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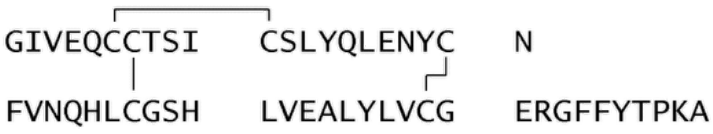
Insulin

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click https://www.uspnf.com/rb-insulin-20190401

Change to read:



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

Insulin (pig) CAS RN®: 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

Time (min)	Solution A (%)	Solution B (%)
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
65	0	100

Time (min)	Solution A (%)	Solution B (%)
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 Reference Standards** ⟨11⟩

▲▲ (RB 1-May-2019)

USP Insulin Pork RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
INSULIN	Jennifer Tong Sun Senior Scientist II	BIO2 Biologics Monographs 2 - Proteins

Chromatographic Database Information: Chromatographic Database

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