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Oxytocin Injection Job Aid to Assist with Laboratory Testing

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usp®

Contact Information

Promoting the Quality of Medicines Plus Program
United States Pharmacopeia
12601 Twinbrook Parkway
Rockville, MD 20852 USA
Tel: +1-301-816-8166
Fax: +1-301-816-8374
Email: PQMplus@USP.org

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The Promoting the Quality of Medicines Plus (PQM+) Program is a five-year cooperative agreement between USAID and USP to sustainably strengthen medical product quality assurance systems in low- and middle-income countries. The program works to improve medical product quality through cross-sectoral and systems strengthening approaches and the application of international quality assurance standards across the pharmaceutical system. By sharing scientific expertise and providing technical support and leadership, PQM+ helps create resilient and robust local health systems that address diseases such as HIV/AIDS, tuberculosis, malaria, and neglected tropical diseases, as well as improve maternal, newborn, and child health.

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Acronyms

ACN	acetonitrile
API	active pharmaceutical ingredient
HPLC	high-performance liquid chromatography
Int. Ph.	International Pharmacopeia
LCMS	liquid chromatography-mass spectrometry
L1	octadecyl silane chemically bonded to porous silica or ceramic microparticles
NLT	no less than
NMT	no more than
Ph. Eur.	European Pharmacopeia
PQM+	Promoting the Quality of Medicines Plus
R	reagent grade
RS	reference standards
RSD	relative standard deviation
TFA	trifluoroacetic acid
TS	test solution
USAID	U.S. Agency for International Development
USP	U.S. Pharmacopeial Convention
UV	ultraviolet

Overview

This document is a job aid to enable quick access to technical information for oxytocin testing. The information consists of assay, pH, identification, storage, and controls. The information herein is adapted from the Promoting the Quality of Medicines (PQM) report “Revisiting the Stability and Storage Specifications of Oxytocin Injection: A Literature Review” (July 2018).

This lab aid is a compilation of information published by USP and the International Pharmacopoeia (Int. Ph.) for oxytocin identification, relative density, assay, pH, bacterial endotoxins, degradants, and related substances to assist in the testing of oxytocin injection. The information should be used to facilitate testing of oxytocin injection.

Aid for Oxytocin Injection: USP Tests

Table 1. USP tests for assay, bacterial toxins, and pH

Tests	USP
Assay by HPLC	ASSAY PREPARATION: Dissolve oxytocin in diluent to obtain approximately 10 USP oxytocin units per mL **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, then mix. **Standard Preparation:** Oxytocin injection is a sterile solution of oxytocin in a suitable diluent. Each mL of oxytocin injection possesses an oxytocic activity of not less than 90.0 percent and not more than 110.0 percent of that stated on the label in USP oxytocin units. CHROMATOGRAPHIC SYSTEM (SEE USP ⟨621⟩) **Detector:** Variable wavelength set at 220 nm **Column:** 12.0 cm × 4.6 mm with 5 µm packing L1 maintained at room temperature **Flow rate:** 1.5 mL per minute. The system is equilibrated with a mixture of 70% mobile phase A and 30% mobile phase B. **Injection volume:** 100 µL **Mobile phase A:** Prepare a buffer solution of 0.1 M monobasic sodium phosphate. **Mobile phase B:** Prepare a filtered and degassed mixture of acetonitrile in water (1:1). After each injection, mobile phase is changed linearly over 20 minutes to 50% mobile phase A and 50%mobile phase B. **Retention time of oxytocin** ≈ 10 minutes **Retention time of chlorobutanol** ≈ 15 and 17 minutes **Resolution:** NLT 1.5 for oxytocin and nearest adjacent peak; **RSD** = NMT 2.0% for oxytocin. Calculate potency in USP Oxytocin Units per mg by the formula: C(rU/rS)(V/W) C = concentration (USP Oxytocin Units per mL) of standard preparation rU and rS = mean peak responses obtained from the assay preparation and the standard preparation, respectively V = volume of sample solution W = amount, in mg, of oxytocin dissolved in the sample solution
Bacterial Endotoxins Test ⟨85⟩	It contains not more than 35.7 Endotoxin Units per USP Oxytocin Unit.
pH	between 3.0 and 5.0.

Aid for Oxytocin Injection: Ph. Int. Tests

Table 2. Ph. Int. tests for identification, assay, related substances, pH, and bacterial endotoxins

<table>
<tr><th>Tests</th><th>USP</th></tr>
<tr><td>Identification</td><td>Either Test A or Test B may be applied.

Test A) Carry out the test as described under 1.14 thin-layer chromatography (TLC) using silica gel R5 as the coating substance chromatography.

mobile phase = 70 volumes of dichloromethane R:30 volumes of methanol R:6 volumes of water R:1 volume of glacial acetic acid R

Solution A = Evaporate 10.0 mL of oxytocin injection to dryness at 30° C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) and dissolve the residue in 1.0 mL of methanol R.

Solution B = Methanol R containing 165.0 µg per mL of oxytocin RS.

Apply 20 µL of each of the following two solutions A and B.

Remove TLC plate from the chromatographic chamber and allow it to dry exhaustively in a current of cool air. Expose the plate to iodine vapor and examine in daylight.

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

Test B) Examine the chromatograms obtained in the assay (1.14.4 high-performance liquid chromatography). 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.</td></tr>
<tr><td>Assay</td><td>Carry out the test under 1.14.4 HPLC using a stainless-steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 15 volumes of acetonitrile R:15 volumes of phosphate buffer:70 volumes of water R

Mobile phase B: 70 volumes of acetonitrile R:15 volumes of phosphate buffer:15 volumes of water R

Phosphate buffer: dissolve 31.2 g of sodium dihydrogen phosphate dihydrate R in 1000 mL of water R.

<table>
<tr><th>Time (min)</th><th>Mobile phase A (% v/v)</th><th>Mobile phase B (% v/v)</th><th>Comments</th></tr>
<tr><td>0–5</td><td>100</td><td>0</td><td>Isocratic</td></tr>
<tr><td>5–20</td><td>100 to 94</td><td>0 to 6</td><td>Linear gradient</td></tr>
<tr><td>20–50</td><td>94 to 60</td><td>6 to 40</td><td>Linear gradient</td></tr>
<tr><td>50–51</td><td>60 to 100</td><td>40 to 0</td><td>Return to initial composition</td></tr>
<tr><td>51–65</td><td>100</td><td>0</td><td>Re-equilibration</td></tr>
</table>
Solution 1: 0.50 mg of the test substance per ml mobile phase A</td></tr>
</table>

Tests	USP
	Solution 2: Dissolve contents of a vial of oxytocin RS in the mobile phase A to obtain a concentration of 16.7 µg per mL **Solution 3:** Use 3 mL of solution 1 and 2 mL of sulfuric acid (~10 g/L) TS. Heat carefully in a boiling water bath for 20 minutes. **Flow rate**=1.0 mL/min **Detector** = UV set at a wavelength of 220 nm. **Column temperature** = 40°C **Injection volume** = 50 µL **Notes:** The assay 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 approximately 0.9 is at least 1.4. 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.
Related substances	Carry out the test described under “Assay” with the following modifications: Prepare the following solutions using mobile phase A as diluent: **Solution 1:** 0.50 mg of the test substance per ml mobile phase A **Solution 2:** Dilute 1 mL of solution 1 to 50 mL. **Solution 3:** Weigh 100 mg of chlorobutanol R into a 20 mL volumetric flask, dissolve in 0.25 mL of acetic acid, glacial R, and dilute with mobile phase A. **Solution 4:** Using 3 mL of solution 1 and 2 mL of sulfuric acid (~10 g/L) TS, heat carefully in a boiling water bath for 20 minutes. **System Suitability**: Inject 50 µL of solution 4. **Note:** The test is not valid unless the resolution between the peak due to oxytocin (retention time ≈ 25 minutes) and the major peak with a relative retention of about 0.9 is at least 1.4. In solution 1, the area of not more than one peak, other than the principal peak, is greater than the area of the principal peak obtained with solution 2 (2 percent). No such peak, other than the principal peak, is greater than 2.5 times the area of the principal peak obtained with solution 2 (5 percent). Disregard any peak obtained in the chromatogram with solution 3.
pH	pH of the injection: 3.0–5.0
Bacterial endotoxins	Carry out the test as described under tUSP <85> test for bacterial endotoxins; contains less than 0.5 IU of endotoxin per IU of oxytocin.

References

United States Pharmacopeia – National Formulary (USP 43–NF 38)

International Pharmacopeia, Ninth Edition, 2019