EUROPEAN PHARMACOPŒIA

COUNCIL OF EUROPE

2nd EDITION

Part II - 3

MAISONNEUVE

EUROPEAN PHARMACOPOEIA

SECOND EDITION

Part II

Third Fascicule

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TEXTS INCLUDED IN THE THIRD FASCICULE

Revised Monographs with their Serial Number

Benzylpenicillinum Kalicum (113)	Benzylpenicillin Potassium
Benzylpenicillinum Natricum (114)	Benzylpenicillin Sodium
Calcii Hydrogenophosphas (116)	Calcium Hydrogen Phosphate
Digitalis Purpureae Folium (117)	Digitalis Leaf
Dinatrii Phosphas Dodecahydricus (118)	Disodium Phosphate Dodecahydrate
Hydrargyri Dichloridum (120)	Mercuric Chloride
Kalii Permanganas (121)	Potassium Permanganate
Radiopharmaceutica (125)	Radiopharmaceutical Preparations

Texts of Part I

V.6.18. Absorption spectrophotometry, infrared

New Monographs with their Serial Number

Aquae Tritiatae[^{3}H] Solutio Iniectabilis (112)	Tritiated[^{3}H] Water Injection
Benzylpenicillinum Procainum (115)	Procaine Benzylpenicillin
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Kryptoni[^{85}Kr] Solutio Iniectabilis (122)	Krypton[^{85}Kr] Injection
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Natrii Pertechnetatis[^{99m}Tc] Fissione Formati Solutio Iniectabilis (124)	Sodium Pertechnetate[^{99m}Tc] Injection (Fission)
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L-Selenomethionini[^{75}Se] Solutio Iniectabilis (127)	L-Selenomethionine[^{75}Se] Injection
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Stanni Pyrophosphatis et Technetii[^{99m}Tc] Solutio Iniectabilis (129)	Technetium[^{99m}Tc] Tin Pyrophosphate Injection
Stibii Sulfidi Colloidalis et Technetii[^{99m}Tc] Solutio Iniectabilis (130)	Technetium[^{99m}Tc] Colloidal Antimony Sulphide Injection
Sulfuris Colloidalis et Technetii [^{99m}Tc] Solutio Iniectabilis (131)	Technetium[^{99m}Tc] Colloidal Sulphur Injection
Unguenta (132)	Topical Semi-solid Preparations
Xenoni[^{133}Xe] Solutio Iniectabilis (133)	Xenon[^{133}Xe] Injection

VIII.9. Water for diluting concentrated haemodialysis solutions.

ERRATA

V.6.3.2. RELATIONSHIP BETWEEN REACTION OF SOLUTION, APPROXIMATE pH AND COLOUR OF CERTAIN INDICATORS. In the sixteenth line of the fourth column, for " 0.5 ml " read " 0.05 ml ".

VII.1.1. REAGENTS. **Sodium hypophosphite.** Replace the molecular formula by " NaH_2PO_2,H_2O ".

AQUAE TRITIATAE[^3H] SOLUTIO INIECTABILIS

Tritiated[^3H] Water Injection

Tritiated[^3H] water injection is water for injections in which some of the water molecules contain tritium atoms in place of protium atoms. It may be made isotonic by the addition of sodium chloride. Tritium[^3H] may be obtained by the neutron irradiation of lithium. The injection contains not less than 90.0 per cent and not more than 110.0 per cent of the declared tritium activity at the date stated on the label.

CHARACTERS

A clear, colourless liquid.

Tritium has a half-life of 12.3 years and emits beta radiation.

IDENTIFICATION

Record the beta-ray spectrum by the method prescribed in the test for radionuclidic purity. The spectrum does not differ significantly from that of a standardised tritiated[^3H] water (1). The maximum energy of the beta radiation is 0.019 MeV.

TESTS

pH (V.6.3.1) The pH of the injection is 4.5 to 7.0.

Radionuclidic purity

(a) Mix 100 µl of a suitable dilution of the injection with 10 ml of a scintillation liquid consisting of 1000 ml of dioxan R, 100 g of naphthalene R, 7 g of diphenyloxazole R and 0.3 g of methylphenyloxazolylbenzene R, the reagents being of an analytical grade suitable for liquid scintillation. Measure the radioactivity of the mixture in a liquid scintillation counter fitted with a discriminator. The count

(1) Standardised tritiated[^3H] water is available from laboratories recognised by the relevant national authority.

should be about 5000 impulses per second at the lowest setting of the discriminator. Record the count at different discriminator settings. For each measurement count at least 10 000 impulses over a period of at least 1 min. Immediately determine in the same conditions the count for a standardised tritiated[^3H] water having approximately the same activity.

Plot the counts at each discriminator setting, correcting for background activity, on semi-logarithmic paper, the discriminator settings being in arbitrary units as the abscissae. The vertical distance between the two curves obtained is constant. They obey the mathematical relationship:

$$\frac{A_1/B_1 - A_2/B_2}{A_1/B_1} \times 100 < 20$$

A_1 = radioactivity recorded for the standardised preparation at the lowest discriminator setting,

B_1 = radioactivity recorded for the preparation to be examined at the lowest discriminator setting,

A_2 = radioactivity recorded for the standard at the discriminator setting such that $A_2 \approx A_1 \times 10^{-3}$,

B_2 = radioactivity recorded for the preparation to be examined at the latter discriminator setting.

(b) Record the gamma-ray spectrum. The instrument registers only background activity.

Radiochemical purity Place a quantity of the injection equivalent to about 2 μCi (74 kBq), diluted to 50 ml with water, in an all-glass distillation apparatus of the type used for the determination of Distillation Range (V.6.8). Determine the radioactive concentration as described in the monograph on Radiopharmaceutica. Distil until about 25 ml of distillate has been collected. Precautions must be taken to avoid contamination of the air. If the test is carried out in a fume cupboard, the equipment must be protected from draughts. Determine the radioactive concentration of the distillate and of the liquid remaining in the distillation flask. Neither of the radioactive concentrations determined after distillation differs by more than 5 per cent from the value determined before distillation.

Sterility It complies with the test for sterility prescribed in the monograph on Radiopharmaceutica.

RADIOACTIVITY

Determine the radioactivity using a liquid scintillation counter as described in the monograph on Radiopharmaceutica.

STORAGE

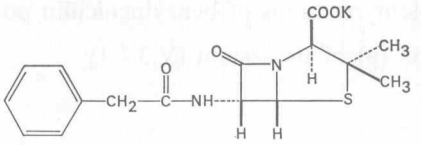
See the monograph on Radiopharmaceutica.

LABELLING

See the monograph on Radiopharmaceutica.

BENZYLPENICILLINUM KALICUM

Benzylpenicillin Potassium



$C_{16}H_{17}KN_2O_4S$ M_r 372.5

Benzylpenicillin potassium is potassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-phenylacetamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate, produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means. It contains not less than 96.0 per cent and not more than the equivalent of 100.5 per cent of penicillins, calculated as $C_{16}H_{17}KN_2O_4S$ with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder with a faint characteristic odour, very soluble in water, practically insoluble in chloroform, in ether, in fatty oils and in liquid paraffin.

IDENTIFICATION

Identification test A may be omitted if identification tests B, C and D are carried out. Identification tests B and C may be omitted if identification tests A and D are carried out.

A. Examine by infrared absorption spectrophotometry (V.6.18). The absorption maxima in the spectrum obtained with the substance to be examined correspond in position and relative intensity to those in the spectrum obtained with benzylpenicillin potassium CRS.

B. Dissolve 0.1 g in 0.067M phosphate buffer solution pH 7.0 R diluted 1 in 10 with carbon dioxide-free water R and dilute to 100 ml with the same diluted buffer solution. Dilute 10 ml of the solution to 100 ml with 0.067M phosphate buffer solution pH 7.0 R diluted 1 in 10 with carbon dioxide-free water R (solution a). To 10 ml of solution (a) add 0.5 ml of penicillinase solution R diluted 1 in 10

and allow to stand at 30 °C for 10 min (solution b). To 5 ml of solution (a) and to 5 ml of solution (b) add 10 ml of acetate buffer solution pH 4.6 R and 5 ml of iodine solution R2. Mix and add 0.1 ml of starch solution R. Solution (a) is blue; solution (b) remains colourless.

C. It gives the colour reactions of benzylpenicillin potassium (V.3.1.5).

D. It gives reaction (a) of potassium (V.3.1.1).

TESTS

pH (V.6.3.1) Dissolve 2.0 g in carbon dioxide-free water R and dilute to 20 ml with the same solvent. The pH of the solution is 5.5 to 7.5.

Specific optical rotation (V.6.6) Dissolve 0.500 g in carbon dioxide-free water R and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 270° to + 300°, calculated with reference to the dried substance.

Absorbance (V.6.19) Dissolve 94.0 mg in water and dilute to 50.0 ml with the same solvent. Measure the absorbance of the solution at 325 nm, 280 nm and at the maximum at 264 nm, measuring at intervals of 0.5 nm and, if necessary, diluting the solution for the measurement at 264 nm. The absorbances at 325 nm and 280 nm do not exceed 0.10 and that at the maximum at 264 nm is 0.82 to 0.93, calculated on the basis of the undiluted (0.188 per cent m/V) solution.

Loss on drying (V.6.22) Not more than 1.0 per cent, determined on 1.00 g by drying in an oven at 100 °C to 105 °C.

ASSAY

Degradation products To 0.250 g add 25 ml of water and 25 ml of acetate buffer solution pH 4.6 R. Shake until dissolution is complete. Titrate immediately at room temperature with 0.02M mercuric nitrate. Determine the end-point potentiometrically (V.6.14) using a mercurous sulphate comparison electrode and a platinum or mercury indicator electrode.

Calculate the percentage content of degradation products (D) as $C_{16}H_{17}KN_2O_4S$ from the expression:

$$\frac{0.7450\ n}{m}$$

m = mass in grams of the substance to be examined,

n = number of millilitres of 0.02M mercuric nitrate used

Penicillins Dissolve 50.0 mg in 5 ml of water. Add 5.0 ml of 1N sodium hydroxide and allow to stand for 15 min. Add 5.0 ml of 1N nitric acid, 20 ml of acetate buffer solution pH 4.6 R and 20 ml of water. Titrate at 35 °C to 40 °C with 0.02M mercuric nitrate. Determine the end-point potentiometrically (V.6.14) using a mercurous sulphate comparison electrode and a platinum or mercury indicator electrode. Titrate slowly so that the titration takes about 15 min. Ignore any preliminary inflexion on the titration curve.

Calculate the percentage content of penicillins as $C_{16}H_{17}KN_2O_4S$ from the expression:

$$\frac{0.7450\, n_1}{m_1} - D$$

m_1 = mass in grams of the substance to be examined,
n_1 = number of millilitres of 0.02M mercuric nitrate used,
D = percentage content of degradation products.

Benzylpenicillin potassium intended for parenteral administration complies with the following additional requirements:

Sterility (V.2.1.1).

Pyrogens (V.2.1.4) Inject per kilogram of the rabbit's mass 1 ml of a solution containing 1.5 mg per millilitre of the substance to be examined in water for injections.

STORAGE

Store in an airtight container, protected from moisture, at a temperature not exceeding 30 °C. If the contents are intended for parenteral administration, the container should be sterile and tamper-proof.

LABELLING

The label on the *container* and the label on the *package* state whether or not the contents are intended for parenteral administration.

VII.1.1. REAGENTS

Mercuric nitrate. — $Hg(NO_3)_2,H_2O$ (M_r 342.6).

Colourless or slightly coloured crystals, hygroscopic, soluble in water in the presence of a small quantity of nitric acid.

Store in an airtight container, protected from light.

VII.2.2. VOLUMETRIC SOLUTIONS

Mercuric nitrate 0.02M Dissolve 6.85 g of mercuric nitrate R in 20 ml of 1N nitric acid and dilute to 1000.0 ml with water.

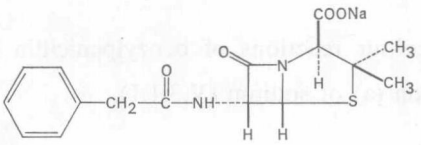
Standardisation Dissolve 15.0 mg of sodium chloride RV in 50 ml of water. Titrate with the mercuric nitrate solution. Determine the end-point potentiometrically (V.6.14), using a mercurous sulphate comparison electrode and a platinum or mercury indicator electrode.

Nitric acid 1N Dilute 96.6 g of nitric acid R to 1000.0 ml with water.

Standardisation Dissolve 2.000 g of sodium carbonate RV in 50 ml of water, add 0.1 ml of methyl orange solution R and titrate with the nitric acid until the solution just becomes reddish-yellow; boil for 2 min. The solution reverts to yellow. Cool and continue the titration until a reddish-yellow colour is obtained.

BENZYLPENICILLINUM NATRICUM

Benzylpenicillin Sodium



$C_{16}H_{17}N_2NaO_4S$ M_r 356.4

Benzylpenicillin sodium is sodium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-phenylacetamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate, produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means. It contains not less than 96.0 per cent and not more than the equivalent of 100.5 per cent of penicillins, calculated as $C_{16}H_{17}N_2NaO_4S$ with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder with a faint characteristic odour, very soluble in water, practically insoluble in chloroform, in ether, in fatty oils and in liquid paraffin.

IDENTIFICATION

Identification test A may be omitted if identification tests B, C and D are carried out. Identification tests B and C may be omitted if identification tests A and D are carried out.

A. Examine by infrared absorption spectrophotometry (V.6.18). The absorption maxima in the spectrum obtained with the substance to be examined correspond in position and relative intensity to those in the spectrum obtained with benzylpenicillin sodium CRS.

B. Dissolve 0.1 g in 0.067M phosphate buffer solution pH 7.0 R diluted 1 in 10 with carbon dioxide-free water R and dilute to 100 ml with the same diluted buffer solution. Dilute 10 ml of the solution to 100 ml with 0.067M phosphate buffer solution pH 7.0 R diluted 1 in 10 with carbon dioxide-free water R (solution a). To 10 ml of

solution (a) add 0.5 ml of penicillinase solution R diluted 1 in 10 and allow to stand at 30 °C for 10 min (solution b). To 5 ml of solution (a) and to 5 ml of solution (b) add 10 ml of acetate buffer solution pH 4.6 R and 5 ml of iodine solution R2. Mix and add 0.1 ml of starch solution R. Solution (a) is blue; solution (b) remains colourless.

C. It gives the colour reactions of benzylpenicillin sodium (V.3.1.5).

D. It gives reaction (a) of sodium (V.3.1.1).

TESTS

pH (V.6.3.1) Dissolve 2.0 g in carbon dioxide-free water R and dilute to 20 ml with the same solvent. The pH of the solution is 5.5 to 7.5.

Specific optical rotation (V.6.6) Dissolve 0.500 g in carbon dioxide-free water R and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 285° to + 310°, calculated with reference to the dried substance.

Absorbance (V.6.19) Dissolve 90.0 mg in water and dilute to 50.0 ml with the same solvent. Measure the absorbance of the solution at 325 nm, 280 nm and at the maximum at 264 nm, measuring at intervals of 0.5 nm and, if necessary, diluting the solution for the measurement at 264 nm. The absorbances at 325 nm and 280 nm do not exceed 0.10 and that at the maximum at 264 nm is 0.82 to 0.93, calculated on the basis of the undiluted (0.180 per cent m/V) solution.

Loss on drying (V.6.22) Not more than 1.0 per cent, determined on 1.00 g by drying in an oven at 100 °C to 105 °C.

ASSAY

Degradation products To 0.250 g add 25 ml of water and 25 ml of acetate buffer solution pH 4.6 R. Shake until dissolution is complete. Titrate immediately at room temperature with 0.02M mercuric nitrate. Determine the end-point potentiometrically (V.6.14), using a mercurous sulphate comparison electrode and a platinum or mercury indicator electrode.

Calculate the percentage content of degradation products (D) as $C_{16}H_{17}N_2NaO_4S$ from the expression:

$$\frac{0.7128\ n}{m}$$

m = mass in grams of the substance to be examined,

n = number of millilitres of 0.02M mercuric nitrate used.

Penicillins Dissolve 50.0 mg in 5 ml of water. Add 5.0 ml of 1N sodium hydroxide and allow to stand for 15 min. Add 5.0 ml of 1N nitric acid, 20 ml of acetate buffer solution pH 4.6 R and 20 ml of water. Titrate at 35 °C to 40 °C with 0.02M mercuric nitrate. Determine the end-point potentiometrically (V.6.14), using a mercurous sulphate comparison electrode and a platinum or mercury indicator electrode. Titrate slowly so that the titration takes about 15 min. Ignore any preliminary inflexion on the titration curve.

Calculate the percentage content of penicillins as $C_{16}H_{17}N_2NaO_4S$ from the expression:

$$\frac{0.7128\ n_1}{m_1} - D$$

m_1 = mass in grams of the substance to be examined,

n_1 = number of millilitres of 0.02M mercuric nitrate used,

D = percentage content of degradation products.

Benzylpenicillin sodium intended for parenteral administration complies with the following additional requirements:

Sterility (V.2.1.1).

Pyrogens (V.2.1.4) Inject per kilogram of the rabbit's mass 1 ml of a solution containing 1.5 mg per millilitre of the substance to be examined in water for injections.

STORAGE

Store in an airtight container, protected from moisture, at a temperature not exceeding 30 °C. If the contents are intended for parenteral administration, the container should be sterile and tamper-proof.

LABELLING

The label on the *container* and the label on the *package* state whether or not the contents are intended for parenteral administration.

VII.1.1. REAGENTS

Mercuric nitrate. — $Hg(NO_3)_2,H_2O$ (M_r 342.6).

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VII.2.2. VOLUMETRIC SOLUTIONS

Mercuric nitrate 0.02M Dissolve 6.85 g of mercuric nitrate R in 20 ml of 1N nitric acid and dilute to 1000.0 ml with water.

Standardisation Dissolve 15.0 mg of sodium chloride RV in 50 ml of water. Titrate with the mercuric nitrate solution. Determine the end-point potentiometrically (V.6.14), using a mercurous sulphate comparison electrode and a platinum or mercury indicator electrode.

Nitric acid 1N Dilute 96.6 g of nitric acid R to 1000.0 ml with water.

Standardisation Dissolve 2.000 g of sodium carbonate RV in 50 ml of water, add 0.1 ml of methyl orange solution R and titrate with the nitric acid until the solution just becomes reddish-yellow; boil for 2 min. The solution reverts to yellow. Cool and continue the titration until a reddish-yellow colour is obtained.