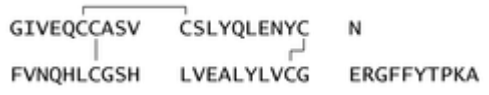
Insulin

$C_{256}H_{381}N_{65}O_{76}S_6$ 5777.54
Insulin (pig) [12584-58-6].



$C_{254}H_{377}N_{65}O_{75}S_6$ 5733.49
Insulin (ox) [11070-73-8].

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 bovine or porcine animals, or both, 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 0.0342 mg of pure Insulin derived from beef or 0.0345 mg of pure Insulin derived from pork.]

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* 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*.]

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 insulin of the appropriate species.
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

• **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 each of USP Insulin Pork RS and USP Insulin Beef RS in 0.01 N hydrochloric acid
Standard solution: 1.5 mg/mL of insulin of the appropriate species, either USP Insulin RS or USP Insulin Beef RS, in 0.01 N hydrochloric acid. For insulin of mixed species prepare a solution containing 1.3 mg/mL of USP Insulin Pork RS and 0.25 mg/mL of USP Insulin Beef RS in 0.01 N hydrochloric acid.
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.
For Insulin derived from a single species, 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 insulin of the appropriate species, either USP Insulin Beef RS or USP Insulin Pork RS, in the *Standard solution* (USP Insulin Units/mL)
C_U = concentration of Insulin in the *Sample solution* (mg/mL)

For Insulin derived from a mixture of beef insulin and pork insulin, calculate the total potency as the sum of the potencies of the beef-derived insulin and pork-derived insulin, determined separately.
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⟩▲ (IRA 1-Jan-2019)
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 insulin of the appropriate species, either USP Insulin RS or USP Insulin Beef RS, in 0.01 N hydrochloric acid. For insulin of mixed species prepare a solution containing 3.2 mg/mL of USP Insulin Pork RS and 0.6 mg/mL of USP Insulin Beef RS in 0.01 N hydrochloric acid.
Standard solution B: 0.375 mg/mL of insulin of the appropriate species, either USP Insulin Beef RS or 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 insulin of the appropriate species, either USP Insulin Beef RS or 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
For Insulin derived from a single species, measure the responses of any peaks corresponding to beef insulin or pork insulin, and calculate the concentration as a percentage of r_T. The amount of cross-contamination is NMT 1.0%.

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.
- **Labeling:** Label it to indicate the one or more animal species to which it is related, as pork, beef, or a mixture of pork and beef. If the Insulin is purified, label it as such.
- **USP Reference Standards** ⟨11⟩
  USP Insulin Beef RS
  USP Insulin Pork RS