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Human Insulin Isophane Suspension and Human Insulin Injection

DEFINITION

Human Insulin Isophane Suspension and Human Insulin Injection is a sterile buffered suspension of Insulin Human, complexed with Protamine Sulfate, in a solution of Insulin Human. Its potency, based on the sum of its insulin and desamido insulin components as determined in the Assay, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Human Units/mL.

IDENTIFICATION

• **A.** The retention time of the major peak of *Sample solution A* or *Sample solution B* corresponds to that of the *Standard solution*, as obtained in the Assay.

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 this 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.

Standard solution: 1.5 mg/mL of [USP Insulin Human RS](#) in 0.01 N [hydrochloric acid](#)

Sample solution A (for Injection labeled as containing 40 USP Insulin Human Units/mL): Add 2.5 µL of 9.6 N [hydrochloric acid](#) to each milliliter of an accurately measured volume of Injection. Allow the suspension to clarify, and mix.

Sample solution B (for Injection labeled as containing 100 USP Insulin Human Units/mL): Add 2.5 µL of 9.6 N [hydrochloric acid](#) to each milliliter of an accurately measured volume of Injection. Allow the suspension to clarify, and mix. [NOTE—Pooling of several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N [hydrochloric acid](#) to volume, and mix.

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 either *Sample solution A* or *Sample solution B*

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

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

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)

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 Human Units/mL

OTHER COMPONENTS

Change to read:

- **Zinc Determination (591):**▲ (IRA 1-JAN-2019) 0.02–0.04 mg for every 100 USP Insulin Human Units

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

- **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 3.0%

SPECIFIC TESTS

• SOLUBLE INSULIN HUMAN CONTENT

[NOTE—Use one of the two methods listed below.]

Method 1

Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Soluble insulin sample solution: Maintain the temperature at $25 \pm 1^\circ$ throughout the *Analysis*. Transfer 5.0 mL of Injection to a centrifuge tube. Add 20 µL of 1 N [sodium hydroxide](#), and adjust with 0.05 N [hydrochloric acid](#) or 0.05 N [sodium hydroxide](#) to a pH of 8.20 ± 0.02 if the total zinc concentration is approximately 20 µg/mL, or adjust to a pH of 8.35 ± 0.02 if the total zinc concentration is approximately 30 µg/mL. Record the volume (V_A), in µL, of acid or base needed to adjust the pH. Allow to stand for 1 h. Centrifuge, transfer the supernatant to another centrifuge tube, and repeat the centrifugation. Transfer 2 mL of the supernatant to another tube, add 5 µL of 9.6 N [hydrochloric acid](#), and mix.

Total insulin sample solution: Transfer 2 mL of Injection to a suitable vessel, add 5 µL of 9.6 N [hydrochloric acid](#), and allow the suspension to clarify. Dilute the resulting solution with 0.01 N [hydrochloric acid](#) to the same theoretical concentration of insulin as the *Soluble insulin sample solution*. [NOTE—For example, if Injection is labeled to contain 20% soluble insulin, the dilution factor is $100/20 = 5$.]

Analysis

Samples: *Soluble insulin sample solution* and *Total insulin sample solution*

Calculate the quantity of soluble insulin human as a percentage of the total insulin content of Injection:

$$\text{Result} = (\Sigma r_s / \Sigma r_t) \times [(V_t + V_A) / V_i] \times (100/D)$$

r_s = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Soluble insulin sample solution*

r_t = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Total insulin sample solution*

V_t = sum of initial volume (5000 µL) of Injection to be transferred to a centrifuge tube and 20 µL of 1 N [sodium hydroxide](#) to be added to Injection for the *Soluble insulin sample solution*, 5020 µL

V_A = volume added to adjust the pH of the *Soluble insulin sample solution* (µL)

V_i = initial volume of Injection to be transferred to a centrifuge tube for the *Soluble insulin sample solution*, 5000 µL

D = dilution factor used to prepare the *Total insulin sample solution*

Acceptance criteria: The percentage of soluble insulin human is in the range $L \pm 5$, where L is the percentage of soluble insulin human stated on the product label.

Method 2

Mobile phase and Chromatographic system: Proceed as directed in the Assay, except use 50 µL for *Injection volume*.

0.1 M tris buffer: Dissolve 3.54 ± 0.01 g of [tris\(hydroxymethyl\)aminomethane hydrochloride](#) and 3.34 ± 0.01 g of [tris\(hydroxymethyl\)aminomethane](#) in 500 mL of water. The pH of this solution must be between 8.15 and 8.35. If the pH is outside of this range, discard the solution and prepare fresh; do not adjust the pH.

System suitability solution: Dissolve about 0.14 mg of insulin human in 1.0 mL of 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.