Insulin Lispro

Insulin Lispro is a 2-chain peptide containing 51 amino acids. The A-chain is composed of 21 amino acids and the B-chain is composed of 30 amino acids. It is identical in primary structure to human insulin, only differing in amino acid sequence at positions 28 and 29 of the B-chain. Human insulin is Pro (B28), Lys (B29), whereas insulin Lispro is Lys (B28), Pro (B29). As in human insulin, insulin Lispro contains 2 interchain disulfide bonds and 1 intrachain disulfide bond.

Insulin Lispro is produced by a method based on recombinant DNA (r-DNA) technology. The potency is not less than 27.0 Insulin Lispro units per mg calculated on the dried basis.

Prior to release, the following tests are carried out on each batch of the final substances.

Residual DNA
The limit is as prescribed by competent authority.

Host cell-derived protein
The limit is not more than 10 ppm.

Single-chain precursor
The limit is not more than 10 ppm. Use a suitably sensitive method.

The content of insulin Lispro is 94.0% to 104.0% of insulin Lispro (dried substance) C\textsubscript{257}H\textsubscript{383}N\textsubscript{65}O\textsubscript{77}S\textsubscript{6}. By convention, for the purpose of labeling insulin lispro preparations, 0.0347mg insulin lispro is equivalent to 1 unit of insulin lispro.

Category
Hypoglycemia
Description
A White or almost white powder.

Solubility
Practically insoluble in water and in ethanol (96 percent). It dissolves in dilute mineral acids and with decomposition in dilute solutions of alkali hydroxides.

Identification
A: In the Assay, principal peak in the chromatogram of the test solution corresponds to that in the chromatogram of the reference solution.

B: Determine by Liquid Chromatography (2.4.14) the peptide fragments, using the following peptide mapping procedure:

Test solution. Prepare a 2.0 mg/ml solution of the substance to be examined in 0.01 M hydrochloric acid and transfer 500 µl of this solution to a clean tube. Add 2 ml of HEPES buffer solution pH 7.5 and 400 µl of a 1 mg/ml solution of Staphylococcus aureus strain V8 protease. Cap the tube and incubate at 25°C for 6 h. Stop the reaction by adding 2.9 ml of sulphate buffer solution pH 2.0.

Reference solution. Prepare at the same time and in the same manner as for the test solution, but using Insulin Lispro RS instead of the substance to be examined.

Chromatographic system
Column:
• size: l= 0.10m, ø =4.6mm
• stationary phase: octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter.
• temperature: 40°C

Mobile phase:
Mobile phase A: mix 200 ml of sulphate buffer solution pH 2.0, 700 ml of water and 100 ml of acetonitrile; filter and degas.

Mobile phase B: mix 200 ml of sulphate buffer solution pH 2.0, 400 ml of water and 400 ml of acetonitrile; filter and degas.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%)</th>
<th>Mobile phase B (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0- 3</td>
<td>95</td>
<td>5</td>
<td>Isocratic</td>
</tr>
<tr>
<td>3- 30</td>
<td>95 → 41</td>
<td>5 → 59</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30- 35</td>
<td>41 → 20</td>
<td>59 → 80</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>35- 40</td>
<td>20 → 95</td>
<td>80 → 5</td>
<td>Return to initial</td>
</tr>
<tr>
<td>40- 50</td>
<td>95</td>
<td>5</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Flow rate: 0.8 ml/min.
Detection: spectrophotometer at 214 nm.
Equilibration: at initial conditions for at least 15 min. Carry out a blank run using the above-mentioned gradient.
Injection: Equal volumes of test solution and reference solution
System suitability:
- the chromatogram obtained with the test solution and the reference solution are qualitatively similar to the chromatogram of insulin lispro digest supplied with insulin lispro RS,
- in the chromatogram obtained with the reference solution, identify the peaks due to digest fragments I, II, III and IV

Symmetry factor: maximum 1.5, for the peaks due to fragments II and III,
Resolution: minimum 3.4, between the peaks due to fragments II and III.

Results: the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

NOTE: Fragment I elutes at the same time in insulin derived from pork and Insulin Human; Fragment II elutes at the same time in all insulins; and Fragment III elutes at the same time in insulin derived from beef and pork.

Fragment I consists of amino acids A5 to A17 and B1 to B13. Fragment II consists of amino acids A18 to A21 and B14 to B21. Fragment III consists of amino acids B22 to B30. Fragment IV consists of amino acids A1 to A4. A refers to the A chain of Insulin Human, and B refers to the B- chain of Insulin Human.

**Bacterial endotoxins** (2.2.3, method D)
It contains not more than 10 Endotoxin Units per mg insulin lispro by Kinetic chromogenic method.

**Loss on drying** (2.4.19)
Dry about 300 mg, accurately weighed, at 105°C for 16 hours in an oven: it loses not more than 10.0% of its weight.

**Sulphated ash** (2.3.18)
Not more than 2.5 percent, determined on 0.3 g (dried substance)

**Zinc**
Under insulin preparations
Not more than 1.0 per cent (dried substance) by Atomic absorption spectrometry

**Impurities with molecular masses greater than that of Insulin Lispro**
Determine by Size Exclusion Chromatography 2.4.16
Proceed as directed in the test for Impurities with molecular masses greater than that of Insulin except that Insulin containing the indicated percentage of high molecular mass proteins may be prepared by allowing insulin powder to stand at room temperature for about 5 days.
Not more than 0.25% is found.

**Related proteins**
Determine by Liquid Chromatography 2.4.14.

Test solution: dissolve 3.5 mg of the substance to be examined in 1.0 ml of 0.01 M hydrochloric acid. Maintain the solution at 2-8°C and use with in 56 h.
Resolution solution: dissolve 3.5 mg of the substance to be examined in 1.0 ml of 0.01 M hydrochloric acid. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% of A21 desamido insulin Lispro.

Chromatographic system
Column:
- Size: l=0.25 m, ø = 4.6 mm
- Stationary phase: octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter.
- Temperature: 40°C

Mobile phase:
Mobile phase A: mix 82 volumes of a 28.4 g/l solution of anhydrous sodium sulfate adjusted to pH 2.3 with phosphoric acid and 18 volumes of acetonitrile; filter and degas

Mobile phase B: mix equal volumes 28.4 g/l solution of anhydrous sodium sulfate adjusted to pH 2.3 with phosphoric acid and acetonitrile; filter and degas.

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<td>84 - 94</td>
<td>81</td>
<td>19</td>
<td>Re-equilibration</td>
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Flow rate: 1 ml/min.
Detection: spectrophotometer at 214 nm.
Injection: 20µl

Retention time: adjust the mobile phase composition to obtain a retention time of about 41 min for insulin Lispro; A21 desamido insulin Lispro elutes near the start of the gradient elution.

System suitability:
Resolution solution: resolution not less than 2.5 between the insulin lispro peak and the A21 desamido insulin lispro peak

Symmetry factor: maximum 2.0 for the peak due to insulin Lispro.

Limits:
- A21 desamido insulin lispro not more than 1.0%;
- any other impurity not more than 0.50%;
- total impurities (excluding A21) not more than 2.0%

Assay
Determine by Liquid Chromatography 2.4.14.
Test solution: dissolve the substance to be examined in 0.01 M hydrochloric acid to obtain a concentration of about 0.8 mg per ml

Reference solution: dissolve insulin Lispro RS in 0.01 M hydrochloric acid to obtain a concentration of about 0.7 mg per ml
Resolution solution: dissolve an accurately weighed quantity of insulin Lispro in 0.01 M hydrochloric acid to obtain a concentration of 1 mg per ml. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% of A21 desamido insulin Lispro.

Column:
- size: \( l = 0.10 \, \text{m}, \, \varnothing = 4.6 \, \text{mm} \)
- stationary phase: octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 \( \mu \text{m} \) in diameter
- temperature: 40°C

Mobile phase: mix 745 ml of 28.4 g/l solution of anhydrous sodium sulfate adjusted to pH 2.3 with phosphoric acid and 255 ml of acetonitrile; filter and degas. Make adjustments if necessary.

Adjust the mobile phase to provide a retention time of about 24 min for main insulin lispro peak.

Flow rate: 0.8 ml/min.
Detection: spectrophotometer at 214 nm.
Injection: 20 \( \mu \text{l} \).

Procedure: separately inject the reference solution, the test solution and the resolution solution into the chromatograph, record the chromatograms and measure the peak areas.

System suitability:
- resolution: between insulin Lispro and A21 desamido insulin Lispro is not less than 3.0
- symmetry factor for insulin Lispro peak is not more than 1.5
- relative standard deviation for 3 replicate injects is not more than 1.1%

Calculate the content of insulin Lispro \( C_{257}H_{383}N_{65}O_{77}S_{6} \) on the as-is basis using the areas of the corresponding peaks in the chromatograms obtained with the test solution and reference solution and the declared content of insulin Lispro in insulin Lispro RS using the formula:

\[
(C_s/C_u)(r_u/r_s)
\]

Where \( C_s \) is the concentration (in insulin lispro units/ml) of insulin Lispro RS in reference solution; \( C_u \) the concentration (in insulin Lispro mg/ml) of insulin Lispro in test solution; and \( r_u \) and \( r_s \) are the insulin Lispro peak areas obtained from the test solution and reference solution respectively. From the value obtained in the test for Loss on drying, calculate the potency on the dried basis.

Storage

In an air tight container, protected from light, at or below -18°C until released by the manufacturer. When thawed, insulin lispro is stored at 5±3°C and used for manufacturing preparations within a short period of time. To avoid absorption of humidity from the air during weighing, insulin lispro must be at room temperature before opening the container.

Labelling

The label states (1) the material is produced by microbial synthesis via recombinant DNA technology; (2) that the material is free from bacterial endotoxins; (3) the storage conditions.