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

Flucloxacillin for Injection

General Notices

Flucloxacillin for Infusion

Action and use

Penicillin antibacterial.

DEFINITION

Flucloxacillin for Injection is a sterile material consisting of Flucloxacillin Sodium Monohydrate 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 flucloxacillin, $C_{19}H_{17}ClFN_3O_5S$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of flucloxacillin sodium *(RS 145)*.
B. Yield reaction B characteristic of *sodium salts*, Appendix VI.

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 10% w/v of flucloxacillin, 5.0 to 7.0, Appendix V L.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in 50% v/v *acetonitrile*. *Prepare the solutions protected from light.*

(1) Disperse a quantity of the contents of the sealed container containing the equivalent of 0.1 g of flucloxacillin in 50% v/v of *acetonitrile* and dilute to 100 mL.
(2) Dilute 1 volume of solution (1) to 100 volumes.
(3) 0.001% w/v of *flucloxacillin impurity D EPCRS* and 0.1% w/v of *flucloxacillin sodium BPCRS*.
(4) 0.1% w/v of *flucloxacillin for peak identification EPCRS*.
(5) Dilute 1 volume of solution (2) to 10 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 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 40°.
(e) Use a detection wavelength of 225 nm.
(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.118% w/v *sodium hexanesulfonate monohydrate for ion-pair chromatography* in a mixture of 0.8 volumes of *concentrated ammonia* and 1000 volumes of *water for chromatography*. Adjust the pH of the resulting solution to pH 2.9 ± 0.1 with *orthophosphoric acid*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	80→45	20→55	linear gradient
30-35	45→35	55→65	linear gradient
35-40	35→80	65→20	linear gradient
40-45	80	20	re-equilibration

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to flucloxacillin (retention time about 18 minutes) are: impurity C, about 0.1; impurity A (isomer 1), about 0.48; impurity A (isomer 2), about 0.50; impurity F, about 0.55; impurity G, about 0.65; impurity B (isomer 1), about 0.75; impurity B (isomer 2), about 0.8; impurity D, about 0.9; impurity H, about 1.2; impurity E, about 1.25; impurity I, about 1.35; impurity J, about 1.55 and impurity K, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (3), the *resolution* between impurity D and flucloxacillin is at least 1.5.

in the chromatogram obtained with solution (5), the *signal-to-noise ratio* of the principal peak is at least 40.

LIMITS

Identify any peak corresponding to impurities B and C in the chromatogram obtained with solution (1), using the chromatogram obtained with solution (4), and multiply the area of these peaks by a correction factor of 1.3 and 4.2 respectively.

In the chromatogram obtained with solution (1):

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

the sum of the areas of any peaks corresponding to impurity B is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the area of any peaks corresponding to impurity C or E is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to impurity H is not greater than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the area of any peaks corresponding to impurity F, I, J or K is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%);

the area of any peaks corresponding to impurity D or G is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%);

the area of any other *secondary peak* is not greater than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);

the sum of the areas of all *secondary peaks* is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (5.0%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (5) (0.1%).

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 9 mg of flucloxacillin per mL (solution A). The endotoxin limit concentration of solution A is less than 0.35 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 prepared in 50% v/v of acetonitrile.

(1) Disperse a quantity of mixed contents of the 10 containers, containing the equivalent of 0.5 g of flucloxacillin in 50% v/v of acetonitrile and dilute to 200 mL. Dilute 1 volume of the resulting solution to 25 volumes.
(2) 0.011% w/v of *flucloxacillin sodium BPCRS*.
(3) 0.0001% w/v of *flucloxacillin impurity D EPCRS* and 0.01% w/v of *flucloxacillin sodium BPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Zorbax SB-C18 is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1.8 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 225 nm.
(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.118% w/v sodium hexanesulfonate monohydrate for ion-pair chromatography in a mixture of 0.8 volumes of *concentrated ammonia* and 1000 volumes of *water*. Adjust the pH of the resulting solution to pH 3.1 ± 0.1 with *orthophosphoric acid*.

Mobile phase B *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-8	65→41	35→59	linear gradient
8-12	41→65	59→35	linear gradient
12-18	65	35	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between impurity D and flucloxacillin is at least 1.5.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{17}ClFN_3O_5S$ in a container of average content weight using the declared content of $C_{19}H_{16}ClFN_3NaO_5S$ in *flucloxacillin sodium BPCRS*. Each mg of $C_{19}H_{16}ClFN_3NaO_5S$ is equivalent to 0.9538 mg of $C_{19}H_{17}ClFN_3O_5S$.

LABELLING

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

IMPURITIES

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