

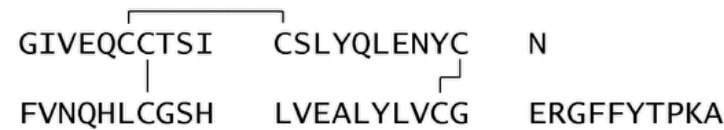
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Insulin

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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

