Comparison: *Ph. Eur. reference spectrum of benzyl benzoate.*

B. To 2 g add 25 mL of *alcoholic potassium hydroxide solution R* and boil under a reflux condenser for 2 h. Remove the ethanol on a water-bath, add 50 mL of *water R* and distill. Collect about 25 mL of distillate and use it for identification test C. Acidify the liquid remaining in the distillation flask with *dilute hydrochloric acid R*. A white precipitate is formed that, when washed with *water R* and dried *in vacuo* melts (*2.2.14*) at 121 °C to 124 °C.

C. To the distillate obtained in identification test B add 2.5 g of *potassium permanganate R* and 5 mL of *dilute sodium hydroxide solution R*. Boil under a reflux condenser for 15 min, cool and filter. Acidify the filtrate with *dilute hydrochloric acid R*. A white precipitate is formed that, when washed with *water R* and dried *in vacuo*, melts (*2.2.14*) at 121 °C to 124 °C.

TESTS

Acidity. Dissolve 2.0 g in *ethanol (96 per cent) R* and dilute to 10 mL with the same solvent. Titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution R* as indicator. Not more than 0.2 mL is required to change the colour of the indicator to pink.

Relative density (*2.2.5*): 1.118 to 1.122.

Refractive index (*2.2.6*): 1.568 to 1.570.

Freezing point (*2.2.18*): minimum 17.0 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

To 2.000 g add 50.0 mL of *0.5 M alcoholic potassium hydroxide* and boil gently under a reflux condenser for 1 h. Titrate the hot solution with *0.5 M hydrochloric acid* using 1 mL of *phenolphthalein solution R* as indicator. Carry out a blank determination.

1 mL of *0.5 M alcoholic potassium hydroxide* is equivalent to 106.1 mg of $C_{14}H_{12}O_2$.

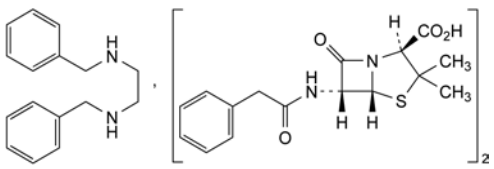
STORAGE

In an airtight, well-filled container, protected from light.

01/2008:0373
corrected 6.0

BENZYLPENICILLIN, BENZATHINE

Benzylpenicillinum benzathinum



$C_{48}H_{56}N_6O_8S_2$ M_r 909
[1538-09-6]

DEFINITION

N,N′-Dibenzylethane-1,2-diamine compound (1:2) with (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.

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

Content:

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

– *N,N′-dibenzylethylenediamine* (benzathine $C_{16}H_{20}N_2$; M_r 240.3): 24.0 per cent to 27.0 per cent (anhydrous substance).

It contains a variable quantity of water. Dispersing or suspending agents may be added.

CHARACTERS

Appearance: white or almost white powder.

Solubility: very slightly soluble in water, freely soluble in dimethylformamide and in formamide, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: A.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *benzathine benzylpenicillin CRS.*

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

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

Reference solution. Dissolve 25 mg of *benzathine benzylpenicillin CRS* in 5 mL of *methanol 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* 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. To 0.1 g add 2 mL of *1 M sodium hydroxide* and shake for 2 min. Shake the mixture with 2 quantities, each of 3 mL, of *ether R*. Evaporate the combined ether layers to dryness and dissolve the residue in 1 mL of *ethanol (50 per cent V/V) R*. Add 5 mL of *picric acid solution R*, heat at 90 °C for 5 min and allow to cool slowly. Separate the crystals and recrystallise from *ethanol (25 per cent V/V) R* containing 10 g/L of *picric acid R*. The crystals melt (*2.2.14*) at about 214 °C.

TESTS

Acidity or alkalinity. To 0.50 g add 100 mL of *carbon dioxide-free water R* and shake for 5 min. Filter through a sintered-glass filter (*2.1.2*). To 20 mL of the filtrate add 0.1 mL of *bromothymol blue solution R1*. The solution is green or yellow. Not more than 0.2 mL of *0.02 M sodium hydroxide* is required to change the colour of the indicator to blue.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use, using sonication (for about 2 min) to dissolve the samples. Avoid any overheating during the sample preparation.*

Test solution. Dissolve 70.0 mg of the substance to be examined in 25 mL of *methanol R* and dilute to 50.0 mL with a solution containing 6.8 g/L of *potassium dihydrogen phosphate R* and 1.02 g/L of *disodium hydrogen phosphate R*.

Reference solution (a). Dissolve 70.0 mg of *benzathine benzylpenicillin CRS* in 25 mL of *methanol R* and dilute to 50.0 mL with a solution containing 6.8 g/L of *potassium dihydrogen phosphate R* and 1.02 g/L of *disodium hydrogen phosphate R*.

Reference solution (b). Dilute 1.0 mL of reference solution (a) to 100.0 mL with mobile phase A.

Column:

- *size*: *l* = 0.25 m, Ø = 4.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: mix 10 volumes of a 34 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with *phosphoric acid R*, 30 volumes of *methanol R* and 60 volumes of *water R*;
- *mobile phase B*: mix 10 volumes of a 34 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.5 with *phosphoric acid R*, 30 volumes of *water R* and 60 volumes of *methanol R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	75	25
10 - 20	75 → 0	25 → 100
20 - 55	0	100
55 - 70	75	25

Flow rate: 1 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 20 µL.

System suitability: reference solution (a):

- *relative retention* with reference to benzylpenicillin: benzathine = 0.3 to 0.4; impurity C = about 2.4; if necessary, adjust the concentration of methanol in the mobile phase.

Limits:

- *impurity C*: not more than twice the sum of the areas of the 2 principal peaks in the chromatogram obtained with reference solution (b) (2 per cent);
- *any other impurity*: for each impurity, not more than the sum of the areas of the 2 principal peaks in the chromatogram obtained with reference solution (b) (1 per cent);
- *disregard limit*: 0.05 times the sum of the areas of the 2 principal peaks in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (*2.5.12*): 5.0 per cent to 8.0 per cent, determined on 0.300 g.

Bacterial endotoxins (*2.6.14, Method E*): less than 0.13 IU/mL, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

Suspend 20 mg in 20 mL of a solution of *0.1 M sodium hydroxide* diluted 1 to 100, shake thoroughly and centrifuge. Examine the supernatant.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Mobile phase: *phosphate buffer solution pH 3.5 R, methanol R, water R* (10:35:55 *V/V/V*).

Injection: test solution and reference solution (a).

Calculate the percentage contents of benzathine and benzathine benzylpenicillin. Calculate the percentage content of benzathine benzylpenicillin by multiplying the percentage content of benzylpenicillin by 1.36.

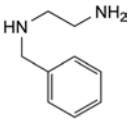
STORAGE

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

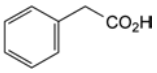
IMPURITIES

Specified impurities: *C*.

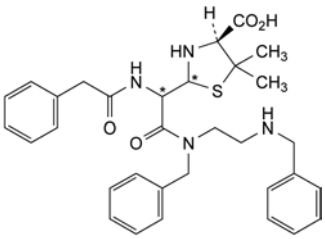
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical 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*): *A, B, D, E, F*.



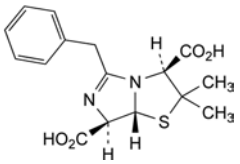
A. monobenzylethylenediamine,



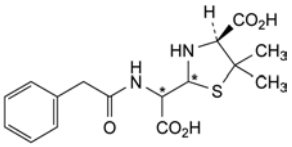
B. phenylacetic acid,



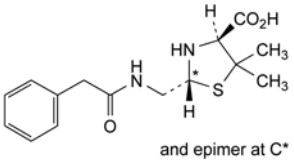
C. benzylpenicilloic acids benzathide,



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),

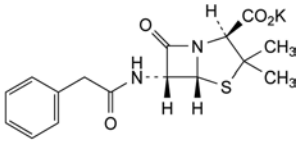


F. (2*RS*,4*S*)-2-[[(phenylacetyl)amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic acids of benzylpenicillin).

01/2008:0113
corrected 6.0

BENZYLPENICILLIN POTASSIUM

Benzylpenicillinum kalicum



$C_{16}H_{17}KN_2O_4S$ M_r 372.5
[113-98-4]

DEFINITION

Potassium (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 potassium 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 potassium CRS* in 5 mL of *water R*.
Reference solution (b). Dissolve 25 mg of *benzylpenicillin potassium 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 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 potassium (*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*): + 270 to + 300 (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 94.0 mg in *water R* and dilute to 50.0 mL with the same solvent. Measure the absorbance of the solution at 325 nm, 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 do not exceed 0.10 and that at the absorption maximum at 264 nm is 0.80 to 0.88, calculated on the basis of the undiluted (1.88 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.