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TEST METHOD of Ampicillin Trihydrate, BP Standard

(Ph Eur monograph 0168)

C_{16}H_{19}N_{3}O_{5}S,3H_{2}O  403.5  7177-48-2

Ph Eur

Definition

Ampicillin trihydrate contains not less than 96.0 per cent and not more than the equivalent of 100.5 per cent of \((2S,5R,6R)-6-\{[2R]-2-amino-2-phenylacetyl]amino\}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, calculated with reference to the anhydrous substance.

Characters

A white, crystalline powder, slightly soluble in water, practically insoluble in alcohol, in ether and in fatty oils. It dissolves in dilute solutions of acids and of alkali hydroxides.

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 *ampicillin trihydrate CRS*.

B. Examine by thin-layer chromatography (2.2.27), using *silanised silica gel H R* as the coating substance.

*Test solution*

Dissolve 25 mg of the substance to be examined in 10 ml of *sodium hydrogen carbonate solution R*.

*Reference solution (a)*

Dissolve 25 mg of ampicillin trihydrate CRS in 10 ml of sodium hydrogen carbonate solution R.

*Reference solution (b)*

Dissolve 25 mg of amoxicillin trihydrate CRS and 25 mg of ampicillin trihydrate CRS in 10 ml of sodium hydrogen carbonate solution R.

Apply separately to the plate 1 ml of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *acetone R* and 90 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 in a water-bath for 1 min; a dark yellow colour develops.

D. It complies with the test for water (see Tests).

**Tests**

**Appearance of solution**

Dissolve 1.0 g in 10 ml of *1m hydrochloric acid*. Separately dissolve 1.0 g in 10 ml of *dilute ammonia R*². Immediately after dissolution, the solutions are not more opalescent than reference suspension II (2.2.1).

**pH (2.2.3)**

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 40 ml with the same solvent. The pH of the solution is 3.5 to 5.5.

**Specific optical rotation (2.2.7)**

Dissolve 62.5 mg in *water R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is +280 to +305, calculated with reference to the anhydrous substance.

**Related substances**

Examine by liquid chromatography (2.2.29) as described under Assay. Inject reference solution (c) and elute isocratically. Inject freshly prepared test solution (b) and start the elution isocratically. Immediately after elution of the ampicillin peak start the following linear gradient.
If the mobile phase composition has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the 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>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Equilibrate the column with the originally chosen mobile phase for 15 min. Inject mobile phase A and use the same elution gradient to obtain a blank. In the chromatogram obtained with test solution (b), the area of any peak, apart from the principal peak and any peak observed in the blank chromatogram, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

N,N-Dimethylaniline (2.4.26, Method B)

Not more than 20 ppm.

Water (2.5.12)

12.0 per cent to 15.0 per cent, determined on 0.100 g by the semi-micro determination of water.

Sulphated ash (2.4.14)

Not more than 0.5 per cent, determined on 1.0 g.

Assay

Examine by liquid chromatography (2.2.29).

*Test solution (a)*

Dissolve 31.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with the same solvent.

*Test solution (b)*

Dissolve 31.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 ml with the same solvent.

*Reference solution (a)*

Dissolve 27.0 mg of *anhydrous ampicillin CRS* in mobile phase A and dilute to 50.0 ml with the same solvent.

*Reference solution (b)*

Dissolve 2.0 mg of *cefaridine CRS* in mobile phase A and dilute to 50 ml with the same solvent. To 5.0 ml of this solution add 5.0 ml of reference solution (a).

*Reference solution (c)*

Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A.

*Reference solution (d)*

Dilute 1.0 ml of reference solution (c) to 25.0 ml with mobile phase A.
The chromatographic procedure may be carried out using:

a column 0.25 m long and 4.6 mm in internal diameter packed with octadecylsilyl silica gel for chromatography R (5 mm),

as mobile phase at a flow rate of 1.0 ml/min:

Mobile phase A A mixture of 0.5 ml of dilute acetic acid R, 50 ml of 0.2 M potassium dihydrogen phosphate R, 50 ml of acetonitrile R diluted to 1000 ml with water R,

Mobile phase B A mixture of 0.5 ml of dilute acetic acid R, 50 ml of 0.2 M potassium dihydrogen phosphate R and 400 ml of acetonitrile R diluted to 1000 ml with water R,

as detector a spectrophotometer set at 254 nm,

a 50 ml loop injector.

Equilibrate the column with a mobile phase with ratio A:B of 85:15. Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is at least 3.0. If necessary, adjust the ratio A:B of the mobile phase. The mass distribution ratio for the first peak (ampicillin) is 2.0 to 2.5. Inject reference solution (d). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3. Inject reference solution (a) 6 times. The test is not valid unless the relative standard deviation for the area of the principal peak is at most 1.0 per cent. Inject alternately test solution (a) and reference solution (a).

Calculate the percentage content of ampicillin.

**Storage**

Store in an airtight container, at a temperature not exceeding 30°C.

**Impurites**

![Impurity Diagram]

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. (2S,5R,6R)-6-[[2S]-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (l-ampicillin),
C. (4S)-2-(3,6-dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid (diketopiperazines of ampicillin),

D. \( R = \text{CO}_2\text{H} \): (4S)-2-[[[(2R)-2-amino-2-phenylacetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of ampicillin),

E. (2R)-2-[[[(2S,5R,6R)-6-[[2R]-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-2-phenylacetic acid (ampicillinyl-\( \alpha \)-phenylglycine),

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

G. (3R,6R)-3,6-diphenylpiperazine-2,5-dione,

H. 3-phenylpyrazin-2-ol,
I. \((2S,5R,6R)-6-[[2R]-2-[[2R]-2-\text{amino-2-phenylacetyl}][\text{amino}]-2-\text{phenylacetyl}][\text{amino}]-3,3-\text{dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (}o\text{-phenylglycylampicillin)},

J. \((2S,5R,6R)-6-[[2,2-\text{dimethylpropanoyl}]\text{amino}]-3,3-\text{dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid},

K. \((2R)-2-[[2,2-\text{dimethylpropanoyl}]\text{amino}]-2-\text{phenylacetic acid},

L. \((2R)-2-\text{amino-2-phenylacetic acid (}o\text{-phenylglycine)},
M. co-oligomers of ampicillin and of penicilloic acids of ampicillin.

**Ph Eur**

**Action and use**

Antibacterial.

**Preparations**

Ampicillin Capsules

Ampicillin Oral Suspension

Co-fluampicil Capsules

Co-fluampicil Oral Suspension