

Stability-indicating HPLC method for the determination of the stability of oxytocin parenteral solutions prepared in polyolefin bags

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ABSTRACT: Oxytocin is very commonly used in clinical settings and is a nonapeptide hormone that stimulates the contraction of uterine smooth muscles. In this study the stability of extemporaneously compounded oxytocin solutions was investigated in polyolefin bags. The sterile preparations of oxytocin were compounded to the strength of 0.02 U/mL in accordance with United States Pharmacopeia (USP) <797> standards. In order to carry out the stability testing of these parenteral products, the solutions were stored under three different temperature conditions of -20°C (frozen), 2-6°C (refrigerated), and 22-25°C (room temperature). Three solutions from each temperature were withdrawn and were assessed for stability on days 0, 7, 15, 21, and 30 as per the USP guidelines. The assay of oxytocin was examined by an HPLC method at each time point. No precipitation, cloudiness or color change was observed during this study at all temperatures. The assay content by HPLC revealed that oxytocin retains greater than at least 90% of the initial concentrations for 21 days. There was no significant change in pH and absorbance values for 21 days under all the conditions of storage. Oxytocin parenteral solutions in the final concentration of 0.02 U/mL and diluted in normal saline are stable for at least 30 days under frozen and refrigerated conditions for 30 days. At the room temperature, the oxytocin solutions were stable for at least 21 days. The stability analysis results show that the shelf-life of 21 days observed in this study was far better than their recommended expiration dates.

Keywords: Oxytocin, stability, parenteral, compounding, HPLC

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1. Introduction

Oxytocin, a nonapeptide hormone, stimulates the contraction of uterine smooth muscles. It is commonly used in the clinical settings for the induction and augmentation of the first stage of labor, mid trimester abortion, and the cessation or prevention of postpartum hemorrhage (1-4). The recommended doses of oxytocin are as follows; for the induction of labor: 10 units per 1,000 mL, for the control of post-partum bleeding: 10 to 40 units per 1,000 mL, and for the treatment of inevitable abortion: 10 units in 500 mL (5). For these purposes, oxytocin is most often used as parenteral solutions and the recommended solvents are saline or 5% dextrose in saline solution (Product Insert, Teva Parenteral Medicines, 2004). The intravenous forms are diluted from the commercially available oxytocin vials, since the final recommended concentrations are commercially unavailable. These intravenous solutions are prepared using aseptic conditions under the laminar flow hood and should be stored suitably until further used.

There are few studies which provide the guidance regarding the short-term stability of these compounded syringes (3,6). In a study by Boothby *et al.* (7), the stability of oxytocin solution was examined in Ringer's lactate solution at 4 and 25°C for over 31 days. In another study by Trissel *et al.* (8), the stability was done for 90 days at room temperature in 5% dextrose injection, normal saline, and lactated Ringer's solution. However, no stability-indicating study has evaluated the stability of oxytocin stored for a period of 30 days under different temperature conditions in normal saline (the most commonly used diluent for oxytocin). Thus, the hospital pharmacy is placed in a unique situation in determining how to prepare the solutions, how long the product should be stored, and how to improve compounding efficiency while at the same time reducing waste in any health-care model.

The objective of the present study was to determine the physical and chemical stability of extemporaneously compounded oxytocin to 0.02 U/mL in normal saline and stored in polyolefin bags. Three different temperature conditions were used for this study; room temperature (22-25°C), frozen (-20°C) or under refrigeration (2-6°C) for up to 30 days.

2. Materials and Methods

2.1. Materials

All chemicals were of analytical grade. Oxytocin, sodium phosphate, sodium chloride, sodium hydroxide, hydrochloric acid, and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile (HPLC grade) was purchased from Fisher Chemicals (Fair Lawn, NJ, USA). Oxytocin vials of 100 U/10 mL (10 U/mL), 10 mL Multiple Dose Vial was purchased from Teva Pharmaceuticals (Teva Pharmaceuticals USA, Inc., Sellersville, PA, USA) and normal saline bags were purchased from B. Braun (Bethlehem, PA, USA).

2.2. HPLC sample preparation using solid phase extraction (SPE) method

All the samples for the HPLC analysis were prepared using Oasis HLB cartridges (60 mg-3 mL; Product No. WAT094226) obtained from Waters Corporation (Milford, MA, USA). The cartridges were pre-washed with 3 mL of acetonitrile and 1.5 mL of water, then conditioned with 1.5 mL of 10 mM phosphate buffer (pH 7.5). The cartridge was not permitted to run dry during the whole conditioning procedure. The sample (3 mL) of oxytocin solution was loaded on the cartridge and was eluted with 0.75 mL 50% of 10 mM phosphate buffer (pH 7.5) and 50% acetonitrile. This sample was immediately centrifuged at $9,000 \times g$ for 3.5 min and 500 μ L of supernatant was transferred to an HPLC vial with a micro-insert.

2.3. HPLC system

All the chromatographic studies were performed on a Dionex Ultimate 3000 HPLC system connected with an absorbance detector. The separations were done on X Bridge™ C8 cartridge column (250 \times 4.6 mm I.D., Waters Associates, Milford, MA, USA) with the column particle diameter of 3.5 μ m. The column effluents were monitored at wavelength of 214 nm for a run time of 16 min at the temperature of 30°C. The absorbance value was selected based on the full range scan of an oxytocin sample that was carried under the same conditions in the HPLC system. The mobile phase was 50% of 10 mM phosphate buffer (pH 7.5) and 50% acetonitrile. The mobile phase was filtered and degassed before use. The flow rate was 0.2 mL/min and the injection volume was 50 μ L.

2.4. Stability-indicating HPLC assay

The suitability of the present HPLC conditions to be used as a stability-indicating method was tested by accelerating the degradation of oxytocin. The stability-indicating capability of the assay was examined by degradation of 0.02 U/mL oxytocin in 1 M HCl and 1

M NaOH solutions and these solutions were heated at 90°C for 1 h. The samples were withdrawn before and after heating each of the solutions and were subjected to SPE. Each sample was analysed by HPLC using the conditions as explained above.

2.5. Oxytocin standard curve

The calibration of HPLC system was performed by constructing a standard curve using five known concentrations of oxytocin. In order to prepare standard curve, fixed amounts (0.01, 0.015, 0.02, 0.03, and 0.04 U/mL) of standard oxytocin were respectively added to the normal saline solutions and these samples were analyzed by subjecting them to SPE and then using the standardized HPLC conditions.

All the samples were analyzed three times (intra-day variation) on three different days (inter-days variation). The accuracy was calculated at each concentration as the ratio of the measured concentration to the nominal concentration multiplied by 100%.

The limit of quantitation (LOQ) of the method was defined as the lowest concentration of that could be quantitatively determined with acceptable precision and accuracy. Acceptance limits were defined as accuracy of 80-120% and precision of $\leq 20\%$.

2.6. Preparation of oxytocin parenteral solutions

Dilutions of the oxytocin parenteral solutions to nominal concentrations of 0.02 U/mL were performed by the Charleston Area Medical Center (CAMC Solutions, Charleston, WV, USA) under aseptic conditions. All the solutions were prepared on the same day. Briefly, 2 mL of solution was withdrawn from a 1,000 mL normal saline bag and was replaced by 2 mL of oxytocin stock (10 U/mL). The bag was then agitated for couple of seconds to ensure uniform mixing.

The solutions were frozen (-20°C), refrigerated ($2-6^{\circ}\text{C}$), or stored at room temperature ($22-25^{\circ}\text{C}$). The solutions at room temperature were exposed to normal fluorescent light. Frozen and refrigerated samples were kept in a dark refrigerator and were exposed to light only during sampling. These samples were allowed to warm at room temperature and no external source of heat was used to warm these parenteral solution bags. Three bags for each of the storage conditions were assessed for physical and chemical stability over 30 days. Stability was assessed on days 0, 7, 15, 21, and 30. The storage temperatures were closely monitored throughout the study.

2.7. Physical evaluation

Physical stability of oxytocin parenteral solutions was assessed by visual examination. The solutions were evaluated against black and white backgrounds for visible particulate matter, cloudiness, and color changes.

To complement the visual inspection of the syringes, absorbance was also measured at the wavelength of 600 nm using spectrophotometer (Hitachi High Technologies America, Inc., CA, USA).

The pH was measured at each sampling point with Corning Pinnacle pH meter (Model 530, Cole-Parmer, IL, USA) fitted with Orion 9102BNWP electrode (single junction Ag/AgCl refillable combination pH), using three-point standardization with buffer solutions (pH of 4.0, 7.0, and 10.0). The pH of each sample was read at least two times. pH is defined as an important parameter that governs the stability of the product as the change in pH can cause the precipitation of the product. Thus pH along with spectroscopy can be a valuable tool for evaluating the stability.

2.8. Oxytocin stability assay

Chromatogram peak heights were used to determine oxytocin concentrations in the parenteral solutions. Three samples were withdrawn on each of the sampling day from each of the storage temperature and each sample was injected at least two times. The percentage of oxytocin remaining in each of the infusion after day zero was calculated based on the oxytocin content at day zero. The drug concentration was considered stable if its concentration was more than 90% of the initial concentration.

2.9. Data Analysis

Student's *t*-test was used to compare the difference between the data of interest with $\alpha = 0.05$ as the minimal level of significance. Wherever possible, the data is presented as mean \pm standard deviation.

3. Results

3.1. Standard curve and method validation

The chromatogram of oxytocin standards in normal saline showed a peak at retention time of 11.6 min (Figure 1). A

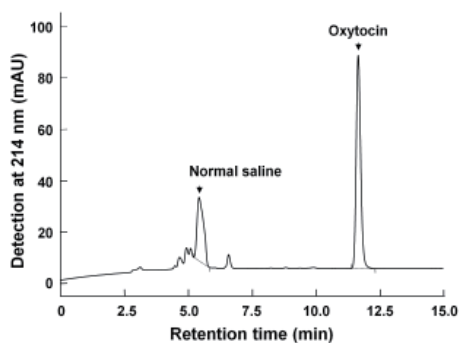


Figure 1. A representative HPLC chromatogram of oxytocin sample (0.02 U/mL) in the saline solution. The chromatogram showed a displacement peak originating from the saline solution at 5.4 min and oxytocin peak at 11.6 min.

blank sample (normal saline only) was also injected to the HPLC system and a peak at 5.4 min was observed from this sample. No peak was observed when sterile water or mobile phase was injected to the HPLC system. Thus the first peak in the chromatogram is because of normal saline and the second peak at 11.6 min is for oxytocin. As shown in Figure 2, a good linearity was exhibited in the concentration range (0.01-0.04 U/mL) by using the presently developed HPLC method. The average coefficient of determination of 0.99 was observed for the standard curve. The slopes of the curves illustrated an excellent agreement with coefficient of variability.

The intra- and inter-day relative standard deviations (RSD) were calculated to be 1.83% and 1.67%, respectively. For each concentration studied, a relative error (RE) of less than 10% was obtained. An acceptable precision and accuracy was acquired by this method for all the standards and quality controls based on the recommended criteria (9). The percentage recovery of oxytocin using the present HPLC method was also calculated from the peak areas obtained. As shown from the data in Table 1, an admirable recovery was accomplished at each of the added concentration. In accordance to the official requirements the LOQ was 0.005 U/mL.

3.2. Stability-indicating HPLC method characterization

Figures 3A and 3B show the degradation of oxytocin after heating at 90°C for 1 h in the presence of 1 M HCl and 1 M NaOH, respectively. As is clear from the chromatograms, multiple degradation product peaks were observed. Under both the conditions, acidic and alkaline, there was significant loss of

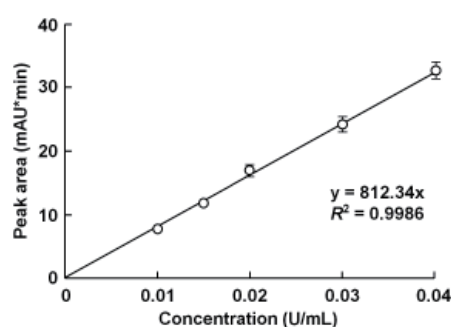


Figure 2. Standard curve of oxytocin assay. The samples were processed by HPLC as described in Section 2.3. For each concentration point 3 set of samples were prepared and this study was done on three different days.

Table 1. Recovery of oxytocin from the standard samples (n = 3)

Concentration added (U/mL)	Concentration obtained (U/mL)	Recovery (%)
0.01	0.012 \pm 0.0008	100.2
0.03	0.032 \pm 0.0011	100.2
0.05	0.053 \pm 0.002	100.3

oxytocin after heating at 90°C for 1 h. There was no interference of the degradation products as these peaks were seen at retention times other than the retention time for oxytocin (11.6 min, arrowhead in Figure 3). These results suggest that the present method is sufficiently specific to the drug and that, based on the chromatograms of forced degradation of oxytocin, this method can simultaneously analyze oxytocin and its degradation products in a sample.

3.3. Physical evaluation

The oxytocin parenteral solution remained clear throughout the study when kept frozen, under refrigeration, or at room temperature. No precipitation was observed. No significant increase ($p > 0.05$) in absorbance was observed as compared to the freshly read sample of the distilled water at the wavelength of 600 nm (data not shown).

The pH value of normal saline before addition of oxytocin was around 4.40. After the addition of oxytocin this value increases to 5.48 ± 0.18 . As shown in Table 2, there was no significant increase or decrease ($p > 0.05$) in this pH value under all the three temperatures used in this study over a period of 30 days.

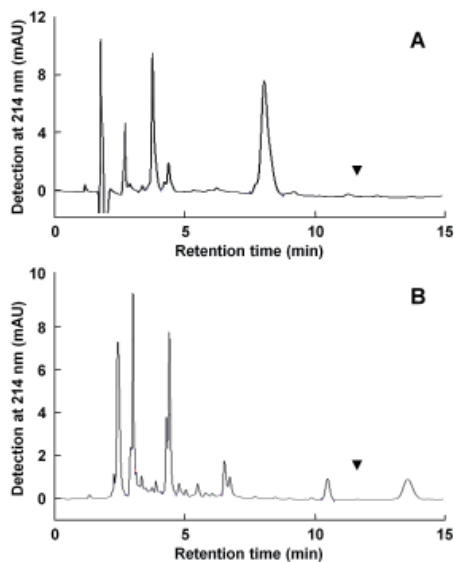


Figure 3. Chromatograms of oxytocin after accelerated degradation. Oxytocin was treated under (A) acidic and (B) alkaline conditions and the samples were processed by HPLC as described in Section 2.3. Arrowhead: expected elution position of oxytocin (11.6 min).

3.4. Drug content

As shown in Figure 4, all the oxytocin parenteral solutions were in the range of 90-110% of the labeled drug amount under frozen (-20°C) and refrigerated ($2-6^{\circ}\text{C}$) conditions for 30 days; at room temperature ($22-25^{\circ}\text{C}$) the oxytocin solutions were stable for at least 21 days. Minimal to no degradation was observed and no new degradation peaks were observed in the stable samples (data not shown).

4. Discussion

The stability data of the routinely used large volume parenteral in the final working condition are unavailable as they are commercially not available. Knowing the stability of these preparations would minimize compounding them just before the administration. A concentration of 0.02 U/mL is commonly used in the clinical setting.

The parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH) suggest that stress studies should be carried out on a drug to ascertain its inherent stability characteristics (10). A proper identification of degradation products would hence support the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability-indicating and be fully validated. In order to analyze the concentration of oxytocin, a stability-indicating HPLC method was validated and used for the present study.

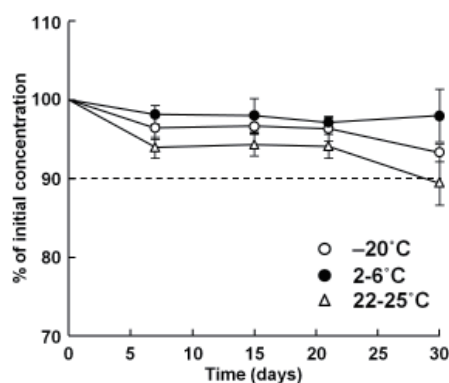


Figure 4. Percentage of initial concentration versus time profile for oxytocin parenteral solutions ($n = 9$). Open circle, -20°C ; closed circle, $2-6^{\circ}\text{C}$; open triangle, $22-25^{\circ}\text{C}$. Dashed line indicates 90% of initial concentration of oxytocin.

Table 2. The results of pH analysis ($n = 9$)

Storage temperature	Day 0	Day 7	Day 15	Day 21	Day 30
-20°C	5.48 ± 0.18	5.17 ± 0.15	5.16 ± 0.07	5.16 ± 0.06	5.10 ± 0.13
$2-6^{\circ}\text{C}$	5.48 ± 0.18	5.22 ± 0.10	5.21 ± 0.04	5.20 ± 0.13	5.08 ± 0.03
$22-25^{\circ}\text{C}$	5.48 ± 0.18	5.36 ± 0.16	5.33 ± 0.06	5.20 ± 0.04	5.28 ± 0.17

The present HPLC method met with all the acceptance criteria and was sensitive and reproducible enough for the acceptable study of oxytocin in unknown samples. As reported by Trissel (11), the failure to recognize the degradation products is the most common point that leads to erroneous reporting of the data on the stability studies.

There are some published studies in the literature that do report the short-term stability of oxytocin (3,6). Gard *et al.* (3) have reported oxytocin is stable in lactated Ringer's solution and lactated Ringer's-dextrose 5% solution over a 24-hour period at 25°C and over a 7-day period at 5°C. In an attempt to measure long term stability, Boothby *et al.* (7) published that oxytocin in lactated Ringer's solution is stable for 31 days at 4 and 25°C. Another comprehensive study done by Trissel *et al.* (8) have examined the extended stability of oxytocin in 5% dextrose injection, 0.9% sodium chloride injection, lactated Ringer's injection bags for 90 days at room temperature. Based on their results they have reported that oxytocin diluted in 5% dextrose or 0.9% sodium chloride is stable to be used for 90 days at room temperature. However, when diluted in lactated Ringer's solution it is advisable to be used only for 28 days when stored at room temperature. The most commonly recommended solvent for the dilution of oxytocin for clinical use is 0.9% sodium chloride. It is very pertinent to establish the stability of commonly used concentration of oxytocin solution at three normally used storage temperatures for parenteral products in the hospitals. Thus for the present study we have used all the three different temperature conditions of room temperature (22-25°C), refrigeration (2-6°C), and frozen (-20°C) and done the stability studies for 30 days.

Based on our results, oxytocin parenteral solutions are stable for at least 30 days when stored under refrigerated or frozen conditions. At room temperature, these solutions were observed to be stable for at least 21 days. Trissel *et al.* (8) have recommended that the use of oxytocin parenteral solutions should be restricted to no greater than 28 days at room temperature, regardless of the diluent used. The recommendation based on our present study is reasonably more than what is presently used.

A major driver of operations within the pharmacy department in a hospital setting is increasing efficiency and reducing waste. Therefore, Charleston Area Medical Center (CAMC) utilizes a consolidated intravenous medication preparation model where a central, USP <797> compliant clean room prepares many compounded sterile products in batches for use within our organization. Thus, the pharmacy is able to mass produce extemporaneously compound parenteral solutions from this facility and ship the finished products to each hospital of our health system, thus allowing the organization to free up its pharmacy team to provide more direct patient care while improving operational efficiencies. One of the challenges to this

model is expiration dating of medications. The aim of the compounding pharmacy is to prepare sterile products that can be compounded in large batches and with long expiration dates. Based on the present study, oxytocin solutions are stable for at least 21 days under different temperature conditions. By consolidating with the pharmacy in a hospital, workload can be removed from each facility thus shortening the turnaround time for the pharmacy to send medications to the patients.

The results of this study show that extended stability for oxytocin parenteral solutions can be assigned. However, assigned stability data should not exceed the mandates from USP <797> in the absence of end product sterility testing according to USP <71>.

5. Conclusion

A stability-indicating method was developed, which separates all the degradation products formed. The present HPLC method meets all the acceptance criteria and was sensitive and reproducible enough for the acceptable study of oxytocin in unknown samples. A good linearity of the standard curves with reproducible slopes was observed with this method. Hence it is recommended for analysis of the drug and degradation products in stability samples. The present method proved to be rapid and simple, and its precision is sufficient for routine quality control of oxytocin.

The oxytocin solutions were stable for at least 21 days under all the temperatures studied in the concentrations normally used in clinical setting when prepared in 0.9% sodium chloride (normal saline) for parenteral use. The extended stability of this preparation can improve operational efficiency, reduce waste, and reduce medication delivery delays.

Acknowledgements

This project was supported by the CAMC Health Education and Research Institute, Inc. and Charleston Area Medical Center Foundation, Inc. of Charleston, WV, USA.

References

1. Balki M, Ronayne M, Davies S, Fallah S, Kingdom J, Windrim R, Carvalho JC. Minimum oxytocin dose requirement after cesarean delivery for labor arrest. *Obstet Gynecol.* 2006; 107:45-50.
2. Elbourne D, Prendiville W, Chalmers I. Choice of oxytocic preparation for routine use in the management of the third stage of labour: An overview of the evidence from controlled trials. *Brit J Obstet Gynecol.* 1988; 95:17-30.
3. Gard JW, Alexander JM, Bawdon RE, Albrecht JT. Oxytocin preparation stability in several common obstetric intravenous solutions. *Am J Obstet Gynecol.* 2002; 186:496-498.
4. Nordström L, Fogelstam K, Fridman G, Larsson A,

- Rydstroem H. Routine oxytocin in the third stage of labour: A placebo controlled randomised trial. *Brit J Obstet Gynecol.* 1997; 104:781-786.
- 5 McEvoy GK. AHFS Drug Information. AHFS Drug Information 2005, Bethesda, MD: American Society of Health-System Pharmacists.
- 6 Chang CH, Ashford WR, Ives DA, *et al.* Stability of oxytocin in various infusion solutions. *Can J Hosp Pharm.* 1972; 25:152.
- 7 Boothby LA, Madabushi R, Kumar V, Breland BD, Hatton RC, Doering PL, Derendorf H. Extended Stability of Oxytocin in Ringer's Lactate Solution at 4° and 25°C. *Hosp Pharm.* 2006; 41:437-441.
- 8 Trissel LA, Zhang Y, Douglass K, Kastango E. Extended stability of oxytocin in common infusion solutions. *Int J Pharm Comp.* 2006; 10:156-158.
- 9 Karnes HT, March C. Precision, accuracy, and data acceptance criteria in biopharmaceutical analysis. *Pharm Res.* 1993; 10:1420-1426.
- 10 Food and Drug Administration, HHS. International Conference on Harmonisation; Stability Data Package for Registration Applications in Climatic Zones III and IV; Stability Testing of New Drug Substances and Products; availability. Notice. *Fed Regist.* 2003; 68:65717-65718.
- 11 Trissel LA. Avoiding common flaws in stability and compatibility studies of injectable drugs. *Am J Hosp Pharm.* 1983; 40:1159-1160.

(Received September 29, 2011; Revised November 13, 2011; Re-revised January 20, 2012; Accepted January 21, 2012)