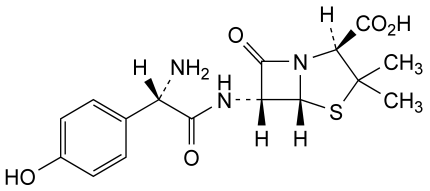
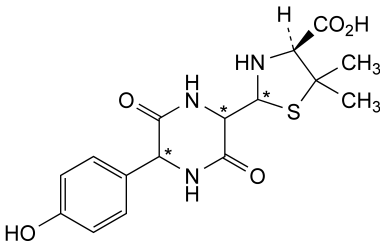
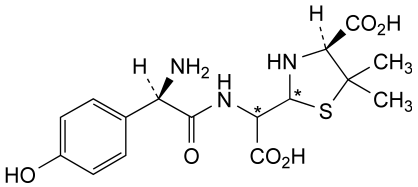


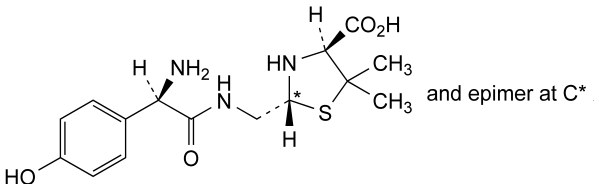
B. (2*S*,5*R*,6*R*)-6-[[(2*S*)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid (L-amoxicillin),



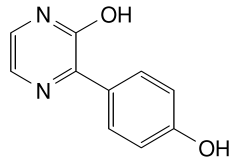
C. (4*S*)-2-[5-(4-hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid (amoxicillin diketopiperazines),



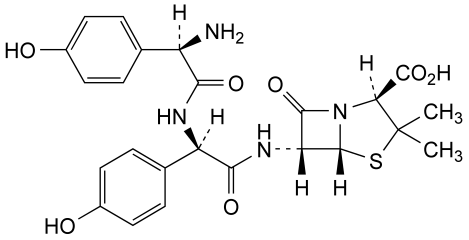
D. (4*S*)-2-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin),



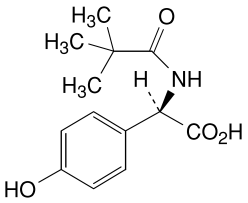
E. (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]-amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of amoxicillin),



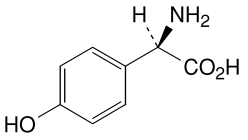
F. 3-(4-hydroxyphenyl)pyrazin-2-ol,



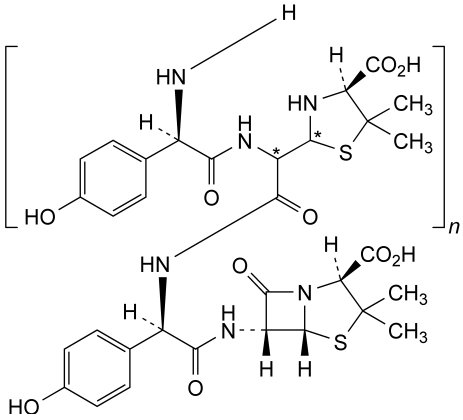
G. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(2*R*)-2-amino-2-(4-hydroxy-phenyl)acetyl]amino]2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (D-(4-hydroxyphenyl)glycylamoxicillin),



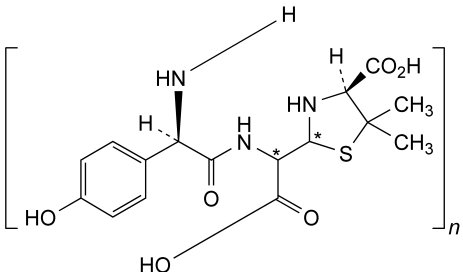
H. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-(4-hydroxyphenyl)acetic acid,



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



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

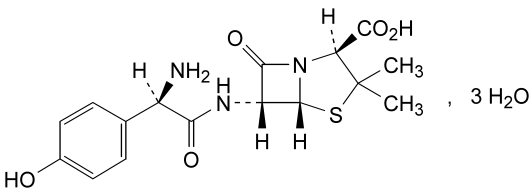


K. oligomers of penicilloic acids of amoxicillin.

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]

DEFINITION

(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-carboxylic acid trihydrate.

Semi-synthetic product derived from a fermentation product.

Content: 95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, very slightly soluble in ethanol (96 per cent), practically insoluble in fatty oils. It dissolves in dilute acids and dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

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.

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. The solutions are not more opalescent than reference suspension II (*2.2.1*).

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.* Examine immediately after dissolution.

pH (*2.2.3*): 3.5 to 5.5 for solution S.

Specific optical rotation (*2.2.7*): + 290 to + 315 (anhydrous substance), determined on solution S.

Related substances. Liquid chromatography (*2.2.29*).

Buffer solution pH 5.0. 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.*

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.

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*: *acetonitrile R,* buffer solution pH 5.0 (1:99 *V/V*);

– *mobile phase B*: *acetonitrile R,* buffer solution pH 5.0 (20:80 *V/V*);

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 composition 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) 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.

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.

Limit:

– *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1 per cent).

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

Water (*2.5.12*): 11.5 per cent to 14.5 per cent, determined on 0.100 g.

Sulphated ash (*2.4.14*): maximum 1.0 per cent, determined on 1.0 g.

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):

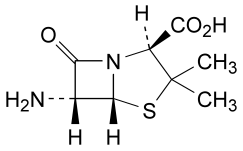
– *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of $C_{16}H_{19}N_3O_5S$ from the declared content of *amoxicillin trihydrate CRS*.

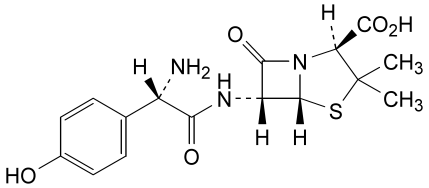
STORAGE

In an airtight container.

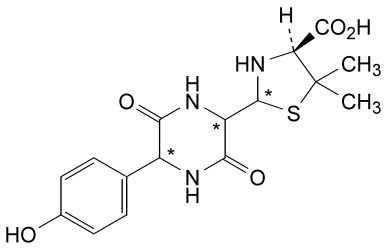
IMPURITIES



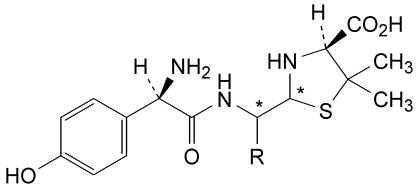
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



B. (2*S*,5*R*,6*R*)-6-[[(2*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),

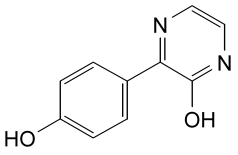


C. (4*S*)-2-[5-(4-hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid (amoxicillin diketopiperazines),

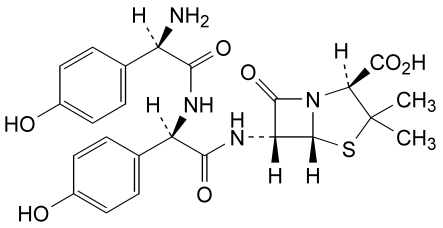


D. R = CO_2H: (4S)-2-[[[(2R)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin),

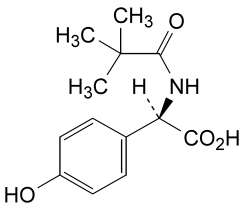
E. R = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-(4-hydroxy-phenyl)acetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of amoxicillin),



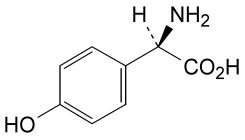
F. 3-(4-hydroxyphenyl)pyrazin-2-ol,



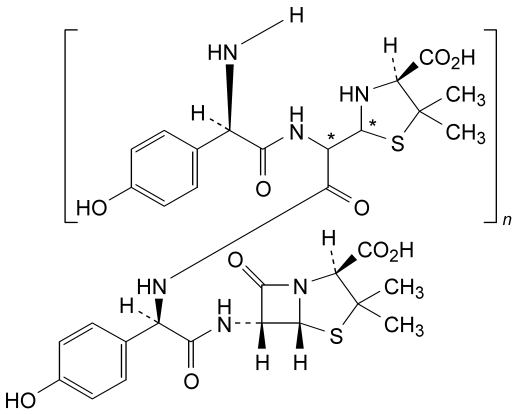
G. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(2*R*)-2-amino-2-(4-hydroxy-phenyl)acetyl]amino]-2-(4-hydroxyphenyl)acetyl]-amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid (D-(4-hydroxyphenyl)glycylamoxicillin),



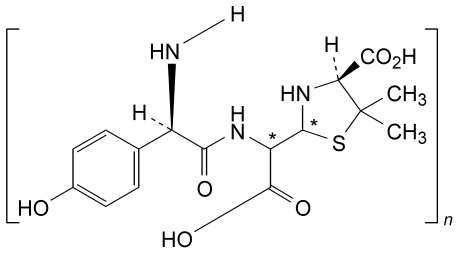
H. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-(4-hydroxyphenyl)acetic acid,



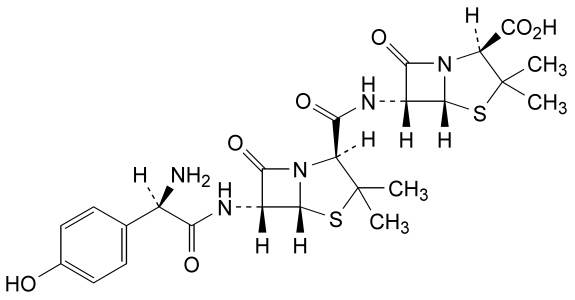
I. (2*R*)-2-amino-2-(4-hydroxyphenyl)acetic acid,



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



K. oligomers of penicilloic acids of amoxicillin,

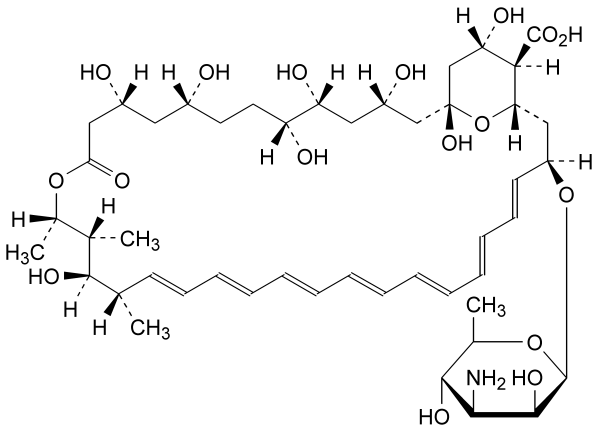


L. (2*S*,5*R*,6*R*)-6-[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-APA amoxicillin amide).

01/2008:1292

AMPHOTERICIN B

Amphotericinum B



$C_{47}H_{73}NO_{17}$ M_r 924

DEFINITION

Amphotericin B is a mixture of antifungal polyenes produced by the growth of certain strains of *Streptomyces nodosus* or by any other means.
It consists mainly of amphotericin B which is (1*R*,3*S*,5*R*,6*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,23*E*, 25*E*, 27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-33-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16, 18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid. The potency is not less than 750 IU/mg, calculated with reference to the dried substance.

CHARACTERS

A yellow or orange powder, practically insoluble in water, soluble in dimethyl sulphoxide and in propylene glycol, slightly soluble in dimethylformamide, very slightly soluble in methanol, practically insoluble in alcohol.

It is sensitive to light in dilute solutions and is inactivated at low pH values.

IDENTIFICATION

A. Dissolve 25 mg in 5 ml of *dimethyl sulphoxide R* and dilute to 50 ml with *methanol R*. Dilute 2 ml of the solution to 200 ml with *methanol R*. Examined between 300 nm and 450 nm (*2.2.25*), the solution shows 3 absorption maxima at 362 nm, 381 nm and 405 nm. The ratio of the absorbance measured at 362 nm to that measured at 381 nm is 0.57 to 0.61. The ratio of the absorbance measured at 381 nm to that measured at 405 nm is 0.87 to 0.93.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *amphotericin B CRS*. If the spectra obtained show differences dry the substance to be examined at 60 °C at a pressure not exceeding 0.7 kPa for 1 h and prepare a new spectrum.

C. To 1 ml of a 0.5 g/l solution in *dimethyl sulphoxide R*, add 5 ml of *phosphoric acid R* to form a lower layer, avoiding mixing the 2 liquids. A blue ring is immediately produced at the junction of the liquids. Mix, an intense blue colour is produced. Add 15 ml of *water R* and mix; the solution becomes pale yellow.

TESTS

Content of tetraenes. Not more than 10.0 per cent and not more than 5.0 per cent if intended for use in the manufacture of parenteral dosage forms, determined by the following method.

Test solution. Dissolve 50.0 mg in 5 ml of *dimethyl sulphoxide R* and dilute to 50.0 ml with *methanol R*. Dilute 4.0 ml of the solution to 50.0 ml with *methanol R*.

Reference solution (a). Dissolve 50.0 mg of *amphotericin B CRS* in 5 ml of *dimethyl sulphoxide R* and dilute to 50.0 ml with *methanol R*. Dilute 4.0 ml of the solution to 50.0 ml with *methanol R*.

Reference solution (b). Dissolve 25.0 mg of *nystatin CRS* in 25 ml of *dimethyl sulphoxide R* and dilute to 250.0 ml with *methanol R*. Dilute 4.0 ml of the solution to 50.0 ml with *methanol R*.

Measure the absorbances (*2.2.25*) of the test solution and of reference solutions (a) and (b) at the maxima at 282 nm and 304 nm respectively, using a 0.8 per cent *V/V* solution of *dimethyl sulphoxide R* in *methanol R* as the compensation liquid. Calculate the specific absorbance of the substance being examined, of *nystatin CRS* and of *amphotericin B CRS* at both wavelengths, each with reference to the dried substance, and calculate the percentage content of tetraenes using the following expression:

$$F + \frac{100\,(B_1S_2 - B_2S_1)}{(N_2B_1 - N_1B_2)}$$

S_1 and S_2 = specific absorbances of the substance to be examined at 282 nm and 304 nm respectively,

N_1 and N_2 = specific absorbance of *nystatin CRS* at 282 nm and 304 nm respectively,

B_1 and B_2 = specific absorbance of *amphotericin B CRS* at 282 nm and 304 nm respectively,

F = declared content of tetraenes in *amphotericin B CRS*.

Loss on drying (*2.2.32*). Not more than 5.0 per cent, determined on 1.000 g by drying in an oven at 60 °C at a pressure not exceeding 0.7 kPa.

Sulphated ash (*2.4.14*). Not more than 3.0 per cent and not more than 0.5 per cent if intended for use in the manufacture of parenteral dosage forms, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*): less than 1.0 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.