatogram of *Solution (1)*, is not more than five times the area under the main peak of *Solution (2)* (0.5%). Disregard peaks with areas up to 0.2 times the area under the main peak in the chromatogram obtained with *Solution (2)* (0.02%).

Selenium. Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Prepare solutions as described below.

2,3-diaminonaphthalene solution: dissolve 0.1 g of 2,3-diaminonaphthalene and 0.5 g of hydroxylamine hydrochloride in 0.1 M hydrochloric acid and complete the volume to 100 mL with the same solvent. Prepare the solution on the day of the test.

Sample solution: with 0.1 g of the sample proceed as described in *Combustion method (5.3.3.3)*, *Flask combustion method at atmospheric pressure*, using 25 mL of diluted nitric acid (1:30) as absorbing solution. Once the sample complete combustion is concluded, wash the cap with a small amount of water and transfer the solution to a beaker, wash the equipment with 25 mL of water and add to the previous solution. Boil gently for 10 minutes and cool to room temperature.

Standard solution: accurately weigh about 40 mg of selenium, dissolve in 100 mL of diluted nitric acid (1:2), if necessary heat in water bath to dissolve, transfer to a 1000 mL volumetric flask with water, complete the volume with water and homogenize. Transfer 5 mL of this solution to a 200 mL volumetric flask and complete the volume with water. Transfer 3 mL of this solution, transfer to a 150 mL beaker, add 25 mL of water and 25 mL of diluted nitric acid (1:30).

Blank solution: mix 25 mL of water and 25 mL of diluted nitric acid (1:30).

Procedure: transfer the *Sample solution*, *Standard solution* and *Blank solution* to separate beakers, adjust the pH to 2.0 ± 0.2 with ammonium hydroxide solution (1:2) and complete the volume to 60 mL with water. Transfer each solution to an amber separating funnel. Wash each beaker with 10 mL of water and add to each separating funnel respectively. Add 0.2 g of hydroxylamine hydrochloride, shake gently to dissolve. Immediately add 5 mL of *2,3-diaminonaphthalene solution*, shake and allow to stand for 100 minutes. Add 5 mL of cyclohexane to each separating funnel, shake for two minutes and separate the phases. Centrifuge the cyclohexane extracts to remove any remaining water. Determine the absorbances of the cyclohexane extracts from the *Standard Solution* and *Sample Solution* at 378 nm. Use cyclohexane extract from the *Blank Solution* for zero adjustment. The absorbance of the *Sample solution* is not more than equal to that of the *Standard solution*.

Heavy metals (5.3.2.3). Use *Method I*. Carry out the test with 12 mL of the 10% w/v solution in water. Prepare standard solution using *Standard lead diluted solution (1 ppm Pb)*. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample in oven at 105 °C for two hours. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS.

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Accurately weigh about 0.25 g of the sample and dissolve in 75 mL of water. Add 15 mL of 0.1 M sodium hydroxide VS and homogenize. Add, under agitation, 30 mL of 0.1 M silver nitrate and 1 mL of bromothymol blue TS. Titrate with 0.1 M sodium hydroxide VS until permanent greenish-blue color. Each mL of 0.1 M sodium hydroxide VS is equivalent to 11.417 mg of $C_4H_6N_2S$.

PACKAGING AND STORAGE

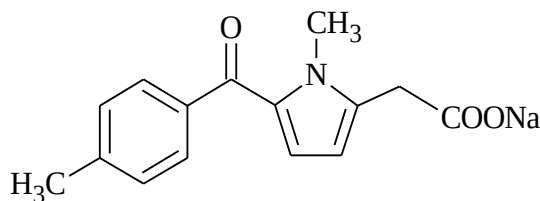
In containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Inhibitor of thyroid hormone synthesis.

TOLMETIN SODIUM*Tolmetinum natricum* $C_{15}H_{14}NNaO_3$; 279.27

tolmetin sodium; 08743

1-Methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-acetic acid sodium salt (1:1)

[35711-34-3]

 $C_{15}H_{14}NNaO_3 \cdot 2H_2O$; 315.30

tolmetin sodium dihydrate; 11397

1-Methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-acetic acid sodium salt dihydrate (1:1:2)

[64490-92-2]

It contains not less than 98.0% and not more than 102.0% of $C_{15}H_{14}NNaO_3$, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Slightly yellowish or orange crystalline powder.

Solubility. Freely soluble in water and in methyl alcohol, slightly soluble in ethyl alcohol.

IDENTIFICATION

A. In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the tolmetin sodium CRS spectrum, prepared identically.

B. In the ultraviolet absorption spectrum (5.2.14), in the range of 200 nm to 400 nm, of 0.001% (w/v) solution in phosphate buffer M/15 pH 7.0, there are maximum points at the same wavelengths observed in similar tolmetin sodium CRS solution.

C. Weigh 1 g of sample and dissolve in 20 mL of water. It meets sodium ion reactions (5.3.1.1).

PURITY TESTS

Related substances. Proceed as described in *Thin layer chromatography* (5.2.17.1), using plate covered with approximately 0.25 mm of silica gel, as support, and mixture of chloroform and glacial acetic acid (95:5) as mobile phase. Apply to the plate, separately, 20 μ L of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.125 g of the sample in 10 mL of methyl alcohol, obtaining a 12.5 mg/mL solution.

Solution (2): dissolve an accurately weighed quantity of tolmetin sodium CRS in methyl alcohol to obtain a 12.5 mg/mL solution. Quantitatively dilute a portion of this solution in methyl alcohol to obtain a 62.5 µg/mL solution.

Develop chromatogram until solvent reaches 3/4 of plate length. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained in the chromatogram with *Solution (1)* corresponds to the one obtained with *Solution (2)*. No secondary stain is more intense than that of *Solution (2)* (0.5%). The total of impurities is not greater than 2%

Volatile organic impurities. Proceed as described in *Gas chromatography (5.2.17.5)*. Use chromatograph equipped with flame ionization detector, using a mixture of nitrogen, synthetic air and hydrogen (1:1:10) as auxiliary gases to the detector flame; capillary column of 30 m in length and 0.53 mm in internal diameter, packed with stationary phase bonded to 5% of phenylpolysiloxane and 95% to methylpolysiloxane, with film thickness of 5 µm; column temperature from 35 °C to 260 °C (35 °C held for five minutes, increased to 175 °C to 8 °C per minute, increased to 260 °C to 35 °C per minute and held at this temperature for not less than 16 minutes), injector temperature of 70 °C and detector temperature of 260 °C; use helium as carrier gas; carrier gas flow rate of 1 mL/minute.

Sample solution: quantitatively dissolve about 1 g the sample in 50 mL of organic compound-free water.

Standard solution: prepare a solution, in organic compound-free water, containing in each milliliter: 10 µg of methylene chloride, 1 µg of chloroform, 2 µg of benzene, 2 µg of dioxane and 2 µg of trichlorethylene.

Inject separately 1 µL of *the Sample solution* and the *Standard solution* in the gas chromatograph. Record the chromatograms and measure the area under the peaks. Identify, based on the retention time, any peaks present in the sample solution chromatogram. The presence and identification of the peaks in the chromatogram must be established by comparing the chromatograms of the *Sample Solution* and *Standard Solution*. Limits: benzene 2 ppm, chloroform 50 ppm, dioxane 100 ppm, methylene chloride 500 ppm and trichlorethylene 80 ppm. Complies with the test.

Heavy metals (5.3.2.3). Use *Method III*. Use 1 g of sample. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of sample. Dry in oven at 60 °C, under reduced pressure, for four hours. Between 10.4% and 12.4%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Accurately weigh approximately 0.3 g of the sample and dissolve, under heating, in 150 mL of glacial acetic acid. Cool to room temperature and titrate with 0.1 M perchloric acid VS, determining the endpoint

potentiometrically. Carry out the blank test and make the necessary corrections. Each mL of 0.1 *M* perchloric acid VS is equivalent to 27.927 mg of C₁₅H₁₄NNaO₃.

PACKAGING AND STORAGE

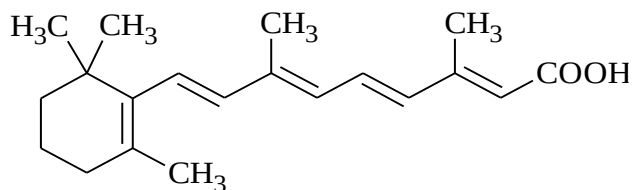
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anti-inflammatory.

TRETINOIN*Tretinoinum*C₂₀H₂₈O₂; 300.44

tretinoin; 08848

Retinoic acid

[302-79-4]

It contains not less than 97.0% and not more than 103.0% of C₂₀H₂₈O₂, in relation to the desiccated substance.

DESCRIPTION

Physicochemical characteristics. Yellow or light orange crystalline powder. Melting temperature (5.2.2): about 182°C, with decomposition.

Solubility. Practically insoluble in water, soluble in chloroform and methyl alcohol, slightly soluble in ethyl ether, very slightly soluble in ethyl alcohol.

IDENTIFICATION

Note: carry out the analyses protected from direct light and use amber glassware.

A. In the infrared absorption spectrum (5.2.14) of the sample dispersed in potassium bromide, previously desiccated, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the tretinoin CRS spectrum, prepared identically.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

PURITY TESTS

Related Substances and Isotretinoin Limit. Proceed as described in method **B.** from *Assay*. Prepare *Solutions (1), (2), (3), (4) and (5)* as described below.

Solution (1): transfer 0.1 g of the sample, accurately weighed, to a 50 mL volumetric flask, dissolve with methyl alcohol, complete the volume with the same solvent and homogenize.

Solution (2): transfer 10 mg of isotretinoin CRS, accurately weighed, to a 10 mL volumetric flask, dissolve with methyl alcohol, complete the volume with the same solvent and homogenize.

Solution (3): transfer 1 mL of *Solution (2)* to a 25 mL volumetric flask, complete the volume with methyl alcohol and homogenize.

Solution (4): transfer 1 mL of *Solution (2)* and 0.5 mL of *Solution (1)* to a 25 mL volumetric flask, mix, complete the volume with methyl alcohol and homogenize.

Solution (5): transfer 0.5 mL of *Solution (1)* to a 100 mL volumetric flask, complete the volume with methyl alcohol and homogenize.

Inject, separately, 10 µL replicates of each one of the *Solutions (1) (2), (3), (4) and (5)*. The standard deviation of the areas under the main peaks is not more than 2.0% in two successive injections. In the *Solution (4)* chromatogram, the resolution between tretinoin and isotretinoin is not less than 2.0.

Procedure: Inject, separately, 10 µL of each one of the *Solutions (1) (2), (3), (4) and (5)*, record the chromatograms and measure the areas under the peaks. In the chromatogram obtained with *Solution (1)* the area under the isotretinoin exact peak is not more than equal to the area under the main peak obtained in the chromatogram of *Solution (3)* (2.0%) and the sum of the areas under all the peaks obtained in *Solution (1)*, except for those under the peaks of tretinoin, the solvent and isotretinoin, is not more than equal to the area under the main peak obtained with *Solution (5)* (0.5 %).

Heavy metals (5.3.2.3). Use *Method I*. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample, at room temperature, under reduced pressure for 16 hours, until constant weight. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of sample. Not more than 0.1%.

BIOLOGICAL SAFETY TESTS.

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Accurately weigh about 0.2 g of the sample and dissolve in 70 mL of acetone. Titrate with 0.1 M tetrabutylammonium hydroxide VS. Determine the endpoint potentiometrically. Each mL of 0.1 M tetrabutylammonium hydroxide VS is equivalent to 30.044 mg of C₂₀H₂₈O₂.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 353 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (10 µm), kept at room temperature; *Mobile phase* flow rate of 1.0 mL/minute. Carry out the analysis protected from direct light.

Mobile phase: methyl alcohol and water (77:23), with 0.5% (v/v) glacial acetic acid; if necessary, adjust to obtain a retention time for tretinoin of about 18 minutes.

Sample solution: dissolve an accurately weighed quantity of the sample in methyl alcohol to obtain a 0.4 mg/mL solution. Transfer 5 mL of this solution to a 50 mL amber volumetric flask and complete the volume with methyl alcohol, obtaining a 40 µg/mL solution.

Standard solution: dissolve an accurately weighed amount of tretinoin CRS in methyl alcohol to obtain a 0.4 mg/mL solution. Transfer 5 mL of this solution to a 50 mL amber volumetric flask and complete the volume with methyl alcohol, obtaining a 40 µg/mL solution.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₂₀H₂₈O₂ in the sample from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In hermetic containers, protected from light, at temperature not exceeding 25 °C.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Keratolytic.

TRETINOIN CREAM

It contains not less than 90.0% and not more than 120.0% of the stated quantity of $C_{20}H_{28}O_2$.

IDENTIFICATION

The retention time of the main peak from the *Sample solution* chromatogram, obtained in Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Keep the sample and its solutions protected from direct light. Use chromatograph equipped with ultraviolet detector at 365 nm; column with 150 mm of length and 3.9 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (4 μ m), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Phosphate buffer: dissolve 1.38 g of sodium phosphate monobasic in 1000 mL of water. Adjust pH to 3.0 with diluted phosphoric acid.

Mobile phase: mixture of *Phosphate buffer* and tetrahydrofuran (55:45). Make the necessary adjustments so that the retention time is 15 minutes.

Diluent: mixture of water and 10% (v/v) phosphoric acid (9:1).

Sample solution: transfer an accurately weighed quantity of cream, equivalent to 1 mg of tretinoin, to a 50 mL amber volumetric flask and add 20 mL of tetrahydrofuran. Shake to disperse the cream, complete the volume with the same solvent. Homogenize and filter. Transfer 5 mL of this solution to a 25 mL amber volumetric flask and complete the volume with mixture of tetrahydrofuran and *Diluent* (3:2). Homogenize and filter.

Standard solution: dissolve an accurately weighed quantity of tretinoin CRS in tetrahydrofuran to obtain a 0.4 mg/mL solution. Carry out successive dilutions of this solution with a mixture of tetrahydrofuran and *Diluent* (3:2), until obtaining a 4 μ g/mL solution.

Inject 25 μ L replicates of the *Standard solution*. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 25 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{20}H_{28}O_2$ in the cream from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

TRETINOIN GEL

It contains not less than 90.0% and not more than 120.0% of the stated quantity of $C_{20}H_{28}O_2$.

IDENTIFICATION

A. In the ultraviolet absorption spectrum (**5.2.14**), in the range of 300 nm to 450 nm of the sample solution obtained in *Assay*, there are maximum and minimum points only at the same wavelengths observed in the standard solution spectrum.

B. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel F_{254} as support, and mixture of cyclohexane and isopropyl alcohol (10:90) as mobile phase. Apply to the plate, separately, 10 μ L of each one of the freshly prepared solutions described below.

Solution (1): dissolve quantity of gel equivalent to approximately 1.25 mg of tretinoin in heated methyl alcohol. Allow to cool to room temperature. Extract with three 50 mL portions of hexane. Wash the extract with 20 mL of water and filter with anhydrous sodium sulfate. Evaporate the filtrate to dryness, in rotary evaporator, at temperature not exceeding 60 °C. Dissolve the residue in 5 mL of methyl alcohol.

Solution (2): 0.25 mg/mL tretinoin CRS solution in methyl alcohol.

Develop chromatogram. Remove plate, allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography* (**5.2.17.4**). Use chromatograph equipped with ultraviolet detector at 353 nm; column with 150 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (10 μ m), kept at room temperature; *Mobile phase* flow rate of 1.4 mL/minute.

Mobile phase: 0.5% (v/v) glacial acetic acid solution in mixture of methyl alcohol and water (77:23).

Solution (1): use *Solution (1)* obtained in method **A.** for *Identification*.

Solution (2): dilute 3 mL of *Solution (1)* with methyl alcohol to 100 mL.

Procedure: inject, separately, 20 μ L of the *Solution (1)* and of *Solution (2)*, record the chromatograms and measure the areas under the peaks. The sum of the areas under the secondary peaks obtained with *Solution (1)* is not greater than the area under the main peak obtained with *Solution (2)* (3.0%).

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

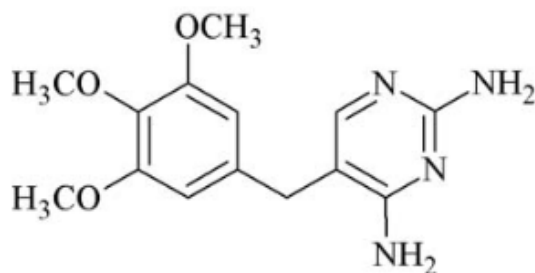
Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Keep the sample and its solutions protected from direct light. Dissolve quantity of gel equivalent to about 0.5 mg of tretinoin in chloroform and complete the volume to 100 mL with the same solvent. Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 365 nm, using chloroform for zero adjustment. Calculate the quantity of $C_{20}H_{28}O_2$ from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 1430$, at 365 nm, in chloroform.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

TRIMETHOPRIM*Trimethoprimum*C₁₄H₁₈N₄O₃; 290.32

trimethoprim; 08921

5-[(3,4,5-Trimethoxyphenyl)methyl]-2,4-pyrimidinediamine

[738-70-5]

It contains not less than 98.5% and not more than 101.0% of C₁₄H₁₈N₄O₃, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White or yellowish-white crystalline powder. Presents polymorphism.

Solubility. Very slightly soluble in water, moderately soluble in methyl alcohol, slightly soluble in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): 199 °C to 203 °C.

IDENTIFICATION

The identification test A. can be omitted if tests B. and C. are carried out.

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the trimethoprim CRS spectrum, prepared identically.

B. Transfer about 0.1 g of the sample to a 100 mL volumetric flask and add 25 mL of ethyl alcohol. Place in ultrasonic bath for 10 minutes and complete the volume with 0.1 M sodium hydroxide. Transfer 2 mL of this solution to a 100 mL volumetric flask and complete the volume with 0.1 M sodium hydroxide to obtain a 0.002% (w/v) solution. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 230 nm to 350 nm, of the 0.002% (w/v) solution, there is maximum point at 287 nm, identical to that observed in the spectrum of similar trimethoprim CRS solution. The maximum absorption should not differ by more than 3.0%.

C. The retention time of the main peak from the *Solution (1)* chromatogram, obtained in *Related substances*, corresponds to the one from the trimethoprim peak in *Solution (2)*.

PURITY TESTS

Related substances. Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 280 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane (5 µm), kept at room temperature; mobile phase flow rate of 1.3 mL/minute.

Perchlorate buffer pH 3.6: dissolve 1.405 g of sodium perchlorate in 950 mL of water, adjust pH to 3.6 with phosphoric acid and dilute to 1000 mL with water.

Mobile phase: mixture of *Perchlorate buffer pH 3.6* and methyl alcohol (7:3). Make adjustments, if necessary.

Solution (1): quantitatively transfer about 25 mg of the sample to a 25 mL volumetric flask with 15 mL of *Mobile Phase*. Place in ultrasonic bath for 10 minutes and complete the volume with the same solvent.

Solution (2): dissolve accurately weighed quantities of trimethoprim CRS and diaveridine in *Mobile phase* and dilute with the same solvent to obtain a solution containing, respectively, 10 µg/mL and 5 µg/mL of each substance.

Inject 20 µL replicates of the *Solution (2)*. The resolution between the diaveridine and trimethoprim CRS peaks is not less than 2.5. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of *Solution (1)*, record the chromatograms for not less than 11 times the main peak retention time and measure the areas under the peaks. Calculate the percentage of each impurity present in the sample according to the formula:

$$100 \left\{ Fr_i / \left[\sum (Fr_i) + Fr_t \right] \right\}$$

where

F = relative response factor, 0.53 for the peak with a relative retention time of 0.9 (4-amino-5-(3,4,5-trimethoxybenzyl)pyrimidin-2-ol); 0.43 for the peak with relative retention time of 2.3((2,4-diaminopyrimidin-5-yl)(3,4,5-trimethoxyphenyl)methanone); 0.66 for the peak with a relative retention time of 2.7 (3,4,5-trimethoxybenzoic acid); 0.5 for the peak with a relative retention time of 10.3 (3,4,5-methyl trimethoxybenzoate) and 1.0 for any other peaks, relative to trimethoprim;
 r_i = area under the peak corresponding to any individual impurity obtained for the *Test solution*;
 r_t = area under the trimethoprim peak obtained for the *Test solution*.

Not more than 0.1% of any individual impurity. The sum of the percentages of all impurities present is not more than 0.2%. Do not consider peaks related to the solvent.

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 105 °C for four hours or until constant weight. Not more than 0.5%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

ASSAY

Proceed as described in *Titrations in non-aqueous medium (5.3.3.5)*. Dissolve 0.3 g of the sample in 60 mL of glacial acetic acid. Titrate with 0.1 M perchloric acid VS. Determine the endpoint potentiometrically. Each mL of 0.1 M perchloric acid VS is equivalent to 29.032 mg of $C_{14}H_{18}N_4O_3$.

PACKAGING AND STORAGE

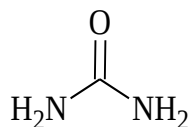
In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antibacterial.

UREA**Ureum**CH₄N₂O; 60.06

urea; 01711

Urea

[57-13-6]

It contains not less than 99.0% and not more than 100.5% of CH₄N₂O, in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. White, crystalline powder or clear, slightly hygroscopic crystals.

Solubility. Freely soluble in water, soluble in ethanol.

Physicochemical constants.

Melting range (5.2.2): 132 °C to 135 °C.

IDENTIFICATION

A. In the infrared absorption spectrum **(5.2.14)** of the sample previously desiccated, dispersed in potassium bromide, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the urea CRS spectrum, prepared identically.

B. Heat 0.5 g of the sample in a test tube. Liquefaction occurs with ammonia release. Continue heating until the liquid becomes turbid and cool. Dissolve the melted mass in 10 mL of water, add 1 mL of sodium hydroxide RS and a drop of cupric sulfate RS. Reddish-violet color develops.

C. Dissolve 0.1 g of the sample in 1 mL of water and add 1 mL of nitric acid RS. A white crystalline precipitate of urea nitrate is produced.

PURITY TESTS

Residue insoluble in ethanol. Dissolve 5 g of the sample in 50 mL of ethanol slightly heated. If any insoluble residue is observed, filter the solution in tared filter paper. Wash the residue and filter paper with 20 mL of slightly heated ethanol and dry in oven at 105 °C for one hour. Not more than 2 mg (0.04%).

Ammonia (5.3.2.6). Dissolve 10 g of the sample in 50 mL of water. Use 0.1 mL of the solution. Not more than 0.05% (500 ppm).

Chlorides (5.3.2.1). Determine in 5 g of the sample. Not more than 0.007% (70 ppm).

Sulfates (5.3.2.2). Determine in 2 g of the sample. Use 0.5 mL of 0.005 M sulfuric acid standard solution. Not more than 0.012% (120 ppm).

Heavy metals (5.3.2.3). Use *Method I*. Determine in 2 g of the sample. Not more than 0.001% (10 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample in oven at 105 °C for two hours. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.1%.

BIOLOGICAL SAFETY TEST

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Weigh, accurately, about 0.5 g of the sample, dissolve in water and dilute to 200 mL with the same solvent. Proceed as described in *Determination of nitrogen by the Kjeldahl method - semi-microdetermination (5.3.3.2.2)*, using 2 mL of the obtained solution. Each mL of 0.005 M sulfuric acid VS is equivalent to 0.303 mg of CH₄N₂O.

PACKAGING AND STORAGE

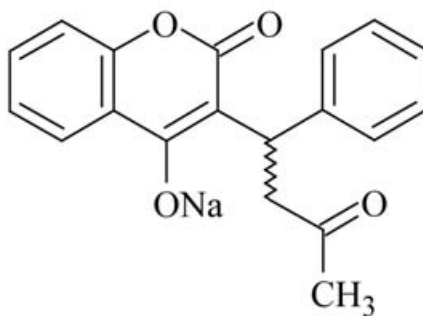
In tightly closed containers.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Keratolytic.

WARFARIN SODIUM*Warfarinum natricum*C₁₉H₁₅NaO₄; 330.31

warfarin sodium; 09101

4-Hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one sodium salt (1:1)

[129-06-6]

It contains not less than 97.0% and not more than 102.0% C₁₉H₁₅NaO₄, in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. White crystalline and hygroscopic powder. Presents polymorphism.

Solubility. Freely soluble in water and in ethyl alcohol.

Physicochemical constants.

Melting range (5.2.2): the precipitate obtained in test **A.** from *Identification* dried in oven at 105 °C, melts at 159 °C to 163 °C.

IDENTIFICATION

A. Dissolve about 1 g of the sample in 25 mL of water. Add 2 mL of hydrochloric acid and filter. Use the filtrate to carry out the test. In the infrared absorption spectrum (**5.2.14**) of the warfarin residue obtained in the filtrate, dispersed in mineral oil, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the warfarin CRS spectrum, prepared identically.

B. The main stain of the chromatogram from *Solution (2)* obtained in *Related substances* corresponds in position, color and intensity to the one obtained with *Solution (4)*.

C. The retention time of the main peak from the *Sample solution* chromatogram, obtained in method **B.** from *Assay*, corresponds to the one from the main peak of the *Standard solution*.

D. Dissolve about 1 g of sample in 10 mL of water. Add 5 mL of nitric acid and filter. Add 2 mL of potassium dichromate RS to the filtrate and shake for five minutes. Allow to stand for 20 minutes. The solution obtained does not present greenish-blue color when compared to the blank.

E. The filtrate obtained in test **A.** from *Identification* meets the sodium ion reactions **(5.3.1.1)**.

PURITY TESTS

Preparation aspect. Dissolve 1 g of the sample in 20 mL of water. The preparation is clear **(5.2.25)** and colorless **(5.2.12)**.

pH (5.2.19). 7.2 to 8.6. Determine in aqueous solution at 1% (w/v).

Related substances. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and mixture of glacial acetic acid, methylene chloride and cyclohexane (20:50:50) as mobile phase. Apply to the plate, separately, 20 µL of each of the freshly prepared solutions described below.

Solution (1): dissolve 0.20 g of the sample in acetone and dilute to 10 mL with the same solvent.

Solution (2): dilute 2 mL of *Solution (1)* in 10 mL of acetone.

Solution (3): dilute 1 mL of *Solution (2)* in 200 mL of acetone.

Solution (4): dissolve 40 mg of warfarin CRS in acetone and dilute to 10 mL with the same solvent.

Solution (5): transfer 10 mg of acenocoumarol CRS and 1 mL of *Solution (1)* to a 10 mL volumetric flask, dilute with acetone to complete the volume with the same solvent.

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). Any secondary stain obtained in the chromatogram with *Solution (1)*, except for the main stain, is not more intense than that obtained in the chromatogram with *Solution (3)* (0.1%). The test is only valid if, in the chromatogram obtained with *Solution (5)*, there are two clearly separated stains and if the stain in the chromatogram obtained with *Solution (3)* is clearly visible.

Phenolic ketones. Weigh, accurately, about 1.25 g of the sample, transfer to a 10 mL volumetric flask and dissolve with 0.5 M sodium hydroxide. Complete the volume with the same solvent and homogenize. Allow the solution to stand for 15 minutes. Measure the absorbance of the resulting solution at 385 nm, using 0.5 M sodium hydroxide for zero adjustment. The absorbance at 385 nm is not more than 0.20.

Heavy metals (5.3.2.3). Use *Method III*. Dissolve 4 g of the sample in 45 mL of water. Add 5 mL of glacial acetic acid and shake vigorously until the precipitate agglomerates. Filter and determine in 25 mL of the obtained solution, using glacial acetic acid to adjust the pH. Not more than 0.001% (10 ppm).

Water (5.2.20.1). Determine in 1 g of the sample. Not more than 4.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Use one of the methods described below.

A. Proceed as described in *Ultraviolet absorption spectrophotometry (5.2.14)*. Quantitatively transfer about 0.1 g of the sample to a 100 mL volumetric flask, dissolve and complete the volume with 0.01 M sodium hydroxide. Dilute to concentration of 0.001% (w/v). Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 308 nm, using 0.01 M sodium hydroxide for zero adjustment. Calculate the content of $C_{19}H_{15}NaO_4$ in the sample from the readings obtained.

B. Proceed as described in *High performance liquid chromatography (5.2.17.4)*, using chromatograph equipped with ultraviolet detector at 280 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group; *Mobile phase* flow rate of 1.0 mL/minute.

Buffer pH 7.4: dissolve 6.8 g of potassium phosphate monobasic in 250 mL of water. Add 160 mL of 0.2 M sodium hydroxide and complete the volume with water to 1000 mL. Adjust pH to 7.4 with sodium hydroxide or with phosphoric acid.

Mobile phase: mixture of methyl alcohol, water and glacial acetic acid (68:32:1).

Diluent: mixture of *Buffer pH 7.4* and acetonitrile (85:15).

Sample solution: dissolve an accurately weighed quantity of the sample in *Diluent* to obtain a 1 mg/mL solution. Dilute in *Mobile phase* to obtain a 100 µg/mL solution.

Standard solution: transfer 25 mg of warfarin CRS to a 25 mL volumetric flask, add 15 mL of *Buffer pH 7.4* and place in ultrasonic bath for five minutes to obtain a 1 mg/mL solution. Complete the volume with *Buffer pH 7.4*. Dilute in *Mobile phase* to obtain a 100 µg/mL solution.

Resolution solution: transfer 0.1 g of propylparaben CRS to a 100 mL volumetric flask, add 50 mL of acetonitrile and place in ultrasonic bath for five minutes. Complete the volume with acetonitrile. Homogenize. Transfer 2.5 mL of this solution to a 25 mL volumetric flask, add 2.5 mL of 1 mg/mL *Standard Solution* and complete the volume with *Mobile Phase*, obtaining a 100 µg/mL solution of propylparaben and warfarin, respectively.

Inject 20 µL replicates of the *Resolution solution*. The resolution between the propylparaben and warfarin peaks is not less than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of $C_{19}H_{15}NaO_4$ in the sample from the responses obtained with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In containers protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Anticoagulant.

WARFARIN SODIUM TABLETS

It contains not less than 92.5% and not more than 107.5% of the declared quantity of $C_{19}H_{15}NaO_4$.

IDENTIFICATION

A. Weigh and pulverize the tablets. Use quantity of powder equivalent to 200 mg of warfarin sodium, add 50 mL of water, centrifuge and filter the supernatant. Extract with 50 mL of ethyl ether and transfer the aqueous phase to a second separating funnel, discarding the organic phase. Adjust the aqueous phase pH to 2.5 with hydrochloric acid. Extract with 50 mL of chloroform and transfer the organic phase to a third separating funnel. Extract with 50 mL of 0.004% (w/v) sodium hydroxide solution, discarding the organic phase. Adjust the aqueous phase pH to 2.5 with hydrochloric acid. Filter and wash the precipitate with four 5 mL portions of water. If the precipitate is not white or off-white, dissolve in a minimum volume of 0.004% (w/v) sodium hydroxide solution, dilute to 50 mL with water and repeat the extraction process. Dry in desiccator, under reduced pressure, for four hours. The precipitate meets test **A**. from *Identification of the Warfarin sodium* monograph.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in *Assay*, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 30 minutes.

Procedure: after the test, remove aliquot from the dissolution medium and filter. Proceed as described in *Assay*. Calculate the quantity of $C_{19}H_{15}NaO_4$ dissolved in the medium, comparing the readings obtained with the warfarin CRS solution at the concentration of 0.0005% (w/v), prepared in *Mobile phase*.

Tolerance: not less than 80% (Q) of the stated quantity of $C_{19}H_{15}NaO_4$ dissolve in 30 minutes.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in method **B.** from *Assay* in the *Warfarin sodium* monograph. Prepare the *Sample solution* as described below.

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 25 mg of warfarin sodium to a 25 mL volumetric flask, add 15 mL of *Diluent* and place in ultrasonic bath for 20 minutes. Complete the volume with *Diluent*, homogenize and filter. Dilute in *Mobile phase* to obtain a 100 µg/mL solution.

Procedure: inject, separately, 20 µL of the *Sample solution* and the *Standard solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₉H₁₅NaO₄ in the tablets from the responses obtained for the *Standard solution* and the *Sample solution*.

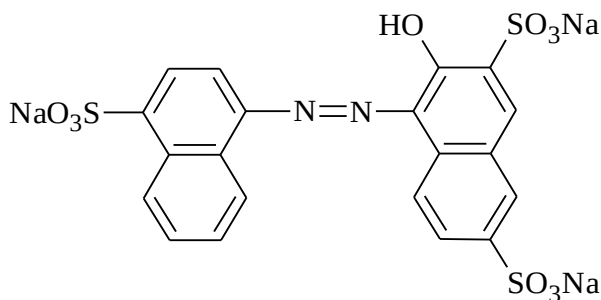
PACKAGING AND STORAGE

In tightly closed containers and protected from light.

LABELING

Comply with current legislation.

AMARANTH RED



$C_{20}H_{11}N_2Na_3O_{10}S_3$; 604.46

CI 16185

amaranth red; 11356

3-Hydroxy-4-[2-(4-sulfo-1-naphthalenyl)diazenyl]-2,7-naphthalenedisulfonic acid, sodium salt (3:1)
[915-67-3]

It contains not less than 85.0% of $C_{20}H_{11}N_2Na_3O_{10}S_3$ in relation to the desiccated substance.

DESCRIPTION

Physical characteristics. Fine, reddish-brown, hygroscopic powder. Burgundy colored aqueous solution.

Solubility. Soluble in water, methyl alcohol and glycerol, slightly soluble in ethyl alcohol, insoluble in ethyl ether and acetone.

IDENTIFICATION

In the visible, ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 700 nm, of 0.001% (w/v) solution in 0.02 M ammonium acetate (pH 5.6), there are maximum points at 519 nm, 330 nm and 217 nm and minimum points at 360 and 310 nm, identical to those observed in the spectrum of similar amaranth red CRS solution.

PURITY TESTS

Subsidiary coloring agents. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support and mixture of butyl alcohol, ethyl alcohol, water and ammonium hydroxide (50:25:25:10) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in water.

Solution (2): 10 mg/mL amaranth CRS solution in water.

Solution (3): dilute 1 mL of *Solution (2)* to 25 mL with water.

Develop chromatogram. Remove plate, allow to air dry. Examine under ambient light and ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. Any secondary stain obtained in the chromatogram with

Solution (1), different from the main stain, is not more intense than that obtained with *Solution (3)* (4.0%).

Lead, copper, tin, zinc. Proceed as described in *Atomic absorption spectrophotometry (5.2.13)*. Weigh 2 g of the sample in silica crucible and burn, slowly, over an asbestos mesh ($\pm 350\text{ }^{\circ}\text{C}$); take to muffle for 12 hours, without exceeding the temperature of $450\text{ }^{\circ}\text{C}$. Remove the crucible and cool. Mix the residue with approximately 2 mL of water and add two drops of 50% (w/v) magnesium nitrate. Dry over electric plate and return to muffle for three to four hours or until the residue is white or yellowish. Then, cool, drip 1 to 2 mL of nitric acid and 1 mL of water and heat over electric plate until it nearly dries. Dissolve the metallic nitrates with 5 mL of water. If necessary, centrifuge. Take to the atomic absorption spectrometer calibrated in advance and read the concentration of each one of the metals. Not more than 0.001% (10 ppm) of lead, 0.002% (20 ppm) of copper, 0.025% (250 ppm) of tin and 0.005% (50 ppm) of zinc.

Chlorides and sulfates. Weigh 0.5 g of the sample, dissolve in 200 mL of water, acidify with 8 mL of 25% (v/v) nitric acid and titrate with 0.1 M silver nitrate VS in potentiometer with combined silver electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 5.844 mg of NaCl.

Weigh 0.5 g of the sample and dissolve with 100 mL of water in water bath. Add 35 g of sulfate-free sodium chloride and shake thoroughly. Transfer to a 200 mL volumetric flask and complete the volume with saturated sodium chloride solution. Homogenize. After one hour, filter in filter paper and transfer aliquot of 100 mL of the filtrate to a 600 mL beaker, dilute to 300 mL with water and acidify with hydrochloric acid RS, adding slight excess. Heat until boiling and drip, with agitation, 25 mL of 12% (w/v) barium chloride or until there no longer is precipitation. Allow to stand for four hours. Separate the barium sulfate by filtration, wash with hot water, dry the paper with residue, transfer to a dry crucible, weighed in advance, and calcinate in muffle at $500\text{ }^{\circ}\text{C}$ for one hour. Cool in desiccator and weigh. Calculate the content of sulfates, according to the formula:

$$\frac{N \times 0,6085 \times 100}{p} = \% \text{sulfatos}$$

where

N = mass in grams of barium sulfate;

p = sample grams used in the precipitation.

Not more than 5.0% of chlorides and sulfates.

Water-insoluble substances. Dissolve 5 g of the sample in 200 mL of hot water ($80\text{ }^{\circ}\text{C}$ to $90\text{ }^{\circ}\text{C}$), with agitation. Cool to room temperature. Filter in filtering plate, dried and weighed in advance. Wash with cold water until the washing waters become colorless. Dry the filter with the residue in oven at $120\text{ }^{\circ}\text{C}$ for four hours and weigh. Not more than 0.5%.

Arsenic (5.3.2.5). Use *Method I*. Determine in 1 g of the sample. Not more than 0.0001% (1 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Determine in 0.5 g of the sample. Not more than 0.004% (40 ppm).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 120 °C for four hours or at 135 °C for three hours. Not more than 10.0%.

ASSAY

Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Prepare sample solution as described in *Identification*. Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 519 nm, using 0.02 M ammonium acetate (pH 5.6) for zero adjustment. Calculate the content of $C_{20}H_{11}N_2Na_3O_{10}S_3$ in the sample from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 564$, at 481 nm, in anhydrous base.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Dye.

AMARANTH RED ALUMINUM LAKE

Dye composed mainly of 3-hydroxy-4-[2-(4-sulfo-1-naphthalenyl)diazenyl]-2,7-naphthalenedisulfonic acid (3:1) sodium salt - amaranth red - on alumina substrate.
amaranth red aluminum lake; 11435
[12227-62-2]

It contains not less than 95% and not more than 105% of the dye content stated on the label.

DESCRIPTION

Physical characteristics. Fine red powder Hygroscopic.

Solubility. Practically insoluble in water and in ethyl alcohol. Soluble in *M* sodium hydroxide, but the dye decomposes slowly in alkaline pH.

IDENTIFICATION

A. In the visible, ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 700 nm, of 0.001% (w/v) sample solution in 0.02 *M* ammonium acetate (pH 5.6), dissolved in advance in *M* sodium hydroxide, there are maximum points at approximately 519 nm, 330 nm and 217 nm and minimum points at 360 nm and 310 nm, identical to those observed in the spectrum of amaranth red CRS solution.

B. Transfer 0.15 g of the sample to a 60 mL beaker and dissolve with about 20 mL of hot 30% (w/v) acetic acid, until it turns only opalescent. Cool and divide the solution into two test tubes. Add to one of them 2 mL of freshly-prepared morin solution at 3 mg/mL in ethyl alcohol. Observe the green fluorescence that develops under the ultraviolet light (254 nm), comparing with the tube with no reactive.

PURITY TESTS

Subsidiary coloring agents. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support and mixture of butyl alcohol, ethyl alcohol, water and concentrated ammonia solution (50:25:25:10) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described below.

Solution (1): 0.25 g of the sample in 10 mL of 0.5 *M* sodium hydroxide.

Solution (2): 0.05 g of amaranth CRS in 10 mL of 0.5 *M* sodium hydroxide.

Solution (3): dilute *Solution (2)* to obtain a 0.2 mg/mL solution, with the same diluent.

Solution (4): dilute *Solution (1)* to obtain a 1 mg/mL solution, with the same diluent.

Develop chromatogram. Remove plate, allow to air dry. Examine under ambient light and ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. The secondary stains obtained with *Solution (1)* must not be more intense than those obtained with *Solution (3)* and *Solution (4)* (4.0%).

Alternatively, a mixture of butyl alcohol, water, glacial acetic acid (20:12:5) can be employed as mobile phase. Chromatographic paper can be used instead of silica gel G, employing the conditions described previously and observing the stains also for transparency.

Chlorides and sulfates. Weigh 10 g of the sample, shake with 250 mL of water, leaving in contact for 30 minutes. Filter. Measure 50 mL of the filtrate, equivalent to 2 g of the sample, dilute to 200 mL with water, acidify with 8 mL of 25% (v/v) nitric acid and titrate with 0.1 M silver nitrate VS, using potentiometer with combined silver electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 5.844 mg of NaCl.

Measure another 50 mL of the filtrate, dilute to 300 mL with water, acidify with hydrochloric acid RS and another 1 mL of excess. Heat until boiling and drip, with agitation, 25 mL of 12% (w/v) barium chloride. Allow to stand for four hours. Separate the barium sulfate by filtration, wash with hot water, dry the paper with residue, transfer to a dry crucible, weighed in advance, and calcinate in muffle at 500 °C for one hour. Cool in desiccator and weigh. Calculate the content of sulfates, according to the formula:

$$\frac{N \times 0,6085 \times 100}{p} = \% \text{sulfatos}$$

where

N = mass in grams of barium sulfate;

p = sample grams used in the precipitation.

Not more than 2.0% of chlorides and sulfates.

Loss on drying (5.2.9.1). Weigh approximately 0.5 g. Dry the sample at 120 °C for four hours or at 135 °C for three hours. Not more than 20.0%

Residue by incineration (5.2.10). Weigh about 0.1 g of the sample in a crucible dried and weighed in advance and incinerate at 800 °C for two hours. It must contain between 40.0% and 55.0%.

ASSAY

Make the dilutions as described in method **A**. from *Identification* and read the absorbance at the absorption maximum at about 519 nm (**5.2.14**). Calculate the dye content, according to the formula:

$$\frac{A \times 100}{436 \times p} = \% \text{ de vermelho amaranato na amostra em 519 nm}$$

where

p = sample weight in grams in the dilution carried out.

Alternatively, it is possible to consider $A(1\%, 1\text{ cm}) = 436$ at 519 nm.

PACKAGING AND STORAGE

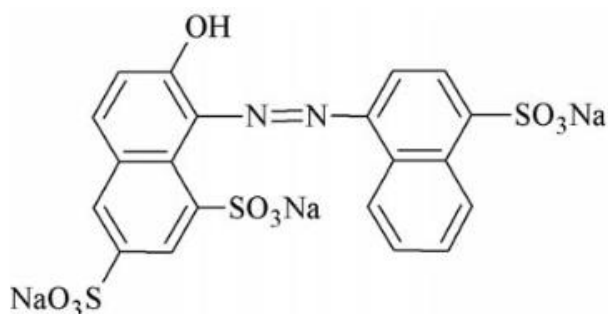
In perfectly closed containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Dye.

PONCEAU RED

$C_{20}H_{11}N_2Na_3O_{10}S_3$; 604.46

CI 16255; E 124

ponceau red; 11248

7-Hydroxy-8-[2-(4-sulfo-1-naphthalenyl)diazenyl]-1,3-naphthalenedisulfonic acid sodium salt (3:1)
[2611-82-7]

It contains not less than 82.0% of $C_{20}H_{11}N_2Na_3O_{10}S_3$ in relation to the anhydrous substance.

DESCRIPTION

Physical characteristics. Fine, red, hygroscopic powder. The aqueous solution is red.

Solubility. Soluble in water and methyl alcohol, insoluble in ethyl alcohol, acetone, ethyl ether and glycerol.

IDENTIFICATION

In the visible, ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 700 nm, of 0.001% (w/v) solution in 0.02 M ammonium acetate (pH 5.6), there are maximum points at 507 nm, 332 nm, 245 nm and 215 nm and minimum points at 375 nm, 300 nm and 238 nm, identical to those observed in the spectrum of similar ponceau red CRS solution.

PURITY TESTS

Subsidiary coloring agents. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support and mixture of butyl alcohol, ethyl alcohol, water and ammonium hydroxide (50:25:25:10) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described below.

Solution (1): 10 mg/mL sample solution in water.

Solution (2): 10 mg/mL ponceau red CRS solution in water.

Solution (3): dilute 1 mL of *Solution (2)* to 50 mL with water.

Develop chromatogram. Remove plate and allow to air dry. Examine under ambient light and ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. Any secondary stain obtained in the

chromatogram with *Solution (1)*, different from the main stain, is not more intense than that obtained with *Solution (3)* (2.0%).

Lead, copper, tin, zinc. Proceed as described in *Atomic absorption spectrophotometry (5.2.13)*. Weigh a silica crucible, add 2 g of the sample and burn, slowly, over an asbestos mesh ($\pm 350\text{ }^{\circ}\text{C}$); take to the muffle for 12 hours, without exceeding the temperature of $450\text{ }^{\circ}\text{C}$. Remove the crucible and cool. Mix the residue with approximately 2 mL of water and add two drops of 50% (w/v) magnesium nitrate. Dry over electric plate and return to muffle for three to four hours or until the residue is white or yellowish. Then, cool, drip 1 to 2 mL of nitric acid and 1 mL of water and heat over electric plate until it nearly dries. Dissolve the metallic nitrates with 5 mL of water. If necessary, centrifuge. Take to the atomic absorption spectrometer calibrated in advance and read the concentration of each one of the metals. Not more than 0.001% (10 ppm) of lead, 0.002% (20 ppm) of copper, 0.025% (250 ppm) of tin and 0.005% (50 ppm) of zinc.

Chlorides and sulfates. Weigh 0.5 g of the sample, dissolve in 200 mL of water, acidify with 8 mL of 25% (v/v) nitric acid and titrate with 0.1 M silver nitrate VS in potentiometer with combined silver electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 5.844 mg of NaCl.

Weigh 0.5 g of the sample and dissolve with 100 mL of water in water bath. Add 35 g of sulfate-free sodium chloride and shake thoroughly. Transfer to a 200 mL volumetric flask and complete the volume with saturated sodium chloride solution. Homogenize. After one hour, filter in filter paper and transfer aliquot of 100 mL of the filtrate to a 600 mL beaker, dilute to 300 mL with water and acidify with hydrochloric acid RS, adding a slight excess. Heat until boiling and drip, with agitation, 25 mL of 12% (w/v) barium chloride, or until there no longer is precipitation. Allow to stand for four hours. Separate the barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to a dry crucible, weighed in advance, and calcinate in muffle at $500\text{ }^{\circ}\text{C}$ for one hour. Cool in desiccator and weigh. Calculate the content of sulfates, according to the formula:

$$\frac{N \times 0,6086 \times 100}{p} = \% \text{sulfatos}$$

where

N = barium sulfate grams;

p = sample grams used in the precipitation.

Not more than 8.0% of chlorides and sulfates.

Water-insoluble substances. Dissolve 5 g of the sample in 200 mL of hot water ($80\text{ }^{\circ}\text{C}$ to $90\text{ }^{\circ}\text{C}$), with agitation. Cool to room temperature. Filter in filtering plate, dried and weighed in advance. Wash with cold water until the washing waters become colorless. Dry the filter with the residue in oven at $120\text{ }^{\circ}\text{C}$ for four hours and weigh. Not more than 0.2%.

Arsenic (5.3.2.5). Use *Method I*. Determine in 3 g of the sample. Not more than 0.0001% (1 ppm).

Heavy metals (5.3.2.3). Use *Method III*. Determine in 0.5 g of the sample. Not more than 0.004% (40 ppm).

Loss on drying (5.2.9.1). Determine in 0.5 g of the sample. Dry in oven at 120 °C for four hours or at 135 °C for three hours. Not more than 10.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Visible absorption spectrophotometry (5.2.14)*. Prepare sample solution as described in *Identification*. Prepare standard solution in the same concentration, using the same solvent. Measure the absorbances of the resulting solutions at 507 nm, using 0.02 M ammonium acetate (pH 5.6) for zero adjustment. Calculate the content of $C_{20}H_{11}N_2Na_3O_{10}S_3$ in the sample from the readings obtained. Alternatively, calculate considering $A(1\%, 1\text{ cm}) = 442.5$, at 507 nm, in 0.02 M ammonium acetate (pH 5.6).

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Dye.

PONCEAU RED ALUMINUM LAKE

Dye composed mainly of 7-hydroxy-8-[2-(4-sulfo-1-naphthalenyl)diazenyl]-1,3-naphthalenedisulfonic acid (3:1) sodium salt - ponceau red - on alumina substrate.

It contains not less than 95% and not more than 105% of the quantity declared on the label.

DESCRIPTION

Physical characteristics. Fine red powder. Hygroscopic.

Solubility. Practically insoluble in water and in ethyl alcohol. Soluble in *M* sodium hydroxide, but the dye decomposes slowly in alkaline pH.

IDENTIFICATION

A. In the visible, ultraviolet absorption spectrum (**5.2.14**), in the range of 200 nm to 700 nm, of 0.001% (w/v) solution in 0.02 *M* ammonium acetate (pH 5.6), there are maximum points at 507 nm, 332 nm, 245 nm and 215 nm and minimum points at 375 nm, 300 nm and 238 nm, identical to those observed in the spectrum of similar ponceau red CRS solution.

B. Transfer 0.15 g of the sample to a 60 mL beaker and dissolve with about 20 mL of hot 30% (w/v) acetic acid, until it turns only opalescent. Cool and divide the solution into two test tubes. Add to one of them 2 mL of freshly-prepared morin solution at 3 mg/mL in ethyl alcohol. Observe the green fluorescence that develops under the ultraviolet light (254 nm), comparing with the tube with no reactive.

PURITY TESTS

Subsidiary coloring agents. Proceed as described in *Thin layer chromatography* (**5.2.17.1**), using silica gel G as support and mixture of butyl alcohol, ethyl alcohol, water and ammonium hydroxide (50:25:25:10) as mobile phase. Apply to the plate, separately, 2 µL of each of the freshly prepared solutions described below.

Solution (1): 0.25 g of the sample in 10 mL of 0.5 *M* sodium hydroxide.

Solution (2): 0.05 g of ponceau red CRS in 10 mL of 0.5 *M* sodium hydroxide.

Solution (3): dilute *Solution (2)* to obtain a 1 mg/mL solution, with the same diluent.

Solution (4): dilute *Solution (1)* to obtain a 0.5 mg/mL solution, with the same diluent.

Develop chromatogram. Remove plate and allow to air dry. Examine under ambient light and ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position, color and intensity to the one obtained with *Solution (2)*. The secondary stains obtained with *Solution (1)* must not be more intense than those obtained with *Solution (3)* and *Solution (4)* (2.0%).

Chlorides and sulfates. Weigh 10 g of the sample, shake with 250 mL of water, leaving in contact for 30 minutes. Filter. Measure 50 mL of the filtrate, equivalent to 2 g of the sample, dilute to 200 mL with water, acidify with 8 mL of 25% (v/v) nitric acid and titrate with 0.1 *M* silver nitrate VS, using

potentiometer with combined silver electrode. Each mL of 0.1 M silver nitrate VS is equivalent to 5.844 mg of NaCl.

Measure another 50 mL of the filtrate, dilute to 300 mL with water, acidify with hydrochloric acid RS and another 1 mL of excess. Heat until boiling and drip, with agitation, 25 mL of 12% (w/v) barium chloride. Allow to stand for four hours. Separate the barium sulfate by filtration, wash with hot water, dry the paper with the residue, transfer to a dry crucible, weighed in advance, and calcinate in muffle at 500 °C for one hour. Cool in desiccator and weigh. Calculate the content of sulfates, according to the formula:

$$\frac{N \times 0,6086 \times 100}{p} = \% \text{sulfatos}$$

where

N = barium sulfate grams;

p = sample grams used in the precipitation.

Not more than 2.0% of chlorides and sulfates.

Loss on drying (5.2.9.1). Weigh approximately 0.5 g. Dry the sample at 120 °C for four hours or at 135 °C for three hours. Not more than 20.0%

Residue by incineration (5.2.10). Determine in 0.1 g of the sample and calcine at (800 ± 25) °C. Between 40.0% and 55.0%.

BIOLOGICAL SAFETY TESTS

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *Visible absorption spectrophotometry* (5.2.14). Make the dilutions as described in method **A**. from *Identification* and read the absorbance at the absorption maximum at about 507 nm. Calculate the dye content, according to the formula:

$$\frac{A \times 100}{442,5 \times p} = \% \text{ de vermelho de ponceau na amostra em 507 nm}$$

where

p = sample weight in grams in the dilution carried out.

PACKAGING AND STORAGE

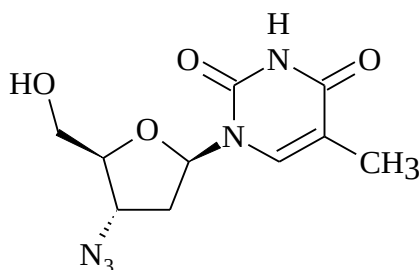
In perfectly closed containers, protected from light.

LABELING

Comply with current legislation.

CATEGORY

Dye.

ZIDOVUDINE***Zidovudinum***C₁₀H₁₃N₅O₄; 267.25

zidovudine; 09256

3'-Azido-3'-deoxythymidine

[30516-87-1]

It contains not less than 97.0% and not more than 102.0% of C₁₀H₁₃N₅O₄, in relation to the desiccated substance.

DESCRIPTION

Physicochemical characteristics. White to yellowish-white crystalline powder. Presents polymorphism. Melting temperature (5.2.2): around 124 °C.

Solubility. Moderately soluble in water, soluble in ethyl alcohol.

Physicochemical constants.

Specific optical rotation (5.2.8): +60,5 to +63,0, at 25 °C, in relation to the desiccated substance. Determine in solution at 1% (w/v) in ethyl alcohol.

IDENTIFICATION

In the infrared absorption spectrum (5.2.14) of the sample, dispersed in potassium chloride, there are maximum absorption points only on the same wavelengths and with the same relative intensities as the ones observed in the zidovudine CRS spectrum, prepared identically.

PURITY TESTS

Solution aspect. Dissolve 0.5 g in 50 mL of water, heating if necessary. The solution is not more stained than the mixture of 1 mL of *Standard color solution SC G (5.2.12)* with 7 mL of water.

Related compounds 1. Proceed as described in *Thin layer chromatography (5.2.17.1)*, using silica gel GF₂₅₄ as support and mixture of methyl alcohol and methylene chloride (10:90) as mobile phase. Apply to the plate, separately, 10 µL of each one of the freshly prepared solutions described below.

Solution (1): dissolve 0.2 mg of sample in methyl alcohol and dilute to 10 mL with the same solvent.

Solution (2): dissolve in methyl alcohol 20 mg of thymine CRS, 20 mg of impurity A (1-[(2*R*,5*S*)-5-hydroxymethyl-2,5-dihydro-2-furyl]-5-methylpyrimidine-2,4(1*H*, 3*H*)-dione), 20 mg of triphenylmethanol, add 1 mL of *Solution (1)* and dilute to 100 mL with methyl alcohol.

Solution (3): dilute 5 mL of *Solution (2)* to 10 mL with methyl alcohol.

Develop chromatogram. Allow the solvent to rise up to 12 cm above the application line. Remove the plate, allow to air dry for five minutes and observe under ultraviolet light (254 nm). In the chromatogram obtained with *Solution (1)*, the stain corresponding to impurity A is not more intense than the corresponding stain obtained in the chromatogram with *Solution (3)* (0.5%) and any stain, except for the main and the ones corresponding to zidovudine and thymine impurities, are not more intense than the stain corresponding to zidovudine in the chromatogram obtained with *Solution (3)* (0.5%). Nebulize the plate with a 1% (w/v) vanillin solution in sulfuric acid. In the chromatogram obtained with *Solution (1)*, any stain corresponding to triphenylmethanol is not more intense than the corresponding stain in the chromatogram obtained with *Solution (3)* (0.5%). The test is valid if the chromatogram obtained with *Solution (2)* presents four clearly separate stains, corresponding to thymine, impurity A, zidovudine and triphenylmethanol, in ascending order of retention factor (*R_f*).

Related compounds 2. Proceed as described in Assay. Prepare solutions as described below.

Solution (1): dissolve an accurately weighed quantity of the sample in *Mobile phase* to obtain a 1 mg/mL solution.

Solution (2): transfer 0.25 mL of *Test Solution* to a 50 mL volumetric flask and complete the volume with *Mobile phase*.

Solution (3): dissolve an accurately weighed quantity of thymine CRS in methyl alcohol to obtain a 0.2 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 20 µg/mL solution.

Solution (4): dissolve accurately weighed quantity of impurity B (1-(3-chloro-2,3-dideoxy-β-D-ribofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione) in *Mobile phase* to obtain a 0.1 mg/mL solution. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*.

Inject 10 µL replicates of the *Resolution solution* described in Assay. The tailing factor is not more than 1.5 for the zidovudine peak and for the impurity B peak. The resolution between zidovudine and impurity B is not less than 1.4. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: separately inject 10 µL of each one of the solutions described above. Record the chromatograms for 1.5 times the retention time of the main peak obtained with *Solution (1)*. The substances are eluted in the following sequence: thymine, zidovudine and impurity B. In the chromatogram obtained with *Solution (1)*, the area under any peak corresponding to thymine is not greater than the area under the peak in the chromatogram obtained with *Solution (3)* (2.0%). The area under the peak corresponding to impurity B in the chromatogram of *Solution (1)* is not greater than the area under the corresponding peak in the chromatogram obtained with *Solution (4)* (1.0%). The area under any other peak obtained with *Solution (1)*, except for that under the main peak, is not greater than the area under the chromatogram peak obtained with *Solution (2)* (0.5%). The sum

of the areas under all peaks, except for that under the main peak, obtained with *Solution (1)*, is not greater than six times the area under the peak obtained with *Solution (2)* (3.0%). Disregard any peaks with an area less than 10% of the area under the peak obtained in the *Solution (2)* chromatogram.

Heavy metals (5.3.2.3). Determine in 1 g of the sample. Transfer to a silica crucible and mix with 0.5 g of magnesium oxide. Incinerate until color turns dark red on flame. Continue until obtaining a homogeneous white or gray mass. If after 30 minutes of ignition the color remains, cool, mix the mass in the crucible with a glass rod, then incinerate again. Incinerate in muffle at 500-600 °C for approximately one hour. The residue obtained is dissolved with two 5 mL portions of 0.2 M hydrochloric acid and add 0.1 mL of phenolphthalein TS. Add 6 M ammonium hydroxide until pink color develops. Cool. Discolor the solution with glacial acetic acid and add another 0.5 mL of the acid. If necessary, filter and dilute the solution with water to a 20 mL volume. Transfer 12 mL of the solution obtained to a Nessler tube, add 2 mL of acetate buffer pH 3.5 and homogenize immediately.

Standard preparation: mix 2 mL of *Standard lead solution (10 ppm Pb)* with 0.5 g of magnesium oxide in a silica crucible. Then dry the mixture in oven at 105 °C, incinerating immediately afterwards. Proceed with the sample preparation technique from “The residue thus obtained is dissolved...”. To 10 mL of the obtained solution, add 2 mL of the sample solution.

Procedure: in each of the tubes related to the sample and the standard, add 1.2 mL of thioacetamide RS. The brown color that develops in the sample should not be more intense than that obtained with the standard. Not more than 0.002% (20 ppm).

Loss on drying (5.2.9.1). Determine in 1 g of the sample. Dry in oven at 100°C to 105 °C until constant weight. Not more than 1.0%.

Residue by incineration (5.2.10). Determine in 1 g of the sample. Not more than 0.25%.

BIOLOGICAL SAFETY TEST

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 265 nm; column with 250 mm of length and 4.6 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 µm); *Mobile phase* flow rate of 1.2 mL/minute.

Mobile phase: mixture of methyl alcohol and water (20:80).

Sample solution: dissolve accurately weighed quantity of the sample in *Mobile phase* to obtain a 1 mg/mL solution. Transfer 10 mL of this solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 0.2 mg/mL solution.

Standard solution: dissolve accurately weighed quantity of zidovudine CRS in *Mobile phase* to obtain a 0.2 mg/mL solution.

Resolution solution: transfer 5 mg of impurity B (1-(3-chloro-2,3-dideoxy- β -D-ribofuranosyl)-5-methylpyrimidine-2,4(1H,3H)-dione) to a 50 mL volumetric flask, add 25 mL of *Standard Solution* and complete the volume with *Mobile Phase*.

Inject 10 μ L replicates of the *Resolution solution*. The tailing factor is not more than 1.5 for the zidovudine peak and for the impurity B peak. The resolution between zidovudine and impurity B is not less than 1.4. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 μ L of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the content of C₁₀H₁₃N₅O₄ in the sample from the responses obtained with the *Standard solution* and *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

THERAPEUTIC CLASS

Antirretroviral.

ZIDOVUDINE CAPSULES

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_{10}H_{13}N_5O_4$.

IDENTIFICATION

A. Weigh the capsules, remove the content and weigh again. Homogenize the content of the capsules. Transfer quantity of powder equivalent to 0.3 g of zidovudine to a 200 mL volumetric flask. Add 50 mL of mixture of methyl alcohol and water (75:25), place in ultrasonic bath for five minutes and complete the volume with methyl alcohol. Allow to decant and dilute the supernatant with a mixture of methyl alcohol and water (75:25) to a concentration of 0.0015% (w/v). In the ultraviolet absorption spectrum (5.2.14) of the resulting solution, in the range of 200 nm to 400 nm, there are maximum and minimum points only at the same wavelengths of similar zidovudine CRS solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

Procedure for content uniformity. Proceed as described in Assay. Prepare *Sample solution* as described below.

Sample solution: weigh each capsule individually, transfer the content to a 100 mL volumetric flask and weigh again. Add 30 mL of mixture of methyl alcohol and water (75:25). Place in ultrasonic bath for 20 minutes and complete the volume with the same solvent. Filter. Dilute, with the same solvent, to obtain a 0.1 mg/mL concentration.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 45 minutes.

Procedure: after the test, remove aliquot from the dissolution medium, filter and dilute, if necessary, in mixture of methyl alcohol and water (75:25) until adequate concentration and proceed as described in Assay. Calculate the quantity of $C_{10}H_{13}N_5O_4$ dissolved in the medium, from the responses obtained with the *Standard solution* and the *Sample solution*.

Tolerance: not less than 75% (Q) of the declared quantity of $C_{10}H_{13}N_5O_4$ dissolve in 45 minutes.

PURITY TESTS

Thymine limit. Proceed as described in Assay. Prepare *Solutions (1)* and *(2)* as described below.

Solution (1): use the *Sample solution* obtained in Assay.

Solution (2): use the *Standard solution* obtained in Assay.

Procedure: inject, separately, 10 µL of *Solutions (1) and (2)*, record the chromatograms and measure the areas under the peaks. Calculate the quantity, in milligrams, of thymine in the sample from the formula:

$$1000 \times C \times \left[\frac{r_1/r_2}{T} \right]$$

where

C = concentration, in mg/mL, of thymine in *Solution (2)*;

r_1 and r_2 = areas under the peaks related to thymine, obtained in *Solutions (1) and (2)*, respectively;

T = quantity of zidovudine, in milligrams, in the mass of powder used to prepare *Solution (1)*, as determined in Assay.

Not more than 3.0.

BIOLOGICAL SAFETY TEST

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in Assay in the *Zidovudine* monograph. Prepare the *Sample solution* and the *Standard solution* as described below.

Sample solution: weigh 20 capsules, remove the content and weigh again. Homogenize the content of the capsules. Transfer quantity of powder equivalent to 100 mg of zidovudine to a 100 mL volumetric flask and add 30 mL of mixture of methyl alcohol and water (75:25). Place in ultrasonic bath for 20 minutes and complete the volume with the same solvent. Filter. Transfer 10 mL of the filtrate to a 100 mL volumetric flask and complete the volume with the same solvent.

Standard solution: dissolve 10 mg of thymine in methyl alcohol and quantitatively transfer to a 50 mL volumetric flask and complete the volume with the same solvent. Transfer 1 mL of this solution and 10 mg of zidovudine CRS to a 100 mL volumetric flask, dissolve in 25 mL of mixture of methyl alcohol and water (75:25) and complete the volume with the same solvent. Homogenize.

Inject 10 µL replicates of the *Standard solution*. The relative retention times are approximately 0.2 for thymine and 1.0 for zidovudine. The resolution between the zidovudine and thymine peaks is not less than 5.0. The tailing factor for the zidovudine peak is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of C₁₀H₁₃N₅O₄ in the capsules from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

ZIDOVUDINE INJECTION SOLUTION

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_{10}H_{13}N_5O_4$.

IDENTIFICATION

A. Transfer volume of the injection solution equivalent to 20 mg of zidovudine to a 200 mL volumetric flask and complete the volume with mixture of methyl alcohol and water (75:25). Transfer 15 mL of this solution to a 100 mL volumetric flask and complete the volume with the same solvent. Homogenize. In the ultraviolet absorption spectrum **(5.2.14)**, in the range of 200 nm to 400 nm of the resulting solution, there are maximum and minimum points at the same wavelengths of similar zidovudine CRS solution.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak of the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.0 to 4.0. Determine in mixture of the injectable solution volume, containing 0.15 g of zidovudine and 5 mL of 0.12 M potassium chloride.

BIOLOGICAL SAFETY TESTS

Sterility (5.5.3.2.1). Complies with the test.

Bacterial endotoxins (5.5.2.2). Not more than 1.0 EU/mg of zidovudine.

PURITY TESTS

Thymine limit. Proceed as described in Assay. Prepare *Solutions (1)* and *(2)* as described below.

Solution (1): use the *Sample solution* obtained in Assay.

Solution (2): use the *Standard solution* obtained in Assay.

Procedure: inject, separately, 10 μ L of *Solutions (1)* and *(2)*, record the chromatograms and measure the areas under the peaks. Calculate the quantity, in milligrams, of thymine in the sample from the formula:

$$1000 \times C \left[\frac{r_1/r_2}{Q} \right]$$

where

C = concentration, in mg/mL, of thymine in *Solution (2)*;

r_1 and r_2 = areas under the peaks related to thymine, obtained in *Solutions (1)* and *(2)*, respectively;

Q = quantity of zidovudine, in milligrams, in the injectable solution volume used to prepare *Solution (1)*.

Not more than 1.0%.

ASSAY

Proceed as described in Assay in the *Zidovudine* monograph. Prepare the *Sample solution* and the *Standard solution* as described below.

Sample solution: quantitatively transfer volume of the injection solution equivalent to 25 mg of zidovudine to a 250 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Standard solution: dissolve 10 mg of thymine CRS in methyl alcohol and dilute to 50 mL with the same solvent. Transfer 1 mL of this solution and 10 mg of zidovudine CRS to a 100 mL volumetric flask, dissolve in 25 mL of mixture of methyl alcohol and water (75:25) and complete the volume with the same solvent. Homogenize.

Inject 10 µL replicates of the *Standard solution*. The relative retention times are approximately 0.2 for thymine and 1.0 for zidovudine. The resolution between the zidovudine and thymine peaks is not less than 5.0. The tailing factor for the zidovudine peak is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of $C_{10}H_{13}N_5O_4$ in the injection solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers, protected from light.

LABELING

Comply with current legislation.

ZIDOVUDINE ORAL SOLUTION

It contains not less than 90.0% and not more than 110.0% of the declared quantity of $C_{10}H_{13}N_5O_4$.

IDENTIFICATION

A. Proceed as described in *Thin-layer chromatography* (5.2.17.1). Use silica gel GF₂₅₄ as support and mixture of butyl acid, n-heptane, acetone and ammonium hydroxide (40:30:30:10) as *Mobile phase*. Apply to the plate, separately and in the form of a band, 5 µL of each of the freshly prepared solutions described below.

Solution (1): 5 mg/mL sample solution in mixture of methyl alcohol and water (75:25).

Solution (2): 5 mg/mL zidovudine CRS solution in mixture of methyl alcohol and water (75:25).

Develop chromatogram. Remove plate and allow to air dry. Examine under ultraviolet light (254 nm). The main stain obtained with *Solution (1)* corresponds in position and intensity to the one obtained with *Solution (2)*.

B. The retention time of the main peak from the *Sample solution* chromatogram, obtained in the Assay, corresponds to the one from the main peak obtained with the *Standard solution*.

CHARACTERISTICS

Determination of volume (5.1.2). Complies with the test.

pH (5.2.19). 3.0 to 4.0. Determine in volume of the oral solution, containing 0.15 g of zidovudine and 5 mL of 0.12 M potassium chloride.

PURITY TESTS

Thymine limit. Proceed as described in Assay. Prepare *Solutions (1)* and *(2)* as described below.

Solution (1): use the *Sample solution* obtained in Assay.

Solution (2): use the *Standard thymine solution* obtained in Assay.

Procedure: inject, separately, 10 µL of *Solutions (1)* and *(2)*, record the chromatograms and measure the areas under the peaks. The area under the thymine peak with *Solution (1)* is not more than 3.0% of the area under the thymine peak obtained with *Solution (2)*.

BIOLOGICAL SAFETY TEST

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography* (5.2.17.4). Use chromatograph equipped with ultraviolet detector at 240 nm; column with 125 mm of length and 4.6 mm of internal

diameter, packed with silica chemically bonded to octadecylsilane group (5 µm), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of 0.04 M sodium acetate, methyl alcohol, acetonitrile and glacial acetic acid (900:90:10:2).

Sample solution: transfer volume of zidovudine oral solution equivalent to 0.1 g of zidovudine to a 100 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize. Transfer 5 mL of this solution to a 50 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Standard stock solution: 1 mg/mL zidovudine CRS solution in *Mobile phase*.

Standard thymine solution: quantitatively transfer about 20 mg of thymine to a 200 mL volumetric flask. Add 150 mL of *Mobile phase* and place in ultrasonic bath for 10 minutes. Complete the volume with *Mobile phase*. Homogenize.

Standard solution: transfer 10 mL of the *Standard stock solution* and 2 mL of *Standard thymine solution* to a 100 mL volumetric flask and complete the volume with *Mobile phase*. Homogenize.

Inject 10 µL replicates of the *Standard solution*. The relative retention times are approximately 0.12 for thymine and 1.0 for zidovudine. The resolution between the zidovudine and thymine peaks is not less than 4.0. The tailing factor for the zidovudine peak is not more than 2.0. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 10 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of zidovudine($C_{10}H_{13}N_5O_4$) in the oral solution from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.

ZIDOVUDINE AND LAMIVUDINE TABLETS

It contains not less than 90.0% and not more than 110.0% of the declared quantity of zidovudine ($C_{10}H_{13}N_5O_4$) and lamivudine ($C_8H_{11}N_3O_3S$).

IDENTIFICATION

The retention times of the main peaks of the *Sample solution* chromatogram, obtained in the Assay, correspond to the ones from the main peaks of the *Standard solution*.

CHARACTERISTICS

Determination of weight (5.1.1). Complies with the test.

Hardness test (5.1.3.1). Complies with the test.

Friability test (5.1.3.2). Complies with the test.

Disintegration test (5.1.4.1). Complies with the test.

Uniformity of unit doses (5.1.6). Complies with the test.

DISSOLUTION TEST (5.1.5)

Dissolution medium: water, 900 mL.

Apparatus: paddles, 50 rpm.

Time: 60 minutes.

Procedure: after the test, remove aliquot from the dissolution medium and dilute, if necessary, in *Mobile phase*, until adequate concentration. Proceed as described in Assay.

Tolerance: not less than 80% (Q) of the stated quantity of zidovudine ($C_{10}H_{13}N_5O_4$) and of lamivudine ($C_8H_{11}N_3O_3S$) dissolve in 60 minutes.

BIOLOGICAL SAFETY TEST

Mesophilic microorganisms total count (5.5.3.1.2). Complies with the test.

Study of pathogenic microorganisms (5.5.3.1.3). Complies with the test.

ASSAY

Proceed as described in *High performance liquid chromatography (5.2.17.4)*. Use chromatograph equipped with ultraviolet detector at 270 nm; column with 125 mm of length and 4 mm of internal diameter, packed with silica chemically bonded to octadecylsilane group (5 μ m), kept at room temperature; *Mobile phase* flow rate of 1 mL/minute.

Mobile phase: mixture of 0.1 M ammonium acetate buffer, methyl alcohol and glacial acetic acid (65:35:0.1).

Sample solution: weigh and pulverize 20 tablets. Transfer quantity of powder equivalent to 75 mg of zidovudine and 37.5 mg of lamivudine to a 100 mL volumetric flask. Add 70 mL of water and place

in ultrasonic bath for 30 minutes. Complete the volume with the same solvent, homogenize and filter. Transfer 1 mL of the filtrate to a 25 mL volumetric flask and complete the volume with *Mobile phase*, obtaining a 30 µg/mL zidovudine solution and a 15 µg/mL lamivudine solution.

Standard stock solution: weigh, accurately, about 75 mg of zidovudine CRS and 37.5 mg of lamivudine CRS. Transfer to a 100 mL volumetric flask, with the aid of 70 mL of water. Place in ultrasonic bath for 15 minutes and complete the volume with the same solvent, obtaining a 0.75 mg/mL zidovudine solution and a 0.375 mg/mL lamivudine solution.

Standard solution: transfer 1 mL of the *Standard stock solution* to a 25 mL volumetric flask and complete with *Mobile phase*, obtaining a 30 µg/mL zidovudine solution and a 15 µg/mL lamivudine solution.

Inject 20 µL replicates of the *Standard solution*. The relative retention times are approximately 1.0 for lamivudine and 1.8 for zidovudine. The relative standard deviation of the replicate areas under the peaks recorded is not more than 2.0%.

Procedure: inject, separately, 20 µL of the *Standard solution* and the *Sample solution*, record the chromatograms and measure the areas under the peaks. Calculate the quantity of zidovudine and of lamivudine in the tablets from the responses obtained with the *Standard solution* and the *Sample solution*.

PACKAGING AND STORAGE

In tightly closed containers.

LABELING

Comply with current legislation.