

Status: Currently Official on 16-Feb-2025
 Official Date: Official as of 01-Dec-2024
 Document Type: USP Monographs
 DocId: GUID-19C2CA26-32EE-45C2-BF4E-A12A875B714B_2_en-US
 DOI: https://doi.org/10.31003/USPNF_M60145_02_01
 DOI Ref: pwnml

© 2025 USPC
 Do not distribute

Oxytocin

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



$\text{C}_{43}\text{H}_{66}\text{N}_{12}\text{O}_{12}\text{S}_2$ 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:

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

Calculate the relative proportion of each amino acid:

$$\text{Relative proportion of each amino acid} = \text{moles of each amino acid} / \text{mean moles of amino acids}$$

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