Injection: 20 µl of reference solutions (b), (c) and (d) with isocratic elution at the initial mobile phase composition and 20 µl of test solution (b) according to the elution gradient described under Mobile phase.

System suitability:

– *resolution*: minimum 10 between the peaks due to impurity A and piperacillin in the chromatogram obtained with reference solution (c); if necessary, adjust the ratio A:B of the mobile phase;

– *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (d);

– *mass distribution ratio*: 2.0 to 3.0 for the peak due to piperacillin in the chromatogram obtained with reference solution (c).

Limit:

– *any impurity*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent).

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

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*): 2.0 per cent to 4.0 per cent, determined on 0.500 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.

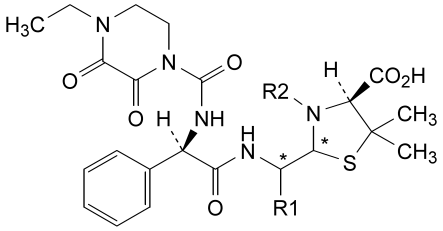
Injection: test solution (a) and reference solution (a).

System suitability: reference solution (a):

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

IMPURITIES

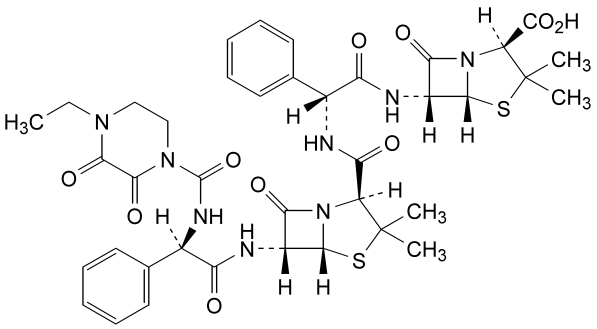
A. ampicillin,



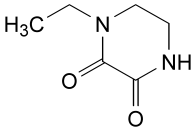
B. R1 = CO_2H, R2 = H: (4*S*)-2-[carboxy[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of piperacillin),

C. R1 = R2 = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of piperacillin),

F. R1 = CO_2H, R2 = CO-CH_3: (4*S*)-3-acetyl-2-[carboxy[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (acetylated penicilloic acids of piperacillin),



D. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[[(2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (piperacillinylampicillin),

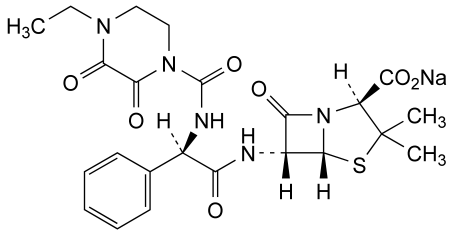


E. 1-ethylpiperazine-2,3-dione.

01/2008:1168
corrected 6.0

PIPERACILLIN SODIUM

Piperacillinum natricum



$C_{23}H_{26}N_5NaO_7S$ M_r 539.5
[59703-84-3]

DEFINITION

Sodium (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]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: 95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, hygroscopic powder.

Solubility: freely soluble in water and in methanol, practically insoluble in ethyl acetate.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: dissolve 0.250 g in *water R*, add 0.5 ml of *dilute hydrochloric acid R* and 5 ml of *ethyl acetate R*; stir and allow to stand for 10 min in iced water. Filter the

crystals through a small sintered-glass filter (40), applying suction. Wash with 5 ml of *water R* and 5 ml of *ethyl acetate R*, then dry in an oven at 60 °C for 60 min.

Comparison: *piperacillin CRS*.

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

TESTS

Solution S. Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 430 nm is not greater than 0.10.

pH (*2.2.3*): 5.0 to 7.0 for solution S.

Specific optical rotation (*2.2.7*): + 175 to + 190 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *acetonitrile R*, 31.2 g/l solution of *sodium dihydrogen phosphate R* (25:75 *V/V*).

Test solution (a). Dissolve 25.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Test solution (b). Prepare the solution immediately before use. Dissolve 40.0 mg of the substance to be examined in the solvent mixture and dilute to 20.0 ml with the solvent mixture.

Reference solution (a). Dissolve 25.0 mg of *piperacillin CRS* in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 25.0 ml with the solvent mixture.

Reference solution (c). Dissolve 10.0 mg of *piperacillin CRS* and 10.0 mg of *anhydrous ampicillin CRS* (impurity A) in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (d). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 50.0 ml with the solvent mixture.

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 576 ml of *water R*, 200 ml of a 31.2 g/l solution of *sodium dihydrogen phosphate R* and 24 ml of an 80 g/l solution of *tetrabutylammonium hydroxide R*; if necessary, adjust to pH 5.5 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*; add 200 ml of *acetonitrile R*;
– *mobile phase B*: mix 126 ml of *water R*, 200 ml of a 31.2 g/l solution of *sodium dihydrogen phosphate R* and 24 ml of an 80 g/l solution of *tetrabutylammonium hydroxide R*; if necessary, adjust to pH 5.5 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*; add 650 ml of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	88	12
t_R - (t_R + 30)	88 → 0	12 → 100
(t_R + 30) - (t_R + 45)	0 → 88	100 → 12

t_R = retention time of piperacillin determined with reference solution (b)

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 220 nm.

Injection: 20 µl of reference solutions (b), (c) and (d) with isocratic elution at the initial mobile phase composition and 20 µl of test solution (b) according to the elution gradient described under Mobile phase.

System suitability:

– *resolution*: minimum 10 between the peaks due to impurity A and piperacillin in the chromatogram obtained with reference solution (c); if necessary, adjust the ratio A:B of the mobile phase;
– *signal-to-noise ratio*: minimum 3 for the principal peak in the chromatogram obtained with reference solution (d);
– *mass distribution ratio*: 2.0 to 3.0 for the peak due to piperacillin in the chromatogram obtained with reference solution (c).

Limit:

– *any impurity*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent).

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

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 0.500 g.

Bacterial endotoxins (*2.6.14*): less than 0.07 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 piperacillin sodium, multiplying the result by 1.042.

STORAGE

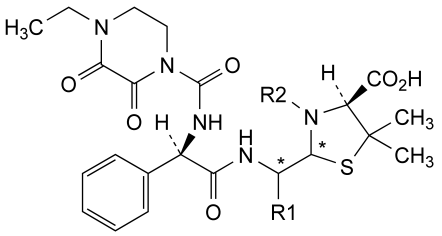
In an airtight container. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities: A, B, C, D, E, F, G.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *H.*

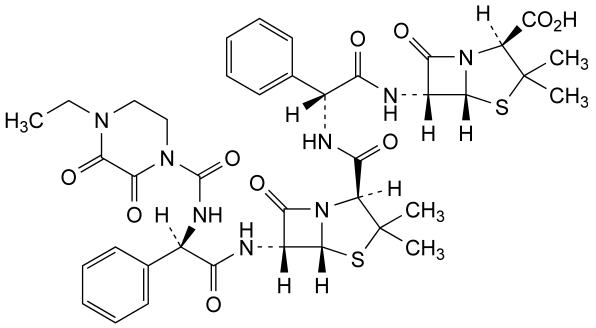
A. ampicillin,



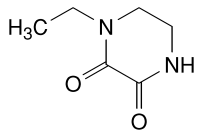
B. R1 = CO_2H, R2 = H: (4*S*)-2-[carboxy[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of piperacillin),

C. R1 = R2 = H: (2*RS*,4*S*)-2-[[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of piperacillin),

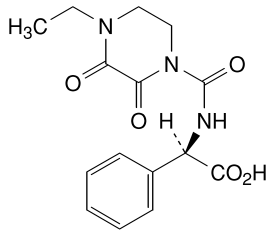
F. R1 = CO_2H, R2 = $CO\text{-}CH_3$: (4*S*)-3-acetyl-2-[carboxy[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (acetylated penicilloic acids of piperacillin).



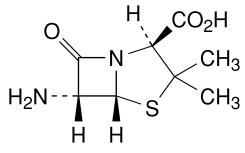
D. (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-[[[(2S,5*R*,6*R*)-6-[[(2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-yl]carbonyl]amino]-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (piperacillinylampicillin),



E. 1-ethylpiperazine-2,3-dione,



G. (2*R*)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino]-2-phenylacetic acid,

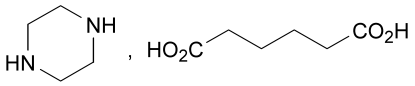


H. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid).

01/2008:0423
corrected 6.0

PIPERAZINE ADIPATE

Piperazini adipas



$C_{10}H_{20}N_2O_4$ M_r 232.3
[142-88-1]

DEFINITION

Piperazine adipate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of piperazine hexanedioate, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white crystalline powder, soluble in water, practically insoluble in alcohol. It melts at about 250 °C, with decomposition.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *piperazine adipate CRS*. Examine the substances prepared as discs.

B. Examine the chromatograms obtained in the test for related substances after spraying with the ninhydrin solutions. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. To 10 ml of solution S (see Tests) add 5 ml of *hydrochloric acid R* and shake with three quantities, each of 10 ml, of *ether R*. Evaporate the combined ether layers to dryness. The residue, washed with 5 ml of *water R* and dried at 100 °C to 105 °C, melts (*2.2.14*) at 150 °C to 154 °C.

TESTS

Solution S. Dissolve 2.5 g in *water 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 B_8 (*2.2.2, Method II*).

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a suitable silica gel as the coating substance.

Test solution (a). Dissolve 1.0 g of the substance to be examined in 6 ml of *concentrated ammonia R* and dilute to 10 ml with *ethanol R*.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R*.

Reference solution (a). Dissolve 0.1 g of *piperazine adipate CRS* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b). Dissolve 25 mg of *ethylenediamine R* in a mixture of 2 volumes of *ethanol R* and 3 volumes of *concentrated ammonia R* and dilute to 100 ml with the same mixture of solvents.