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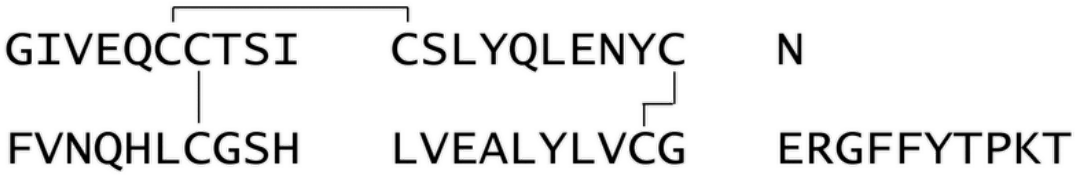
Insulin Human



$C_{257}H_{383}N_{65}O_{77}S_6$ 5807.57

Insulin (human) CAS RN®: 11061-68-0; UNII: 1Y17CTI5SR.

DEFINITION

Insulin Human is a two-chain peptide hormone consisting of 51 amino acids, and its structure corresponds to native insulin produced in vivo by the beta cells of the pancreas. The A-chain is composed of 21 amino acids, and the B-chain is composed of 30 amino acids. It is either produced by methods based on recombinant DNA technology or derived by enzymatic modification of insulin from porcine pancreas to change the amino acid sequence appropriately. The presence of host cell DNA in Insulin Human 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 27.5 USP Insulin Human Units/mg, calculated on the dried basis.

[NOTE—One USP Insulin Human Unit is equivalent to 0.0347 mg of pure Insulin Human.]

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, except use the following *Mobile phase* and *System suitability*. It meets the requirements.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
60	30	70
65	0	100
70	0	100
71	90	10
86	90	10

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 Human RS.

ASSAY

• **Procedure**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.

Mobile phase: Acetonitrile and *Solution A* (26:74). [Note—The acetonitrile is warmed to NLT 20° to avoid precipitation.]

System suitability solution: 1.5 mg/mL of Insulin Human in 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin human.

[Note—The *Standard solution* and *Sample solution* may be stored at room temperature for up to 12 h, or in a refrigerator for up to 48 h.]

Standard solution: 1.5 mg/mL of USP Insulin Human RS in 0.01 N hydrochloric acid

Sample solution: 1.5 mg/mL of Insulin Human in 0.01 N hydrochloric acid

Chromatographic system

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

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between insulin human and A-21 desamido insulin human, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin human peak, *System suitability solution*

Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the peak responses for insulin human and A-21 desamido insulin human.

Calculate the potency on the undried basis, in USP Insulin Human Units/mg, of Insulin Human in the *Sample solution*:

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

r_U = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Sample solution*

r_S = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Standard solution*

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

C_U = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: NLT 27.5 USP Insulin Human Units/mg, on the dried basis

OTHER COMPONENTS

Change to read:

• **Zinc Determination ⟨591⟩**

▲▲ (USP 1-May-2021)

Acceptance criteria: NMT 1.0% on the dried basis

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **Related Substances**

Solvent: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.

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

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

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	78	22
36	78	22
61	36	64
67	36	64
68	78	22
78	78	22

System suitability solution: 1.5 mg/mL of Insulin Human in 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin human.

Standard solution A: 3.75 mg/mL of USP Insulin Human RS in 0.01 N hydrochloric acid

Standard solution B: Pipet 1 mL of *Standard solution A* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix (0.375 mg/mL).

Standard solution C: Pipet 1 mL of *Standard solution B* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix (0.0375 mg/mL).

[NOTE—*Standard solutions A–C* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Sample solution: 3.75 mg/mL of Insulin Human in 0.01 N hydrochloric acid. Prepare the solution in a capped vial, cap the vial, and shake gently to dissolve. Store the solution at room temperature for NMT 2 h, or in a refrigerator for NMT 12 h.

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 between 15 and 25 min for the main insulin human peak, with A-21 desamido insulin human eluting just before the start of the gradient elution phase.

Samples: *System suitability solution*, *Standard solution A*, *Standard solution B*, and *Standard solution C*

Suitability requirements for the *System suitability solution*

Resolution: NLT 2.0 between insulin human and A-21 desamido insulin human

Tailing factor: NMT 1.8 for the insulin human peak

Suitability requirements for *Standard solutions A–C*

Calculate the factor X_1:

$$X_1 = (r_B/r_A) \times D$$

r_B = peak response from *Standard solution B*

r_A = peak response from *Standard solution A*

D = dilution factor, 10

Result: Between 0.91 and 1.09

Calculate the factor X_2:

$$X_2 = (r_C/r_A) \times D$$

r_C = peak response from *Standard solution C*

r_A = peak response from *Standard solution A*

D = dilution factor, 100

Result: Between 0.7 and 1.3

Analysis

Sample: *Sample solution*

Calculate the percentage of insulin human, A-21 desamido insulin human, and other impurities in the portion of Insulin Human taken.

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

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

r_I = peak response of insulin human 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 human (%*D*):

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

r_D = peak response of A-21 desamido insulin human from the *Sample solution*

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

Calculate the percentage of other insulin human-related substances:

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

Acceptance criteria

Individual impurities: NMT 2.0% of A-21 desamido insulin human

Total impurities: NMT 2.0%, excluding A-21 desamido insulin human

• **Physicochemical Analytical Procedures for Insulins ⟨121.1⟩, *Limit of High Molecular Weight Proteins*:** Meets the requirements

Acceptance criteria: NMT 1.0%

PROCESS-RELATED IMPURITIES

• **Single-Chain Precursor Content:** The single-chain precursor content of Insulin Human produced by recombinant DNA technology or the proinsulin content of Insulin Human derived from porcine 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*:** Meets the requirements

• **Loss on Drying ⟨731⟩**

Sample: 200 mg

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

Acceptance criteria: NMT 10.0%

Change to read:

• **Bacterial Endotoxins Test ⟨85⟩:** ▲The level of bacterial endotoxins are such that the requirement under the relevant dosage form monograph(s) in which Insulin Human is used can be met. Where the label states Insulin Human must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins are such that the requirement under the relevant dosage form monograph(s) in which Insulin Human is used can be met.▲ (USP 1-May-2021)

Change to read:

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** ▲The total aerobic microbial count does not exceed 3×10^2 cfu/g and the total combined yeasts and molds count does not exceed 5×10^1 cfu/g,▲ (USP 1-May-2021) the test being performed on a portion of 0.2 g, accurately weighed.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store in a freezer and protect from light.

Change to read:

• **Labeling:** Label it to indicate that it has been produced by methods based on recombinant DNA technology or that it is derived by enzymatic modification of insulin from porcine pancreas. ▲Where Insulin Human must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.▲ (USP 1-May-2021)

• **USP Reference Standards** ⟨11⟩

USP Insulin Human RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
INSULIN HUMAN	Jennifer Tong Sun Senior Scientist II	BIO2 Biologics Monographs 2 - Proteins

Chromatographic Database Information: Chromatographic Database

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