Insulin Injection

DEFINITION

Insulin Injection is an isotonic, sterile solution of Insulin. Its potency, based on the sum of the insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

• **A.** The retention time of the insulin peak of *Sample solution A* or *Sample solution B* corresponds to that of the appropriate species of the *Identification solution*, as obtained in the *Assay*. [NOTE—It may be necessary to inject a mixture of *Sample solution* and *Identification solution.*]

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 of the appropriate species, either insulin beef or insulin pork, in 0.01 N hydrochloric acid. For insulin of mixed species, prepare a solution containing 1.3 mg/mL of insulin beef and 0.25 mg/mL of insulin pork 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.
[NOTE—The *Identification solution*, *Standard solution*, and *Sample solutions* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Identification solution: 0.6 mg/mL each of USP Insulin Beef RS and USP Insulin Pork RS in 0.01 N hydrochloric acid

Standard solution: 1.5 mg/mL of either USP Insulin Beef RS or USP Insulin Pork RS in 0.01 N hydrochloric acid. For insulin of mixed species, prepare a solution containing 1.3 mg/mL of USP Insulin Beef RS and 0.25 mg/mL of USP Insulin Pork RS in 0.01 N hydrochloric acid.

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

Sample solution B (for Injection labeled as containing 100 USP Insulin Units/mL)**:** Add 2.5 µL of 9.6 N hydrochloric acid for each milliliter of an accurately measured volume of Injection. Allow the suspension, if present, to clarify, and mix. [NOTE—Pooling 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 and A-21 desamido insulin, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis
Samples: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*
Measure the peak responses for insulin and A-21 desamido insulin using the chromatogram of the *Identification solution* to identify the insulin peaks.
For Injection prepared from a single species, calculate the potency, in USP Insulin Units/mL, in the portion 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 and A-21 desamido insulin from the *Sample solution*
r_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*
C_S = concentration of either USP Insulin Beef RS or USP Insulin Pork RS in the *Standard solution* (USP Insulin Units/mL)
D = dilution factor used to prepare the *Sample solution*

For Injection prepared from a mixture of insulin beef and insulin pork, calculate the total potency as the sum of the potencies of insulin beef and insulin pork, determined separately, as directed above.

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

Change to read:

▲• **ZINC DETERMINATION** ⟨591⟩**:**▲ (IRA 1-Jan-2019) 10–40 µg for every 100 USP Insulin Units of the appropriate species

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 2.0%

SPECIFIC TESTS

• **PH** ⟨791⟩**:** 7.0–7.8
• **PARTICULATE MATTER IN INJECTIONS** ⟨788⟩**:** Meets the requirements for small-volume injections
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units per 100 USP Insulin Units
• **STERILITY TESTS** ⟨71⟩**,** *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements
• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in the unopened, multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.
• **LABELING:** Label it to indicate the one or more animal species to which it is related, as porcine, bovine, or a

mixture of porcine and bovine. If the Insulin Injection is made from insulin that is purified, label it as such. 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 Units/mL.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Insulin Beef RS
USP Insulin Pork RS