Benzylpenicillin potassium

EUROPEAN PHARMACOPOEIA 5.0

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: 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 by the semi-micro determination of water.

Bacterial endotoxins (2.6.14, Method E). Suspend 20 mg of the substance to be examined in 20 ml of a solution of 0.1 M sodium hydroxide diluted 1 to 100, shake thoroughly and centrifuge. The supernatant contains 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.

ASSAY

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


Injection: 20 µl; inject the test solution and reference solution (a).

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

STORAGE

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

LABELLING

The label states:

- where applicable, the name and quantity of any added dispersing or suspending agent,
- where applicable, that the substance is free from bacterial endotoxins.

IMPURITIES

A. monobenzylethylenediamine,

B. phenylacetic acid,

C. benzylpenicilloic acids benzathide,

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

E. (4S)-2-[carboxy[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of benzylpenicillin),

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

BENZYLPCENICILLIN POTASSIUM

Benzylpenicillinum kalicum

C₁₆H₁₇KN₂O₄S

M, 372.5

DEFINITION

Benzylpenicillin potassium is potassium (2S,5R,6R)-3,3-dimethyl-7-oxo-6-[(phenylacetylamino)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate, a substance produced by the growth of certain strains of Penicillium notatum or related organisms, or obtained by any other
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Benzylpencillin potassium

means. It contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of benzylpencillin potassium, calculated with reference to the dried substance.

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

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

A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with benzylpencillin potassium CRS.

B. Examine by thin-layer chromatography (2.2.27), using a TLC silica gel plate R.

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

Reference solution (a). Dissolve 25 mg of benzylpencillin potassium CRS in 5 ml of water R.

Reference solution (b). Dissolve 25 mg of benzylpencillin potassium CRS and 25 mg of phenoxybenzylpenicillin potassium CRS in 5 ml of water R.

Apply to the plate 1 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of acetic acid R and 70 volumes of a 154 g/l solution of ammonium acetate R, the pH of which has been adjusted to 5.0 with glacial acetic acid R. Allow the plate to dry in air and expose it to iodine vapour until the spots appear. Examine in daylight. The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 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. It gives reaction (a) of potassium (2.3.1).

TESTS

**pH** (2.2.3). 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** (2.2.7). 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** (2.2.25). Dissolve 94.0 mg in water R 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, diluting the solution, if necessary, 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 0.80 to 0.88, calculated on the basis of the undiluted (1.88 g/l) solution. Verify the resolution of the apparatus (2.2.25); the ratio of the absorbances is at least 1.7.

**Related substances.** Examine by liquid chromatography (2.2.29) as described under Assay. Inject 20 µl of reference solution (d) and elute isocratically with the chosen mobile phase. Inject 20 µl of test solution (b) and start the elution isocratically. Immediately after elution of the benzylpencillin peak start the following linear gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 20</td>
<td>70 → 0</td>
<td>30 → 100</td>
<td>linear gradient</td>
</tr>
<tr>
<td>20 - 35</td>
<td>0 → 100</td>
<td></td>
<td>isocratic</td>
</tr>
<tr>
<td>35 - 50</td>
<td>70 → 0</td>
<td>30</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

Inject water R and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b), the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1 per cent).

**Loss on drying** (2.2.32). Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 100-105 °C.

**Bacterial endotoxins** (2.6.14, Method E): less than 0.16 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY
Examine by liquid chromatography (2.2.29). Prepare the solutions immediately before use.

Test solution (a). Dissolve 50.0 mg of the substance to be examined in water R and dilute to 50.0 ml with the same solvent.

Test solution (b). Dissolve 80.0 mg of the substance to be examined in water R and dilute to 20.0 ml with the same solvent.

Reference solution (a). Dissolve 50.0 mg of benzylpencillin sodium CRS in water R and dilute to 50.0 ml with the same solvent.

Reference solution (b). Dissolve 10 mg of benzylpencillin sodium CRS and 10 mg of phenoxybenzylpenicillin CRS in water R and dilute to 50 ml with the same solvent.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with water R. Dilute 1.0 ml of the solution to 50.0 ml with water R.

Reference solution (d). Dilute 4.0 ml of reference solution (a) to 100.0 ml with water R.

The chromatographic procedure may be carried out using:
- a column 0.25 m long and 4.6 mm in internal diameter packed with octadecylsilyle silica gel for chromatography R (5 µm),
- as mobile phase at a flow rate of 1.0 ml/min:
  - **Mobile phase A.** Mix 10 volumes of a 68 g/l solution of potassium dihydrogen phosphate R adjusted to pH 3.5 with a 500 g/l solution of dilute phosphoric acid R, 30 volumes of methanol R and 60 volumes of water R.
  - **Mobile phase B.** Mix 10 volumes of a 68 g/l solution of potassium dihydrogen phosphate R adjusted to pH 3.5 with a 500 g/l solution of dilute phosphoric acid R, 40 volumes of water R and 50 volumes of methanol R,
- as detector a spectrophotometer set at 225 nm.

Equilibrate the column with a mobile phase ratio A:B of 70:30. Inject 20 µl of reference solution (b). The test is not valid unless the resolution between the 2 principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the mass distribution ratio for the second peak (benzylpencillin) is 4.0 to 6.0. Inject 20 µl of reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.
Calculate the percentage content of benzylpenicillin potassium by multiplying the percentage content of benzylpenicillin sodium by 1.045.

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

LABELLING
The label states, where applicable, that the substance is free from bacterial endotoxins.

IMPURITIES

A. (2S,5R,6R)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),

B. phenylacetic acid,

C. (2S,5R,6R)-6-[[4-hydroxyphenyl]acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,

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

E. (4S)-2-[carboxyl(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin),

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