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SUBSTANCES, Vol. 4

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Comments on this draft document are invited and should be forwarded to WHO, Pharmaceuticals, attention Dr A. Mechkovski, 1211 Geneva 27, Switzerland, within 3 months of the date of distribution of the document.

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1. Introduction

The selection of monographs for the International Pharmacopoeia is determined by the WHO Model List of Essential Drugs, which is periodically updated. The majority of substances from this list are represented by monographs in volumes 2 and 3 of the International Pharmacopoeia. Added substances from the fifth WHO Model List of Essential Drugs, (TRS 770) will appear in volume 4. The attached 26 draft monographs have been prepared for substances not included in previous volumes.

We would be grateful if, for formulating your comments, you would refer to the "Guidelines for preparing and commenting on monographs for the International Pharmacopoeia".<sup>1</sup>

<sup>1</sup>Annex 5, WHO Expert Committee on Specifications for Pharmaceutical Preparations, twenty-ninth report, TRS 704, 1984.

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2. Monographs2.1 List of draft monographs

Acidum amidotrizoicum	S.0149
Acidum iopanoicum	S.0910
Acidum iotroxicum	S.0933
Acidum lacticum	S.1101
Aluminii sulfas	S.0173
Calaminum	S.03132
Cisplatinum	S.03131
Dactinomycinum	S.0401
Homatropini methylbromidum	S.0824
Imipramini hydrochloridum	S.0902
Iohexolum	S.0911
Ketamini hydrochloridum	S.1003
Magnesii sulfatis heptahydras	S.1286
Medroxyprogesteroni acetat	S.1287
Megluminum	S.1289
Mercaptopurinum	S.1218
Natrii amidotrizoas	S.1856
Natrii hydroxydum	S.18126
Norethisteroni enantas	S.1339
Podophylli resina	S.1536
Propylidodum	S.15108
Tamoxifeni citras	S.1971
Thiopentalum natricum	S.1951
Timololi maleas	S.1967
Vinblastini sulfas	S.2103
Insulinum	S.0921

2.2 Draft monographs

ACIDUM AMIDOTRIZOICUM

Amidotrizoic acid

Molecular formula.  $C_{11}H_9I_3N_2O_4$  (anhydrous);  $C_{11}H_9I_3N_2O_4 \cdot 2H_2O$  (dihydrate).

Relative molecular mass. 613.9 (anhydrous); 649.9 (dihydrate).

Graphic formula.

Chemical name. 3,5-Bis(acetylamino)-2,4,6-triiodobenzoic acid;  
3,5-diacetamido-2,4,6-triiodobenzoic acid; CAS Reg. No. 117-96-4 (anhydrous).  
3,5-Bis(acetylamino)-2,4,6-triiodobenzoic acid dihydrate;  
3,5-diacetamido-2,4,6-triiodobenzoic acid dihydrate;  
CAS reg. No. 50978-11-5 (dihydrate).

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

Solubility. Very slightly soluble in water and ethanol (~750 g/l)TS;  
freely soluble in dimethylformamide R; soluble in solutions of alkali  
hydroxides; sparingly soluble in methanol R; practically insoluble in  
chloroform R and ether R.

Category: Used in the preparation of meglumine amidotrizoate injection;  
radiocontrast medium.

Storage. Amidotrizoic acid should be kept in a well-closed container,  
protected from light.

Labelling. The designation on the container of amidotrizoic acid should state  
whether the substance is the dihydrate or is in the anhydrous form.

REQUIREMENTS

General requirement. Amidotrizoic acid contains not less than 98.0 % and not  
more than 102.0 % of  $C_{11}H_9I_3N_2O_4$ , 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). For amidotrizoic acid dihydrate, the substance must be previously dried at 105 °C for 4 hours. The infrared absorption spectrum is concordant with the spectrum obtained from amidotrizoic acid RS or with the reference spectrum of amidotrizoic acid.

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 20 volumes of chloroform R, 10 volumes of methanol R and 2 volumes of ammonia (~260 g/l)TS as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in a mixture of 0.8 g of sodium hydroxide R dissolved in 1000 ml of methanol R containing (A) 1.0 mg of the test substance per ml and (B) 1.0 mg of amidotrizoic acid 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.

C. Heat about 0.5 g in a suitable crucible; violet vapours are evolved.

D. About 10 mg yields the reaction described for the identification of primary aromatic amines under "General identification tests" (vol. 1, p.111), producing a red-purple precipitate.

Heavy metals. Suspend 10.0 g in 10 ml of water and add in small portions with stirring 1.5 ml of sodium hydroxide (~400 g/l)TS. When dissolved, adjust the pH between 7.0 and 7.5 with sodium hydroxide (~80 g/l)TS or hydrochloric acid (~70 g/l)TS, and dilute with water to 20 ml. Use 2.0 ml of this solution and determine the heavy metals content as described under "Limit test for heavy metals", according to method A (vol. 1, p. 119) (keep the remaining solution for the test of iodine and iodides); not more than 20 µg/g.

Halides. Dissolve 2.5 g in a mixture of 20 ml of water and 2.5 ml of ammonia (~100 g/l)TS. Add 20 ml of nitric acid (~130 g/l)TS, dilute with sufficient water to produce 100 ml, allow to stand for 15 minutes with occasional shaking, and filter. Eliminate the first 10 ml of the filtrate and proceed with 25 ml of the filtrate as described under "Limit test for chlorides" (vol. 1, p. 116); the content of halides, expressed as chlorides, does not exceed 35 µg/g.

Iodine and iodides. Place 4.0 ml of the solution prepared above for the test of heavy metals into a 50-ml centrifuge tube, add 20 ml of water, 5 ml of toluene R and 5 ml of sulfuric acid (~100 g/l)TS. Shake and centrifuge; the toluene layer shows no red colour. Add 2 ml of sodium nitrite (10 g/l)TS, shake well and centrifuge. Similarly prepare a reference solution containing 0.5 mg of potassium iodide R in 22 ml of water; any red colour in the toluene layer is not darker than that obtained from the reference solution (0.2 mg I/g).

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 4 hours; anhydrous Amidotrizoic acid loses not more than 10 mg/g and the dihydrate loses not less than 45 mg/g and not more than 70 mg/g.

Primary aromatic amines. Dissolve 0.20 g in a mixture of 5 ml of water and 1 ml of sodium hydroxide (~80 g/l)TS. Add 4 ml of sodium nitrite (10 g/l)TS, 10 ml of hydrochloric acid (1 mol/l)VS, shake and allow to stand for 2 minutes. Add 5 ml of ammonium sulfamate (25 g/l)TS, shake well, allow to stand for 1 minute and add 0.4 ml of 1-naphthol/ethanol TS, 15 ml of sodium hydroxide (~80 g/l)TS and dilute with water to 50 ml. Measure the absorbance at about 485 nm against a solvent cell containing the reagents prepared in a similar manner; the absorbance is not greater than 0.15.

Assay. Place about 0.3 g, accurately weighed, in a 125-ml conical flask, add 30 ml of sodium hydroxide (50 g/l)TS and 0.5 g of zinc R powder. Connect the flask to a reflux condenser and boil for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 ml of water into the flask and filter the mixture. Rinse the flask and the filter thoroughly and add the rinsing to the filtrate. Add 5 ml of glacial acetic acid R and 1 ml of tetrabromo-phenolphthalein ethyl ester TS and titrate with silver nitrate (0.05 mol/l)VS until the yellow precipitate just changes to green. Each ml of silver nitrate (0.05 mol/l)VS is equivalent to 10.23 mg of  $C_{11}H_9I_3N_2O_4$ .

ACIDUM IOPANOICUM  
Iopanoic acid

Molecular formula.  $C_{11}H_{12}I_3NO_2$

Relative molecular mass. 570.9

Graphic formula.

Chemical name. 3-Amino- $\alpha$ -ethyl-2,4,6-triiodohydrocinnamic acid; 3-amino- $\alpha$ -ethyl-2,4,6-triiodobenzenepropanoic acid; CAS Reg. No. 96-83-3.

Description. A light yellowish white powder; odour, faint, characteristic.

Solubility. Practically insoluble in water; soluble in ethanol (~750 g/l)TS, chloroform R and acetone R. Soluble in solutions of alkali hydroxides.

Category. Radiocontrast medium.

Storage. Iopanoic acid should be kept in a tightly-closed container, protected from light.

Additional information. Iopanoic acid is gradually affected by light.

REQUIREMENTS

General requirement. Iopanoic acid contains not less than 97.0 % and not more than 101.0 % of  $C_{11}H_{12}I_3NO_2$ , calculated with reference to the dried 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 iopanoic acid RS or with the reference spectrum of iopanoic acid.

B. Heat strongly about 0.05 g in a suitable crucible; violet vapours are evolved.

C. Melting temperature, about 155 °C with decomposition.

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.

Halides. Dissolve 0.80 g in a minimum quantity of sodium hydroxide (10 g/l)TS, dilute to 10 ml with water, add drop by drop sufficient nitric acid (~130 g/l)TS until complete precipitation is obtained and add an excess of 3 ml. Filter, wash the precipitate with 5 ml of water; to the filtrate add 1 ml of hydrogen peroxide (~330 g/l)TS and 1 ml of chloroform R, and shake. To serve as a reference solution, treat similarly 2 ml of iodide standard (20 µg I/ml)TS with 3 ml of nitric acid (~130 g/l)TS and sufficient water to equal the volume of the solution to be tested. The content of halides, expressed as iodides, does not produce a solution with any purple colour that is more intense than the reference solution.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 1 hour; it loses not more than 10 mg/g.

Assay. Place about 0.4 g, accurately weighed, in a 125-ml conical flask, add 30 ml of sodium hydroxide (50 g/l)TS and 0.5 g of zinc R powder. Connect the flask to a reflux condenser and boil for 30 minutes. Cool the flask to room temperature, rinse the condenser with 20 ml of water into the flask and filter the mixture. Rinse the flask and the filter thoroughly and add the rinsing to the filtrate. Add 5 ml of glacial acetic acid R and 1 ml of tetrabromophenolphthalein ethyl ester TS and titrate with silver nitrate (0.05 mol/l)VS until the yellow precipitate just changes to green. Each ml of silver nitrate (0.05 mol/l)VS is equivalent to 9.516 mg of  $C_{11}H_{12}I_3NO_2$ .

ACIDUM IOTROXICUM

Iotroxic acid

Molecular formula.  $C_{22}H_{18}I_6N_2O_9$

Relative molecular mass. 1215.8

Graphic formula.

Chemical name. 3,3'-[Oxybis(ethyleneoxymethylenecarbonylimino)]bis[2,4,6-triiodobenzoic acid]; CAS Reg. No. 51022-74-3.

Description. An almost white powder.

Solubility. Practically insoluble in water, benzene R and ether R; freely soluble in methanol R and dimethylformamide R; soluble in alkali hydroxide solutions.

Category. Used in the preparation of meglumine iotroxate as a radiocontrast medium.

Storage. Iotroxic acid should be kept in a well-closed container, protected from light.

REQUIREMENTS

General requirement. Iotroxic acid contains not less than 98.0 % and not more than 102.0 % of  $C_{22}H_{18}I_6N_2O_9$ , 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 spectrum obtained from iotroxic acid RS or with the reference spectrum of iotroxic acid.

B. Heat about 0.05 g in 2 ml of sulfuric acid (~1760 g/l)TS in a suitable crucible; violet vapours are evolved.

Heavy metals. For the preparation of the test solution used 1.0 g and add 3.0 ml of meglumine (100 g/l)TS as described under "Limit test for heavy metals", Procedure 1 (vol. 1, p. 118); determine the heavy metals content according to method A (vol. 1, p. 119); not more than 10 µg/g.

Halides. Dissolve 10 g in about 30 ml of meglumine (100 g/l)TS and titrate potentiometrically with silver nitrate (0.001 mol/l)VS. Each ml of silver nitrate (0.001 mol/l)VS is equivalent to 0.1269 mg of I; the content of halides, expressed as iodides, does not exceed 40 µg/g.

Solution in alkali. Dissolve 5.0 g in 5.0 ml of sodium hydroxide (~80 g/l)TS and add 2.0 ml of water; the solution is 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 0.4 g of the substance; the water content is not less than 10 mg/g and not more than 30 mg/g.

Foreign substances. Carry out the test as described under "Thin-layer chromatography" (vol. 1, p. 83), using silica gel R6 as the coating substance and a mixture of 62 volumes of chloroform R, 32 volumes of methanol R, 2 volumes of anhydrous formic acid R and 6 volumes of water as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol R containing (A) 100 mg of the test substance per ml and (B) 0.50 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of air at room temperature 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.

Primary aromatic amines. Transfer about 1 g, accurately weighed, to a 50-ml volumetric flask, dissolve in 2.5 ml of sodium hydroxide (1 mol/l)VS and add 12.5 ml of water (= solution A). Dissolve 5.0 mg of 3-amino-2,4,6-triiodobenzoic acid RS, accurately weighed, in 0.20 ml of sodium hydroxide (0.1 mol/l)VS and dilute with sufficient water to produce 10.0 ml. Introduce 2.0 ml of this solution to a 50-ml volumetric flask, add 3.0 ml of water and 10.0 ml of sodium hydroxide (0.1 mol/l)VS (= solution B). For the blank solution transfer 5.0 ml of water to a 50-ml volumetric flask and add 10 ml of sodium hydroxide (0.1 mol/l)VS. Note: Proceed with the three solutions concomitantly, strictly observing the instructions.

Add 25 ml of dimethyl sulfoxide R, close the flask and swirl to mix. Allow to stand in the dark in an ice bath for 5 minutes. Continue the procedure in the dark. Add while shaking 2.0 ml of hydrochloric acid (~420 g/l)TS, allow to stand in the ice bath for 5 minutes and add while shaking 2.0 ml of freshly prepared sodium nitrite (20 g/l)TS. Start the timing using a stopwatch readable to 1 second and allow to stand in the ice bath for exactly 5 minutes. Add 1.0 ml of freshly prepared sulfamic acid (80 g/l)TS, start the timing again and shake till no more gas evolves. Allow to stand in the ice bath for exactly 5 minutes. Then add 2.0 ml of freshly prepared N-(1-naphthyl)ethylenediamine hydrochloride/propylene glycol TS, timing with the stopwatch allow to stand in a water-bath at 22-25 °C for exactly 10 minutes, and dilute to volume with water. Immediately measure the absorbances of solutions A and B against the blank solution at a wavelength of 465 nm. The absorbance of solution A should not be greater than that of solution B.

Assay. Carry out the combustion as described under "Oxygen flask method" (vol. 1, p. 124), but using 3-5 mg of the test substance and allowing the absorbing liquid after rinsing to stand for 20-30 minutes. Titrate the liberated iodine with sodium thiosulfate (0.02 mol/l)VS. Each ml of sodium thiosulfate (0.02 mol/l)VS is equivalent to 0.675 mg of  $C_{22}H_{18}I_6N_2O_9$ .

#### ACIDUM LACTICUM

##### Lactic acid

Composition. Lactic acid is a mixture of lactic acid, its condensation products and water, the equilibrium between the components being dependent on the concentration and temperature.

Molecular formula.  $C_3H_6O_3$

Relative molecular mass. 90.08

Graphic formula.

Chemical name. 2-Hydroxypropanoic acid; CAS Reg. No. 50-21-5.

Description A colourless or slightly yellow, clear, syrupy, caustic liquid; odourless or with a slight characteristic odour.

Miscibility. Miscible with water, ethanol (~750 g/l)TS and ether R; practically immiscible with chloroform R.

Category. Used in the preparation of sodium lactate solution.

Storage. Lactic acid should be kept in a tightly closed container.

Additional information. Lactic acid as described, is not suitable for parenteral administration (haemodialysis). Lactic acid is usually a racemate (RS), but the (+)-(S)-isomer may predominate; it is hygroscopic.

#### REQUIREMENTS

General requirement. Lactic acid contains not less than 88.0 % m/m and not more than 92.0 % m/m of  $C_3H_6O_3$ .

#### Identity tests

A. Heat 5 drops with 5 ml of potassium permanganate (10 g/l)TS to boiling; the solution decolorizes.

B. A mixture of 1.0 ml and 9 ml of water shows an acid reaction with pH-indicator paper R.

C. Relative density,  $d_{20}^{20} = 1.20 - 1.21$ .

Heavy metals. Use 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 1 (vol. 1, p. 118); determine the heavy metals content according to Method A (vol. 1, p. 119); not more than 10  $\mu\text{g/g}$ .

Iron. Use 1.0 g; the solution complies with the "Limit test for iron" (vol. 1, p. 121); not more than 40 µg/g.

Calcium. Dissolve 5 g in 42 ml of sodium hydroxide (1 mol/l)VS and dilute to 50 ml with distilled water. Dilute 5 ml to 15 ml with distilled water (keep the remaining solution for the tests below).

To 0.2 ml of ethanolic calcium standard (100 µg/ml Ca)TS add 1 ml of ammonium oxalate (50 g/l)TS and allow to stand for 1 minute. Add a mixture of 1 ml of acetic acid (~60 g/l)TS and the prepared 15 ml of solution to be tested. Similarly prepare a reference solution but using 10 ml of calcium standard (10 µg/ml)TS and 5 ml of water. Allow both solutions to stand for 15 minutes. Any opalescence in the solution tested is not more intense than that of the reference solution (200 µg/g).

Chlorides. Dissolve 0.1 g in 10 ml of water, acidify with nitric acid (~130 g/l)TS and add a few drops of silver nitrate (40g/l)TS; no opalescence is produced immediately.

Sulfates. Take 25 ml of the solution prepared for the limit test for calcium and proceed as described under "Limit test for sulfates" (vol. 1, p. 116); the sulfate content is not more than 0.2 mg/g.

Sugars and other reducing substances. To 1 ml of the solution prepared for the limit test for calcium add 1 ml of hydrochloric acid (1 mol/l)VS, heat to boiling, cool,, add 1.5 ml of sodium hydroxide (1 mol/l)VS and 2 ml of potassium-cupric tartrate TS. Heat to boiling; no red or greenish precipitate is produced.

Volatile fatty acids. Heat 5 g cautiously in a glass-stoppered flask at 50 °C for 10 minutes; no unpleasant odour resembling that of the lower fatty acids is detectable immediately after opening the flask.

Methanol and methyl esters. Place 2.0 g in a round-bottomed flask and add 10 ml of water. Cool in ice, add cautiously a mixture of 7.5 ml of water with 22.5 ml of potassium hydroxide (400 g/l)TS and cool in ice for a further 10 - 15 minutes. Adjust to a suitable condenser and steam distil. Collect the distillate in a 10-ml graduated flask containing 1 ml of ethanol (~750 g/l)TS and distil until a volume of at least 9.5 ml is obtained. Dilute to 10.0 ml with water. To 1.0 ml of this solution add 5 ml of potassium permanganate/phosphoric acid TS and mix. After 15 minutes add 2 ml

of oxalic acid/sulfuric acid TS, stir with a glass rod until the solution is colourless and then add 5 ml of decolorized fuchsin TS. Allow to stand for 2 hours. The solution is not more intensely coloured than a similarly prepared reference solution, but using instead of the distillate 1.0 ml of a solution containing 100 µg of methanol R and 0.1 ml of dehydrated ethanol R per ml (500 µg/g of methanol).

Citric, oxalic, phosphoric and tartaric acid. To 1 g dissolved in 10 ml of water add 40 ml of calcium hydroxide TS, and boil for 2 minutes; no turbidity is produced.

Ether-insoluble substances. Dissolve 1.0 g in 25 ml of ether R; the solution is not more opalescent than 25 ml of ether R.

Colour. It is 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.

Assay. To 1 g, accurately weighed, add 10 ml of water and 20.0 ml of sodium hydroxide (1 mol/l)VS. Stopper the flask and allow to stand for 30 minutes. Titrate with hydrochloric acid (1 mol/l)VS using 0.5 ml of phenolphthalein/ethanol TS as indicator. Each ml of sodium hydroxide (1 mol/l)VS is equivalent to 90.1 mg of  $C_3H_6O_3$ .

ALUMINII SULFAS  
Aluminium sulfate

Molecular formula.  $Al_2(SO_4)_3 \cdot nH_2O$ .

Relative molecular mass. 342.1(anhydrous).

Chemical name. Aluminium sulfate (2:3); sulfuric acid, aluminium salt (3:2); CAS Reg. No. 10043-01-3 (anhydrous); CAS Reg.No. 17927-65-0 (2-3 hydrates).

Description. Colourless, lustrous crystals or crystalline masses, or a white crystalline powder; odourless.

Solubility. Soluble in cold water; freely soluble in hot water; practically insoluble in ethanol (~750 g/l)TS.

Category. Used in the preparation of aluminium acetate solution; astringent.

Storage. Aluminium sulfate should be kept in a well-closed container.

Additional information. Aluminium sulfate contains a variable quantity of water of crystallization.

#### REQUIREMENTS

General requirement. Aluminium sulfate contains not less than 51.0 % and not more than 59.0 % of  $Al_2(SO_4)_3$ .

#### Identity tests

A. Dissolve 0.20 g in 2 ml of water, add about 0.5 ml of hydrochloric acid (~70 g/l)TS and about 0.5 ml of thioacetamide TS; no precipitate is formed. Add drop by drop sodium hydroxide (~80 g/l)TS; a gelatinous, white precipitate is formed which dissolves on further addition of sodium hydroxide. Gradually add ammonium chloride (100 g/l)TS; a gelatinous, white precipitate reappears.

B. A 0.10 g/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 1 (vol. 1, p. 118); determine the heavy metals content according to method A (vol. 1, p. 119); not more than 50 µg/g.

Iron. Use 0.4 g; the solution complies with the "Limit test for iron" (vol. 1, p. 121); not more than 100 µg/g.

Alkali and alkaline-earth metals. Dissolve 1 g in 100 ml of water, heat, add 0.1 ml of methyl red/ethanol TS and sufficient ammonia (~100 g/l)TS until the colour of the solution changes to yellow. Dilute to 150 ml with water, heat to boiling and filter. Evaporate 75 ml of the filtrate to dryness on a water-bath and ignite to constant mass; the residue weighs not more than 2 mg (0.4 %).

Colour and clarity of solution. A solution of 0.50 g in 10 ml of water is not more intense than opalescence standard TS2 and colourless.

pH value. pH of a 20 mg/ml solution in carbon-dioxide-free water R, 2.5 - 4.0.

Assay. Dissolve about 0.5 g, accurately weighed, in 20 ml of water, and proceed as described under "Complexometric titrations" for aluminium (vol. 1, p. 128). Each ml of disodium edetate (0.05 mol/l)VS is equivalent to 8.554 mg of  $\text{Al}_2(\text{SO}_4)_3$ .

#### CALAMINUM

##### Calamine

Composition. Calamine is zinc oxide with a small proportion of ferric oxide.

Description. A fine, amorphous pink or reddish brown powder; odourless.

Solubility. Practically insoluble in water; soluble with effervescence in mineral acids.

Category. Used in the preparation of calamine lotion; antipruritic.

Storage. Calamine should be kept in a well-closed container.

Additional information. Attention should be paid to the microbiological quality since Calamine is of natural origin.

#### REQUIREMENTS

General requirement. Calamine contains not less than 98.0 % and not more than 100.5 % of ZnO, calculated with reference to the ignited substance.

#### Identity tests

A. Shake 1 g with 10 ml of hydrochloric acid (~70 g/l)TS and filter. To 5 ml of the filtrate add 0.3 ml of sodium hydroxide (~80 g/l)TS; a white precipitate is formed. Add a further 2 ml of sodium hydroxide (~80 g/l)TS; the precipitate dissolves. Add 10 ml of ammonium chloride (100 g/l)TS; the solution remains clear. Add 0.1 ml of sodium sulfide TS; a flocculent, white precipitate is formed.

B. To 1 g add 10 ml of hydrochloric acid (~70 g/l)TS, heat to boiling and filter. To the filtrate add a few drops of ammonium thiocyanate (75 g/l)TS; a reddish colour is produced.

Calcium or magnesium. Digest 1 g in 25 ml of hydrochloric acid (~70 g/l)TS for 30 minutes and filter. To the filtrate add ammonia (~100 g/l)TS until the precipitate first formed is redissolved, then add 5 ml more of ammonia (~100 g/l)TS. To 10 ml of this solution add 2 ml of ammonium oxalate (25 g/l)TS; not more than a slight turbidity is produced. To a further 10 ml portion add 2 ml of disodium hydrogen phosphate (100 g/l)TS; not more than a slight turbidity is produced.

Lead. Dissolve 2.0 g in a mixture of 20 ml of water and 5 ml of glacial acetic acid R, filter, and add 0.10 ml of potassium chromate (100 g/l)TS to the filtrate; the solution remains clear for 5 minutes.

Acid-insoluble substances. Dissolve 2.0 g in 50 ml of hydrochloric acid (~70 g/l)TS, and filter. Wash the residue with water and dry it to constant weight at 105 °C; the residue weighs not more than 40 mg (2.0 %).

Alkaline substances. Digest 1.0 g with 20 ml of water and warm on a water-bath for 15 minutes. Filter and add 2 drops of phenolphthalein/ethanol TS to the filtrate; if a red colour is produced not more than 0.20 ml of sulfuric acid (0.05 mol/l)VS is required to discharge it.

Ethanol-soluble dyes. Shake 1.0 g with 10 ml of ethanol (~710 g/l)TS and filter; the filtrate is colourless.

Water-soluble dyes. Shake 1.0 g with 10 ml of water and filter; the filtrate is colourless.

Loss on ignition. Weigh 2 g and ignite at 500 °C to constant weight; not more than 20 mg/g (keep the freshly ignited material for the assay).

Assay. Weigh about 1.5 g of freshly ignited material (see loss on ignition), accurately weighed, add 50.0 ml of sulfuric acid (0.5 mol/l)VS, heat gently until no further solution occurs and filter. Wash the residue with hot water until the last washing is neutral to litmus paper R. To the combined filtrate and washings add 2.5 g of ammonium chloride R, cool, and titrate with sodium hydroxide (1 mol/l)VS using methyl orange/ethanol TS as indicator. Each ml of sulfuric acid (0.5 mol/l)VS is equivalent to 40.69 mg of ZnO.

## CISPLATINUM

## Cisplatin

Molecular formula.  $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$

Relative molecular mass. 300.0

Graphic formula.

Chemical name. cis-Diamminedichloroplatinum; CAS Reg. No. 15663-27-1.

Description. White to yellowish crystals or a yellow powder.

Solubility. Slightly soluble in water; sparingly soluble in dimethylformamide R; practically insoluble in methanol R and chloroform R.

Category. Cytotoxic drug.

Storage. Cisplatin should be kept in a tightly closed container, protected from light, and stored at a temperature between 2 and 8 °C.

Additional information. **CAUTION:** Cisplatin must be handled with care avoiding contact with the skin and inhaling of airborne particles. When heated, it blackens at about 270 °C with decomposition.

REQUIREMENTS

General requirement. Cisplatin contains not less than 96.0 % and not more than 102.0 % of  $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$ , calculated with reference to the anhydrous substance.

Identity tests

. Either test A and B 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 cisplatin RS or with the reference spectrum of cisplatin.

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 0.050 g in a glass dish, add 2 ml of sodium hydroxide (~80 g/l)TS and evaporate to dryness on a water-bath in a mixture of 0.5 ml of nitric acid (~1000 g/l)TS and 1.5 ml of hydrochloric acid (~420 g/l TS), and again evaporate to dryness; the residue is orange. Dissolve it in 0.5 ml of water and add 0.5 ml of ammonium chloride (100 g/l)TS; a yellow, crystalline precipitate is produced.

Clarity and colour of solution. Dissolve 25.0 mg in 25.0 ml of a solution composed of 0.22 g of sodium chloride R dissolved in 25.0 ml of carbon-dioxide-free water R; the solution is clear and not more intensely coloured than standard colour Gn3 when compared as described under "Colour of liquids (vol.1, p.50). (Keep the solution for the pH-value as described below).

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 10 mg/g.

pH value. The pH of the solution prepared for the "Clarity and colour of solution" and measured immediately, 4.5 - 6.0.

Related substances. Carry out the test as described under "Thin-layer chromatography (vol.1, p.83), using cellulose R previously activated by heating at 150 °C as the coating substance and mixture of 1 volume of water and 9 volumes of acetone R as the mobile phase. Apply separately to the plate 2.5 µl of each of 2 solutions in a mixture of equal volumes of dimethylformamide R and water containing (A) 2.0 mg of the test substance per ml, and (B) 2.0 mg of cisplatin RS per ml. Also apply 5 µl of each of 2 solutions in dimethylformamide R containing (C) 20 mg of the test substance per ml and (D) 0.40 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in a current of warm air, spray it with stannous chloride/hydrochloric acid TS and allow to dry again. With solution C no spot appears lower than the principal spot. Any other spot obtained with solution C, other than the principal spot, is not more intense than that obtained with solution D.

Ultraviolet absorbance ratio. (Prior to use, clean all glassware with a mixture of 3 volumes of hydrochloric acid (~420 g/l)TS and 1 volume of nitric acid (~1000 g/l)TS, rinse thoroughly with water and dry. Note: Do not use dichromate for cleaning and do not use acetone or pressurized air for drying. Protect the test solutions from light and use them within 1 hour after preparation). Transfer about 98.5 mg, accurately weighed, to a 100-ml volumetric flask and dissolve in sufficient hydrochloric acid (0.1 mol/l)VS to produce 100 ml. Stir with a magnetic bar at a high speed for 5 minutes or place in an ultrasonic bath for 10 seconds or until completely dissolved. The ratio of the absorbance measured in a 2-cm layer against hydrochloric acid (0.1 mol/l)VS at the maximum wavelength of about 301 nm to that at the minimum wavelength of about 246 nm is not less than 4.5.

Silver. Determine by atomic absorption spectrophotometry (vol.1, p.45) at a wavelength of 328 nm using a silver hollow cathode lamp, an air-acetylene flask, and at a slit width of 0.5 nm; as a standard solution use silver standard (5 µg Ag/ml)TS. For the test solution dissolve 0.10 g in 15 ml of nitric acid (~1000 g/l)TS while heating at 80 °C and dilute with water to 25 ml; not more than 250 µg of Ag per g.

Assay. Dissolve about 25 mg, accurately weighed in sufficient hydrochloric acid (~70 g/l)TS to produce 25 ml. Dilute 1.0 ml of this solution with the same solvent to 25 ml. Transfer 5 ml to a glass-stoppered 25-ml conical flask and add 10 ml of hydrochloric acid (~70 g/l)TS. Place into a second flask 15 ml of hydrochloric acid (~70 g/l)TS to serve as a blank. Add 2.5 ml of stannous chloride/hydrochloric acid TS2 and dilute to volume with hydrochloric acid (~70 g/l)TS. Mix and allow to stand for 30 minutes. Measure the absorbance of a 2-cm layer at the maximum at about 402 nm against a solvent cell containing the blank. Calculate the amount of  $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$  in the substance being tested by comparison with cisplatin RS, similarly and concurrently examined.

#### DACTINOMYCINUM

Dactinomycin

Molecular formula.  $\text{C}_{62}\text{H}_{86}\text{N}_{12}\text{O}_{16}$

Relative molecular mass. 1255

Graphic formula.

Chemical name. Actinomycin D; CAS Reg. No. 50-76-0.

Description. An orange-red to red, crystalline powder.

Solubility. Soluble in water at 10 °C and slightly soluble in water at 37 °C; freely soluble in ethanol (~750 g/l)TS and methanol R; very slightly soluble in ether R.

Category. Cytotoxic drug.

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

Additional information. Dactinomycin is hygroscopic and is affected by light and heat. CAUTION. Dactinomycin must be handled with care, avoiding contact with the skin and inhaling of airborne particles.

REQUIREMENTS

General requirement. Dactinomycin contains not less than 95.0 % and not more than 103.0 % of  $C_{62}H_{86}N_{12}O_{16}$ , calculated with reference to the dried substance.

Identity tests

A. The absorption spectrum of a 25 µg/ml solution in methanol R, when observed between 220 nm and 500 nm, exhibits 2 maxima at about 240 nm and 445 nm. The absorbance of a 1-cm layer at the maximum wavelength of 445 nm is about 0.83; the ratio of the absorbance at 240 nm to that at 445 nm is between 1.30 and 1.50.

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 1-butanol R, 2 volumes of water and 1 volume of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in acetone R containing (A) 10 mg of the test substance per ml and (B) 10 mg of dactinomycin 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.

C. Add about 1 mg to a solution of 10 mg of paraformaldehyde R in 1 ml of sulfuric acid (~1760 g/l)TS; a red-violet colour is produced.

Melting range. 235 - 237 °C.

Specific optical rotation. Use a 10 mg/ml solution in methanol R and calculated with reference to the dried substance;  $[\alpha]_D^{20} = -292$  to  $-317^\circ$ .

Sulfated ash. Not more than 10 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 50 mg/g.

pH value. pH of a saturated solution, 5.5 - 7.0.

Assay. Carry out the test as described under "High performance liquid chromatography" (vol. 3, p. 373), using a column 30 cm long and 3.9 mm in internal diameter packed with porous silica gel or ceramic microparticles, 5-10 µm in diameter, the surface of which has been modified with chemically bonded octadecyl silane groups.

As the mobile phase, use a mixture of 46 volumes of acetonitrile R, 25 volumes of sodium acetate (0.04 mol/l)VS and 25 volumes of acetic acid (0.07 mol/l)VS, filter through a membrane filter (porosity of 1 µm or finer) and degas the resulting solvent mixture. (Note: the concentration of acetonitrile may have to be adjusted to provide a suitable chromatogram and elution time.)

Prepare the following solutions immediately before use in the above-mentioned mobile phase, and store them protected from light, containing:

- (A) 1.20 mg of the test substance per ml, and
- (B) 1.20 mg of dactinomycin RS per ml.

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

Make 3 replicate injections of solution B, each of 20 µl to determine the peak responses. The relative standard deviation of the peaks is not more than 1.0 %.

Inject 20 µl of each of solutions A and B. Measure the peak responses. (The retention time for dactinomycin is about 25 minutes.) Calculate the content in % of  $C_{62}H_{86}N_{12}O_{16}$  using the following formula:  
 $0.25(M/W)(A_1/A_2)$ , in which  $M$  is the concentration of the reference solution of mg per ml,  $W$  is the weight in mg of the substance to be tested, and  $A_1$  and  $A_2$  are the peak responses of the test substance and reference substance, respectively.

Pyrogens. Carry out the test as described under "Test for pyrogens" (vol.1, p.155) injecting, per kg of the rabbit's mass 1 ml of a solution in sterile water R containing 0.2 mg of the substance to be examined per ml.

#### HOMATROPINI METHYLBROMIDUM

Homatropine methylbromide

Molecular formula.  $C_{17}H_{24}BrNO_3$

Relative molecular mass. 370.3

Graphic formula.

Chemical name. 8-Methyltropinium bromide mandelate; 3 $\alpha$ -hydroxy-8-methyl-1 $\alpha$ H,5 $\alpha$ H-tropanium bromide mandelate; endo-3-[(hydroxyphenylacetyl)oxy]-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide; CAS Reg. No. 80-49-9.

Description. A white, crystalline powder; odourless.

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

Category. Mydriatic.

Storage. Homatropine methylbromide should be kept in a tightly closed container, protected from light.

Additional information. Homatropine methylbromide darkens on exposure to light.

REQUIREMENTS

General requirements. Homatropine methylbromide contains not less than 98.5 % and not more than 101.0 % of  $C_{17}H_{24}BrNO_3$ , calculated with reference to the dried substance.

Identity tests

A. Dissolve 10 mg in 1 ml of water, add ammonia (~100 g/l)TS to render the solution slightly alkaline, and shake with 5 ml of chloroform R. Evaporate the chloroform layer to dryness on a water-bath and add 1.5 ml of mercuric chloride/ethanol TS to the residue; no yellow or red colour is produced (distinction from homatropine, atropine and other solanaceous alkaloids).

B. A 20 mg/ml solution yields reaction A described under "General identification tests" as characteristic of bromides (vol.1, p.112).

C. Melting temperature, about 190 °C.

Sulfated ash. Not more than 2.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

pH value. pH of a 10 mg/ml solution, 4.5 - 6.5.

Related substances. Carry out the test as described under "Thin-layer chromatography" (vol.1, p.83); using silica gel R5 as the coating substance and a mixture of 6 volumes of 1-propanol R, 3 volumes of water, 2 volumes of methanol 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 a mixture of 9 volumes of methanol R and 1 volume of water containing (A) 40 mg of the test substance per ml, and (B) 0.4 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air and spray it first with potassium iodobismuthate TS<sub>2</sub>, then with sodium nitrite (50 g/l)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.

Assay. Dissolve about 0.7 g, accurately weighed, in 50 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/l)VS, determining the endpoint potentiometrically 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 37.03 mg of  $C_{17}H_{24}BrNO_3$ .

## IMIPRAMINI HYDROCHLORIDUM

Imipramine hydrochloride

Molecular formula.  $C_{19}H_{24}N_2 \cdot HCl$

Relative molecular mass. 316.9

Graphic formula.

Chemical name. 5-[3-(Dimethylamino)propyl]-10,11-dihydro-5H-dibenz[b,f]azepine monohydrochloride; 10,11-dihydro-N,N-dimethyl-5H-dibenz[b,f]azepine-5-propanamine monohydrochloride; CAS Reg. No. 113-52-0.

Other name. Imizine.

Description. A white or slightly yellowish, crystalline powder; odourless or almost odourless.

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

Category. Psychotherapeutic drug.

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

Additional information. Even in the absence of light, Imipramine hydrochloride is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

REQUIREMENTS

General requirement. Imipramine hydrochloride contains not less than 98.0 % and not more than 102.0 % of  $C_{19}H_{24}N_2.HCl$ , calculated with reference to the dried 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 imipramine hydrochloride RS or with the reference spectrum of imipramine hydrochloride.
- B. Dissolve about 2 mg in 2.0 ml of water and add 2 ml of nitric acid (~1000 g/l)TS; an intense blue colour is produced (based on BT).
- C. Dissolve about 0.05 g in 3 ml of water and add 0.05 ml of quinhydrone/methanol TS; no red colour develops within 15 minutes.
- D. A 0.05 g/ml solution yields reaction B described under "General identification tests" as characteristic of chlorides (vol. 1, p. 113).

Melting range. 170 - 174 °C, with decomposition.

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. Dissolve rapidly by shaking and triturating with a glass rod 1.0 g in 10 ml of carbon-dioxide-free water R; the solution is clear. (Keep a portion of this solution for the test of the pH value.) Immediately dilute the solution with an equal volume of water; it is 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 to constant weight at 105 °C; it loses not more than 5.0 mg/g.

pH value. pH of the solution prepared for the "Clarity of solution", measured immediately after preparation, 3.6 - 5.0.

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 5 volumes of hydrochloric acid (~250 g/l)TS, 5 volumes of water, 35 volumes of glacial acetic acid R and 55 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of 3 solutions in methanol R prepared immediately before use containing (A) 25 mg of the test substance per ml, (B) 0.05 mg of the test substance per ml, and (C) 0.05 mg of iminodibenzyl R per ml. After removing the plate from the chromatographic chamber, allow it to dry for 5 minutes, spray with a solution of 0.5 g of potassium dichromate R dissolved in a mixture of 4 volumes of water and 1 volume of sulfuric acid (~1760 g/l)TS. Examine the plate immediately in daylight. Any spot obtained with solution A, other than the principal spot which is blue in colour and the spot corresponding to iminodibenzyl as obtained with solution C, is not more intense than the spot obtained with solution B. Furthermore, any spot obtained with solution A corresponding to iminodibenzyl is not more intense than the spot obtained with solution C.

Assay. Dissolve about 0.3 g, accurately weighed, in 80 ml of glacial acetic acid R1, add 10 ml of mercuric acetate/acetic acid TS, and titrate 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 31.69 mg of  $C_{19}H_{24}N_2, HCl$ .

#### IOHEXOLUM

iohexol

Molecular formula.  $C_{19}H_{26}I_3N_3O_9$

Relative molecular mass. 821.1

Graphic formula.

Chemical name.

N,N'-bis(2,3-Dihydroxypropyl)-5-[N-(2,3-dihydroxypropyl)acetamido]-2,4,6-triiodoisophthalamide; CAS Reg. No. 66108-95-0.

Description. A white to greyish, amorphous powder.

Solubility. Very soluble in water and in methanol R; practically insoluble in chloroform R.

Category. Radiocontrast medium.

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

Additional information. The bulk is isolated by evaporation of an aqueous solution; melting range, 177-187 °C.

#### REQUIREMENTS

General requirement. Iohexol contains not less than 98.5 % and not more than 101.5 % of  $C_{19}H_{26}I_3N_3O_9$ , 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 iohexol RS or with the reference spectrum of iohexol.

B. The absorption spectrum of a 10 µg/ml solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 245 nm; the absorbance of a 1-cm layer at the maximum wavelength is about 0.36.

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. Heat about 0.05 g in a suitable crucible; violet vapours are evolved.

Aluminium. Separately, place into four different separatory funnels 1.0 ml and 2.0 ml of a freshly prepared aluminium standard (10 µg Al<sup>3+</sup>/ml)TS, 10 ml of a 0.5 g/ml solution of the substance to be examined in water, and

10 ml of water to serve as a blank. To each funnel add 5 ml of ammonium chloride buffer, pH 10.5, TS and dilute to 25 ml with water. To every funnel add 5 ml of 8-hydroxyquinoline/chloroform TS and shake for 2 minutes. Allow the phases to separate and measure the absorbance of the extracts against the extracted blank solution at a maximum at about 395 nm; the aluminium content is not more than 4 µg/g.

Copper. Separately, place into four different separatory funnels 0.20 ml and 1.0 ml of a freshly prepared copper standard (10 µg/ml Cu)TS, 10 ml of a 0.5 g/ml solution of the substance to be examined in water, and 15 ml of water to serve as a blank. To each funnel add 1.0 ml of ammonium pyrrolidinedithiocarbamate (10 g/l)TS, 5 ml of acetate buffer, pH 4.5, TS and dilute to 25 ml with water. To every funnel add 5 ml of isobutyl methyl ketone R and shake for 2 minutes. Allow the phases to separate and measure the absorbance of the extracts against the extracted blank solution at a maximum at about 435 nm; the copper content is not more than 0.5 µg/g.

Halides. Dissolve 5 g in about 20 ml of water and titrate with silver nitrate (0.001 mol/l)VS determining the endpoint potentiometrically, using a silver/silver chloride electrode system. Repeat the operation without the substance being tested. Each ml of silver nitrate (0.001 mol/l)VS is equivalent to 0.1269 mg of I; the content of halides, calculated as iodides, does not exceed 20 µg/g.

Colour of solution. Dissolve 6.47 g of the substance in water to make 10.0 ml (make correction for water content). The concentration of the solution is 64.7 %, eqv. to 300 mg I/ml. Filter through Millipore filter 0.22 µm, and measure the absorbance in a 1 cm cell with distilled water as blank at 400 nm, 420 nm and 450 nm. The absorbance is not greater than 0.200, 0.050 and 0.025, respectively.

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; the water content is not more than 50 mg/g.

Related substances. Carry out the test as described under "Thin-layer chromatography" (vol. 1, p. 83), using silica gel R6 as the coating substance and a mixture of 50 volumes of 1-butanol R, 11 volumes of acetic acid (~300 g/l)TS and 25 volumes of water as the mobile phase. Apply separately to the plate 10 µl of each of 4 solutions in methanol R containing (A) 10 mg

of the test substance per ml, (B) 10 mg of iohexol RS per ml, (C) 20 mg of the test substance per ml, and (D) 40 µg of the test substance 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, other than the principal spot, is not more intense than that obtained with solution D.

Primary aromatic amines

. The solutions must be protected from light throughout the procedure.

To 0.20 g add 15 ml of water, mix to dissolve and allow to stand in an ice-bath for 5 minutes. Add 1.5 ml of hydrochloric acid (~250 g/l)TS and 2.0 ml of sodium nitrite (10 g/l)TS, mix, and return the flask to the ice-bath for exactly 4 minutes. Add 1.0 ml of sulfamic acid (50 g/l)TS and again place the flask in the ice-bath for exactly 1 minute. Remove the flask from the bath, add 0.5 ml of freshly prepared N-(1-naphthyl)ethylenediamine hydrochloride/propylene glycol TS, mix and dilute to 25.0 ml with water. Within 20 minutes measure the absorbance of a 5-cm layer (see Annex, p. 86) at the maximum at about 495 nm against a solvent cell containing a solution prepared by treating 15 ml of water in a similar manner; the absorbance is not greater than 0.21.

Assay. Carry out the combustion as described under "Oxygen flask method" (vol. 1, p. 124), but using 5-10 mg of the test substance. Titrate the liberated iodine with sodium thiosulfate (0.01 mol/l)VS. Each ml of sodium thiosulfate (0.01 ml/l)VS is equivalent to 0.456 mg of  $C_{19}H_{26}I_3N_3O_9$ .

KETAMINI HYDROCHLORIDUM

Ketamine hydrochloride

Molecular formula.  $C_{13}H_{16}ClNO, HCl$

Relative molecular mass. 274.2

Graphic formula.

Chemical name. 2-(2-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride; (±)-2-(o-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride; CAS Reg. No. 1867-66-9.

Description. A white, crystalline powder; odour, characteristic.

Solubility. Freely soluble in water and methanol R; soluble in ethanol (~750 g/l)TS; sparingly soluble in chloroform R; practically insoluble in ether R.

Category. General anaesthetic.

Storage. Ketamine hydrochloride should be kept in a well-closed container.

#### REQUIREMENTS

General requirement. Ketamine hydrochloride contains not less than 98.5 % and not more than 101.0 % of  $C_{13}H_{16}ClNO, HCl$ .

#### 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 ketamine hydrochloride RS or with the reference spectrum of ketamine hydrochloride.

B. The absorption spectrum of a 0.33 mg/ml solution in hydrochloric acid (0.1 mol/l)VS, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 269 nm and 276 nm. The ratio of the absorbance at 269 nm to that at 276 nm is between 1.10 and 1.22.

C. Dissolve 1 g in 10 ml of water, add 1 ml of sulfuric acid (~100 g/l)TS and 1 ml of ammonium reineckate (10 g/l)TS; a light red precipitate is produced.

D. A 0.1 g/ml solution yields reaction B described under "General identification tests" as characteristic of chlorides (vol. 1, p. 113).

Melting range. 258-261 °C.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 1 (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. A solution of 2.0 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

pH value. pH of a 0.10 g/ml solution, 3.5 - 4.1.

Related substances. Carry out the test as described under "Thin-layer chromatography" (vol. 1, p. 83), using silica gel R1 and a mixture of 49 volumes of cyclohexane R and 1 volume of isopropylamine R as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in methanol R containing (A) 50 mg of the test substance per ml and (B) 0.25 mg of the test substance per ml. Develop the plate for a distance of 10 cm. After removing the plate from the chromatographic chamber, allow it to dry in air and spray the plate evenly with modified Dragendorff reagent TS, dry and spray with hydrogen peroxide (~60 g/l)TS. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.5 g, accurately weighed, in 1 ml of formic acid (~1080 g/l)TS, add 70 ml of a mixture of 6 volumes of acetic anhydride R and 1 volume of glacial acetic acid R1. Add 10 ml of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l)VS, determining the endpoint potentiometrically 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 27.42 mg of  $C_{13}H_{16}ClNO, HCl$ .

MAGNESII SULFATIS HEPTAHYDRAS

Magnesium sulfate heptahydrate

Molecular formula.  $MgSO_4 \cdot 7H_2O$

Relative molecular mass. 246.5

Chemical name. Magnesium sulfate (1:1) heptahydrate; sulfuric acid magnesium salt (1:1), heptahydrate; CAS Reg. No. 10034-99-8.

Other name. Epsom salt.

Description. Brilliant, colourless crystals or a white, crystalline powder; odourless.

Solubility. Freely soluble in water; practically insoluble in ethanol (~750 g/l)TS.

Category. Cathartic.

Storage. Magnesium sulfate heptahydrate should be kept in a well-closed container.

Additional information. Magnesium sulfate heptahydrate effloresces in warm, dry air.

#### REQUIREMENTS

General requirements. Magnesium sulfate heptahydrate contains not less than 99.0 % and not more than 100.5 % of  $MgSO_4$ , calculated with reference to the dried substance.

#### Identity tests

A. Dissolve 10 mg in 2 ml of water, add 1.0 ml of ammonia (~100 g/l)TS; a white precipitate is produced which redissolves after adding 1.0 ml of ammonium chloride (100 g/l)TS. Add 1.0 ml of disodium hydrogen phosphate (40 g/l)TS; a white, fine crystalline precipitate is formed.

B. 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 1 (vol. 1, p. 118); determine the heavy metals content according to method A (vol. 1, p. 119); not more than 10 µg/g.

Arsenic. Use a solution of 5.0 g in 35 ml of water and proceed as described under "Limit test for arsenic" (vol. 1, p. 122); the arsenic content is not more than 2 µg/g.

Chlorides. Dissolve 0.85 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 0.30 mg/g.

Iron. Use 2.0 g; the solution complies with the "Limit test for iron" (vol. 1, p. 121); not more than 20 µg/g.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Loss on drying. Dry 0.5 g at 110-120 °C for 1 hour and then at 400 °C to constant mass; it loses not less than 0.48 g/g and not more than 0.52 g/g.

Acidity or alkalinity. Dissolve 1.0 g in 10 ml of water and add 0.05 ml of phenol red/ethanol TS; not more than 0.2 ml of hydrochloric acid (0.01 mol/l)VS or sodium hydroxide (0.01 mol/l)VS is required to attain the midpoint of the indicator (pink).

Assay. Dissolve about 0.25 g, accurately weighed, in 100 ml of water, and proceed with the titration as described under "Complexometric titrations" for magnesium (vol. 1, p. 129). Each ml of disodium edetate (0.05 mol/l)VS is equivalent to 6.018 mg of MgSO<sub>4</sub>.

MEDROXYPROGESTERONI ACETAS

Medroxyprogesterone acetate

Molecular formula. C<sub>24</sub>H<sub>34</sub>O<sub>4</sub>

Relative molecular mass. 386.5

Graphic formula.

Chemical name. 17-Hydroxy-6α-methylpregn-4-ene-3,20-dione acetate;  
(6α)-17-(acetyloxy)-6-methylpregn-4-ene-3,20-dione; CAS Reg. No. 71-58-9.

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

Solubility. Practicallly insoluble in water; freely soluble in chloroform R; soluble in acetone R and dioxan R; slightly soluble in ethanol (~750 g/l)TS, methanol R and ether R.

Category. Progestogen.

Storage. Medroxyprogesterone acetate should be kept in a well-closed container, protected from light.

#### REQUIREMENTS

General requirement. Medroxyprogesterone acetate contains not less than 97.0 % and not more than 103.0 % of  $C_{24}H_{34}O_4$ , 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 medroxyprogesterone acetate RS or with the reference spectrum of medroxyprogesterone acetate.

B. Carry out the test as described under "Thin-layer chromatography" (vol. 1, p. 83) preparing 2 plates and using kieselguhr R1 as the coating substance and a mixture of 1 volume of propylene glycol R and 9 volumes of acetone R to impregnate the plate, dipping it about 5 mm into the liquid. After the solvent has reached a height of at least 16 cm, remove the plate from the chromatographic chamber and allow it to stand at room temperature until the solvent has completely evaporated. Use the impregnated plate within 2 hours, carrying out the chromatography in the same direction as the impregnation. Use a mixture of equal volumes of cyclohexane R and light petroleum R1 as the mobile phase. (Keep 1 plate for the test of related substance.) Apply separately to one plate 2  $\mu$ l of each of 3 solutions in a mixture of 9 volumes of chloroform R and 1 volume of methanol R containing (A) 2.5 mg of the test substance per ml, (B) 2.5 mg of medroxyprogesterone acetate RS per ml

and (C) a mixture of equal volumes of solutions A and B. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 15 minutes and spray the hot plate with sulfuric acid/ethanol TS. Heat at 120 °C for a further 10 minutes, allow to cool, and examine the chromatogram in daylight and in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B. A single spot is obtained with solution C.

C. Melting temperature, about 204°C.

D. Use 20 mg; it yields the reaction described under "General identification tests" as characteristic of acetylated substances (vol. 1, p. 111).

Specific optical rotation. Use a 10 mg/ml solution in dioxan R;

$$[\alpha]_D^{20 \text{ } ^\circ\text{C}} = +45 \text{ to } +51 \text{ } ^\circ.$$

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry at 105 °C for 3 hours; it loses not more than 10 mg/g.

Related substances. To the remaining plate prepared for the identity test and using the same mobile phase apply separately 5 µl of each of 3 solutions in chloroform R containing (A) 5.0 mg of the test substance per ml, (B) 0.15 mg of the test substance per ml and (C) 0.050 mg of the test substance per ml. After removing the plate from the chromatographic chamber, allow it to dry in air until the solvents have evaporated, heat at 120 °C for 30 minutes and spray with 4-toluenesulfonic acid/ethanol TS. Heat again at 120 °C for 10 minutes, expose the plate to iodine vapours for 10 minutes, and examine the chromatogram. Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B. Not more than 1 such spot is more intense than that obtained with solution C.

Assay. Dissolve about 0.1 g, accurately weighed, in sufficient ethanol (~750 g/l)TS to produce 100 ml; dilute 1.0 of this solution to 100 ml with the same solvent. Measure the absorbance of the diluted solution in a 1-cm layer at the maximum at about 241 nm and calculate the content of

$\text{C}_{24}\text{H}_{34}\text{O}_4$  using the absorptivity value of 42.6 ( $\frac{1\%}{1\text{ cm}} = 426$ ).

MEGLUMINUM

Meglumine

Molecular formula.  $C_{7}H_{17}NO_{5}$

Relative molecular mass. 195.2

Graphic formula.

Chemical name. 1-Deoxy-1-(methylamino)-D-glucitol;

CAS Reg. No. 6284-40-8.

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

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

Category. Used in the preparation of meglumine amidotrizoate injection; radiocontrast medium.

Storage. Meglumine should be kept in a well-closed container.

REQUIREMENTS

General requirement. Meglumine contains not less than 99.0 % and not more than 100.5 % of  $C_{7}H_{17}NO_{5}$ , calculated with reference to the dried substance.

Identity tests

A. To 5 ml of water add 0.5 ml of paraldehyde R and 0.5 ml of sulfuric acid (~190 g/l)TS. Shake and warm carefully until a cloudy solution appears, then allow to cool for 15 minutes. To 1 ml of this solution add 0.2 ml of a freshly prepared solution of sodium nitroprusside R containing 0.10 g/ml, then

add 50 mg of the substance to be tested and 2 ml of a solution of sodium tetraborate R containing 50 mg/ml; a blue colour is slowly developed, which becomes more intense with time.

B. Dissolve 0.2 g in 2 ml of water, add 0.05 ml of methyl red/ethanol TS and neutralize with sulfuric acid (0.25 mol/l)VS. Add 1.0 ml of sodium hydroxide (0.1 mol/l)VS and 1.0 g of boric acid R; the solution is acid.

Melting range. 128 - 131 °C.

Specific optical rotation. Use a 0.10 g/ml solution;

$$[\alpha]_D^{20} = -15.7 \text{ to } -17.3 \text{ }^\circ.$$

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

Reducing sugars. Dissolve 0.25 g in 5 ml of water, add 5 ml of potassio-cupric tartrate TS and boil for 2 minutes; no red-brown precipitate is produced.

Clarity and colour of solution. A solution of 1.0 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 10 mg/g.

Assay. Dissolve about 0.5 g, accurately weighed, in 40 ml of water and titrate with hydrochloric acid (0.1 mol/l)VS using methyl red/ethanol TS as indicator. Each ml of hydrochloric acid (0.1 mol/l)VS is equivalent to 19.52 mg of  $C_7H_{17}NO_5$ .

MERCAPTOPURINUM  
Mercaptopurine

Molecular formula.  $C_5H_4N_4S.H_2O$

Relative molecular mass. 170.2

Graphic formula.

Chemical name. 1,7-Dihydro-6H-purine-6-thione monohydrate; purine-6-thiol monohydrate; CAS Reg. No. 6112-76-1.

Description. A yellow, crystalline powder.

Solubility. Practically insoluble in water and ether R; slightly soluble in ethanol (~750 g/l)TS; soluble in solutions of alkali hydroxides.

Category. Cytotoxic drug.

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

Additional information. **CAUTION:** Mercaptopurine must be handled with care avoiding contact with the skin and inhaling airborne particles. It melts at a temperature exceeding 308 °C with decomposition.

REQUIREMENTS

General requirement. Mercaptopurine contains not less than 97.0 % and not more than 102.0 % of  $C_5H_4N_4S$ , calculated with reference to the anhydrous substance.

Identity tests

A. Dissolve 20 mg in 5 ml of dimethyl sulfoxide R and dilute to 100 ml with hydrochloric acid (0.1 mol/l)VS. Dilute 5 ml of this solution to 200 ml with hydrochloric acid (0.1 mol/l)VS. The absorption spectrum of the diluted solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 325 nm.

B. Dissolve 20 mg in 20 ml of warm ethanol (~750 g/l)TS and add 1 ml of a saturated solution of mercuric acetate R in ethanol (~750 g/l)TS; a white precipitate is produced.

C. Dissolve 20 mg in 20 ml of warm ethanol (~750 g/l)TS and add 1 ml of a solution containing 10 mg/ml of lead acetate R in ethanol (~750 g/l)TS; a yellow precipitate is produced.

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.

Water. Determine as described under "Determination of water by the Karl Fischer method", method A (vol. 1, p. 135), using about 0.15 g of the substance; the water content is not less than 100 mg/g and not more than 120 mg/g.

Hypoxanthine. 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 90 volumes of acetone R, 7 volumes of water and 3 volumes of ammonia (~260 g/l)TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions containing (A) 0.050 g of the test substance dissolved in 1 ml of dimethyl sulfoxide R and diluted to 10 ml with methanol R, and (B) 10 mg of hypoxanthine R dissolved in 10 ml of dimethyl sulfoxide R and diluted to 100 ml with methanol R. 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.

Assay. Dissolve about 0.3 g, accurately weighed, in 80 ml of dimethylformamide R, add 5 drops of thymol blue/dimethylformamide TS and titrate with sodium methoxide (0.1 mol/l)VS to a blue endpoint, as described under "Non-aqueous titration", Method B (vol. 1, p. 132). Each ml of sodium methoxide (0.1 mol/l)VS is equivalent to 15.22 mg of  $C_5H_4N_4S$ .

NATRII AMIDOTRIZOAS  
Sodium amidotrizoate

Molecular formula.  $C_{11}H_8I_3N_2NaO_4$

Relative molecular mass. 635.9

Graphic formula.

Chemical name. 3,5-Bis(acetylamino)-2,4,6-triiodobenzoic acid monosodium salt; 3,5-diacetamido-2,4,6-triiodobenzoate monosodium; CAS Reg. No. 737-31-5.

Other names. Sodium diatrizoate; diatrizoate sodium.

Description. A white powder; odourless or almost odourless.

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

Category. Radiocontrast medium.

Storage. Sodium amidotrizoate should be kept in a well-closed container, protected from light.

REQUIREMENTS

General requirement. Sodium amidotrizoate contains not less than 98.0 % and not more than 102.0 % of  $C_{11}H_8I_3N_2NaO_4$ , calculated with reference to the anhydrous substance.

Identity tests

. Either test A 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 sodium amidotrizoate RS or with the reference spectrum of sodium amidotrizoate.

B. Carry out the test as described under "Thin-layer chromatography" (vol. 1, p. 83), using silica gel R4 as the coating substance and 20 volumes of chloroform R, 10 volumes of methanol R and 2 volumes of ammonia (~260 g/l)TS as the mobile phase. Apply separately to the plate 10  $\mu$ l of each of 2 solutions in a mixture of 0.8 volumes of sodium hydroxide (~80 g/l)TS and 1000 ml of methanol R containing (A) 1 mg of the test substance per ml and (B) 1 mg of sodium amidotrizoate 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.

C. Place 20 mg into a flask, add 5 ml of sodium hydroxide (~80 g/l)TS and boil gently under a reflux condenser for 10 minutes. Cool, add 5 ml of hydrochloric acid (~70 g/l)TS and cool in ice for 5 minutes. Add 4 ml of sodium nitrite (10 g/l)TS, cool in ice for 5 minutes, add 0.3 g of sulfamic acid R, swirl gently until effervescence ceases and add 2 ml of N-(1-naphthyl)ethylenediamine hydrochloride (5 g/l)TS; an orange-red colour is produced.

D. Heat about 0.1 g in a suitable crucible; violet vapours are evolved.

E. 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 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals"; Procedure 1 (vol. 1, p. 118); determine the heavy metals content according to Method A (vol. 1, p. 119); not more than 20  $\mu$ g/g.

Halides. Dissolve 0.8 g in 10 ml of water, add drop by drop sufficient nitric acid (~130 g/l)TS until complete precipitation is obtained and add an excess of 3 ml. Filter, wash the precipitate with 5 ml of water; to the filtrate add 1 ml of hydrogen peroxide (~330 g/l)TS, 1 ml of chloroform R and shake. To serve as a reference solution, treat similarly 2 ml of iodide standard (20  $\mu$ g I/ml)TS with 3 ml of nitric acid (~130 g/l)TS and sufficient water to equal the volume of the solution to be tested. The content of halides, expressed as iodides, does not produce a solution with any purple colour that is more intense than the reference solution.

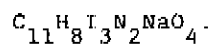
Clarity and colour of solution. A solution of 0.20 g in 10 ml of water is clear and colourless.

Water. Determine as described under "Determination of water by the Karl Fischer method", method A (vol. 1, p. 135), using about 0.4 g of the substance; the water content is not more than 100 mg/g.

pH value. pH of a 0.50 g/ml solution, 7.5 - 9.5.

Primary aromatic amines. Place 1.0 g in a glass-stoppered graduated flask, add 5 ml of water, 10 ml of sodium hydroxide (0.1 mol/l)VS and 25 ml of dimethyl sulfoxide R. Stopper the flask, mix the contents by gentle swirling and cool in ice, protected from light. After 5 minutes, add slowly 2 ml of hydrochloric acid (~250 g/l)TS, mix and allow to stand for 5 minutes. Add 1.5 ml of sodium nitrite (35 g/l)TS, mix and allow to stand for 5 minutes. Add 2 ml of sulfamic acid (50 g/l)TS, mix and allow to stand for 5 minutes. Add 2 ml of N-(1-naphthyl)ethylenediamine hydrochloride/1-propanol TS and mix. Remove the flask from the ice and allow to stand in water at about 22-25 °C for 10 minutes with occasional gentle shaking. Dilute to 50 ml with dimethyl sulfoxide R and mix. Measure the absorbance at about 470 nm, within 5 minutes after the addition of the last reagent, against a solvent cell containing the reagents prepared in a similar manner. The absorbance is not greater than 0.40.

Assay. To about 0.30 g, accurately weighed, add 30 ml of sodium hydroxide (50 g/l)TS and 0.50 g of zinc R powder. Boil under a reflux condenser for 1 hour. Cool the flask to room temperature, rinse the condenser with 20 ml of water and filter. Rinse the flask and filter thoroughly adding the rinsings to the filtrate. Add 5 ml of glacial acetic acid R and 1 ml of tetrabromophenolphthalein ethyl ester TS and titrate with silver nitrate (0.05 mol/l)VS until the yellow precipitate just turns green. Each ml of silver nitrate (0.05 mol/l)VS is equivalent to 10.60 mg of



## NATRII HYDROXYDUM

Sodium hydroxide

Molecular formula. NaOH

Relative molecular mass. 40.0

Chemical name. Sodium hydroxide; CAS Reg. No. 1310-73-2.

Description. White or almost white fused masses, sticks, pellets or flakes; they are hard and brittle, showing a crystalline fracture.

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

Category. Alkalinizing agent; used in the preparation of sodium lactate solution.

Storage. Sodium hydroxide should be kept in a tightly closed container.

Additional information. **CAUTION:** Sodium hydroxide must be handled with care avoiding contact with the skin. It is very deliquescent, strongly alkaline and corrosive. It rapidly absorbs carbon dioxide.

REQUIREMENTS

General requirement. Sodium hydroxide contains not less than 97.5 % of total alkali, calculated as NaOH and not more than 2.5 % of  $\text{Na}_2\text{CO}_3$ .

Identity tests

A. 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.

B. A solution is strongly alkaline.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 1 (vol. 1, p. 118); determine the heavy metals content according to Method A (vol. 1, p. 119); not more than 10  $\mu\text{g/g}$ .

Aluminium, iron and matter insoluble in hydrochloric acid. Boil 5 g with 70 ml of hydrochloric acid (~70 g/l)TS. Cool, make alkaline with ammonia (~100 g/l)TS, boil, filter and wash with a mixture of equal volumes of water and ammonium nitrate (50 g/l)TS. Ignite the residue to constant weight; not more than 5 mg.

Potassium. Dissolve 0.25 g in 5 ml of water, acidify with acetic acid (~60 g/l)TS and add 5 drops of sodium cobaltinitrite (100 g/l)TS; no precipitate is formed.

Chlorides. Dissolve 0.35 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 0.7 mg/g.

Sulfates. Dissolve 0.40 g in 20 ml of water, add 6 ml of hydrochloric acid (~70 g/l)TS, and proceed as described under "Limit test for sulfates" (vol. 1, p. 116); the sulfate content is not more than 1.2 mg/g.

Assay. Dissolve 2 g in 25 ml of carbon-dioxide-free water R, add 5 ml of barium chloride (50 g/l)TS and titrate with hydrochloric acid (1 mol/l)VS using phenolphthalein/ethanol TS as indicator. Note the consumption of acid. Add bromophenol blue/ethanol TS to the solution and continue the titration with hydrochloric acid (1 mol/l)VS. Each ml of hydrochloric acid (1 mol/l)VS used in the second titration is equivalent to 52.99 mg of  $\text{Na}_2\text{CO}_3$ . Each ml of hydrochloric acid (1 mol/l)VS used in the combined titration is equivalent to 40.00 mg of total alkali, calculated as NaOH.

NORETHISTERONI ENANTAS  
Norethisterone enantate

Molecular formula.  $\text{C}_{27}\text{H}_{38}\text{O}_3$

Relative molecular mass. 410.6

Graphic formula.

Chemical names. 17-Hydroxy-19-nor-17 $\alpha$ -pregn-4-en-20-yn-3-one enantate;  
17 $\alpha$ -ethinyl-17-hydroxyestr-4-en-3-one enantate; CAS Reg. No. 3836-23-5.

Description. A white to creamy white, crystalline powder; odourless.

Solubility. Practically insoluble in water; freely soluble in acetone R, methanol R, dehydrated ethanol R, dioxan R, ether R and chloroform R; slightly soluble in light petroleum R.

Category. Contraceptive.

Storage. Norethisterone enantate should be kept in a tightly closed container, protected from light.

#### REQUIREMENTS

General requirement. Norethisterone enantate contains not less than 96.0 % and not more than 104.0 % of  $C_{27}H_{38}O_3$ , calculated with reference to the dried 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 norethisterone enantate RS or with the reference spectrum of norethisterone enantate.

B. The absorption spectrum of a 13.5 µg/ml solution in methanol R, when observed between 210 nm and 290 nm exhibits a maximum at about 240 nm.

C. Dissolve about 1 mg in 1 ml of dehydrated ethanol R and add 0.5 ml of sulfuric acid (~1760 g/l)TS; a violet solution is produced with a red fluorescence.

Melting range. 68 - 73 °C.

Specific optical rotation. Use a 20 mg/ml solution in chloroform R;

$$[\alpha]_D^{20} = -10.0 \text{ to } -15.0^\circ.$$

Solution in chloroform. A solution of 0.20 g in 10 ml of chloroform R is clear and almost colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over silica gel, desiccant, R at ambient temperature 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 R6 as the coating substance and a mixture of 2 volumes of cyclohexane R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in chloroform R containing (A) 20 mg of the test substance per ml and (B) 0.10 mg of the test substance 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). Spray the plate with antimony trichloride TS, heat at 110 °C for 15 minutes 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.

Free enantiic acid. Dissolve 0.30 g in 10 ml of ethanol (~750 g/l)TS which has been previously neutralized against sodium hydroxide (0.01 mol/l)VS to a light blue colour, using bromothymol blue/ethanol TS as indicator. Titrate the solution quickly with sodium hydroxide (0.01 mol/l)VS to a light blue endpoint; not more than 0.3 ml (corresponding to 1.3 mg/g of enantiic acid).

Assay. Dissolve about 13.5 mg, accurately weighed, in sufficient methanol R to produce 100 ml; dilute 10 ml of this solution to 100 ml with the same solvent. Measure the absorbance of the diluted solution in a 1-cm layer at the maximum at about 240 nm and calculate the content of  $C_{27}H_{38}O_3$  using the absorptivity value of 42.8 ( $\frac{1\%}{1\text{ cm}} = 428$ ).

PODOPHYLLI RESINA

Podophyllum resin

Composition. Podophyllum resin is a mixture of resins obtained from the rhizomes and roots of Podophyllum hexandrum Royle (P. emodi Wall.) or Podophyllum peltatum L. after percolation with ethanol and precipitation from water or very diluted acids.

Other name. Podophyllinum.

Description. Light brown to greenish yellow or brownish grey masses or an amorphous powder; odour, characteristic.

Solubility. Practically insoluble in cold water; partially soluble in hot water, chloroform R and ether R; soluble in ethanol (~750 g/l)TS.

Category. Keratolytic agent.

Storage. Podophyllum resin should be kept in a tightly closed container, protected from light and stored at a temperature between 2 and 15 °C.

Labelling. The designation on the container should state the botanical source.

Additional information. **CAUTION:** Podophyllum resin must be handled with care avoiding contact with the skin, mucous membranes and inhaling airborne particles. On exposure to light or to temperatures above 25 °C, it becomes darker in colour.

#### REQUIREMENTS

General requirement. Podophyllum resin contains not less than 40.0 % and not more than 52.5 % of podophyllum toxin ( $\alpha$  and  $\beta$  peltatum), calculated with reference to the dried substance.

#### Identity tests

- A. Dissolve about 10 mg in 2 ml of ethanol (~750 g/l)TS and add 1 drop of ferric chloride (25 g/l)TS; a deep dark green colour is produced and the solution appears black in reflected light.
- B. Add 0.4 g, finely powdered, to 3 ml of ethanol (~535 g/l)TS, then add 0.5 ml of potassium hydroxide (1 mol/l)VS, shake gently and allow to stand; the resin of P. hexandrum produces a stiff jelly, whereas the resin of P. peltatum does not gelatinize.
- C. To two separate tubes add a few mg of the test substance, and dissolve either in potassium hydroxide (1 mol/l)VS or sodium hydroxide (1 mol/l)VS; a yellow solution is formed which becomes darker on standing. Add a few drops of hydrochloric acid (~250 g/l)TS; the resin precipitates.

Matter insoluble in ethanol (~750 g/l)TS. Shake 1 g, finely powdered, with 20 ml of ethanol (~750 g/l)TS for 5 minutes. Filter through a sintered-glass crucible (approx. porosity 40  $\mu$ ), wash the filter with ethanol (~750 g/l)TS and dry at 105 °C; the residue weighs not more than 25 mg.

Matter insoluble in ammonia (~100 g/l)TS. Shake 0.5 g, finely powdered, with 30 ml of ammonia (~100 g/l)TS for 30 minutes at about 20 °C. Filter through a sintered-glass crucible (approx. porosity 40  $\mu$ ) and wash the flask and filter with 30 ml of water, the time taken for filtering and washing being not more than 10 minutes. Dry the filter with the residue to constant weight at 105 °C; the residue from the resin of *P. hexandrum* weighs not less than 0.18 g and not more than 0.30 g, whereas the residue from the resin of *P. peltatum* weighs not more than 50 mg.

Sulfated ash. Not more than 15 mg/g.

Loss on drying. Dry to constant weight at 105 °C; it loses not more than 50 mg/g.

Assay. Transfer about 0.45 g, finely powdered and accurately weighed, to a glass-stoppered 50-ml flask, add 15 ml of chloroform R and shake for 30 minutes. Filter, transfer 10 ml of the filtrate to a tared 100-ml flask containing 80 ml of light petroleum R. Filter the formed precipitate through a sintered-glass crucible (approx. porosity 40  $\mu$ ), previously dried to constant weight at 70 °C, wash the flask and the filter with 20 ml of light petroleum R and dry the flask and the filter at 70 °C for 1 hour. Cool and weigh the residue.

PROPYLIODONUM

Propyliodone

Molecular formula.  $C_{10}H_{11}I_2NO_3$

Relative molecular mass. 447.0

Graphic formula.

Chemical name. Propyl-3,5-diiodo-4-oxo-1(4H)pyridineacetate; 3,5-diiodo-4-oxo-1(4H)-pyridineacetic acid propyl ester; CAS Reg. No. 587-61-1.

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

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

Category. Radiocontrast medium.

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

#### REQUIREMENTS

General requirement. Propyliodone contains not less than 99.0 % and not more than 101.0 % of  $C_{10}H_{11}I_2NO_3$ , calculated with reference to the dried substance.

#### Identity tests

A. The absorption spectrum of a 20 µg/ml solution in dehydrated ethanol R, when observed between 230 nm and 350 nm, exhibits 2 maxima at about 239 nm and 281 nm; the absorbances of a 1-cm layer at those wavelengths are about 0.64 and 0.52, respectively.

B. Heat about 0.1 g with a few drops of sulfuric acid (~1760 g/l)TS in a suitable crucible; violet vapours are evolved.

Melting range. 187 - 190 °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.

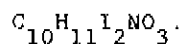
Halides. Shake 2.4 g with 30 ml of water for 15 minutes and filter. To 10 ml of the filtrate add 1 ml of nitric acid (~130 g/l)TS, 2 ml of sodium nitrite (1 g/l)TS and 2 ml of chloroform R, shake well and centrifuge. To serve as a reference solution treat similarly 2 ml of iodide standard (20 µg I/ml)TS with 8 ml of water. The content of halides, expressed as iodides, does not produce a solution with any purple colour that is more intense than the reference solution.

Sulfated ash. Not more than 1.0 mg/g.

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

Acidity. Dissolve 1.0 g in 40 ml of hot 1-propanol R, previously neutralized to phenolphthalein/ethanol TS, cool and allow to stand for 15 minutes with frequent shaking. Filter, wash the residue with neutralized 1-propanol R and titrate the combined filtrate and washings with sodium hydroxide (0.05 mol/l)VS, using phenolphthalein/ethanol TS as indicator, until a pink colour persists for 15 seconds; not more than 0.15 ml of sodium hydroxide (0.05 mol/l)VS is required.

Assay. Carry out the combustion as described under "Oxygen flask method" for iodine (vol. 1, p. 125), using 15 mg, accurately weighed, of the test substance but titrating with sodium thiosulfate (0.02 mol/l)VS. Each ml of sodium thiosulfate (0.02 mol/l)VS is equivalent to 0.7450 mg of



TAMOXIFENI CITRAS  
Tamoxifen citrate

Molecular formula.  $C_{26}H_{29}NO, C_6H_8O_7$

Relative molecular mass. 563.6

Graphic formula.

Chemical name. (Z)-2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-N,N-dimethyl-ethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1);  
(Z)-2-[p-(1,2-(diphenyl 1-butenyl)phenoxy)-N,N-dimethylethylamine citrate (1:1); CAS Reg. No. 54965-24-1.

Description. A white or almost white, crystalline powder.

Solubility. Slightly soluble in water and acetone R; soluble in methanol R.

Category. Anti-estrogen.

Storage. Tamoxifen citrate should be kept in a well-closed container, protected from light.

#### REQUIREMENTS

General requirement. Tamoxifen citrate contains not less than 99.0 % and not more than 101.0 % of  $C_{26}H_{29}NO_7$ , calculated with reference to the dried substance.

#### Identity tests

. Either test 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 tamoxifen citrate RS or with the reference spectrum of tamoxifen citrate.

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 9 volumes of toluene R and 1 volume of triethylamine R as the mobile phase. Apply separately to the plate 5  $\mu$ l of each of 2 solutions in methanol R containing (A) 10 mg of the test substance per ml, and (B) 10 mg of tamoxifen citrate 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.

C. To 10 mg add 4 ml of pyridine R and 2 ml of acetic anhydride R, and shake; a yellow colour is immediately produced. Heat on a water-bath for 2 minutes; a light pink to red colour is produced.

D. Melting temperature, about 142 °C with decomposition.

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 10 µg/g.

Sulfate ash. Not more than 2.0 mg/g.

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

E-isomer and related substances. Carry out the test as described under "High performance liquid chromatography" (vol. 3, p. 373), using a stainless steel column 20 cm long and 5 mm in internal diameter packed with particles of silica gel, 5 µm in diameter, the surface of which has been modified with chemically-bonded octadecyl silyl groups. As the mobile phase use a mixture of 300 volumes of acetonitrile R, 125 volumes of water, 75 volumes of tetrahydrofuran R and 2 volumes of ammonia (~260 g/l)TS.

Use the following 3 solutions: (A) Dissolve 25 mg of the substance being tested in a mixture of 12 volumes of acetonitrile R, 5 volumes of water and 3 volumes of tetrahydrofuran R, and dilute to 10 ml with the same solvent mixture. (B) Prepare similarly 25 mg of tamoxifen citrate impurity standard RS. For solution (C) dilute 1 volume of solution A to 100 volumes with the same solvent mixture.

Operate at a flow rate of 1.5 ml per minute. As detector use an ultraviolet spectrophotometer at a wavelength of about 240 nm, fitted with a low volume flow cell (10 µl is suitable), and a suitable recorder.

Determine experimentally the volumes of the solutions to be injected to produce an adequate response.

In the chromatogram obtained with solution B a peak due to the E-isomer immediately follows the peak due to Z-tamoxifen. Adjust the sensitivity of the instrument so that the height of the peak due to E-tamoxifen is about 15 % of full-scale deflection on the chart paper. Measure the height of the peak due to E-tamoxifen by dropping a perpendicular from the apex of the peak to a line drawn tangentially between the troughs on each side of the E-isomer peak or the trough between the E- and Z-isomer peaks and the baseline, whichever is appropriate. The height of the trough separating the peaks due to E- and Z-tamoxifen in the chromatogram obtained with solution A must be less than 7 % of full-scale deflection on the chart paper and the retention time of the main peak is not more than 30 minutes. (The retention time decreases with increasing concentration of ammonia in the mobile phase).

Calculate the content of E-isomer using the declared content of E-isomer in tamoxifen citrate impurity standard RS; not more than 10 mg/g. Furthermore, the area of any secondary peak obtained with solution A, other than the peak due to the E-isomer, is not greater than half that of the peak due to tamoxifen obtained with solution C, and the sum of these areas is not greater than the peak due to tamoxifen obtained with solution C.

Assay. Dissolve about 1 g, accurately weighed, in 150 ml of glacial acetic acid R1, add 0.25 ml of 1-naphtholbenzein/acetic acid TS, and titrate 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 56.36 mg of  $C_{26}H_{29}NO, C_6H_8O_7$ .

#### THIOPENTALUM NATRICUM

Thiopental sodium

Molecular formula.  $C_{11}H_{17}N_2NaO_2S$

Relative molecular mass. 264.3

Graphic formula.

Chemical name. 5-Ethyl-5-(1-methylbutyl)-2-thioxo-4,6-(1H,5H)-pyrimidinedione monosodium salt; sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate; CAS Reg. No. 71-73-8.

Description. A yellowish white powder; odour, characteristic.

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

Category. General anaesthetic.

Storage. Thiopental sodium should be kept in a tightly closed container, protected from light.

Additional information. Thiopental sodium is hygroscopic. Even in the absence of light, it is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

### REQUIREMENTS

General requirement. Thiopental sodium contains not less than 92.0 % and not more than 95.3 % of  $C_{11}H_{17}N_2NaO_2S$ , calculated with reference to the dried substance.

#### Identity tests

. Either test 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 thiopental sodium RS or with the reference spectrum of thiopental sodium.

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. Fuse 0.20 g with 1.0 g of sodium hydroxide R in a test-tube until the glass glows red; the melt turns red-brown and vapours are evolved. Insert moistened pH-indicator paper R into the vapours; its coloration is changed to an alkaline range. Cool and add 5 ml of water to the melt, mix well and filter. Acidify the filtrate with sulfuric acid (~100 g/l)TS and heat gently; the vapours evolved turn a strip of lead nitrate paper R to brown and then to black.

D. 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 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. A solution of 1.0 g in 10 ml of water is clear and not more intensely coloured than standard colour solution G<sub>5</sub> when compared as described under "Colour of liquids" (vol. 1, p. 50).

Loss on drying. Dry at 80 °C for 4 hours; it loses not more than 20 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 ammonia (~260 g/l)TS, 15 volumes of ethanol (~750 g/l)TS and 80 volumes of chloroform R as the mobile phase. Apply separately to the plate 20 µl of each of 3 solutions containing (A) 10 mg of the test substance per ml (disregard any slight residue), (B) 10 mg of thiopental sodium RS per ml, and (C) 0.05 mg of the test substance per ml. After removing the plate from the chromatographic chamber, examine the chromatogram immediately 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. Disregard any spot at the starting point.

Assay. Dissolve about 0.15 g, accurately weighed, in 5 ml of water, add 2 ml of sulfuric acid (~100 g/l)TS and extract with four 10-ml quantities of chloroform R. Filter the combined chloroform extracts, evaporate the filtrate to dryness on a water-bath and dissolve the residue in 30 ml of dimethylformamide R previously neutralized with lithium methoxide (0.1 mol/l)VS. Titrate immediately with lithium methoxide (0.1 mol/l)VS, using 0.1 ml of thymol blue/methanol TS as indicator, until a blue colour is obtained. Protect the solution from atmospheric carbon dioxide during the titration. Each ml of lithium methoxide (0.1 mol/l)VS is equivalent to 26.43 mg of  $C_{11}H_{17}N_2NaO_2S$ .

#### TIMOLOLI MALEAS

Timolol maleate

Molecular formula.  $C_{13}H_{24}N_4O_3S, C_4H_4O_4$

Relative molecular mass. 432.5

Graphic formula.

Chemical name. (S)-1-[(1,1-Dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol (Z)-2-butenedioate (1:1) (salt); (-)-1-(tert-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol maleate (1:1) (salt); CAS Reg No. 26921-17-5.

Description. A white or almost white powder; odourless or almost odourless.

Solubility. Soluble in water and ethanol (~150 g/l)TS; sparingly soluble in chloroform R; practically insoluble in ether R.

Category. Antiglaucoma drug.

Storage. Timolol maleate should be kept in a well closed container.

#### REQUIREMENTS

General requirement. Timolol maleate contains not less than 98.0 % and not more than 101.0 % of  $C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$ , 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 timolol maleate RS or with the reference spectrum of timolol maleate.

B. The absorption spectrum of a 25 µg/ml solution in sulfuric acid (0.05 mol/l)VS, when observed between 230 nm and 350 nm, exhibits a maximum at about 295 nm; the absorbance of a 1-cm layer at this wavelength is about 0.52.

C. Dissolve 0.2 g in 3 ml of water, add 2 ml of sodium hydroxide (~200 g/l)TS and shake with 3 quantities each of 3 ml of ether R. Warm the aqueous layer in a water-bath for 10 minutes, add 2 ml of bromine TS1, boil and cool. Add 0.2 ml of this solution to 10 mg of resorcinol R dissolved in 3 ml of sulfuric acid (~1760 g/l)TS, and heat in a water-bath for 15 minutes; a bluish black colour is produced.

Specific optical rotation. Use a 50 mg/ml solution in hydrochloric acid (1 mol/l)VS and measure the rotation at 405 nm;  $[\alpha]_D^{20} = -11.7$  to  $-12.5$ °.

Clarity and colour of solution. A solution of 0.20 g in 10 ml of water is clear and colourless.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant weight at 100 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it loses not more than 5.0 mg/g.

pH value. pH of a 20 mg/ml solution, 3.8 - 4.3.

Related substances. Carry out the test as described under "Thin-layer chromatography" (vol. 1, p. 83), using silica gel R6 (a precoated plate from a commercial source is suitable) and a mixture of 80 volumes of dichloromethane R, 20 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 3 solutions in methanol R containing (A) 50 mg of the test substance per ml, (B) 0.20 mg of the test substance per ml and (C) 0.10 mg of the test substance 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). Then expose the plate to iodine vapours for 2 hours and examine the chromatogram in daylight. Using both methods of visualization, any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B, and not more than two such spots are more intense than that obtained with solution C.

Assay. Dissolve about 0.85 g, accurately weighed, in 90 ml of glacial acetic acid R1, add 3 drops of 1-naphtholbenzein/acetic acid TS as indicator and titrate 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 43.25 mg of  $C_{13}H_{24}N_4O_3 \cdot C_4H_4O_4$ .

VINBLASTINI SULFAS  
Vinblastine sulfate

Molecular formula.  $C_{46}H_{58}N_4O_9 \cdot H_2SO_4$

Relative molecular mass. 909.1

Graphic formula.

Chemical name. Vincalukoblastine sulfate (1:1) (salt);

CAS Reg. No. 143-67-9.

Description. A white to slightly yellow, amorphous or crystalline powder.

Solubility. Freely soluble in water; sparingly soluble in chloroform R; very slightly soluble in ethanol (~750 g/l)TS; practically insoluble in ether R.

Category. Cytotoxic drug.

Storage. Vinblastine sulfate should be kept in a tightly closed container, protected from light, and stored at a temperature between 2 and 8 °C.

Additional information. **CAUTION:** Vinblastine sulfate must be handled with care avoiding contact with the skin and inhaling of airborne particles. It is very hygroscopic and unstable. Before the bottle is opened, it should be allowed to come to room temperature in a desiccator.

#### REQUIREMENTS

General requirement. Vinblastine sulfate contains not less than 96.0 % and not more than 101.0 % of  $C_{46}H_{58}N_4O_9 \cdot H_2SO_4$ , calculated with reference to the dried substance.

#### Identity tests

. Either test 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 vinblastine sulfate RS or with the reference spectrum of vinblastine sulfate.

B. See the test described below under "Related alkaloids". The principal spot obtained with solution A corresponds in position, appearance and intensity with that obtained with solution C.

C. To about 1 mg add 0.2 ml of vanillin/hydrochloric acid TS and allow to stand for 1 minute; a pink colour is produced (distinction from vincristine sulfate).

D. A 20 mg/ml solution yields reaction A described under "General identification tests" as characteristic of sulfates (vol. 1, p. 115).

Specific optical rotation. Use a 20 mg/ml solution in methanol R and calculate with reference to the dried substance;

$$[\alpha]_D^{20 \text{ } ^\circ\text{C}} = -28 \text{ to } -35^\circ.$$

Clarity of solution. A solution of 30 mg in 10 ml of water is clear.

Loss on drying. Dry at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 16 hours; it loses not more than 170 mg/g.

pH value. pH of a 1.5 mg/ml solution, 3.5-5.0.

Related alkaloids. 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 80 volumes of toluene R, 40 volumes of chloroform R and 6 volumes of diethylamine R as the mobile phase. Apply separately to the plate 5 µl of each of 3 solutions in methanol R containing (A) 10 mg of the test substance per ml, (B) 0.20 mg of vincristine sulfate RS per ml and (C) 10 mg of vinblastine sulfate 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 than that obtained with solution B.

Assay. Dissolve about 10 mg, accurately weighed, in sufficient methanol R to produce 500 ml. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 267 nm. Calculate the content of

$C_{46}H_{58}N_4O_9 \cdot H_2SO_4$ , using the absorptivity value of 18.5 ( $A_1^{1\%} \frac{1}{cm} = 185$ ).

## INSULINUM

### Insulin

Composition. Insulin is the natural antidiabetic hormone obtained from beef or pork pancreas and purified; CAS Reg. No. 11070-73-8 (beef); 12584-58-6 (pork).

Description. A white or almost white powder; odourless.

Solubility. Practically insoluble in water, chloroform R, ethanol (~750 g/l)TS and ether R. It dissolves in dilute solutions of mineral acids and alkali hydroxides.

Category. Antidiabetic agent.

Storage. Insulin should be kept in a tightly closed container, protected from light and stored at a temperature not exceeding  $-20^{\circ}\text{C}$ .

Labelling. The designation on the container should state the animal source of the insulin; expiry date.

Additional information. Insulin is hygroscopic; it degrades in solutions of alkali hydroxides. Attention should be paid to the microbiological quality since Insulin is of natural origin.

The quality of insulin, which represents a unique case among preparations in the WHO Model List of Essential Drugs, cannot be adequately controlled by methods adopted for the International Pharmacopoeia. Therefore, it is advised to refer to methods described in other pharmacopoeias, such as: European Pharmacopoeia, United States Pharmacopoeia, Chinese Pharmacopoeia, Japanese Pharmacopoeia, etc.

Reagents

Acetic acid (0.07 mol/l)VS. A solution prepared by diluting 4.2 ml of glacial acetic acid R to 1000 ml with water.

Alum R.  $KAl(SO_4)_2 \cdot 12H_2O$  (SRIP, 1963, p. 29).

Aluminium standard (10 µg Al<sup>3</sup>/ml)TS.

Procedure. Dissolve 17.6 mg of alum R in 5 ml of sulfuric acid (0.05 mol/l)VS and dilute with sufficient water to produce 100 ml.

Amidotrizoic acid RS. International Chemical Reference Substance.

3-Amino-2,4,6-triiodobenzoic acid RS. International Chemical Reference Substance.

Ammonium chloride buffer, pH 10.5, TS. A buffer mixture of pH 10.5.

Procedure. Dissolve 6.95 g of ammonium chloride R in 75 ml of ammonia (~260 g/l)TS and dilute with sufficient water to produce 100 ml.

Ammonium pyrrolidinedithiocarbamate R. Ammonium tetramethylenedithiocarbamate;  $C_5H_{12}N_2S_2$ . Reagent grade quality.

Ammonium pyrrolidinedithiocarbamate (10 g/l)TS.

Procedure. Immediately before use, wash 10 g of ammonium pyrrolidinedithiocarbamate R three times, each with 25 ml of isobutyl methyl ketone R. Then dissolve 1.0 g in sufficient water to produce 10 ml.

Bismuth subnitrate R. Bismuth subnitrate is a basic salt, the composition of which varies with the conditions under which it is prepared. It contains not less than 71.5 % and not more than 74.5 % of Bi, calculated with reference to the dried substance.

Description. A white powder.

Solubility. Practically insoluble in water and ethanol (~750 g/l)TS; soluble in hydrochloric acid (~250 g/l)TS and nitric acid (~1000 g/l)TS.

Cisplatin RS. International Chemical Reference Substance.

Dactinomycin RS. International Chemical Reference Substance.

Dragendorff reagent TS.

Procedure. Shake vigorously to dissolve 0.85 g of bismuth subnitrate R in 10 ml of glacial acetic acid R and 40 ml of water (solution A). Dissolve 8 g of potassium iodide R in 20 ml of water (solution B). Immediately before use, mix equal volumes of solutions A and B as well as glacial acetic acid R.

Storage. Solutions A and B must be protected from light.

Dragendorff reagent, modified TS.

Procedure. Add 20 ml of acetic acid (~60 g/l)TS to 4 ml of a mixture of equal volumes of solutions A and B of Dragendorff reagent TS. Prepare this solution immediately before use.

Ethanol (~535 g/l)TS.

Procedure. Dilute 623 ml of ethanol (~750 g/l)TS with water to 1000 ml.

8-Hydroxyquinoline R.  $C_9H_7NO$ .

Description. A white to yellowish white, crystalline powder.

Solubility. Practically insoluble in water and ether R; freely soluble in ethanol (~750 g/l)TS, acetone R and chloroform R.

Melting point. About 74 °C.

8-Hydroxyquinoline/chloroform TS.

Procedure. Dissolve 1 g of 8-hydroxyquinoline R in sufficient chloroform R to produce 100 ml.

Hypoxanthine R.  $C_5H_4N_4O$ .

Description. A white, crystalline powder.

Solubility. Very slightly soluble in water; sparingly soluble in boiling water; soluble in dilute acids and alkali hydroxide solutions.

Imipramine hydrochloride RS. International Chemical Reference Substance.

Iodide standard (20 µg I/ml)TS.

Procedure. Dissolve 26.0 mg of potassium iodide R in sufficient water to produce 100 ml. Dilute 10 ml of this solution to 100 ml with water.

Iohexol RS. International Chemical Reference Substance.

Iopanoic acid RS. International Chemical Reference Substance.

Iotrox acid RS. International Chemical Reference Substance.

Isobutyl methyl ketone R.  $C_6H_{12}O$ .

Description. A clear, colourless liquid; odour, characteristic.

Boiling point. About 115 °C.

Mass density.  $\rho_{20}$  = about 0.80 kg/l.

Ketamine hydrochloride RS. International Chemical Reference Substance.

Lead nitrate paper R.

Procedure. Dip some strips of filter paper into a solution of 10 g of lead nitrate R dissolved in 100 ml of water, and let them dry.

Megroxyprogesterone acetate RS. International Chemical Reference Substance.

Meglumine R.  $C_7H_{17}NO_5$ . Meglumine as described in the monograph, p. 36.

Meglumine (100 g/l)TS. A solution of meglumine R containing about 100 g of  $C_7H_{17}NO_5$  per litre.

Note: Meglumine (100 g/l)TS must be freshly prepared.

1-Naphthol/ethanol TS.

Procedure. Dissolve 0.05 g of 1-naphthol R in 60 ml of ethanol (~750 g/l)TS and add sufficient water to produce 100 ml.

N-(1-Naphthyl)ethylenediamine hydrochloride/propylene glycol TS.

Procedure. Dissolve 0.1 g of N-(1-naphthyl)ethylenediamine hydrochloride R in 30 ml of water and dilute to 100 ml with propylene glycol R.

Note: N-(1-Naphthyl)ethylenediamine hydrochloride/propylene glycol TS must be freshly prepared.

N-(1-Naphthyl)ethylenediamine hydrochloride/1-propanol TS.

Procedure. Mix 7 ml of N-(1-naphthyl)ethylenediamine hydrochloride (1 g/l)TS with 3 ml of 1-propanol R.

Norethisterone enantate RS. International Chemical Reference Substance.

Oxalic acid/sulfuric acid TS.

Procedure. Dissolve 5 g of oxalic acid R in a sufficient quantity of a cooled mixture of equal volumes of sulfuric acid (~1760 g/l)TS and water to produce 100 ml.

Paraldehyde R.  $C_6H_{12}O_3$ .

Description. Liquid; odour, characteristic, aromatic.

Boiling point. About 124 °C.

Mass density.  $\rho_{20} = 0.994$  kg/l.

Potassium permanganate/phosphoric acid TS.

Procedure. Dissolve 3 g of potassium permanganate R in a mixture of 15 ml of phosphoric acid (~1440 g/l)TS and 70 ml of water, and dilute to 100 ml with water.

Quinhydrone R.  $C_{12}H_{10}O_4$ .

Description. Dark green lustrous crystals or crystalline powder.

Melting point. About 171 °C.

Quinhydrone/methanol TS.

Procedure. Dissolve 2.5 g of quinhydrone R in sufficient methanol R to produce 100 ml.

Silver nitrate (0.001 mol/l)VS. Silver nitrate R, dissolved in water to contain 0.1699 g of  $AgNO_3$  in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under silver nitrate (0.1 mol/l)VS, vol. 1, p. 202.

Silver nitrate (0.05 mol/l)VS. Silver nitrate R, dissolved in water to contain 8.494 g of  $AgNO_3$  in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under silver nitrate (0.1 mol/l)VS, vol. 1, p. 202.

Silver standard (5 µg Ag/ml)TS.

Procedure. Dissolve 39.5 mg of silver nitrate R in sufficient water to produce 100 ml. Dilute 1.0 ml of this solution to 100 ml with water.

Sodium acetate (0.04 mol/l)VS. Sodium acetate R, dissolved in water to contain 3.281 g of  $C_2H_3NaO_2$  in 1000 ml.

Sodium amidotrizoate RS. International Chemical Reference Substance.

Sodium nitrite (20 g/l)TS. A solution of sodium nitrite R containing about 20 g of  $NaNO_2$  per litre.

Sodium nitrite (50 g/l)TS. A solution of sodium nitrite R containing about 50 g of  $NaNO_2$  per litre.

Stannous chloride/hydrochloric acid TS2.

Procedure. Dissolve 10 g of stannous chloride R in sufficient hydrochloric acid (~70 g/l)TS to produce 100 ml.

Stannous chloride/hydrochloric acid TS1.

Procedure. Dissolve 5 g of stannous chloride R in 100 ml of hydrochloric acid (~420 g/l)TS.

Sulfamic acid (80 g/l)TS. A solution of sulfamic acid R containing about 80 g of  $H_3NO_3S$  per litre.

Note: Sulfamic acid (80 g/l)TS must be freshly prepared.

Tamoxifen citrate RS. International Chemical Reference Substance.

Tamoxifen citrate impurity standard RS. International Chemical Reference Substance.

Tetrabromophenolphthalein ethyl ester R.  $C_{22}H_{14}Br_4O_4$ . Use a suitable reagent grade.

Tetrabromophenolphthalein ethyl ester TS.

Procedure. Dissolve 100 mg of tetrabromophenolphthalein ethyl ester R in sufficient glacial acetic acid R to produce 100 ml.

Note: The solution should be freshly prepared.

Thiopental sodium RS. International Chemical Reference Substance.

Timolol maleate RS. International Chemical Reference Substance.

Triethylamine R.  $C_6H_{15}N$ .

Description. A colourless liquid; odour, ammoniacal.

Boiling range. 89-90 °C.

Mass density.  $\rho_{20}$  = about 0.73 kg/l.

Refractive index.  $n_D^{20}$  = 1.4003.

Vinblastine sulfate RS. International Chemical Reference Substance.

\* \* \*