Status: Currently Official on 15-Feb-2025
Official Date: Official as of 01-May-2018
Document Type: USP Monographs
DocId: GUID-BC041DA0-C4B1-44A1-B631-143BC6BC6F41_3_en-US
DOI: https://doi.org/10.31003/USPNF_M35790_03_01
DOI Ref: lz303

Gonadorelin Hydrochloride

5-oxoP H W S Y G L R P G—NH_2 • *x* HCl

$C_{55}H_{75}N_{17}O_{13}$×HCl 1182.30 (free base)

Gonadorelin hydrochloride;

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosylglycyl-L-leucyl-L-arginyl-L-prolylglycinamide hydrochloride salt CAS RN®: 51952-41-1; UNII: 3PFC574ITA.

DEFINITION

Gonadorelin Hydrochloride is a synthetic polypeptide hormone having the property of stimulating the release of the luteinizing hormone from the hypothalamus. It contains NLT 95.0% and NMT 102.0% of gonadorelin ($C_{55}H_{75}N_{17}O_{13}$), calculated on the anhydrous and hydrochloride-free basis.

[**Caution—**Gonadorelin Hydrochloride is extremely hygroscopic. Protect from exposure to moisture, and store in a desiccator.]

IDENTIFICATION

• **A.** The monoisotopic mass by *Mass Spectrometry* ⟨736⟩ is 1181.6 ± 1 mass units.

[Note—This quantity corresponds to m/z = 1182.6 ± 1 for $(M + H)^+$ or m/z = 591.8 ± 0.5 for $(M + 2H)^{2+}$.]

• **B. HPLC**

Buffer, Mobile phase, 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.

ASSAY

• **Procedure**

[Note—Perform all manipulations involving the weighing of the gonadorelin hydrochloride and the Reference Standard in a low-humidity glove box.]

Buffer: Dissolve 6.8 g of monobasic potassium phosphate in water and dilute to 1 L. Adjust with 1 N potassium hydroxide to a pH of 6.5.

Mobile phase: Acetonitrile and *Buffer* (18:82)

Standard solution: 0.10 mg/mL of USP Gonadorelin Hydrochloride RS in *Mobile phase*

[Note—*Standard solution* may be stored in a refrigerator for 2 months. Remove suitable portions and warm to room temperature before use.]

Sample solution: 0.10 mg/mL of Gonadorelin Hydrochloride in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

[Note—Condition the column with *Mobile phase* until a stable baseline is obtained.]

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Retention time: 8–11 min for the gonadorelin peak

Tailing factor: NMT 2.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of gonadorelin ($C_{55}H_{75}N_{17}O_{13}$) in the portion of Gonadorelin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times [100/(100 - W - \text{HCl})] \times 100$$

r_U = peak response of gonadorelin from the *Sample solution*

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

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

C_U = concentration of Gonadorelin Hydrochloride in the *Sample solution* (mg/mL)

F = conversion factor, (1182.3/1255.2)

W = water content in Gonadorelin Hydrochloride sample (%)

HCl = hydrochloride content in Gonadorelin Hydrochloride sample (%) [NOTE—%HC = %Cl × (36.46/35.45).]

Acceptance criteria: 95.0–102.0% on the anhydrous and hydrochloride-free basis

OTHER COMPONENTS

• **COUNTER ION: CHLORIDE**

Sample: 25 mg of Gonadorelin Hydrochloride

Titrimetric system

(See *Titrimetry ⟨541⟩*.)

Mode: Direct titration

Titrant: 0.01 N silver nitrate VS

Blank: To 1 mL of methanol, add 10 mL of water and 1 drop of glacial acetic acid.

Endpoint detection: Potentiometric

Analysis: Dissolve the *Sample* in 1 mL of methanol, and add 10 mL of water and 1 drop of glacial acetic acid. Titrate with *Titrant* and perform a blank determination.

Calculate the percentage of chloride (Cl^-) in the portion of Gonadorelin Hydrochloride taken:

$$\text{Result} = [(V_S - V_B) \times N \times F \times 100]/W$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (Eq/L = mEq/mL)

F = equivalency factor for chloride, 35.45 mg/mEq

W = *Sample* weight (mg)

Acceptance criteria: 4.0%–6.0%

IMPURITIES

• **GONADORELIN RELATED IMPURITIES**

[NOTE—The *Sample solution* may be stored at room temperature for up to 20 min or at 4° for up to 8 h.]

Solution A: Dissolve 6.8 g of monobasic potassium phosphate in water and dilute to 1 L. Adjust with 1 N potassium hydroxide to a pH of 6.5.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	82	18
20	82	18
30	30	70
35	30	70
38	100	0

[Note—Pump *Solution A* through the column at a flow rate of 1 mL/min for 30 min or until a stable baseline is obtained, then inject 100 µL of *Solution A*, and run the gradient elution program to completion to condition the column. Again inject 100 µL of *Solution A*, and run the gradient elution program to completion.]

Standard stock solution: 1 mg/mL of USP Gonadorelin Hydrochloride RS in *Solution A*

Standard solution: 5 µg/mL of USP Gonadorelin Hydrochloride RS in *Solution A* from *Standard stock solution*

System suitability solution: 0.01 mg/mL of USP Gonadorelin Acetate Related Compound A RS and 1 mg/mL of USP Gonadorelin Hydrochloride RS in *Solution A*

Sample solution: 1 mg/mL of Gonadorelin Hydrochloride in *Solution A*

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 100 µL

Autosampler temperature: 4°

System suitability

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

[Note—The relative retention times for gonadorelin related compound A and gonadorelin are 0.98 and 1.0, respectively.]

Suitability requirements

Retention time: 24–30 min for the gonadorelin peak, *Standard solution*

Resolution: The valley point between gonadorelin related compound A and gonadorelin is visible, *System suitability solution*.

Tailing factor: NMT 2.0, *Standard solution*

[Note—If necessary, adjust the flow rate to between 0.8 and 2 mL/min, or, alternatively, change by NMT 3% the percentages of *Solution A* and *Solution B* at 3 min and at 20 min.]

Analysis

Samples: *Solution A*, *Standard solution*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Gonadorelin Hydrochloride taken:

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

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

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

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

C_U = concentration of Gonadorelin Hydrochloride in the *Sample solution* (mg/mL)

F = conversion factor, 0.001 mg/µg

Acceptance criteria

Any individual impurity: NMT 2.0%

Total impurities: NMT 3.0%

- **Acetic Acids in Peptides ⟨503⟩:** NMT 1.0%
- **Limit of Trifluoroacetic Acid**

Perform if trifluoroacetic acid is used in the manufacturing process.

Buffer: 7.0 mL/L of phosphoric acid and 5.0 mL/L of ammonium hydroxide in water

Solution A: Methanol and *Buffer* (2:100)

Solution B: Acetonitrile and water (1:1)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
6	0	100
14	0	100
15	100	0
25	100	0

Diluent: 5 mL/L of phosphoric acid in water

Trifluoroacetic acid stock solution: 10 mg/mL of trifluoroacetic acid in water prepared as follows. Add about 50 mL of water to a 100-mL volumetric flask with a stopper. Tare the stoppered flask on an analytical balance until there is no further significant drift in the reading. Transfer 670 µL of trifluoroacetic acid to the flask, stopper immediately, and weigh. Dilute with water to volume.

Standard solutions: Transfer 10 mL, 2 mL, and 100 µL of the *Trifluoroacetic acid stock solution* into three separate 100-mL volumetric flasks, and dilute each with *Diluent* to obtain *Standard solutions* having known concentrations, respectively, of about 1 mg/mL, 0.2 mg/mL, and 0.01 mg/mL of trifluoroacetic acid in *Diluent*.

Sample solution: 4.0 mg/mL of Gonadorelin Hydrochloride in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solutions*

Suitability requirements

Correlation coefficient (r^2): NLT 0.995, determined by plotting the peak areas of the *Standard solutions* versus concentration, in mg/mL, and determining the regression line using the least-squares method

Relative standard deviation: NMT 2.0%, the most concentrated *Standard solution*

Analysis

Samples: *Standard solutions* and *Sample solution*

From the graph of the *Standard solutions* and the concentration of the *Sample solution*, determine the percentages of trifluoroacetic acid in the sample.

Acceptance criteria: NMT 0.25%

- **Limit of Fluoride**

Perform if fluoride is used in the manufacturing process. Use polypropylene vessels for preparation of sample and Standards.

Standard solutions: Prepare a series of calibration standards containing 0.05, 0.1, 1, and 10 ppm of fluoride dissolved in an ionic strength adjustment buffer suitable for the electrode in use (pH of 5).

Sample solution: 2.2–3.6 mg/mL of Gonadorelin Hydrochloride in the same buffer as that used to prepare the *Standard solutions*

Analysis: Using a fluoride ion-selective electrode connected to a pH/ion meter, measure the potential of each *Standard solution*, and plot the response versus the logarithm of the concentration. Determine the regression line using the least-squares method. The slope of the calibration curve is −54 to −60 mV/decade, and the square of the correlation coefficient curve (r^2) is NLT 0.995.

From the calibration curve and the concentration of the *Sample solution*, determine the amount of fluoride in the sample.

Acceptance criteria: NMT 0.1%

SPECIFIC TESTS

- **Water Determination, *Method I*⟨921⟩:** NMT 7.0%
- **Bacterial Endotoxins Test ⟨85⟩:** NMT 70 USP Endotoxin Units/mg
- **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count is NMT 100 cfu/g. The total yeast and mold count is NMT 100 cfu/g.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, well-sealed containers.
- **USP Reference Standards ⟨11⟩**

USP Gonadorelin Hydrochloride RS

USP Gonadorelin Acetate Related Compound A RS

Gonadorelin free acid.

$C_{55}H_{74}N_{16}O_{14}$ 1183.3

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

Topic/Question	Contact	Expert Committee
GONADORELIN HYDROCHLORIDE	Ying Han Associate Science & Standards Liaison	BIO12020 Biologics Monographs 1 - Peptides

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 40(4)

Current DocID: GUID-BC041DA0-C4B1-44A1-B631-143BC6BC6F41_3_en-US
Previous DocID: GUID-BC041DA0-C4B1-44A1-B631-143BC6BC6F41_1_en-US
DOI: https://doi.org/10.31003/USPNF_M35790_03_01
DOI ref: lz303