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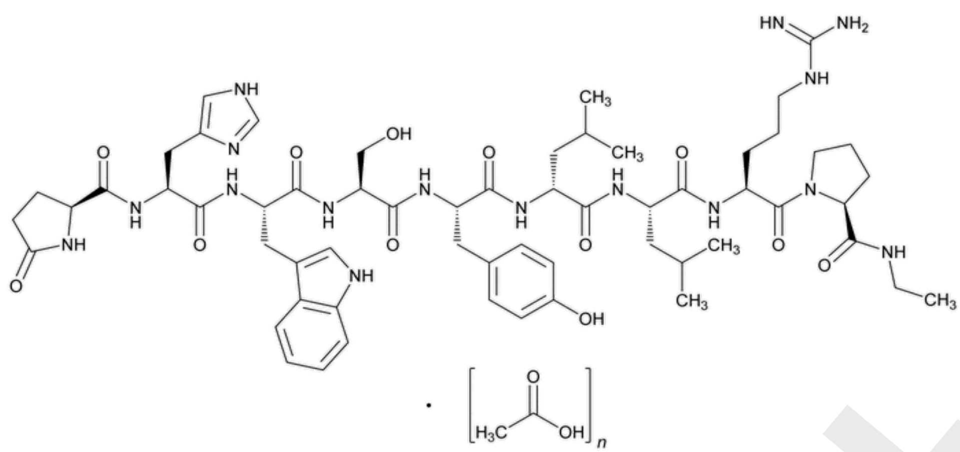
Leuprolide Acetate



$C_{59}H_{84}N_{16}O_{12} \cdot (C_2H_4O_2)_n$, n= 1–2 1209.41 (as free base)

Luteinizing hormone-releasing factor, 6-D-leucine-9-(*N*-ethyl-L-prolinamide)-10-desglycinamide acetate (salt);

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide acetate (salt);

Free base CAS RN®: 53714-56-0; UNII: EFY6W0M8TG.

Acetate salt CAS RN®: 74381-53-6; UNII: 37JNS02E7V.

DEFINITION

Leuprolide Acetate is a synthetic nonapeptide agonist analog of luteinizing hormone-releasing factor. It contains NLT 97.0% and NMT 103.0% of leuprolide ($C_{59}H_{84}N_{16}O_{12}$), calculated on the anhydrous, acetic acid-free basis.

[Note—Due to the hygroscopic nature of this material, analyses are performed immediately after opening the container in a glove box under dry nitrogen purge.]

[**Caution—**Leuprolide Acetate is a potent hormonal manipulator. Avoid skin contact and inhalation of dusts and mists.]

IDENTIFICATION

• **A. HPLC**

Solution A, Solution B, Mobile phase, Standard solution, Degradation 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*, the *Sample solution*, and the *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 of the *Identity sample solution* elutes as a single peak.

• **B. Amino Acid Analysis**

[Note—The following method is given for informational purposes; any validated amino acid analysis method can be used.]

Standard solutions: Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. Prepare a separate, equimolar solution of L-tryptophan.

Sample solution: Transfer about 6.4 mg of Leuprolide Acetate to a suitable vacuum hydrolysis tube. Add 2.0 mL of 6 N hydrochloric acid to the tube, evacuate, and seal. Heat at 120° for 16 h. Allow to cool. Remove the solvent under vacuum. Dissolve in, and dilute to a suitable volume in, a buffer solution suitable for amino acid analysis.

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Analysis: Standardize the instrument with the *Standard solutions*. Inject suitable volumes of the *Standard solutions* and the *Sample solution* into the amino acid analyzer. Record and measure the responses for each amino acid peak. Express the content of each amino acid in nmol.

Calculate the mean nmol of each of the amino acids taken:

Result = (nmol found in the *Analysis* for Glu, Pro, Tyr, His, Arg, Leu)/7

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

Acceptance criteria

Glutamic acid, proline, tyrosine, histidine, and **arginine:** 0.85–1.1

Leucine: 1.8–2.2

Serine and tryptophan: Present

ASSAY

• **Procedure**

Solution A: 15.2 mg/mL of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0.

Solution B: Acetonitrile and *n*-propyl alcohol (3:2)

Mobile phase: *Solution A* and *Solution B* (17:3)

Standard stock solution: 1 mg/mL of USP Leuprolide Acetate RS in *Mobile phase*

Standard solution: 50 µg/mL of USP Leuprolide Acetate RS in *Mobile phase* prepared from the *Standard stock solution*

Degradation standard solution: Dilute the *Standard stock solution* with water to 0.1 mg/mL. Transfer 5 mL of the solution into a scintillation vial. Add 100 µL of 1 N sodium hydroxide solution, cap tightly, and shake vigorously. Place in an oven at 100° for 60 min. Remove, allow to cool, add 50 µL of 1 M phosphoric acid, recap, and shake vigorously to mix.

Sample solution: 50 µg/mL of Leuprolide Acetate in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 1–1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Mobile phase*, *Standard solution*, and *Degradation standard solution*

[Note—Chromatograph the *Mobile phase* and verify that no extraneous peaks are present.]

[Note—The relative retention times for the degradation product and leuprolide are about 0.90 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between leuprolide and the degradation product, *Degradation standard solution*

Tailing factor: 0.8–1.5, *Standard solution*

Retention time: 41–49 min for leuprolide, *Degradation standard solution*

Relative standard deviation: NMT 1.5% for leuprolide acetate, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of leuprolide ($C_{59}H_{84}N_{16}O_{12}$) in the portion of Leuprolide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of USP Leuprolide Acetate RS in the *Standard solution* (µg/mL)

C_U = concentration of Leuprolide Acetate in the *Sample solution* (µg/mL) on the anhydrous and acetic acid-free basis

Acceptance criteria: 97.0%–103.0% on the anhydrous and acetic acid-free basis

OTHER COMPONENTS

• **Acetic Acid in Peptides ⟨503⟩:** 4.7%–9.0%

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PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **Leuprolide-Related Impurities**

Solution A, Solution B, Mobile phase, Standard stock solution, Degradation standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of USP Leuprolide Acetate RS in *Mobile phase* prepared by diluting the *Standard stock solution*

Sample solution: 1 mg/mL of Leuprolide Acetate in *Mobile phase*

System suitability

Samples: *Mobile phase*, *Degradation standard solution*, and *Standard solution*

[Note—Chromatograph the *Mobile phase* and verify that no extraneous peaks are present.]

[Note—The relative retention times for the degradation product and leuprolide are about 0.90 and 1.0, respectively.]

Suitability requirements

Resolution, Tailing factor, and **Retention time:** Proceed as directed in the *Assay*.

Relative standard deviation: NMT 1.5% for leuprolide acetate, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

[Note—Record the chromatograms for 90 min.]

Calculate the percentage of each impurity in the portion of Leuprolide Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response of leuprolide from the *Standard solution*

C_S = concentration of USP Leuprolide Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Leuprolide Acetate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Ser-leuprolide[a]	0.8	0.5
D-His-leuprolide[b]	0.9	0.5
Leuprolide	1.0	—
L-Leu^6-leuprolide[c]	1.2	0.5
(*O*-Acetyl-L-ser)-leuprolide[d]	1.5	1.0
Any other individual impurity	—	0.5
Total impurities	—	2.5

[a] 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-D-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.

[b] 5-Oxo-L-prolyl-D-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.

[c] 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-L-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.

[d] 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-(*O*-acetyl)-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.

PROCESS-RELATED IMPURITIES

• **Trifluoroacetic Acid (TFA) in Peptides ⟨503.1⟩:** NMT 0.25%. [Note—Perform this test if trifluoroacetic acid is used in the manufacturing process.]

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SPECIFIC TESTS

- **Water Determination ⟨921⟩**, *Method I, Method Ic*: NMT 8.0%
- **Bacterial Endotoxins Test ⟨85⟩**: It contains NMT 166.7 USP Endotoxin Units/mg of leuprolide acetate.
- **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩**: The total aerobic microbial count does not exceed 10^2 cfu/g. The total yeast and mold count does not exceed 10^2 cfu/g.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store at a temperature not higher than 30°.
- **USP Reference Standards ⟨11⟩**
  USP Leuprolide Acetate RS

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

Topic/Question	Contact	Expert Committee
LEUPROLIDE ACETATE	Ying Han Associate Science & Standards Liaison	BIO12020 Biologics Monographs 1 - Peptides
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO12020 Biologics Monographs 1 - Peptides

Chromatographic Database Information: Chromatographic Database

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