E. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S. Dissolve 5.0 g in *alcohol (50 per cent V/V) R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

pH (*2.2.3*). Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent. Disregard any slight residue. The pH of the solution is not more than 11.0.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution. Dissolve 1.0 g of the substance to be examined in *alcohol R* and dilute to 100 ml with the same solvent.

Reference solution. Dilute 0.5 ml of the test solution to 100 ml with *alcohol R*.

Apply separately to the plate 20 µl of each solution. Develop over a path of 15 cm using the lower layer of a mixture of 5 volumes of *concentrated ammonia R*, 15 volumes of *alcohol R* and 80 volumes of *chloroform R*. Examine the plate immediately in ultraviolet light at 254 nm. Spray with *diphenylcarbazone mercuric reagent R*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution R* diluted 1 in 5 with *aldehyde-free alcohol R*. Heat at 100 °C to 105 °C for 5 min and examine immediately. When examined in ultraviolet light and after spraying, any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent). Disregard any spot at the starting-point.

Loss on drying (*2.2.32*). Not more than 3.0 per cent, determined on 0.50 g by drying in an oven at 130 °C.

ASSAY

Dissolve 0.200 g in 5 ml of *ethanol R*. Add 0.5 ml of *thymolphthalein solution R* and 10 ml of *silver nitrate solution in pyridine R*. Titrate with *0.1 M ethanolic sodium hydroxide* until a pure blue colour is obtained. Carry out a blank titration.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 24.83 mg of $C_{11}H_{17}N_2NaO_3$.

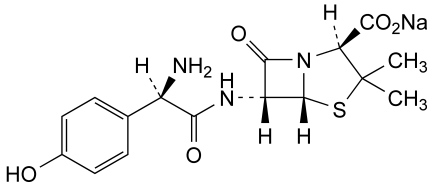
STORAGE

Store in an airtight container.

01/2008:0577
corrected 6.0

AMOXICILLIN SODIUM

Amoxicillinum natricum



$C_{16}H_{18}N_3NaO_5S$ M_r 387.4
[34642-77-8]

DEFINITION

Sodium (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-carboxylate.

Semi-synthetic product derived from a fermentation product.

Content: 89.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, very hygroscopic, powder.

Solubility: very soluble in water, sparingly soluble in anhydrous ethanol, very slightly soluble in acetone.

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: dissolve 0.250 g in 5 ml of *water R*, add 0.5 ml of *dilute acetic acid R*, swirl and allow to stand for 10 min in iced water. Filter the crystals and wash with 2-3 ml of a mixture of 1 volume of *water R* and 9 volumes of *acetone R*, then dry in an oven at 60 °C for 30 min.

Comparison: *amoxicillin trihydrate CRS*.

B. Thin-layer chromatography (*2.2.27*).

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*.

Plate: *TLC silanised silica gel plate R*.

Mobile phase: mix 10 volumes of *acetone R* and 90 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 the chromatogram obtained with reference solution (a).

C. Place about 2 mg in a test-tube about 150 mm long and about 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 gives reaction (a) of sodium (*2.3.1*).

TESTS

Appearance of solution. The solution is not more opalescent than reference suspension II (*2.2.1*), it may show an initial, but transient, pink colour, and after 5 min, its absorbance (*2.2.25*) at 430 nm is not greater than 0.20.

Dissolve 1.0 g in *water R* and dilute to 10.0 ml with the same solvent. Examine immediately after dissolution.

pH (*2.2.3*): 8.0 to 10.0.

Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Specific optical rotation (*2.2.7*): + 240 to + 290 (anhydrous substance).

Dissolve 62.5 mg in a 4 g/l solution of *potassium hydrogen phthalate R* and dilute to 25.0 ml with the same solution.

Related substances. 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 mobile phase A.

Test solution (b). Dissolve 30.0 mg of the substance to be examined in mobile phase A and dilute to 20.0 ml with mobile phase A. *Prepare immediately before use.*

Reference solution (a). Dissolve 30.0 mg of *amoxicillin trihydrate CRS* in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (b). Dissolve 4.0 mg of *cefadroxil CRS* in mobile phase A and dilute to 50 ml with mobile phase A. 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 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.

Reference solution (d). To 0.20 g of *amoxicillin trihydrate R* add 1.0 ml of *water R*. Shake and add dropwise *dilute sodium hydroxide solution R* to obtain a solution. The pH of the solution is about 8.5. Store the solution at room temperature for 4 h. Dilute 0.5 ml of this solution to 50.0 ml with mobile phase A.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: mix 1 volume of *acetonitrile R* and 99 volumes of a 25 per cent *V/V* solution of *0.2 M potassium dihydrogen phosphate R* adjusted to pH 5.0 with *dilute sodium hydroxide solution R*;
– *mobile phase B*: mix 20 volumes of *acetonitrile R* and 80 volumes of a 25 per cent *V/V* solution of *0.2 M potassium dihydrogen phosphate R* adjusted to pH 5.0 with *dilute sodium hydroxide solution R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	92	8
t_R - (t_R + 25)	92 → 0	8 → 100
(t_R + 25) - (t_R + 40)	0	100
(t_R + 40) - (t_R + 55)	92	8

t_R = retention time of amoxicillin determined with reference solution (c)

If the mobile phase has been adjusted to achieve the required resolution, the adjusted composition will apply at time zero in the gradient and in the assay.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 50 µl of reference solutions (b) and (c) with isocratic elution at the initial mobile phase composition and 50 µl of test solution (b) and reference solution (d) according to the elution gradient described under Mobile phase; inject mobile phase A as a blank according to the elution gradient described under Mobile phase.

Identification of impurities: use the chromatogram obtained with reference solution (d) to identify the 3 principal peaks eluted after the main peak corresponding to impurity C, amoxicillin dimer (impurity J; *n* = 1) and amoxicillin trimer (impurity J; *n* = 2).

Relative retention with reference to amoxicillin: impurity C = about 3.4; impurity J (*n* = 1) = about 4.1; impurity J (*n* = 2) = about 4.5.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to amoxicillin and cefadroxil; if necessary, adjust the ratio A:B of the mobile phase.

Limits:

– *impurity J* (*n* = 1): not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (3 per cent);
– *any other impurity*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2 per cent);
– *total*: not more than 9 times the area of the principal peak in the chromatogram obtained with reference solution (c) (9 per cent);
– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

***N*,*N*-Dimethylaniline** (*2.4.26, Method A or B*): maximum 20 ppm.

2-Ethylhexanoic acid (*2.4.28*): maximum 0.8 per cent *m/m*.

Heavy metals (*2.4.8*): maximum 20 ppm.

1.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 3.0 per cent, determined on 0.400 g.

Bacterial endotoxins (*2.6.14*): less than 0.25 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

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

Mobile phase: initial composition of the mixture of mobile phases A and B, adjusted where applicable.

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

System suitability: reference solution (a):

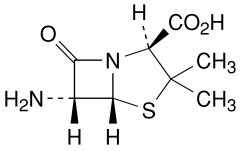
– *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of amoxicillin sodium by multiplying the percentage content of amoxicillin by 1.060.

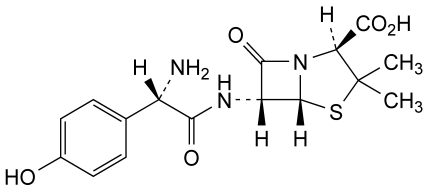
STORAGE

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

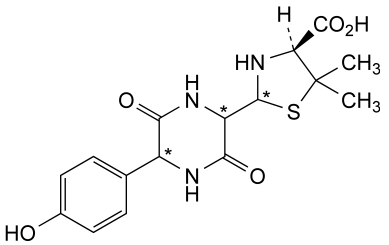
IMPURITIES



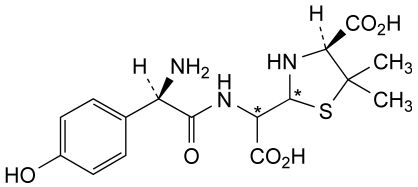
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



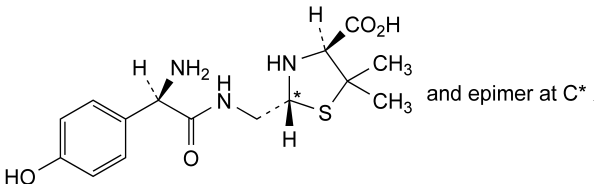
B. (2*S*,5*R*,6*R*)-6-[[(2*S*)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid (L-amoxicillin),



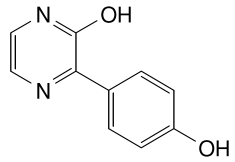
C. (4*S*)-2-[5-(4-hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid (amoxicillin diketopiperazines),



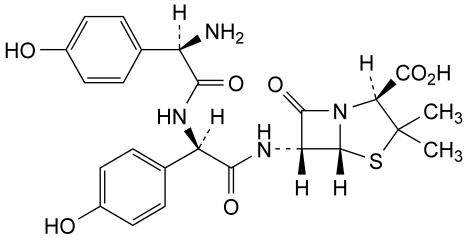
D. (4*S*)-2-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin),



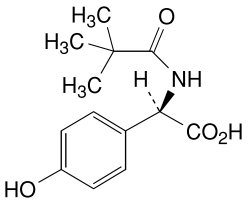
E. (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]-amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of amoxicillin),



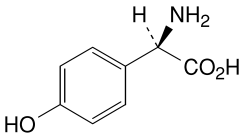
F. 3-(4-hydroxyphenyl)pyrazin-2-ol,



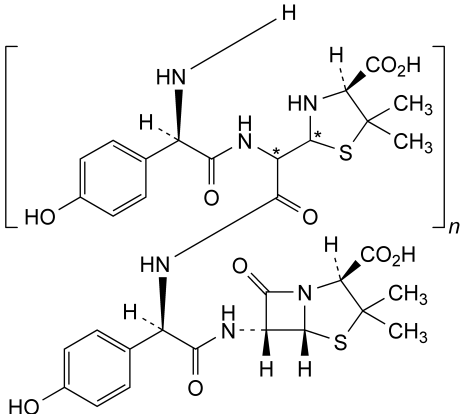
G. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(2*R*)-2-amino-2-(4-hydroxy-phenyl)acetyl]amino]2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (D-(4-hydroxyphenyl)glycylamoxicillin),



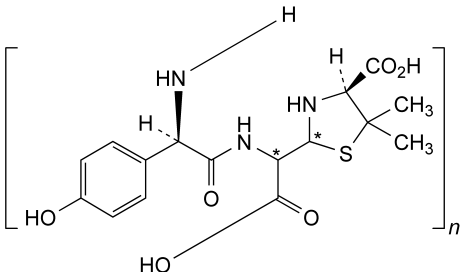
H. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-(4-hydroxyphenyl)acetic acid,



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



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

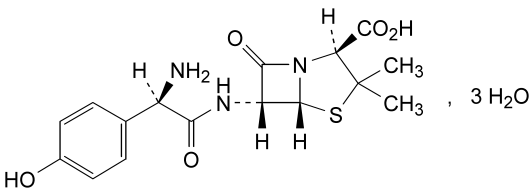


K. oligomers of penicilloic acids of amoxicillin.

01/2008:0260
corrected 6.0

AMOXICILLIN TRIHYDRATE

Amoxicillinum trihydricum



$C_{16}H_{19}N_3O_5S,3H_2O$ M_r 419.4
[61336-70-7]