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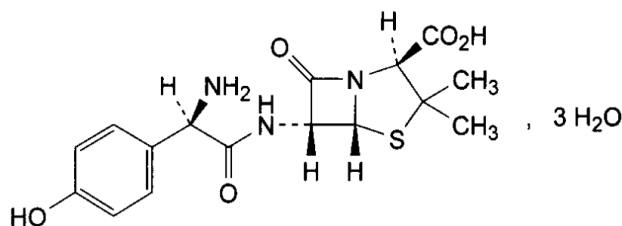
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Test Method of Amoxicillin Trihydrate

(Ph Eur monograph 0260)



$C_{16}H_{19}N_3O_5S \cdot 3H_2O$ 419.4 61336-70-7

Ph Eur

DEFINITION

Amoxicillin trihydrate contains not less than 95.0 per cent and not more than the equivalent of 100.5 per cent of (2*S*,5*R*,6*R*)-6-[[*(2R)*-2-amino-2-(4-hydroxyphenyl)acetyl]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 or almost white, crystalline powder, slightly soluble in water and in alcohol, practically insoluble in ether and in fatty oils. It dissolves in dilute acids and dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification

A.

Second identification

B, C.

A. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with *amoxicillin trihydrate CRS*.

B. Examine by thin-layer chromatography (2.2.27), using *silanised silica gel HR* 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 *amoxicillin 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 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.

TESTS

Solution S

With the aid of ultrasound or gentle heating, dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution

Dissolve 1.0 g in 10 ml of 0.5 M *hydrochloric acid*. Dissolve separately 1.0 g in 10 ml of *dilute ammonia R2*. Immediately after dissolution, the solutions are not more opalescent than reference suspension II (2.2.1).

pH (2.2.3)

The pH of solution S is 3.5 to 5.5.

Specific optical rotation (2.2.7)

+ 290 to + 315, determined on solution S and calculated with reference to the anhydrous substance.

Related substances

Examine by liquid chromatography (2.2.29) adjusting the ratio A:B of the mobile phase and the attenuation as described under Assay. Inject reference solution (d). Inject a freshly prepared test solution (b) and start the elution isocratically with the chosen mobile phase. Immediately after elution of the amoxicillin peak start a linear gradient elution to reach a mobile phase A:B of 0:100 over a period of 25 min. Continue the chromatography with mobile phase B for 15 min, then equilibrate the column for 15 min with the mobile phase chosen originally. 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 (d) (1 per cent).

N,N-Dimethylaniline (2.4.26, Method B)

Not more than 20 ppm.

Water (2.5.12)

11.5 per cent to 14.5 per cent, determined on 0.100 g by the semi-micro determination of water.

Sulphated ash (2.4.14)

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

ASSAY

Examine by liquid chromatography (2.2.29).

Test solution (a)

Dissolve 30.0 mg of the substance to be examined in mobile phase A and dilute to 50.0 ml with the same mobile phase.

Test solution (b)

Dissolve 30.0 mg of the substance to be examined in mobile phase A and dilute to 20.0 ml with the same mobile phase.

Reference solution (a)

Dissolve 30.0 mg of *amoxicillin trihydrate CRS* in mobile phase A and dilute to 50.0 ml with the same mobile phase.

Reference solution (b)

Dissolve 4.0 mg of *cefadroxil CRS* in mobile phase A and dilute to 50 ml with the same mobile phase. To 5.0 ml of this solution add 5.0 ml of reference solution (a) and dilute to 100 ml with mobile phase A.

Reference solution (c)

Dilute 1.0 ml of reference solution (a) to 20.0 ml with mobile phase A. Dilute 1.0 ml of this solution to 50.0 ml with mobile phase A.

Reference solution (d)

Dilute 2.0 ml of reference solution (a) to 20.0 ml with mobile phase A. Dilute 5.0 ml of this solution to 20.0 ml with mobile phase A.

The chromatographic procedure may be carried out using:

a stainless steel 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 1 volume of *acetonitrile R* and 99 volumes of buffer solution pH 5.0,

Mobile phase B

A mixture of 20 volumes of *acetonitrile R* and 80 volumes of buffer solution pH 5.0,

Prepare the buffer solution as follows: to 250 ml of *0.2 M potassium dihydrogen phosphate R* add *dilute sodium hydroxide solution R* to pH 5.0 and dilute to 1000.0 ml with *water R*,

as detector a spectrophotometer set at 254 nm,

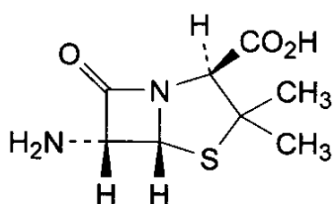
a 50 ml loop injector.

Equilibrate the column with a mobile phase with ratio A:B of 92:8. Inject reference solution (b). The assay is not valid unless the resolution between the 2 principal peaks is at least 2.0. If necessary, adjust the ratio A:B of the mobile phase. The mass distribution ratio for the first peak (amoxicillin) is 1.3 to 2.5. Inject reference solution (c). 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).

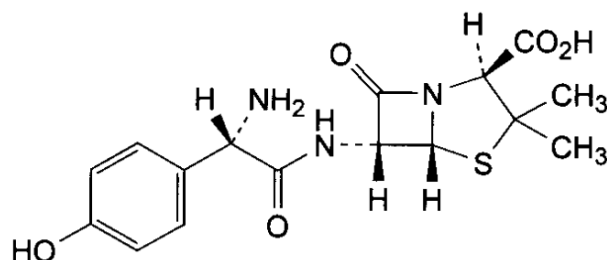
STORAGE

Store in an airtight container .

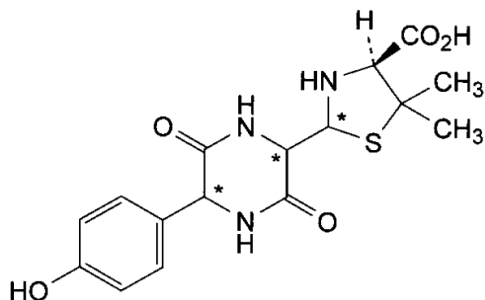
IMPURITIES



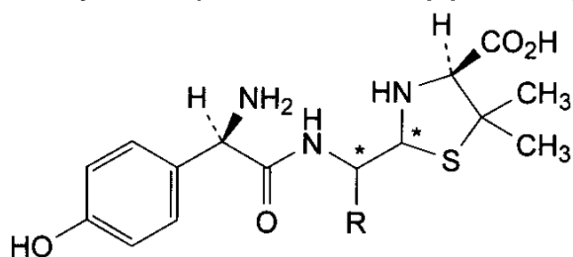
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



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

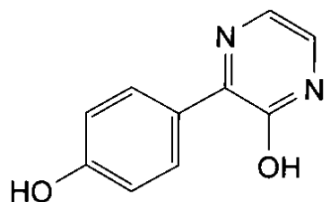


C. (4*S*)-2-[5-(4-hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid (amoxicillin diketopiperazines),

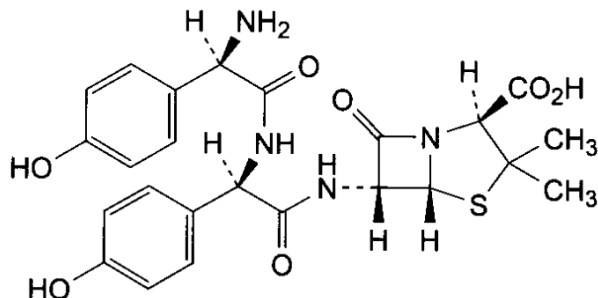


D. R = CO₂H: (4S)-2-[[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin),

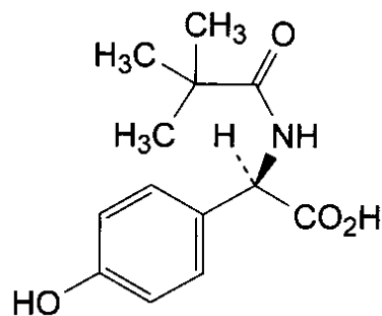
E. R = H: (2RS,4S)-2-[[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of amoxicillin),



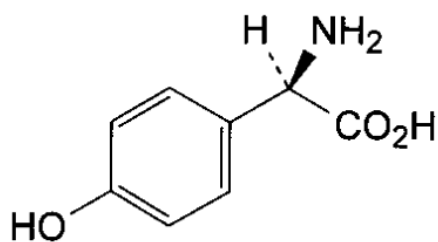
F. 3-(4-hydroxyphenyl)pyrazin-2-ol,



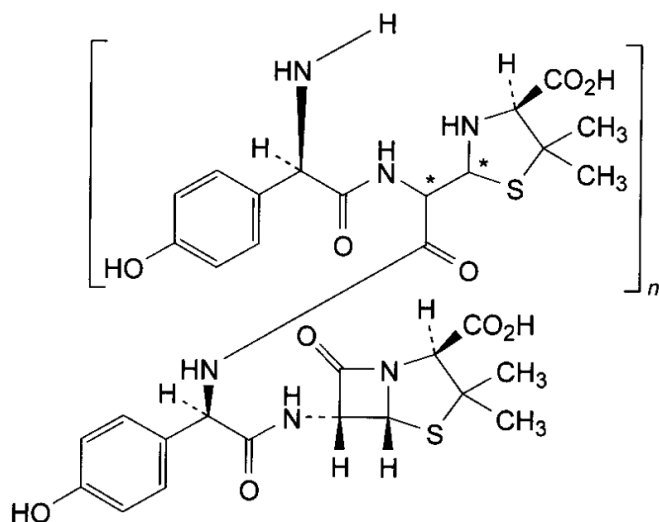
G. (2S,5R,6R)-6-[[[(2R)-2-[[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (D-(4-hydroxyphenyl)glycylamoxicillin),



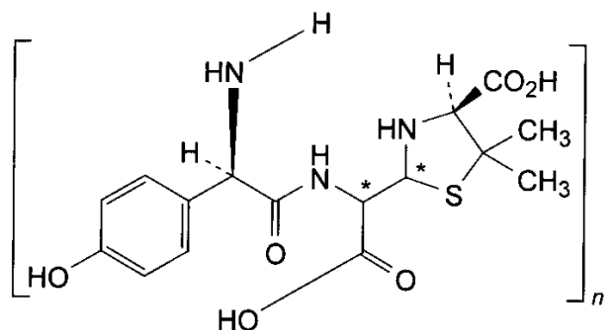
H. (2R)-2-[(2,2-dimethylpropanoyl)amino]-2-(4-hydroxyphenyl)acetic acid,



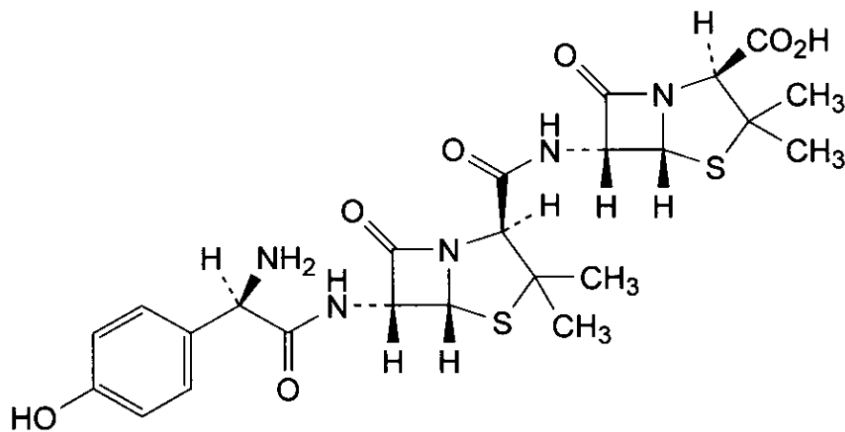
I. (2R)-2-amino-2-(4-hydroxyphenyl)acetic acid,



J. co-oligomers of amoxicillin and of penicilloic acids of amoxicillin,



K. oligomers of penicilloic acids of amoxicillin,



L. (2*S*,5*R*,6*R*)-6-[[[(2*S*,5*R*,6*R*)-6-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-APA amoxicillin amide).

Ph Eur

Action and use

Antibacterial.

Preparations

Amoxicillin Capsules

Amoxicillin Oral Suspension

Co-amoxiclav Tablets