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

Insulin Aspart Injection

General Notices

Action and use

Hormone; treatment of diabetes mellitus.

DEFINITION

Insulin Aspart Injection is a sterile, neutral, aqueous solution of Insulin Aspart.

The injection complies with the requirements stated under Injectable Insulin Preparations with the modifications described below.

Content of insulin aspart, $C_{256}H_{381}N_{65}O_{79}S_6$

90.0 to 110.0% of the stated amount.

CHARACTERISTICS

A colourless liquid, free from turbidity and foreign matter; during storage traces of a very fine sediment may be deposited.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Related proteins

Carry out the method for *liquid chromatography*, Appendix III D, as described under Assay using the *normalisation procedure*.

LIMITS

In the chromatogram obtained with solution (1), the area of the peak corresponding to B28isoAsp insulin aspart is not more than 2.5%, the total area of the peaks corresponding to A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart is not more than 5%, and the total area of other peaks corresponding to impurities is not more than 3.5%.

Total zinc

Not more than 40 µg per 100 units of insulin aspart, determined by *atomic absorption spectrometry*, Appendix II D Method I.

Test solution Shake the preparation gently and dilute a volume containing 100 units of insulin aspart to 25.0 mL with 0.01M *hydrochloric acid*. Dilute, if necessary, to a suitable concentration of zinc (for example 0.1 µg to 1.0 µg of Zn per millilitre) with 0.01M *hydrochloric acid*.

Reference solutions Use solutions containing a suitable range of concentrations, for example 0.20 µg, 0.40 µg, 0.60 µg, 0.80 µg and 1.00 µg of Zn per millilitre, freshly prepared by diluting *zinc standard solution (5 mg/mL Zn)* with 0.01M *hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 80 IU of endotoxin per 100 units of insulin aspart.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to produce a solution containing 100 units/mL of insulin aspart (equivalent to approximately 0.35% w/v insulin aspart). Add 4 µL of *6M hydrochloric acid* per mL to this solution to obtain a clear acid solution. Maintain the solution at 2° to 8° and use within 48 hours.
(2) Dissolve the contents of a vial of *insulin aspart EPCRS* in 0.01M *hydrochloric acid* to produce a solution containing 0.4% w/v insulin aspart. Maintain the solution at 2° to 8° and use within 48 hours.
(3) Use an appropriate solution with a content of B3Asp insulin aspart and A21Asp insulin aspart of not less than 1%. This may be achieved by storing solution (2) at room temperature for 1 to 3 days. Maintain the solution at 2° to 8° and use within 72 hours.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP18 is suitable).
(b) Use gradient elution and the mobile phases described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 35°.
(e) Use a detection wavelength of 214 nm.
(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 70 g of *anhydrous sodium sulfate* in approximately 4500 mL of *water*; add 6.5 mL of *orthophosphoric acid* and adjust to pH 3.4, if necessary, with *dilute sodium hydroxide solution*. Dilute to 5000 mL with *water*; filter and degas. Mix 9 volumes of the solution with 1 volume of *acetonitrile R1*.

Mobile phase B Mix equal volumes of *water* and *acetonitrile R1*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-35	58	42	Isocratic
35-40	58 → 20	42 → 80	Linear gradient
40-45	20	80	Isocratic
45-46	20 → 58	80 → 42	Linear gradient
46-60	58	42	Re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retention times with reference to insulin aspart (retention time, 20 to 26 minutes) are: B28isoAsp insulin aspart, approximately 0.9; B3Asp insulin aspart plus A21Asp insulin aspart (generally co eluted), approximately 1.3; B3isoAsp insulin aspart, approximately 1.5.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peak due to insulin aspart and the peak due to A21Asp insulin aspart plus B3Asp insulin aspart is greater than 1.6.

The test is not valid unless the *symmetry factor* of the principal peak in the chromatogram obtained with solution (2) is less than 1.8.

DETERMINATION OF CONTENT

Calculate the content of insulin aspart $C_{256}H_{381}N_{65}O_{79}S_6$, together with the content of B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart using the areas of the corresponding peaks in the chromatograms obtained with solution (1) and solution (2) and the declared content of insulin aspart together with the content of B28isoAsp insulin aspart, A21Asp insulin aspart, B3Asp insulin aspart and B3isoAsp insulin aspart[1] in *insulin aspart EPCRS*.

LABELLING

The label states the potency in units per mL.

1 100 units are equivalent to 3.50 mg of insulin aspart.