

In the chromatogram obtained with the test solution, verify that there are no peaks with the same retention time as the standards.

01/2005:0705

Limits:

- *benzaldehyde*: not more than the difference between the area of the peak due to benzaldehyde in the chromatogram obtained with reference solution (b) and the area of the peak due to benzaldehyde in the chromatogram obtained with the test solution (0.05 per cent).
- *cyclohexylmethanol*: not more than the difference between the area of the peak due to cyclohexylmethanol in the chromatogram obtained with reference solution (b) and the area of the peak due to cyclohexylmethanol in the chromatogram obtained with the test solution (0.10 per cent).
- *total of other peaks with a relative retention less than that of benzyl alcohol*: not more than twice the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (b) (0.02 per cent).
- *total of peaks with a relative retention greater than that of benzyl alcohol*: not more than the area of the peak due to dicyclohexyl in the chromatogram obtained with reference solution (b) (0.2 per cent).
- *disregard limit*: 0.01 times the area of the peak due to ethylbenzene in the chromatogram obtained with reference solution (b) (0.0001 per cent).

Residue on evaporation: maximum 0.05 per cent.

After ensuring that the substance to be examined complies with the test for peroxide value, evaporate 10.0 g to dryness on a water-bath, dry at 100–105 °C for 1 h and allow to cool in a desiccator. The residue weighs a maximum of 5 mg.

ASSAY

To 0.900 g (m g) add 15.0 ml of a freshly prepared mixture of 1 volume of *acetic anhydride R* and 7 volumes of *pyridine R* and boil under a reflux condenser for 30 min. Cool and add 25 ml of *water R*. Using 0.25 ml of *phenolphthalein solution R* as indicator, titrate with 1 M *sodium hydroxide* (n_1 ml). Carry out a blank titration (n_2 ml).

Calculate the percentage content of C_7H_8O from the expression:

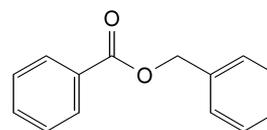
$$\frac{10.81 (n_2 - n_1)}{m}$$

STORAGE

In an airtight container, under nitrogen, protected from light at a temperature between 2 °C and 8 °C.

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

BENZYL BENZOATE**Benzylis benzoas** $C_{14}H_{12}O_2$ M_r 212.2**DEFINITION**

Benzyl benzoate contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of phenylmethyl benzoate.

CHARACTERS

Colourless or almost colourless crystals or a colourless or almost colourless, oily liquid, practically insoluble in water, miscible with alcohol, with methylene chloride and with fatty and essential oils.

It boils at about 320 °C.

IDENTIFICATION

First identification: A.

Second identification: B, C.

- A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the *Ph. Eur. reference spectrum of benzyl benzoate*.
- B. To 2 g add 25 ml of *alcoholic potassium hydroxide solution R* and boil under a reflux condenser for 2 h. Remove the ethanol on a water-bath, add 50 ml of *water R* and distil; collect about 25 ml of distillate and use it for identification test C. Acidify the liquid remaining in the distillation flask with *dilute hydrochloric acid R*; a white precipitate is formed, which, when washed with *water R* and dried *in vacuo*, melts (2.2.14) at 121 °C to 124 °C.
- C. To the distillate obtained in identification test B add 2.5 g of *potassium permanganate R* and 5 ml of *dilute sodium hydroxide solution R*. Boil under a reflux condenser for 15 min, cool and filter. Acidify the filtrate with *dilute hydrochloric acid R*; a white precipitate is formed which, when washed with *water R* and dried *in vacuo*, melts (2.2.14) at 121 °C to 124 °C.

TESTS

Acidity. Dissolve 2.0 g in *alcohol 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.

Freezing point (2.2.18): Not less than 17.0 °C.

Sulphated ash (2.4.14): Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

To 2.000 g add 50.0 ml of 0.5 M *alcoholic potassium hydroxide* and boil gently under a reflux condenser for 1 h. Titrate the hot solution with 0.5 M *hydrochloric acid* using 1 ml of *phenolphthalein solution R* as indicator. Carry out a blank determination.

1 ml of 0.5 M alcoholic potassium hydroxide is equivalent to 106.1 mg of C₁₄H₁₂O₂.

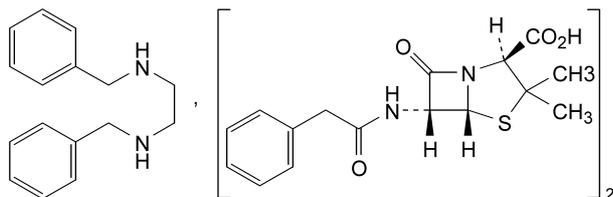
STORAGE

Store in an airtight, well-filled container, protected from light.

01/2005:0373

BENZYLPENICILLIN, BENZATHINE

Benzylpenicillinum benzathinum

C₄₈H₅₆N₆O₈S₂M_r 909

DEFINITION

Benzathine benzylpenicillin is *N,N'*-dibenzylethane-1,2-diamine compound (1:2) with (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of benzathine benzylpenicillin and not less than 24.0 per cent and not more than 27.0 per cent of *N,N'*-dibenzylethylenediamine (benzathine C₁₆H₂₀N₂; M_r 240.3), both calculated with reference to the anhydrous substance. It contains a variable quantity of water. Dispersing or suspending agents may be added.

CHARACTERS

A white powder, very slightly soluble in water, freely soluble in dimethylformamide and in formamide, slightly soluble in alcohol.

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

- A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with benzathine benzylpenicillin CRS.
- B. Examine by thin-layer chromatography (2.2.27), using a TLC silanised silica gel plate R.

Test solution. Dissolve 25 mg of the substance to be examined in 5 ml of methanol R.

Reference solution. Dissolve 25 mg of benzathine benzylpenicillin CRS in 5 ml of methanol R.

Apply to the plate 1 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of acetone R and 70 volumes of a 154 g/l solution of ammonium acetate R, the pH of which has been adjusted to 7.0 with ammonia R. Allow the plate to dry in air and expose it to iodine vapour until the spots appear. Examine in daylight. The two principal spots in the chromatogram obtained with the test solution are similar in position, colour and size to the two principal spots in the chromatogram obtained with the reference solution. The test is not valid unless the chromatogram obtained with the reference solution shows two clearly separated spots.

- 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 two quantities, each of 3 ml, of ether R. Evaporate the combined ether layers to dryness and dissolve the residue in 1 ml of alcohol (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 alcohol (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. 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 over heating 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,

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 10	75	25
10 - 20	75 → 0	25 → 100
20 - 55	0	100
55 - 70	75	25

Flow rate: 1 ml/min.

Detection: spectrophotometer at 220 nm.

Injection: 20 µl; inject the test solution and the reference solutions.