Insulin Lispro Injection

DEFINITION

Insulin Lispro Injection is an isotonic, sterile solution of Insulin Lispro in Water for Injection. It has a potency of NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Lispro Units/mL.

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: 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.3.

Mobile phase: Acetonitrile and *Solution A* (51:149)

System suitability solution: 1 mg/mL of insulin lispro in 0.01 N hydrochloric acid. Allow to stand at room temperature to obtain a solution containing 0.8%–11% of A-21 desamido insulin lispro.

Standard solution: 0.7 mg/mL of USP Insulin Lispro RS in 0.01 N hydrochloric acid

Sample solution: Acidify each milliliter of Injection with 3 µL of 9.6 N hydrochloric acid. Quantitatively dilute a portion of the acidified solution with 0.01 N hydrochloric acid to obtain a solution containing 20 USP Insulin Lispro Units/mL.

Chromatographic system

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

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; packing L1

Column temperature: 40°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Adjust the *Mobile phase* to obtain a retention time of about 24 min for the main insulin lispro peak.

Sample: *System suitability solution* (3 replicate injections)

Suitability requirements

Resolution: NLT 3.0 between insulin lispro and A-21 desamido insulin lispro

Tailing factor: NMT 1.5 for the insulin lispro peak

Relative standard deviation: NMT 1.1% for the insulin lispro peak

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

r_U = peak response of insulin lispro from the *Sample solution*

r_S = peak response of insulin lispro from the *Standard solution*

C_S = concentration of USP Insulin Lispro RS in the *Standard solution* (USP Insulin Lispro Units/mL)

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

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Lispro Units/mL

OTHER COMPONENTS

Change to read:

▲• **ZINC DETERMINATION** ⟨591⟩:▲ (IRA 1-Jan-2019) 14–35 µg for every 100 USP Insulin Lispro Units

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

Change to read:

• **RELATED SUBSTANCES**

Solvent: 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Adjust with phosphoric acid to a pH of 2.3.

Solution A: Acetonitrile and *Solvent* (18:82)

Solution B: Acetonitrile and *Solvent* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	81	19
60	81	19
83	51	49
84	81	19
94	81	19

System suitability solution: 3.5 mg/mL of insulin lispro in 0.01 N hydrochloric acid. Allow to stand at room temperature to obtain a solution containing 0.8%–11% of A-21 desamido insulin lispro.

Sample solution: Acidify each milliliter of Injection with 3 µL of 9.6 N hydrochloric acid.

Chromatographic system

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

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Adjust the *Mobile phase* composition and the duration of the isocratic elution to obtain a retention time of about 41 min for the main insulin lispro peak, with A-21 desamido insulin lispro eluting just prior to the start of the gradient elution phase.

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 2.5 between insulin lispro and A-21 desamido insulin lispro

Tailing factor: NMT 2.0 for the insulin lispro peak

Analysis

Sample: *Sample solution*

Calculate the percentage of insulin lispro, A-21 desamido insulin lispro, and other impurities in the portion of ▲Injection▲ (ERR 1-Sep-2018) taken.

Calculate the percentage of insulin lispro (%*I*):

$$\text{Result} = (r_I/r_T) \times 100$$

r_I = peak response of insulin lispro from the *Sample solution*

r_T = sum of the responses of all the peaks from the *Sample solution*

Calculate the percentage of A-21 desamido insulin lispro (%*D*):

$$\text{Result} = (r_D/r_T) \times 100$$

r_D = peak response of A-21 desamido insulin lispro from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Calculate the percentage of other insulin lispro-related substances:

$$\text{Result} = 100 - (\%I + \%D)$$

Acceptance criteria
Individual impurities: NMT 1.50% of A-21 desamido insulin lispro
Total impurities: NMT 4.00%, excluding A-21 desamido insulin lispro

- **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩**,** *Limit of High Molecular Weight Proteins*

Proceed as directed in the chapter, except for the *Sample solution*. It meets the requirements.
Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid to each milliliter of an accurately measured volume of Injection, and mix.
Acceptance criteria: NMT 1.50%

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 7.0–7.8
- **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**,** *Photometric Quantitative Techniques, Chromogenic Technique***:** NMT 80 USP Endotoxin Units per 100 USP Insulin Lispro Units, using the kinetic-chromogenic assay
- **STERILITY TESTS** ⟨71⟩**,** *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, multiple-dose containers. Dispense in the unopened, multiple-dose container provided by the manufacturer. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **LABELING:** Label it to indicate that it has been prepared with Insulin Lispro produced by methods based on recombinant DNA technology. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Lispro Units/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Insulin Lispro RS