Insulin Aspart Injection

DEFINITION

Insulin Aspart Injection is an isotonic, sterile solution of Insulin Aspart in Water for Injection. It has a potency of NLT 95% and NMT 105% of the potency stated on the label, expressed in USP Insulin Aspart Units/mL. [NOTE—1 USP Insulin Aspart Unit is equivalent to 0.0350 mg of pure insulin aspart.]

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Dissolve 70 g of anhydrous sodium sulfate in approximately 4500 mL of water, add 6.5 mL of phosphoric acid, and adjust with sodium hydroxide TS to a pH of 3.4. Dilute with water to 5000 mL. Mix 900 mL of this solution with 100 mL of acetonitrile.

Solution B: Acetonitrile and water (1:1)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	56	44
35	56	44
40	20	80
45	20	80
46	56	44
60	56	44

[NOTE—If necessary, adjust the *Mobile phase* composition to obtain a retention time of insulin aspart of 20–26 min and to ensure that the preservatives are well separated from B28isoAsp insulin aspart peak. If necessary, adjust the start time of the gradient to ensure that B3isoAsp insulin aspart is eluted before the gradient starts.]

System suitability solution: Use an appropriate solution with a content of B3Asp insulin aspart and A21Asp insulin aspart of NLT 1%. This may be achieved by storing the *Standard solution* at room temperature for about 1–3 days.

Standard solution: Dissolve the contents of 1 vial of USP Insulin Aspart RS in 0.01 N hydrochloric acid to obtain a known concentration of 100 USP Insulin Aspart Units/mL. Add 4 µL of 6 N hydrochloric acid per milliliter, and mix.

Sample solution: Acidify each milliliter of Injection with 4 µL of 6 N hydrochloric acid. Dilute, if necessary, a portion of the acidified solution with 0.01 N hydrochloric acid to obtain a solution containing about 100 USP Insulin Aspart Units/mL.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.0-mm × 25.0-cm; 5-µm packing L1

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for B28isoAsp insulin aspart, insulin aspart, B3Asp insulin aspart plus A21Asp insulin aspart (generally coelute), and B3isoAsp insulin aspart are about 0.9, 1.0, 1.3, and 1.5 min, respectively.]

Suitability requirements

Resolution: NLT 1.6 between the peak for insulin aspart and the peak for A21Asp insulin aspart and for B3Asp insulin aspart, *System suitability solution*

Relative standard deviation: NMT 1.4% for 5 replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency, in USP Insulin Aspart Units/mL, of Injection taken:

$$\text{Result} = C_S \times D \times (r_U/r_S)$$

C_S = concentration of insulin aspart in the *Standard solution* (USP Insulin Aspart Units/mL)

D = dilution factor used to prepare the *Sample solution*

r_U = peak area of insulin aspart (sum of B28isoAsp insulin aspart, insulin aspart, B3Asp insulin aspart, A21Asp insulin aspart, and B3isoAsp insulin aspart peak areas) from the *Sample solution*

r_S = peak area of insulin aspart (sum of B28isoAsp insulin aspart, insulin aspart, B3Asp insulin aspart, A21Asp insulin aspart, and B3isoAsp insulin aspart peak areas) from the *Standard solution*

Acceptance criteria: 95%–105% of the potency stated on the label, expressed in USP Insulin Aspart Units/mL

IMPURITIES

• **RELATED PROTEINS**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay* using the normalization procedure.

Acceptance criteria

Individual impurities: NMT 2.5% of B28isoAsp insulin aspart; NMT 5.0% total of the peaks due to A21Asp insulin aspart, B3Asp insulin aspart, and B3isoAsp insulin aspart

Total of other impurities: NMT 3.5%

• **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩**,** *Limit of High Molecular Weight Proteins***:** Meets the requirements

Sample solution: Acidify each milliliter of Injection with 4 µL of 6 N hydrochloric acid. Dilute, if necessary, a portion of the acidified solution with 0.01 N hydrochloric acid to obtain a solution containing about 100 USP Insulin Aspart Units/mL.

Acceptance criteria: NMT 1.5%

SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units per 100 USP Insulin Aspart Units

• **STERILITY TESTS** ⟨71⟩**,** *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements

• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections

• **pH** ⟨791⟩**:** 7.0–7.8, determined potentiometrically

Change to read:

▲• **Zinc Determination** ⟨591⟩:▲ (IRA 1-Jan-2019) 10–40 µg for every 100 USP Insulin Aspart Units
• **Injections and Implanted Drug Products** ⟨1⟩**:** It meets the requirements.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in the unopened, multi-dose container provided by the manufacturer. Store in a refrigerator, protect from sunlight, and avoid freezing.
• **Labeling:** The label states that it has been prepared with insulin aspart obtained from microbial synthesis; it is to be stored in a refrigerator and that freezing is to be avoided; the potency, expressed in USP Insulin Aspart Units/mL.
• **USP Reference Standards** ⟨11⟩
USP Insulin Aspart RS