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Exenatide

HGEGTFTSDL SKQMEEEAVR LFIEWLKNNG PSSGAPPPS —NH₂

C₁₈₄H₂₈₂N₅₀O₆₀S 4186.57

L-Histidylglycyl-L-glutamylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-aspartyl-L-leucyl-L-seryl-L-lysyl-L-glutaminyl-L-methionyl-L-glutamyl-L-glutamyl-L-glutamyl-L-alanyl-L-valyl-L-arginyl-L-leucyl-L-phenylalanyl-L-isoleucyl-L-glutamyl-L-tryptophyl-L-leucyl-L-lysyl-L-asparaginyglycylglycyl-L-prolyl-L-seryl-L-serylglycyl-L-alanyl-L-prolyl-L-prolyl-L-prolyl-L-serinamide CAS RN®: 141758-74-9.

DEFINITION

Exenatide is a 39 amino acid synthetic peptide agonist for glucagon-like peptide-1 (GLP-1) receptor. Exenatide contains NLT 95% and NMT 105% of exenatide (C₁₈₄H₂₈₂N₅₀O₆₀S), calculated on the anhydrous, acetic acid-free basis. Exenatide is a white to off-white powder.

[NOTE—Exenatide is very hygroscopic. Protect from exposure to moisture.]

IDENTIFICATION

• A. HPLC

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and

System suitability: Proceed as directed in the Assay.

Identity sample solution: Mix equal volumes of the *Standard solution* and the *Sample solution*.

Analysis

Samples: *Standard solution*, *Sample solution*, and *Identity sample solution*

Examine the chromatograms of the *Standard solution*, *Sample solution*, and *Identity sample solution*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the major peak from the *Identity sample solution* elutes as a single peak.

• B. AMINO ACID ANALYSIS

For further discussion of the theory and applications, see [Biotechnology-Derived Articles—Amino Acid Analysis \(1052\)](#), which may be a helpful, but not mandatory, resource. [NOTE—Use a suitable, validated hydrolysis and separation and calculation procedure including amino acids used in the calculation (see [Biotechnology-Derived Articles—Amino Acid Analysis \(1052\)](#).)]

Standardize the instrument with a mixture containing equal molar per volume amounts (except for L-cystine which is half the molar amount) of glycine and the L-form of the following amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, norleucine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, and valine.

Hydrolysis solution: 6 N hydrochloric acid containing 4% of phenol

Sample solution: Accurately weigh out between 0.4 and 1.0 mg of Exenatide in glass ampuls. Add a minimum of 1.0 mL of *Hydrolysis solution*, freeze the sample ampul, and flame seal under vacuum. Hydrolyze at 110° for about 22 h. After hydrolysis, dry the test sample under vacuum to remove any residual acid. To the ampul add 2 mL of a buffer solution that is suitable for the amino acid analyzer, and pass through a filter of 0.45-µm pore size.

Procedure: Prepare a co-injection of the *Standard solution* and the test sample. Inject a suitable volume into the amino acid analyzer, and record and measure the responses for each amino acid peak in the *Standard solution*. Express the content of each amino acid in nanomoles.

Calculate the mean nanomole of the amino acids:

Result = (nmol found in the *Analysis* for Ala, Arg, Asx, Glx, Gly, His, Ile, Leu, Lys, Phe, Pro, Trp, Val)/30

Divide the nanomole of each amino acid by the *Result* to determine the amino acid ratios that must meet the *Acceptance criteria*.

Acceptance criteria: See [Table 1](#).

Table 1

Name	Acceptance Criteria (amino acid ratio)
Aspartic acid, threonine, phenylalanine, lysine, alanine	1.5–2.5
Serine	4.2–5.5
Glutamic acid	5.2–6.8
Proline	3.5–4.5
Glycine	4.5–5.5
Valine	0.5–1.5
Methionine, isoleucine, histidine, arginine	0.5–1.5
Leucine	2.5–3.5
Tryptophan	0.5–1.5

- **C.** The average mass by [Mass Spectrometry \(736\)](#) is 4186.6 ± 1.0 mass units.

ASSAY

• PROCEDURE

Solution A: 10 mM ammonium hydrogen carbonate, pH 9.5, 0.0375% ammonia

Solution B: Acetonitrile and *Solution A* (90:10)

Mobile phase: See [Table 2](#).

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	74	26
0.5	74	26
33	63	37
35	10	90
36	10	90
36.1	74	26
42	74	26

System suitability solution: 1.0 mg/mL of [USP Exenatide RS](#) and 0.005 mg/mL each of USP [Glu¹³]-Exenatide RS and USP [Met(O)¹⁴]-Exenatide RS

Standard solutions: 1.0 mg/mL of [USP Exenatide RS](#) in water, prepared in duplicate. [NOTE—The retention time for the exenatide peak is approximately 14–20 min.]

Sample solutions: 1.0 mg/mL of Exenatide in water, prepared in duplicate

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Temperatures

Column: 60°

Autosampler: 10°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solutions*

Suitability requirements

Mean peak area: Maximum difference between the mean peak area of the two *Standard solutions* is ±2%.

Resolution: NLT 1.0 between the [Glu¹³]-exenatide and [Met(O)¹⁴]-exenatide peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for the exenatide peak area and retention time from three replicate injections of the two *Standard solutions*

Analysis

Samples: *Standard solutions* and *Sample solutions*

Calculate the percentage of exenatide (C₁₈₄H₂₈₂N₅₀O₆₀S) in the portion of Exenatide taken:

Result = (r_U/r_S) × (C_S/C_U) × 100

r_U = peak response of exenatide from the *Sample solutions*

r_S = peak response of exenatide from the *Standard solutions*

C_S = concentration of [USP Exenatide RS](#) in the *Standard solutions* (mg/mL)

C_U = concentration of Exenatide in the *Sample solutions* (mg/mL), calculated on the anhydrous, acetic acid-free basis

Acceptance criteria: 95%–105% on the anhydrous, acetic acid-free basis

IMPURITIES

• **PROCEDURE 1: EXENATIDE RELATED SUBSTANCES AND IMPURITIES**

[NOTE—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Mobile phase, System suitability solution, Standard solutions, Sample solutions, and Chromatographic system: Proceed as directed in the Assay.

Acceptance criteria

Individual impurities: See [Table 3](#).

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
[Glu ¹³]-exenatide	0.65–0.68	0.50
Sum of [Asp ²⁸]-exenatide and [Met(O) ¹⁴]-exenatide	0.68–0.76	0.50
Exenatide	1.00	—
Unspecified impurities	—	0.50

Total impurities: NMT 3.0% from *Procedure 1*

Reporting limit: 0.05%

• **PROCEDURE 2: N-ACETYL HIS¹-EXENATIDE**

[NOTE—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]