

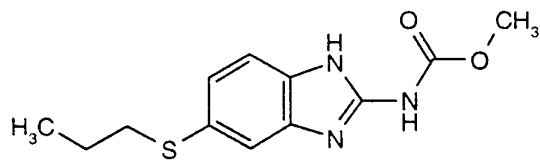
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Monographs for  
pharmaceutical substances



## Albendazolum

### Albendazole



$C_{12}H_{15}N_3O_2S$

**Relative molecular mass.** 265.3

**Chemical name.** Methyl 5-(propylthio)-2-benzimidazolecarbamate; CAS Reg. No. 54965-21-8.

**Description.** A white or almost white powder.

**Solubility.** Practically insoluble in water; soluble in glacial acetic acid R; slightly soluble in acetone R; very slightly soluble in ethanol (~750 g/l) TS.

**Category.** Anthelmintic.

**Storage.** Albendazole should be kept in a well-closed container, protected from light.

**Additional information.** Melting temperature, about 210°C, with decomposition.

### Requirements

Albendazole contains not less than **98.0%** and not more than **101.0%** of  $C_{12}H_{15}N_3O_2S$ , calculated with reference to the dried substance.

### Identity tests

- *Either test A alone or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from albendazole RS or with the *reference spectrum* of albendazole.

- B. See the test described below under “Related substances”. The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.
- C. Ignite about 0.1 g; fumes are evolved, staining lead acetate paper R black.
- D. Add about 0.1 g to 3 ml of sulfuric acid (~100 g/l) TS and warm to dissolve. Add about 1 ml of potassium iodobismuthate/acetic acid TS; a reddish brown precipitate is produced.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry at 105 °C for 4 hours; it loses not more than 5.0 mg/g.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R2 as the coating substance and a mixture of 6 volumes of dichloromethane R, 1 volume of ether R, and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of 5 solutions in a mixture of 9 volumes of dichloromethane R and 1 volume of anhydrous formic acid R containing (A) 10.0 mg of Albendazole per ml, (B) 1.0 mg of Albendazole per ml, (C) 1.0 mg of albendazole RS per ml, (D) 0.05 mg of albendazole RS per ml, and (E) 0.025 mg of albendazole RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, and examine the chromatogram in ultraviolet light (254 nm).

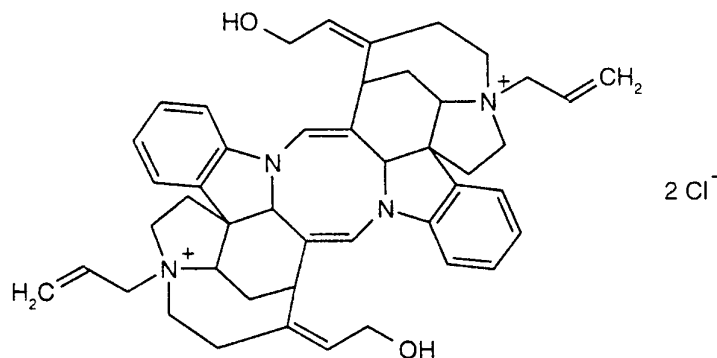
Any spot obtained with solution A, other than the principal spot, is not more intense than the principal spot obtained with solution D (0.5%), and only one spot may be more intense than the principal spot obtained with solution E (0.25%).

**Assay.** Dissolve about 0.25 g, accurately weighed, in 3 ml of anhydrous formic acid R, and add 40 ml of glacial acetic acid R1. Then add 0.2 ml of 1-naphtholbenzein/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS until a green colour is obtained as described under “Non-aqueous titration”, Method A (Vol. 1, p. 131).

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 26.53 mg of  $C_{12}H_{15}N_3O_2S$ .

Alcuronii chloridum

Alcuronium chloride



**Relative molecular mass.** 737.8

**Chemical name.** *N,N'*-Diallylnortoxiferinium dichloride; CAS Reg. No. 15180-03-7.

**Other name.** Alcuronium dichloride.

**Description.** A white to yellow-white, crystalline powder.

**Solubility.** Soluble in water and ethanol (~750 g/l) TS.

**Category.** Muscle relaxant.

**Storage.** Alcuronium chloride should be kept in a tightly closed container at room temperature.

**Labelling.** The designation Alcuronium chloride for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

**Additional information.** *CAUTION:* Alcuronium chloride is highly toxic. It must be handled with care, avoiding contact with the skin and inhalation of airborne particles. It is hygroscopic.

## Requirements

Alcuronium chloride contains not less than **98.0%** and not more than **101.0%** of  $C_{44}H_{50}Cl_2N_4O_2$ , calculated with reference to the anhydrous substance.

*Note:* All tests must be carried out immediately after opening the container, and as rapidly as possible.

### **Identity tests**

- *Either tests A and D or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from alcuronium chloride RS or with the *reference spectrum* of alcuronium chloride.
- B. The absorption spectrum of a 14 µg/ml solution in phosphate buffer, pH 7.0 (0.067 mol/l) TS, when observed between 230 nm and 350 nm, exhibits a maximum at about 293 nm and a minimum at about 237 nm; the absorbance of a 1-cm layer at the maximum wavelength is about 0.9.
- C. See the test described below under “Related substances”. The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.
- D. A 20 mg/ml solution yields reaction A described under “General identification tests” as characteristic of chlorides (Vol. 1, p. 112).

**Specific optical rotation.** Use a 10 mg/ml solution, measured within 10 minutes of preparation, and calculate with reference to the anhydrous substance;  $[\alpha]_D^{20} = -430^\circ$  to  $-451^\circ$ .

**Heavy metals.** Use 2.5 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method B (Vol. 1, p. 119); not more than 20 µg/g.

**Clarity and colour of solution.** A solution of 0.10 g in 10 ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Sulfated ash.** Not more than 1.0 mg/g.

**Water.** Determine as described under “Determination of water by the Karl Fischer method”, Method A (Vol. 1, p. 135), using about 0.5 g of the substance; the water content is not more than 0.050 g/g.

**pH value.** pH of a 10 mg/ml solution in carbon-dioxide-free water R, 6.0–8.5.

**Related substances.** Carry out the test protected from daylight until the start of detection as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R6 as the coating substance (a precoated plate from a commercial source is suitable) and a mixture of 1 volume of methanol R and 1 volume of ammonium nitrate TS as the mobile phase. Apply separately to the plate 5 µl of each of 4 solutions in methanol R containing (A) 40 mg of Alcuronium chloride per ml, (B) 40 mg of alcuronium chloride RS per ml, (C) 0.20 mg of alcuronium chloride RS per ml, and (D) 0.10 mg of alcuronium chloride RS per ml. Prior to development allow the plate to dry in a current of cold air and place in a chromatographic chamber. After removing the plate from the chromatographic chamber, allow it again to dry in a current of cold air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (0.5%), and no more than 3 of these spots are greater than the spot obtained with solution D (0.25%).

**Assay.** To about 0.3 g, accurately weighed, add 70 ml of acetic anhydride R and place the mixture in an ultrasonic bath for 15 seconds. Titrate the turbid solution with perchloric acid (0.1 mol/l) VS as described under “Non-aqueous titration”, Method A (Vol. 1, p. 131).

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 36.89 mg of  $C_{44}H_{50}Cl_2N_4O_2$ .

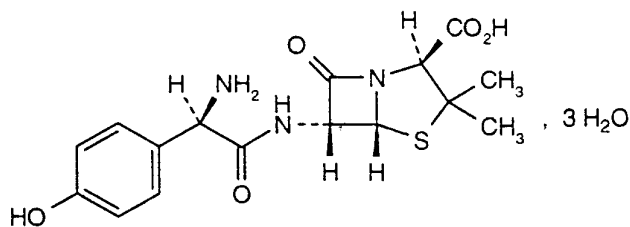
### ***Additional requirements for Alcuronium chloride for parenteral use***

*Complies with the monograph for “Parenteral preparations” (see Vol. 4, p. 36).*

**Bacterial endotoxins.** Carry out the test as described under “Test for bacterial endotoxins” (p. 30); contains not more than 17.5 IU of endotoxin RS per mg.

## *Amoxicillinum trihydricum*

### *Amoxicillin trihydrate*



**Relative molecular mass.** 419.5

**Chemical name.** (-)-6-[2-Amino-2-(*p*-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid trihydrate; (2*S*,5*R*,6*R*)-6-[(*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; 6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; CAS Reg. No. 61336-70-7.

**Description.** A white or almost white, crystalline powder; odourless.

**Solubility.** Slightly soluble in water and methanol R; very slightly soluble in ethanol (~750 g/l) TS, ether R, and fatty oils; soluble in dilute acids and dilute solutions of alkali hydroxides.

**Category.** Antibacterial drug.

**Storage.** Amoxicillin trihydrate should be kept in a tightly closed container, and at a temperature not exceeding 30 °C.

## Requirements

Amoxicillin trihydrate contains not less than **95.0%** and not more than the equivalent of **102.0%** of C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S, calculated with reference to the anhydrous substance.

### Identity tests

- *Either test A alone or tests B and C may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from amoxicillin trihydrate RS or with the *reference spectrum* of amoxicillin trihydrate.
- B. Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silanized silica gel R3 as the coating substance and a mixture of 9 volumes of a solution containing 15.4 g of ammonium acetate R in 100 ml, the pH of which has been adjusted to 5.0 with glacial acetic acid R, and 1 volume of acetone R as the mobile phase. Apply separately to the plate 1 µl of each of 3 solutions in sodium hydrogen carbonate (40 g/l) TS containing (A) 2.5 mg of Amoxicillin trihydrate per ml, (B) 2.5 mg of amoxicillin trihydrate RS per ml, and (C) a mixture of 2.5 mg of amoxicillin trihydrate RS and 2.5 mg of ampicillin trihydrate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the

solvents have evaporated. Expose the plate to iodine vapours until the spots appear and examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is valid only if the chromatogram obtained with solution C shows two clearly separated spots.

- C. Place about 2 mg in a test-tube (150 mm × 15 mm), moisten with 1 drop of water, and add about 2 ml of sulfuric acid (~1760 g/l) TS. Mix the contents of the tube by swirling; the solution remains practically colourless. Place the tube in a water-bath for 1 minute; a dark yellow colour develops.

**Specific optical rotation.** Use a 2.0 mg/ml solution in carbon-dioxide-free water R and calculate with reference to the anhydrous substance;  $[\alpha]_D^{20} = +290^\circ$  to  $+315^\circ$ .

**Solution in hydrochloric acid and ammonia.** Prepare a solution of 1.0 g in 10 ml of hydrochloric acid (0.5 mol/l) VS. Prepare a second solution of 1.0 g in 10 ml of ammonia (~100 g/l) TS. Examine both solutions immediately.

Neither of these solutions are more opalescent than opalescence standard TS3.

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 µg/g.

**Sulfated ash.** Not more than 10 mg/g.

**Water.** Determine as described under "Determination of water by the Karl Fischer method", Method A (Vol. 1, p. 135), using about 0.1 g of Amoxicillin trihydrate; the water content is not less than 0.115 g/g and not more than 0.145 g/g.

**pH value.** pH of a 2 mg/ml solution in carbon-dioxide-free water R, 3.5–5.5.

**Related substances.** Carry out the test as described under "High-performance liquid chromatography" (p. 264), using a stainless steel column (25 cm × 4.6 mm) packed with *stationary phase A* (5 µm). Prepare the following pH 5 buffer solution to be used in the mobile phases: to 250 ml of potassium dihydrogen phosphate (27.2 g/l) TS add sodium hydroxide (~80 g/l) TS until a pH of 5.0 is reached, and dilute the solution with sufficient water to produce 1000 ml. As mobile phase A use a mixture of 99 volumes of buffer solution pH 5.0 and 1 volume of acetonitrile R. As mobile phase B use a mixture of 8 volumes of buffer solution pH 5.0 and 2 volumes of acetonitrile R.

Prepare the following solutions in mobile phase A: solution (A) 1.5 mg of Amoxicillin trihydrate per ml; solution (B) 0.015 mg of amoxicillin trihydrate RS per ml; and solution (C) 0.15 µg of amoxicillin trihydrate RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Using a 50-µl loop injector, inject solution B. Start the elution isocratically with the mobile phase mixture used for the equilibration. Immediately after elution of the amoxicillin peak start a linear gradient elution to reach a ratio of mobile phase A:B of 0:100 over a period of 25 minutes. Adjust the sensitivity of the system so that the height of the principal peak is at least 50% of the full scale of the recorder. Continue the chromatography with mobile phase B for 15 minutes, then equilibrate the column for 15 minutes with the mobile phase originally used for the equilibration. The mass distribution ratio for the first peak (amoxicillin) is 1.3–2.5. Inject mobile phase A using the 50-µl loop injector and use the same elution gradient to obtain a blank. Inject solution C using the 50-µl loop injector. Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

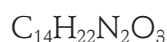
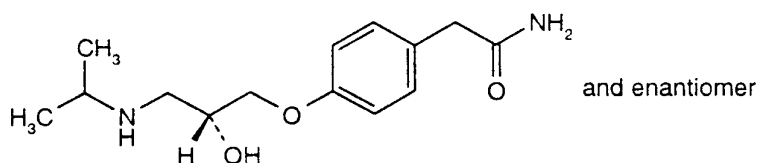
Using the 50-µl loop injector, inject solution A. Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak and any peak obtained in the blank chromatogram, is not greater than that of the principal peak obtained with solution B (1%).

**Assay.** Dissolve about 0.06 g, accurately weighed, in sufficient water to produce 500 ml. Simultaneously, prepare a reference solution containing 0.06 g of amoxicillin trihydrate RS. Transfer 10.0 ml of one solution to a 100-ml volumetric flask and 10.0 ml of the other solution to a second 100-ml volumetric flask. To each add 10 ml of buffer borate, pH 9.0, TS and 1 ml of acetic anhydride/dioxan TS, mix, allow to stand for 5 minutes at room temperature, and dilute to volume with water. Transfer two 2.0 ml aliquots of each solution to separate stoppered test-tubes. To one tube containing the test solution, and to the other, containing the reference solution, add 10 ml of imidazole/mercuric chloride TS, mix, stopper the tubes, and place them in a water-bath at 60°C for exactly 25 minutes. Cool the tubes rapidly to 20°C (*solution A*). To the remaining tubes add 10 ml of water and mix (*solution B*). Without delay, measure the absorbances of a 1-cm layer at the maximum at about 325 nm of both *solutions A*, using as a blank a mixture of 2.0 ml of water and 10 ml of imidazole/mercuric chloride TS placed in the solvent cell. For *solutions B* use water as a blank placed in the solvent cell.

From the difference between the absorbances of *solutions A* and *solutions B*, calculate the percentage content of  $C_{16}H_{19}N_3O_5S$  by comparison with amoxicillin trihydrate RS, with reference to the anhydrous substance.

## Atenololum

### Atenolol



**Relative molecular mass.** 266.3

**Chemical name.** 2-[*p*-[2-Hydroxy-3-(isopropylamino)propoxy]phenyl]acetamide (racemate); CAS Reg. No. 29122-68-7.

**Description.** A white or almost white powder.

**Solubility.** Sparingly soluble in water; soluble in ethanol (~750 g/l) TS; slightly soluble in dichloromethane R.

**Category.** Cardiovascular agent;  $\beta$ -adrenoreceptor blocking agent.

**Storage.** Atenolol should be kept in a tightly closed container.

### Requirements

Atenolol contains not less than **99.0%** and not more than **101.0%** of  $C_{14}H_{22}N_2O_3$ , calculated with reference to the dried substance.

### Identity tests

- *Either tests A and D or tests B, C, and D may be applied.*
- A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from atenolol RS or with the *reference spectrum* of atenolol.

- B. The absorption spectrum of a 0.10 mg/ml solution in methanol R, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 275 nm and 282 nm. The ratio of the absorbance at 275 nm to that at 282 nm is between 1.15 and 1.20.
- C. Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 99 volumes of methanol R and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol R containing (A) 10 mg of Atenolol per ml, and (B) 10 mg of atenolol RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

- D. Melting temperature, about 154 °C.

**Chlorides.** Dissolve 0.25 g in a mixture of 2 ml of nitric acid (~130 g/l) TS and 20 ml of water, and proceed as described under “Limit test for chlorides” (Vol. 1, p. 116); the chloride content is not more than 1.0 mg/g.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry to constant mass at 105 °C; it loses not more than 5.0 mg/g.

**Related substances.** Carry out the test as described under “High-performance liquid chromatography” (p. 264), using a stainless steel column (15 cm × 4.6 mm) packed with *stationary phase A* (5 µm). Prepare the following solution to be used as the mobile phase: dissolve 1.0 g of sodium octanesulfonate R and 0.4 g of tetrabutylammonium hydrogen sulfate R in 1000 ml of a mixture of 80 volumes of a 3.4 mg/ml solution of potassium dihydrogen phosphate R, the pH of the solution adjusted to 3.0 with phosphoric acid (~1440 g/l), 18 volumes of methanol R, and 2 volumes of tetrahydrofuran R.

Prepare the following solutions: for solution (A) dissolve 10 mg of Atenolol in 5 ml of mobile phase; for solution (B) dissolve 0.05 g of Atenolol in 0.10 ml of dimethyl sulfoxide R, if necessary applying gentle heat by placing the flask in a water-bath for a few seconds, and dilute with sufficient mobile phase to produce 25 ml; for solution (C) dilute 0.5 ml of solution A with sufficient mobile phase to produce 100 ml; and for solution (D) dissolve 0.05 g of atenolol for column validation RS in 0.10 ml of dimethyl sulfoxide R, if necessary applying gentle heat by placing the flask in a water-bath for a few seconds, and dilute with sufficient mobile phase to produce 25 ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 226 nm.

Inject 10 µl of solution C. Adjust the sensitivity of the system so that the height of the principal peak is at least 50% of the full scale of the recorder.

Inject 10 µl of solution D. The tracing obtained is similar to that of the specimen chromatogram provided with atenolol for column validation RS, where the peak due to the bis-ether precedes and is separated from the tertiary amine which normally appears as a doublet. If necessary, adjust the concentration of sodium octanesulfonate R in the mobile phase: a higher concentration would increase the retention time of the tertiary amine.

Inject alternately 10 µl each of solutions A and C. Continue the recording of the chromatogram for four times the retention time of the principal peak.

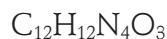
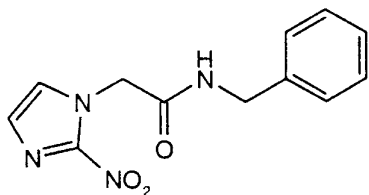
Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than half the area of the principal peak obtained with solution C (0.25%). The sum of the areas of all the peaks, other than the principal peak, is not greater than that of the principal peak obtained with solution C (0.5%). Disregard any peak with an area less than 0.1 times that of the principal peak obtained with solution C. If the content of bis-ether in Atenolol is greater than 0.15%, repeat the chromatography with 10 µl of solution B to confirm its compliance.

**Assay.** Dissolve about 0.2 g, accurately weighed, in 80 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under "Non-aqueous titration", Method A (Vol. 1, p. 131), determining the end-point potentiometrically.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 26.63 mg of  $C_{14}H_{22}N_2O_3$ .

## *Benznidazolium*

### *Benznidazole*



**Relative molecular mass.** 260.3

**Chemical name.** *N*-Benzyl-2-nitroimidazole-1-acetamide; *N*-benzyl-2-nitro-1-imidazole-acetamide; CAS Reg. No. 22994-85-0.

**Description.** A yellowish powder; odourless or almost odourless.

**Solubility.** Practically insoluble in water; sparingly soluble in acetone R; slightly soluble in methanol R; very slightly soluble in ethanol (~750 g/l) TS.

**Category.** Antiprotozoal drug.

**Storage.** Benznidazole should be kept in a well-closed container, protected from light.

## Requirements

Benznidazole contains not less than **98.5%** and not more than the equivalent of **101.5%** of  $C_{12}H_{12}N_4O_3$ , calculated with reference to the dried substance.

### Identity tests

- *Either test A alone or tests B and C may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from benznidazole RS or with the *reference spectrum* of benznidazole.
- B. See the test described below under “Related substances”. The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.
- C. Melting temperature, about 190°C.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry at 105°C for 4 hours; it loses not more than 5.0 mg/g.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R2 as the coating substance and a mixture of 40 volumes of chloroform R, 40 volumes of ethyl acetate R, 15 volumes of methanol R, and 5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 20 µl of each of 3 solutions in acetone R containing (A) 25 mg of Benznidazole per ml, (B) 25 mg of benznidazole RS per ml, and (C) 125 µg of benznidazole RS per ml. After removing the plate from

the chromatographic chamber, allow it to dry in air until the solvents have evaporated, and heat at 110 °C for 10 minutes. Allow it to cool and examine the chromatogram in ultraviolet light (254 nm).

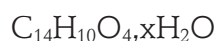
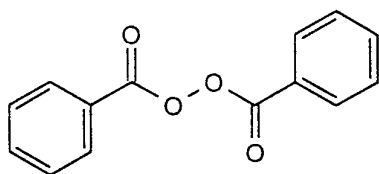
Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (0.5%).

**Assay.** Dissolve about 0.2 g, accurately weighed, in 75 ml of acetic anhydride R, and titrate with perchloric acid (0.1 mol/l) VS as described under “Non-aqueous titration”, Method A (Vol. 1, p. 131), determining the end-point potentiometrically.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 26.03 mg of  $C_{12}H_{12}N_4O_3$ .

### *Benzoylis peroxidum cum aqua*

#### *Hydrous Benzoyl peroxide*



**Relative molecular mass.** 242.2 (anhydrous)

**Chemical name.** Dibenzoyl peroxide; CAS Reg. No. 94-36-0.

**Description.** A white, amorphous or granular powder.

**Solubility.** Practically insoluble in water; soluble in acetone R; soluble in dichloromethane R with separation of water; slightly soluble in ethanol (~750 g/l) TS.

**Category.** Keratolytic agent.

**Storage.** Hydrous Benzoyl peroxide should be kept in a container that has been treated to reduce static discharge and that has a device for the release of excess pressure. Store at a temperature between 2 and 8 °C, protected from light.

**Additional information.** *CAUTION:* Hydrous Benzoyl peroxide may explode at temperatures higher than 60 °C or if its water content is too low. It may burst into flame in the presence of reducing substances. Unused material must not be returned to the original container but destroyed by treating with sodium hydroxide (~80 g/l) TS to a point where no iodine is liberated after acidifying with hydrochloric acid (~70 g/l) TS and adding a crystal of potassium iodide R.

Hydrous Benzoyl peroxide loses water rapidly on exposure to air. It must be handled with care, avoiding contact with the skin and mucous membranes and inhalation of airborne particles.

## Requirements

Hydrous Benzoyl peroxide contains not less than **70.0%** and not more than **77.0%** of  $C_{14}H_{10}O_4$ , and not less than **20.0%** of water.

*Note:* Before carrying out any tests, thoroughly mix the entire sample.

## Identity tests

- *Either test A alone or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the *reference spectrum* of benzoyl peroxide.
- B. Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 50 volumes of toluene R, 2 volumes of dichloromethane R, and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 10.0 mg of Hydrous Benzoyl peroxide per ml, and (B) a solution of hydrous benzoyl peroxide R containing the equivalent of 10.0 mg of benzoyl peroxide per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).
- The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.
- C. Dissolve about 25 mg in 2 ml of acetone R, add 1 ml of diethylphenylenediamine sulfate TS, and mix; a red colour is produced which turns rapidly to dark violet within 5 minutes.
- D. To 1 g add 5 ml of ethanol (~750 g/l) TS, 5 ml of sodium hydroxide (~80 g/l) TS, and 10 ml of water. Boil the mixture under a reflux condenser for 20 minutes and cool. To 1 ml of the resulting solution add 0.5 ml of ferric

chloride (65 g/l) TS; a dull yellow precipitate is produced which is soluble in ether R.

**Chlorides.** Dissolve a quantity containing the equivalent of 0.5 g of anhydrous Benzoyl peroxide in 15 ml of acetone R. Add, while stirring, 50 ml of nitric acid (0.05 mol/l) VS, allow to stand for 10 minutes, and filter. Wash the residue with two quantities, each of 10 ml, of nitric acid (0.05 mol/l) VS, combining the filtrate and the washings. Dilute this solution to 100 ml with nitric acid (0.05 mol/l) VS. Using 2.5 ml of this solution, proceed as described under "Limit test for chlorides" (Vol. 1, p. 116); the chloride content does not exceed 4 mg/g.

**Water.** Determine as described under "Determination of water by the Karl Fischer method", Method A (Vol. 1, p. 135), using 5.0 ml of solution A as prepared below under "Assay". Add 3 ml of a solution containing 0.10 g of potassium iodide R in dimethylformamide R. Stir for 5 minutes before starting the titration. Repeat the procedure using 5 ml of dimethylformamide R in place of solution A and make any necessary corrections. Calculate the content of water as a percentage.

**Acidity.** Dissolve a quantity containing the equivalent of 1.0 g of anhydrous Benzoyl peroxide in 25 ml of acetone R, add 75 ml of water, and filter. Wash the residue with two quantities of 10 ml of water. Combine the filtrate and washings, and titrate with sodium hydroxide (0.1 mol/l) VS, using 0.25 ml of phenolphthalein/ethanol TS as indicator, until the change in colour is observed. Repeat the procedure without the substance being examined. The difference between the titrations represents the amount of sodium hydroxide required; not more than 1.25 ml of sodium hydroxide (0.1 mol/l) VS.

**Related substances.** Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 40 volumes of light petroleum R1, 20 volumes of toluene R, 15 volumes of acetone R, and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of 4 freshly prepared solutions in acetone R containing (A) a quantity equivalent to 40 mg of anhydrous Benzoyl peroxide per ml, (B) 0.4 mg of anhydrous Benzoyl peroxide per ml, (C) 0.6 mg of benzoic acid R per ml, and for solution (D) mix 0.4 ml of benzyl benzoate R with 5 ml of acetone R and dilute to 10 ml with the same solvent. To 1.0 ml of this solution add 1.0 ml of solution A and dilute to 10 ml with acetone R. After removing the plate from the chromatographic chamber, allow it to dry in air for 20 minutes, and examine the chromatogram in ultra-violet light (254 nm).

Any spot corresponding to benzoic acid obtained with solution A is not more intense than that obtained with solution C (1.5%). Any spot obtained with solution A, other than the principal spot and the spot corresponding to benzoic

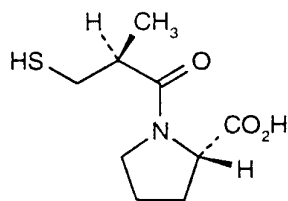
acid, is not more intense than that obtained with solution B (1%). The test is not valid unless the chromatogram obtained with solution D shows two clearly separated principal spots.

**Assay.** Immediately before testing dissolve 2.5 g in sufficient dimethylformamide R to produce 100 ml (*solution A*). To 5.0 ml of *solution A* add 20 ml of acetone R and 5 ml of potassium iodide (300 g/l) TS. Mix, allow to stand for 1 minute, and titrate with sodium thiosulfate (0.1 mol/l) VS until the solution is colourless. Repeat the procedure using 5 ml of dimethylformamide R in place of *solution A* and make any necessary corrections.

Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 12.11 mg of  $C_{14}H_{10}O_4$ .

## *Captoprilum*

### *Captopril*



$C_9H_{15}NO_3S$

**Relative molecular mass.** 217.3

**Chemical name.** 1-[(2*S*)-3-Mercapto-2-methylpropionyl]-L-proline; 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline; CAS Reg. No. 62571-86-2.

**Description.** A white or almost white, crystalline powder.

**Solubility.** Freely soluble in water, dichloromethane R, and methanol R.

**Category.** Cardiovascular agent; angiotensin-converting enzyme inhibitor.

**Storage.** Captopril should be kept in a tightly closed container, protected from light.

**Additional information.** Captopril may exist in different polymorphic forms.

## Requirements

Captopril contains not less than **98.0%** and not more than **102.0%** of  $C_9H_{15}NO_3S$ , calculated with reference to the dried substance.

### Identity tests

- *Either tests A and D or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from captopril RS or with the *reference spectrum* of captopril.
- B. Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 75 volumes of toluene R, 25 volumes of glacial acetic acid R, and 1 volume of methanol R as the mobile phase. Apply separately to the plate 2  $\mu$ l of each of 2 solutions in dichloromethane R containing (A) 5.0 mg of Captopril per ml, and (B) 5.0 mg of captopril RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with 5,5'-dithiobis-2-nitrobenzoic acid/methanol TS. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

- C. Dissolve 25 mg in 2 ml of ethanol (~750 g/l) TS, add a few crystals of sodium nitrite R and 10 ml of sulfuric acid (~100 g/l) TS, and shake; a red colour is produced.
- D. Melting temperature, about 107 °C.

**Specific optical rotation.** Use a 10 mg/ml solution in dehydrated ethanol R and calculate with reference to the dried substance;  $[\alpha]_D^{20} = -125^\circ$  to  $-134^\circ$ .

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20  $\mu$ g/g.

**Sulfated ash.** Not more than 2.0 mg/g.

**Loss on drying.** Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 10 mg/g.

**Related substances.** Carry out the test as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (12.5 cm × 4 mm) packed with *stationary phase A* (5 μm). Prepare the following solution to be used as the mobile phase: mix 0.05 volumes of phosphoric acid (~1440 g/l) TS with 50 volumes of methanol R and 50 volumes of water.

Prepare the following solutions in the mobile phase: solution (A) 0.5 mg of Captopril per ml; solution (B) 10 μg of Captopril per ml; and for solution (C) dissolve 10 mg of Captopril in the mobile phase, add 1 ml of iodine (0.05 mol/l) VS, and dilute to 100 ml with the mobile phase; further dilute 10 ml of this solution to 100 ml with the mobile phase.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 220 nm.

Inject 20 μl of solution B and adjust the sensitivity of the system so that the height of the principal peak is not less than 40% of the full scale of the recorder. Inject 20 μl of solution C. The test is not valid unless three peaks are obtained and the resolution between the last two eluting principal peaks is at least 2.0.

Inject alternately 20 μl each of solutions A and B. Continue the chromatography for three times the retention time of the principal peak obtained with solution A.

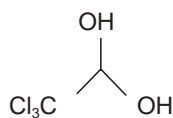
Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than half the area of the principal peak obtained with solution B (1.0%). The sum of the areas of all the peaks, other than the principal peak, is not greater than the area of the peak obtained with solution A (2.0%). Disregard any peak with a retention time of less than 1.4 minutes or with an area less than 0.1 times that of the peak obtained with solution B.

**Assay.** Dissolve about 0.3 g, accurately weighed, in 100 ml of water, add 10 ml of sulfuric acid (~190 g/l) TS and 1 g of potassium iodide R. Mix and titrate with potassium iodate (0.01 mol/l) VS, using starch TS as indicator. Repeat the operation without the substance being examined. The difference between the titrations represents the amount of potassium iodate required.

Each ml of potassium iodate (0.01 mol/l) VS is equivalent to 13.04 mg of C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S.

Chlorali hydras

Chloral hydrate



**Relative molecular mass.** 165.4

**Chemical name.** 2,2,2-Trichloroethane-1,1-diol; CAS Reg. No. 302-17-0.

**Description.** Colourless, transparent or white crystals; odour, aromatic, pungent and characteristic.

**Solubility.** Very soluble in water; freely soluble in ethanol (~750 g/l) TS and ether R.

**Category.** Premedication.

**Storage.** Chloral hydrate should be kept in a tightly closed container.

**Additional information.** Melting temperature, about 55 °C; when exposed to air it slowly volatilizes.

## Requirements

Chloral hydrate contains not less than **98.5%** and not more than **101.0%** of  $\text{C}_2\text{H}_3\text{Cl}_3\text{O}_2$ .

*Note:* Prepare the following test solution for use in “Identity tests A and B”, and for “Clarity and colour”. Dissolve 2.5 g in sufficient carbon-dioxide-free water R to produce 25 ml.

### Identity tests

- To 1.0 ml of the test solution add 2.0 ml of sodium sulfide TS; a yellow colour develops which quickly becomes reddish brown. On standing, a red precipitate may be produced.
- Transfer 10 ml of the test solution to a conical flask and add 10 ml of 1-ethylquinaldinium iodide (15 g/l) TS that has previously been filtered through a 0.45- $\mu\text{m}$  filter. Then add 60 ml of 2-propanol R, 5 ml of monoethanolamine (0.1 mol/l) VS, and 15 ml of water. Mix, and heat in a water-bath at 60 °C for 15 minutes; a blue colour develops.

**Chlorides.** Dissolve 2.5g in a mixture of 2ml of nitric acid (~130g/l) TS and 20ml of water, and proceed as described under "Limit test for chlorides" (Vol. 1, p. 115); the chloride content is not more than 0.1 mg/g.

**Chloral alcoholate.** Warm 1.0g with 10 ml of sodium hydroxide (~80g/l) TS. Filter the upper layer and add iodine (0.05 mol/l) VS a drop at a time until a yellow colour is obtained; no precipitate is produced within 1 hour.

**Clarity and colour of solution.** The test solution is clear and colourless.

**Sulfated ash.** Not more than 1.0 mg/g.

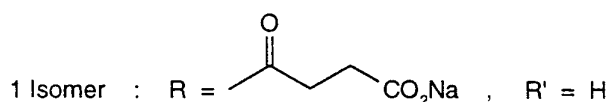
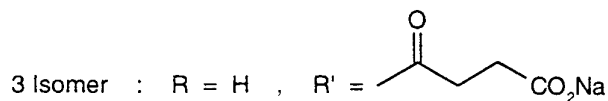
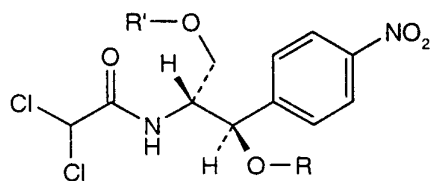
**pH value.** pH of a 0.10g/ml solution in carbon-dioxide-free water R, 3.5–5.5.

**Assay.** Dissolve about 4g, accurately weighed, in 10 ml of carbon-dioxide-free water R and add 30.0ml of carbonate-free sodium hydroxide (1 mol/l) VS. Allow the mixture to stand for 2 minutes and titrate with sulfuric acid (0.5 mol/l) VS, using phenolphthalein/ethanol TS as indicator. Repeat the procedure without the Chloral hydrate being examined and make any necessary corrections.

Each ml of carbonate-free sodium hydroxide (1 mol/l) VS is equivalent to 0.1654g of  $C_2H_3Cl_3O_2$ .

### *Chloramphenicoli natrii succinas*

### *Chloramphenicol sodium succinate*



**Relative molecular mass.** 445.2

**Chemical name.** A mixture in variable proportions of (2*R*,3*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-3-(4-nitrophenyl)propyl succinate (3 isomer) and of sodium (1*R*,2*R*)-2-(2,2-dichloroacetamido)-3-hydroxy-1-(4-nitrophenyl)propyl succinate (1 isomer); [*R*-(*R*\*,*R*\*)]-mono[2-[(2,2-dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl] ester, butanedioic acid, monosodium salt; *D*-*threo*-(-)-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide α-(sodium succinate); CAS Reg. No. 982-57-0.

**Description.** A white or yellowish white powder.

**Solubility.** Very soluble in water; freely soluble in ethanol (~750 g/l)TS.

**Category.** Antibacterial drug.

**Storage.** Chloramphenicol sodium succinate should be kept in a tightly closed container, protected from light.

**Labelling.** The designation Chloramphenicol sodium succinate for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

**Additional information.** Chloramphenicol sodium succinate is hygroscopic. Even in the absence of light, Chloramphenicol sodium succinate gradually degrades when exposed to a humid atmosphere; decomposition is more rapid at higher temperatures.

## Requirements

Chloramphenicol sodium succinate contains not less than **98.0%** and not more than the equivalent of **102.0%** of  $C_{15}H_{15}Cl_2N_2NaO_8$ , calculated with reference to the anhydrous substance.

## Identity tests

- A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from chloramphenicol sodium succinate RS or with the *reference spectrum* of chloramphenicol sodium succinate.
- B. See the test described below under "Chloramphenicol and chloramphenicol disodium disuccinate, test B". The two principal spots obtained with solution A correspond in position and appearance with those obtained with solution B. The positions of the spots obtained with solutions A and B are different from that of the principal spot obtained with solution C.

- C. Dissolve 10 mg in 2.0 ml of ethanol (~750 g/l) TS, add 0.2 g of zinc R powder, 1.0 ml of sulfuric acid (~100 g/l) TS, and allow to stand for 10 minutes. Filter. To the filtrate add 0.5 ml of sodium nitrite (10 g/l) TS, and allow to stand for 2 minutes. Then add 1.0 g of urea R and a solution containing 10 mg of 2-naphthol R in 2 ml of sodium hydroxide (~80 g/l) TS; a red colour is produced. Repeat the test omitting the zinc R powder; no red colour is produced.
- D. Dissolve 5 mg in 5 ml of water and add a few drops of silver nitrate (40 g/l) TS; no precipitate is produced. Heat 0.05 g with 2.0 ml of potassium hydroxide/ethanol TS1 on a water-bath for 15 minutes, add 15 mg of charcoal R, shake, and filter. The filtrate yields reaction A described under "General identification tests" as characteristic of chlorides (Vol. 1, p. 112).
- E. When tested for sodium as described under "General identification tests" (Vol. 1, p. 115), it yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

**Specific optical rotation.** Use a 50 mg/ml solution and calculate with reference to the anhydrous substance;  $[\alpha]_D^{20} = +5.0^\circ$  to  $+8.0^\circ$ .

**Clarity of solution.** A solution of 1.0 g in 3.0 ml of carbon-dioxide-free water R is clear.

**Water.** Determine as described under "Determination of water by the Karl Fischer method", Method A (Vol. 1, p. 135), using about 0.5 g of Chloramphenicol sodium succinate; the water content is not more than 0.20 g/g.

**pH value.** pH of a 0.25 g/ml solution in carbon-dioxide-free water R, 6.4–7.0.

### **Chloramphenicol and chloramphenicol disodium disuccinate**

- *Either test A or test B may be applied.*

A. Carry out the test as described under "High-performance liquid chromatography" (p. 264), using a stainless steel column (25 cm × 4.6 mm) packed with *stationary phase A* (5 μm). As the mobile phase, use a mixture of 55 volumes of water, 40 volumes of methanol R, and 5 volumes of phosphoric acid (~20 g/l) TS.

Prepare the following solutions in the mobile phase: solution (A) 0.25 mg of Chloramphenicol sodium succinate per ml; solution (B) 5.0 μg of chloramphenicol RS per ml; solution (C) 5.0 μg of chloramphenicol disodium disuccinate RS per ml; and for solution (D) dissolve 25 mg of Chloramphenicol sodium succinate in the mobile phase, add 0.5 mg of chloramphenicol RS

and 0.5 mg of chloramphenicol disodium disuccinate RS and dilute to 100 ml with the mobile phase.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 275 nm.

Using a 20- $\mu$ l loop injector inject solution D. Inject alternately solutions A, B, C, and D. The test is not valid unless the two peaks in the chromatogram obtained with solution D, corresponding to those in the chromatograms obtained with solutions B and C, are clearly separated from the peaks corresponding to the two principal peaks in the chromatogram obtained with solution A. If necessary, adjust the methanol content of the mobile phase.

Measure the areas of the peak responses obtained in the chromatograms from solutions A, B, and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak corresponding to chloramphenicol is not greater than that of the principal peak obtained with solution B (2.0%). The area of any peak corresponding to chloramphenicol disodium disuccinate is not greater than that of the principal peak obtained with solution C (2.0%).

- B. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 85 volumes of dichloromethane R, 14 volumes of methanol R, and 1 volume of acetic acid (~60 g/l) TS as the mobile phase. Apply separately to the plate 2  $\mu$ l of each of 3 solutions in acetone R containing (A) 10 mg of Chloramphenicol sodium succinate per ml, (B) 10 mg of chloramphenicol sodium succinate RS per ml, and (C) 10 mg of chloramphenicol RS per ml. Then apply separately 10  $\mu$ l of solution (A) as prepared above and 1  $\mu$ l of solution (D) containing 0.20 mg of chloramphenicol RS per ml of acetone R. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with the second application of solution A, other than the principal spot, is not more intense than that obtained with solution D (2.0%).

**Assay.** Dissolve about 0.2 g, accurately weighed, in sufficient water to produce 500 ml; dilute 5.0 ml of this solution to 100 ml with water. Measure the absorbance of the diluted solution in a 1-cm layer at the maximum at about 276 nm and calculate the percentage content of  $C_{15}H_{15}Cl_2N_2NaO_8$  using the absorptivity value of 22.0 ( $A_{1\text{cm}}^{1\%} = 220$ ), and with reference to the anhydrous substance.

### ***Additional requirements for Chloramphenicol sodium succinate for parenteral use***

Complies with the monograph for "Parenteral preparations" (see Vol. 4, p. 36).

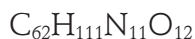
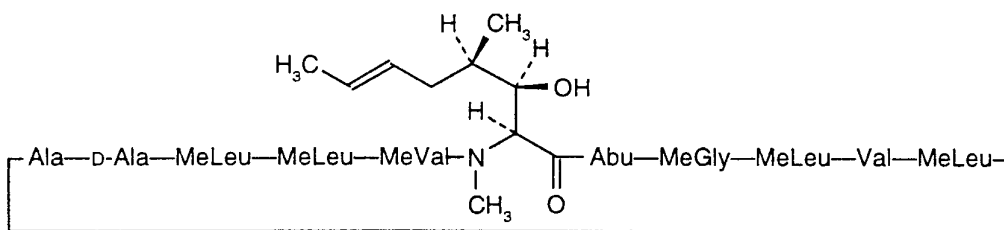
**Storage.** Sterile Chloramphenicol sodium succinate should be kept in a sterile, tightly closed, and tamper-proof container, protected from light.

**Bacterial endotoxins.** Carry out the test as described under "Test for bacterial endotoxins" (p. 30); contains not more than 0.2 IU of endotoxin RS per mg.

**Sterility.** Complies with the "Sterility testing of antibiotics", Membrane filtration test procedure (Vol. 1, p. 152).

## Ciclosporinum

### *Ciclosporin*



**Relative molecular mass.** 1203

**Chemical name.** Cyclo[[*(E)*-(2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenyl]-L-2-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl]; cyclosporin A; CAS Reg. No. 59865-13-3.

**Other name.** Cyclosporin.

**Description.** A white or almost white powder.

**Solubility.** Practically insoluble in water; freely soluble in ethanol (~750 g/l) TS and dichloromethane R.

**Category.** Immunosuppressant drug.

**Storage.** Ciclosporin should be kept in a well-closed container, protected from light.

## Requirements

Ciclosporin contains not less than **98.5%** and not more than **101.5%** of  $C_{62}H_{111}N_{11}O_{12}$ , calculated with reference to the dried substance.

### Identity tests

- *Either test A alone or tests B and C may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from ciclosporin RS or with the *reference spectrum* of ciclosporin.
- B. See the test described below under “Related substances” and under “Assay”. The principal peak obtained with solution A corresponds in retention time to that obtained with solution B.
- C. Dissolve 5 mg in 5 ml of methanol R, and 1 drop of potassium permanganate (10 g/l) TS, and allow to stand; the blue-red colour is gradually discharged.

**Specific optical rotation.** Use a 5.0 mg/ml solution in methanol R and calculate with reference to the dried substance;  $[\alpha]_D^{20} = -185^\circ$  to  $-193^\circ$ .

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 µg/g.

**Clarity and colour of solution in ethanol.** A solution of 1.0 g in 10 ml of ethanol (~750 g/l) TS is clear and not more intensely coloured than standard colour solution Yw3 or Rd1 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 20 mg/g.

**Related substances.** Carry out the test as described below under “Assay”.

Inject alternately 20 µl each of solutions A and C. Continue the recording of the chromatogram for 1.7 times the retention time of the principal peak.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than 0.7 times the area of the principal peak obtained with solution C (0.7%), and the sum of these areas is not greater than 1.5 times the area of the principal peak of the chromatogram obtained with solution C (1.5%).

**Assay.** Determine as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (25 cm × 4 mm) packed with *stationary phase A* (3–5 µm). The column is connected to the injection port by a steel capillary tube about 1 m long with an internal diameter of 0.25 mm. Maintain the temperature of the column and of the steel capillary at 80 °C. As the mobile phase, use a mixture of 52 volumes of water, 43 volumes of acetonitrile R, 5 volumes of *tert*-butyl methyl ether R, and 0.1 volume of phosphoric acid (~1440 g/l) TS.

Prepare the following solutions in a solvent mixture of equal volumes of acetonitrile R and water: solution (A) 1.2 mg of Ciclosporin per ml; solution (B) 1.2 mg of ciclosporin RS per ml; for solution (C) dilute 2.0 ml of solution B to 200 ml with the solvent mixture; and for solution (D) dissolve 3 mg of ciclosporin U RS in 2.5 ml of the solvent mixture and add 2.5 ml of solution B.

Operate with a flow rate of about 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 210 nm.

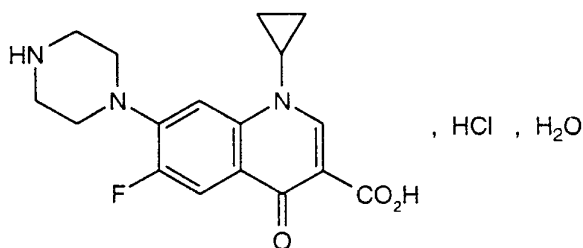
Inject 20 µl of solution D. The assay is valid only if the relative standard deviation of the area of the principal peak is not more than 1.0%, unless the resolution between the two principal peaks is 1.0 and 1.8. The assay is not valid unless the retention time of the principal peak is between 25 and 30 minutes.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>.

*Ciprofloxacini hydrochloridum*

*Ciprofloxacin hydrochloride*



$C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$

**Relative molecular mass.** 385.8

**Chemical name.** 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid monohydrochloride monohydrate; CAS Reg. No. 86393-32-0.

**Description.** A pale yellow, crystalline powder.

**Solubility.** Soluble in water; slightly soluble in methanol R; very slightly soluble in ethanol (~750 g/l) TS; practically insoluble in acetone R and dichloromethane R.

**Category.** Antibacterial.

**Storage.** Ciprofloxacin hydrochloride should be kept in a tightly closed container, protected from light.

### Requirements

Ciprofloxacin hydrochloride contains not less than **98.0%** and not more than **102.0%** of  $C_{17}H_{18}FN_3O_3 \cdot HCl$ , calculated with reference to the anhydrous substance.

### Identity tests

- A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from ciprofloxacin hydrochloride RS or with the *reference spectrum* of ciprofloxacin hydrochloride.
- B. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 4 volumes

of methanol R, 4 volumes of dichloromethane R, 2 volumes of ammonia (~260 g/l) TS, and 1 volume of acetonitrile R as the mobile phase. Apply separately to the plate as 1-cm bands, 5 µl of each of 2 solutions containing (A) 10 mg of Ciprofloxacin hydrochloride per ml, and (B) 10 mg of ciprofloxacin hydrochloride RS per ml. Place an evaporating-dish containing 50 ml of ammonia (~260 g/l) TS in the chromatographic chamber. Expose the plate to the ammonia vapour in the closed chamber for 15 minutes. Withdraw the plate and transfer to another chromatographic chamber containing the mobile phase to develop. After removing the plate from the chromatographic chamber, allow it to dry in air for about 15 minutes, and examine the chromatogram in ultraviolet light (254 nm and 365 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. A 0.1 g/ml solution yields reaction B described under “General identification tests” as characteristic of chlorides (Vol. 1, p. 112).

**Heavy metals.** For the preparation of the test solution dissolve 0.25 g in water and dilute to 30 ml with the same solvent. Carry out the prefiltration. Determine the heavy metals content in the filtrate as described under “Limit test for heavy metals”, Method B (Vol. 1, p. 119); not more than 20 µg/g.

**Clarity and colour of solution.** A solution of 0.25 g in 10 ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour solution Gn4 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Sulfated ash.** Not more than 1.0 mg/g.

**Water.** Determine as described under “Determination of water by the Karl Fischer method”, Method B (Vol. 1, p. 135), using about 0.2 g of the substance; the water content is between 0.047 g/g and 0.067 g/g.

**pH value.** pH of a 25 mg/ml solution in carbon-dioxide-free water R, 3.0–4.5.

**Fluoroquinolonic acid.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 4 volumes of methanol R, 4 volumes of dichloromethane R, 2 volumes of ammonia (~260 g/l) TS, and 1 volume of acetonitrile R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 10 mg of Ciprofloxacin hydrochloride per ml, and for solution (B) dissolve 10 mg of fluoroquinolonic acid RS in a mixture of 0.10 ml of ammonia (~100 g/l) TS and 90 ml of water, and dilute to 100 ml with water. Dilute 2.0 ml of this solution to 10 ml with water. Place an evaporating-dish containing 50 ml

of ammonia (~260 g/l) TS in the chromatographic chamber. Expose the plate to the ammonia vapour in the closed chamber for 15 minutes. Withdraw the plate and transfer to another chromatographic chamber containing the mobile phase to develop. After removing the plate from the chromatographic chamber, allow it to dry in air for about 15 minutes, and examine the chromatogram in ultraviolet light (254 nm).

The spot corresponding to fluoroquinolonic acid in the chromatogram obtained with solution A is not more intense than that obtained with solution B (0.2%).

**Related substances.** Carry out the test as described below under “Assay”.

Inject 50 µl each of solutions A and F. Record the chromatogram for twice the retention time of ciprofloxacin.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and F, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the areas of the peaks corresponding to the ethylenediamine compound and the by-compound A are not greater than the corresponding peaks in the chromatogram obtained with solution F (0.2%); the area of any other peak is not greater than the area of the peak corresponding to the ethylenediamine compound in the chromatogram obtained with solution F (0.2%); the sum of the areas of all the peaks, other than the principal peak, is not greater than 2.5 times the area of the peak corresponding to the ethylenediamine compound in the chromatogram obtained with solution F (0.5%). Disregard any peak with an area less than 0.25 times the area of the peak corresponding to the ethylenediamine compound in the chromatogram obtained with solution F (0.05%).

**Assay.** Determine as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (25 cm × 4.6 mm) packed with *stationary phase A* (5 µm). As the mobile phase, use a mixture of 87 volumes of phosphoric acid (~2.45 g/l) TS, adjusted to a pH of 3.0 with triethylamine R and 13 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase to produce 50 ml: solution (A) contains 0.50 mg of Ciprofloxacin hydrochloride per ml; solution (B) contains 0.50 mg of ciprofloxacin hydrochloride RS per ml; solution (C) contains 0.050 mg of 1-cyclopropyl-1,4-dihydro-4-oxo-7-(1-piperazin-1-yl)quinoline-3-carboxylic acid RS per ml (desfluoro compound); solution (D) contains 0.050 mg of 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid RS (ethylenediamine compound) per ml; solution (E) contains 0.050 mg of 7-chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(piperazin-1-yl)quinoline-3-carboxylic acid RS (by-compound A) per ml. For solution (F) mix 0.1 ml of solution A with 1.0 ml of solution C, 1.0 ml of solution D, 1.0 ml of solution E, and dilute to 50 ml with the mobile phase.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 278 nm. Maintain the temperature of the column at 40 °C.

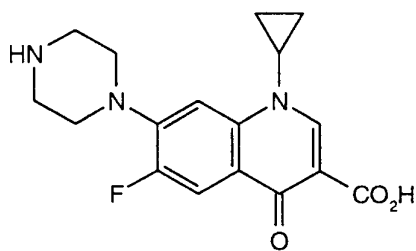
Inject 50 µl of solution F. The following order of elution is obtained: desfluoro compound, ethylenediamine compound, ciprofloxacin and by-compound A. The retention time of ciprofloxacin is about 9 minutes. Adjust the sensitivity of the system so that the height of the peak due to the ethylenediamine compound is at least 40% of the full scale of the recorder. The assay is not valid unless the resolution between the peaks corresponding to the desfluoro compound and the ethylenediamine compound is at least 1.3, and the resolution between the peaks corresponding to ciprofloxacin and the by-compound A is at least 3.0. Inject 10 µl of solution B. The assay is not valid unless the relative standard deviation of the peak area of ciprofloxacin is at most 1.0%.

Inject alternately 10 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of  $C_{17}H_{18}FN_3O_3 \cdot HCl$ .

## *Ciprofloxacinum*

### *Ciprofloxacin*



**Relative molecular mass.** 331.4

**Chemical name.** 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid; CAS Reg. No. 85721-33-1.

**Description.** A white to pale yellow, crystalline powder.

**Solubility.** Practically insoluble in water; very slightly soluble in ethanol (~750 g/l) TS and dichloromethane R.

**Category.** Antibacterial.

**Storage.** Ciprofloxacin should be kept in a well-closed container, protected from light.

**Additional information.** Ciprofloxacin exists in different polymorphic forms.

## Requirements

Ciprofloxacin contains not less than **98.0%** and not more than **102.0%** of  $C_{17}H_{18}FN_3O_3$ , calculated with reference to the dried substance.

### Identity test

Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from ciprofloxacin RS or with the *reference spectrum* of ciprofloxacin.

**Heavy metals.** For the preparation of the test solution dissolve 0.5 g in acetic acid (~60 g/l) TS and dilute to 30 ml with the same solvent. Carry out the pre-filtration. To the filtrate add 2.0 ml of water and determine the heavy metals content as described under “Limit test for heavy metals”, Method B (Vol. 1, p. 119); not more than 20 µg/g.

**Clarity and colour of solution.** A solution of 0.25 g in 20 ml of hydrochloric acid (0.1 mol/l) VS is clear and not more intensely coloured than standard colour solution Gn4 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry to constant mass at 120 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury); it loses not more than 10 mg/g.

**Fluoroquinolonic acid.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 4 volumes of methanol R, 4 volumes of dichloromethane R, 2 volumes of ammonia (~260 g/l) TS, and 1 volume of acetonitrile R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 10 mg of Ciprofloxacin per ml of acetic acid (~60 g/l) TS, and for solution (B) dissolve 10 mg of fluoroquinolonic acid RS in a mixture of 0.10 ml of ammonia (~100 g/l) TS and 90 ml of water, and dilute to 100 ml with water. Dilute 2.0 ml of this solution to 10 ml with water. Place an evaporating-dish containing 50 ml of ammonia (~260 g/l) TS in the chromatographic chamber. Expose the plate to the ammonia vapour in the closed chamber for 15 minutes. Withdraw the plate and transfer to another chromatographic chamber containing the mobile phase

to develop. After removing the plate from the chromatographic chamber, allow it to dry in air for about 15 minutes, and examine the chromatogram in ultraviolet light (254 nm).

The spot corresponding to fluoroquinolonic acid in the chromatogram obtained with solution A is not more intense than that obtained with solution B (0.2%).

**Related substances.** Carry out the test as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (25 cm × 4.6 mm) packed with base-deactivated *stationary phase A* (5 μm). As the mobile phase, use a mixture of 87 volumes of phosphoric acid (~2.8 g/l) TS adjusted to a pH of 3.0 with triethylamine R and 13 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase. For solution (A) add 0.2 ml of phosphoric acid (~105 g/l) TS to 25 mg of Ciprofloxacin, dilute to 50 ml, and treat in an ultrasonic bath until a clear solution is obtained. For solution (B) dilute 0.10 ml of solution A to 50 ml. For solution (C) use 2.5 mg of 1-cyclopropyl-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid RS (desfluoro compound) and dilute to 50 ml (this solution is also used to prepare solution F), further dilute 1.0 ml of this solution to 50 ml with the mobile phase. For solution (D) use 2.5 mg of 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid RS (ethylenediamine compound) and dilute to 50 ml (this solution is also used to prepare solution F), further dilute 1.0 ml of this solution to 50 ml with the mobile phase. For solution (E) use 2.5 mg of 7-chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(piperazin-1-yl)quinoline-3-carboxylic acid RS (by-compound A) and dilute to 50 ml (this solution is also used to prepare solution F), further dilute 1.0 ml of this solution to 50 ml with the mobile phase. For solution (F) mix 0.1 ml of solution A with 1.0 ml of each of solutions C, D, and E, prior to dilution as described above, and dilute to 50 ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 278 nm. Maintain the temperature of the column at 40 °C.

Inject alternately 50 μl each of solutions B, C, D, E, and F. The retention time of ciprofloxacin is about 9 minutes. Adjust the sensitivity of the system so that the height of the peak due to the ethylenediamine compound obtained with solution F is at least 40% of the full scale of the recorder. The test is not valid unless the resolution between the peaks corresponding to the desfluoro compound and the ethylenediamine compound in the chromatogram obtained with solution F is at least 1.3, and the resolution between the peaks corresponding to ciprofloxacin and the by-compound A is at least 3.0.

Inject alternately 50 μl each of solutions A, D, and E. Record the chromatogram for twice the retention time of ciprofloxacin.

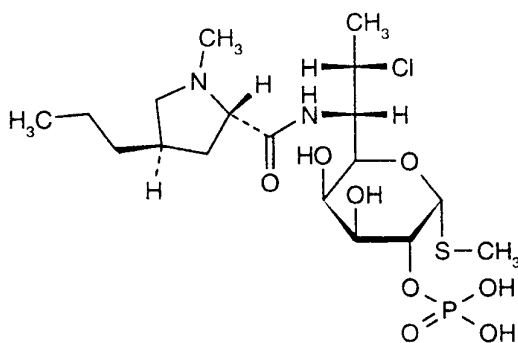
Measure the areas of the peak responses obtained in the chromatograms from solutions A, D, and E, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the areas of the peaks corresponding to the ethylenediamine compound and by-compound A are not greater than the corresponding peaks obtained with solutions D and E (0.2%). The area of any other peak is not greater than the area of the peak obtained with solution D (0.2%). The sum of the areas of all the peaks, other than the principal peak, is not greater than 2.5 times the area of the peak in the chromatogram obtained with solution D (0.5%). Disregard any peak with an area less than 0.25 times the area of the peak obtained with solution D (0.05%).

**Assay.** Dissolve about 0.3g, accurately weighed, in 80ml of glacial acetic acid R1, and titrate with perchloric acid (0.1mol/l) VS as described under "Non-aqueous titration", Method A (Vol. 1, p. 131), determining the end-point potentiometrically.

Each ml of perchloric acid (0.1mol/l) VS is equivalent to 33.14mg of  $C_{17}H_{18}FN_3O_3$ .

### Clindamycini phosphas

### Clindamycin phosphate



$C_{18}H_{34}ClN_2O_8PS$

**Relative molecular mass.** 505.0

**Chemical name.** (2*S*-*trans*)-Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-*threo*- $\alpha$ -D-*galacto*-octopyranoside 2-(dihydrogen phosphate); CAS Reg. No. 24729-96-2.

**Description.** A white or almost white, crystalline powder.

**Solubility.** Freely soluble in water; very slightly soluble in ethanol (~750 g/l) TS and acetone R.

**Category.** Antibacterial drug.

**Storage.** Clindamycin phosphate should be kept in a tightly closed container and stored at a temperature not exceeding 30°C.

**Labelling.** The designation Clindamycin phosphate for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

**Additional information.** Clindamycin phosphate is slightly hygroscopic.

## Requirements

Clindamycin phosphate contains not less than **95.0%** and not more than **100.5%** of  $C_{18}H_{34}ClN_2O_8PS$ , calculated with reference to the anhydrous substance.

### Identity tests

- *Either tests A and D or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from clindamycin phosphate RS or with the *reference spectrum* of clindamycin phosphate.
- B. Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R3 as the coating substance and a mixture of 6 volumes of 1-butanol R, 2 volumes of water, and 2 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions in methanol R containing (A) 2.0 mg of Clindamycin phosphate per ml, (B) 2.0 mg of clindamycin phosphate RS, and for solution (C) dissolve 10 mg of lincomycin hydrochloride RS in 5 ml of solution B. After removing the plate from the chromatographic chamber, allow it to dry at 105°C for 30 minutes, and spray with potassium permanganate (1 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity to that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows two clearly separated spots.

- C. Dissolve 10 mg in 2 ml of hydrochloric acid (~70 g/l) TS and heat directly in a flame for 1 minute; a disagreeable sulfurous odour is perceptible. Cool,

add 4 ml of sodium carbonate (75 g/l) TS and 0.5 ml of sodium nitroprusside (45 g/l) TS; a violet-red ring is formed that fades quickly.

D. Boil 0.1 g under a reflux condenser with a mixture of 5 ml of sodium hydroxide (~400 g/l) TS and 5 ml of water for 90 minutes. Cool and add 5 ml of nitric acid (~1000 g/l) TS. Extract with three 15-ml quantities of dichloromethane R, and discard the extracts. Filter the aqueous layer through a paper filter; the filtrate yields reaction B described under “General identification tests” as characteristic of orthophosphates (Vol. 1, p. 114).

**Specific optical rotation.** Use a 10 mg/ml solution and calculate with reference to the anhydrous substance;  $[\alpha]_D^{20\text{ }^\circ\text{C}} = +115^\circ$  to  $+130^\circ$ .

**Clarity and colour of solution.** A solution of 0.040 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

**Water.** Determine as described under “Determination of water by the Karl Fischer method”, Method A (Vol. 1, p. 135), using 0.5 g of the substance; the water content is not more than 0.060 g/g.

**pH value.** pH of a 10 mg/ml solution in carbon-dioxide-free water R, 3.5–4.5.

**Related substances.** Carry out the test as described below under “Assay”.

Inject alternately 20 µl each of solutions A and D. Continue the recording of the chromatogram until clindamycin is eluted.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and D, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak or any peak corresponding to the solvent, is not greater than 2.5 times the area of the principal peak obtained with solution D (2.5%). The sum of the areas of all the peaks, other than the principal peak or any peak corresponding to the solvent, is not greater than 4 times the peak corresponding to clindamycin phosphate obtained with solution D (4.0%).

**Assay.** Determine as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (25 cm × 4.6 mm) packed with *stationary phase A* (5–10 µm). As the mobile phase, use a mixture of 8 volumes of potassium dihydrogen phosphate (13.6 g/l) TS adjusted to pH 2.5 with phosphoric acid (~105 g/l) TS and 2 volumes of acetonitrile R.

Prepare the following solutions in the mobile phase: solution (A) 3.0 mg of Clindamycin phosphate per ml; solution (B) 3.0 mg of clindamycin phosphate RS per ml; for solution (C) dissolve 5 mg of lincomycin hydrochloride RS and 15.0 mg of clindamycin hydrochloride RS in 5.0 ml of solution B and dilute with

sufficient mobile phase to produce 100 ml; and for solution (D) dilute 1.0 ml of solution B with sufficient mobile phase to produce 100 ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 210 nm.

Inject 20 µl of solution C.

The assay is not valid unless the first peak (lincomycin) is clearly separated from the solvent peak, and the resolution between the second peak (clindamycin phosphate) and the third peak (clindamycin) is at least 6.0. The assay is valid only if the symmetry factor of the clindamycin phosphate peak is not greater than 1.5.

Inject 20 µl of solution B. If necessary adjust the integrator parameters.

Inject alternately 20 µl each of solutions A and B.

Measure the areas of the peak responses obtained with solutions A and B, and calculate the percentage content of  $C_{18}H_{34}ClN_2O_8PS$ .

### ***Additional requirements for Clindamycin phosphate for parenteral use***

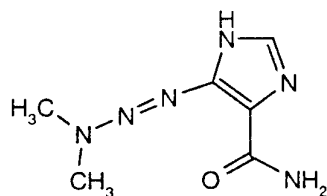
*Complies with the monograph for "Parenteral preparations" (see Vol. 4, p. 36).*

**Bacterial endotoxins.** Carry out the test as described under "Test for bacterial endotoxins" (p. 30); contains not more than 0.6 IU of endotoxin RS per mg of clindamycin.

**Sterility.** Complies with the "Sterility testing of antibiotics" (Vol. 1, p. 152), applying the membrane filtration test procedure and using a solution in water containing 150 mg of Clindamycin phosphate per ml.

## *Dacarbazinum*

### *Dacarbazine*



$C_6H_{10}N_6O$

**Relative molecular mass.** 182.2

**Chemical name.** 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide; 5-(3,3-dimethyl-1-triazenyl)-1*H*-imidazole-4-carboxamide; CAS Reg. No. 4342-03-4.

**Description.** A colourless or pale yellow, crystalline powder.

**Solubility.** Slightly soluble in water and ethanol (~750 g/l) TS.

**Category.** Cytotoxic drug.

**Storage.** Dacarbazine should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 8 °C.

**Additional information.** *CAUTION:* Dacarbazine must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

## Requirements

Dacarbazine contains not less than **97.0%** and not more than **102.0%** of  $C_6H_{10}N_6O$ , calculated with reference to the dried substance.

### Identity tests

- *Either test A alone or tests B, C, and D may be applied.*
- Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from dacarbazine RS or with the *reference spectrum* of dacarbazine.
  - The absorption spectrum of a 6 µg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 323 nm and a pronounced shoulder at 275 nm. The absorbance of a 1-cm layer at the maximum wavelength of 323 nm is about 0.64.
  - Dissolve 25 mg in 5 ml of water, add 1 drop of cobalt(II) chloride (30 g/l) TS and 1 drop of ammonia (~100 g/l) TS; a violet-red solution is produced.
  - Dissolve 25 mg in 5 ml of hydrochloric acid (~70 g/l) TS, add about 0.2 g of zinc R powder and allow to stand for 5 minutes. Filter, and to the filtrate add 3 drops of sodium nitrite (10 g/l) TS and 0.5 ml of ammonium sulfamate (5 g/l) TS. After the reaction has subsided add 5 drops of

*N*-(1-naphthyl)ethylenediamine hydrochloride/ethanol TS; a deep red solution is produced.

**Clarity and colour of solution.** A solution of 0.20 g in 10 ml of citric acid (20 g/l) TS is clear and not more intensely coloured than standard colour solution Yw2 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry at 60 °C to constant mass under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5 mg/g.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R2 as the coating substance and 5 volumes of 1-butanol R, 2 volumes of water and 1 volume of acetic acid (~300 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of the 3 following solutions in methanol R containing (A) 0.04 g of Dacarbazine per ml, (B) 0.4 mg of dacarbazine related compound A RS per ml, and (C) 0.4 mg of dacarbazine related compound B RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense or greater in size than that obtained with solution B (1%) and solution C (1%).

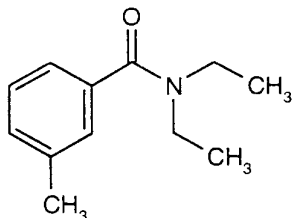
### **Assay**

*Note:* The solutions must be protected from light throughout the assay.

Dissolve about 30 mg, accurately weighed, in sufficient hydrochloric acid (0.1 mol/l) VS to produce 50 ml of stock solution. For solution S<sub>1</sub> dilute 1.0 ml of the stock solution to 100 ml with hydrochloric acid (0.1 mol/l) VS. For solution S<sub>2</sub> dilute a further 1.0 ml aliquot of the stock solution to 100 ml with phosphate buffer, pH 7.0, TS. Measure the absorbance of a 1-cm layer of solution S<sub>1</sub> at the maximum at about 323 nm against a solvent cell containing hydrochloric acid (0.1 mol/l) VS. Measure the absorbance of a 1-cm layer of solution S<sub>2</sub> at the maximum at about 329 nm against a solvent cell containing phosphate buffer, pH 7.0, TS. Calculate the percentage content of C<sub>6</sub>H<sub>10</sub>N<sub>6</sub>O.

*Diethyltoluamidum*

*Diethyltoluamide*



C<sub>12</sub>H<sub>17</sub>NO

**Relative molecular mass.** 191.3

**Chemical name.** *N,N*-Diethyl-*m*-toluamide; *N,N*-diethyl-3-methylbenzamide; CAS Reg. No. 134-62-3.

**Description.** Colourless or faintly yellow liquid.

**Solubility.** Practically immiscible in water and glycerol R; miscible with ethanol (~750 g/l) TS and ether R.

**Category.** Insect repellent.

**Storage.** Diethyltoluamide should be kept in a tightly closed container.

**Additional information.** *CAUTION:* Diethyltoluamide is an irritant to eyes and mucous membranes.

## Requirements

Diethyltoluamide contains not less than **97.0%** and not more than **103.0%** of C<sub>12</sub>H<sub>17</sub>NO, calculated with reference to the anhydrous substance.

### Identity tests

- *Either test A alone or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from diethyltoluamide RS or with the *reference spectrum* of diethyltoluamide.
- B. Refractive index,  $n_D^{20} = 1.520\text{--}1.524$ .

- C. To about 2 ml, add 25 ml of hydrochloric acid (~250 g/l) TS and heat under a reflux condenser for 1 hour. Neutralize the solution with sodium hydroxide (~200 g/l) TS, cool, and extract with three quantities, each of 30 ml, of ether R. (Keep the aqueous layer for test D.) Carefully evaporate the ether layer to dryness on a water-bath, and dissolve the residue in 5 ml of sodium nitrite (100 g/l) TS. Allow to stand at 5 °C for 10 minutes, add 10 ml of water, and extract with 20 ml of ether R. Evaporate the ether layer and add to the residue 1.0 g of phenol R. Cool and add about 1 ml of sulfuric acid (~1760 g/l) TS; an intense green solution is produced. Pour the mixture into water; the colour turns to red. Add sodium hydroxide (~80 g/l) TS; the colour changes to green.
- D. Acidify the aqueous layer obtained in test C with hydrochloric acid (~70 g/l) TS, extract with two quantities, each of 20 ml of ether R, and carefully evaporate the ether layer. Dry the residue at 60 °C; the melting temperature of the residue is about 108 °C.

**Mass density.**  $\rho_{20} = 0.996\text{--}1.002$ .

**Sulfated ash.** Not more than 1.0 mg/g.

**Water.** Determine as described under “Determination of water by the Karl Fischer method”, Method A (Vol. 1, p. 135), using about 0.5 g of the substance; the water content is not more than 5.0 mg/g.

**Acidity.** Dissolve 10.0 g in 50 ml of neutralized ethanol TS, titrate with sodium hydroxide (0.01 mol/l) VS using phenolphthalein/ethanol TS as indicator; not more than 4.0 ml of sodium hydroxide (0.01 mol/l) VS is required to obtain the midpoint of the indicator (pink).

**Assay.** Carry out Method A as described under “Determination of nitrogen” (Vol. 1, p. 136), using about 0.3 g, accurately weighed, and 7 ml of nitrogen-free sulfuric acid (~1760 g/l) TS, and proceed with the distillation. Titrate with sulfuric acid (0.05 mol/l) VS using methyl red/ethanol TS as indicator. Repeat the procedure without the Diethyltoluamide being examined and make any necessary corrections.

Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 19.13 mg of  $C_{12}H_{17}NO$ .

### *Dinitrogenii oxidum*

### *Dinitrogen oxide*

$N_2O$

**Relative molecular mass.** 44.01

**Chemical name.** Nitrous oxide; CAS Reg. No. 10024-97-2.

**Other name.** Nitrous oxide.

**Description.** A colourless gas; odourless.

**Solubility.** One volume dissolves in about 1.5 volumes of water at a pressure of 101.3 kPa and a temperature of 20 °C.

**Category.** Inhalational anaesthetic gas.

**Storage.** Dinitrogen oxide should be kept as compressed gas or liquid at very low temperatures, in appropriate containers complying with the safety regulations of the national authority.

**Labelling.** An ISO standard<sup>1</sup> requires that cylinders containing Dinitrogen oxide intended for medical use should bear the name of the contents in legible and permanent characters and, preferably, also the molecular formula N<sub>2</sub>O.

**Additional information.** In the analysis of medicinal gases certain tests are not intended for hospital pharmacists. They are applicable solely by laboratories equipped with specialized apparatus.

Valves or taps should not be lubricated with oil or grease. It is recommended that cylinders marked as described above are not used for other gases.

## **Requirements**

Dinitrogen oxide contains not less than **98.0% v/v** of N<sub>2</sub>O in the gaseous phase, when sampled at 15 °C.

*Note:* If the test is performed on a cylinder, keep the cylinder of the gas to be examined at room temperature for at least 6 hours before carrying out the tests. Keep the cylinder in the vertical position with the outlet valve uppermost.

The test for carbon monoxide should be carried out on the first portion of gas drawn from the container and the tests for nitrogen monoxide and nitrogen dioxide immediately thereafter.

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<sup>1</sup> *International Standard 32. Gas cylinders for medical use – marking for identification content.* International Organization for Standardization, Switzerland, 1977.

### Identity tests

- Either test A alone or tests B, C, and D may be applied.
- A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the *reference spectrum* of dinitrogen oxide.
  - B. Place a glowing splinter of wood into the gas; the splinter bursts into flame.
  - C. Shake the gas with alkaline pyrogallol TS; it is not absorbed and the solution does not become brown (distinction from oxygen).
  - D. Mix the gas with an equal volume of nitrogen monoxide R; no red fumes are produced (distinction from oxygen).

### Carbon monoxide

- Either test A, test B, or test C may be applied.

*Note:* The tests should be carried out on the first portion of gas released from the container.

- A. The apparatus (Fig. 6) consists of the following parts connected in series:
  - a U-tube (U1) containing desiccant silica gel R impregnated with chromium trioxide R;
  - a wash bottle (F1) containing 100 ml of potassium hydroxide (~400 g/l) TS;
  - a U-tube (U2) containing pellets of potassium hydroxide R;
  - a U-tube (U3) containing phosphorus pentoxide R dispersed on previously granulated, fused pumice;
  - a U-tube (U4) containing 30 g of recrystallized iodine pentoxide R in granules, previously dried at 200 °C and kept at a temperature of 120 °C (T) during the test. The iodine pentoxide is packed in the tube in 1-cm columns separated by 1-cm columns of glass wool to give an effective length of 5 cm;
  - a reaction tube (F2) containing 2.0 ml of potassium iodide (160 g/l) TS and 0.15 ml of starch TS.

Flush the apparatus with 5.0 litres of argon R. If necessary, discharge the blue colour in tube F2 containing potassium iodide (160 g/l) TS by adding a sufficient volume of freshly prepared sodium thiosulfate (0.002 mol/l) VS. Continue flushing with gas until not more than 0.045 ml of sodium thiosulfate (0.002 mol/l) VS is required after the passage of 5.0 litres of argon R. Pass 5.0 litres of Dinitrogen oxide from the container through the apparatus. Flush the last traces of liberated iodine into the reaction tube by passing 1.0 litre of argon R through the apparatus. Titrate the liberated iodine with

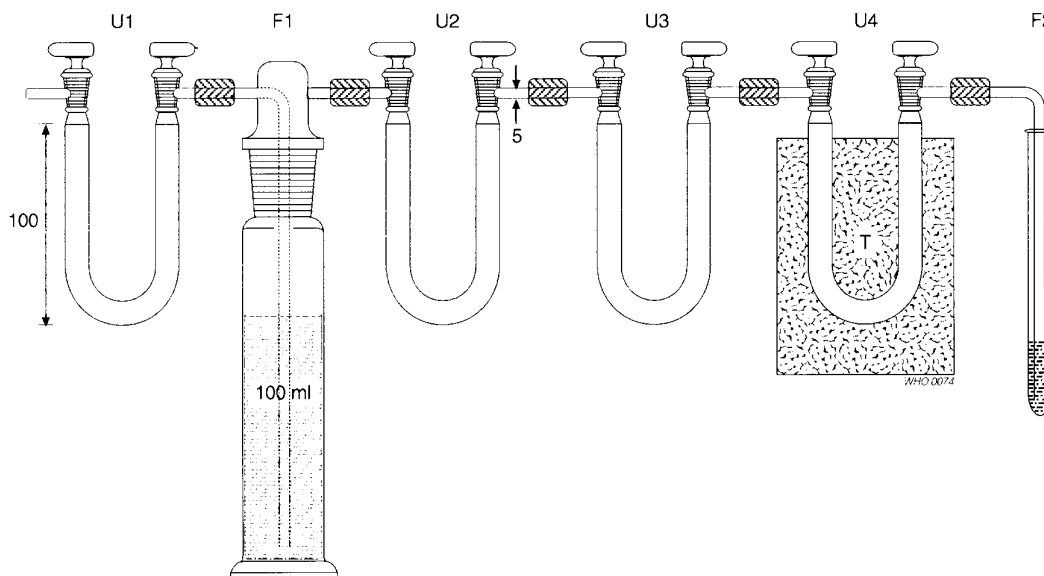


Figure 6. Apparatus for the determination of carbon monoxide in medicinal gases  
Measurements in mm.

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European Directorate for the Quality of Medicines, Council of Europe.

sodium thiosulfate (0.002 mol/l) VS. Repeat the procedure using 5.0 litres of argon R.

The difference between the volumes of sodium thiosulfate (0.002 mol/l) VS used in the titrations is not more than 0.25 ml (5 µl/l).

- B. Carry out the test as described under “Gas chromatography” (Vol. 1, p. 94), using a stainless steel column (2 m × 4 mm) packed with a 0.5- nm molecular sieve (e.g. X13, obtainable from a commercial source). Maintain the column at 80 °C, and the injection port and the detector at room temperature. Use helium R as the carrier gas at a flow rate of 60 ml per minute, and a helium ionization detector.

Use the following gases: (1) Dinitrogen oxide; and (2) a mixture containing 5 µl of carbon monoxide R in 1 litre of dinitrogen oxide R as the reference gas.

Inject a suitable volume of both gases (1) and (2). Adjust the volume, as well as the conditions specified above, to produce a peak response for carbon monoxide obtained with the reference gas (2) that gives a height of not less than 5% on the recorder.

Measure the areas of the peak responses obtained in the chromatograms from injections 1 and 2 and calculate the content of carbon monoxide in

Dinitrogen oxide (1) by comparing with the peak response for carbon monoxide obtained from the reference gas (2); not more than 5 µl/l.

- C. Determine the content using a carbon monoxide detector tube. Pass the required volume of Dinitrogen oxide through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of Dinitrogen oxide to purge the tubing to an appropriate flow. Fit the carbon monoxide detector tube to the metering pump according to the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and pump a suitable volume of Dinitrogen oxide through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 5 µl/l.

*Note:* For the following tests – Nitrogen monoxide and nitrogen dioxide, Carbon dioxide Test A, Halogens and hydrogen sulfide, and Acidity and alkalinity – pass the gas to be tested through the appropriate reagent contained in a hermetically closed flat-bottomed glass cylinder, with dimensions such that 50 ml of liquid reaches a height of 12–14 cm, that is fitted with (a) a delivery tube terminated by a capillary 1 mm in internal diameter and placed within 2 mm of the bottom of the cylinder; and (b) an outlet tube.

Prepare the reference solutions in identical cylinders.

### **Nitrogen monoxide and nitrogen dioxide**

- *Either test A or test B may be applied.*

*Note:* This test should be performed after release of the 5.0 litres of gas as described above under "Carbon monoxide, test A".

- A. Pass Dinitrogen oxide through two of the cylinders connected in series as described above under "Carbon monoxide, test A". To obtain the liquid phase invert the gas cylinder; the liquid vaporizes on leaving the valve.

To 50 ml of water add 1.2 ml of sulfuric acid (~1760 g/l) TS and dilute with sufficient water to produce 100 ml. To 15 ml of this solution add 375 mg of potassium permanganate R, mix, and transfer to the first cylinder (*solution A*).

Dissolve 1 g of sulfanilic acid R in a mixture of 180 ml of water and 10 ml of glacial acetic acid R (*solution 1*). Separately dissolve 0.2 g of *N*-(1-naphthyl)ethylenediamine hydrochloride R in a mixture of 4 ml of glacial acetic acid R and 5 ml of water, heat gently, and dilute to 200 ml with water

(solution 2). Mix 1 volume of solution 2 with 9 volumes of solution 1 and transfer 20 ml of this mixture to the second cylinder (*solution B*).

Connect the outlet tube of the first cylinder to the delivery tube of the second cylinder containing solution B. Pass 2.5 litres of Dinitrogen oxide through the reagents at a rate of 15.0 litres per hour.

Prepare a reference solution by adding 0.25 ml of a solution containing 61.6 µg/ml of sodium nitrite R in water to 20 ml of solution B as prepared above. Allow the test solution and reference solution to stand for 10 minutes.

Examine the gaseous and the liquid phases separately.

For both gaseous and liquid phases, any red colour produced from the solution of Dinitrogen oxide is not more intense than that from the reference solution (2 µl/l of NO + NO<sub>2</sub>).

- B. Determine the content using a nitrogen monoxide and nitrogen dioxide detector tube. Pass the required volume of Dinitrogen oxide through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of Dinitrogen oxide to purge the tubing to an appropriate flow. Fit the nitrogen monoxide and nitrogen dioxide detector tube to the metering pump following the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and pump a suitable volume of Dinitrogen oxide through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 2 µl/l.

### **Carbon dioxide**

- *Either test A, test B, or test C may be applied.*

- A. Pass 1.0 litre of Dinitrogen oxide through 50 ml of a clear solution of barium hydroxide (0.15 mol/l) VS. Similarly prepare a reference solution by adding 1.0 ml of a 1.1 mg/ml solution of sodium hydrogen carbonate R in carbon-dioxide-free water R to 50 ml of barium hydroxide (0.15 mol/l) VS.

Any turbidity in the solution after the passage of the Dinitrogen oxide is not more intense than that of the reference solution (300 µl/l).

- B. Carry out the test as described under "Gas chromatography" (Vol. 1, p. 94), using a stainless steel column (3.5 m × 2 mm) packed with ethylvinyl-

benzenedivinybenzene copolymer. Maintain the column at 40 °C and the detector at 90 °C. Use helium R as the carrier gas at a flow rate of 15 ml per minute, and a thermal conductivity detector.

Use the following gases: (1) Dinitrogen oxide; and (2) a mixture containing 300 µg of carbon dioxide R in 1 litre of dinitrogen oxide R as the reference gas.

Inject a suitable volume of both gases (1) and (2). Adjust the volume, as well as the conditions specified above, to obtain a peak response for carbon dioxide obtained with the reference gas (2) of a height of not less than 35% on the recorder.

Measure the areas of the peak responses obtained in the chromatograms from the injections of gases 1 and 2 and calculate the content of carbon dioxide in Dinitrogen oxide (1) by comparing with the peak response for carbon dioxide obtained from the reference gas (2); not more than 300 µl of CO<sub>2</sub> per litre.

- C. Determine the content using a carbon dioxide detector tube. Pass the required volume of Dinitrogen oxide through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a suitable pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of Dinitrogen oxide to purge the tubing to an appropriate flow. Fit the carbon dioxide detector tube to the metering pump according to the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and pump a suitable volume of Dinitrogen oxide through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 300 µl/l.

**Halogens and hydrogen sulfide.** Pass 20.0 litres of Dinitrogen oxide through a mixture of 1 ml of silver nitrate (40 g/l) TS and 49 ml of water at a flow rate not exceeding 15 litres per hour.

Prepare the reference solution as follows: to 1.0 ml of silver nitrate (40 g/l) TS add 40 ml of chloride standard (5 µg/ml) TS and 0.15 ml of nitric acid (~130 g/l) TS, dilute to 50 ml with water, and allow to stand protected from light for 5 minutes. For the blank solution, repeat the procedure passing Dinitrogen oxide through 50 ml of water.

Compare a 100-mm layer of the solution as described under "Colour of liquids" (Vol. 1, p. 50).

The solution of Dinitrogen oxide does not darken when compared with the blank. Any opalescence is not more intense than that of the reference solution (10 µg Cl per litre of dinitrogen oxide).

### **Water**

- *Either test A or test B may be applied.*

A. The apparatus consists of either an electrolytic hygrometer as described below, an appropriate humidity detector tube, or a capacity hygrometer.

The measuring cell consists of a thin film of phosphoric anhydride placed between two coiled platinum wires which act as electrodes. The water vapour in Dinitrogen oxide is absorbed by the phosphoric anhydride to form phosphoric acid which acts as an electrical conductor.

Before introducing Dinitrogen oxide into the device, allow the gas to stabilize at room temperature and make sure that the temperature is constant throughout the apparatus. Apply a continuous voltage across the electrodes to produce electrolysis of the water and regeneration of phosphoric anhydride. Measure the resulting electric current, which is proportional to the water content in Dinitrogen oxide. (This is a self-calibrating system that obeys Faraday's law.)

Calculate the content of water; not more than 60 µg/l.

B. Determine the content using a water vapour detector tube. Pass the required volume of Dinitrogen oxide through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a suitable pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of Dinitrogen oxide to purge the tubing to an appropriate flow. Fit the water vapour detector tube to the metering pump according to the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and pump a suitable volume of Dinitrogen oxide through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 60 µl/l.

**Acidity and alkalinity.** Pass 2.0 litres of Dinitrogen oxide through a mixture of 0.10 ml of hydrochloric acid (0.01 mol/l) VS and 50 ml of carbon-dioxide-free water R.

For *reference solution 1*, use 50 ml of carbon-dioxide-free water R. For *reference solution 2*, use a mixture of 0.20 ml of hydrochloric acid (0.01 mol/l) VS and 50 ml of carbon-dioxide-free water R.

To each solution add 0.1 ml of methyl red/ethanol TS; the intensity of the colour in the Dinitrogen oxide solution is between that of reference solutions 1 and 2.

**Assay.** Determine as described under "Gas chromatography" (Vol. 1, p. 94), using a stainless steel column (2 m × 2 mm) packed with silica gel for chromatography R (250–355 μm). Maintain the column at 60 °C and the detector at 130 °C. Use helium R as the carrier gas at a flow rate of 50 ml per minute, and a thermal conductivity detector.

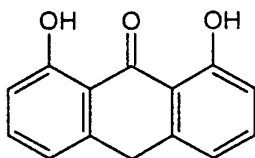
Use the following gases: (1) Dinitrogen oxide; and (2) dinitrogen oxide R as the reference gas.

Inject a suitable volume of both gases (1) and (2). Adjust the volume, as well as the conditions specified above, to produce a peak response for dinitrogen oxide obtained with reference gas (2) that gives a height of not less than 35% on the recorder.

Measure the areas of the peak responses obtained in the chromatograms from the injections of gases (1) and (2), and calculate the percentage content of Dinitrogen oxide.

## *Dithranolum*

### *Dithranol*



**Relative molecular mass.** 226.2

**Chemical name.** 1,8,9-Anthratriol; CAS Reg. No. 1143-38-0.

**Other name.** Anthralin.

**Description.** A yellow or brownish yellow, crystalline powder.

**Solubility.** Practically insoluble in water; soluble in dichloromethane R; sparingly soluble in acetone R; slightly soluble in ethanol (~750 g/l) TS and ether R.

**Category.** Keratolytic agent.

**Storage.** Dithranol should be kept in a tightly closed container, protected from light.

## Requirements

Dithranol contains not less than **98.5%** and not more than **101.0%** of  $C_{14}H_{10}O_3$ , calculated with reference to the dried substance.

## Identity tests

- *Either tests A and D or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from dithranol RS or with the *reference spectrum* of dithranol.
- B. The absorption spectrum of a 10 µg/ml solution in dichloromethane R, when observed between 250 nm and 450 nm, exhibits 3 maxima at about 256 nm, 288 nm, and 356 nm. The absorbance of a 1-cm layer at the maximum wavelength at 356 nm is about 0.46 and at 288 nm about 0.49.
- C. Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R3 as the coating substance and a mixture of equal volumes of hexane R and dichloromethane R as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in dichloromethane R containing (A) 1.0 mg of Dithranol per ml, (B) 1.0 mg of dithranol RS, and for solution (C) dissolve 5 mg of dantron R in 5 ml of solution B. After removing the plate from the chromatographic chamber, allow it to dry in air. Place the plate in a chamber saturated with ammonia vapour until the spots appear. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The test is not valid unless the chromatogram obtained with solution C shows two clearly separated spots.

D. Melting temperature, about 180°C.

**Chlorides.** Dissolve 2.5 g in a mixture of 2.0 ml of nitric acid (~130 g/l) TS and 30 ml of water, and proceed as described under “Limit test for chlorides” (Vol. 1, p. 116); the chloride content is not more than 0.1 mg/g.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry to constant mass at 105 °C; it loses not more than 5 mg/g.

**pH value.** Shake 1.5 g with 30 ml of carbon-dioxide-free water R for 1 minute and filter; pH of the filtrate, 6.0–7.6.

### Related substances

A. Carry out the test as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (25 cm × 4.6 mm) packed with *stationary phase C* (5 μm). As the mobile phase, use a mixture of 82 volumes of hexane R, 5 volumes of dichloromethane R, and 1 volume of glacial acetic acid R.

Prepare the following solutions. For solution (A) dissolve 0.20 g of Dithranol in 20 ml of dichloromethane R, add 1.0 ml of glacial acetic acid R, and dilute to 100 ml with hexane R. For solution (B) dissolve 10.0 mg of each of anthrone R, dantron R, 9,9'-bisanthracene-10,10'(9*H*,9'*H*)-dione RS, and dithranol RS in dichloromethane R, and dilute to 10.0 ml with the same solvent. To 1.0 ml of this solution add 19 ml of dichloromethane R and 1.0 ml of glacial acetic acid R, and dilute to 50 ml with hexane R.

Operate with a flow rate of 2.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 260 nm.

Inject 20 μl each of solutions A and B. Continue the chromatography for 1.5 times the retention time of the peak due to 9,9'-bisanthracene-10,10'(9*H*,9'*H*)-dione obtained with solution B. Adjust the sensitivity of the system so that the height of the peak due to dithranol in the chromatogram obtained with solution B is about 70% of the full scale of the recorder. The peaks are eluted in the following order: dithranol, dantron, anthrone and 9,9'-bisanthracene-10,10'(9*H*,9'*H*)-dione. The test is not valid unless, in the chromatogram obtained with solution B, the resolution between the peaks due to dithranol and dantron is greater than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak corresponding to anthrone, dantron or 9,9'-bisanthracene-10,10'(9*H*,9'*H*)-dione is not greater than that of the corresponding peak in the chromatogram obtained with solution B (1.0%). The area of any peak, other than the principal peak and any peaks due to anthrone, dantron or 9,9'-bisanthracene-10,10'(9*H*,9'*H*)-dione, is not greater than that of

the peak due to dithranol in the chromatogram obtained with solution B (1.0%).

- B. Carry out the test as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (20 cm × 4.6 mm) packed with *stationary phase A* (5 μm). As the mobile phase, use a mixture of 60 volumes of water, 40 volumes of tetrahydrofuran R, and 2.5 volumes of glacial acetic acid R.

Prepare the following solutions in the mobile phase: solution (A) 1.0 mg of Dithranol per ml; and for solution (B) dissolve 0.5 mg of 1-hydroxy-9-anthrone RS and 0.5 mg of dithranol RS per ml, and dilute 1.0 ml of this solution to 20 ml with the mobile phase.

Operate with a flow rate of about 0.9 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject 20 μl each of solutions A and B. Continue the chromatography for 3 times the retention time of the peak due to dithranol. The test is not valid unless, in the chromatogram obtained with solution B, the resolution between the peaks due to 1-hydroxy-9-anthrone and dithranol is greater than 2.5.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak corresponding to 1-hydroxy-9-anthrone is not greater than that of the corresponding peak in the chromatogram obtained with solution B (2.5%).

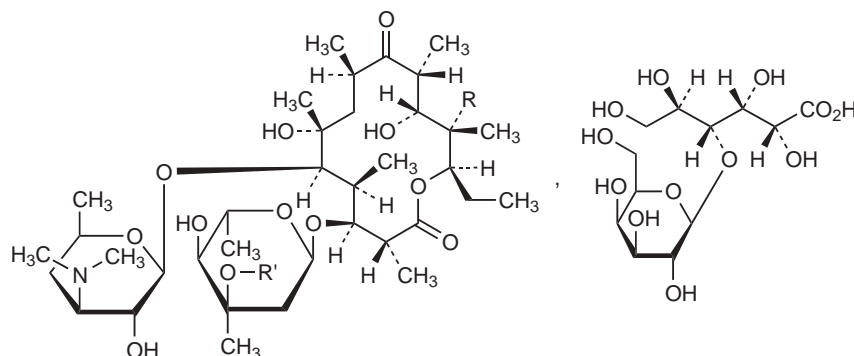
The total content of related substances as determined in tests A and B is not more than 3.0%.

**Assay.** Dissolve about 0.2 g, accurately weighed, in 50 ml of anhydrous pyridine R and titrate under an atmosphere of nitrogen with tetrabutylammonium hydroxide (0.1 mol/l) VS as described under “Non-aqueous titration”, Method B (Vol. 1, p. 132), determining the end-point potentiometrically.

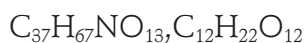
Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 22.62 mg of C<sub>14</sub>H<sub>10</sub>O<sub>3</sub>.

*Erythromycini lactobionas*

*Erythromycin lactobionate*



Erythromycin	R	R'
A	OH	CH <sub>3</sub>
B	H	CH <sub>3</sub>
C	OH	H



**Relative molecular mass.** 1092

**Chemical name.** Erythromycin lactobionate (1:1) (salt); erythromycin mono(4-O-β-D-galactopyranosyl-D-gluconate) (salt); CAS Reg. No. 3847-29-8.

**Description.** White or slightly yellow crystals or a white, crystalline powder; odour, faint.

**Solubility.** Freely soluble in water, ethanol (~750 g/l) TS and methanol R; slightly soluble in acetone R; practically insoluble in ether R.

**Category.** Antibacterial drug.

**Storage.** Erythromycin lactobionate should be kept in a tightly closed container, protected from light.

**Additional information.** Each mg of erythromycin lactobionate is equivalent to 0.6722 mg of erythromycin.

### Identity tests

- Either test A alone or tests B, C, and D may be applied.
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from erythromycin lactobionate RS or with the *reference spectrum* of erythromycin lactobionate.
- B. See the test described below under “Related substances”. One of the two principal spots obtained with solution A corresponds in position with the principal spot obtained with solution B. The other principal spot corresponds in position with the principal spot obtained with solution D.
- C. Dissolve 20 mg in 2.0 ml of water and cautiously add about 1 ml of sulfuric acid (~1760 g/l) TS to form a lower layer; a red-brown ring appears at the interface of the two liquids. Shake; a dark red-brown solution is produced.
- D. Dissolve about 10 mg in 5 ml of hydrochloric acid (~250 g/l) TS; a yellowish green colour develops.

**Clarity and colour of solution.** A solution of 0.85 g in 10 ml of water is clear or not more opalescent than opalescence standard TS1, and colourless or not more intensely coloured than standard colour solution Yw1 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Water.** Determine as described under “Determination of water by the Karl Fischer method”, Method A (Vol. 1, p. 135), using about 0.5 g of Erythromycin lactobionate; the water content is not more than 0.050 g/g.

**Sulfated ash.** Not more than 20 mg/g.

**pH value.** pH of a 0.05 g/ml solution in carbon-dioxide-free water R, 6.0–7.5.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silanized silica gel R3 as the coating substance and a mixture of 5 volumes of methanol R and 3 volumes of ammonium acetate (50 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 4 solutions in methanol R containing (A) 3 mg of Erythromycin lactobionate per ml, (B) 2 mg of erythromycin RS per ml, (C) 0.10 mg of erythromycin RS per ml, and (D) 0.66 mg of lactobionic acid R per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray with anisaldehyde TS, heat at 110 °C for 5 minutes, and allow to cool. Examine the chromatogram in daylight.

Disregard the spot corresponding to lactobionic acid. Any spot obtained with solution A, other than the principal spot and any spot with a lower  $R_f$ -value,

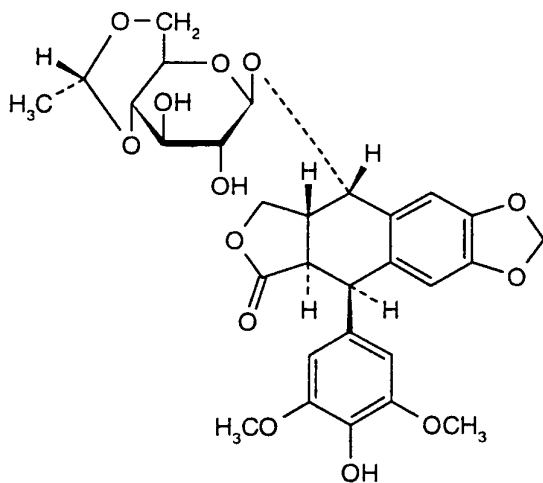
is not more intense than that obtained with solution B. Any spot obtained with solution A, other than the principal spot and any spot with a higher  $R_f$ -value, is not more intense than that obtained with solution C.

**Assay.** Carry out the determination as described under "Microbiological assay of antibiotics" (Vol. 1, p. 145), using either (a) *Bacillus pumilus* (NCTC 8241 or ATCC 14884) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0, TS, an appropriate concentration of erythromycin (usually between 5 and 25 IU per ml), and an incubation temperature of 35–39 °C, or (b) *Micrococcus luteus* (ATCC 9341) as the test organism, culture medium Cm1 with a final pH of 8.0–8.1, sterile phosphate buffer, pH 8.0, TS1, or TS2, an appropriate concentration of erythromycin (usually between 0.5 and 1.5 IU per ml), and an incubation temperature of 32–35 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency ( $P = 0.95$ ) are not less than 95% and not more than 105% of the estimated potency.

The potency is not less than 600 IU of erythromycin per mg, calculated with reference to the anhydrous substance.

## Etoposidum

### Etoposide



$C_{29}H_{32}O_{13}$

**Relative molecular mass.** 588.6

**Chemical name.** 4'-Demethylepipodophyllotoxin 9-(4,6-O-ethylidene-β-D-glucopyranoside); [5R-[5α,5aβ,8α,9β(R\*)]]-9-[(4,6-O-ethylidene-β-D-glucos-

<http://www.femeba.org.ar/fundacion/>

pyranosyl)oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3',4':6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one; CAS Reg. No. 33419-42-0.

**Description.** A white or almost white, crystalline powder.

**Solubility.** Practically insoluble in water; sparingly soluble in methanol R; slightly soluble in ethanol (~750 g/l) TS and dichloromethane R.

**Category.** Cytotoxic drug.

**Storage.** Etoposide should be kept in a tightly closed container.

**Labelling.** The designation Etoposide for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

**Additional information.** *CAUTION:* Etoposide must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

## Requirements

Etoposide contains not less than **98.0%** and not more than the equivalent of **102.0%** of  $C_{29}H_{32}O_{13}$ , calculated with reference to the dried substance.

### Identity tests

- *Either test A alone or tests B, C, and D may be applied.*
- A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from etoposide RS or with the *reference spectrum* of etoposide.
- B. See the test described below under "Related substances". The principal band obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.
- C. Transfer about 5 mg to a test-tube and dissolve in 5 ml of glacial acetic acid R, add about 0.1 ml of ferric chloride (50 g/l) TS, and mix. Cautiously add about 2 ml of sulfuric acid (~1760 g/l) TS. Without mixing allow to stand for about 30 minutes; a pink to reddish brown ring develops at the interface and the upper layer is yellow.
- D. Dissolve 5 mg in 5 ml of methanol R, add 5 ml of hydrochloric acid (~70 g/l) TS, and evaporate to dryness on a water-bath. To the residue add 20 ml of water and 10 ml of dichloromethane R, and shake vigorously. Allow to sep-

arate and to 1.0 ml of the aqueous layer add 2.0 ml of anthrone TS2 and mix; a blue-green colour is produced.

**Specific optical rotation.** Dissolve 0.050 g in 10 ml of a mixture of 1 volume of methanol R and 9 volumes of dichloromethane R;  $[\alpha]_D^{20} = -106^\circ$  to  $-114^\circ$ , calculated with reference to the dried substance.

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 µg/g.

**Solution in methanol/dichloromethane.** Dissolve 0.6 g in 20 ml of a mixture of 1 volume of methanol R and 9 volumes of dichloromethane R; the solution is clear and not more intensely coloured than standard colour solution 2 of the most appropriate hue, when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry at 105 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 30 mg/g.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 100 volumes of dichloromethane R, 20 volumes of acetone R, 8 volumes of glacial acetic acid R, and 1.5 volumes of water as the mobile phase. Apply separately to the plate 5 µl, spread to form 10-mm bands, of each of 5 solutions in a mixture of 1 volume of methanol R and 9 volumes of dichloromethane R containing (A) 0.050 g of Etoposide per ml, (B) 5 mg of Etoposide per ml, (C) 5 mg of etoposide RS per ml, (D) 0.25 mg of etoposide RS per ml, and (E) 0.10 mg of etoposide RS per ml. After removing the plate from the chromatographic chamber, dry it in a current of warm air for 5 minutes. Spray with a mixture of 1 volume of sulfuric acid (~1760 g/l) TS and 9 volumes of ethanol (~750 g/l) TS, and heat at 140 °C for 15 minutes. Cover the plate immediately with a glass plate of the same size. Examine the chromatogram in daylight.

Any band obtained with solution A, other than the principal band, is not more intense than that obtained with solution D. Furthermore, not more than two such bands are more intense than the band obtained with solution E (0.2%).

**Assay.** Determine as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (25 cm × 4.6 mm) packed with *stationary phase A* (10 µm). Prepare a diluted solution of acetic acid as follows to be used in the mobile phase and for the preparation of solution C: to 96 ml of water

add 4 ml of glacial acetic acid R. As the mobile phase, use a mixture of 76 volumes of the diluted acetic acid solution and 24 volumes of acetonitrile R.

Prepare the following solutions in methanol R: solution (A) 1.0 mg of Etoposide per ml; solution (B) 1.0 mg of etoposide RS per ml; and for solution (C) add 0.1 ml of the diluted acetic acid solution described above and 0.1 ml of phenolphthalein/ethanol TS to 10 ml of solution A, then add sodium hydroxide (1 mol/l) VS until the solution becomes faintly pink (about 0.15 ml), allow to stand for 15 minutes, and add 0.1 ml of the diluted acetic acid solution described above.

Operate with a flow rate of 2.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 285 nm.

Inject 10 µl of solution C. Allow the chromatography to continue until the peak corresponding to phenolphthalein is eluted. Phenolphthalein has a retention time relative to etoposide of about 2.7. Disregard any peak due to phenolphthalein. The assay is not valid unless the chromatogram shows two principal peaks and the resolution between these peaks is at least 1.5. If necessary, reduce the concentration of acetonitrile R in the mobile phase or reduce the flow rate to achieve the required resolution.

Inject alternately 10 µl each of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of  $C_{29}H_{32}O_{13}$ .

### ***Additional requirements for Etoposide for parenteral use***

*Complies with monograph for "Parenteral preparations" (see Vol. 4, p. 36).*

**Bacterial endotoxins.** Carry out the test as described under "Test for bacterial endotoxins" (p. 30); contains not more than 2.0 IU of endotoxin RS per mg.

## *Heparinum calcicum*

### *Heparin calcicum*

**Composition.** Heparin calcium is a preparation containing the calcium salt of a sulfated glucosaminoglycan present in mammalian tissues. It has the characteristic property of delaying the clotting of fresh blood; CAS Reg. No. 37270-89-6.

**Description.** A white or almost white powder.

**Solubility.** Freely soluble in water.

**Category.** Anticoagulant.

**Storage.** Heparin calcium should be kept in a tightly closed container.

**Labelling.** The designation Heparin calcium for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. The label should also state the name and quantity of any added substances, and the source of the material (lung or mucosal). Expiry date.

**Additional information.** Heparin calcium may be prepared from the lungs of oxen or the intestinal mucosae of oxen, pigs, or sheep. Attention should be paid to minimize or eliminate microbiological contamination and substances lowering blood pressure. Heparin calcium is moderately hygroscopic.

## Requirements

Heparin calcium intended for the manufacture of a parenteral dosage form contains not less than **150IU per mg**, and Heparin calcium not intended for use in the manufacture of a parenteral dosage form contains not less than **120IU per mg**, both calculated with reference to the dried substance.

### Identity tests

- A. Delays the clotting of fresh blood.
- B. Specific optical rotation, use a 40 mg/ml solution;  $[\alpha]_D^{20^\circ\text{C}}$  = not less than  $+35^\circ$ .
- C. A 20 mg/ml solution yields the reactions described under "General identification tests" as characteristic of calcium (Vol. 1, p. 112).

**Heavy metals.** Use 0.5 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 30 µg/g.

**Calcium.** Proceed with about 0.2 g, accurately weighed, as described under "Complexometric titrations" for calcium (Vol. 1, p. 128). Each ml of disodium edetate (0.05 mol/l) VS is equivalent to 2.004 mg of Ca; 95–115 mg/g, calculated with reference to the dried substance. (As an alternative, determine the content of calcium by atomic absorption spectrophotometry.)

**Nitrogen.** Carry out Method A as described under “Determination of nitrogen” (Vol. 1, p. 136), using about 0.1 g, accurately weighed, and 5 ml of nitrogen-free sulfuric acid (~1760 g/l) TS. Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 1.401 mg of nitrogen; not more than 25 mg/g, with reference to the dried substance.

**Protein and nucleotidic impurities.** Measure the absorbance of a 1-cm layer of a 4 mg/ml solution at a wavelength of 260 nm and 280 nm; at 260 nm not greater than 0.20 and at 280 nm not greater than 0.15.

**Clarity and colour of solution.** A solution containing 5000 IU per ml is clear and not more intensely coloured than standard colour solution Yw3 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Sulfated ash.** Use 0.2 g; 0.32–0.40 g/g, with reference to the dried substance.

**Loss on drying.** Dry to constant mass at 60 °C under reduced pressure (not exceeding 0.5 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 0.080 g/g.

**pH value.** pH of 10 mg/ml solution in carbon-dioxide-free water R, 5.5–8.0.

**Assay.** The anticoagulant activity of heparin is determined *in vitro* using a biological assay to compare its ability to delay the clotting of recalcified citrated sheep plasma with that of the reference substance. The following method is suitable for carrying out the assay (*other methods may also be applicable*).

The onset of clotting is determined either as a change in optical density (by direct visual inspection, preferably using indirect illumination against a matt black background, or by spectrophotometry, recording at a wavelength of approximately 600 nm) or as a change in fluidity (by visual detection while manually tilting the tube or by mechanical recording while stirring, taking care to cause the minimum disturbance of the solution during the initial phase of clotting). Use appropriate tubes according to the chosen technique.

Prepare a solution of Heparin calcium and a solution of heparin RS in sodium chloride (9 g/l) TS, each containing an accurately known number of IU of heparin per ml. Using sodium chloride (9 g/l) TS prepare from each solution a series of dilutions in geometric progression such that the clotting time obtained with the lowest concentration is not less than 1.5 times the blank recalcification time, and the clotting time obtained with the highest concentration is such as to give a satisfactory log dose–response curve, as determined in a preliminary test.

Place in an ice-bath 12 labelled tubes for each dilution: T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, etc. for Heparin calcium and S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, etc. for heparin RS. To each tube add 1.0 ml of

thawed plasma substrate R and 1.0 ml of the appropriate dilution, either from Heparin calcium or heparin RS, mixing each tube carefully, and not allowing bubbles to form. (The detection technique employed may require the addition of different volumes of plasma substrate, consequently the appropriate adjustment of all tubes would be needed.) Transfer all the tubes to a water-bath at 37°C, and allow to equilibrate for about 15 minutes. Add to each tube, mixing after each addition, 1 ml of a dilution of cephalin TS and 1 ml of kaolin suspension TS freshly prepared just before use. (A suitable dilution of cephalin TS is one that, under the conditions of the assay, gives a blank recalcification time of not more than 60 seconds.) After exactly 2 minutes, add 1.0 ml of calcium chloride (3.7 g/l) TS. Record in seconds the interval between this addition and the onset of clotting, determined according to the chosen technique. Similarly determine the blank recalcification time at the beginning and at the end of the procedure, using 1.0 ml of sodium chloride (9 g/l) TS in place of one of the heparin dilutions; the two values for the blank should not differ significantly. Repeat the procedure using fresh dilutions of the initial solutions and carrying out the incubation in the reverse order (first tubes S, then tubes T).

Transform the clotting times to logarithms using the mean values for the duplicate tubes and calculate the results by standard statistical methods.

Carry out not fewer than 3 independent assays. For each assay prepare fresh solutions of Heparin calcium and heparin RS, and use a different, freshly-thawed portion of the stored plasma substrate R.

Calculate the potency of Heparin calcium by combining the results of the assays by standard statistical methods. If the variance is significant ( $P = 0.01$ ), due to differences between assays, it is possible to obtain a combined estimate by calculating the non-weighted mean of potency estimates.

The estimated potency is not less than 90% and not more than 111% of the stated potency. The fiducial limits of error of the estimated potency ( $P = 0.95$ ) are not less than 80% and not more than 125% of the stated potency.

### ***Additional requirements for Heparin calcium for parenteral use***

*Complies with the monograph for "Parenteral preparations" (see Vol. 4, p. 36).*

**Bacterial endotoxins.** Carry out the test as described under "Test for bacterial endotoxins" (p. 30); Heparin calcium intended for the manufacture of a parenteral dosage form, without further appropriate procedure for the removal of bacterial endotoxins, contains not more than 0.01 IU of endotoxin RS per IU of heparin activity. The addition of divalent cations may be necessary in order to fulfil the validation criteria.

*Heparinum natricum*

*Heparin sodium*

**Composition.** Heparin sodium is a preparation containing the sodium salt of a sulfated glucosaminoglycan present in mammalian tissues. It has the characteristic property of delaying the clotting of fresh blood; CAS Reg. No. 9041-08-1.

**Description.** A white or almost white powder.

**Solubility.** Freely soluble in water.

**Category.** Anticoagulant.

**Storage.** Heparin sodium should be kept in a tightly closed container.

**Labelling.** The designation Heparin sodium for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. The label should also state the name and quantity of any added substances, and the source of the material (lung or mucosal). Expiry date.

**Additional information.** Heparin sodium may be prepared from the lungs of oxen or the intestinal mucosae of oxen, pigs, or sheep. Attention should be paid to minimize or eliminate microbiological contamination and substances lowering blood pressure. Heparin sodium is moderately hygroscopic.

## Requirements

Heparin sodium intended for the manufacture of a parenteral dosage form contains not less than **150IU per mg**, and Heparin sodium not intended for use in the manufacture of a parenteral dosage form contains not less than **120IU per mg**, both calculated with reference to the dried substance.

## Identity tests

- A. Delays the clotting of fresh blood.
- B. Specific optical rotation, use a 40 mg/ml solution;  $[\alpha]_D^{20^\circ\text{C}}$  is not less than  $+35^\circ$ .
- C. When tested for sodium as described under "General identification tests" (Vol. 1, p. 115) yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.

**Heavy metals.** Use 0.5 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 30 µg/g.

**Sodium.** Determine by atomic absorption spectrophotometry (Vol. 1, p. 43) at a wavelength of 330.3 nm, using a sodium hollow cathode lamp and a flame of suitable composition (e.g. 11 litres of air and 2 litres of acetylene per minute). Prepare a solution of 5 mg in 10 ml of hydrochloric acid (0.1 mol/l) VS containing 1.27 mg/ml of caesium chloride R. As a reference solution use sodium standard (200 µg of Na per ml) TS and use dilutions containing 25, 50, and 75 µg of Na per ml in the same mixture of caesium chloride and hydrochloric acid as prepared above; 95–125 mg of Na per g, calculated with reference to the dried substance.

**Nitrogen.** Carry out Method A as described under “Determination of nitrogen” (Vol. 1, p. 136), using about 0.1 g, accurately weighed, and 5 ml of nitrogen-free sulfuric acid (~1760 g/l) TS. Each ml of sulfuric acid (0.05 mol/l) VS is equivalent to 1.401 mg of nitrogen; not more than 25 mg/g, with reference to the dried substance.

**Protein and nucleotidic impurities.** Measure the absorbance of a 1-cm layer of a 4 mg/ml solution at a wavelength of 260 nm and 280 nm; at 260 nm not greater than 0.20 and at 280 nm not greater than 0.15.

**Clarity and colour of solution.** A solution containing 5000 IU per ml is clear and not more intensely coloured than standard colour solution Yw3 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Sulfated ash.** Use 0.2 g; 0.30–0.43 g/g, with reference to the dried substance.

**Loss on drying.** Dry to constant mass at 60 °C under reduced pressure (not exceeding 0.5 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 0.080 g/g.

**pH value.** pH of 10 mg/ml solution in carbon-dioxide-free water R, 5.5–8.0.

**Assay.** The anticoagulant activity of heparin is determined *in vitro* using a biological assay to compare its ability to delay the clotting of recalcified citrated sheep plasma with that of the reference substance. The following method is suitable for carrying out the assay (*other methods may also be applicable*).

The onset of clotting is determined either as a change in optical density (by direct visual inspection, preferably using indirect illumination against a matt black background, or by spectrophotometry, recording at a wavelength of approximately 600 nm) or as a change in fluidity (by visual detection while

manually tilting the tube or by mechanical recording while stirring, taking care to cause the minimum disturbance of the solution during the initial phase of clotting). Use appropriate tubes according to the chosen technique.

Prepare a solution of Heparin sodium and a solution of heparin RS in sodium chloride (9 g/l) TS, each containing an accurately known number of IU of heparin per ml. Using sodium chloride (9 g/l) TS prepare from each solution a series of dilutions in geometric progression such that the clotting time obtained with the lowest concentration is not less than 1.5 times the blank recalcification time, and the clotting time obtained with the highest concentration is such as to give a satisfactory log dose–response curve, as determined in a preliminary test.

Place in an ice-bath 12 labelled tubes for each dilution:  $T_1, T_2, T_3$ , etc. for Heparin sodium and  $S_1, S_2, S_3$ , etc. for heparin RS. To each tube add 1.0 ml of thawed plasma substrate R and 1.0 ml of the appropriate dilution, either from Heparin sodium or heparin RS, mixing each tube carefully, and not allowing bubbles to be formed. (The detection technique employed may require the addition of different volumes of plasma substrate, consequently the appropriate adjustment of all tubes would be needed.) Transfer all the tubes to a water-bath at 37 °C, and allow to equilibrate for about 15 minutes. Add to each tube, mixing after each addition, 1 ml of a dilution of cephalin TS and 1 ml of kaolin suspension TS freshly prepared just before use. (A suitable dilution of cephalin TS is one that, under the conditions of the assay, gives a blank recalcification time of not more than 60 seconds.) After exactly 2 minutes, add 1.0 ml of calcium chloride (3.7 g/l) TS. Record in seconds the interval between this addition and the onset of clotting, determined according to the chosen technique. Similarly determine the blank recalcification time at the beginning and at the end of the procedure, using 1.0 ml of sodium chloride (9 g/l) TS in place of one of the heparin dilutions; the two values for the blank should not differ significantly. Repeat the procedure using fresh dilutions of the initial solutions and carrying out the incubation in the reverse order (first tubes S, then tubes T).

Transform the clotting times to logarithms using the mean values for the duplicate tubes and calculate the results by standard statistical methods.

Carry out not fewer than 3 independent assays. For each assay prepare fresh solutions of Heparin sodium and heparin RS, and use a different, freshly-thawed portion of the stored plasma substrate R.

Calculate the potency of Heparin sodium by combining the results of the assays by standard statistical methods. If the variance is significant ( $P = 0.01$ ), due to differences between assays, it is possible to obtain a combined estimate by calculating the non-weighted mean of potency estimates.

The estimated potency is not less than 90% and not more than 111% of the stated potency. The fiducial limits of error of the estimated potency ( $P = 0.95$ ) are not less than 80% and not more than 125% of the stated potency.

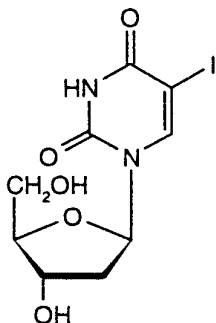
***Additional requirements for Heparin sodium for parenteral use***

*Complies with the monograph for "Parenteral preparations" (see Vol. 4, p. 36).*

**Bacterial endotoxins.** Carry out the test as described under "Test for bacterial endotoxins" (p. 30); Heparin sodium intended for the manufacture of a parenteral dosage form, without further appropriate procedure for the removal of bacterial endotoxins, contains not more than 0.01 IU of endotoxin RS per IU of heparin activity. The addition of divalent cations may be necessary in order to fulfil the validation criteria.

*Idoxuridinum*

*Idoxuridine*



**Relative molecular mass.** 354.1

**Chemical name.** 2'-Deoxy-5-iodouridine; CAS Reg. No. 54-42-2.

**Description.** A white or almost white, crystalline powder.

**Solubility.** Slightly soluble in water, ethanol (~750 g/l) TS and hydrochloric acid (~70 g/l) TS; freely soluble in sodium hydroxide (~80 g/l) TS.

**Category.** Anti-infective agent.

**Storage.** Idoxuridine should be kept in a well-closed container, protected from light.

**Labelling.** The designation Idoxuridine for sterile non-injectable use indicates that the substance complies with the additional requirement and may be used for sterile applications. Expiry date.

**Additional information.** Melting temperature, about 180 °C, with decomposition.

## Requirements

Idoxuridine contains not less than **98.0%** and not more than **101.0%** of  $C_9H_{11}N_2O_5$ , calculated with reference to the dried substance.

## Identity tests

- *Either test A alone or tests B, C, and D may be applied.*
- Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from idoxuridine RS or with the *reference spectrum* of idoxuridine.
  - Dissolve about 2 mg in 50 ml of sodium hydroxide (0.01 mol/l) VS; the absorption spectrum, when observed between 230 nm and 350 nm, exhibits a maximum at about 279 nm.
  - See the test described below under “Related substances”. The principal spot obtained with solution D corresponds in position, appearance, and intensity to that obtained with solution E.
  - Heat about 5 mg in a test-tube over an open flame; violet vapours are evolved.

**Specific optical rotation.** Use a 0.10 g/ml solution in sodium hydroxide (1 mol/l) VS and determine the rotation immediately after preparation. Calculate with reference to the dried substance;  $[\alpha]_D^{20} = +28^\circ$  to  $+32^\circ$ .

**Iodine and iodide.** For solution (A) dissolve 0.10 g in a mixture of 20 ml of water and 5 ml of sodium hydroxide (~80 g/l) TS, and immediately add 5 ml of sulfuric acid (~100 g/l) TS. Cool in an ice-bath, allow to stand for 10 minutes, shaking occasionally, and filter. For solution (B) dissolve 0.111 g of potassium iodide R in sufficient water to produce 1000 ml. To 1.0 ml of this solution add 19 ml of water, 5 ml of sodium hydroxide (~80 g/l) TS, and 5 ml of sulfuric acid (~100 g/l) TS, mix, and filter. Transfer both filtrates from solutions A and B to

separate comparison tubes, to each add 10 ml of dichloromethane R and 3 drops of potassium iodate (0.05 mol/l) VS, shake for 30 seconds, and allow to stand. The colour in the dichloromethane layer of solution A is not more intense than that produced with solution B when compared as described under "Colour of Liquids" (Vol. 1, p. 50).

**Solution in alkali.** A solution of 0.10 g in 10 ml of sodium hydroxide (1 mol/l) VS is clear and colourless.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry about 1 g to constant mass at 60°C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) over phosphorus pentoxide R; it loses not more than 10 mg/g.

**Related substances.** Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 5 volumes of 2-propanol R, 4 volumes of dichloromethane R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions in a mixture of 5 volumes of methanol R and 1 volume of ammonia (~260 g/l) TS containing (A) 40 mg of Idoxuridine per ml, (B) 0.20 mg of Idoxuridine per ml, (C) 0.10 mg of Idoxuridine per ml, (D) 4 mg of Idoxuridine per ml, and (E) 4 mg of idoxuridine RS per ml. After removing the plate from the chromatographic chamber, dry it in a current of cold air and repeat the development. After removing the plate following the second development from the chromatographic chamber, dry it in a current of cold air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Not more than 3 such spots are more intense than the spot obtained with solution C (0.25%).

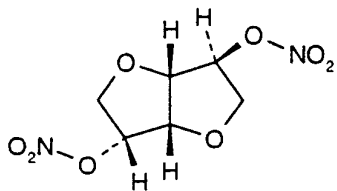
**Assay.** Dissolve about 0.3 g, accurately weighed, in 50 ml of dimethylformamide R and titrate with tetrabutylammonium hydroxide (0.1 mol/l) VS as described under "Non-aqueous titration", Method B (Vol. 1, p. 132), determining the end-point potentiometrically.

Each ml of tetrabutylammonium hydroxide (0.1 mol/l) VS is equivalent to 35.41 mg of  $C_9H_{11}IN_2O_5$ .

### ***Additional requirement for sterile non-injectable Idoxuridine***

*Complies with the "Test for sterility of non-injectable preparations" (see page 32).*

*Isosorbidi dinitras dilutus*  
*Diluted Isosorbide dinitrate*



**CAUTION:** To permit safe handling, Isosorbide dinitrate is mixed with a suitable inert diluent, such as lactose or mannitol.

Appropriate precautions in handling undiluted Isosorbide dinitrate need to be observed, since it can explode if subjected to percussion or excessive heat. Only exceedingly small amounts should be isolated.

**Relative molecular mass.** 236.1

**Chemical name.** 1,4:3,6-Dianhydrosorbitol 2,5-dinitrate; CAS Reg. No. 87-33-2.

**Description.** Undiluted Isosorbide dinitrate is a fine, white, crystalline powder.

**Solubility.** Undiluted Isosorbide dinitrate is very slightly soluble in water; sparingly soluble in ethanol (~750 g/l) TS; very soluble in acetone R; freely soluble in dichloromethane R.

The solubility of the diluted product depends on the diluent and its concentration.

**Category.** Antianginal drug.

**Storage.** Diluted Isosorbide dinitrate should be kept in a tightly closed container, protected from light.

**Labelling.** The designation on the container should state the percentage content of Isosorbide dinitrate,  $C_6H_8N_2O_8$ .

**Additional information.** Diluted Isosorbide dinitrate may contain a suitable stabilizer, such as up to 1% of ammonium phosphate.

## Requirements

Diluted Isosorbide dinitrate contains not less than **95.0%** and not more than **105.0%** of the amount of  $C_6H_8N_2O_8$  stated on the label. It usually contains 20–50% of Isosorbide dinitrate.

### Identity tests

- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the *reference spectrum* of isosorbide dinitrate. (*Instructions for the preparation of the spectrum will be given on the reference spectrum.*)
- B. Dissolve in a test-tube a quantity equivalent to 10mg of Isosorbide dinitrate in a mixture of 1.0ml of water and about 2.0ml of sulfuric acid (~1760g/l) TS and cool. Introduce slowly 3.0ml of ferrous sulfate (15g/l) TS to form two layers; a brown colour is formed at the interface of the two liquids.
- C. Shake a quantity equivalent to 25mg of Isosorbide dinitrate with 10ml of acetone R and filter. Evaporate the filtrate to dryness at a temperature not exceeding 40°C and dry the residue under reduced pressure (not exceeding 0.6kPa or about 5mm of mercury) over phosphorus pentoxide R for 16 hours; melting point of the residue, about 71°C.

**Heavy metals.** Use 1.0g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method B (Vol. 1, p. 119); not more than 10µg/g.

**Loss on drying.** Dry at ambient temperature under reduced pressure (not exceeding 0.6kPa or about 5mm of mercury) over phosphorus pentoxide R for 16 hours; it loses not more than 10mg/g.

**Inorganic nitrates.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R3 as the coating substance and a mixture of 6 volumes of toluene R, 3 volumes of ethyl acetate R, and 1.5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 5µl of each of the two following solutions. For solution (A) shake a quantity equivalent to 0.10g of Isosorbide dinitrate in 5ml of ethanol (~750g/l) TS and filter. Solution (B) must be freshly prepared by dissolving 10mg of potassium nitrate R in 1ml of water and diluting to 100ml with ethanol (~750g/l) TS. After removing the plate from the chromatographic chamber, dry it in a current of air, and spray with freshly prepared potassium iodide/starch TS1. Expose the plate to ultraviolet light for 15 minutes. Examine the chromatogram in daylight.

Any spot corresponding to the nitrate ion obtained with solution A is not more intense than that obtained with solution B (0.5%, calculated as potassium nitrate).

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 8 volumes of toluene R and 2 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 20 µl of each of the following 2 solutions. For solution (A) shake a quantity equivalent to 0.20 g of Isosorbide dinitrate with 5 ml of acetone R and filter. For solution (B) dilute 1 volume of solution A to 200 volumes with acetone R. After removing the plate from the chromatographic chamber, dry it in a current of air, and spray with diphenylamine/sulfuric acid TS. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%).

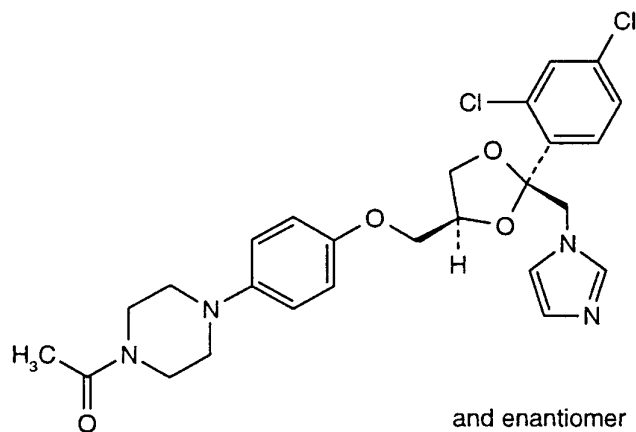
**Assay.** Shake a quantity equivalent to about 25 mg of Isosorbide dinitrate, accurately weighed, with 15 ml of glacial acetic acid R for 15 minutes. Dilute with sufficient glacial acetic acid R to produce 25 ml and filter. To 1.0 ml of the filtrate add 2 ml of phenoldisulfonic acid TS, allow to stand for 15 minutes, add 50 ml of water, make alkaline with ammonia (~260 g/l) TS, cool, and add sufficient water to produce 100 ml (solution A). Prepare similarly a solution containing 0.20 g of potassium nitrate R, previously dried at 105 °C, in 5 ml of water and add sufficient glacial acetic acid R to produce 25 ml. To 5 ml of the resulting solution add sufficient glacial acetic acid R to produce 50 ml. To 1.0 ml of this solution add 2 ml of phenoldisulfonic acid TS, allow to stand for 15 minutes, add 50 ml of water, make alkaline with ammonia (~260 g/l) TS, cool, and add sufficient water to produce 100 ml (solution B).

Measure the absorbance of a 1-cm layer at the maximum at about 405 nm of solution A against a solvent cell containing solution B, and calculate the content of  $C_6H_8N_2O_8$ .

Each ml of the potassium nitrate solution is equivalent to 0.934 mg of  $C_6H_8N_2O_8$ .

## Ketoconazolum

### Ketoconazole



**Relative molecular mass.** 531.4

**Chemical name.**  $\pm$ -*cis*-1-Acetyl-4-[*p*-[2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine; *cis*-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine; *cis*-1-acetyl-4-[*p*-[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-piperazine; CAS Reg. No. 65277-42-1.

**Description.** A white or almost white powder.

**Solubility.** Practically insoluble in water; freely soluble in dichloromethane R; soluble in methanol R; sparingly soluble in ethanol (~750 g/l) TS.

**Category.** Antifungal drug.

**Storage.** Ketoconazole should be kept in a well-closed container, protected from light.

### Requirements

Ketoconazole contains not less than **99.0%** and not more than the equivalent of **101.0%** of  $\text{C}_{26}\text{H}_{28}\text{Cl}_2\text{N}_4\text{O}_4$ , calculated with reference to the dried substance.

### Identity tests

- Either test A alone or tests B, C, and D may be applied.

<http://www.femeba.org.ar/fundacion/>

- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from ketoconazole RS or with the *reference spectrum* of ketoconazole.
- B. See the test described below under “Related substances”. The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.
- C. Place 1 ml of nitric acid (~1000 g/l) TS in a porcelain dish and add 10 mg of the substance; a clear orange-red solution is produced.
- D. Place 30 mg in a porcelain dish, add 0.3 g of anhydrous sodium carbonate R, and heat over an open flame for 10 minutes. Allow to cool, add 5 ml of nitric acid (~130 g/l) TS to the residue, stir, and filter. To 1 ml of the filtrate add 1 ml of water. The solution yields reaction A described under “General identification tests” as characteristic of chlorides (Vol. 1, p. 112).

**Melting range.** 148–152 °C.

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 µg/g.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry to constant mass at 105 °C; it loses not more than 5 mg/g.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 4 volumes of dioxan R, 4 volumes of methanol R, and 2 volumes of ammonium acetate TS as the mobile phase. Apply separately to the plate 5 µl of each of 5 solutions in the mobile phase containing (A) 6 mg of Ketoconazole per ml, (B) 6 mg of ketoconazole RS per ml, for (C) prepare a mixture of 6 mg of each of ketoconazole RS and econazole nitrate RS per ml, (D) 30 µg of ketoconazole RS per ml, and (E) 15 µg of ketoconazole RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air for 15 minutes. Expose the plate to iodine vapours until the spots appear and examine the chromatogram in daylight.

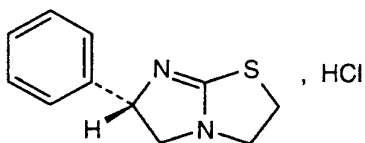
The test is not valid unless solution C shows two clearly separated spots. Any spot obtained with solution A, other than the principal spot, is not more intense than the principal spot obtained with solution D (0.5%) and only one such spot is more intense than that obtained with solution E (0.25%).

**Assay.** Dissolve about 0.2 g, accurately weighed, in 70 ml of a mixture of 1 volume of glacial acetic acid R1 and 7 volumes of ethylmethylketone R, and titrate with perchloric acid (0.1 mol/l) VS as described under “Non-aqueous titration”, Method A (Vol. 1, p. 131), determining the end-point potentiometrically.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 26.57 mg of  $C_{26}H_{28}Cl_2N_4O_4$ .

### *Levamisoli hydrochloridum*

### *Levamisole hydrochloride*



$C_{11}H_{12}N_2S, HCl$

**Relative molecular mass.** 240.8

**Chemical name.** (-)-2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]thiazole monohydrochloride; (S)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole monohydrochloride; CAS Reg. No. 16595-80-5.

**Description.** A white or almost white, crystalline powder.

**Solubility.** Freely soluble in water; soluble in ethanol (~750 g/l) TS; slightly soluble in dichloromethane R.

**Category.** Anthelmintic drug.

**Storage.** Levamisole hydrochloride should be kept in a well-closed container, protected from light.

### **Requirements**

Levamisole hydrochloride contains not less than **98.5%** and not more than **101.0%** of  $C_{11}H_{12}N_2S, HCl$ , calculated with reference to the dried substance.

### **Identity tests**

- *Either tests A and D or tests B, C, and D may be applied.*

- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from levamisole hydrochloride RS or with the *reference spectrum* of levamisole hydrochloride.
- B. See the test described below under “Related substances”. The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution D.
- C. Dissolve about 0.06g in 20ml of water, add 2ml of sodium hydroxide (~80g/l) TS, boil for 10 minutes, and cool. Add a few drops of sodium nitroprusside (45g/l) TS; a red colour is produced which fades on standing.
- D. A 0.05g/ml solution yields reaction B described under “General identification tests” as characteristic of chlorides (Vol. 1, p. 112).

**Specific optical rotation.** Use a 0.050g/ml solution in carbon-dioxide-free water R and calculate with reference to the dried substance;  $[\alpha]_D^{20^\circ\text{C}} = -121.5^\circ$  to  $-128^\circ$ .

**Clarity and colour of solution.** A solution of 0.50g in 10ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour Yw1 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Sulfated ash.** Not more than 1.0mg/g.

**Loss on drying.** Dry to constant mass at 105°C; it loses not more than 5.0mg/g.

**pH value.** pH of a 0.05g/ml solution, 3.5–5.0.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R2 as the coating substance and a mixture of 60 volumes of toluene R, 40 volumes of acetone R, and 1 volume of ammonia (~260g/l) TS as the mobile phase. Apply separately to the plate 10µl of each of 4 solutions in methanol R containing (A) 50mg of Levamisole hydrochloride per ml, (B) 5.0mg of Levamisole hydrochloride per ml, (C) 0.25mg of Levamisole hydrochloride per ml, and (D) 5.0mg of levamisole hydrochloride RS per ml. After removing the plate from the chromatographic chamber, dry it at 105°C for 15 minutes, and examine the chromatogram in ultraviolet light (254nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (0.5%).

Expose the plate to iodine vapour in a tightly closed chamber for 15 minutes and examine the chromatogram in daylight.

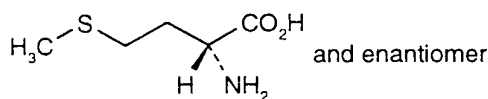
Any spot obtained with solution A, other than any spot with a very low  $R_f$  value, is not more intense than that obtained with solution C (0.5%).

**Assay.** Dissolve about 0.2 g, accurately weighed, in 30 ml of ethanol (~750 g/l) TS and add 5 ml of hydrochloric acid (0.01 mol/l) VS. Titrate with sodium hydroxide (0.1 mol/l) VS, determining the two inflection points potentiometrically. Record the volume, in ml, of sodium hydroxide (0.1 mol/l) VS consumed between the two inflection points.

Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 24.08 mg of  $C_{11}H_{12}N_2S, HCl$ .

### DL-Methioninum

#### DL-Methionine



$C_5H_{11}NO_2S$

**Relative molecular mass.** 149.2

**Chemical name.** (*RS*)-2-Amino-4-(methylthio)butyric acid; CAS Reg. No. 59-51-8.

**Description.** An almost white, crystalline powder or small flakes.

**Solubility.** Sparingly soluble in water; very slightly soluble in ethanol (~750 g/l) TS. It dissolves in dilute acids and in dilute solutions of the alkali hydroxides.

**Category.** Antidote.

**Storage.** DL-Methionine should be protected from light.

**Additional information.** Melting temperature, about 270 °C.

## Requirements

DL-Methionine contains not less than **99.0%** and not more than **101.0%** of  $C_5H_{11}NO_2S$ , calculated with reference to the dried substance.

### Identity tests

- *Either tests A and D or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from DL-methionine RS or with the *reference spectrum* of DL-methionine.
- B. See the test described below under “Related substances”. The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.
- C. Dissolve together about 0.1 g of DL-Methionine with 0.1 g of glycine R in 4.5 ml of sodium hydroxide (~80 g/l) TS, add 1 ml of 25 mg/ml solution of sodium nitroprusside R, heat at 40 °C for 10 minutes, and allow to cool. Add 2 ml of a mixture of 1 volume of phosphoric acid (~1440 g/l) TS and 9 volumes of hydrochloric acid (~420 g/l) TS; a deep-red colour is produced.
- D. Use a 0.050 g/ml solution in hydrochloric acid (1 mol/l) VS and measure the angle of optical rotation as described under “Determination of optical rotation and specific rotation” (Vol. 1, p. 31);  $[\alpha]_D^{20\text{ }^\circ\text{C}} = -0.05^\circ$  to  $+0.05^\circ$ .

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 µg/g.

**Chlorides.** Dissolve 1.2 g in a mixture of 5 ml of nitric acid (~130 g/l) TS and 35 ml of water, and proceed as described under “Limit test for chlorides” (Vol. 1, p. 116); the chloride content is not more than 0.2 mg/g.

**Sulfates.** Dissolve 1.0 g in 20 ml of water for injections R by heating to 60 °C, cool to 10 °C, and filter. Proceed with the filtrate as described under “Limit test for sulfates” (Vol. 1, p. 116); the sulfate content is not more than 0.2 mg/g.

**Clarity and colour of solution.** A solution of 0.20 g in 10 ml of carbon-dioxide-free water R is clear and colourless.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry to constant mass at 105 °C; it loses not more than 5.0 mg/g.

**pH value.** pH of a 20 mg/ml solution in carbon-dioxide-free water R, 5.4–6.1.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 6 volumes of 2-butanol R, 2 volumes of glacial acetic acid R, and 2 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of 4 solutions containing (A) 20 mg of DL-Methionine per ml, (B) 0.40 mg of DL-Methionine per ml, (C) 0.40 mg of DL-methionine RS per ml, and (D) 0.040 mg of DL-methionine RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with triketohydrindene/butanol/acetic acid TS and heat at 105 °C for 15 minutes. Examine the chromatogram in daylight.

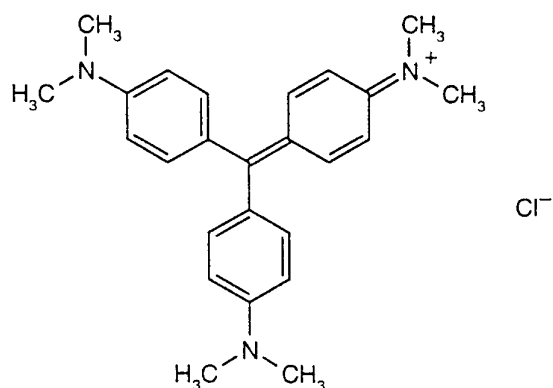
Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution D (0.2%).

**Assay.** Dissolve about 0.14 g, accurately weighed, in 3 ml of anhydrous formic acid R and add 30 ml of glacial acetic acid R1. Without delay titrate with perchloric acid (0.1 mol/l) VS as described under “Non-aqueous titration”, Method A (Vol. 1, p. 131), determining the end-point potentiometrically.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 14.92 mg of C<sub>5</sub>H<sub>11</sub>NO<sub>2</sub>S.

*Methylrosanilini chloridum*

*Methylrosanilinium chloride*



C<sub>25</sub>H<sub>30</sub>ClN<sub>3</sub>

**Relative molecular mass.** 408.0

**Chemical name.** C.I. Basic violet 3; *N*-[4-[bis[4-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-*N*-methylmethanaminium chloride; CAS Reg. No. 548-62-9.

**Other names.** Crystal violet, gentian violet.

**Description.** A dark green powder or greenish, glistening pieces having a metallic lustre; odourless or almost odourless.

**Solubility.** Sparingly soluble in water; soluble in ethanol (~750 g/l) TS and glycerol R; practically insoluble in ether R.

**Category.** Anti-infective drug.

**Storage.** Methylrosanilinium chloride should be kept in a tightly closed container, protected from light.

## Requirements

Methylrosanilinium chloride contains not less than **96.0%** and not more than the equivalent of **101.0%** of  $C_{25}H_{30}ClN_3$ , calculated with reference to the anhydrous substance.

### Identity tests

- A. See the test described below under "Related substances". The principal spot obtained with solution A corresponds to the spot with the lowest  $R_f$ -value of the three distinct spots obtained with solution B. A spot other than the principal spot may be present on the chromatogram obtained with solution A; this other spot corresponds to the spot with intermediate  $R_f$ -value obtained with solution B.
- B. Dissolve about 20 mg in 10 ml of water and add 5 drops of hydrochloric acid (~420 g/l) TS. To 5 ml of this solution add tannic acid (50 g/l) TS, drop by drop; a blue precipitate is produced (keep the remaining solution for test C).
- C. To the remaining solution from test B add 0.5 g of zinc R powder, and warm the mixture; the solution discolours rapidly. Place on a filter-paper 1 drop of this solution adjacent to 1 drop of ammonia (~100 g/l) TS; a blue colour is produced at the zone of contact.

**Ethanol-insoluble matter.** Add 1.0 g to 50 ml of ethanol (~750 g/l) TS and boil under reflux for 15 minutes. Filter through a tared filtering crucible, wash the residue on the filter with hot ethanol (~750 g/l) TS until no violet colour appears

in the washings, and dry the crucible at 105 °C for 1 hour; the residue weighs not more than 10 mg (1.0%).

**Sulfated ash.** Not more than 15 mg/g.

**Water.** Determine as described under “Determination of water by the Karl Fischer method”, Method A (Vol. 1, p. 135), using 0.5 g of Methylrosanilinium chloride; the water content is not more than 0.075 g/g.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R2 as the coating substance and a mixture of 100 volumes of 1-butanol R, 5 volumes of ammonium chloride (20 g/l) TS, and 0.5 volume of formic acid (~1080 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 1 mg of Methylrosanilinium chloride per ml, and (B) 1 mg of methyl violet 2B R per ml; also apply to the plate 10 µl of each of 4 solutions in methanol R containing (C) 10 mg of Methylrosanilinium chloride per ml, (D) 2.5 mg of Methylrosanilinium chloride per ml, (E) 0.05 mg of 4,4'-bis(dimethylamino)benzophenone R per ml, and (F) 0.05 mg of Methylrosanilinium chloride per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm).

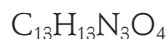
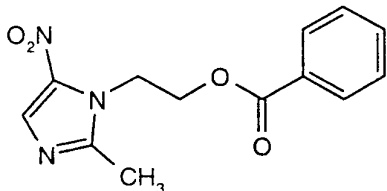
Any spot obtained with solution C corresponding to 4,4'-bis(dimethylamino)benzophenone is not more intense than that obtained with solution E (0.5%). Any spot obtained with solution D, other than the principal spot or any spot due to 4,4'-bis(dimethylamino)benzophenone, is not more intense than that obtained with solution F (2.0%).

**Assay.** Transfer about 0.4 g, accurately weighed, to a 300-ml conical flask, add 25 ml of water and 10 ml of hydrochloric acid (~420 g/l) TS. Replace the air in the flask with carbon dioxide R and maintain a stream of carbon dioxide R through the flask during the determination. Add 50.0 ml of titanium trichloride (0.1 mol/l) VS, heat to boiling, continuing to boil gently for 10 minutes, swirling the liquid occasionally. Cool, add 5 ml of ammonium thiocyanate (10 g/l) TS, and titrate with ferric ammonium sulfate (0.1 mol/l) VS until a faint red colour is produced. Repeat the procedure without the Methylrosanilinium chloride being examined and make any necessary corrections.

Each ml of titanium trichloride (0.1 mol/l) VS is equivalent to 20.40 mg of  $C_{25}H_{30}ClN_3$ .

*Metronidazoli benzoas*

*Metronidazole benzoate*



**Relative molecular mass.** 275.3

**Chemical name.** 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl benzoate; 2-methyl-5-nitro-1*H*-imidazole-1-ethanol benzoate; CAS Reg. No. 13182-89-3.

**Description.** A white or slightly yellowish, crystalline powder.

**Solubility.** Practically insoluble in water; freely soluble in dichloromethane R; soluble in acetone R; slightly soluble in ethanol (~750 g/l) TS; very slightly soluble in ether R.

**Category.** Anti-infective drug.

**Storage.** Metronidazole benzoate should be kept in a well-closed container, protected from light.

## Requirements

Metronidazole benzoate contains not less than **98.5%** and not more than **101.0%** of  $C_{13}H_{13}N_3O_4$ , calculated with reference to the dried substance.

### Identity tests

- *Either tests A and D or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from metronidazole benzoate RS or with the *reference spectrum* of metronidazole benzoate.
- B. See the test described below under “Related substances”. The principal spot obtained with solution B corresponds in position, appearance, and intensity with that obtained with solution C.

C. To about 10mg add 10mg of zinc R powder, 1 ml of water, and about 0.3 ml of hydrochloric acid (~420 g/l) TS. Heat on a water-bath for 5 minutes and cool. The solution yields the reaction described for the identification of primary aromatic amines under “General identification tests” (Vol. 1, p. 111), producing a red precipitate.

D. Melting temperature, about 101 °C.

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 µg/g.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry at 80 °C for 3 hours; it loses not more than 5.0 mg/g.

**pH value.** pH of a 20 mg/ml suspension in carbon-dioxide-free water R, 5.0–7.0.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R2 as the coating substance. Heat to activate the plate at 110 °C for 1 hour and cool before use. As the mobile phase, use ethyl acetate R. Apply separately to the plate 10 µl of each of 8 solutions in acetone R containing (A) 20 mg of Metronidazole benzoate per ml, (B) 2.0 mg of Metronidazole benzoate per ml, (C) 2.0 mg of metronidazole benzoate RS per ml, (D) 0.10 mg of Metronidazole benzoate per ml, (E) 0.040 mg of Metronidazole benzoate per ml, (F) 0.10 mg of metronidazole RS per ml, (G) 0.10 mg of 2-methyl-5-nitroimidazole R per ml, and for solution (H) dissolve 10 mg of metronidazole RS and 10 mg of 2-methyl-5-nitroimidazole R in sufficient acetone R to produce 50 ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot corresponding to metronidazole or to 2-methyl-5-nitroimidazole obtained with solution A is not more intense than the corresponding spot obtained with solutions F and G (0.5%). Any spot obtained with solution A, other than the principal spot and the spots corresponding to metronidazole and to 2-methyl-5-nitroimidazole, is not more intense than that obtained with solution D (0.5%), and not more than one such spot is more intense than that obtained with solution E (0.2%). The test is not valid unless the chromatogram obtained with solution H shows two clearly separated principal spots.

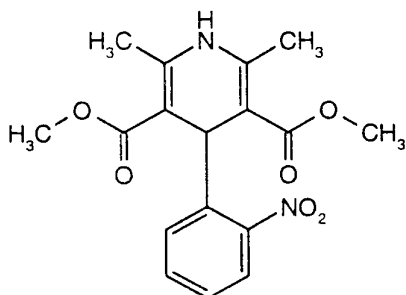
**Assay.** Dissolve about 0.25 g, accurately weighed, in 50 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS as described under

“Non-aqueous titration”, Method A (Vol. 1, p. 131), determining the end-point potentiometrically.

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 27.53 mg of  $C_{13}H_{13}N_3O_4$ .

## *Nifedipinum*

### *Nifedipine*



$C_{17}H_{18}N_2O_6$

**Relative molecular mass.** 346.3

**Chemical name.** 1,4-Dihydro-2,6-dimethyl-4-(*o*-nitrophenyl)-3,5-pyridine-dicarboxylate dimethyl ester; 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester; CAS Reg. No. 21829-25-4.

**Description.** A yellow, crystalline powder.

**Solubility.** Nifedipine is practically insoluble in water; freely soluble in acetone R; sparingly soluble in dehydrated ethanol R.

**Category.** Cardiovascular drug; calcium-channel blocking agent.

**Storage.** Nifedipine should be kept in a tightly closed container, protected from light.

**Additional information.** *CAUTION:* Nifedipine decomposes on exposure to daylight, artificial light of certain wavelengths, and ultraviolet light.

## Requirements

Nifedipine contains not less than **98.0%** and not more than **102.0%** of  $C_{17}H_{18}N_2O_6$ , calculated with reference to the dried substance.

*Note:* Throughout the monograph perform the tests and the assay in the dark or under a suitable fluorescent light, using low-actinic glassware.

### Identity tests

- *Either tests A and D or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from nifedipine RS or with the *reference spectrum* of nifedipine.
- B. See the test described below under “Related substances, Test B”. The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.
- C. Using gentle heat dissolve 25 mg in 10 ml of a mixture of 5 volumes of ethanol (~750 g/l) TS, 3.5 volumes of water, and 1.5 volumes of hydrochloric acid (~420 g/l) TS. Add 0.5 g of granulated zinc R and allow to stand for 5 minutes, swirling occasionally, and filter. To the filtrate add 5 ml of sodium nitrite (10 g/l) TS and allow to stand for 2 minutes. Add 2.0 ml of ammonium sulfamate (50 g/l) TS, shake vigorously but carefully, and add 2.0 ml of *N*-(1-naphthyl)ethylenediamine hydrochloride (5 g/l) TS; an intense red colour is produced which does not fade within 5 minutes.
- D. Melting temperature, about 173 °C.

**Sulfated ash.** Use an ignition temperature of 600 °C; not more than 1.0 mg/g.

**Loss on drying.** Dry at 105 °C for 2 hours; it loses not more than 5.0 mg/g.

### Related substances

- *Either test A or test B may be applied.*
- A. Carry out the test as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (15 cm × 4.6 mm) packed with *stationary phase A* (5–10 μm). As the mobile phase, use a mixture of 55 volumes of water, 36 volumes of methanol R, and 9 volumes of acetonitrile R.

Prepare the following solutions in methanol R: for solution (A) dissolve 0.20 g of Nifedipine in 20 ml of methanol R and dilute to 50 ml with the mobile phase; solution (B) 0.4 mg of dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate RS per ml; solution (C) 0.4 mg of dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate RS

per ml; and for solution (D) mix 1.0ml each of solutions B and C and 0.10ml of solution A, dilute to 20ml with the mobile phase, then dilute 2.0ml of the resulting solution to 10ml with the mobile phase.

Operate with a flow rate of 1.0ml per minute. As a detector use an ultra-violet spectrophotometer set at a wavelength of about 235 nm; the use of an electronic integrator is advisable.

Inject 20 µl of solution D. The peaks are eluted in the following order: dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate; dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate; and nifedipine. The retention time of nifedipine is about 15.5 minutes.

The test is not valid unless, in the chromatogram obtained with solution D:

- the resolution between the peaks corresponding to dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate and dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate is greater than 1.5; and
- the resolution between the peaks corresponding to dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate and nifedipine is greater than 1.5.

Adjust the sensitivity of the system so that the height of the peak corresponding to dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate is not less than 20% of the full scale of the recorder.

Inject alternately 20 µl each of solutions A and D. Record the chromatogram for twice the retention time of nifedipine.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and D, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, none of the peaks, other than the principal peak and the peaks corresponding to dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate and dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate, has an area greater than that of the peak corresponding to nifedipine in the chromatogram obtained with solution D (0.1%). The areas of the peaks corresponding to dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate and dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate are not greater than the corresponding peaks in the chromatogram obtained with solution D (0.1%). The total amount of related substances does not exceed 0.3%. Disregard any peak with an area less than 10% of the area of the peak corresponding to nifedipine in the chromatogram obtained with solution D (0.01%).

- B. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), in an unsaturated chamber, using silica gel R6 as the coating substance and a mixture of 6 volumes of cyclohexane R and 4 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions in methanol R containing (A) 1.0 mg of Nifedipine per ml, (B) 1.0 mg of nifedipine RS per ml, and (C) 10 µg of Nifedipine per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

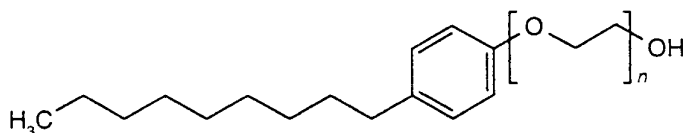
Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution C (1.0%).

**Assay.** Dissolve about 0.13 g, accurately weighed, in a mixture of 25 ml of *tert*-butanol R and 25 ml of perchloric acid TS, and titrate with ceric ammonium sulfate (0.1 mol/l) VS, using 0.1 ml of ferroin TS as indicator until the pink colour is discharged, titrating slowly towards the end-point. Repeat the procedure without the Nifedipine being examined and make any necessary corrections.

Each ml of ceric ammonium sulfate (0.1 mol/l) VS is equivalent to 17.32 mg of C<sub>17</sub>H<sub>18</sub>N<sub>2</sub>O<sub>6</sub>.

## *Nonoxinolum 9*

### *Nonoxinol 9*



(Average value of  $n = 9$ , with a possible range of 4–16.)

**Composition.** Nonoxinol 9 is an anhydrous liquid mixture containing mainly monononylphenyl ethers of macrogols.

**Chemical name.** Polyethylene glycol mono(*p*-nonylphenyl) ether;  $\alpha$ -(4-nonylphenyl)- $\omega$ -hydroxypoly-(oxy-1,2-ethanediyl); CAS Reg. No. 26027-38-3.

**Description.** A clear, colourless to light yellow, viscous liquid.

**Solubility.** Miscible with water, ethanol (~750 g/l) TS and olive oil R.

**Category.** Adjunctive contraceptive agent.

**Storage.** Nonoxinol 9 should be kept in a tightly closed container.

**Additional information.** Nonoxinol 9 should be kept away from oxidizing agents.

## **Requirements**

The content is not less than **95.0%** and not more than **105.0%** of Nonoxinol 9, calculated with reference to the anhydrous substance.

### **Identity tests**

- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the *reference spectrum* of nonoxinol 9.
- B. See the test described below under “Assay”. The retention time of the major peak in the chromatogram obtained with solution A corresponds to that in the chromatogram obtained with solution B.

**Acid value** (Vol. 1, p. 140). Not more than 0.2.

**Water.** Determine as described under “Determination of water by the Karl Fischer method”, Method A (Vol. 1, p. 135), using about 0.5 g of the substance; the water content is not more than 5.0 mg/g.

**Macrogol.** Transfer about 10 g, accurately weighed, to a 250-ml beaker. Add 100 ml of ethyl acetate R and allow to dissolve using a magnetic stirrer. Transfer to a 500-ml separatory funnel fitted with a glass stopper with the aid of 100 ml of sodium chloride (300 g/l) TS. Insert the stopper and shake vigorously for 1 minute. Remove the stopper carefully. Immerse a thermometer into the mixture and place the funnel so that it is partially immersed in a water-bath maintained at 50 °C. Swirl the funnel gently while letting the internal temperature rise to between 40 and 45 °C. Once this is reached remove the funnel from the bath immediately, dry the outside surface, and drain the aqueous layer into another 500-ml separatory funnel. Extract the organic layer with 100 ml of sodium chloride (300 g/l) TS a second time, combining the two aqueous extracts. Discard the organic layer. Wash the combined aqueous layers with 100 ml of ethyl acetate R and separate the aqueous layer into another 500-ml separatory funnel. Discard the organic layer. Extract the aqueous layer with two successive 100-ml portions of dichloromethane R, filtering the organic layers through a folded filter paper (e.g. grade Whatman 2V) and combining them in a 250-ml beaker. Evaporate to dryness on a water-bath and continue heating until the odour of dichloromethane is no longer perceptible. Allow the beaker

to cool. Add 25 ml of acetone R and allow the residue to dissolve using a magnetic stirrer. Filter into a tared 250-ml beaker, rinsing with two 25-ml quantities of acetone R. Evaporate to dryness on a water-bath. Dry under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) at 60 °C for 1 hour. Allow the beaker to cool, and weigh; not more than 16 mg/g.

**Cloudiness of solution.** Transfer 1.0 g to a 250-ml beaker, add 99 g of water, and mix to dissolve. Pour about 30 ml of the solution into a 70-ml test-tube. Place the test-tube into a water-bath and stir the contents constantly with a thermometer until the solution becomes cloudy, then immediately remove the test-tube from the bath, so that the temperature does not rise further by more than 2 °C, and continue stirring. The temperature at which the solution becomes sufficiently clear and when the entire thermometer bulb is clearly visible is between 52 and 56 °C.

**Ethylene oxide and dioxan.** Carry out the test as described under “Gas chromatography” (Vol. 1, p. 94), with the apparatus equipped with an injection system for the performance of head-space chromatography. Use a capillary glass or quartz column (30 m × 0.32 mm), the inner surface of which is coated with a thick layer of polydimethylsiloxane R (1.0 μm). Maintain the temperature of the column at 50 °C for 5 minutes. Increase the temperature at a rate of 5 °C per minute to 180 °C, and then increase the temperature again at a rate of 30 °C per minute to 230 °C, and maintain it at this point for 5 minutes. Maintain the temperature of the injection port at 150 °C and that of the detector at 250 °C. Use helium R or nitrogen R as the carrier gas with a linear velocity of about 20 cm per second and a split ratio of 1:20; use a flame-ionization detector.

Prepare the following solutions: for solution (A) weigh 1.0 g of Nonoxinol 9, add 1.0 ml of water, mix to obtain a homogeneous solution, and allow to stand at 70 °C for 45 minutes; for solution (B) weigh 1.0 g of Nonoxinol 9, add 0.5 ml of ethylene oxide TS and 0.5 ml of dioxan TS, mix to obtain a homogeneous solution, and allow to stand at 70 °C for 45 minutes; for solution (C) add to 0.5 ml of ethylene oxide TS 0.10 ml of a freshly prepared 10 mg/l solution of acetaldehyde R and 0.10 ml of dioxan TS, mix to obtain a homogeneous solution, and allow to stand at 70 °C for 45 minutes.

Inject 1.0 ml of the gaseous phase of solution C. Adjust the sensitivity of the system so that the heights of the peaks corresponding to ethylene oxide and acetaldehyde in the chromatogram obtained are at least 15% of the full scale of the recorder. The test is not valid unless the resolution between the peaks corresponding to acetaldehyde and ethylene oxide is at least 2.0 and the peak of ethylene oxide is detected with a signal-to-noise ratio of at least 5.

Inject separately 1.0 ml each of the gaseous phases of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B. The mean areas of the ethylene oxide and dioxan peaks obtained with solution A are not greater than half the mean area of the corresponding peak obtained with solution B (1 µg/g of ethylene oxide and 50 µg/g of dioxan).

**Assay.** Determine by “High-performance liquid chromatography” (p. 257), using a stainless steel column (25 cm × 4 mm) packed with *stationary phase C*, the surface of which has been modified with chemically bonded dihydroxypropane groups (diol) (10 µm). As mobile phase A, use a mixture of 2 volumes of ethyl acetate R and 8 volumes of hexane R. As mobile phase B, use a mixture of 2.5 volumes of methanol R and 97.5 volumes of ethyl acetate R.

Prepare the following solutions in mobile phase A: solution (A) 2.0 mg of Nonoxinol 9 per ml; and solution (B) 2.0 mg of nonoxinol 9 RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 280 nm.

Use the following gradient elution system:

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0–2	100	0	equilibration
2–10	100 → 84	0 → 16	linear gradient
10–20	84 → 70	16 → 30	linear gradient
20–30	70 → 62	30 → 38	linear gradient
30–40	62 → 57	38 → 43	linear gradient
40–50	57 → 54	43 → 46	linear gradient
50–70	54 → 50	46 → 50	linear gradient
70–75	50 → 50	50 → 50	isocratic
75–76	50 → 100	50 → 0	re-equilibration

Inject 100 µl each of solutions A and B. The nonoxinol oligomers elute as sharp distinct peaks, and their areas should be included in the peak response for nonoxinol 9.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B. The sum of the areas of any peaks corresponding to nonoxynols with chain lengths  $n < 4$  or  $n > 16$  is not greater than 1.0% of the sum of the areas of the peaks corresponding to nonoxynols with chain lengths  $n = 4$  to  $n = 16$ . Calculate the content of Nonoxinol 9 as a percentage, with reference to the anhydrous substance.

## Oxygenium

### Oxygen

O<sub>2</sub>

**Relative molecular mass.** 32.00

**Chemical name.** Oxygen; CAS Reg. No. 7782-44-7.

**Description.** A colourless gas; odourless.

**Solubility.** One volume dissolves in about 32 volumes of water and in about 7 volumes of ethanol (~750 g/l) TS, both at a pressure of 101.3 kPa and 20 °C.

**Category.** Gas for inhalation.

**Storage.** Oxygen should be kept as compressed gas or liquid at cryogenic temperature, in appropriate containers complying with the safety regulations of the national authority.

**Labelling.** An ISO standard<sup>1</sup> requires that cylinders containing oxygen intended for medical use should bear the name of the contents in legible and permanent characters and, preferably, also the molecular formula O<sub>2</sub>.

**Additional information.** In the analysis of medicinal gases certain tests are not intended for hospital pharmacists. They are solely applicable by laboratories equipped with the specialized apparatus.

Valves or taps should not be lubricated with oil or grease. It is recommended that cylinders marked as described above are not used for other gases.

## Requirements

Oxygen contains not less than **99.5% v/v** of O<sub>2</sub>.

### Identity tests

- A. Place a glowing splinter of wood into Oxygen; the splinter bursts into flame.
- B. Shake Oxygen with alkaline pyrogallol TS; it is absorbed and the solution becomes dark brown (distinction from Dinitrogen oxide).

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<sup>1</sup> *International Standard 32. Gas cylinders for medical use – marking for identification of content.* International Organization for Standardization, Switzerland, 1977.

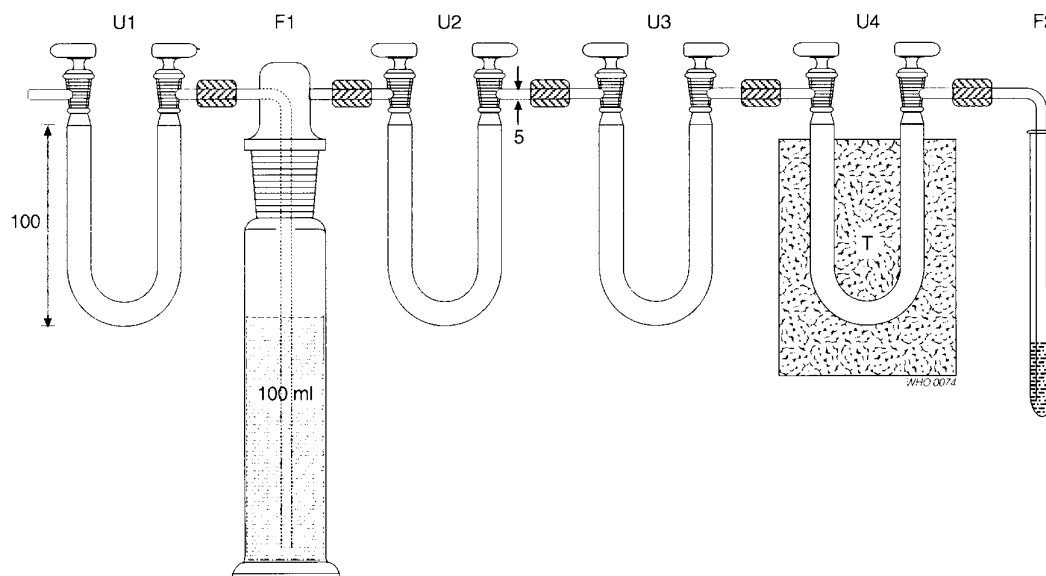


Figure 7. Apparatus for the determination of carbon monoxide in medicinal gases  
Measurements in mm.

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- *Oxygen labelled as having been produced by the air-liquefaction process may be exempted from the requirements of the tests for carbon monoxide and carbon dioxide.*

*Note:* For the following tests deliver the gas to be examined at a rate of 4 litres per hour.

### Carbon monoxide

- *Either test A or test B may be applied.*
- A. The apparatus (Fig. 7) consists of the following parts connected in series:
- a U-tube (U1) containing desiccant silica gel R impregnated with chromium trioxide R;
  - a wash bottle (F1) containing 100 ml of potassium hydroxide (~400 g/l) TS;
  - a U-tube (U2) containing pellets of potassium hydroxide R;
  - a U-tube (U3) containing phosphorus pentoxide R dispersed on previously granulated, fused pumice;
  - a U-tube (U4) containing 30 g of recrystallized iodine pentoxide R in granules, previously dried at 200 °C and kept at a temperature of 120 °C (T) during the test. The iodine pentoxide is packed in the tube in 1-cm columns separated by 1-cm columns of glass wool to give an effective length of 5 cm;

- a reaction tube (F2) containing 2.0 ml of potassium iodide (160 g/l) TS and 0.15 ml of starch TS.

Flush the apparatus with 5.0 litres of argon R. If necessary, discharge the blue colour in tube F2 containing potassium iodide (160 g/l) TS by adding a sufficient volume of freshly prepared sodium thiosulfate (0.002 mol/l) VS. Continue flushing with gas until not more than 0.045 ml of sodium thiosulfate (0.002 mol/l) VS is required after the passage of 5.0 litres of argon R. Pass 7.5 litres of Oxygen from the container through the apparatus. Flush the last traces of liberated iodine into the reaction tube by passing 1.0 litre of argon R through the apparatus. Titrate the liberated iodine with sodium thiosulfate (0.002 mol/l) VS. Repeat the procedure using 7.5 litres of argon R.

- B. Determine the content using a carbon monoxide detector tube. Pass the required volume of Oxygen through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of Oxygen to purge the tubing to an appropriate flow. Fit the carbon monoxide detector tube to the metering pump following the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and operate the pump sufficiently to pass a suitable volume of Oxygen through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 5 µl/l.

*Note:* For the following tests – “Carbon dioxide”, “Oxidizing substances”, and “Acidity and alkalinity” – pass the gas to be tested through the appropriate reagent contained in a hermetically closed flat-bottomed glass cylinder (with dimensions such that 50 ml of liquid reaches a height of 12–14 cm) that is fitted with (a) a delivery tube terminated by a capillary 1 mm in internal diameter and placed within 2 mm of the bottom of the cylinder; and (b) an outlet tube.

Prepare the reference solutions in identical cylinders.

### **Carbon dioxide**

- *Either test A or test B may be applied.*
- A. Pass 1.0 litre of Oxygen through 50 ml of a clear solution of barium hydroxide (0.15 mol/l) VS. Similarly prepare a reference solution by adding 1.0 ml of a 1.1 mg/ml solution of sodium hydrogen carbonate R in carbon-dioxide-free water R to 50 ml of barium hydroxide (0.15 mol/l) VS.

Any turbidity in the solution after the passage of the Oxygen is not more intense than that of the reference solution (300 µl/l).

- B. Determine the content using a carbon dioxide detector tube. Pass the required volume of Oxygen through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a suitable pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of Oxygen to purge the tubing to an appropriate flow. Fit the carbon dioxide detector tube to the metering pump following the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and operate the pump sufficiently to pass a suitable volume of Oxygen through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 300 µl/l.

**Oxidizing substances.** To two cylinders add 50 ml of freshly prepared potassium iodide/starch TS1 and about 0.2 ml of glacial acetic acid R. Protect the cylinders from light. Pass 5.0 litres of Oxygen into one of the solutions and compare the colour produced.

The solutions in both cylinders remain colourless.

#### **Water**

- *Either test A or test B may be applied.*

- A. The apparatus consists either of an electrolytic hygrometer as described below, an appropriate humidity detector tube, or a capacity hygrometer.

The measuring cell consists of a thin film of phosphoric anhydride placed between two coiled platinum wires that act as electrodes. The water vapour in Oxygen is absorbed by the phosphoric anhydride to form phosphoric acid, which acts as an electrical conductor.

Before introducing Oxygen into the device, allow the gas to stabilize at room temperature and make sure that the temperature is constant throughout the apparatus. Apply a continuous voltage across the electrodes to produce electrolysis of the water and regeneration of phosphoric anhydride. Measure the resulting electrical current, which is proportional to the water content in Oxygen. (This is a self-calibrating system that obeys Faraday's law.)

Calculate the content of water; not more than 60 µg/l.

- B. Determine the content using a water vapour detector tube. Pass the required volume of Oxygen through the tube, the calibration of which is verified according to the manufacturer's instructions.

The gas supply is connected to a pressure regulator and needle valve. Connect the flexible tubing fitted with a Y-piece to the valve and adjust the flow of Oxygen to purge the tubing to an appropriate flow. Fit the water vapour detector tube to the metering pump following the manufacturer's instructions. Connect the open end of the tube to the short leg of the tubing and operate the pump sufficiently to pass a suitable volume of Oxygen through the tube. Read the value corresponding to the length of the coloured layer or the intensity of the colour on the graduated scale; not more than 60 µl/l.

**Acidity and alkalinity.** Pass 2.0 litres of Oxygen through a mixture of 0.10 ml of hydrochloric acid (0.01 mol/l) VS and 50 ml of carbon-dioxide-free water R.

For *reference solution 1*, use 50 ml of carbon-dioxide-free water R. For *reference solution 2*, use a mixture of 0.20 ml of hydrochloric acid (0.01 mol/l) VS and 50 ml of carbon-dioxide-free water R.

To each solution add 0.1 ml of methyl red/ethanol TS; the intensity of the colour in the solution of Oxygen is between those of reference solutions 1 and 2.

### **Assay**

- *Either method A or method B may be applied.*

A. For the determination use a 25-ml capacity gas burette (Fig. 8) in the form of a chamber with at its upper end, a tube graduated in 0.2% between 95 and 100, and isolated at each end by a tap with a conical barrel. The lower tap is joined to a tube with an olive-shaped nozzle and is used to introduce the Oxygen into the apparatus. A cylindrical funnel above the upper tap is used to introduce the absorbent solution. Wash the burette with water and dry. Open the two taps. Connect the nozzle to the container of Oxygen and set the flow rate to 1 litre per minute. Flush the burette by passing the gas through it for 1 minute. Close the upper tap of the burette and immediately afterwards the lower tap. Rapidly disconnect the burette from the container of Oxygen, and give a half turn to the upper tap to eliminate any excess pressure in the burette. Keeping the burette vertical, fill the funnel with a freshly prepared mixture of 21 ml of potassium hydroxide (~560 g/l) TS and 130 ml of sodium dithionite (200 g/l) TS. Open the upper tap slowly. The solution absorbs the oxygen and enters the burette. Allow to stand for 10 minutes without shaking.

Read the level of the liquid meniscus on the graduated part of the burette; the figure represents the content of oxygen as a percentage in v/v.

B. Oxygen in medicinal gases can also be determined using a paramagnetic analyser, which measures electronically the molecule's interaction with magnetic fields.

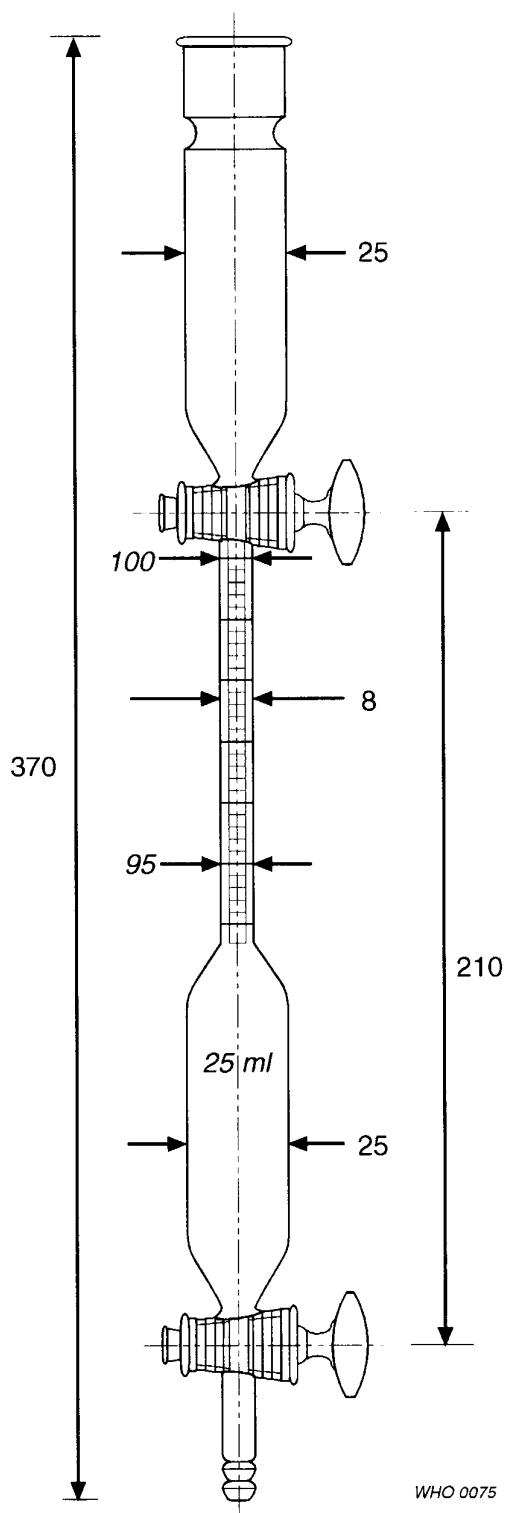
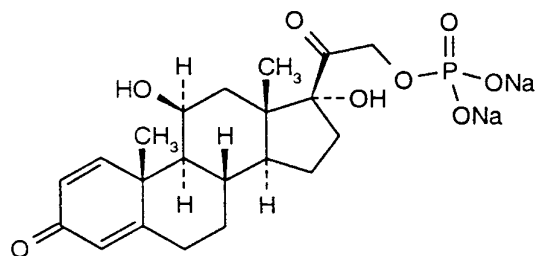


Figure 8. Burette used for the assay of oxygen  
Measurements in mm.

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Carry out the method according to the instrument manufacturer's instructions.

*Prednisoloni natrii phosphas*  
*Prednisolone sodium phosphate*



$C_{21}H_{27}Na_2O_8P$

**Relative molecular mass.** 484.4

**Chemical name.** 11 $\beta$ ,17,21-Trihydroxypregna-1,4-diene-3,20-dione 21-(disodium phosphate); (11 $\beta$ ) 11,17-dihydroxy-21-(phosphonooxy)-pregna-1,4-diene-3,20-dione disodium salt; CAS Reg. No. 125-02-0.

**Description.** A white to light yellow, crystalline powder or granules.

**Solubility.** Freely soluble in water; soluble in methanol R; very slightly soluble in ethanol (~750 g/l) TS and acetone R.

**Category.** Corticosteroid.

**Storage.** Prednisolone sodium phosphate should be kept in a tightly closed container, protected from light.

**Labelling.** The designation Prednisolone sodium phosphate for sterile non-injectable use indicates that the substance complies with the additional requirement and may be used for sterile applications. Expiry date.

**Additional information.** Prednisolone sodium phosphate is hygroscopic.

## Requirements

Prednisolone sodium phosphate contains not less than **96.0%** and not more than **103.0%** of  $C_{21}H_{27}Na_2O_8P$ , calculated with reference to the anhydrous substance.

### Identity tests

- Either tests A, D, and E or tests B, C, D, and E may be applied.
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from prednisolone sodium phosphate RS or with the *reference spectrum* of prednisolone sodium phosphate.
- B. Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R1 as the coating substance and a freshly prepared mixture of 3 volumes of 1-butanol R, 1 volume of acetic anhydride R, and 1 volume of water as the mobile phase. Apply separately to the plate 2 µl of each of 4 solutions in methanol R containing (A) 2.5 mg of Prednisolone sodium phosphate per ml, (B) 2.5 mg of prednisolone sodium phosphate RS per ml, (C) a mixture of equal volumes of solutions A and B, and (D) equal volumes of solution A and a solution of 2.5 mg of dexamethasone sodium phosphate RS per ml of methanol R. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, spray with a mixture of 10 ml of sulfuric acid (~1760 g/l) TS and 90 ml of ethanol (~750 g/l) TS, heat at 120 °C for 10 minutes, allow to cool, and examine the chromatogram in ultraviolet light (365 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B. The principal spot obtained with solution C appears as a single compact spot, whereas the chromatogram obtained with solution D shows two spots which may not be completely separated.

- C. To about 2 mg add 2 ml of sulfuric acid (~1760 g/l) TS and shake to dissolve; an intense red colour is produced within 5 minutes. Examine under ultraviolet light (365 nm); a reddish brown fluorescence is observed. Add the solution to 10 ml of water and mix; the colour fades and a greenish yellow fluorescence is produced.
- D. When tested for sodium as described under “General identification tests” (Vol. 1, p. 115), it yields the characteristic reactions. If reaction B is to be used, prepare a 20 mg/ml solution.
- E. To 1 ml of a 20 mg/ml solution add 3 ml of nitric acid (~130 g/l) TS; it yields reaction A described under “General identification tests” as characteristic of orthophosphates (Vol. 1, p. 114).

**Specific optical rotation.** Use a 10 mg/ml solution and calculate with reference to the anhydrous substance;  $[\alpha]_D^{20} = +95^\circ$  to  $+102^\circ$ .

**Clarity and colour of solution.** A solution of 0.5 g in 10 ml of carbon-dioxide-free water R is clear and not more intensely coloured than standard colour Bn1 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Water.** Determine as described under “Determination of water by the Karl Fischer Method”, Method A (Vol. 1, p. 135), using about 0.2 g of the substance; not more than 0.080 g/g.

**pH value.** pH of a 0.05 g/ml solution in carbon-dioxide-free water R, 7.5–9.0.

**Inorganic phosphates.** Dissolve 0.050 g in sufficient water to produce 100 ml. To 10 ml add 5 ml of ammonium molybdate/vanadate TS, mix and allow to stand for 5 minutes; any yellow colour produced is not more intense than that of a reference solution prepared similarly using 10 ml of phosphate standard (5 µg/ml) TS.

#### **Free prednisolone and other related substances**

- *Either test A or test B may be applied.*

A. Carry out the test as described under “High-performance liquid chromatography” (p. 257), using a stainless steel column (15 cm × 4.6 mm) packed with *stationary phase A* (5 µm). As the mobile phase, use a mixture prepared as follows: weigh 1.36 g of potassium dihydrogen phosphate R and 0.60 g of hexylamine R, transfer to a 250-ml conical flask, mix, and allow to stand for 10 minutes, and then dissolve in 185 ml of water. Add 65 ml of acetonitrile R, mix, and filter.

Prepare the following solutions in the mobile phase: solution (A) 2.5 mg of Prednisolone sodium phosphate per ml; solution (B) 2.5 mg of prednisolone sodium phosphate RS and 2.5 mg of prednisolone RS per ml, dilute 1.0 ml of this solution to 25 ml with the mobile phase; and for solution (C) dilute 1.0 ml of solution A to 50 ml with the mobile phase.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultra-violet spectrophotometer set at a wavelength of 254 nm.

Inject 20 µl of solution B. Adjust the sensitivity of the system so that the heights of the principal peaks in the chromatogram obtained with solution B are 70–90% of the full scale of the recorder. The retention times for prednisolone sodium phosphate are about 6.5 minutes, and for prednisolone about 8.5 minutes. The test is not valid unless the resolution between the peaks corresponding to prednisolone sodium phosphate and prednisolone is not less than 4.5.

Inject alternately 20 µl each of solutions A and C. Continue the chromatography for 3 times the retention time of the principal peak.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the contents of free prednisolone and other related substances as a percentage. In the chromatogram obtained from solution A, the area of any peak, other than the principal peak, is not greater than that of the principal peak obtained with solution C (2.0%), and not more than one such peak has an area greater than half the area of the principal peak obtained with solution C (1.0%). The sum of the areas of all the peaks, other than the principal peak, is not greater than 1.5 times the area of the principal peak obtained with solution C (3.0%). Disregard any peak due to the solvent and any peak with an area less than 0.025 times the area of the principal peak obtained with solution C.

- B. Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R1 as the coating substance and methanol R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in methanol R containing (A) 10 mg of Prednisolone sodium phosphate per ml, and (B) 0.20 mg of prednisolone RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air for 5 minutes, spray with a solution of 3 g of zinc chloride R in 10 ml of methanol R, heat at about 125 °C for 1 hour, and examine the chromatogram in ultraviolet light (365 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (2.0%).

**Assay.** Dissolve about 0.1 g, accurately weighed, in sufficient water to produce 100 ml. Dilute 5 ml to 250 ml with water and measure the absorbance of this solution in a 1-cm layer at the maximum at about 247 nm.

Calculate the percentage content of  $C_{21}H_{27}Na_2O_8P$  using the absorptivity value of 31.2 ( $A_{1\text{cm}}^{1\%} = 312$ ).

### ***Additional requirement for sterile non-injectable Prednisolone sodium phosphate***

*Complies with the “Test for sterility of non-injectable preparations” (see page 32).*

### ***Protamini sulfas***

#### ***Protamine sulfate***

**Composition.** Protamine sulfate is a mixture of sulfates of purified proteins extracted from the sperm or roe of fish usually belonging to the family *Clupeidae* and *Salmonidae*; CAS Reg. No. 9009-65-8.

**Description.** A white or almost white powder; hygroscopic.

**Solubility.** Soluble in water; practically insoluble in ethanol (~750 g/l) TS and ether R.

**Category.** Drug affecting blood coagulation.

**Storage.** Protamine sulfate should be kept in a tightly closed and tamper-proof container.

**Labelling.** The designation Protamine sulfate for parenteral use indicates that the substance complies with the additional requirements and may be used for parenteral administration. Expiry date.

**Additional information.** Protamine sulfate binds with heparin in solution, inhibiting its anticoagulant activity. It is prepared in conditions designed to minimize the risk of microbial contamination.

## Requirements

The quantity of **1 mg** of Protamine sulfate precipitates not less than **100 IU** of heparin sodium activity, calculated with reference to the dried substance.

### Identity tests

- A. Use a 10 mg/ml solution in hydrochloric acid (0.1 mol/l) VS. Measure the optical rotation and calculate with reference to the dried substance;  $[\alpha]_D^{20^\circ\text{C}} = -65^\circ$  to  $-85^\circ$ .
- B. Dissolve 0.1 g in 5 ml of water, add 4.5 ml of water, 1.0 ml of sodium hydroxide (~80 g/l) TS, and 2.0 ml of 1-naphthol TS1. Cool the mixture to 5°C and add 0.5 ml of sodium hypobromite TS; an intense red colour is produced.
- C. Dissolve 0.04 g in 2 ml of water and heat in a water-bath at 60°C. Add 0.1 ml of mercuric sulfate TS and mix; no precipitate is formed. Cool the mixture in an ice-bath; a precipitate is formed.
- D. A 20 mg/ml solution yields reaction A described under “General identification tests” as characteristic of sulfates (Vol. 1, p. 115).

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under “Limit test for heavy metals”, Procedure 4 (Vol. 1, p. 119); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 µg/g.

**Sulfates.** Transfer 0.15 g to a beaker and dissolve in 15 ml of water. Add 5 ml of hydrochloric acid (~70 g/l) TS, heat to boiling and slowly add to the boiling solution 10 ml of barium chloride (100 g/l) TS. Cover the beaker and heat in a water-bath for 1 hour. Filter, and wash the precipitate several times with small quantities of hot water. Dry and ignite the residue at 600 °C to constant mass. Each g of residue is equivalent to 0.412 g of sulfates (SO<sub>4</sub>), calculated with reference to the dried substance; 0.16–0.24 g/g.

**Clarity and colour of solution.** A solution of 0.50 g in 10 ml of water is not more opalescent than opalescence standard TS2 and not more intensely coloured than standard colour solution Yw2 when compared as described under “Colour of liquids” (Vol. 1, p. 50).

**Loss on drying.** Dry at 105 °C for 3 hours; it loses not more than 0.050 g/g.

**Light absorbance.** Dissolve 0.050 g in 5 ml of water and measure the absorbance of a 1-cm layer at a wavelength between 260 nm and 280 nm; not greater than 0.1.

**Nitrogen.** Proceed as described under “Determination of nitrogen”, Method B (Vol. 1, p. 137), using 10 mg of Protamine sulfate; the content of nitrogen is not less than 0.23 g/g and not more than 0.27 g/g, calculated with reference to the dried substance.

**Assay.** Prepare the following solutions: for solution (A) dissolve 15.0 mg of Protamine sulfate in sufficient water to produce 100 ml; for solution (B) dilute 2.0 ml of solution A to 3.0 ml with water; for solution (C) dilute 1.0 ml of solution A to 3.0 ml with water.

As titrant use a solution of heparin RS in water containing about 170 IU/ml. Titrate each of solutions A, B, and C in duplicate and carry out 3 independent assays. Measure accurately 1.5 ml of one of the solutions and introduce it to a cell of a suitable spectrophotometer set at 420 nm. Add small volumes of the titrant until a sharp change in transmittance is observed and note the volume of titrant added.

For each individual titration, calculate the number of International Units of heparin in the volume of titrant added, per mg of Protamine sulfate. Average the 18 values and test the linearity of the response using the usual statistical methods. The assay is not valid unless the relative standard deviations calculated for the results obtained with each solution are less than 5% of the average result.

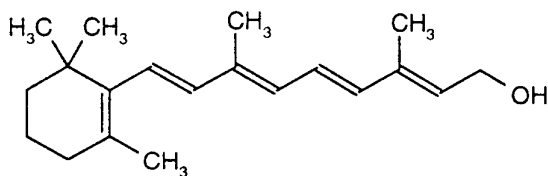
### ***Additional requirements for Protamine sulfate for parenteral use***

Complies with the monograph for "Parenteral preparations" (see Vol. 4, p. 36).

**Bacterial endotoxins.** Carry out the test as described under "Test for bacterial endotoxins (p. 30); contains not more than 7.0IU of endotoxin RS per mg.

### ***Retinolum densatum oleosum***

#### ***Retinol concentrate, oily form***



**Composition.** The oily form of Retinol concentrate consists of an ester or a mixture of esters (acetate, propionate, or palmitate) of retinol (C<sub>20</sub>H<sub>30</sub>O), usually prepared by synthesis. It may be diluted in a suitable vegetable oil.

**Chemical name.** 3,7 Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol; CAS Reg. No. 68-26-8.

**Other name.** Vitamin A concentrate (oily form).

**Description.** A yellow to brownish yellow, oily liquid.

**Solubility.** Practically insoluble in water; soluble or partly soluble in dehydrated ethanol R; miscible with organic solvents.

**Category.** Vitamin.

**Storage.** The oily form of Retinol concentrate should be kept in a well-closed and well-filled container, protected from light, and stored at a temperature between 8 and 15 °C.

**Labelling.** The designation on the container should state the name of the ester or esters, whether any additional agents are added and their quantities, as well as the method of solubilizing the liquid if partial crystallization has occurred.

**Additional information.** Even in the absence of light, the oily form of Retinol concentrate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Partial crystallization may occur in concentrated solutions and upon refrigeration. It may contain suitable antimicrobial agents and stabilizing agents such as antioxidants.

## **Requirements**

The declared content of retinol is not less than **500 000 IU/g**. Retinol concentrate contains not less than **95.0%** and not more than **110.0%** of the amount of  $C_{20}H_{30}O$  stated on the label.

*Note:* Once the container has been opened its contents should be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

## **Identity tests**

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 8 volumes of cyclohexane R and 2 volumes of ether R as the mobile phase. Apply separately to the plate 2  $\mu$ l of each of 4 solutions in cyclohexane R containing (A) 2 mg of Retinol per ml, (B) 2 mg of retinol acetate RS per ml, (C) 2 mg of retinol propionate RS per ml, and (D) 2 mg of retinol palmitate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with antimony trichloride TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds to one or more of the spots obtained with solutions B, C, and D.

B. Dissolve a small drop in about 1 ml of dichloromethane R and add 5 ml of antimony trichloride TS; a blue colour is immediately produced which turns gradually to violet-red.

**Acid value.** Not more than 2.0.

**Peroxides.** For solution (A) dissolve 0.30 g in 25 ml of a mixture of 4 volumes of methanol R and 6 volumes of toluene R. For solution (B) prepare a solution containing 0.27 g of ferric chloride R per ml, and add 1.0 ml to 99 ml of a mixture of 4 volumes of methanol R and 6 volumes of toluene R. Dilute 2.0 ml to 100 ml with the same solvent mixture.

Place in 2 separate test-tubes in the following order, mixing after each addition, 3 ml of a solution containing 18 mg of ammonium thiocyanate R per ml, 10 ml

of methanol R, 0.3 ml of ferrous sulfate/hydrochloric acid TS, and 15 ml of toluene R. Then add 1.0 ml of solution A into one tube and 1.0 ml of solution B into the other, shake, and allow to stand for 5 minutes. The colour produced with solution A is not more intense than that produced with solution B.

### **Assay**

*Note:* Carry out the assay as rapidly as possible, avoiding exposure to actinic light and oxidizing agents, and maintaining whenever possible an atmosphere of nitrogen above the solution.

The spectrophotometric measurements should be made at 20–25 °C. Before each series of measurements, check the wavelength scale of the spectrophotometer as well as the absorbance scale (Vol. 1, p. 33). The cells filled with 2-propanol R must not differ from each other in absorbance by more than 0.002 at each of the following wavelengths: 300 nm, 325 nm, 350 nm, and 370 nm.

Carry out each determination in duplicate, using separately weighed amounts of Retinol concentrate. Prepare a dilution series containing 25–100 mg of Retinol concentrate in 5 ml of *n*-pentane R and dilute with 2-propanol R to a presumed concentration of 10–15 IU per ml. Verify that the absorption maximum of the solution to be examined, measured against a solvent cell containing 2-propanol R, lies between 325 nm and 327 nm. Measure the absorbances at 300 nm, 326 nm, 350 nm, and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio  $A_{\lambda}/A_{326}$  for each wavelength. If the ratios do not exceed 0.592 at 300 nm, 0.537 at 350 nm, and 0.142 at 370 nm, calculate the content of retinol in International Units per gram from the expression:  $A_{326} \times V \times 1900/100 m$ , where  $A_{326}$  is the absorbance at 326 nm,  $V$  is the total volume used for the dilution to give 10–15 IU per ml,  $m$  is the mass of Retinol concentrate in g, and 1900 is the factor to convert the specific absorbances of ester of retinol into IU per g.

## *Selenii disulfidum*

### *Selenium disulfide*

SeS<sub>2</sub>

**Relative molecular mass.** 143.1

**Chemical name.** Selenium sulfide; CAS Reg. No. 7488-56-4.

**Description.** A bright orange to reddish brown powder.

**Solubility.** Selenium disulfide is practically insoluble in water and organic solvents.

**Category.** Antifungal drug.

**Storage.** Selenium disulfide should be kept in a well-closed container.

## **Requirements**

Selenium disulfide contains not less than **52.0%** and not more than **55.5%** of Se.

### **Identity tests**

- A. Gently boil 0.05 g with 5 ml of nitric acid (~1000 g/l) TS for 30 minutes, dilute to 50 ml with water, and filter. To 5 ml of the filtrate add 10 ml of water and 5 g of urea R, boil, cool, and add 2.0 ml of potassium iodide (80 g/l) TS; a yellow to orange colour is produced which darkens rapidly on standing. (Keep this solution for test B.)
- B. Allow the coloured solution obtained in test A to stand for 10 minutes, and filter through kieselguhr R1. The filtrate yields the reactions described under "General identification tests" as characteristic of sulfates (Vol. 1, p. 115).

**Sulfated ash.** Not more than 2.0 mg/g.

**Soluble selenium compounds.** For solution A, use 10 g of Selenium disulfide, add 100 ml of water, mix well, allow to stand for 1 hour with frequent shaking, and filter. For solution B, use a solution of selenious acid R containing 5 µg of selenium per ml. To 10 ml of each of solutions A and B, add 2 ml of a solution containing about 1 ml of formic acid (~1080 g/l) TS in 10 ml of water, and dilute both solutions to 50 ml with water. If necessary, adjust the pH to  $2.5 \pm 0.5$  with the diluted formic acid as prepared above. Then add 2.0 ml of freshly prepared 3,3'-diaminobenzidine tetrahydrochloride (5 g/l) TS, allow to stand for 45 minutes, and adjust the pH to  $6.5 \pm 0.5$  with ammonia (~100 g/l) TS. Shake both solutions for 1 minute with 10 ml of toluene R, and allow to separate. Measure the absorbances of a 1-cm layer of the toluene layers at 420 nm against a solvent cell containing the same reagents treated as described above. The absorbance of solution A is not more than that of solution B (5 µg of Se per g).

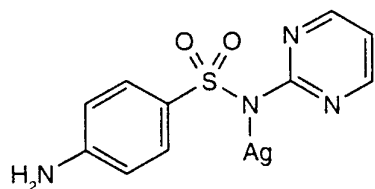
**Assay.** To about 0.1 g, accurately weighed, add 25 ml of fuming nitric acid R, heat on a water-bath for 1 hour, cool, and dilute to 100 ml with water. To 25 ml of this solution add 50 ml of water and 5 g of urea R, and heat to boiling. Cool, add 7 ml of potassium iodide (80 g/l) TS, 3 ml of starch TS, and titrate immediately with sodium thiosulfate (0.1 mol/l) VS. Repeat the procedure

without the Selenium disulfide being examined and make any necessary corrections.

Each ml of sodium thiosulfate (0.1 mol/l) VS is equivalent to 1.974 mg of Se.

## *Sulfadiazinum argentum*

### *Sulfadiazine silver*



**Relative molecular mass.** 357.1

**Chemical name.** *N*<sup>1</sup>-2-Pyrimidinylsulfanilamide monosilver(1+) salt; 4-amino-*N*-2-pyrimidinylbenzenesulfonamide monosilver(1+) salt; CAS Reg. No. 22199-08-2.

**Description.** A white or almost white, crystalline powder.

**Solubility.** Sulfadiazine silver is practically insoluble in water and ethanol (~750 g/l) TS; slightly soluble in acetone R and ether R; soluble in ammonia (~260 g/l) TS.

**Category.** Anti-infective drug.

**Storage.** Sulfadiazine silver should be kept in a well-closed container, protected from light.

## **Requirements**

Sulfadiazine silver contains not less than **98.0%** and not more than **102.0%** of  $\text{C}_{10}\text{H}_9\text{AgN}_4\text{O}_2\text{S}$ , calculated with reference to the dried substance.

## **Identity tests**

- *Either tests A and D or tests B, C, and D may be applied.*

Prepare the following residue to be used in tests A, B, and C: dissolve 0.5 g in 5 ml of nitric acid (~1000 g/l) TS, add 20 ml of water and 20 ml of sodium chloride (400 g/l) TS, mix, and filter. Neutralize the filtrate with sodium hydroxide (~80 g/l) TS using phenolphthalein/ethanol TS as indicator, and add 2.0 ml of acetic acid (~60 g/l) TS; a white precipitate is produced. Filter, wash the precipitate on the filter with water, and dry it at 105 °C for 1 hour.

- A. Carry out the examination with the residue as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from sulfadiazine RS or with the *reference spectrum* of sulfadiazine.
- B. About 10 mg of the residue yields the reaction described for the identification of primary aromatic amines under “General identification tests” (Vol. 1, p. 111), producing an orange-red precipitate.
- C. Dissolve about 0.1 g of the residue in 3 ml of water, add 3 ml of sodium hydroxide (50 g/l) TS, shake, and filter. To a portion of the filtrate add 1 drop of copper(II) sulfate (160 g/l) TS; a yellowish green precipitate is produced that on standing turns to brownish red.
- D. To about 0.1 g add 20 ml of water, 2 ml of nitric acid (~130 g/l) TS, and mix; a curdy, white precipitate is produced which is soluble in ammonia (~100 g/l) TS.

**Loss on drying.** Dry to constant mass at 80 °C; it loses not more than 5.0 mg/g.

**pH value.** Heat 1.0 g with 50 ml of carbon-dioxide-free water R to 70 °C for 5 minutes, cool rapidly, and filter. pH of the filtrate, 5.5–7.0.

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 7 volumes of dichloromethane R, 4 volumes of methanol R, and 1 volume of ammonia (~260 g/l) TS as the mobile phase. (*Note:* Mix the dichloromethane and methanol before adding the ammonia.) Apply separately to the plate 10 µl of each of the following 2 solutions. For solution (A) dissolve 50 mg of Sulfadiazine silver in 3.0 ml of ammonia (~260 g/l) TS and dilute with sufficient methanol R to produce 10 ml. For solution (B) dilute 1.0 ml of solution A with a mixture containing 4 volumes of methanol R and 1 volume of ammonia (~260 g/l) TS to produce 100 ml. Allow the spots to dry before development. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

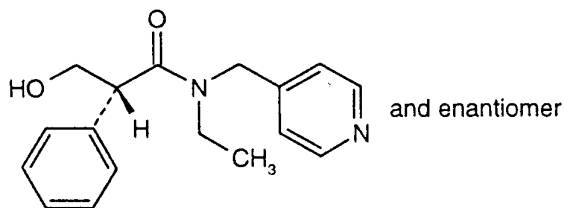
Any spot obtained with solution A, other than the principal spot, is not larger and more intense than that obtained with solution B (1.0%).

**Assay.** Transfer into a stoppered flask about 0.5 g, accurately weighed, and dissolve in 8 ml of nitric acid (~130 g/l) TS. Add 50 ml of water and titrate with ammonium thiocyanate (0.1 mol/l) VS, using ferric ammonium sulfate (45 g/l) TS as indicator. Repeat the procedure without the Sulfadiazine silver being examined and make any necessary corrections.

Each ml of ammonium thiocyanate (0.1 mol/l) VS is equivalent to 35.71 mg of  $C_{10}H_9AgN_4O_2S$ .

## Tropicamidum

### Tropicamide



$C_{17}H_{20}N_2O_2$

**Relative molecular mass.** 284.4

**Chemical name.** *N*-Ethyl-2-phenyl-*N*-(4-pyridylmethyl)hydracrylamide; *N*-ethyl- $\alpha$ -(hydroxymethyl)-*N*-(4-pyridinylmethyl)-benzeneacetamide; CAS Reg. No. 1508-75-4.

**Description.** A white or almost white, crystalline powder.

**Solubility.** Slightly soluble in water; freely soluble in dichloromethane R and ethanol (~750 g/l) TS.

**Category.** Mydriatic.

**Storage.** Tropicamide should be kept in a tightly closed container, protected from light.

**Labelling.** The designation Tropicamide for sterile non-injectable use indicates that the substance complies with the additional requirement and may be used for sterile applications. Expiry date.

## Requirements

Tropicamide contains not less than **99.0%** and not more than **101.0%** of  $C_{17}H_{20}N_2O_2$ , calculated with reference to the dried substance.

### Identity tests

- *Either test A alone or tests B, C, and D may be applied.*
- A. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from tropicamide RS or with the *reference spectrum* of tropicamide.
- B. The absorption spectrum of a 0.04 mg/ml solution in hydrochloric acid (0.1 mol/l) VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 254 nm; the absorbance of a 1-cm layer at this wavelength is about 0.72.
- C. Dissolve 5 mg in 3 ml of a mixture of 9 ml of acetic anhydride R, 1 ml of acetic acid (~300 g/l) TS, and 0.10 g of citric acid R. Heat on a water-bath for 5–10 minutes: a reddish yellow colour is produced.
- D. Melting temperature, about 97 °C.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry at 80 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 4 hours; it loses not more than 5.0 mg/g.

**Tropic acid.** To 10.0 mg add 5 mg of sodium tetraborate R and 0.35 ml of a freshly prepared solution containing 1.0 g of dimethylaminobenzaldehyde R in 10 ml of a mixture of 9 volumes of sulfuric acid (~1760 g/l) TS and 1 volume of water. Heat on a water-bath for 3 minutes. Cool in ice-water and add 5 ml of acetic anhydride R; no violet-red colour develops (0.05%).

**Related substances.** Carry out the test as described under “Thin-layer chromatography” (Vol. 1, p. 83), using silica gel R4 as the coating substance and a mixture of 95 volumes of dichloromethane R, 5 volumes of methanol R, and 0.5 volume of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in dichloromethane R containing (A) 20 mg of Tropicamide per ml, (B) 0.10 mg of Tropicamide per ml, and (C) 40 µg of Tropicamide per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Not more than one such spot is more intense than that obtained with solution C (0.2%).

**Assay.** Dissolve about 0.2 g, accurately weighed, in 50 ml of glacial acetic acid R1, and titrate with perchloric acid (0.1 mol/l) VS, using 1-naphtholbenzein/ acetic acid TS as indicator until the colour changes from orange to green as described under “Non-aqueous titration”, Method A (Vol. 1, p. 131).

Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 28.44 mg of  $C_{17}H_{20}N_2O_2$ .

***Additional requirement for sterile non-injectable Tropicamide***

*Complies with the “Test for sterility of non-injectable preparations” (see page 32).*