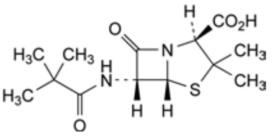
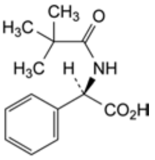
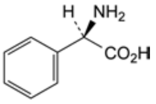


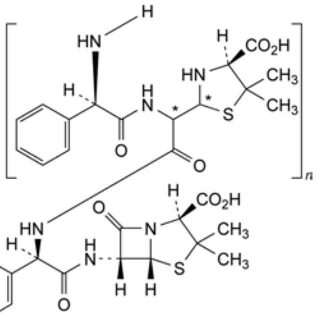
J. (2*S*,5*R*,6*R*)-6-[(2,2-dimethylpropanoyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



K. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-phenylacetic acid,



L. (2*R*)-2-amino-2-phenylacetic acid (D-phenylglycine),

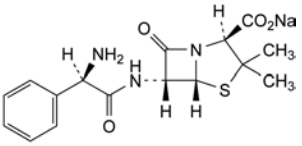


M. co-oligomers of ampicillin and of penicilloic acids of ampicillin.

01/2008:0578
corrected 6.0

AMPICILLIN SODIUM

Ampicillinum natricum



$C_{16}H_{18}N_3NaO_4S$ M_r 371.4
[69-52-3]

DEFINITION

Sodium (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]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: 91.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder, hygroscopic.

Solubility: freely soluble in water, sparingly soluble in acetone, practically insoluble in fatty oils and in liquid paraffin.

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 through a small sintered-glass filter (40) (*2.1.2*), applying suction, 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: *ampicillin 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 *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*.

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 *sulfuric 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. Solutions A and B are not more opalescent than reference suspension II (*2.2.1*) and the absorbance (*2.2.25*) of solution B at 430 nm is not greater than 0.15.

Place 1.0 g in a conical flask and add slowly and with continuous swirling 10 mL of *1 M hydrochloric acid* (solution A). Separately dissolve 1.0 g in *water R* and dilute to 10.0 mL with the same solvent (solution B). 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. Measure 10 min after dissolution.

Specific optical rotation (*2.2.7*): + 258 to + 287 (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 solvent.

Related substances. 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 mobile phase A.

Test solution (b). Dissolve 31.0 mg of the substance to be examined in mobile phase A and dilute to 10.0 mL with mobile phase A. *Prepare immediately before use.*

Reference solution (a). Dissolve 27.0 mg of *anhydrous ampicillin CRS* in mobile phase A and dilute to 50.0 mL with mobile phase A.

Reference solution (b). Dissolve 2.0 mg of *cefradine 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).

Reference solution (c). Dilute 1.0 mL of reference solution (a) to 20.0 mL with mobile phase A.

Reference solution (d). To 0.20 g of the substance to be examined add 1.0 mL of *water R*. Heat the solution at 60 °C for 1 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 0.5 mL of *dilute acetic acid R*, 50 mL of *0.2 M potassium dihydrogen phosphate R* and 50 mL of *acetonitrile R*, then dilute to 1000 mL with *water R*;
- *mobile phase B*: mix 0.5 mL of *dilute acetic acid R*, 50 mL of *0.2 M potassium dihydrogen phosphate R* and 400 mL of *acetonitrile R*, then dilute to 1000 mL with *water R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	85	15
t_R - (t_R + 30)	85 → 0	15 → 100
(t_R + 30) - (t_R + 45)	0	100
(t_R + 45) - (t_R + 60)	85	15

t_R = retention time of ampicillin 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) 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 peaks: use the chromatogram obtained with reference solution (d) to identify the peaks due to ampicillin and ampicillin dimer.

Relative retention with reference to ampicillin: ampicillin dimer = about 2.8.

System suitability: reference solution (b):

- *resolution*: minimum 3.0 between the peaks due to ampicillin and cefradin; if necessary adjust the ratio A:B of the mobile phase.

Limits:

- *ampicillin dimer*: not more than 4.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (4.5 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).

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

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

Methylene chloride. Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 1.0 mL of *ethylene chloride R* in *water R* and dilute to 500.0 mL with the same solvent.

Test solution (a). Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 10.0 mL with the same solvent.

Test solution (b). Dissolve 1.0 g of the substance to be examined in *water R*, add 1.0 mL of the internal standard solution and dilute to 10.0 mL with *water R*.

Reference solution. Dissolve 1.0 mL of *methylene chloride R* in *water R* and dilute to 500.0 mL with the same solvent. To 1.0 mL of this solution add 1.0 mL of the internal standard solution and dilute to 10.0 mL with *water R*.

Column:

- *material*: glass;
- *size*: l = 1.5 m, Ø = 4 mm;
- *stationary phase*: *diatomaceous earth for gas chromatography R* impregnated with 10 per cent *m/m* of *macrogol 1000 R*.

Carrier gas: *nitrogen for chromatography R*.

Flow rate: 40 mL/min.

Temperature:

- *column*: 60 °C;
- *injection port*: 100 °C;
- *detector*: 150 °C.

Detection: flame ionisation.

Calculate the content of methylene chloride taking its density at 20 °C to be 1.325 g/mL.

Limit:

- *methylene chloride*: maximum 0.2 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 2.0 per cent, determined on 0.300 g.

Bacterial endotoxins (*2.6.14*): less than 0.15 IU/mg, if intended for use in the manufacture of parenteral preparations 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):

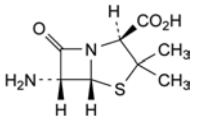
- *repeatability*: maximum relative standard deviation of 1.0 per cent after 6 injections.

Calculate the percentage content of ampicillin sodium by multiplying the percentage content of ampicillin by 1.063.

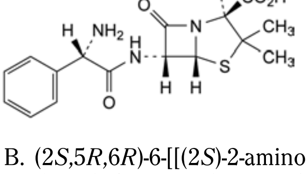
STORAGE

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

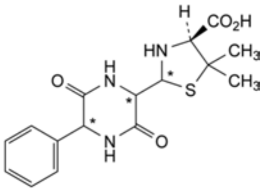
IMPURITIES



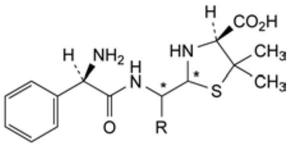
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



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

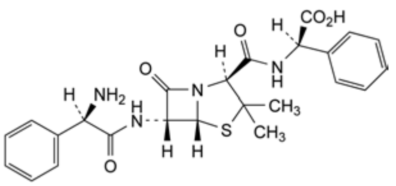


C. (4*S*)-2-(3,6-dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid (diketopiperazines of ampicillin),

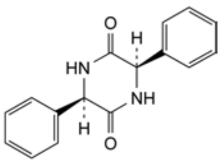


D. R = CO_2H: (4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of ampicillin),

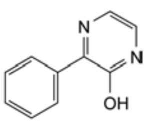
F. R = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-amino-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of ampicillin),



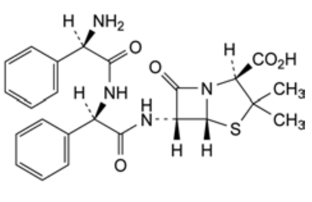
E. (2*R*)-2-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-2-phenylacetic acid (ampicillinyl-D-phenylglycine),



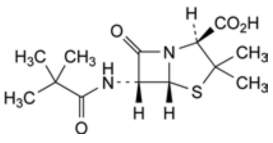
G. (3*R*,6*R*)-3,6-diphenylpiperazine-2,5-dione,



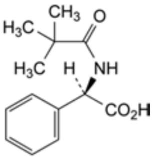
H. 3-phenylpyrazin-2-ol,



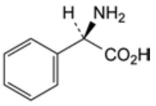
I. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(2*R*)-2-amino-2-phenylacetyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (D-phenylglycylampicillin),



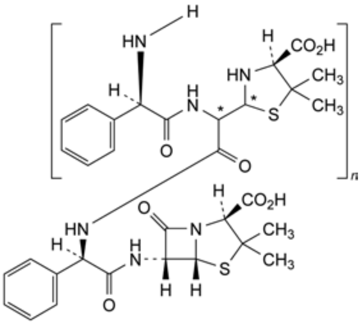
J. (2*S*,5*R*,6*R*)-6-[(2,2-dimethylpropanoyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



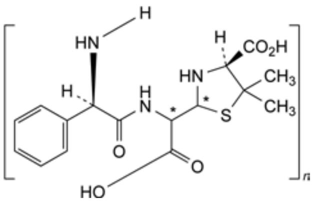
K. (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-phenylacetic acid,



L. (2*R*)-2-amino-2-phenylacetic acid (D-phenylglycine),



M. co-oligomers of ampicillin and of penicilloic acids of ampicillin,

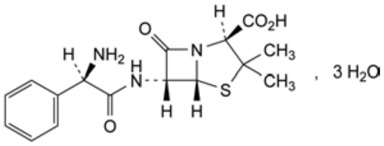


N. oligomers of penicilloic acids of ampicillin.

01/2008:0168
corrected 6.0

AMPICILLIN TRIHYDRATE

Ampicillinum trihydricum



$C_{16}H_{19}N_3O_4S,3H_2O$ M_r 403.5
[7177-48-2]

DEFINITION

(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-Amino-2-phenylacetyl]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: 96.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, practically insoluble in ethanol (96 per cent) 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. Infrared absorption spectrophotometry (*2.2.24*).