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Benzylpenicillin Infusion

General Notices

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Penicillin antibacterial.

DEFINITION

Benzylpenicillin Infusion is a sterile solution containing Benzylpenicillin Sodium and a suitable buffer. It is supplied as a ready-to-use solution.

The infusion complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the infusion also complies with the requirements stated under Unlicensed Medicines.

Content of benzylpenicillin, $C_{16}H_{18}N_2O_4S$

90.0 to 105.0% of the stated amount.

CHARACTERS

A clear, colourless solution.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in *water*.
(1) Dilute a volume of the infusion to produce a solution containing the equivalent of 0.6% w/v of benzylpenicillin.
(2) 0.64% w/v of *benzylpenicillin sodium EPCRS*.
(3) 0.5% w/v of each of *benzylpenicillin sodium EPCRS* and *phenoxymethylpenicillin EPCRS*.

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 the 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 5.0 with *glacial acetic acid*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, colour and size to that in the chromatogram obtained with solution (2).
B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 6.0 to 7.5, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water*, prepared immediately before use.

(1) Dilute a volume of the infusion to produce a solution containing the equivalent of 0.6% w/v of benzylpenicillin.
(2) Dilute 1 volume of solution (1) to 100 volumes.
(3) Dissolve 5 mg of *benzylpenicillin for system suitability EPCRS* in 0.35 mL of *methanol R1* and add 0.65 mL of *water*.
(4) Dilute 1 volume of solution (2) to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (3 µm) (YMC-Pack Pro is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1.5 mL per minute.
(d) Use a column temperature of 50°.
(e) Use a detection wavelength of 225 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.4 with a 50% w/v solution of *orthophosphoric acid*, 30 volumes of *methanol R1* and 60 volumes of *water*.

Mobile phase B 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate* adjusted to pH 3.4 with a 50% w/v solution of *orthophosphoric acid*, 35 volumes of *water* and 55 volumes of *methanol R1*.

Time (min)	Mobile Phase A (% V/V)	Mobile Phase B (% V/V)	Comment
0 - 7	70	30	isocratic
7 - 17	70 → 0	30 → 100	linear gradient
17 - 22	0	100	isocratic

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to benzylpenicillin (retention time, about 7 minutes) are: impurity A, about 0.22; impurity D, about 0.33; impurity C, about 0.35; impurity E, about 0.48 and 0.55; impurity B, about 0.62; impurity F, about 0.81 and 0.83; impurity G, about 1.47; impurity H, about 1.90.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution* between the peaks due to the epimers of impurity F is at least 1.2;

the *resolution* between the peaks due to impurities D and C is at least 1.5.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, D, E and F using the chromatogram obtained with solution (3) and multiply the areas of these peaks by the following correction factors: impurity A, 1.3; impurity D, 0.6; impurity E, 2.0; impurity F, 1.7.

In the chromatogram obtained with solution (1);

the sum of the areas of any peaks corresponding to the isomers of impurity E is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the sum of the areas of any peaks corresponding to the epimers of impurity F is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);

the area of any peak corresponding to impurity B is not greater than half the area of the principal 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 principal peak in the chromatogram obtained with solution (4) (0.2%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%).

Disregard any peak with an area less than 0.75 times the area of the principal peak in the chromatogram obtained with solution (4) (0.15%).

Bacterial endotoxins

Dilute the infusion with *water BET* to contain the equivalent of 10 mg of benzylpenicillin per mL (solution A). The endotoxin limit concentration of solution A is 1.6 IU per mL, Appendix XIV C.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in *water* prepared immediately before use.

(1) Dilute a volume of the infusion to produce a solution containing the equivalent of 0.12% w/v of benzylpenicillin.
(2) 0.13% w/v of *benzylpenicillin sodium EPCRS*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used but using isocratic elution, a flow rate of 1.2 mL per minute and an injection volume of 10 µL.

MOBILE PHASE

30 volumes of mobile phase B and 70 volumes of mobile phase A as described under Related substances.

DETERMINATION OF CONTENT

Calculate the content of $C_{16}H_{18}N_2O_4S$ in the infusion using the declared content of $C_{16}H_{17}N_2NaO_4S$ in *benzylpenicillin sodium EPCRS*. Each mg of $C_{16}H_{17}N_2NaO_4S$ is equivalent to 0.9383 mg of $C_{16}H_{18}N_2O_4S$.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of benzylpenicillin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Benzylpenicillin Sodium.