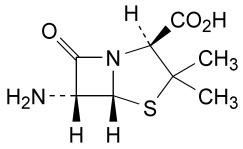
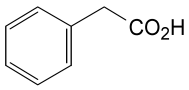
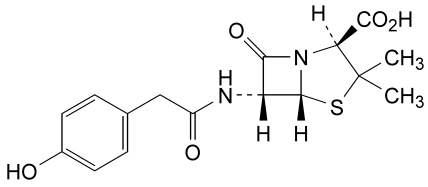
IMPURITIES



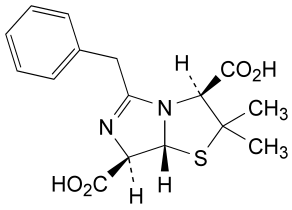
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



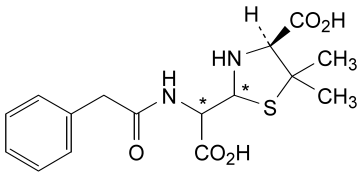
B. phenylacetic acid,



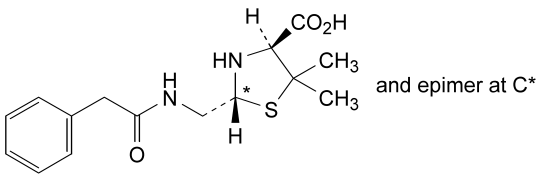
C. (2*S*,5*R*,6*R*)-6-[[(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



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),

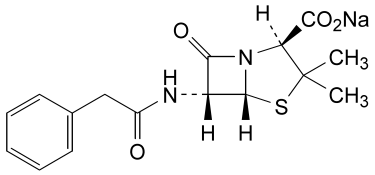


F. (2*RS*,4*S*)-2-[[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin).

01/2005:0114
corrected

BENZYLPENICILLIN SODIUM

Benzylpenicillinum natricum



$C_{16}H_{17}N_2NaO_4S$ M_r 356.4

DEFINITION

Benzylpenicillin sodium is 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, a substance produced by the growth of certain strains of *Penicillium notatum* or related organisms, or obtained by any other means. It contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of benzylpenicillin sodium, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, very soluble in water, practically insoluble in fatty oils and in liquid paraffin.

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *benzylpenicillin sodium CRS*.

B. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silanised silica gel plate R*.

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*.

Apply to the plate 1 µl of each solution. Develop over a path of 15 cm using a mixture of 30 volumes of *acetone R* and 70 volumes of a 154 g/l solution of *ammonium acetate R*, the pH of which has been adjusted to 5.0 with *glacial acetic acid R*. Allow the plate to dry in air and expose it to iodine vapour until the spots appear. Examine in daylight. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

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 *sulphuric 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*). Dissolve 2.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent. The pH of the solution is 5.5 to 7.5.

Specific optical rotation (*2.2.7*). Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 285 to + 310, calculated with reference to the dried substance.

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 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 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*) as described under Assay. Inject 20 µl of reference solution (d) and elute isocratically with the chosen mobile phase. Inject 20 µl of test solution (b) and start the elution isocratically. Immediately after elution of the benzylpenicillin peak start the following linear gradient:

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Comment
0 - 20	70 → 0	30 → 100	linear gradient
20 - 35	0	100	isocratic
35 - 50	70	30	re-equilibration

Inject *water R* and use the same elution pattern to obtain a blank. In the chromatogram obtained with test solution (b), the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (d) (1 per cent).

2-Ethylhexanoic acid (*2.4.28*). Not more than 0.5 per cent *m/m*.

Loss on drying (*2.2.32*). Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 100-105 °C.

Bacterial endotoxins (*2.6.14, Method E*): less than 0.16 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*). *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* in *water R* and dilute to 50 ml with the same solvent.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 20.0 ml with *water R*. Dilute 1.0 ml of the solution to 50.0 ml with *water R*.

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

The chromatographic procedure may be carried out using:

- a column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),
- as mobile phase at a flow rate of 1.0 ml/min:

  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*.
- as detector a spectrophotometer set at 225 nm.

Equilibrate the column with a mobile phase ratio A:B of 70:30. Inject 20 µl of reference solution (b). The test is not valid unless the resolution between the 2 principal peaks is at least 6.0 (if necessary, adjust the ratio A:B of the mobile phase) and the mass distribution ratio for the second peak (benzylpenicillin) is 4.0 to 6.0. Inject 20 µl of reference solution (c). Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

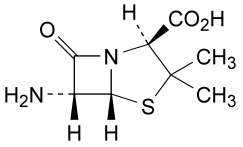
STORAGE

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

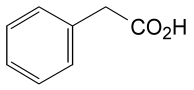
LABELLING

The label states, where applicable, that the substance is free from bacterial endotoxins.

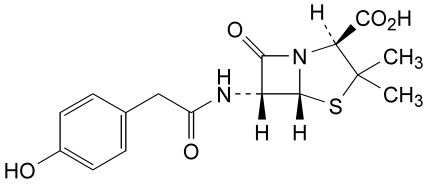
IMPURITIES



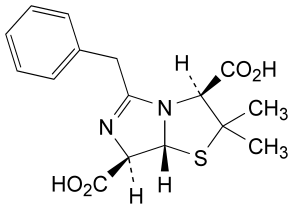
A. (2*S*,5*R*,6*R*)-6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid),



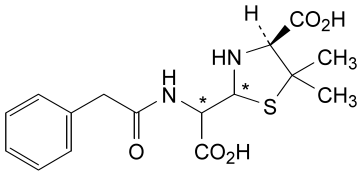
B. phenylacetic acid,



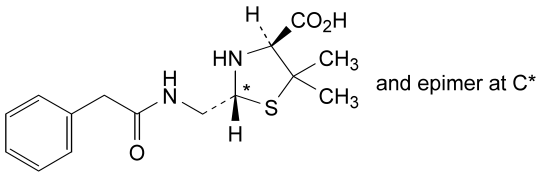
C. (2*S*,5*R*,6*R*)-6-[[(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia- 1-azabicyclo[3.2.0]heptane-2-carboxylic acid,



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),

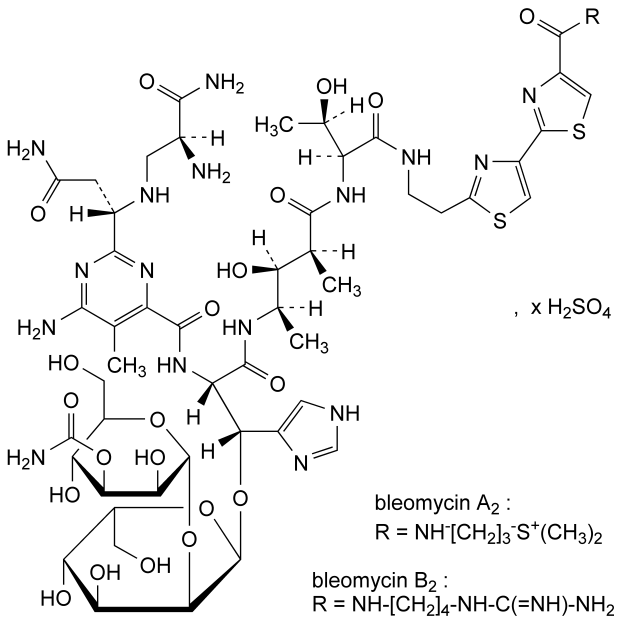


F. (2*RS*,4*S*)-2-[[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin).

01/2005:0976
corrected

BLEOMYCIN SULPHATE

Bleomycini sulfas



DEFINITION

Bleomycin sulphate is the sulphate of a mixture of glycopeptides produced by *Streptomyces verticillus* or by any other means; the two principal components of the mixture are *N*-[3-(dimethylsulphonio)propyl]bleomycinamide (bleomycin A_2) and *N*-[4-(carbamimidoylamino)butyl]bleomycinamide (bleomycin B_2). The potency is not less than 1500 IU/mg, calculated with reference to the dried substance.

CHARACTERS

A white or yellowish-white powder, very hygroscopic, very soluble in water, slightly soluble in ethanol, practically insoluble in acetone.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for composition. The retention times and sizes of the two principal peaks in the chromatogram obtained with the test solution are approximately the same as those of the two principal peaks in the chromatogram obtained with reference solution (a).

B. It gives the reactions of sulphates (*2.3.1*).

TESTS

Appearance of solution. Dissolve 0.200 g in *water R* and dilute to 10.0 ml with the same solvent. The solution is clear (*2.2.1*). The absorbance (*2.2.25*) measured at 430 nm is not greater than 0.10.

pH (*2.2.3*). Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent. The pH of the solution is 4.5 to 6.0.

Composition. Examine by liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (a). Dissolve 25.0 mg of *bleomycin sulphate CRS* in *water R* and dilute to 50.0 ml with the same solvent.

Reference solution (b). Dilute 1.5 ml of reference solution (a) to 100.0 ml with *water R*.

The chromatographic procedure may be carried out using:

– a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (7 µm),

– gradient elution at a flow rate of 1.2 ml/min with a mobile phase initially composed of 10 per cent *V/V* of *methanol R* and 90 per cent *V/V* of a mixture prepared as follows: dissolve 0.960 g of *sodium pentanesulphonate R* in 900 ml of acetic acid (4.8 g/l $C_2H_4O_2$), add 1.86 g of *sodium edetate R*, dilute to 1000 ml with the same solvent and adjust to pH 4.3 using *ammonia R*; increasing the proportion of *methanol R* to 40 per cent *V/V* over 60 min and continuing with the final mixture for about 20 min, until demethylbleomycin A_2 is eluted (retention time 1.5 to 2.5, relative to bleomycin A_2),

– as detector a spectrophotometer set at 254 nm,

– a 20 µl loop injector.

Inject reference solution (a). The test is not valid unless the resolution between the two principal peaks is at least 5. Inject reference solution (b). The test is not valid unless the signal-to-noise ratio calculated for the principal peak is at least 20.

Inject reference solution (a) six times. The test is not valid unless the relative standard deviation of the area of the principal peak is at most 2 per cent.

Inject the test solution. The composition, calculated by the normalisation procedure and disregarding any peak with an area less than 0.1 per cent of the total, is: bleomycin A_2 (first principal peak) 55 per cent to 70 per cent; bleomycin B_2 (second principal peak) 25 per cent to 32 per cent; sum of bleomycin A_2 and bleomycin B_2 not less than 85 per cent;