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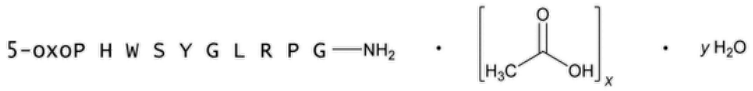
Gonadorelin Acetate



$C_{55}H_{75}N_{17}O_{13} \cdot xC_2H_4O_2 \cdot yH_2O$ 1374.46

Luteinizing hormone-releasing factor acetate (salt) hydrate.

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosylglycyl-L-leucyl-L-arginyl-L-prolylglycinamide acetate (salt) hydrate CAS RN®: 52699-48-6; 33515-09-2; UNII: 2RG1XQ1NYJ.

» Gonadorelin Acetate is a synthetic polypeptide hormone having the property of stimulating the release of the luteinizing hormone from the hypothalamus. It contains not less than 80 percent by weight of $C_{55}H_{75}N_{17}O_{13}$, the remainder being acetic acid and water.

[NOTE—Gonadorelin Acetate is extremely hygroscopic. Protect from exposure to moisture, and store in a desiccator.]

Packaging and storage—Preserve in tight, well-sealed containers, protected from moisture. Store at a temperature of not more than 8°.

Labeling—Label it to indicate it is for veterinary use only.

USP REFERENCE STANDARDS ⟨11⟩—

USP Gonadorelin Acetate RS $C_{55}H_{75}N_{17}O_{13} \cdot xC_2H_4O_2 \cdot yH_2O$ 1182.3
(acetate free)

USP Gonadorelin Acetate Related Compound A RS
Gonadorelin free acid.
$C_{55}H_{74}N_{16}O_{14}$ 1183.3

Identification—

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

B: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution,* as obtained in the test for *Related compounds.*

SPECIFIC ROTATION ⟨781S⟩: between −54° and −66°, at 20°, calculated with reference to the peptide content determined in the *Assay*.

Test solution: 10 mg per mL, in 1% (v/v) acetic acid.

WATER DETERMINATION, *Method Ic* ⟨921⟩: not more than 7.0%, determined by directly introducing not less than 2 mg of the solid substance into the titrator.

Limit of fluoride—[NOTE—Use polypropylene vessels for preparation of solutions and standards.]

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

Test solution—Dissolve between 3 and 5 mg of Gonadorelin Acetate in 1.375 mL of the same buffer as that used for the preparation of the *Standard solutions.*

Procedure—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 test is considered valid if the slope of the curve is in the range of −54 to −60 mV per decade and the regression curve has a square of the correlation coefficient, r^2, not less than 0.995. From the calibration curve and the concentration of the *Test solution,* determine the amount of fluoride in the sample: not more than 0.1% (w/w) is found.

Acetic acid and trifluoroacetic acid—

Solution A—To 900 mL of water add 7.0 mL of phosphoric acid and 5.0 mL of concentrated ammonia. Mix, and dilute with water to 1000 mL, pass through a 0.45-µm filter, and degas. Add 20 mL of methanol, mix, and degas for an additional 2 minutes.

Solution B—Prepare a degassed mixture of acetonitrile and water (1:1).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography ⟨621⟩*).

Diluent—Dilute 5.0 mL of phosphoric acid with water to 1000 mL, and mix thoroughly.

Trifluoroacetic acid stock solution—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. Carefully add 670 µL of trifluoroacetic acid to the flask, stopper immediately, and weigh. Dilute with water to volume.

Standard solutions—Accurately weigh out 150, 75, and 10 mg of sodium acetate trihydrate into three separate 100-mL volumetric flasks. Add 10 mL, 2 mL, and 100 µL, respectively, of the *Trifluoroacetic acid stock solution* to the flasks, and dilute each with *Diluent* to the 100-mL mark. Calculate the concentration, in mg per mL, of acetic acid in each *Standard solution* using the following equation:

$$0.00434W_A$$

in which W_A is the weight, in mg, of sodium acetate trihydrate taken. Calculate the concentration, in mg per mL, of trifluoroacetic acid in each *Standard solution* using the following equation:

$$0.0001(W_T V)$$

in which W_T is the weight, in mg, of trifluoroacetic acid used for preparation of the *Trifluoroacetic acid stock solution;* and V is the volume, in mL, of *Trifluoroacetic acid stock solution* used to prepare the *Standard solution*.

Test solution—Prepare duplicate samples by accurately weighing out two separate aliquots of about 4.0 mg of Gonadorelin Acetate and dissolving each with 1 mL of the *Diluent*.

Chromatographic system (see Chromatography ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column containing 5-µm packing L1. The flow rate is approximately 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–5	100	0	isocratic
5–6	100→0	0→100	linear
6–14	0	100	isocratic
14–15	0→100	100→0	return to initial
15–25	100	0	re-equilibration

Chromatograph the *Standard solutions,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 2000 theoretical plates for the trifluoroacetic acid peak and not less than 10,000 for the acetic acid peak; and the relative standard deviation for six replicate injections of the most concentrated *Standard solution* is not more than 2.0%.

Procedure—Inject in duplicate equal volumes (about 20 µL) of each of the *Standard solutions* followed by the duplicate *Test solutions*. Plot the peak areas of each of the components in the *Standard solutions* versus concentration, in mg per mL, and determine the regression line using the least squares method. The test is considered valid if the regression curves for both acetic acid and trifluoroacetic acid have a square of the correlation coefficient, r^2, not less than 0.995. From the resulting graph, determine the percentages of acetic acid and trifluoroacetic acid in the *Test solution:* between 8% and 12.5% of acetic acid is found, and not more than 0.25% of trifluoroacetic acid is found.

Related compounds—

Standard solution—Dissolve an accurately weighed quantity of USP Gonadorelin Acetate RS in water to obtain a solution having a known concentration of about 0.5 mg per mL.

System suitability solution—Dissolve an accurately weighed quantity of USP Gonadorelin Acetate Related Compound A RS in water to obtain a solution having a known concentration of about 0.5 mg per mL. Mix equal volumes of this solution and the *Standard solution*.

Test solution—Dissolve an accurately weighed quantity of Gonadorelin Acetate in water to obtain a solution having a known concentration of about 0.5 mg per mL.

SYSTEM 1—

Solvent 1—Mix 1 mL of trifluoroacetic acid with 1 L of water. Pass through a 0.45-µm filter, and degas.

Solvent 2—Mix 1 mL of trifluoroacetic acid with 1 L of acetonitrile.

Solution A—Prepare a mixture of *Solvent 1* and *Solvent 2* (95:5).

Solution B—Prepare a mixture of *Solvent 2* and *Solvent 1* (60:40).

Chromatographic system (see Chromatography ⟨621⟩)— The HPLC is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is approximately 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	91	9	initial
0–25	91→45	9→55	linear
25	45→91	55→9	return to initial
25–30	91	9	re-equilibrium

SYSTEM 2—

Mobile phase—Add 47 mL of phosphoric acid and 55 mL of triethylamine to 4 L of water, and adjust with phosphoric acid or triethylamine to a pH of 2.5, as appropriate. Pass through a 0.45-µm filter, and degas. Add acetonitrile to obtain a 13% (v/v) concentration of acetonitrile.

Chromatographic system (see Chromatography ⟨621⟩)— The HPLC is equipped with a 215-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is approximately 1.5 mL per minute using isocratic elution and having a run time of 50 minutes. Using both *System 1* and *System 2* chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure.* The *Standard solution* is used only to identify the gonadorelin acetate peak. Chromatograph the *System suitability solution,* and record the peak responses as directed for *Procedure:* the resolution, *R,* between gonadorelin acetate and gonadorelin acetate related compound A is not less than 2.0; the column efficiency is not less than 75,000 theoretical plates for *System 1* and not less than 3000 theoretical plates for *System 2;* the tailing factor is not more than 2.0 for both *System 1* and *System 2;* and the relative standard deviation for five replicate injections is not more than 2.0%.

Procedure—Inject equal volumes (about 20 µL) of each of the *Standard solution,* the *System suitability solution,* and the *Test solution,* followed by a co-injection of the *Test solution* with the *Standard solution,* into both *System 1* and *System 2*. Include blank injections between the different solutions. Integrate all peaks in order to obtain a baseline similar to that in the blank chromatograms, disregarding any peaks due to the solvent, counter-ion, and baseline artifacts. Using peak areas, and including all peaks greater than 0.05%, calculate the percentage of each impurity in the portion of Gona dorelin Acetate taken: not more than 1% of any single impurity is found, and not more than 2% of total impurities is found.

Amino acid analysis—Proceed as directed in the *Assay*. Express the content of each amino acid in µmoles, and calculate the total number of µmoles of Gonadorelin Acetate in the test sample as directed in the *Assay*. By dividing the number of µmoles of each amino acid by the total number of µmoles of Gonadorelin Acetate in the test sample, the relative proportions of amino acids are found: serine, 0.7 to 1.05; glutamic acid, 0.95 to 1.05; proline, 0.95 to 1.05; glycine, 1.9 to 2.1; leucine, 0.9 to 1.1; tyrosine, 0.7 to 1.05; histidine, 0.95 to 1.05; and arginine, 0.95 to 1.05. Isoleucine and lysine are absent; not more than traces of other amino acids except tryptophan are detected.

Assay—(see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩). [Note—The following method is given for informational purposes; any validated amino acid analysis method can be used.]

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: lysine, threonine, alanine, leucine, histidine, serine, valine, tyrosine, arginine, glutamic acid, methionine, phenylalanine, aspartic acid, proline, isoleucine, tryptophan, and cystine.

Assay preparation (see *Protein Hydrolysis, Method 1* ⟨1052⟩)—Accurately weigh out between 0.4 and 1.0 mg of Gonadorelin Acetate in glass ampuls. Add a minimum of 1.0 mL of *Hydrolysis Solution* containing 4% phenol, freeze the sample ampul, and flame seal under vacuum. Hydrolyze at 110° for about 22 hours. 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 having a 0.45-µm porosity.

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. Express the content of each amino acid in µmoles. The total number of µmoles of gonadorelin acetate in the test sample is calculated by summing the number of µmoles for glutamic acid, proline, glycine, leucine, tyrosine, histidine, and arginine, and dividing by eight. Calculate the percentage of $C_{55}H_{75}N_{17}O_{13}$ in the portion of Gonadorelin Acetate taken by the formula:

$$118.23(N/W)$$

in which N is the total number of µmoles of gonadorelin acetate; and W is the weight of the sample in mg.

Topic/Question	Contact	Expert Committee
GONADORELIN ACETATE	Julie Zhang Associate Science & Standards Liaison	BIO12020 Biologics Monographs 1 - Peptides

Chromatographic Database Information: Chromatographic Database

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