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).

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Bacterial endotoxins (*2.6.14, Method E*): less than 0.16 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.

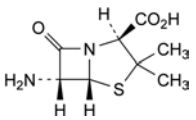
Injection: test solution (a) and reference solution (a).

Calculate the percentage content of $C_{16}H_{17}KN_2O_4S$ by multiplying the percentage content of benzylpenicillin sodium by 1.045.

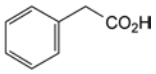
STORAGE

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

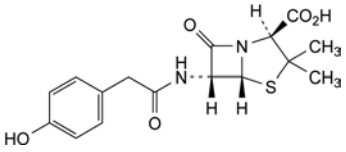
IMPURITIES



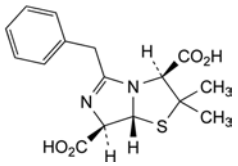
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



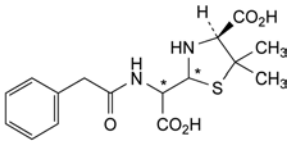
B. phenylacetic acid,



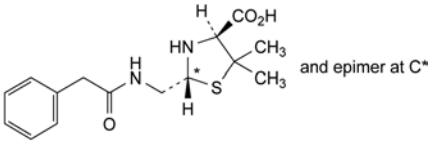
C. (2*S*,5*R*,6*R*)-6-[[(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



D. (3*S*,7*R*,7a*R*)-5-benzyl-2,2-dimethyl-2,3,7,7a-tetrahydroimidazo[5,1-*b*]thiazole-3,7-dicarboxylic acid (penillic acid of benzylpenicillin),



E. (4*S*)-2-[carboxy[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of benzylpenicillin),

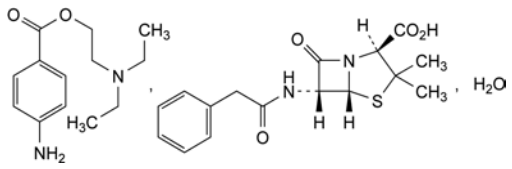


F. (2*RS*,4*S*)-2-[[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin).

01/2008:0115
corrected 6.0

BENZYLPENICILLIN, PROCAINE

Benzylpenicillinum procainum



$C_{29}H_{38}N_4O_6S,H_2O$ M_r 588.7
[6130-64-9]

DEFINITION

(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with 2-(diethylamino)ethyl 4-aminobenzoate monohydrate.

Substance produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Content:

– *procaine benzylpenicillin*: 96.0 per cent to 102.0 per cent (anhydrous substance);

– *procaine* ($C_{13}H_{20}N_2O_2$; M_r 236.3): 39.0 per cent to 42.0 per cent (anhydrous substance).

Dispersing or suspending agents (for example, lecithin and polysorbate 80) may be added.

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *procaine benzylpenicillin CRS.*

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

Test solution. Dissolve 25 mg of the substance to be examined in 5 mL of *acetone R.*

Reference solution. Dissolve 25 mg of *procaine benzylpenicillin CRS* in 5 mL of *acetone R.*

Plate: *TLC silanised silica gel plate R.*

Mobile phase: mix 30 volumes of *acetone R* and 70 volumes of a 154 g/L solution of *ammonium acetate R* previously adjusted to pH 7.0 with *ammonia 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:

– the chromatogram shows 2 clearly separated spots.

Results: the 2 principal spots in the chromatogram obtained with the test solution are similar in position, colour and size to the 2 principal spots in the chromatogram obtained with the reference solution.

C. Place about 2 mg in a test-tube about 150 mm long and 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 on a water-bath for 1 min; a reddish-brown colour develops.

D. Dissolve 0.1 g in 2 mL of *dilute hydrochloric acid R* and use the solution which may be turbid. The solution gives the reaction of primary aromatic amines (*2.3.1*).

TESTS

pH (*2.2.3*): 5.0 to 7.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 15 mL with the same solvent. Shake until dissolution is complete.

Specific optical rotation (*2.2.7*): + 165 to + 180 (anhydrous substance).

Dissolve 0.250 g in a mixture of 2 volumes of *water R* and 3 volumes of *acetone R*, then dilute to 25.0 mL with the same mixture of solvents.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution (a). Dissolve 70.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Test solution (b). Dissolve 70.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a). Dissolve 70.0 mg of *procaine benzylpenicillin CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (b). Dissolve 4 mg of *4-aminobenzoic acid R* (impurity A) in reference solution (a) and dilute to 25 mL with reference solution (a).

Reference solution (c). Dissolve 16.8 mg of *4-aminobenzoic acid R* (impurity A) in *water R* and dilute to 50.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10.0 mL with *water R*. To 1.0 mL of this solution, add 1.0 mL of test solution (a) and dilute to 100.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 250 mL of *acetonitrile R1*, 250 mL of *water R* and 500 mL of a solution containing 14 g/L of *potassium dihydrogen phosphate R* and 6.5 g/L of *tetrabutylammonium hydroxide solution (400 g/L) R* adjusted to pH 7.0 with *1 M potassium hydroxide*; if necessary, adjust the mixture to pH 7.2 with *dilute phosphoric acid R*.

Flow rate: 1.75 mL/min.

Detection: spectrophotometer at 225 nm.

Injection: 10 µL of test solution (a) and reference solutions (b) and (c).

Run time: 1.5 time the retention time of benzylpenicillin.

Elution order: impurity A, procaine, benzylpenicillin.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to impurity A and procaine; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

– *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.024 per cent);

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

Water (*2.5.12*): 2.8 per cent to 4.2 per cent, determined on 0.500 g.

Bacterial endotoxins (*2.6.14, Method E*): less than 0.10 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.

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

System suitability: reference solution (a):

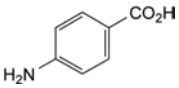
– *repeatability*: maximum relative standard deviation of 1.0 per cent for the 2 principal peaks after 6 injections.

Calculate the percentage contents of procaine and procaine benzylpenicillin.

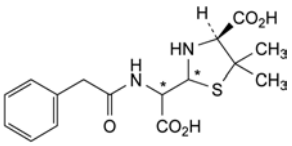
STORAGE

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

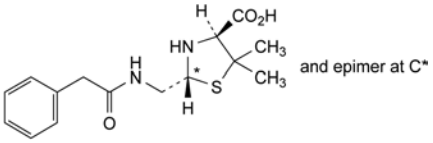
IMPURITIES



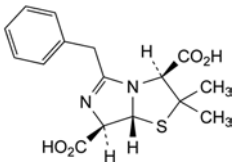
A. 4-aminobenzoic acid,



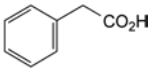
B. (4*S*)-2-[carboxy[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of benzylpenicillin),



C. (2*RS*,4*S*)-2-[[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin),



D. (3*S*,7*R*,7a*R*)-5-benzyl-2,2-dimethyl-2,3,7,7a-tetrahydroimidazo[5,1-*b*]thiazole-3,7-dicarboxylic acid (penillic acid of benzylpenicillin),

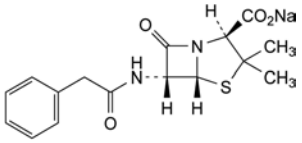


E. phenylacetic acid.

01/2008:0114
corrected 6.0

BENZYLPENICILLIN SODIUM

Benzylpenicillinum natricum



$C_{16}H_{17}N_2NaO_4S$ M_r 356.4
[69-57-8]

DEFINITION

Sodium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)-amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Substance produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means.

Content: 96.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: very soluble in water, 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*).
Comparison: *benzylpenicillin sodium CRS.*

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 25 mg of the substance to be examined in 5 mL of *water R.*
Reference solution (a). Dissolve 25 mg of *benzylpenicillin sodium CRS* in 5 mL of *water R.*
Reference solution (b). Dissolve 25 mg of *benzylpenicillin sodium CRS* and 25 mg of *phenoxymethylpenicillin potassium CRS* in 5 mL of *water R.*
Plate: *TLC silanised silica gel plate R.*
Mobile phase: mix 30 volumes of *acetone R* and 70 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 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 on a water-bath for 1 min; a reddish-brown colour develops.

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

TESTS

pH (*2.2.3*): 5.5 to 7.5.

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*): + 285 to + 310 (dried substance).

Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Absorbance (*2.2.25*). Dissolve 90.0 mg in *water R* and dilute to 50.0 mL with the same solvent. Measure the absorbance of the solution at 325 nm, at 280 nm and at the absorption maximum at 264 nm, diluting the solution, if necessary, for the measurement at 264 nm. The absorbances at 325 nm and 280 nm are not greater than 0.10 and the absorbance at the absorption maximum at 264 nm is 0.80 to 0.88, calculated on the basis of the undiluted (1.80 g/L) solution. Verify the resolution of the apparatus (*2.2.25*); the ratio of the absorbances is at least 1.7.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution (a). Dissolve 50.0 mg of the substance to be examined in *water R* and dilute to 50.0 mL with the same solvent.

Test solution (b). Dissolve 80.0 mg of the substance to be examined in *water R* and dilute to 20.0 mL with the same solvent.

Reference solution (a). Dissolve 50.0 mg of *benzylpenicillin sodium CRS* in *water R* and dilute to 50.0 mL with the same solvent.

Reference solution (b). Dissolve 10 mg of *benzylpenicillin sodium CRS* and 10 mg of *phenylacetic acid R* (impurity B) in *water R*, then dilute to 50 mL with the same solvent.

Reference solution (c). Dilute 4.0 mL of reference solution (a) to 100.0 mL with *water R.*

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 10 volumes of a 68 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with a 500 g/L solution of *dilute phosphoric acid R*, 30 volumes of *methanol R* and 60 volumes of *water R*;
– *mobile phase B*: mix 10 volumes of a 68 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with a 500 g/L solution of *dilute phosphoric acid R*, 40 volumes of *water R* and 50 volumes of *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - t_R	70	30
t_R - (t_R + 20)	70 → 0	30 → 100
(t_R + 20) - (t_R + 35)	0	100
(t_R + 35) - (t_R + 50)	70	30

t_R = retention time of benzylpenicillin 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 225 nm.

Injection: 20 µL of reference solutions (b) and (c) 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; inject *water R* as a blank according to the elution gradient described under Mobile phase.

System suitability: reference solution (b):
– *resolution*: minimum 6.0 between the peaks due to impurity B and benzylpenicillin; if necessary, adjust the ratio A:B of the mobile phase.