Insulin

Type of Posting	Revision Bulletin
Posting Date	29–Mar–2019
Official Date	01–May–2019
Expert Committee	Biologics Monographs 1–Peptides
Reason for Revision	Omission of USP Insulin Beef RS and requirements related to bovine insulin due to the absence of commercial bovine insulin products in the United States

In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Biologics Monographs 1–Peptides Expert Committee has revised the Insulin monograph. The purpose for the revision is to remove the USP Insulin Beef RS and requirements related to bovine insulin because there are no approved manufacturers of therapeutic bovine insulin in the United States and suitable reference materials are not available.

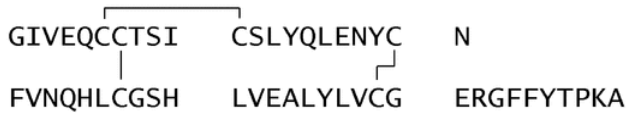
Although USP Insulin Beef RS is used as a standard for *Identification*, *Assay*, and the test for *Product-Related Substances* for insulins of bovine origin, the only use for non-bovine products is as an *Identification solution*, where the USP Insulin Pork RS and USP Insulin Beef RS are mixed together and used as a standard for identification based on retention time. The USP Insulin Beef RS and USP Insulin Pork RS are well resolved in the *Assay*, with retention times of approximately 14 and 21 min, respectively, so the USP Insulin Beef RS is not necessary for identification of insulin pork. Furthermore, *Identification B*, *Peptide Mapping*, can easily distinguish between bovine and porcine insulin. Therefore, references to bovine insulin and to the USP Insulin Beef RS have been removed from the *Identification*, *Assay*, and the test for *Product-Related Substances*, and associated changes have also been made to the chemical information, *Definition*, *Labeling*, and *USP Reference Standards*.

The Insulin Revision Bulletin supersedes the currently official monograph.

Should you have any questions or concerns, please contact Diane McCarthy, Senior Manager (301-692-3637 or diane.mccarthy@usp.org).

Insulin

Change to read:



$C_{256}H_{381}N_{65}O_{76}S_6$ 5777.54

Insulin (pig) [12584-58-6].

▲▲ (RB 1-May-2019)

DEFINITION

Change to read:

▲Insulin 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.▲ USP 1-May-2019 It is obtained from the pancreas of healthy ▲▲ (RB 1-May-2019) porcine animals, ▲▲ (RB 1-May-2019) used for food by humans. Its potency is NLT 26.5 USP Insulin Units/mg, calculated on the dried basis; Insulin labeled as purified contains NLT 27.0 USP Insulin Units/mg, calculated on the dried basis. ▲▲ USP 1-May-2019

[NOTE—1 USP Insulin Unit is equivalent to ▲▲ (RB 1-May-2019) 0.0345 mg of pure Insulin derived from pork.]

IDENTIFICATION

Change to read:

• **A.** The retention time of the major peak in the *Sample solution* corresponds to that ▲▲ (RB 1-May-2019) of the *Identification solution*, as obtained in the *Assay* ▲and no other significant peaks are observed.▲ (RB 1-May-2019)

[NOTE—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

Delete the following:

▲B. PEPTIDE MAPPING

Sulfate buffer: 2.0 M ammonium sulfate and 0.5 M sulfuric acid (1:1)

Enzyme solution: 500 units/mL of *Staphylococcus aureus* V-8 protease activity in water

HEPES buffer: 0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid). Adjust with 5 M sodium hydroxide to a pH of 7.5 before diluting with water to a final volume.

Solution A: Acetonitrile, water, and *Sulfate buffer* (100:700:200)

Solution B: Acetonitrile, water, and *Sulfate buffer* (400:400:200)

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

Standard digest solution: 2 mg/mL of USP Insulin RS of the appropriate species in 0.01 N hydrochloric acid. Transfer 500 µL of the resulting solution to a clean vial. Add 2.0 mL of *HEPES buffer* and 400 µL of *Enzyme solution*, and incubate at 25° for 6 h. Quench the digestion by adding 2.9 mL of *Sulfate buffer*.

Sample digest solution: 2 mg/mL of Insulin in 0.01 N hydrochloric acid, mix to dissolve. Transfer 500 µL of the resulting solution to a clean vial. Add 2.0 mL of *HEPES buffer* and 400 µL of *Enzyme solution*, and incubate at 25° for 6 h. Quench the digestion by adding 2.9 mL of *Sulfate buffer*.

Chromatographic system

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

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; packing L1

Column temperature: 40°

Flow rate: 1 mL/min

System suitability

Sample: *Standard digest solution*

Suitability requirements

Chromatogram comparability: The chromatogram of the *Standard digest solution* corresponds to that of the reference chromatogram provided with USP Insulin RS of the appropriate species.

Resolution: NLT 1.9 between digest fragments II and III.

[NOTE—Fragment I elutes at the same time in insulin derived from pork and Insulin Human; Fragment II elutes at the same time in Insulin Human and insulin derived from beef and pork; and Fragment III elutes at the same time in insulin derived from beef and pork.]

Tailing factor: NMT 1.5

Analysis

Samples: *Standard digest solution* and *Sample digest solution*

Using the gradient program, run a blank. Separately inject equal volumes of the *Standard digest solution* and the *Sample digest solution*, and record the responses of each peak.

Acceptance criteria: The chromatographic profile of the *Sample digest solution* corresponds to that of the *Standard digest solution*.▲ USP 1-May-2019

Add the following:

▲• B. PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS ⟨121.1⟩**,** *Peptide Mapping***:** Proceed as directed in the chapter, except for the *Mobile phase* and *System suitability*.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
60	30	70

Table 1 *(continued)*

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

System suitability
Sample: *Standard solution*
Suitability requirements
Resolution: NLT 1.9 between digest fragments II and III
[NOTE—Fragment I elutes at the same time in insulin derived from pork and Insulin Human; Fragment II elutes at the same time in Insulin Human and insulin derived from beef and pork; and Fragment III elutes at the same time in insulin derived from beef and pork.]
Tailing factor: NMT 1.5 for digest fragments II and III
Chromatogram similarity: The chromatogram of the *Standard solution* corresponds to that of the reference chromatogram provided with USP Insulin Pork RS.
Acceptance criteria: Meets the requirements▲ USP 1-May-2019

Add the following:

▲• **C. INSULIN ASSAYS** ⟨121⟩**,** *Assay, Bioidentity Test***:** Meets the requirements▲ USP 1-May-2019

ASSAY

Change to read:

• **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 a temperature of NLT 20° to avoid precipitation.]
System suitability solution: 1.5 mg/mL of Insulin 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 solution* 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 of USP Insulin Pork RS ▲▲ (RB 1-May-2019) in 0.01 N hydrochloric acid
Standard solution: 1.5 mg/mL of ▲USP Insulin Pork RS▲ (RB 1-May-2019) in 0.01 N hydrochloric acid▲▲ (RB 1-May-2019)
Sample solution: 1.5 mg/mL of Insulin 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 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 *Sample solution*
Measure the peak responses for insulin and A-21 desamido insulin, using the chromatogram of the *Identification solution* to identify the insulin peaks.
▲▲ (RB 1-May-2019) Calculate the potency on the undried basis, in USP Insulin Units/mg, of Insulin 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 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 ▲▲ (RB 1-May-2019) USP Insulin Pork RS in the *Standard solution* (USP Insulin Units/mL)
C_U = concentration of Insulin in the *Sample solution* (mg/mL)

▲▲ (RB 1-May-2019)
Acceptance criteria: NLT 26.5 USP Insulin Units/mg on the dried basis; Insulin labeled as purified contains NLT 27.0 USP Insulin Units/mg on the dried basis.

OTHER COMPONENTS

Change to read:

• **ZINC DETERMINATION** ⟨591⟩
▲**Acceptance criteria:** NMT 1.0% on the dried basis▲ USP 1-May-2019

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

Change to read:

▲• **PRODUCT-RELATED SUBSTANCES**▲ USP 1-May-2019
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.
Solution B: Acetonitrile and *Solution A* (18:82)
Solution C: Acetonitrile and *Solution A* (50:50)
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution B (%)	Solution C (%)
0	81	19
60	81	19
85	36	64
91	36	64
92	81	19

System suitability solution: 1.5 mg/mL of Insulin 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—*Standard solutions A–C* may be stored at room temperature for up to 12 h and in a refrigerator for up to 48 h.]

Standard solution A: 3.75 mg/mL of ▲USP Insulin Pork RS▲ (RB 1-May-2019) in 0.01 N hydrochloric acid▲▲ (RB 1-May-2019)

Standard solution B: 0.375 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid prepared as follows. Pipet 1 mL of *Standard solution A* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

Standard solution C: 0.0375 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid prepared as follows. Pipet 1 mL of *Standard solution B* into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

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

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
Samples: *System suitability solution*, *Standard solution A*, *Standard solution B*, and *Standard solution C*
[NOTE—Adjust the *Mobile phase* composition and the duration of the isocratic elution to obtain a retention time of about 31 min for insulin, with the A-21 desamido insulin eluting just prior to the start of the gradient elution phase.]
Suitability requirements for the System suitability solution
Resolution: NLT 2.0 between insulin and A-21 desamido insulin
Tailing factor: NMT 1.8 for the insulin peak
Suitability requirements for the Standard solutions
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, A-21 desamido insulin, and other ▲insulin-related substances▲ USP 1-May-2019 in the portion of Insulin taken:
Calculate the percentage of Insulin (%I):

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

r_I = peak response of insulin 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 (%D):

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

r_D = peak response of A-21 desamido insulin from the *Sample solution*
r_T = sum of the responses of all the peaks from the *Sample solution*

Calculate the percentage of other insulin-related ▲substances:▲ USP 1-May-2019

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

Acceptance criteria: NMT 10.0% of A-21 desamido insulin, and NMT 5.0% of other insulin-related ▲substances▲ USP 1-May-2019
▲▲ (RB 1-May-2019)

Change to read:

▲• **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩**,** *Limit of High Molecular Weight Proteins***:** Meets the requirements▲ USP 1-May-2019
Acceptance criteria: NMT 1.0%

PROCESS-RELATED IMPURITIES

Add the following:

▲• **PROINSULIN CONTENT:** NMT 10 ng/mg, determined by a validated method▲ USP 1-May-2019

SPECIFIC TESTS

Delete the following:

▲• **INSULIN ASSAYS** ⟨121⟩**,** *Assay, Bioidentity Test***:** Meets the requirements▲ USP 1-May-2019
• **LOSS ON DRYING** ⟨731⟩
Sample: 200 mg
Analysis: Dry the *Sample* at 105° for 16 h.
Acceptance criteria: NMT 10.0%

Delete the following:

▲• **ZINC DETERMINATION** ⟨591⟩**,** *Procedure, Dithizone Method*
Sample: 10 mg
Acceptance criteria: NMT 1.0% on the dried basis▲ USP 1-May-2019
• **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 10 USP Endotoxin Units/mg of insulin
• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total bacterial count does not exceed 3×10^2 cfu/g, the test being performed on a portion of about 0.2 g, accurately weighed.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store, protected from light, in a freezer.

Change to read:

• **LABELING:** Label it ▲▲ (RB 1-May-2019) as pork▲▲ (RB 1-May-2019). If the Insulin is purified, label it as such.

Change to read:

USP Insulin Pork RS

- **USP REFERENCE STANDARDS** ⟨11⟩
▲ ▲ (RB 1-May-2019)