



## OXYTOCIN

### Draft proposal for The International Pharmacopoeia

(August 2007)

*DRAFT FOR COMMENT*

This document was provided by a contracted quality control laboratory. Please send any comments to Dr S. Kopp with a copy to Ms M.-L. Rabouhans, Quality Assurance and Safety: Medicines, Medicines Policy and Standards, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or e-mail: [kopps@who.int](mailto:kopps@who.int) and [rabouhansm@who.int](mailto:rabouhansm@who.int) by **10 November 2007**.

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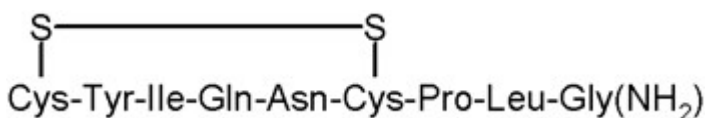
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**SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/07.241**  
*International Pharmacopoeia monograph on oxytocin*

	<b>Date</b>
Preliminary draft monograph prepared by laboratory	August 2007
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Collation of comments received	October 2007
Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations	October 2007
Further follow-up action as required	October 2007 - ...

**Draft proposal for *The International Pharmacopoeia*  
(September 2007)**

**OXYTOCINUM**  
**OXYTOCIN**



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

**Relative molecular mass.** 1007

**Chemical name.** L-Cysteinyl-L-tyrosyl-L-isoleucyl-L-glutamyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide cyclic (1→6)-disulfide; CAS Reg. No. 50-56-6.

**Other name.** Alpha-hypophamine.

**Description.** White or almost white powder.

**Solubility.** Very soluble in water. It dissolves in dilute solutions of acetic acid and of ethanol.

**Category.** Uterine-stimulating (Oxytocic).

**Storage.** Oxytocin should be kept in an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

**Labelling.** The designation on the container should state the oxytocin peptide content ( $C_{43}H_{66}N_{12}O_{12}S_2$ ).

**Additional information.** Oxytocin is hygroscopic.

### Requirements

**Definition.** Oxytocin is a synthetic cyclic nonapeptide having the structure of the hormone produced by the posterior lobe of the pituitary gland that stimulates contraction of the uterus and milk ejection in receptive mammals. It is available in the freeze-dried form as an acetate.

Oxytocin contains not less than 93.0% and not more than 102.0% of  $C_{43}H_{66}N_{12}O_{12}S_2$ , calculated with reference to the anhydrous and acetic acid-free substance.

By convention, for the purpose of labelling oxytocin preparations, 1 mg of oxytocin peptide ( $C_{43}H_{66}N_{12}O_{12}S_2$ ) is equivalent to 600 IU of biological activity.

### Identity tests

- Either tests A and B, or tests C and D may be applied.
- A. Examine the chromatograms obtained in the assay. The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.
- B. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from oxytocin RS or with the *reference spectrum* of oxytocin.
- C. Carry out test C.1. or, where UV detection is not available, test C.2.

C.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 70 volumes of dichloromethane R, 30 volumes of methanol R, 6 volumes of purified water and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10  $\mu$ l of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of oxytocin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R5 as the coating substance and a mixture of 70 volumes of dichloromethane R, 30 volumes of methanol R, 6 volumes of purified water and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10  $\mu$ l of each of 2 solutions in methanol containing (A) 5 mg of the test substance per ml and (B) 5 mg of oxytocin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Spray with ninhydrin. Heat the plate for a few minutes at 120°C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

- D. The absorption spectrum of a 0.30 mg/ml solution, when observed between 240 nm and 330 nm, exhibits a maximum at about 275 nm; the specific absorbance ( $A_{1\text{cm}}^{1\%}$ ) is 14 to 16, calculated with reference to the anhydrous and acetic acid-free substance.

**Specific optical rotation.** Use a 5.0 mg/ml solution and calculate with reference to the anhydrous and acetic acid-free substance;  $[\alpha]_{\text{D}}^{20\text{ }^\circ\text{C}} = -24.0^\circ$  to  $-28.0^\circ$ .

**pH value.** pH of a 20 mg/ml solution in carbon-dioxide-free water R, 3.0-6.0.

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.10 g of the substance; the water content is not more than 50 mg/g.

**Acetic acid content.** Determine by 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with octadecylsilyl silica gel for chromatography R (5  $\mu\text{m}$ ).

Use the following conditions for gradient elution:

Mobile phase A: Dilute 0.7 ml of phosphoric acid (~1440 g/l) TS with 900 ml purified water; adjust the pH to 3.0 with sodium hydroxide (~200 g/l) TS and dilute to 1000 ml with purified water.

Mobile phase B: Methanol R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0 – 5	95	5	Isocratic
5 – 10	95 to 50	5 to 50	Linear gradient
10 – 20	50	50	Isocratic
20 – 22	50 to 95	50 to 5	Linear gradient
22 – 30	95	5	Isocratic re-equilibration

Prepare the following solutions:

For solution (1), dissolve 15.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

For solution (2), prepare a 0.10 g/l solution of glacial acetic acid R in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A.

Operate with a flow rate of 1.2 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 210 nm.

Inject alternatively 10 µl each of solutions (1) and (2). In the chromatograms obtained, the peak corresponding to acetic acid has a retention time of 3-4 min. The baseline presents a steep rise after the start of the linear gradient, which corresponds to the elution of oxytocin from the column. Calculate the acetic acid content; not less than 60 mg/g and not more than 100 mg/g.

**Related substances.** Carry out the test as described under 1.14.4 High performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with octadecylsilyl silica gel for chromatography R (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 15 volumes of acetonitrile R, 15 volumes of phosphate buffer and 70 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 15 volumes of phosphate buffer and 15 volumes of purified water.

Prepare the phosphate buffer by dissolving 31.2 g of sodium dihydrogen phosphate dihydrate R in 1000 ml of purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0 – 5	100	0	Isocratic
5 – 20	100 to 94	0 to 6	Linear gradient
20 – 50	94 to 60	6 to 40	Linear gradient
50 – 51	60 to 100	40 to 0	Linear gradient
51-65	100	0	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 0.50 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 5.0 µg of oxytocin per ml.

For the system suitability test: prepare solution (3) using 3 ml of solution (1) and 2 ml of sulfuric acid (10 g/l), heat carefully in a boiling water-bath for 20 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 220 nm.

Maintain the column temperature at 40 °C.

Inject 50 µl of solution (3). The test is not valid unless the resolution between the peak due to oxytocin (retention time about 25 minutes) and the peak with a relative retention of about 0.9 is not less than 1.4.

Inject alternatively 50 µl each of solutions (1) and (2).

In the chromatogram obtained with solution (1), the area of any peak, other than the principal peak, is not greater than 1.5 times the area of the principal peak obtained with solution (2) (1.5%). The sum of the areas of all peaks, other than the principal peak, is not greater than 5.0 times the area of the principal peak obtained with solution (2) (5%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

### Assay

- Either method A or method B may be applied.
- A. Determine by High performance liquid chromatography as described in the test for related substances with the following modifications.
- Prepare solution (2) as follows. Dissolve the contents of a vial of oxytocin RS in mobile phase A to obtain a concentration of 0.50 mg/ml.
- Inject alternatively 50 µl each of solutions (1) and (2).
- Calculate the content of oxytocin ( $C_{43}H_{66}N_{12}O_{12}S_2$ ) from the declared content of  $C_{43}H_{66}N_{12}O_{12}S_2$  in oxytocin RS.
- B. Dissolve about 30 mg, accurately weighed, in sufficient purified water to produce 100 ml. Measure the absorbance of this solution in a 1-cm layer at the maximum at about 275 nm, and calculate the content of  $C_{43}H_{66}N_{12}O_{12}S_2$ , using the absorptivity value of 1.49 ( $A_{1cm}^{1\%} = 14.9$ ).

### *Additional requirement for Oxytocin for parenteral use*

Complies with the monograph "Parenteral preparations".

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 300 IU of endotoxin RS per mg.

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