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Benzylpenicillin for Injection

General Notices

Action and use

Penicillin antibacterial.

DEFINITION

Benzylpenicillin for Injection is a sterile material consisting of Benzylpenicillin Potassium or Benzylpenicillin Sodium with or without excipients. It is supplied in a sealed container.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of penicillins, calculated as $C_{16}H_{18}N_2O_4S$

95.0 to 105.0% of the content of benzylpenicillin stated on the label.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the spectrum of *benzylpenicillin potassium EPCRS* or *benzylpenicillin sodium EPCRS* as appropriate.
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 of a solution containing the equivalent of 10.0% w/v of benzylpenicillin, 5.5 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) Dissolve a quantity of the contents of a sealed container to produce a solution containing the equivalent of 0.4% 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 0.2; impurity D, about 0.3; impurity C, about 0.4; impurity E, about 0.48 and 0.55; impurity B, about 0.6; impurity F, about 0.81 and 0.83; impurity G, about 1.5 and impurity H, about 1.9.

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

LIMITS

In the chromatogram obtained with solution (1):

identify any peaks corresponding to impurities A to H using the chromatogram supplied with *benzylpenicillin for system suitability EPCRS* and the chromatogram obtained with solution (3). Multiply the area of any peaks corresponding to impurity A, impurity D, impurity E and impurity F by the following correction factors respectively: 1.3, 0.6, 2.0 and 1.7.

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 *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%).

Loss on drying

When dried to constant weight at 105°, lose not more than 1.0% of their weight. Use 1 g.

Bacterial endotoxins

Carry out the test for *bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing the equivalent of 10 mg of benzylpenicillin per mL (solution A). The endotoxin limit of solution A is 1.6 IU per mL.

ASSAY

Determine the weight of the contents of 10 containers as described in the test for *uniformity of weight*, Appendix XII C1, Powders for Parenteral Administration.

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

(1) Dissolve a quantity of the mixed contents of the 10 containers to produce a solution containing the equivalent of 0.1% w/v of benzylpenicillin.
(2) 0.11% w/v of *benzylpenicillin sodium EPCRS*.

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 isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.2 mL per minute.
(d) Use a column temperature of 50°.
(e) Use a detection wavelength of 225 nm.
(f) Inject 10 µL of each solution.

MOBILE PHASE

Mix 10 volumes of a 6.8% w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 3.5 with a 50% w/v solution of *dilute orthophosphoric acid*, 36 volumes of *methanol* and 54 volumes of *water*.

DETERMINATION OF CONTENT

Calculate the content of penicillins, as $C_{16}H_{18}N_2O_4S$, in a container of average content weight 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 $C_{16}H_{18}N_2O_4S$.

STORAGE

The sealed container should be stored at a temperature not exceeding 30°.

LABELLING

The label of the sealed container states (1) whether the contents are Benzylpenicillin Potassium or Benzylpenicillin Sodium; (2) the quantity of Benzylpenicillin Potassium or Benzylpenicillin Sodium contained in it in terms of the equivalent amount of benzylpenicillin.

IMPURITIES

The impurities limites by the requirements of this monograph include those listed under Benzylpenicillin Potassium and Benzylpenicillin Sodium.