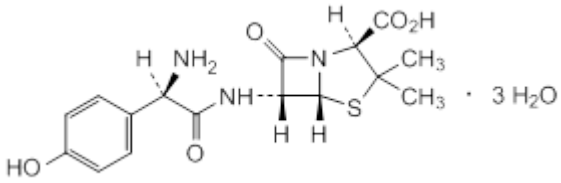
Amoxicillin trihydrate (Amoxicillinum trihydricum)

2018-01

Molecular formula. $C_{16}H_{19}N_3O_5S.3H_2O$

Relative molecular mass. 419.45

Graphic formula



Chemical names. (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate (*IUPAC*); 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-, hydrate (1:3), (2*S*,5*R*,6*R*) (*CAS*); CAS Reg. No. 61336-70-7.

Description. A white or almost white, crystalline powder.

Solubility. Slightly soluble in water and methanol R; very slightly soluble in ethanol (~750 g/L) TS, practically insoluble in fatty oils.

Category. Antibacterial.

Storage. Amoxicillin trihydrate should be kept in a tightly closed container.

Additional information. Amoxicillin trihydrate is a semi-synthetic product derived from a fermentation product.

Requirements

Definition. Amoxicillin trihydrate contains not less than 95.0% and not more than 102.0% of $C_{16}H_{19}N_3O_5S$, calculated with reference to the anhydrous substance.

Manufacture. The method of production is validated to demonstrate that the substance, if tested, would comply with a limit of not more than 20 µg/g of *N,N*-dimethylaniline using a suitable method.

Identity tests

-Either test A alone or any two of tests B, C and D may be applied.

A. Carry out the examination as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum is concordant with the spectrum obtained from amoxicillin trihydrate RS or with the *reference spectrum* of amoxicillin trihydrate.

B. Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using silanized silica gel R3 as the coating substance and a mixture of 9 volumes of a solution containing 15.4 g of ammonium acetate R in 100 mL, the pH of which has been adjusted to 5.0 with glacial acetic acid R, and 1 volume of acetone R as the mobile phase. Apply separately to the plate 1 µL of each of 3 solutions in sodium hydrogen carbonate (40 g/L) TS containing (A) 2.5 mg of the test substance per mL, (B) 2.5 mg of amoxicillin trihydrate RS per mL and (C) a mixture of 2.5 mg of amoxicillin trihydrate RS and 2.5 mg of ampicillin RS per mL. After removing the plate from the chromatographic chamber allow it to dry in air until the solvents are evaporated. Expose the plate to iodine vapours until the spots appear and examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B). The test is valid only if the chromatogram obtained with solution (C) shows two clearly separated spots.

C. Place about 2 mg of the test substance in a test-tube (150 mm × 15 mm), moisten it with 1 drop of water and add about 2 mL of sulfuric acid (~1760 g/L) TS. Mix the contents of the tube by swirling; the solution remains practically colourless. Place the tube in a water-bath for 1 minute; a dark yellow colour develops.

D. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using the conditions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

Specific optical rotation (*1.4*). Use a solution containing 2.0 mg of the test substance per mL of water R and calculate with reference to the anhydrous substance; $[\alpha]_D^{20}$ = +290 to +315.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under *2.2.3 Limit test for heavy metals*, Procedure 3; determine the heavy metals content according to method A; not more than 20 µg/g.

Sulfated ash (*2.3*). Not more than 10 mg/g.

Water. Determine as described under *2.8 Determination of water by the Karl Fischer method*, Method A, using 0.100 g of the test substance; the water content is not less than 0.115 g/g and not more than 0.145 g/g.

pH value (*1.13*). pH of a solution containing 2mg of the test substance per mL of carbon-dioxide-free water R, 3.5–5.5.

Related substances. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).

Prepare the following buffer solution pH 5.0. Adjust the pH of 250 mL of potassium dihydrogen phosphate (27.2 g/L) TS with sodium hydroxide (~80 g/L) TS to 5.0 and dilute to 1000 mL with water R.

Use the following conditions for gradient elution:

mobile phase A: 1 volume of acetonitrile R and 99 volumes of buffer solution pH 5.0;

mobile phase B: 20 volumes of acetonitrile R and 80 volumes of buffer solution pH 5.0.

Time (minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-t_R	92	8	Isocratic
t_R–(t_R+25)	92 to 0	8 to 100	Linear gradient
(t_R+25)–t_R-(t_R+40)	0	100	Isocratic
(t_R+40)–t_R-(t_R+55)	0 to 92	100 to 8	Return to initial composition
(t_R+55)–t_R-(t_R+70)	92	8	Re-equilibration

t_R = retention time of amoxicillin determined with solution (2)

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Prepare the following solutions immediately before use in mobile phase A. For solution (1) dissolve about 30 mg of the test substance and dilute to 20.0 mL. For solution (2) dilute 2.0 mL of solution (1) to 20.0 mL. Dilute 2.0 mL of this solution to 20.0 mL. For solution (3) dissolve 4.0 mg of cefadroxil R and dilute to 50.0 mL. To 5.0 mL of this solution add 2.0 mL of solution (1) and dilute to 100.0 mL.

Inject 50 µL of solution (3).The test assay is not valid unless in the chromatogram the resolution between the peaks due to amoxicillin and cefadroxil is at least 2.0. Cefadroxil is eluted with a relative retention of about 1.3 with reference to amoxicillin (retention time about 10 minutes).

Inject alternately 50 µL each of solution (1) and (2).

In the chromatogram obtained with solution (1):

-the area of any impurity peak is not greater than the area of the peak due to amoxicillin in the chromatogram obtained with solution (2) (1.0%).

Assay. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using the conditions given under “Related substances”, with the following modifications:

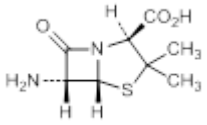
Perform an isocratic solution and use as the mobile phase the initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Prepare the following solutions immediately before use in mobile phase. For solution (1) dissolve 30.0 mg of the test substance and dilute to 50.0 mL. For solution (2) dissolve 30.0 mg of amoxicillin trihydrate RS and dilute to 50.0 mL.

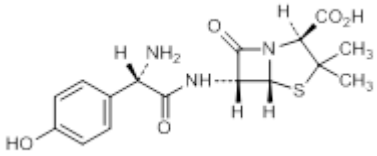
Inject alternately 50 µL each of solution (1) and (2) and record the chromatograms.

Measure the areas of the peaks corresponding to amoxicillin obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of amoxicillin ($C_{16}H_{19}N_3O_5S$), using the declared content of amoxicillin ($C_{16}H_{19}N_3O_5S$) in amoxicillin trihydrate RS.

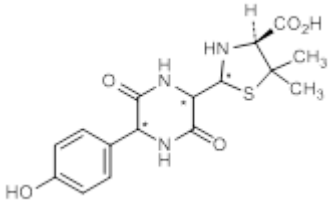
Impurities



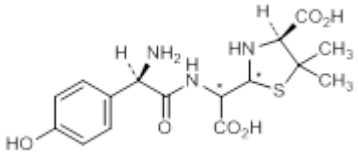
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid) (synthesis-related impurity),



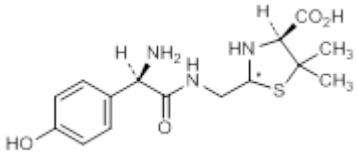
B. (2*S*,5*R*,6*R*)-6-[(2*S*)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (l-amoxicillin) (synthesis-related impurity, degradation product),



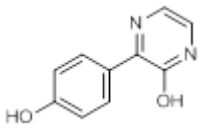
C. (4*S*)-2-[5-(4-hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (amoxicillin diketopiperazine) (degradation products),



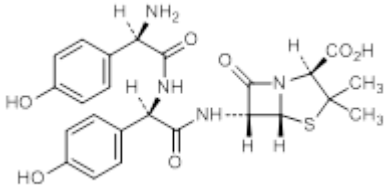
D. (4*S*)-2-{[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl}-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin) (degradation products),



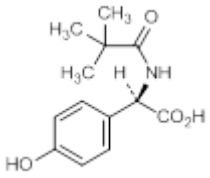
E. (2*RS*,4*S*)-2-{[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]methyl}-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin) (degradation products),



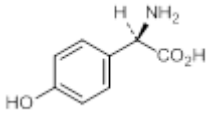
F. 3-(4-hydroxyphenyl)pyrazin-2-ol (synthesis-related impurity),



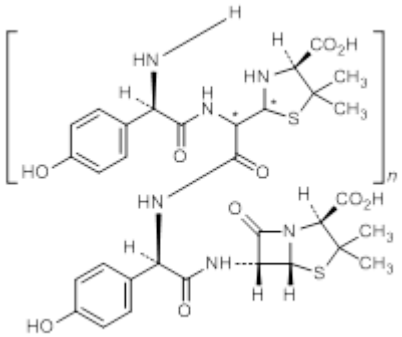
G. (2*S*,5*R*,6*R*)-6-{(2*R*)-2-[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (d-(4-hydroxyphenyl)glycylamoxicillin) (synthesis-related impurity),



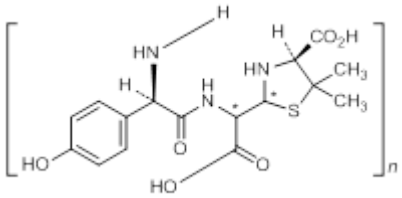
H. (2*R*)-2-(2,2-dimethylpropanamido)-2-(4-hydroxyphenyl)acetic acid (synthesis related impurity),



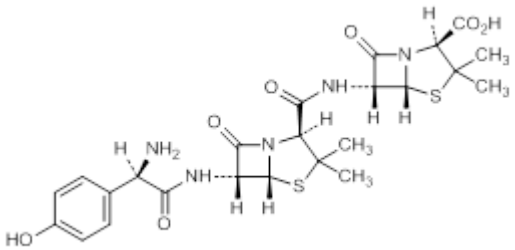
I. (2*R*)-2-(4-hydroxyphenyl)acetic acid (synthesis related impurity),



J. co-oligomers of amoxicillin and penicilloic acids of amoxicillin (degradation products),



K. oligomers of penicilloic acids of amoxicillin (degradation products),



L. (2*S*,5*R*,6*R*)-6-({(2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetamido]-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).