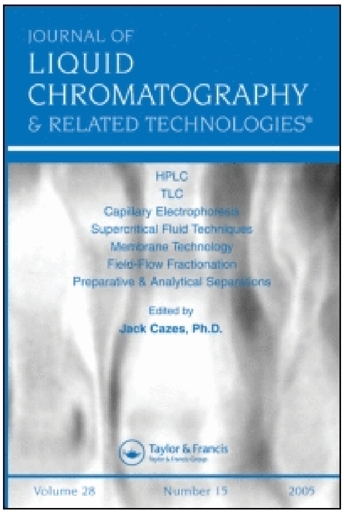

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A STABILITY-INDICATING HPLC METHOD FOR THE DETERMINATION OF OXYTOCIN ACETATE IN OXYTOCIN INJECTION, USP, SYNTHETIC[†]

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ABSTRACT

A gradient high performance liquid chromatographic (HPLC) procedure employing ultraviolet (UV) detection for the analysis of oxytocin acetate in Oxytocin Injection, USP, Synthetic is reported. The method requires no sample pretreatment and is accurate, reproducible and selective. The peak area versus oxytocin acetate concentration is linear over the range of 50-150% of its label claim of 10 Oxytocin USP Units/mL. The mean absolute recovery of oxytocin acetate using the described method is 98.3 ± 0.6%, (mean ±SD, n = 9). The precision, relative standard deviation (RSD), of label claim, amongst five sample preparations is not more than 0.4%. Intermediate precision, as determined from fifteen sample preparations, generated by two Analysts on different HPLC systems over three days, exhibits an RSD of 1.9%. The Standard and Assay Preparations are stable for up to 48 hours at room temperature.

The selectivity was evaluated by subjecting the finished product (Oxytocin Injection, USP, Synthetic) to thermal, acidic, basic, oxidative and fluorescent radiation stress conditions. No interference in the analysis of oxytocin acetate was observed from degradation products or from the preservative, chlorobutanol, showing the method is stability-indicating.

INTRODUCTION

Oxytocin is an endogenous nonapeptide hormone produced in the supraoptic and paraventricular nuclei of the hypothalamus and stored in the posterior pituitary.[1] The release of oxytocin from the posterior pituitary causes contractions in uterine, vascular, and other smooth muscle.[1] Synthetic oxytocin is the active component of the finished product, Oxytocin Injection, USP, Synthetic, which is administered to aid in the progression of labor and delivery in modern obstetrics.

The analysis of oxytocin is primarily determined immunologically[2-4] or chromatographically.[5-11] Although several methods exist for the determination of oxytocin, a computer search (Medline and Chemical Abstracts) indicated only two methods applicable to parenteral solutions.[9,10] The first method by Krummen and Frei[9] does not provide selectivity data for various stress conditions of the finished product, while the second method by Brown and Jenke[10] requires postcolumn derivatization with fluorescamine, which is time consuming and costly. The method described herein is a modification of the United States Pharmacopeia (USP) method,[11] whereby the effect of Mobile Phase A pH on resolution, column length and selectivity were examined.

This manuscript describes a sensitive, accurate, and reproducible, gradient reversed phase HPLC method for the determination of oxytocin acetate in Oxytocin Injection, USP, Synthetic. Moreover, this method was determined to be stability-indicating.

According to the USP 23 <1225> guidelines, analytical methods for the quantitation of major components of bulk drug substance or preservatives in finished pharmaceutical products fall under Assay Category I.[12] Data elements required for Assay Category I include precision, accuracy, specificity, range, linearity, and ruggedness. The method for oxytocin acetate in Oxytocin Injection, USP, Synthetic satisfies all of these requirements.

EXPERIMENTAL

Chemicals and Reagents

Oxytocin Injection, USP, Synthetic was formulated at Fujisawa USA, Inc. (Melrose Park, IL, USA). Oxytocin acetate was an in-house reference standard, while chlorobutanol was obtained from Akzo Nobel (Edison, NJ, USA). ACS reagent grade glacial acetic acid, sodium acetate trihydrate, sodium phosphate monobasic, sodium hydroxide, hydrochloric acid, and hydrogen peroxide were purchased from Mallinckrodt (Paris, KY, USA), while phosphoric acid was purchased from J.T. Baker (Phillipsburg, NJ, USA). HPLC grade acetonitrile was purchased from Baxter (Deerfield, IL, USA) and ethyl alcohol, absolute, was purchased from McCormick, Distilling Co. (Perkin, IL, USA). The water was deionized and distilled. All reagents were used without further purification.

Apparatus

The chromatographic system consisted of a Waters 680 Automated Gradient Controller, two 510 pumps, a WISP 717 autosampler, and a 486 variable-wavelength UV detector set at 220 nm (Waters Associates, Milford, MA, USA).

Two columns were evaluated; an Alltech Hypersil ODS (12 cm x 4.6 mm, 5 μm, Alltech, Deerfield, IL, USA) and a Beckman Ultrasphere ODS column (15 cm x 4.6 mm, 5 μm, Beckman, Fullerton, CA, USA). Both were maintained at ambient temperature.

Mobile Phases

Mobile Phase A consisted of 100 mM sodium phosphate monobasic. The pH of Mobile Phase a was varied from 3.1 to 4.5 in 0.1 pH intervals by adjustment with phosphoric acid. Mobile Phase B consisted of acetonitrile-water (1:1, v/v). Each mobile phase was filtered through a 0.45 μm filter and degassed. The gradient conditions are provided in Table 1. The flow rate was 1.5 mL/minute with a typical operating pressure of *ca.* 850 psi. Under these conditions, the retention times of oxytocin acetate and chlorobutanol were 10.2 and 21.1 minutes, respectively.

Table 1

Gradient Parameters

Interval (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	70	30
30	50	50
35	50	50
45	70	30
60	70	30

Preparation of Solutions

A. Diluent

Dissolve 5 g/L chlorobutanol, 5 mL/L glacial acetic acid, 5 g/L ethyl alcohol, and 1.82 g/L sodium acetate trihydrate in water, and mix.

B. Oxytocin standard stock solution

Dissolve enough Oxytocin Standard in Diluent to yield a concentration of 200 Oxytocin USP Units/mL.

C. Standard preparation

Dilute the Oxytocin Standard Stock Solution with Diluent to yield a concentration of 10 Oxytocin USP Units/mL.

D. Assay preparation

The concentration of Oxytocin Injection, USP, Synthetic is 10 Oxytocin USP Units/mL so, consequently, no dilution is required.

E. Resolution solution

Transfer 15.0 mL of Oxytocin Standard Stock Solution into a 100 mL volumetric flask, dilute to volume with Diluent, mix, and adjust the pH to 2.0 ± 0.1 with 0.1N hydrochloric acid. Heat the solution at 1000C for 40 minutes, then adjust the pH to 4.0 ± 0.1 with 0.1N sodium hydroxide.

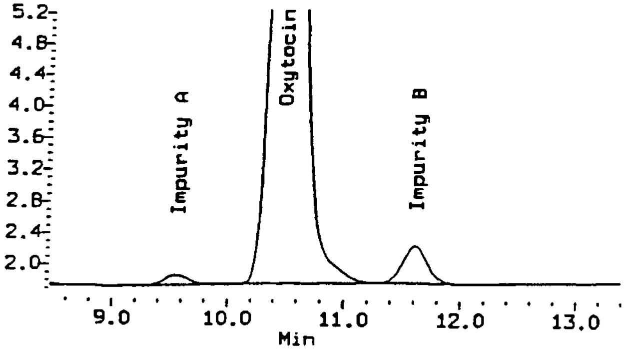

Figure 1. Typical chromatogram of the Resolution Solution.

System Suitability

The system suitability results were calculated according to the USP 23 <621> from typical chromatograms.[13] The instrument precision as determined by five successive injections of the Standard Preparation should provide a relative standard deviation (RSD) not more than (NMT) 2.0%. The column efficiency should be greater than 6000 theoretical plates. The tailing factor should not exceed 1.5 at 5% peak height. Finally, inject 100 μL of the Resolution Solution. The resolution between oxytocin acetate and the nearest adjacent peak (Impurity A or Impurity B) is not less than (NLT) 1.5 (Figure 1).

Specificity

The specificity of the method was studied through the analysis of stressed Oxytocin Injection, USP, Synthetic (finished product, 10 Oxytocin USP Units/mL) and stressed Placebo Solutions (finished product without oxytocin acetate). The finished product was subjected to thermal, acidic, basic, oxidative and fluorescent light environments to cause oxytocin degradation of 10-30%, as determined by peak area percent.

Five mL aliquots of the finished product and Placebo solution were sealed in transparent glass containers with equal head space and exposed to various stress conditions. Thermal stressed samples were stored at 105°C. Acid stressed samples were adjusted to pH 2 with concentrated HCl.

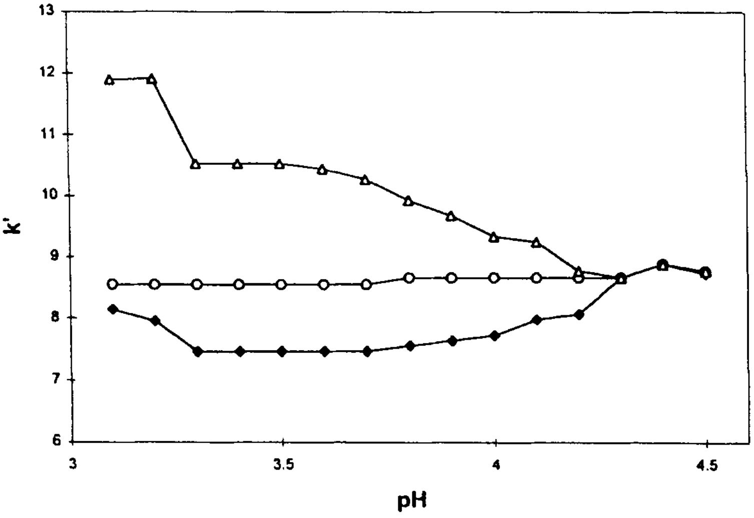

Figure 2. Effect of pH on k*, where o = oxytocin, ● = impurity A and ■ = impurity B.

Base stressed samples were adjusted to pH 12 with 50% NaOH. Oxidative stressed samples were subjected to 30% H_2O_2. Fluorescent stressed samples were subjected to 500-700 foot-candles of radiation.

Data Acquisition

The peak areas of oxytocin acetate were measured using PE Nelson 6000 (Perkin-Elmer Corporation, Cupertino, CA, USA). The chromatographic data was automatically processed for peak area followed by an unweighted linear regression analysis.

RESULTS AND DISCUSSION

Effect of Mobile Phase A pH on Resolution

The unadjusted pH of 100 mM sodium phosphate monobasic is about 4.5, which results in an resolution of less than 1.5 between oxytocin acetate and the nearest adjacent peak (Figure 2). Consequently, the Resolution Solution of oxytocin acetate at different pH values (pH 3.1 - 4.5 in 0.1 intervals) of Mobile Phase A were analyzed.

Table 2

Effect of Column on Resolution and Sensitivity

Column	Rs Between Impurity A & Oxytocin	Rs Between Impurity B & Oxytocin	S/N Ratio of Sensitivity Solution
Alltech 12 cm x 4.6 mm	1.9	1.9	15.0
Beckman 15 cm 4.6 mm	2.4	2.7	14.8

From Figure 2, it is apparent that the resolution is significantly affected by the pH of Mobile Phase A. Based on these results, a pH range of 4.0 ± 0.1 was selected.

Column Choice

The USP method[11] for Oxytocin Injection utilizes a 12 cm column, and due to its odd length needs to be specially ordered. This could result in delay times since the column is not a standard length and, furthermore, costs more.

Hence, a standard 15 cm x 4.6 mm column (Beckman ODS, 5 μm) was evaluated. The 15 cm column was selected since the resolution of oxytocin acetate from the nearest adjacent peak was higher, while maintaining sensitivity (Table 2).

Chromatography

Typical chromatograms obtained from a 100 μL injection of a Standard Preparation, Assay Preparation and Placebo using Mobile Phase A (pH 4.0 ± 0.1) and a 15 cm column are illustrated in Figures 3 (a-c), respectively. The retention times of oxytocin acetate and chlorobutanol were 10.2 and 21.1 minutes, respectively. The overall chromatographic run time was 60 minutes.

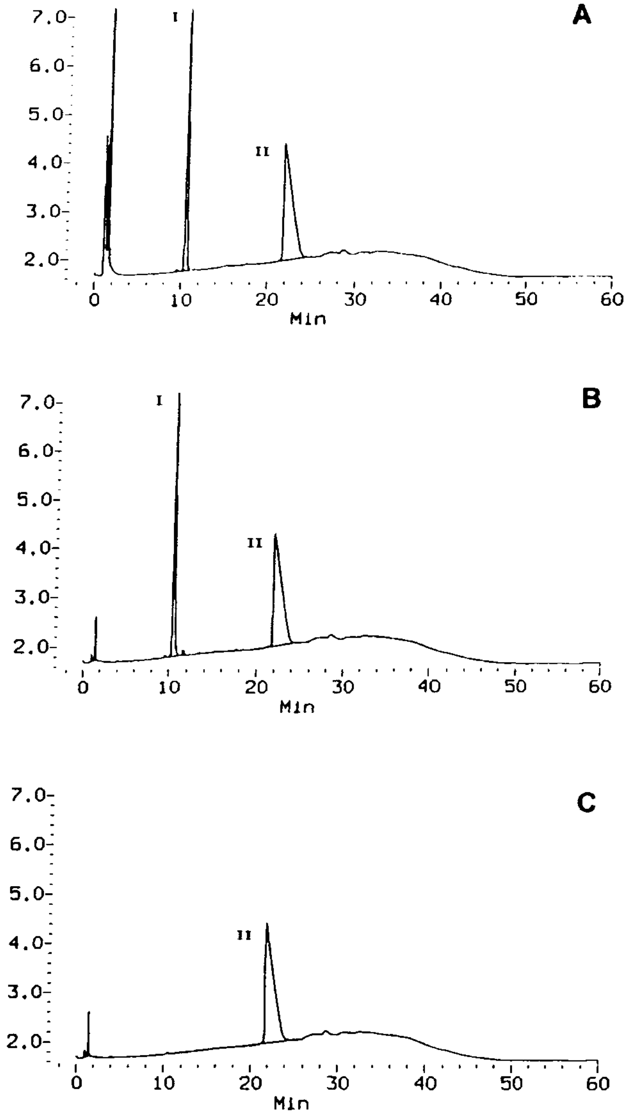

Figure 3. Typical chromatograms of (a) a Standard Solution of Oxytocin, (b) Oxytocin Injection, USP, Synthetic and (c) Placebo (Oxytocin Injection, USP, Synthetic not containing oxytocin acetate). I = oxytocin acetate and II = chlorobutanol.

Table 3

System Suitability*

Run	%RSD (n=5)	Tailing Factor Limit: NMT 2.0	Theoretical Plates Limit: NLT 2000	Resolution Limit:NLT 1.5	
				Impurity A	Impurity B
1	0.3	1.1	7861	2.0	2.7
2	1.8	1.1	8341	2.4	3.1
3	0.5	1.1	9801	1.9	2.2
4	0.2	1.3	10625	1.6	1.8
5	1.2	1.3	12407	1.8	2.4
6	0.1	1.1	9604	2.0	2.7
7	0.4	1.2	9523	2.0	2.4
8	0.2	1.2	8281	2.1	2.4
9	0.1	1.2	7798	2.0	2.4
10	0.2	1.0	8649	2.1	3.1

System Suitability

In all cases, the column efficiency for oxytocin acetate was greater than 7700 theoretical plates. The tailing factors of oxytocin acetate were not more than 1.3. The resolution between Impurity A or Impurity B and oxytocin acetate was not less than 1.6 and 1.8, respectively. The instrument precision, determined by 5 replicate injections of the Standard Preparation, exhibited a maximum RSD of 1.8%. Table 3 illustrates the system suitability results obtained over 10 independent runs spanning 3 months.

Precision

The precision (repeatability and intermediate precision) of the method was determined from one lot of finished product.

A. Repeatability

Five Assay Preparations were analyzed in a single session by Chemist I with HPLC System I. The RSD of the five results was 0.4%, which was within the 2% limit (Table 4).

Table 4

Assay Precision*

Run	Assay Value (USP Units/mL)	Average Assay (n=5) (USP Units/mL)	RSD (%)
1 Chemist I HPLC System I	10.18 10.26 10.27 10.20 10.25	10.23	0.4
2 Chemist I HPLC System I	9.89 9.85 9.90 9.89 9.90	9.89	0.2
3 Chemist II HPLC System II	10.32 10.32 10.27 10.31 10.30	10.30	0.2
Immediate Precision (n=15)		10.14	1.9

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* Repeatability Acceptance Criteria: NMT 2%.

B. Intermediate Precision

Intermediate precision was evaluated using Chemist I/HPLC System I to independently analyze another five Assay Preparations from the same lot of finished product, and to have another analyst using a different chromatographic system (Chemist II/HPLC System II) analyze another five Assay Preparations from the same lot. The RSD of each individual precision run was not more than 2% (Table 4).

Table 5

Assay Accuracy*

Approximate % Claim of Sample	Amount Determined (% Claim)	Theoretical Amount (% Claim)	Amount Recovered (%)	Average Recovered (n = 3)	RSD (%)
	50.86	51.97	97.86		
50	50.45	51.07	98.78	98.15	0.6
	47.33	48.38	97.83		
	101.80	103.94	97.94		
100	101.20	102.14	99.08	98.21	0.8
	94.46	96.77	97.61		
	153.20	155.90	98.27		
150	152.00	153.22	99.20	98.55	0.6
	152.50	145.15	98.17		
Overall Recovery (n = 9)				98.30	0.6

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*Accuracy Acceptance Criteria: 97.0 to 103.0%. Precision Acceptance Criteria: 3% within each level.

Furthermore, the average assay values obtained were 10.23, 9.89, and 10.30 Oxytocin USP Units/mL for runs 1, 2, and 3, respectively (Table 4). This yields an intermediate precision RSD value of 1.9% ($\overline{X}$=10.14 Oxytocin USP Units/mL, n=15) amongst the three runs. The low scatter in the data supports the high degree of robustness of the analytical method.

Accuracy

The accuracy of the method was shown by analyzing spiked finished product samples of a known concentration, in Placebo, and comparing the analytical result to the known added value. The average percent recovery was calculated at each concentration level. The average amounts recovered were 98.15, 98.21, and 98.55% for concentrations of about 50, 100, and 150% of label claim, respectively. This yields an overall average recovery of 98.30% (n=9) for the analytical method in general (Table 5). Since the results obtained are within the acceptable range of 97.0 to 103.0%, the method is deemed to be accurate.

Table 6

Linearity of Oxytocin Response*

% Label Claim	Average Peak Area Response
51.97	236953
83.15	378900
103.94	476167
124.72	565728
155.90	713080

slope, m = 4569.2
y - intercept, b = -742.9
correlation, r = 1.000
Bias = 0.2%

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*Coefficient of correlation acceptance criteria: NLT 0.999. Bias acceptance criteria: ± 3.0%.

Table 7

Stability of Analytical Solutions

Time (Hours)	Potency (Oxytocin USP Units/mL) Standard Preparation	% Change	Assay Preparation	% Change
Zero Time	10.09	NA	10.69	NA
25	10.15	0.6	10.79	0.9
49	9.95	-1.4	10.51	-1.7

Linearity

A linear response in peak area for oxytocin acetate over the range of 50-150% of its label claim in Oxytocin Injection, USP, Synthetic was observed. The correlation coefficient was 1.000 and the bias was -0.2% (Table 6).

Table 8

Specificity Results

Stress Condition of Finished Product	% Degradation	Peak Homogeneity Limit: NLT 990
Thermal (105°C, 5 hours)	22.2	998.4
Acid (20 hours)	29.8	999.9
Base (20 hours)	13.3	998.0
Oxidation (16 hours)	20.0	999.9
Fluorescence (500-700 ft-candles, 48 hours)	0.0	1000

Range

The range of the assay method has been set at 50 to 150% of the finished product label claim (10 Oxytocin USP Units/mL), since the method has been shown to be precise, accurate, and linear within this region.

Stability of Analytical Solutions

The stability of the analytical solutions was determined from the Standard Preparation (prepared from In-house Reference Standard) and Assay Preparation (prepared from finished product) at room temperature. These solutions were analyzed at 0, 24, and 48 hours. Solutions were analyzed against a freshly prepared standard at each time interval. The oxytocin acetate concentrations were examined as a function of time (Table 7). These data were evaluated for percent change of potency from time zero. The Standard Preparation and Assay Preparation were found to be stable for 48 hours, respectively. Since the percent change in potency is within ± 2%, the solutions are considered stable at room temperature.

Specificity

Oxytocin Injection, USP, Synthetic was stressed by thermal, acidic, basic, oxidative and fluorescent radiation for up to 48 hours or until approximately 10-30% degradation of oxytocin acetate was achieved, as determined by peak area percent. The results of the stress study are presented in Table 8. No interfering peaks at the retention time of oxytocin acetate were observed in any of the stressed sample.

Peak Homogeneity

The control sample, degradation samples, test samples, and placebo samples were analyzed using an HPLC equipped with a photodiode array detector. The oxytocin acetate peak was determined to be homogeneous since a purity value ≥ 990 was obtained in all cases (Table 8).

CONCLUSION

The described gradient HPLC method for the analysis of oxytocin acetate in Oxytocin Injection, USP, Synthetic has been evaluated for system suitability, linearity, precision, accuracy, stability of solutions, and specificity. The oxytocin acetate peak response has been shown to be linear, precise and accurate in the range of 50 to 150% label claim. Precision between two chemists on two different chromatographic systems was demonstrated to be within 1.9%. The Standard and Assay Preparations were found to be stable for 48 hours, respectively, at room temperature. Finally, the method has proven to be specific under a variety of stress conditions, while maintaining peak homogeneity. Consequently, the validated method for the determination of oxytocin acetate in Oxytocin Injection, USP, Synthetic is regarded as stability-indicating.

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