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 ± 1° 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.

Soluble insulin sample solution: Dilute a suitable volume of Injection with *0.1 M tris buffer* to obtain a solution containing about 6 USP Insulin Human Units/mL of soluble insulin (e.g., 2 mL of 70/30 Injection containing 100 USP Insulin Human Units/mL would be diluted with 8 mL of *0.1 M tris buffer* to obtain a filtrate that contains 6 USP Insulin Human Units/mL of soluble insulin). Immerse the container in a water bath at 25 ± 1° for 30 ± 2 min. Immediately pass this solution through a filter of 0.2-µm pore size using a disposable syringe. Transfer 2 parts of the filtrate to a suitable vessel, and add 1 part 0.2 N hydrochloric acid. [NOTE—For example, the dilution factor for the *Soluble insulin sample solution* that contains 30% soluble insulin is (5 × 3)/2 = 7.5.]

Total insulin sample solution: For each milliliter of Injection add 3.0 µL of 9.6 N hydrochloric acid, mix, and allow the suspension to clarify. Dilute the resulting solution with 0.01 N hydrochloric acid to 4 USP Insulin Human Units/mL (e.g., if the product is labeled to contain a total of 100 USP Insulin Human Units/mL, the dilution factor is 25).

System suitability

Make adjustments as necessary to obtain a retention time for insulin human between 10 and 17 min.

Samples: *System suitability solution* (5 replicate injections)

[NOTE—The retention time for human insulin is between 10 and 17 min.]

Suitability requirements

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

Tailing factor: Between 0.8 and 1.5 for the insulin human peak

Relative standard deviation: NMT 1.6%

Analysis

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

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

$$\text{Result} = (\Sigma r_S/\Sigma r_T) \times (D_S/D_T) \times 100$$

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*

D_S = dilution factor used to prepare the *Soluble insulin sample solution*

D_T = 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.

- **pH** ⟨791⟩**:** 7.0–7.8
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 80 USP Endotoxin Units per 100 USP Insulin Human Units
- **Sterility Tests** ⟨71⟩**,** *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements when tested as directed in the chapter and the Injection being filtered immediately after it has been put into a solution using a validated suitable solvent.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **Labeling:** The Injection container label states that the Injection is to be properly resuspended before use. Label it to indicate that it has been prepared with Insulin Human produced by methods based on recombinant DNA technology or that it is derived by enzymatic modification of insulin from porcine pancreas. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Human Units/mL and the percent ratio of Human Insulin Isophane Suspension to soluble Human Insulin Injection.
- **USP Reference Standards** ⟨11⟩
  USP Insulin Human RS