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Development and validation of a stability-indicating analytical method for the quantitation of oxytocin in pharmaceutical dosage forms

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Abstract

A single stability-indicating assay for oxytocin (OT) in pharmaceutical dosage forms using gradient elution over 21 min has been reported in the literature. Furthermore, published and compendial methods for the analysis of OT containing dosage forms also involve using HPLC with gradient elution and complicated mobile phases that include hydrophobic ion pairing agents. A simple isocratic and stability-indicating assay was developed and validated. The conditions are as follows, column: Phenomenex® C_{18} Hypersil, 5 μm packing, 4.6 mm × 150 mm with acetonitrile–phosphate buffer (pH 5; 0.08 M) (20:80) as the mobile phase with UV detection at 220 nm The method was found to be specific for OT in the presence of degradation products and chlorbutol (preservative) with an overall analytical run time of 16 min. Accuracy was determined to be 0.77–1.18% bias for all samples tested. Intra-assay precision (repeatability) was found to be 0.22–1.04%R.S.D. while the inter-day precision (intermediate precision) was found to be 1.27–1.68%R.S.D. for the samples studied. The calibration curve was found to be linear with the equation $y = 1.81x + 0.02$ and a linear regression coefficient of 0.9991 over the range 0.4–12.0 IU/ml. The LOD and the LOQ were determined to be 0.1 and 0.4 IU/ml, respectively. Syntocinon®, a commercially available dosage form of OT was assayed resulting in 100.5–106.6% recovery of the label claim and an average of 10.04 IU/ml.

Keywords: Oxytocin; Pharmaceutical dosage forms; HPLC; Isocratic; Stability indicating

1. Introduction

Oxytocin (OT) is a cyclic neurohypophyseal nonapeptide. The primary structure of OT is shown in Fig. 1. OT possesses a 20 membered cyclic portion that is linked by a disulphide bridge between the two-cysteine residues. It is synthesised by the hypothalamus and is released from the posterior lobe of the pituitary gland. It possesses uterotonic and galactogenic activity in mammals [1]. The main use of OT in clinical practice is the induction, and the augmentation of labour, control of postpartum haemorrhage and uterine hypotonicity in the third stage of labour. OT is also used to stimulate lactation [2].

The stability properties of OT have been previously reported by Nachtmann et al. [1]. The stability of OT is dependent on the pH and the optimal pH is between 3 and 5. In strongly acidic solutions, the peptide linkages undergo hydrolysis. Dimeric and polymeric compounds form under neutral and alkaline conditions and this occurs by the conversion of intramolecular disulphide bridges to intermolecular bridges resulting in the deactivation of OT [1]. Stability is important from a quality control perspective in the industry and therefore any analytical method developed should preferably be stability indicating.

Previously reported assays for OT have mainly used HPLC as the preferred analytical tool. Krummen and Frei [3,4] described isocratic methods for the quantitation of OT in which both C_8 and C_{18} columns and relatively high flow rates in excess of 1.5 ml/min and up to 4.0 ml/min were used. However, a major disadvantage of using high flow rates is high backpressure on the analytical column [5], which has a negative impact on both the column and solvent delivery module, reducing the working lifetime. Pask-Hughes et al. published a method that uses sodium tetradecyl sulphate as a hydrophobic ion-pairing agent in the mobile phase [6]. The main disadvantage for the use of the ion pairing agents is the long time that is needed to equilibrate the column and the remove the ion pair agent from the column [7]. Ohta et al. [8] and Dudkiewicz-Wilczyńska et al. [9] pub-

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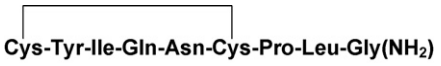

Fig. 1. Primary sequence of OT.

lished simple isocratic methods for the quantitating OT in dosage forms. Maxl and Siehr published a gradient method for the quality control of OT [10]. The compendial methods in the current United States Pharmacopeia [11] and British Pharmacopeia [12] are both gradient elution methods with phosphate buffer and acetonitrile. There is no information published in the aforementioned compendia with respect to the stability-indicating status of their methods. A stability indicating analytical method for the analysis of OT using gradient elution and a flow rate of 1.5 ml/min with resultant retention times of OT and chlorbutanol of 10.2 and 21.1 min, respectively, has been reported [13].

The objective of these studies was to develop a simple isocratic method for the quantitative analysis of OT that was also stability indicating. The method that is reported is sensitive, selective, precise and accurate with a retention time of 4.7 min for OT. Furthermore analysis of a commercially available product containing OT revealed that the preservative, chlorbutol eluted at approximately 14.6 min.

2. Experimental

2.1. Reagents and chemicals

OT was obtained from PolyPeptide Laboratories s.r.o. (Hostiva, Czech Republic). The potency was 541 IU/mg. All reagents used were of analytical grade. Acetonitrile (HPLC grade, far UV) was purchased from Romil Ltd. (Cambridge, UK). Sodium hydroxide pellets and *ortho*-phosphoric acid (85%, w/w) were obtained from Merck Chemicals Ltd. (Johannesburg, RSA). HPLC grade water was purified using a Milli-Ro® -15 Water purification system (Millipore, Bedford, MA, USA), which is made up of a Super-C® carbon cartridge, two Ion-X® ion-exchange cartridges and an Organex-Q® cartridge. The water was filtered through a 0.22 μm Millipak® stack filter before use. Syntocinon® (Novartis, Johannesburg, South Africa), a locally available dosage form of OT at a concentration of 10 IU/ml was purchased from a local pharmacy.

2.2. HPLC

The modular HPLC system consisted of a Spectra-Physics Isochrom LC pump (Spectra-physics, San Jose, CA, USA), a Waters WISP 712 Autosampler (Waters Chromatography Division, Milford, MA, USA), a Linear Model 200 EZ Chrom UV–vis variable wavelength detector (Linear Instruments Corp., Reno, NV, USA) and a Model 561 strip chart recorder (Hitachi, Tokyo, Japan). A Phenomenex® (Torrance, CA, USA) Hypersil column, 5 μm, 4.6 mm × 150 mm was used at ambient temperature. Separation was achieved under isocratic conditions using a mobile phase of 20% (v/v) acetonitrile in 80 mM phosphate buffer (pH 5) at a flow rate of 1.5 ml/min and UV detection at 220 nm. pH of solutions were measured using a Crison Model GLP 21 pH meter (Crison, Barcelona, Spain). The volume of injection was 20 μl.

2.3. Standards and sample solutions

Approximately 10 mg of OT (541 IU/mg) was accurately weighed and transferred into a 100 ml A-grade volumetric flask and was made up to volume with HPLC grade water. The concentration of the resultant solution was 54.1 IU/ml. The standard solutions were prepared in the concentration ranges 1–12 IU/ml using serial dilution of the stock solution using A-grade glassware.

2.4. Preparation of buffers

Buffer solutions were prepared by pipetting appropriate volumes of 85% *ortho*-phosphoric acid into A-grade volumetric flasks and making up to volume with HPLC grade water. The pH was titrated to the required value using sodium hydroxide pellets.

2.5. Method development and validation

A previously published method [3] was optimised for the conditions in our laboratory in terms of the composition of the mobile phase, flow rate, pH and the molarity of the buffer. The method was validated according to USP [14] and ICH [15,16] guidelines. The validation parameters addressed were specificity, precision, accuracy, linearity, limits of detection and quantification and the stability of OT in the mobile phase.

2.6. Stress testing of OT solution

In order to ensure that the analytical assay was stability indicating, stress studies were performed as outlined by Snyder et al. [17]. All solutions prepared and used in these studies had an initial starting concentration of 10 IU/ml.

2.6.1. Acid degradation studies

A 2 ml of 0.1 M hydrochloric acid was added to 8 ml of a 10 IU/ml solution of OT. The resultant concentration was 8 IU/ml. This solution was allowed to stand for 1 h.

2.6.2. Alkali degradation studies

A 2 ml of 0.1 M sodium hydroxide was added to 8 ml of a 10 IU/ml solution of OT. The resultant concentration was 8 IU/ml. This solution was allowed to stand for 1 h.

2.6.3. Temperature stress studies

A 10 IU/ml OT solution was heated to and maintained at 50 °C for 10 min.

2.6.4. Oxidation studies

A 2 ml of a 3% hydrogen peroxide solution was added to 8 ml of a 10 IU/ml solution of OT. The resultant concentration was 8 IU/ml. This solution was placed in a dark locker for 2 h.

2.6.5. Photostability studies

A 10 ml OT solution at a concentration of 10 IU/ml in a clear volumetric flask was exposed to natural sunlight for 8 h. The solution was placed by a laboratory window for the duration of the experiment.

2.7. Preparation of Syntocinon® for assay

A 1.0 ml aliquot of the parenteral preparation was diluted to 5.0 ml with HPLC grade water and was assayed using the developed method.

3. Results and discussion

3.1. HPLC method development

An HPLC method reported in the literature [3] was optimised for the conditions in our laboratory with respect to the choice of analytical column used, the composition of the mobile phase, pH and molarity in addition to mobile phase flow rate. The reported method used C_8 columns at 67 mM phosphate buffer at pH 7 and flow rates of 2 or 3 ml/min.

C_8 columns are less hydrophobic than C_{18} columns. For organic non-polar molecules, the sample retention increases with increase in the length of the bonded phases [18]. However, OT is a very polar peptide that is ionised at physiological pH. In theory, comparing the equilibrium of OT between a C_8 column and the mobile phase and that between a C_{18} column and the mobile phase, there is going to be a higher ratio of OT in the mobile phase for the C_{18} column. For this reason OT would be eluted quicker from the C_{18} column than the C_8 column. The longer retention of OT in the C_8 column resulted in the need for higher flow rates or higher proportions of organic modifier being utilised in order to decrease the retention time of OT. It was our intention to use lower flow rates as high flow rates decrease the lifetime of both the column and the pump. Higher pressures in the column generate heat as the higher pressure is forcing the mobile phase through the column. The heat generated as a result degrades the column and reduces column efficiency and selectivity [5]. Increase in the content of the organic modifier resulted in a decrease in the retention time of the drug. At a composition of 30% (v/v) acetonitrile, OT eluted with the solvent front. At compositions less than 20% (v/v) acetonitrile, the retention time of OT was greater than 10 min at both flow rates of 1 and 1.5 ml/min, which were compared. The optimal conditions for OT were considered to be 20% (v/v) acetonitrile in buffer at a flow rate of 1.5 ml/min as this gave the peaks with the best shape and a retention time of 6.8 min. It is desirable to have retention times less than 10 min as this allows multiple analyses to be carried out in a reduced time. Changing the pH of the buffer affected both the retention times and the shape of the peaks produced. Decreasing the pH resulted in shorter retention times and gave sharper peaks. A pH of 5 was considered to be optimal as this gave a good compromise between retention time (5 min) and peak shape. The buffer molarity was changed and the choice of the optimal buffer strength was based on the theoretical plate number. A buffer strength of 80 mM gave the highest plate number while higher concentration resulted in a decrease in the plate number. The retention time shifted to 4.7 min. A typical representative chromatogram is shown in Fig. 2.

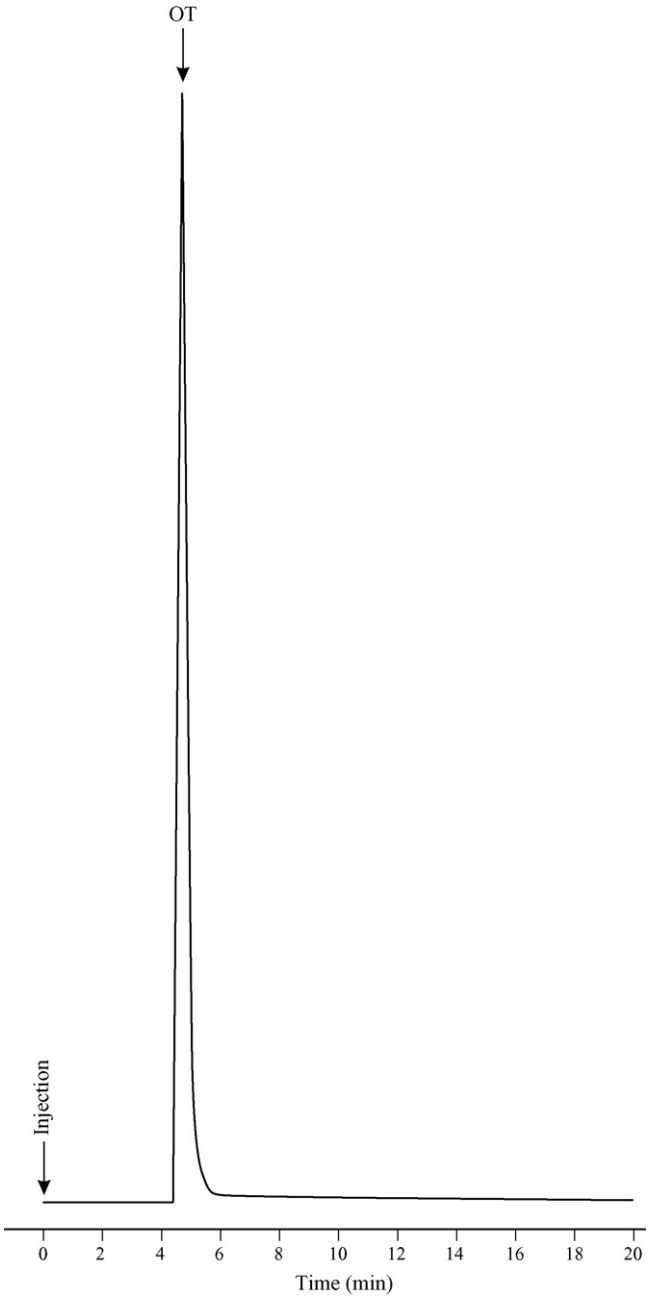

Fig. 2. Typical chromatogram showing the separation of OT.

3.2. Method validation

3.2.1. Specificity and stress studies

The results of the stress studies indicated the specificity of the method that has been developed. After exposure of OT solutions to stress conditions, an assay of OT was performed on the resultant solutions. Typical chromatograms obtained for these analyses are shown in Figs. 3–6.

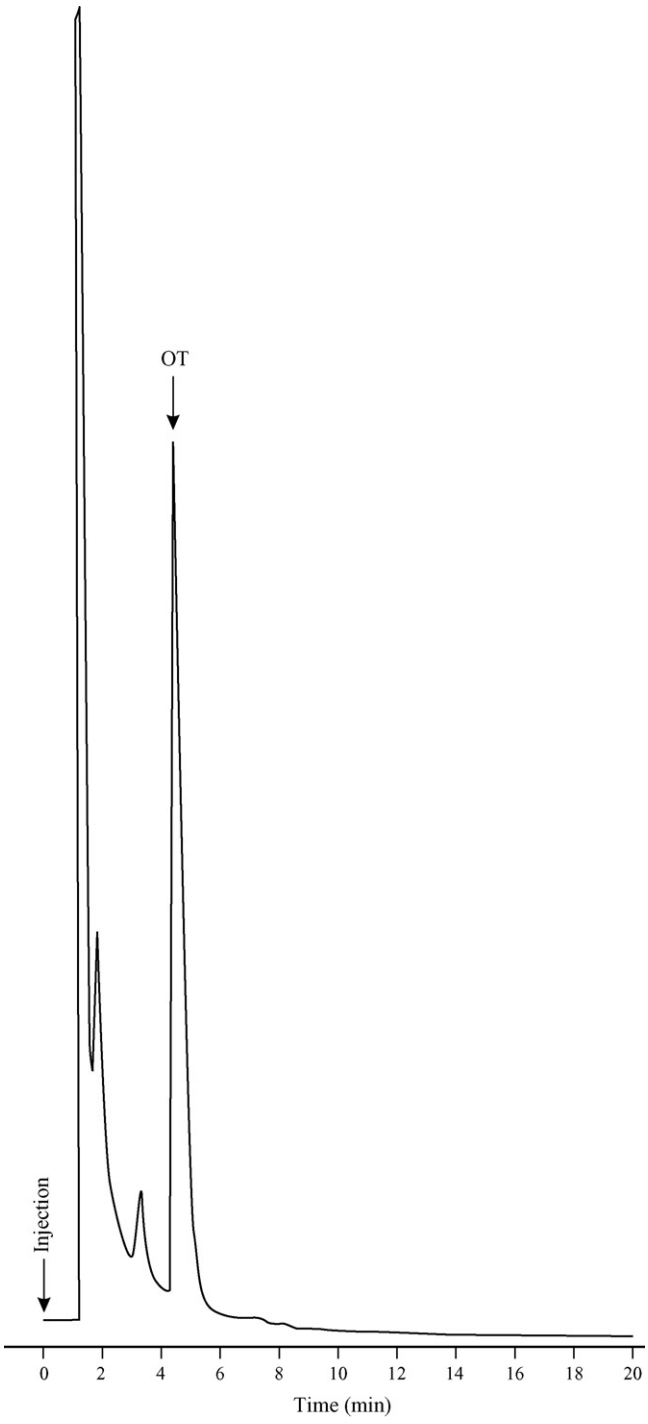

Fig. 3. Resultant chromatogram obtained following the exposure of OT solution to 0.1 M HCl showing the separation of OT and degradation products.

It is clearly evident that when exposed to acidic conditions, OT undergoes degradation. The peaks of the resultant degradants were well resolved from the OT peak as shown in Fig. 3. OT is highly unstable in alkali conditions [1] and following exposure of OT to the alkali conditions previously described, almost all the OT had degraded with only a small peak being visible in the chromatogram as shown in Fig. 4. Following exposure to temperatures of 50 °C for 10 min the resultant peak of degradation product was well resolved from the OT peak and a typical

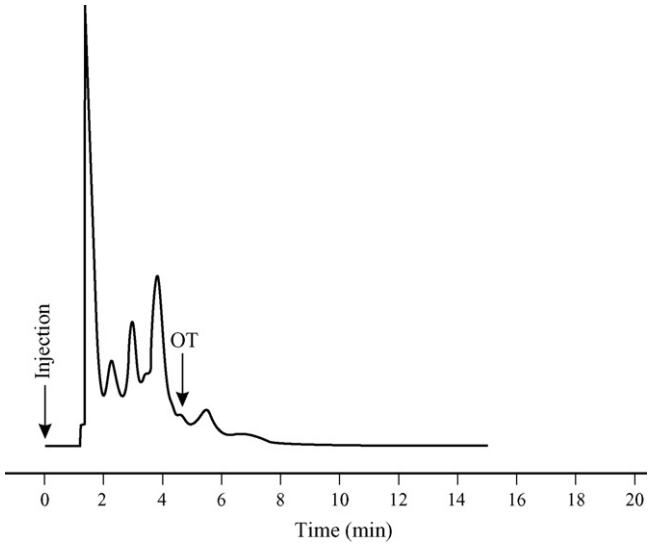

Fig. 4. Resultant chromatogram obtained following the exposure of OT solution to 0.1 M NaOH showing the separation of OT and degradation products.

chromatogram from these studies is shown in Fig. 5. OT also degraded in the presence of 3% hydrogen peroxide and the resultant OT peak was resolved from that of the degradation products as seen in Fig. 6. OT was found to be stable under the light conditions studied.

The assay of a product must also show specificity with regard to the potential interference that might be a result of the presence of excipients in a formulation. In the case of Syntocinon®, chlorbutol is used as a preservative and the HPLC method that has been developed reveals that the preservative is well resolved from that of OT with a retention time of 14.6 min, thus indicating the assay procedure is specific for OT. A typical chromatogram developed during the analysis of Syntocinon® is shown in Fig. 7.

3.2.2. Linearity

Calibration curves that were constructed for OT were linear over the range 0.4–12 IU/ml (see Table 1). The peak height (cm) of OT was plotted versus the concentration (IU/ml) of OT. The equation for the resultant calibration curve was $y = 1.81x + 0.02$ with a linear regression coefficient of 0.9991.

Table 1
Linearity and precision data

Concentration ($n = 5$)	Peak height	%R.S.D.
0	0	0
0.4	0.53	5.17
0.5	0.69	3.80
1.0	1.81	1.24
2.0	3.63	1.23
4.0	7.79	0.54
6.0	11.04	0.20
8.0	14.45	0.35
10.0	18.02	0.15
12.0	21.48	0.31

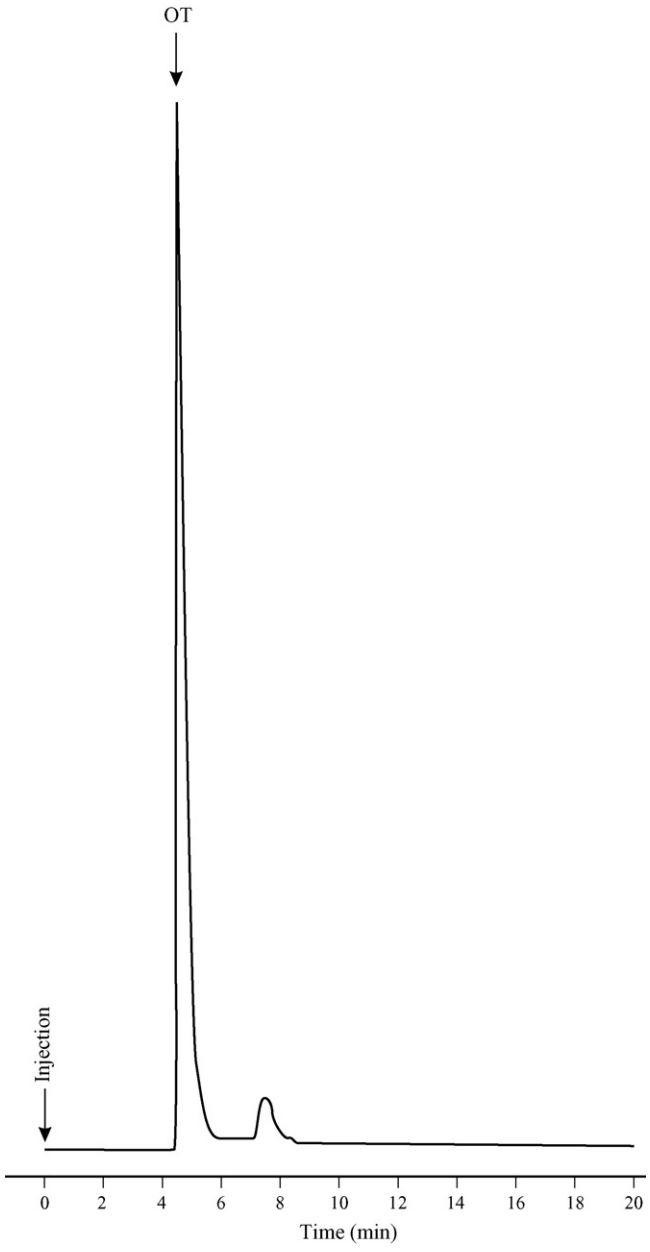

Fig. 5. Resultant chromatogram obtained following the exposure of OT solution elevated temperatures showing the separation of OT peak and degradation products.

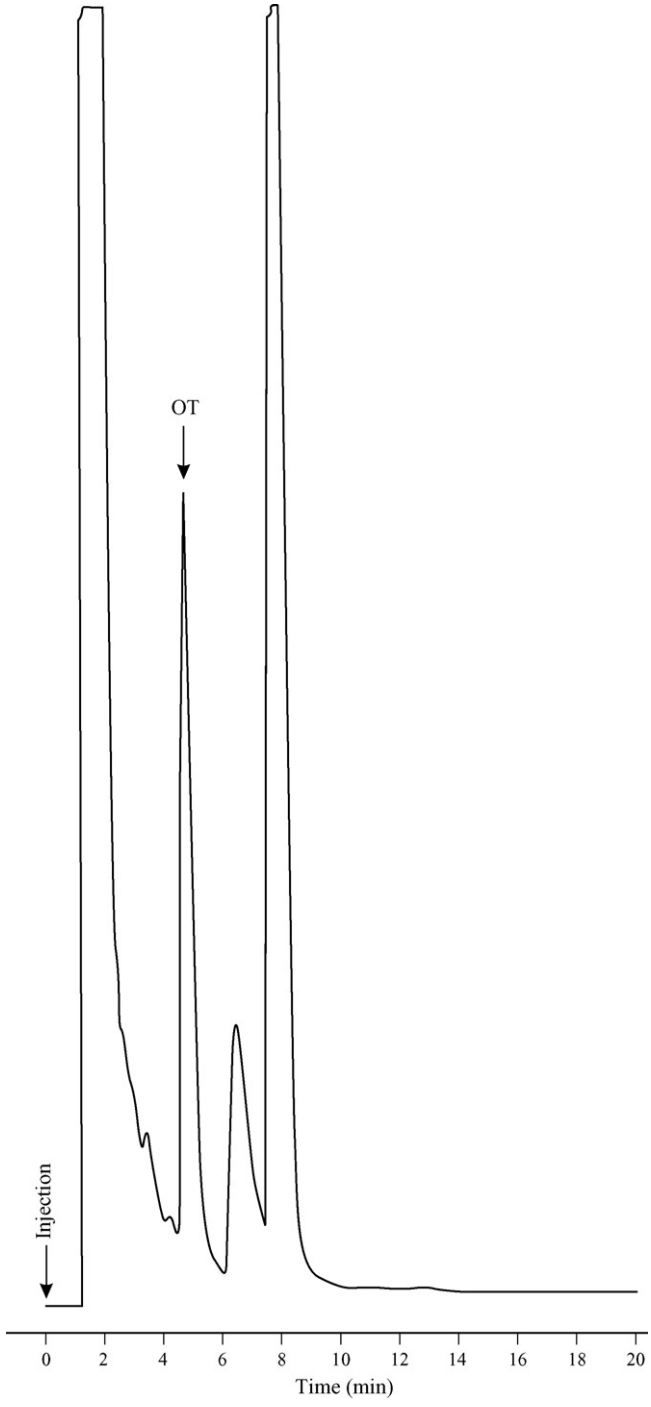

Fig. 6. Resultant chromatogram obtained following the exposure of OT solution to 3% hydrogen peroxide solution showing the separation of OT and degradation products.

3.2.3. Precision

Intra-assay precision (repeatability) and inter-day (intermediate) precision were determined. The analyses were performed using concentrations at three levels, 3, 7 and 11 IU/ml. Each concentration was analysed in triplicate ($n = 3$) and intra-assay precision was found to be less than 1.5%R.S.D. for all samples on all days. Inter-day precision %R.S.D. for analyses conducted on three separate days was found to be 1.51, 1.68 and 1.27%R.S.D. for the low, middle and high concentrations studied, respectively. The results of these studies are summarized in Table 2.

3.2.4. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was determined by using the signal to noise ratio method. The concentration that resulted in a signal to noise ratio of 3:1 was found to be 0.1 IU/ml and was determined to be the LOD. A signal to noise ratio of 10:1 was used to determine the LOQ and was the concentration that elicited a response that could be accurately and reliably measured. This concentration

Table 2
Intra-assay and inter-day precision

OT (IU/ml)	Day 1		Day 2		Day 3		Intermediate precision
	Peak height	%R.S.D. ($n=3$)	Peak height	%R.S.D. ($n=3$)	Peak height	%R.S.D. ($n=3$)	%R.S.D. ($n=3$)
3	5.37	0.51	5.43	0.53	5.45	1.04	1.51
7	12.56	0.33	12.83	0.45	12.98	0.22	1.68
11	19.68	0.34	20.05	0.86	20.17	0.38	1.27

was found to be 0.4 IU/ml. The precision of the LOQ determination ($n=6$) was found to be 4.84%R.S.D.

3.2.5. Accuracy

Accuracy was determined at three concentrations, similar to those used to assess the precision of the method. Each of the solutions was injected ($n=5$) and the percentage bias was determined. The methods show a low percentage bias of less than 1.20% for all solutions tested and the results are summarized in Table 3.

Table 3
Accuracy and percent bias

Concentration (IU/ml)	Determined concentration	% Bias
3	2.96	1.18
7	6.95	0.77
11	10.89	1.01

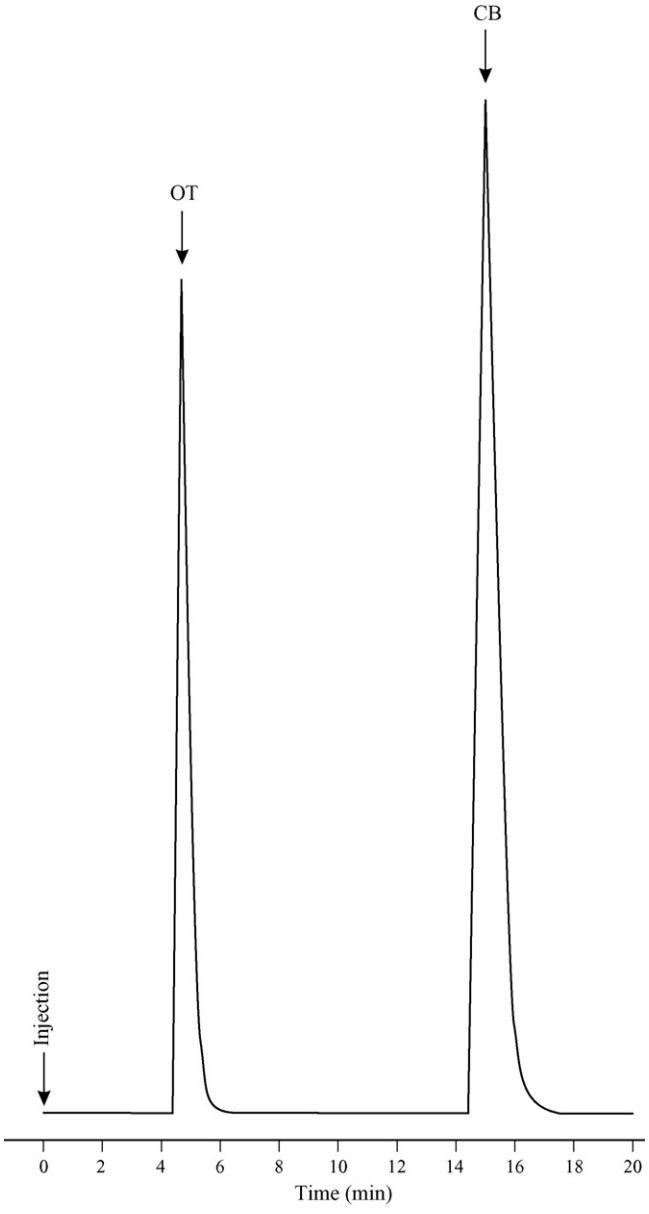

Fig. 7. Resultant chromatogram obtained following the analysis of Syntocinon® showing the separation of OT and chlorbutol.

3.3. Assay of Syntocinon®

An assay of the commercially available OT parenteral preparation ($n=3$) injected in triplicate resulted in 100.2–106.6% of the label claim of 10.00 IU/ml and an average assay result of 10.38 IU/ml with the precision of the assay ranging between 0.76 and 1.49%R.S.D. for each set of determinations.

4. Conclusion

A simple isocratic and stability-indicating assay was developed and validated. The method is sensitive, selective, precise and accurate and was successfully applied to the analysis of commercially available OT products. The method has the advantage over a previously reported stability-indicating assay [13] in that it is isocratic method with a total analytical run time, when analysing OT in commercially available products, of approximately 16 min as opposed to the 21.1 min achieved using gradient elution chromatography.

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