BENZYL BENZOATE

Benzylis benzoas

C_{14}H_{12}O_{2} \quad M, 212.2

DEFINITION
Phenylmethyl benzoate.
Content: 99.0 per cent to 100.5 per cent.

CHARACTERS
Appearance: colourless or almost colourless crystals or colourless or almost colourless, oily liquid.
Solubility: practically insoluble in water, miscible with ethanol (96 per cent), with methylene chloride and with fatty and essential oils.
Eb: about 320 °C.

IDENTIFICATION
First identification: A.
Second identification: B, C.
A. Infrared absorption spectrophotometry (2.2.24).
B. Thin-layer chromatography (2.2.27).
Test solution. Dissolve 25 mg of the substance to be examined in 5 ml of methanol (96 per cent).
Reference solution. Dissolve 25 mg of benzyl benzoate CRS in 5 ml of methanol.

TESTS
Acidity. Dissolve 2.0 g in ethanol (96 per cent) R and dilute to 10 ml with the same solvent. Titrate with 0.1 M sodium hydroxide using phenolphthalein solution R as indicator. Not more than 0.2 ml is required to change the colour of the indicator to pink.
Relative density (2.2.5): 1.118 to 1.122.
Refractive index (2.2.6): 1.568 to 1.570.

BENZYL PENICILLIN, BENZATHINE

Benzylpenicillinum benzathinum

C_{48}H_{56}N_{6}O_{8}S_{2} \quad M, 909

DEFINITION
N,N′-Dibenzylethane-1,2-diamine compound (1:2) with (2S,5R,6R)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.
Substance produced by the growth of certain strains of Penicillium notatum or related organisms, or obtained by any other means.
Content:
– benzathine benzylpenicillin: 96.0 per cent to 102.0 per cent (anhydrous substance);
– N,N′-dibenzylethylenediamine (benzathine C_{16}H_{20}N_{2}; M = 240.3): 24.0 per cent to 27.0 per cent (anhydrous substance).
It contains a variable quantity of water. Dispersing or suspending agents may be added.

CHARACTERS
Appearance: white or almost white powder.
Solubility: very slightly soluble in water, freely soluble in dimethylformamide and in formamide, slightly soluble in ethanol (96 per cent).

IDENTIFICATION
First identification: A.
Second identification: B, C, D.
A. Infrared absorption spectrophotometry (2.2.24).
Comparison: benzathine benzylpenicillin CRS.
B. Thin-layer chromatography (2.2.27).
Test solution. Dissolve 25 mg of the substance to be examined in 5 ml of methanol.
Reference solution. Dissolve 25 mg of benzathine benzylpenicillin CRS in 5 ml of methanol.
Benzylicillin, benzathine

**Plates:** TLC silanised silica gel plate R.

**Mobile phase:** mix 30 volumes of acetone R and 70 volumes of a 154 g/l solution of ammonium acetate R adjusted to pH 7.0 with ammonia R.

**Application:** 1 µl.

**Development:** over a path of 15 cm.

**Drying:** in air.

**Detection:** expose to iodine vapour until the spots appear and examine in daylight.

**System suitability:** reference solution:
- the chromatogram shows 2 clearly separated spots.

**Results:** the 2 principal spots in the chromatogram obtained with the test solution are similar in position, colour and size to the 2 principal spots in the chromatogram obtained with the reference solution.

**C.** Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of water R and add 2 ml of sulphuric acid-formaldehyde reagent R. Mix the contents of the tube by swirling; the solution is practically colourless. Place the test-tube on a water-bath for 1 min; a reddish-brown colour develops.

**D.** To 0.1 g add 2 ml of 1 M sodium hydroxide and shake for 2 min. Shake the mixture with 2 quantities, each of 3 ml, of ether R. Evaporate the combined ether layers to dryness and dissolve the residue in 1 ml of ethanol (50 per cent V/V) R. Add 5 ml of picric acid solution R, heat at 90 °C for 5 min and allow to cool slowly. Separate the crystals and recrystallise from ethanol (25 per cent V/V) R containing 10 g/l of picric acid R. The crystals melt (2.2.14) at about 214 °C.

**Tests**

**Acidity or alkalinity.** To 0.50 g add 100 ml of carbon dioxide-free water R and shake for 5 min. Filter through a sintered-glass filter (2.1.2). To 20 ml of the filtrate add 0.1 ml of bromothymol blue solution R1. The solution is green or yellow. Not more than 0.2 ml of 0.02 M sodium hydroxide is required to change the colour of the indicator to blue.

**Related substances.** Liquid chromatography (2.2.29).

Prepare the solutions immediately before use, using sonication (for about 2 min) to dissolve the samples. Avoid any overheating during the sample preparation.

**Test solution.** Dissolve 70.0 mg of the substance to be examined in 25 ml of methanol R and dilute to 50.0 ml with a solution containing 6.8 g/l of potassium dihydrogen phosphate R and 1.02 g/l of disodium hydrogen phosphate R.

**Reference solution (a).** Dissolve 70.0 mg of benzathine benzylpenicillin CRS in 25 ml of methanol R and dilute to 50.0 ml with a solution containing 6.8 g/l of potassium dihydrogen phosphate R and 1.02 g/l of disodium hydrogen phosphate R.

**Reference solution (b).** Dilute 1.0 ml of reference solution (a) to 100.0 ml with mobile phase A.

**Column:**
- size: l = 0.25 m, Ø = 4.0 mm;
- stationary phase: end-capped octadecylsilyl silica gel for chromatography R (5 µm);
- temperature: 40 °C.

**Mobile phase:**
- mobile phase A: mix 10 volumes of a 34 g/l solution of potassium dihydrogen phosphate R adjusted to pH 3.5 with phosphoric acid R, 30 volumes of methanol R and 60 volumes of water R;
- mobile phase B: mix 10 volumes of a 34 g/l solution of potassium dihydrogen phosphate R adjusted to pH 3.5 with phosphoric acid R, 30 volumes of water R and 60 volumes of methanol R.

**Flow rate:** 1 ml/min.

**Detection:** spectrophotometer at 220 nm.

**Injection:** 20 µl.

**System suitability:** reference solution (a):
- relative retention with reference to benzylpenicillin: benzathine = 0.3 to 0.4; impurity C = about 2.4; if necessary, adjust the concentration of methanol in the mobile phase.

**Limits:**
- impurity C: not more than twice the sum of the areas of the 2 principal peaks in the chromatogram obtained with reference solution (b) (2 per cent);
- any other impurity: for each impurity, not more than the sum of the areas of the 2 principal peaks in the chromatogram obtained with reference solution (b) (1 per cent);
- disregard limit: 0.05 times the sum of the areas of the 2 principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

**Water** (2.5.12): 5.0 per cent to 8.0 per cent, determined on 0.300 g.

**Bacterial endotoxins** (2.6.14, Method E): less than 0.13 IU/ml, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

Suspend 20 mg in 20 ml of a solution of 0.1 M sodium hydroxide diluted 1 to 100, shake thoroughly and centrifuge. Examine the supernatant.

**ASSAY**

Liquid chromatography (2.2.29) as described in the test for related substances with the following modifications.

**Mobile phase:** phosphate buffer solution pH 3.5 R, methanol R, water R (10:35:55 V/V/V).

**Injection:** test solution and reference solution (a).

Calculate the percentage contents of benzathine and benzathine benzylpenicillin. Calculate the percentage content of benzathine benzylpenicillin by multiplying the percentage content of benzylpenicillin by 1.36.

**Storage**

In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

**Impurities**

Specified impurities: C.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to
identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use: A, B, D, E, F.

A. monobenzylethylenediamine,

B. phenylacetic acid,

C. benzylpenicilloic acids benzathide,

D. \((3S,7R,7aR)\)-5-benzyl-2,2-dimethyl-2,3,7,7a-tetrahydroimidazo[5,1-b]thiazole-3,7-dicarboxylic acid (penillic acid of benzylpenicillin),

E. \((4S)-2\text{[carboxy[(phenylacetyl)amino]methyl]}\)-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin),

F. \((2RS,4S)-2\text{[(phenylacetyl)amino]methyl]}\)-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin).

DEFINITION
Potassium \((2S,5R,6R)-3,3\text{-dimethyl-7-oxo-6-}\text{[phenylacetyl]-amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.}

Substance produced by the growth of certain strains of Penicillium notatum or related organisms, or obtained by any other means.

Content: 96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance: white or almost white, crystalline powder.
Solubility: very soluble in water, practically insoluble in fatty oils and in liquid paraffin.

IDENTIFICATION
First identification: A, D.
Second identification: B, C, D.

A. Infrared absorption spectrophotometry (2.2.24).
Comparison: benzylpenicillin potassium CRS.

B. Thin-layer chromatography (2.2.27).
Test solution. Dissolve 25 mg of the substance to be examined in 5 ml of water R.
Reference solution (a). Dissolve 25 mg of benzylpenicillin potassium CRS in 5 ml of water R.
Reference solution (b). Dissolve 25 mg of benzylpenicillin potassium CRS and 25 mg of phenoxymethylpenicillin potassium CRS in 5 ml of water R.
Plate: TLC silanised silica gel plate R.
Mobile phase: a mixture of 30 volumes of acetone R and 70 volumes of a 154 g/l solution of ammonium acetate R previously adjusted to pH 5.0 with glacial acetic acid R.
Application: 1 µl.
Development: over a path of 15 cm.
Drying: in air.
Detection: expose to iodine vapour until the spots appear and examine in daylight.
System suitability: reference solution (b):
the chromatogram shows 2 clearly separated spots.
Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and 15 mm in diameter. Moisten with 0.05 ml of water R and add 2 ml of sulphuric acid-formaldehyde reagent R.