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Ticarcillin and Clavulanic Acid Infusion

General Notices

Ticarcillin and Clavulanic Acid Intravenous Infusion
Ticarcillin Sodium and Potassium Clavulanate Intravenous Infusion
Ticarcillin and Clavulanic Acid for Intravenous Infusion

Action and use

Penicillin antibacterial + beta-lactamase inhibitor.

DEFINITION

Ticarcillin and Clavulanic Acid Infusion is a sterile solution containing Ticarcillin Sodium and Potassium Clavulanate. It is prepared by dissolving Ticarcillin and Clavulanic Acid for Infusion with a suitable diluent in accordance with the manufacturer's instructions.

The infusion complies with the requirements stated under Parenteral Preparations.

STORAGE

Ticarcillin and Clavulanic Acid Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

TICARCILLIN AND CLAVULANIC ACID FOR INFUSION

DEFINITION

Ticarcillin and Clavulanic Acid for Infusion is a sterile material consisting of Ticarcillin Sodium and Potassium Clavulanate with or without excipients. It is supplied in a sealed container.

PRODUCTION

The methods of production, extraction and purification of Potassium Clavulanate used in the formulation of Ticarcillin and Clavulanic Acid Infusion are such that potassium clavam-2-carboxylate is eliminated or present at a level not exceeding 0.01%.

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 ticarcillin, $C_{15}H_{16}N_2O_6S_2$

90.0 to 105.0% of the stated amount.

Content of clavulanic acid, $C_8H_9NO_5$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Dissolve a quantity of the contents of a sealed container containing the equivalent of 1.5 g of ticarcillin in 5 mL of *water*, add 0.5 mL of *hydrochloric acid R1*, swirl and allow to stand in a bath of iced water for 10 minutes. Filter the precipitate (Whatman GF/C is suitable), wash with 5 mL of *water* and dissolve in a mixture of 1 volume of *water* and 9 volumes of *acetone*. Evaporate the solvent almost to dryness under a stream of nitrogen and then dry in an oven at 60° for 1 hour. The *infrared absorption spectrum*, Appendix II A, is concordant with the *reference spectrum* of ticarcillin (*RS 458*).
B. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions in a mixture of 4 volumes of *methanol* and 6 volumes of *0.1M mixed phosphate buffer pH 7.0*.
(1) Dissolve a quantity of the contents of a sealed container to produce a solution containing the equivalent of 0.4% w/v of clavulanic acid.
(2) 0.4% w/v of *lithium clavulanate EPCRS*.
(3) 0.4% w/v of *ticarcillin monosodium BPCRS*.
(4) Mix equal volumes of solutions (2) and (3).

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F_{254} plate* (Merck silica gel 60 F_{254} plates are suitable). Impregnate the plate by spraying it with a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and allow to dry overnight. Activate the plate by heating at 105° for 1 hour just prior to use.
(b) Use the mobile phase described below.
(c) Apply 1 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

1 volume of *butan-1-ol*, 2 volumes of a 0.1% w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, 6 volumes of *glacial acetic acid* and 10 volumes of *butyl acetate*.

SYSTEM SUITABILITY

In the chromatogram obtained with solution (4), the spot with the lower Rf value corresponds to the principal spot in the chromatogram obtained with solution (2) and the spot with the higher Rf value corresponds to the principal spot in the chromatogram obtained with solution (3) and the spots are clearly separated.

CONFIRMATION

In the chromatogram obtained with solution (1), the spots corresponding to clavulanic acid and ticarcillin correspond in position and colour to the principal spots in the chromatogram obtained with solution (4).

C. In the Assay, the retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to those of the two principal peaks in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH of a solution containing the equivalent of 10% w/v of ticarcillin, 5.5 to 8.0, Appendix V L.

Clavulanate polymer and other fluorescent impurities

Carry out the method for *fluorescence spectrophotometry*, Appendix II E, using the following freshly prepared solutions.

(1) To a quantity of the contents of a sealed container containing the equivalent of 0.1 g of clavulanic acid add 50 mL of a 0.1M phosphate buffer solution pH 5.0, prepared as described below, shake vigorously for 1 minute and then shake with the aid of ultrasound for 5 minutes; add sufficient of the buffer solution to produce 100 mL and filter through a 0.45-µm filter. To prepare the buffer solution dissolve 15.6 g of *sodium dihydrogen orthophosphate* in 800 mL of *water*, adjust the pH to 5.0 using 1M *sodium hydroxide* and add sufficient *water* to produce 1000 mL.
(2) Prepare a solution containing 0.42 µg per mL of *quinine sulfate BPCRS* in 0.5M *sulfuric acid*. [*NOTE:* The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.]

PROCEDURE

Measure the *fluorescence* of the solutions using an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell.

LIMITS

The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid).

Related substances (ticarcillin)

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a solution prepared by dissolving 10.35 g of *sodium dihydrogen orthophosphate monohydrate* in 1500 mL of *water* and adjusting the pH to 6.4 using 10M *sodium hydroxide* (solvent A). The solutions should be freshly prepared.

(1) Dissolve a quantity of the contents of a sealed container in sufficient solvent A to produce a solution containing the equivalent of 0.2% w/v of ticarcillin.
(2) 0.004% w/v of *ticarcillin monosodium BPCRS*.
(3) Dissolve 11 mg of *ticarcillin monosodium BPCRS* and 2.9 mg of *ticarcillin impurity A BPCRS* in 10 mL of 0.1M *sodium hydroxide* and allow to stand for 15 minutes (generation of ticarcillin impurity D). Transfer the solution into a 500 mL volumetric flask quantitatively, washing the beaker out with solvent A. Add 400 mL of solvent A, ensuring that all traces of the degraded solution are washed from the neck of the flask. Add 11 mg of *ticarcillin impurity A BPCRS*, 10 mg of *ticarcillin impurity B BPCRS*, 10 mg of *ticarcillin impurity C BPCRS* and 55 mg of *ticarcillin monosodium BPCRS* to the flask and dilute to 500 mL with solvent A.
(4) Dilute 1 volume of solution (2) to 40 volumes with solvent A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *methylsilyl silica gel for chromatography* (5 µm) with a pore size of 6 nm (ES Industries Chromegabond TMS C1 5u 60A is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 230 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A 54 volumes of *acetonitrile* and 246 volumes of a buffer solution prepared as described below; adjust the pH of the mixture to 3.0 with 2M *orthophosphoric acid*. To prepare the buffer solution dissolve 10.35 g of *sodium dihydrogen orthophosphate monohydrate* and 4.83 g of *tetrabutylammonium bromide* in 3000 mL of *water* and mix.

Mobile phase B Equal volumes of *acetonitrile* and buffer solution; adjust the pH of the mixture to 3.0 with 2M *orthophosphoric acid*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-25	100	0	isocratic
25-40	100→0	0→100	linear gradient
40-48	0	100	isocratic
48-49	0→100	100→0	linear gradient
49-57	100	0	isocratic

When the chromatograms are recorded under the prescribed conditions, the retention time of ticarcillin is about 14 minutes. If necessary, adjust the content of *acetonitrile* in mobile phase A to achieve the stated retention time. Retention times relative to ticarcillin are: ticarcillin impurity B, about 0.37; ticarcillin impurity C, about 0.42; ticarcillin impurity D, about 0.5; ticarcillin impurity A, about 1.6.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to impurity B and impurity C is at least 1.6 and the *resolution factor* between the peaks due to impurity C and impurity D is at least 1.6.

LIMITS

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities A, B, C and D using the chromatogram obtained with solution (3). Multiply the area of any peak corresponding to impurity B by the following correction factor: 0.5; multiply the area of any peak corresponding to impurity C by the following correction factor: 0.6.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (4%);

the area of any peak corresponding to impurity D is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (6%);

the area of any other *secondary peak* is not greater than 1.25 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%);

the sum of the areas of all the *secondary peaks* is not greater than six times the area of the principal peak in the chromatogram obtained with solution (2) (12%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

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 ticarcillin per mL (solution A). The endotoxin limit concentration of solution A is 0.7 IU of endotoxin per mL.

Water

Not more than 4.5% w/w, Appendix IX C. Use 0.3 g.

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 a solution prepared by dissolving 7.8 g of *sodium dihydrogen orthophosphate* in 1000 mL of *water* and adjusting the pH to 6.4 using 5M *sodium hydroxide* (solvent B). The solutions should be stored at 2° to 8° and used within 4 hours.

(1) Dissolve a quantity of the mixed contents of the 10 containers in sufficient *water* to produce a solution containing the equivalent of 1.4% w/v of ticarcillin. Dilute 1 volume to 10 volumes with solvent B.
(2) 0.15% w/v of *ticarcillin monosodium BPCRS* and 0.01% w/v of *lithium clavulanate EPCRS* in solvent B.
(3) 0.1% w/v of each of *ampicillin trihydrate BPCRS* and *ticarcillin monosodium BPCRS* in solvent B.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with *end-capped phenylethyl silica gel for chromatography* (4 µm) (Phenomenex Synergi 4µ Polar-RP 80A is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use a column temperature of 30°.
(e) Use a detection wavelength of 230 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 31.2 g of *sodium dihydrogen orthophosphate* in 4000 mL of *water* and adjust the pH to 3.9 using 1M *orthophosphoric acid*.

Mobile phase B *acetonitrile*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	100	0	isocratic
1-1.5	100→92	0→8	linear gradient
1.5-6	92	8	isocratic
6-7	92→50	8→50	linear gradient
7-8	50→100	50→0	linear gradient
8-9	100	0	isocratic

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks due to ampicillin and ticarcillin is at least 4.0 and the *symmetry factor* of the peak corresponding to ticarcillin is less than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{15}H_{16}N_2O_6S_2$ and $C_8H_9NO_5$ in a container of average content weight using the declared content of $C_{15}H_{15}N_2NaO_6S_2$ in *ticarcillin monosodium BPCRS* and the declared content of $C_8H_8LiNO_5$ in *lithium clavulanate EPCRS*. Each mg of $C_{15}H_{15}N_2NaO_6S_2$ is equivalent to 0.9459 mg of $C_{15}H_{16}N_2O_6S_2$. Each mg of $C_8H_8LiNO_5$ is equivalent to 0.9711 mg of $C_8H_9NO_5$

LABELLING

The label of the sealed container states the quantity of Ticarcillin Sodium contained in it, in terms of the equivalent amount of ticarcillin, and the quantity of Potassium Clavulanate, in terms of the equivalent amount of clavulanic acid.

The label of the sealed container states that the preparation contains penicillin.

IMPURITIES

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