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Cilastatin and Imipenem for Infusion

Action and use
Dehydropeptidase-I inhibitor; inhibition of the renal metabolism of imipenem; Carbapenem antibacterial.

DEFINITION
Cilastatin and Imipenem for Infusion is a sterile mixture of Cilastatin Sodium and Imipenem 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 cilastatin, $C_{16}H_{25}N_2NaO_5S$
95.0 to 105.0% of the stated amount.

Content of imipenem, $C_{12}H_{17}N_3O_4S$
95.0 to 105.0% of the stated amount.

CHARACTERISTICS
White to light yellow powder.

IDENTIFICATION
A. To 1g of the powder for injection, add 4 ml of methanol and shake for 5 minutes. Add a further 3 ml of methanol and shake for a further 10 minutes. Filter the resulting suspension and reserve the filtrate for test B. The infrared absorption spectrum of the dried residue, Appendix II A, is concordant with the reference spectrum of Imipenem Monohydrate.

B. Dry the filtrate reserved in test A at a temperature not exceeding 40°. The infrared absorption spectrum of the dried filtrate, Appendix II A, is concordant with the reference spectrum of Cilastatin Sodium.

TESTS

Acidity or alkalinity
pH of the reconstituted powder, 6.5 to 8.5, Appendix V L.

Colour of solution
A solution containing the equivalent of 1% w/v of cilastatin in *water* is not more intensely coloured than solution, Y_6, Appendix IV B.

Related substances
Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions in a 0.9% w/v solution of *Sodium Chloride* in *Water*. Prepare the solutions immediately before use.

(1) Dissolve a quantity of the contents of a sealed container containing the equivalent of 32 mg of cilastatin in *water*, and dilute to 20 ml.

(2) Dilute 1 volume of solution (1) to 200 volumes.

(3) 0.15% w/v *Cilastatin for system suitability 1 EPCRS*

(4) 0.15% w/v *Cilastatin for system suitability 2 EPCRS*

(5) Dissolve 5 mg of Imipenem EPCRS in 8 mL of a 0.006% w/v Sulphuric acid solution. Allow to settle at room temperature for 5 minutes, add 10 mg sodium carbonate and dilute to 10 mL with water.

(6) Dilute 1 volume of solution 2 to 5 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (YMC Pack ODS-AQ 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 30°.

(e) Use a detection wavelength of 210 nm.

(f) Use an autosampler temperature of 5 °.

(g) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 1.36 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust the pH to 5.6 with a 10% w/v solution of *Potassium hydroxide*.

Mobile phase B Equal volumes of acetonitrile and a phosphate buffer solution pH 3.0 prepared in the following manner.

Dissolve 1.36 g of *potassium dihydrogen orthophosphate* in 1000 mL of *water* and adjust the pH to 3.0 with a 5% v/v solution of *orthophosphoric acid.*

Time (Minutes)	Mobile phase A%	Mobile phase B%	Comment
0-3	100	0	isocratic
3-30	100→90	0→10	linear gradient
30-68	90→60	10→40	linear gradient
68-78	60→30	40→70	Linear gradient
78-88	30	70	isocratic

When the chromatograms are recorded under the prescribed conditions the retention times relative to cilastatin (retention time, about 48 minutes) are: imipenem impurity B, about 0.08 and 0.09; imipenem impurity A, about 0.18; imipenem, about 0.22; impurity A, about 0.64 and 0.66; impurity F, about 0.98; impurity G, about 1.04 and 1.07; impurity D, about 1.15; impurity H, about 1.20; impurity B, about 1.30; impurity C, about 1.40.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *peak-to-valley ratio* is at least 1.8 where H_p is the height above the baseline of the peak due to Impurity G (epimer 1) and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity G (epimer 2).

The test is not valid unless, in the chromatogram obtained with solution (4), the *peak-to-valley ratio* is at least 1.8 where H_p is the height above the baseline of the peak due to impurity B (epimer 1) and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity B (epimer 2).

The test is not valid unless, in the chromatogram obtained with solution (6), the *signal to noise ratio* for the peak due to imipenem is not less than 25 and the *signal to noise ratio* for the peak due to cilastatin is not less than 40.

LIMITS

for imipenem

Identify any peaks in the chromatogram obtained with solution (1) corresponding to imipenem impurities A and B using the chromatogram obtained with solution (5) and the relative retention times.

Multiply the area of any peak corresponding to imipenem impurity A by a correction factor of 2.4.

In the chromatogram obtained with solution (1):

the area of any peak corresponding to imipenem impurity A is not greater than twice the area of the peak due to imipenem in the chromatogram obtained with solution (2) (1.0%);

the area of any peak corresponding to imipenem impurity B is not greater than 0.6 times the area of the peak due to imipenem in the chromatogram obtained with solution (2) (0.3% each);

the sum of the areas of any secondary peaks due to imipenem is not greater than 4 times the area of the peak due to imipenem in the chromatogram obtained with solution (2) (2.0%).

Disregard any peak with an area less than the area of the peak due to imipenem in the chromatogram obtained with solution (6) (0.1%) and any peak eluting after 20 minutes.

for cilastatin

Identify any peaks in the chromatogram obtained with solution (1) corresponding to cilastatin impurities A, B, C, D, E, F and G using the chromatograms obtained with solutions (3) and (4) and the relative retention times.

Multiply the area of any peak corresponding to cilastatin impurity C by a correction factor of 1.3.

Multiply the area of any peak corresponding to cilastatin impurity E by a correction factor of 3.3.

Multiply the area of any peak corresponding to cilastatin impurity G by a correction factor of 1.6.

the sum of the areas of any peak corresponding to cilastatin impurity A is not greater than the area of the peak due to cilastatin in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to cilastatin impurity C is not greater than 0.8 times the area of the peak due to cilastatin in the chromatogram obtained with solution (2) (0.4%);

the area of any peak corresponding to cilastatin impurity E is not greater than 0.6 times the area of the peak due to cilastatin in the chromatogram obtained with solution (2) (0.3%);

the area of any peak corresponding to cilastatin impurity B, F, G or H is not greater than 1.5 times the area of the peak due to cilastatin in the chromatogram obtained with solution (6) (0.15% each);

the area of any other *secondary peak* is not greater than the area of the peak due to cilastatin in the chromatogram obtained with solution (6) (0.1%);

the sum of the areas of any secondary peaks due to cilastatin is not greater than 3 times the area of the peak due to cilastatin in the chromatogram obtained with solution (2) (1.5%);

Disregard any peak with an area less than the area of the peak due to cilastatin in the chromatogram obtained with solution (6) (0.1%), any peak due to impurity D and any peak eluting before 20 minutes.

Loss on drying

When dried *in vacuo* at 60° at a pressure not exceeding 0.67 kPa for 3 hours, lose not more than 3.5% of their weight. 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 the diluent.

diluent Prepare a mixture of 0.002% w/v sodium bicarbonate, 0.18% w/v sodium chloride, 0.0135% w/v *potassium dihydrogen orthophosphate* previously adjusted to pH 6.8 with *sodium hydroxide* or *orthophosphoric acid*.

(1) Disperse a quantity of mixed contents of the 10 containers containing the equivalent of 0.5 g of cilastatin and 0.5g of imipenem in diluent and dilute to 100 mL. Dilute 1 volume of this solution to 10 volumes.

(2) 0.05% w/v of *imipenem EPCRS*.

(3) 0.05% w/v of *cilastatin sodium EPCRS*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Hypersil-ODS is suitable).

(b) Use isocratic elution and the mobile phase described below.

(c) Use a flow rate of 2 mL per minute.

(d) Use a column temperature of 50 °.

(e) Use a detection wavelength of 254 nm.

(f) Inject 10 µL of each solution.

MOBILE PHASE

Dissolve 0.54g *Potassium dihydrogen orthophosphate* and 2.0g of *Sodium hexanesulphonate* in 800 mL water. Adjust the pH to pH 6.8 with 0.5 M *sodium hydroxide* or 0.5 M *orthophosphoric acid*. Dilute to 1000 mL with water.

SYSTEM SUITABILITY

The Assay is not valid unless, in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to imipenem is not greater than 2.0.

The Assay is not valid unless, in the chromatogram obtained with solution (3), the *symmetry factor* of the peak due to cilastatin is not greater than 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{12}H_{17}N_3O_4S,H_2O$ in a container of average content weight from the chromatograms obtained and from the declared content of $C_{12}H_{17}N_3O_4S,H_2O$ in *Imipenem EPCRS.*

Calculate the content of $C_{16}H_{25}N_2NaO_5S$ in a container of average content weight from the chromatograms obtained and from the declared content of $C_{16}H_{25}N_2NaO_5S$ in *cilastatin sodium EPCRS.*

IMPURITIES

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