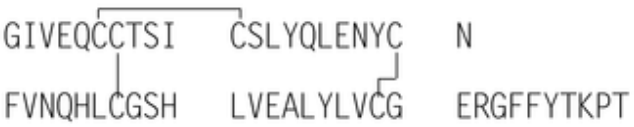
Insulin Lispro



$C_{257}H_{383}N_{65}O_{77}S_6$ 5807.57
Insulin (human), 28^B-L-lysine-29^B-L-proline-;
28^B-L-Lysine-29^B-L-prolineinsulin (human) [133107-64-9].

DEFINITION

Insulin Lispro is identical in structure to Insulin Human, except that it has lysine and proline at positions 28 and 29, respectively, of the B-chain, whereas this sequence is reversed in Insulin Human. Insulin Lispro is produced by methods based on recombinant DNA technology. The presence of host cell DNA in Insulin Lispro is process-specific. The capability of the process to clear host-derived DNA requires validation and is determined by validated methods. Its potency is NLT 27.0 USP Insulin Lispro Units/mg, calculated on the dried basis.
[NOTE—1 USP Insulin Lispro Unit is equivalent to 0.0347 mg of pure Insulin Lispro.]

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*.
- **B. PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩**,** *Peptide Mapping*

Proceed as directed in the chapter, except for the *Flow rate* in the *Chromatographic system* and *System suitability*.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Flow rate: 0.8 mL/min
System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 3.4 between digest fragments II and III
Tailing factor: NMT 1.5 for digest fragments II and III
Chromatogram similarity: Identify the peaks due to digest fragments I, II, III, and IV in the *Standard solution*. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with USP Insulin Lispro RS.
Acceptance criteria: Meets the requirements

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: About 0.7 mg/mL of USP Insulin Lispro RS in 0.01 N hydrochloric acid
Sample solution: About 0.8 mg/mL of Insulin Lispro in 0.01 N hydrochloric acid
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 on the undried basis, in USP Insulin Lispro Units/mg, of insulin lispro in the *Sample solution*:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Insulin Lispro RS in the *Standard solution* (USP Insulin Lispro Units/mL)
C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NLT 27.0 USP Insulin Lispro Units/mg on the dried basis

OTHER COMPONENTS

Change to read:

▲• **ZINC DETERMINATION** ⟨591⟩▲ (IRA 1-Jan-2019)
Acceptance criteria: 0.30%–0.60% on the dried basis

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

- **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: 3.5 mg/mL of Insulin Lispro in 0.01 N hydrochloric acid. [NOTE—Store this solution for NMT 56 h in a refrigerator.]
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 before 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 Insulin Lispro 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 1.00% of A-21 desamido insulin lispro

Other individual impurities: NMT 0.50% of any insulin lispro-related substance

Total impurities: NMT 2.00%, excluding A-21 desamido insulin lispro

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

Acceptance criteria: NMT 0.25%

PROCESS-RELATED IMPURITIES

- **SINGLE-CHAIN PRECURSOR CONTENT:** The single-chain precursor content of Insulin Lispro is NMT 10 ng/mg, determined by a validated method.
- **HOST CELL PROTEIN:** The residual host cell protein content is NMT 10 ng/mg, determined by a validated method or demonstrated by a validated process.

SPECIFIC TESTS

- **INSULIN ASSAYS** ⟨121⟩**,** *Assay, Bioidentity Test*

Analysis: Proceed as directed in the chapter, except obtain the first blood specimen at 45 min, instead of 1 h, after the time of injection.

Acceptance criteria: Meets the requirements

- **LOSS ON DRYING** ⟨731⟩

Sample: 300 mg

Analysis: Dry the *Sample* at 105° for 16 h.

Acceptance criteria: NMT 10.0%

- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**,** *Photometric Quantitative Techniques, Chromogenic Technique***:** NMT 10 USP Endotoxin Units/mg of Insulin Lispro, using the kinetic-chromogenic assay
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count does not exceed 10^2 cfu/g, the test being performed on a portion of about 0.3 g, accurately weighed.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a freezer and protect from light.
- **LABELING:** Label it to indicate that it has been produced by methods based on recombinant DNA technology.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP Insulin Lispro RS