EUROPEAN PHARMACOPŒIA

COUNCIL OF EUROPE

2nd EDITION

Part II - 4

MAISONNEUVE

EUROPEAN PHARMACOPOEIA

SECOND EDITION

Part II

Fourth Fascicule

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TEXTS INCLUDED IN THE FOURTH FASCICULE

Revised Monographs with their Serial Number

Apomorphini Hydrochloridum (136)	Apomorphine Hydrochloride
Estradioli Benzoas (139)	Estradiol Benzoate
Ethinylestradiolum (140)	Ethinylestradiol
Isoniazidum (146)	Isoniazid
Phenoxymethylpenicillinum (148)	Phenoxymethylpenicillin
Tuberculini Derivatum Proteinosum Purificatum ad Usum Humanum (151)	Tuberculin Purified Protein Derivative for Human Use
Tuberculinum Pristinum ad Usum Humanum (152)	Old Tuberculin for Human Use
Vaccina ad Usum Humanum (153)	Vaccines for Human Use
Vaccinum Cholerae (154)	Cholera Vaccine
Vaccinum Cholerae Cryodesiccatum (155)	Freeze-dried Cholera Vaccine
Vaccinum Febris Typhoidi (156)	Typhoid Vaccine
Vaccinum Febris Typhoidi Cryodesiccatum (157)	Freeze-dried Typhoid Vaccine
Vaccinum Influenzae Inactivatum (159)	Influenza Vaccine (Inactivated)

Vaccinum Pertussis (160)	Pertussis Vaccine
Vaccinum Pertussis Adsorbatum (161)	Pertussis Vaccine (Adsorbed)
Vaccinum Tuberculosis (BCG) Cryodesiccatum (163)	Freeze-dried BCG Vaccine
Vaccinum Variolae Cryodesiccatum Dermicum (164)	Freeze-dried Smallpox Vaccine (Dermal)

Texts of Part I

V.6.20.3. Gas chromatography

VIII.1. Table of relative atomic masses

New Monographs with their Serial Number

Adeps Lanae (134)	Wool Fat
Adeps Lanae cum Aqua (135)	Hydrous Wool Fat
Chlorambucilum (137)	Chlorambucil
Dimeticonum (138)	Dimeticone
Ethionamidum (141)	Ethionamide
Ethisteronum (142)	Ethisterone
Histamini Dihydrochloridum (143)	Histamine Dihydrochloride
Histamini Phosphas (144)	Histamine Phosphate
Immunoserum contra Venena Viperarum Europaearum (145)	European Viper Venom Antiserum
Naphazolini Nitras (147)	Naphazoline Nitrate
Phenoxymethylpenicillinum Kalicum (149)	Phenoxymethylpenicillin Potassium
Titanii Dioxidum (150)	Titanium Dioxide
Vaccinum Influenzae ex Virorum Fragmentis Praeparatum (158)	Influenza Vaccine (Split Virion)
Vaccinum Rubellae Vivum (162)	Rubella Vaccine (Live)

Texts of Part I

V.6.20.4. Liquid chromatography

VI.1.3.1. Silicone oil used as a lubricant

ADEPS LANAE

Wool Fat

Wool fat is a purified, anhydrous, waxy material obtained from the wool of the sheep *(Ovis aries)*. It may contain not more than 200 parts per million of butylated hydroxytoluene.

CHARACTERS

A pale-yellow, unctuous substance with a characteristic odour. When melted, it is a clear or almost clear, yellow liquid. It is practically insoluble in water, soluble in chloroform and in ether and slightly soluble in boiling ethanol. A solution in light petroleum is opalescent.

IDENTIFICATION

A. In a test-tube, dissolve 0.5 g in 5 ml of chloroform R and add 1 ml of acetic anhydride R and 0.1 ml of sulphuric acid R. A green colour develops.

B. Dissolve 50 mg in 5 ml of chloroform R, add 5 ml of sulphuric acid R and shake. A red colour develops and an intense green fluorescence appears in the lower layer.

TESTS

Water-soluble acid or alkaline substances Melt 5.0 g on a water-bath and shake vigorously for 2 min with 75 ml of water previously heated to 90 °C to 95 °C. Allow to cool and filter through filter paper previously rinsed with water. To 60 ml of the filtrate (which may not be clear) add 0.25 ml of bromothymol blue solution R1. Not more than 0.2 ml of 0.02N hydrochloric acid or 0.15 ml of 0.02N sodium hydroxide is required to change the colour of the indicator.

Drop point (V.6.11.4) 38 °C to 44 °C. To fill the metal cup, melt the wool fat on a water-bath, cool to about 50 °C, pour into the cup and allow to stand at 15 °C to 20 °C for 24 h.

Water-absorption capacity Place 10 g in a mortar. Add water in portions of 0.2 ml to 0.5 ml from a burette, stirring vigorously after each addition

to incorporate the water. The end-point is reached when visible droplets remain which cannot be incorporated. Not less than 20 ml of water is absorbed.

Acid value (V.3.4.1) Not more than 1.0, determined on 5.0 g dissolved in 25 ml of the prescribed mixture of solvents.

Peroxide value (V.3.4.5) Not more than 20.

Saponification value (V.3.4.6) 90 to 105, determined on 2.00 g. Heat under reflux for 4 h.

Water-soluble oxidisable substances To 10 ml of the filtrate obtained in the test for water-soluble acid or alkaline substances add 1 ml of dilute sulphuric acid R and 0.1 ml of 0.1N potassium permanganate. After 10 min, the solution is not completely decolorised.

Butylated hydroxytoluene Not more than 200 ppm. Examine by gas chromatography (V.6.20.3), using methyl decanoate R as the internal standard.

Internal standard solution Dissolve 0.2 g of methyl decanoate R in carbon disulphide R and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 10.0 ml with carbon disulphide R.

Test solution (a) Dissolve 1.0 g of the substance to be examined in carbon disulphide R and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 1.0 g of the substance to be examined in carbon disulphide R, add 1.0 ml of the internal standard solution and dilute to 10.0 ml with carbon disulphide R.

Reference solution Dissolve 0.2 g of butylated hydroxytoluene R in carbon disulphide R and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with carbon disulphide R. To 1.0 ml of this solution add 1.0 ml of the internal standard solution and dilute to 10.0 ml with carbon disulphide R.

The chromatography may be carried out using:

— a column 1.5 m long and 4 mm in internal diameter packed with silanised diatomaceous earth for gas chromatography R impregnated with 10 per cent *m/m* of polydimethylsiloxane R; the column is preceded by a column containing silanised glass wool,

— nitrogen for chromatography R as the carrier gas at a flow rate of 40 ml per minute,

— a flame-ionisation detector.

Maintain the temperature of the column at 150 °C, that of the injection port at 180 °C and that of the detector at 300 °C.

Inject the selected volumes of test solutions (a) and (b) and of the reference solution.

Paraffins *The tap and cotton plugs used should be free from grease.* Prepare a column of anhydrous aluminium oxide 230 mm long and 20 mm in diameter by adding a slurry of anhydrous aluminium oxide R and light petroleum R1 to a glass tube fitted with a tap and containing light petroleum R1. Allow to settle and reduce the depth of the layer of solvent above the column to about 40 mm. Dissolve 3.0 g of the substance to be examined in 50 ml of warm light petroleum R1, cool, pass the solution through the column at a flow rate of 3 ml per minute and wash with 250 ml of light petroleum R1. Concentrate the combined eluate and washings to low bulk by distillation, evaporate to dryness on a water-bath and heat the residue at 105 °C for periods of 10 min until two successive weighings do not differ by more than 1 mg. The residue weighs not more than 30 mg.

Chlorides Boil 1.0 g with 20 ml of alcohol (90 per cent *V/V*) under a reflux condenser for 5 min. Cool, add 40 ml of water and 0.5 ml of nitric acid R and filter. To the filtrate add 0.15 ml of a 1 per cent *m/V* solution of silver nitrate R in alcohol (90 per cent *V/V*). After 5 min protected from light, any opalescence in the solution is not more intense than that in a standard prepared at the same time by adding 0.15 ml of a 1 per cent *m/V* solution of silver nitrate R in alcohol (90 per cent *V/V*) to a mixture of 0.2 ml of 0.02N hydrochloric acid, 20 ml of alcohol (90 per cent *V/V*), 40 ml of water and 0.5 ml of nitric acid R (150 ppm).

Loss on drying (V.6.22) Not more than 0.5 per cent, determined on 1.00 g by drying in an oven at 100 °C to 105 °C for 1 h.

Sulphated ash (V.3.2.14) Not more than 0.15 per cent. Ignite 5.0 g and use the residue to determine the sulphated ash.

STORAGE

Store in a well-closed container, at a temperature not exceeding 25 °C.

LABELLING

The label on the *container* states the concentration of any added butylated hydroxytoluene.

1982.

ADEPS LANAE CUM AQUA

Hydrous Wool Fat

Hydrous wool fat is a mixture of 75 per cent *m/m* of wool fat and 25 per cent *m/m* of water. It is obtained by the gradual addition of water to melted wool fat with continuous stirring. It may contain not more than 150 parts per million of butylated hydroxytoluene.

CHARACTERS

A pale-yellow, unctuous substance with a faint, characteristic odour.

IDENTIFICATION

A. In a test-tube, dissolve 0.5 g in 5 ml of chloroform R and add 1 ml of acetic anhydride R and 0.1 ml of sulphuric acid R. A green colour develops.

B. Dissolve 50 mg in 5 ml of chloroform R, add 5 ml of sulphuric acid R and shake. A red colour develops and an intense green fluorescence appears in the lower layer.

TESTS

Water-soluble acid or alkaline substances Melt 6.7 g on a water-bath and shake vigorously for 2 min with 75 ml of water previously heated to 90 °C to 95 °C. Allow to cool and filter through filter paper previously rinsed with water. To 60 ml of the filtrate (which may not be clear) add 0.25 ml of bromothymol blue solution R1. Not more than 0.2 ml of 0.02N hydrochloric acid or 0.15 ml of 0.02N sodium hydroxide is required to change the colour of the indicator.

Drop point (V.6.11.4) 38 °C to 44 °C, determined on the residue obtained in the test for wool-fat content. To fill the metal cup, melt the residue on a water-bath, cool to about 50 °C, pour into the cup and allow to stand at 15 °C to 20 °C for 24 h.

Water-absorption capacity Place 10 g of the residue obtained in the test for wool-fat content in a mortar. Add water in portions of 0.2 ml to 0.5 ml from a burette, stirring vigorously after each addition to incorporate the water. The end-point is reached when visible droplets remain which cannot be incorporated. Not less than 20 ml of water is absorbed.

Acid value (V.3.4.1) Not more than 0.8, determined on 5.0 g dissolved in 25 ml of the prescribed mixture of solvents.

Peroxide value (V.3.4.5) Not more than 15.

Saponification value (V.3.4.6) 67 to 79, determined on 2.00 g. Heat under reflux for 4 h.

Water-soluble oxidisable substances To 10 ml of the filtrate obtained in the test for water-soluble acid or alkaline substances add 1 ml of dilute sulphuric acid R and 0.1 ml of 0.1N potassium permanganate. After 10 min, the solution is not completely decolorised.

Butylated hydroxytoluene Not more than 150 ppm. Examine by gas chromatography (V.6.20.3), using methyl decanoate R as the internal standard.

Internal standard solution Dissolve 0.2 g of methyl decanoate R in carbon disulphide R and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with carbon disulphide R.

Test solution (a) Dissolve 1.0 g of the residue obtained in the test for wool-fat content in carbon disulphide R and dilute to 10.0 ml with the same solvent.

Test solution (b) Dissolve 1.0 g of the residue obtained in the test for wool-fat content in carbon disulphide R, add 1.0 ml of the internal standard solution and dilute to 10.0 ml with carbon disulphide R.

Reference solution Dissolve 0.2 g of butylated hydroxytoluene R in carbon disulphide R and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 10.0 ml with carbon disulphide R. To 1.0 ml of this solution add 1.0 ml of the internal standard solution and dilute to 10.0 ml with carbon disulphide R.

The chromatography may be carried out using:

— a column 1.5 m long and 4 mm in internal diameter packed with silanised diatomaceous earth for gas chromatography R impregnated with 10 per cent *m/m* of polydimethylsiloxane R; the column is preceded by a column containing silanised glass wool,

— nitrogen for chromatography R as the carrier gas at a flow rate of 40 ml per minute,

— a flame-ionisation detector.

Maintain the temperature of the column at 150 °C, that of the injection port at 180 °C and that of the detector at 300 °C.

Inject the selected volumes of test solutions (a) and (b) and of the reference solution.

Paraffins *The tap and cotton plugs used should be free from grease.* Prepare a column of anhydrous aluminium oxide 230 mm long and 20 mm in diameter by adding a slurry of anhydrous aluminium oxide R and light petroleum R1 to a glass tube fitted with a tap and containing light petroleum R1. Allow to settle and reduce the depth of the layer of solvent above the column to about 40 mm. Dissolve 3.0 g of the residue obtained in the test for wool-fat content in 50 ml of warm light petroleum R1, cool, pass the solution through the column at a rate of 3 ml per minute and wash with 250 ml of light petroleum R1. Concentrate the combined eluate and washings to low bulk by distillation, evaporate to dryness on a water-bath and heat the residue at 105 °C for periods of 10 min until two successive weighings do not differ by more than 1 mg. The residue weighs not more than 30 mg.

Chlorides Boil 1.3 g with 20 ml of alcohol (90 per cent *V/V*) under a reflux condenser for 5 min. Cool, add 40 ml of water and 0.5 ml of nitric acid R and filter. To the filtrate add 0.15 ml of a 1 per cent *m/V* solution of silver nitrate R in alcohol (90 per cent *V/V*). After 5 min protected from light, any opalescence in the solution is not more intense than that in a standard prepared at the same time by adding 0.15 ml of a 1 per cent *m/V* solution of silver nitrate R in alcohol (90 per cent *V/V*) to a mixture of 0.2 ml of 0.02N hydrochloric acid, 20 ml of alcohol (90 per cent *V/V*), 40 ml of water and 0.5 ml of nitric acid R (115 ppm).

Sulphated ash (V.3.2.14) Not more than 0.1 per cent. Ignite 5.0 g and use the residue to determine the sulphated ash.

Wool-fat content 72.5 per cent to 77.5 per cent. In a suitable tared dish containing a glass rod, heat 30.0 g to constant mass on a water-bath, stirring continuously. Weigh the residue.

STORAGE

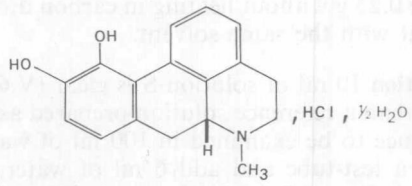
Store in a well-closed container, at a temperature not exceeding 25 °C.

LABELLING

The label on the *container* states the concentration of any added butylated hydroxytoluene.

APOMORPHINI HYDROCHLORIDUM

Apomorphine Hydrochloride



$C_{17}H_{18}ClNO_2,\frac{1}{2}H_2O$ M_r 312.8

Apomorphine hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of (6a*R*)-6-methyl-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de*,*g*]quinoline-10,11-diol hydrochloride, calculated with reference to the dried substance.

CHARACTERS

White or faintly yellow to green-tinged greyish, crystalline powder or crystals; on exposure to air and light the green tinge becomes more pronounced; sparingly soluble in water and in alcohol, very slightly soluble in ether, practically insoluble in chloroform.

IDENTIFICATION

A. Dissolve 10.0 mg in 0.1N hydrochloric acid and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of the solution to 100.0 ml with 0.1N hydrochloric acid. Examined between 230 nm and 350 nm (V.6.19), the solution shows an absorption maximum at 273 nm and a shoulder at 300 nm to 310 nm. The specific absorbance at the maximum is 530 to 570.

B. To 5 ml of solution S (see Tests) add a few millilitres of sodium bicarbonate solution R until a permanent, white precipitate is formed. The precipitate slowly becomes greenish. Add 0.25 ml of 0.1N iodine and shake. The precipitate becomes greyish-green. Collect the precipitate. The precipitate dissolves in ether R giving a purple solution, in chloroform R giving a violet-blue solution and in alcohol R giving a blue solution.

C. To 2 ml of solution S add 0.1 ml of nitric acid R. Mix and filter. The filtrate gives reaction (a) of chlorides (V.3.1.1).

TESTS

Solution S Dissolve 0.25 g without heating in carbon dioxide-free water R and dilute to 25 ml with the same solvent.

Appearance of solution 10 ml of solution S is clear (V.6.1) and not more intensely coloured than a reference solution prepared as follows: dissolve 5 mg of the substance to be examined in 100 ml of water, transfer 1 ml of the solution to a test-tube and add 6 ml of water, 1 ml of sodium bicarbonate solution R and 0.5 ml of 0.1N iodine; allow to stand for 30 s, add 0.6 ml of 0.1N sodium thiosulphate and dilute to 10 ml with water.

pH (V.6.3.1) The pH of solution S is 4.0 to 5.0.

Specific optical rotation (V.6.6) Dissolve 0.25 g in 0.02N hydrochloric acid and dilute to 25.0 ml with the same acid. The specific optical rotation is – 48° to – 52°, calculated with reference to the dried substance.

Morphine Examine by thin-layer chromatography (V.6.20.2), using silica gel G R as the coating substance.

Test solution Dissolve 20 mg of the substance to be examined in methanol R and dilute to 10 ml with the same solvent.

Reference solution Dilute 1 ml of the test solution to 50 ml with methanol R.

Apply separately to the plate 5 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of acetonitrile R, 30 volumes of ethyl acetate R, 30 volumes of methylene chloride R, 5 volumes of anhydrous formic acid R and 5 volumes of water. Dry the plate in a current of cold air until all traces of solvent have disappeared and spray with a 3 per cent *m/V* solution of sodium nitrite R. Expose the plate to ammonia vapour for a few minutes and allow to stand in daylight for about 1 h. In the chromatogram obtained with the test solution, no reddish-orange spot with an R_f value 0.3 to 0.5 times that of the principal spot appears (about 2 per cent of morphine). The test is not valid unless there is a clearly visible spot in the chromatogram obtained with the reference solution.

Loss on drying (V.6.22) 2.5 per cent to 4.2 per cent, determined on 0.50 g by drying in an oven at 100 °C to 105 °C.

Sulphated ash (V.3.2.14) Not more than 0.1 per cent, determined on 0.5 g.

ASSAY

Dissolve 0.250 g in 5 ml of anhydrous formic acid R. Add 30 ml of anhydrous acetic acid R and 7 ml of mercuric acetate solution R. Carry out the non-aqueous titration of halogen salts of organic bases (V.3.5.5), titrating with 0.1N perchloric acid and determining the end-point potentiometrically (V.6.14).

1 ml of 0.1N perchloric acid is equivalent to 30.38 mg of $C_{17}H_{18}ClNO_2$.

STORAGE

Store in an airtight container, protected from light.

VII.1.1. REAGENTS

Acetonitrile. — C_2H_3N (M_r 41.05). Methyl cyanide.

A clear, colourless liquid, miscible with water, with chloroform, with ether and with methanol.

Acidity or alkalinity A 10 per cent *m/V* solution is neutral to litmus paper R.

d_{20}^{20}: about 0.781.

Distillation range (V.6.8) Not less than 95 per cent distils between 80 °C and 82 °C.