Edition: BP 2025 (Ph. Eur. 11.6 update)

Procaine Benzylpenicillin Injection

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Procaine Benzylpenicillin Injection is a sterile suspension of Benzylpenicillin (Procaine) Monohydrate in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of total penicillins, calculated as $C_{13}H_{20}N_2O_2$,$C_{16}H_{18}N_2O_4S$, H_2O

90.0 to 110.0% of the stated amount of Procaine Benzylpenicillin.

Content of procaine, $C_{13}H_{20}N_2O_2$

36.0 to 44.0% of the stated amount of Procaine Benzylpenicillin.

CHARACTERISTICS

A white suspension.

IDENTIFICATION

A. Dilute a volume of the well-shaken suspension containing 10 mg of Procaine Benzylpenicillin to 10 mL with *water* and add 0.5 mL of *neutral red solution*. Add sufficient 0.01M *sodium hydroxide* to produce a permanent orange colour and then add 1 mL of *penicillinase solution*. A red colour is produced rapidly.
B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Shake a volume of the well-shaken suspension containing 50 mg of Procaine Benzylpenicillin with 5 mL of *methanol*, add a small quantity of *water* to dissolve any residue and dilute to 10 mL with *water*.
(2) 0.5% w/v of *procaine benzylpenicillin BPCRS* in *acetone*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *TLC silica gel silanised plate* (Merck silanised silica gel 60 plates are suitable).
(b) Use the mobile phase as described below.
(c) Apply 1 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, allow it to dry in air, expose to iodine vapour until spots appear and examine in daylight.

MOBILE PHASE

30 volumes of *acetone* and 70 volumes of a 15.4% w/v solution of *ammonium acetate* adjusted to pH 7.0 with 10M *ammonia*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (2) shows two clearly separated spots.

CONFIRMATION

The two principal spots in the chromatogram obtained with solution (1) are similar in position, colour and size to those in the chromatogram obtained with solution (2).

C. Yields the reaction characteristic of *primary aromatic amines*, Appendix VI, producing a bright orange-red precipitate.

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) To a quantity of the well-shaken suspension containing 70 mg of Procaine Benzylpenicillin add sufficient mobile phase to produce 50 mL, mix, filter and use the filtrate.
(2) Mix 1 mL of solution (1) and 1 mL of a 0.007% w/v solution of *4-aminobenzoic acid* and add sufficient mobile phase to produce 100 mL.
(3) Dissolve 4 mg of *4-aminobenzoic acid* in 25 mL of a solution containing 0.070% w/v of *procaine benzylpenicillin BPCRS* in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrospher ODS is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.5 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 225 nm.
(f) Inject 20 µL of each solution.
(g) For solution (1) allow the chromatography to proceed for 1.5 times the retention time of the peak due to benzylpenicillin.

MOBILE PHASE

250 volumes of *acetonitrile*, 250 volumes of water and 500 volumes of a freshly prepared solution containing 1.4% w/v of *potassium dihydrogen orthophosphate* and 0.65% w/v of *tetrabutylammonium hydroxide*, adjusted to pH 7.0 with 1M *potassium hydroxide*. Adjust the pH of the mixture to 7.2 with 2M *orthophosphoric acid*, if necessary.

For solution (3), when the chromatogram is recorded under the prescribed conditions the substances elute in the following order: 4-aminobenzoic acid, procaine, benzylpenicillin.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to 4-aminobenzoic acid and procaine is at least 2.0. If necessary, adjust the concentration of *acetonitrile* in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak due to 4-aminobenzoic acid is not greater than 10 times the area of the corresponding peak in the chromatogram obtained with solution (2) (0.5%);

the area of any other *secondary peak* is not greater than the area of the peak corresponding to benzylpenicillin in the chromatogram obtained with solution (2) (1%).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C, Method C. Dilute a quantity of the well-shaken suspension, if necessary, with *water BET* to produce a solution containing 3 mg of Procaine Benzylpenicillin per mL (solution A). The endotoxin limit concentration of solution A is 0.3 IU per mL. Carry out the test using a suitable dilution of solution A as described under Method C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add to a quantity of the well-shaken suspension containing 70 mg of Procaine Benzylpenicillin sufficient mobile phase to produce 100 mL, mix, filter and use the filtrate.
(2) 0.07% w/v of *procaine benzylpenicillin BPCRS* in the mobile phase.
(3) Dissolve 4 mg of *4-aminobenzoic acid* in 25 mL of solution (2).

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

For solution (3), when the chromatogram is recorded under the prescribed conditions the substances elute in the following order: 4-aminobenzoic acid, procaine, benzylpenicillin.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to 4-aminobenzoic acid and procaine is at least 2.0. If necessary, adjust the concentration of *acetonitrile* in the mobile phase.

DETERMINATION OF CONTENT

Calculate the content of $C_{13}H_{20}N_2O_2$ and of $C_{13}H_{20}N_2O_2,C_{16}H_{18}N_2O_4S,H_2O$ in the injection from the chromatograms obtained and using the declared content of $C_{13}H_{20}N_2O_2$ and of $C_{13}H_{20}N_2O_2,C_{16}H_{18}N_2O_4S,H_2O$ in *procaine benzylpenicillin BPCRS*.

3 g of Procaine Benzylpenicillin is approximately equivalent to 2 g of benzylpenicillin.