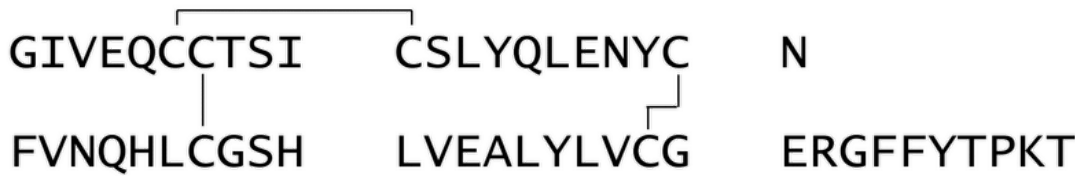


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Insulin Human



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