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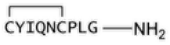
Oxytocin

(*This monograph has been updated to the current USP style. No revisions or changes to tests have been made.*)



$C_{43}H_{66}N_{12}O_{12}S_2$ 1007.19

Oxytocin CAS RN®: 50-56-6; UNII: 1JQS135EYN.

DEFINITION

Oxytocin is a nonapeptide hormone having the property of causing the contraction of uterine smooth muscle and myoepithelial cells within the mammary gland. It is prepared by synthesis. Its oxytocic activity is NLT 400 USP Oxytocin Units/mg.

IDENTIFICATION

[Note—Perform either *Identification A* and *B* or *Identification A* and *C* tests.]

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. Nuclear Magnetic Resonance:** [Note—Concentrations of Oxytocin in both the *Standard solution* and the *Sample solution* must be the same (within 5% of each other) but can be adjusted based on the quality of the spectrum obtained. The spectra must be acquired under the same conditions for both the *Standard solution* and the *Sample solution*. The spectra obtained are of sufficient quality to allow quantification of the integrals of the resonances specified below to be obtained. Integrals and spectra of both the *Standard solution* and the *Sample solution* can be repeated and averaged.]

Buffer: Dissolve 27.6 g of monobasic sodium phosphate in 900 mL of water, adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 5.0 ± 0.1, dilute with *water* to 1000 mL, and mix.

Standard solution: Prepare a 10-mg/mL solution (approximately 1 mL) of USP Oxytocin Identification RS in *Buffer*. Lyophilize to dryness, redissolve in deuterium oxide, lyophilize again, redissolve in deuterium oxide, and lyophilize once again (to replace exchangeable hydrogens with deuterium). Dissolve in 1 mL of deuterium oxide containing 0.5% v/v 2,2,3,3-(d4)-3-(trimethylsilyl) propionic acid sodium salt (TSP) as a chemical shift reference.

Sample solution: Prepare a 10-mg/mL solution (approximately 1 mL) of Oxytocin in *Buffer*. Proceed as directed for the *Standard solution*.

Analysis

Samples: *Standard solution* and *Sample solution*

Obtain a proton NMR spectrum of both the *Standard solution* and the *Sample solution*. Identify any other resonances in the spectrum of the *Sample solution* that are not present in the spectrum of the *Standard solution*.

Acceptance criteria: The spectra from both the *Standard solution* and the *Sample solution* are qualitatively and quantitatively similar, and all of the resonances from the spectrum of the *Standard solution* are present in the spectrum of the *Sample solution* and have the same chemical shift values (±0.1 ppm).

The integrals of the acetate and deuterium oxide peaks at 1.9 and 4.9 ppm can differ quantitatively in the spectra of the *Standard solution* and the *Sample solution*.

• **C. Amino Acid Content:** Use a suitable, validated procedure (see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩).

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. For the validation of the method, use an appropriate internal standard, such as norleucine. Prepare a separate, equimolar solution of L-tryptophan.

Sample solution: [Note—The following hydrolysis conditions and concentrations can be modified depending on the method of analysis chosen.] Transfer about 64 mg of Oxytocin, accurately weighed, to a suitable vessel, and dissolve in 1.0 mL of water. Transfer 0.10 mL of this solution to a vacuum hydrolysis tube, add 2.0 mL of 6 N hydrochloric acid, evacuate the tube, and heat for 16 h at 120°. Transfer 0.10 mL of the hydrolysate so obtained to a suitable vessel, add 1 mL of water, and lyophilize. Dissolve in and dilute to a suitable volume in a buffer solution suitable for amino acid analysis.

Analysis

Samples: *Standard solutions* and *Sample solution*

Inject equal volumes of the *Standard solutions* and the *Sample solution* into the amino acid analyzer, measure and record the responses for each amino acid peak. Express the content of each amino acid in moles.

Calculate the mean moles of the amino acids:

Mean moles of amino acids = (sum of moles found in the *Analysis* for aspartic acid, glutamic acid, proline, glycine, isoleucine, and leucine)/6

Calculate the relative proportion of each amino acid:

Relative proportion of each amino acid = moles of each amino acid/mean moles of amino acids

Acceptance criteria: See *Table 1*.

Table 1

Amino Acid	Acceptance Criteria
Aspartic acid	0.90–1.10
Glutamic acid	0.90–1.10
Proline	0.90–1.10
Glycine	0.90–1.10
Leucine	0.90–1.10
Isoleucine	0.90–1.10
Tyrosine	0.7–1.05
Half-cystine	1.4–2.1
Other amino acids	NMT traces of other amino acids are present.

ASSAY

• **Procedure**

Solution A: 0.1 M monobasic sodium phosphate

Solution B: Acetonitrile in water (1:1). Filter and degas before use. Make adjustments if necessary (see *Chromatography ⟨621⟩, System Suitability*).

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	70	30
20	50	50

Diluent: Dissolve 5.0 g of chlorobutanol in 5.0 mL of glacial acetic acid. Add 5.0 g of alcohol, 1.1 g of sodium acetate, and 1000 mL of water, and mix.

Standard solution: Dissolve the entire contents of a vial of USP Oxytocin RS in a known volume of *Diluent*. [Note—The solution may be diluted as necessary to a working concentration range for the *Assay*.]

Sample solution: 10 USP Oxytocin Units/mL of Oxytocin in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 12.0-cm; 5-µm packing L1

Column temperature: Room temperature

Flow rate: About 1.5 mL/min

Injection volume: About 100 µL

System suitability

Sample: *Standard solution*

[NOTE—Adjust the flow rate or the composition of the *Mobile phase* such that the retention time of Oxytocin is approximately 10 min and between 15 and 17 min for chlorobutanol.]

Suitability requirements

Resolution: NLT 1.5 between Oxytocin and the nearest adjacent peak

Relative standard deviation: NMT 2.0% for oxytocin from replicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Separately inject three equal volumes of the *Standard solution* and the *Sample solution* into the chromatograph, and record the chromatograms as described in *Chromatographic system*. Identify the peaks, and determine the area of the Oxytocin peak.

Calculate the potency of Oxytocin in USP Oxytocin Units/mg:

$$\text{Result} = (r_U/r_S) \times (V/W) \times C$$

r_U = mean peak response from the *Sample solution*

r_S = mean peak response from the *Standard solution*

V = volume of the *Sample solution* in which the sample was dissolved (mL)

W = amount of oxytocin dissolved in the *Sample solution* (mg)

C = concentration of the *Standard solution* (USP Oxytocin Units/mL)

Acceptance criteria: NLT 400 USP Oxytocin Units/mg

OTHER COMPONENTS

• **ACETIC ACID IN PEPTIDES** ⟨503⟩

Sample solution: Transfer about 15 mg of Oxytocin, accurately weighed, to a 10-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

Acceptance criteria: 6%–10%

IMPURITIES

• **ORDINARY IMPURITIES**

Solution A, Solution B, Mobile phase, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Sample: *Sample solution*

Acceptance criteria: The sum of the responses of impurities from the *Sample solution* is NMT 5% of the area of the oxytocin peak.

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total bacterial count does not exceed 200 cfu/g. For products of animal origin, it also meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, preferably of Type I glass, in a refrigerator.

• **USP REFERENCE STANDARDS** ⟨11⟩

USP Oxytocin RS

USP Oxytocin Identification RS

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

Topic/Question	Contact	Expert Committee
OXYTOCIN	Jennifer Tong Sun Senior Scientist II	BIO12020 Biologics Monographs 1 - Peptides

Topic/Question	Contact	Expert Committee
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO12020 Biologics Monographs 1 - Peptides

Chromatographic Database Information: Chromatographic Database

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