www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.2, pp 1340-1343, April-June 2010

RP-HPLC Method Development and Validation for the Estimation of Oxytocin in Milk

K.Suresh Kumar, M. P. Kavitha, J.Dharuman, B.Dhandapani*

[1]Department of Pharmaceutical Analysis, K.M.C.H. College of Pharmacy, Kovai Medical Center Research and Educational Trust, Kovai Estate, Kalapatti Road, Coimbatore - 641045, Tamilnadu, India

*Corres.author: pharmsuki@gmail.com

Abstract: A simple, precise, accurate and validated reverse phase HPLC method has been developed for the estimation of oxytocin in milk by the use of phenyl hexyl column (250mm x 4. 6mm id, 5µm).The mobile phase used was a mixture of acetonitrile and 0.03 M phosphate buffer, pH 3.5 (21:79) at a flow rate of 1ml/min.The detection of oxytocin was carried out at a wavelength of 197 nm. The retention time was found to be 4. 78 and recovery was between 99 and 101 % which was performed by standard addition method.
Key words: Oxytocin in Milk ,RP-HPLC Method.

Introduction

Oxytocin is a cyclic octapeptide hormone released by the posterior pituitary and showing uterotonic and galactogenic activity. It is chemically L-Hemi-cystinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-hemi-cystinyl-L-propyl-L-leucyl glycinamide.Its 20 membered ring consists of five amino acids- cysteine, tyrosine, isoleucine, glutamine, and asparagines, further three amino acids, proline, leucine and glycinamide. [Klaus Florey, Analytical profile of drug substances] Oxytocin is involved in the contraction of uterus and milk ejection in receptive mammals. In the brain oxytocin is classically viewed as primarily involved in the milk let down reflex and in the stimulation of uterine smooth muscle during parturition. New inventions prove that when oxytocin is injected into cows, there is a result over production of milk and traces of oxytocin can be found in the same. When excess of oxytocin is found to be present in milk it may cause headache, nausea, abdominal pain, drowsiness etc .to the user. [Collin Dollery, Therapeutic Drugs, 2nd edition] [1-7]

The aim of the present study is to develop a method for estimation of oxytocin in milk. For this two different brands of milk has been chosen. Though the reported methods [8-11] for the estimation of oxytocin in milk is very few, the present study aims a different method for the estimation of content of oxytocin in milk.

Materials and Methods

A reverse phase HPLC system (Schimadzu Prominence 20 AT) consisting of LC-20 AT pump, M-20A PDA (Photo Diode Array) detector, Phenyl hexyl column (250mm x 4.6mm id, 5µm), 20µl Rheodyne injection syringe and LC Solution software was used for analysis. Pure sample of oxytocin was obtained from Analytical Laboratory, KMCH College of pharmacy, Coimbatore. All other chemicals used were HPLC grade and AR grade. Optimized chromatographic conditions were listed in Table.1

Standard stock solution was prepared in 10 ml volumetric flask by dissolving accurately weighed quantities of oxytocin (10 mg) in HPLC grade water followed by dilution up to the mark with HPLC grade water(1000 µg / ml). From this further dilution was prepared to get the concentration of 0.5 µg / ml,1 µg / ml,1.5 µg / ml,2 µg / ml, and 2.5 µg / ml. These concentrations were taken for studying Linearity range of oxytocin.

Analysis of Sample

Sample concentration of milk was prepared by taking 1 ml of sample solution with 1 ml of ice cold solution of acetone. This solution was mixed and centrifuged at 3500 rpm in the centrifugal apparatus. After centrifugation process, the acetone layer was taken and mixed with 1 ml of petroleum ether. This solution was mixed and kept for 5 minutes. After 5 minutes, the ether layer was discarded and the lower layer was evaporated to dryness, to this 0.2 ml buffer solution was added and filtered. The filtered solution was injected . Fig.1 shows the standard chromatogram for oxytocin. Fig.2 shows the sample chromatogram of oxytocin.

Method Validation

The method was validated in terms of linearity, accuracy, Intra-day and inter-day, reproducibility and specificity. The limit of detection (LOD) and limit of quantification (LOQ) were also determined. The accuracy of the method was evaluated by carrying out recovery studies. For that, known concentration of the standard solution was added to the sample solution and recovery was calculated. The intra-day precision was determined by analyzing standard solutions in the linearity range of the calibration curve in triplicate on the same day. While inter- day precision was calculated by analyzing corresponding standard solutions daily, for a period of one week. The relative standard deviation of < 2.5% was observed. The validated data was shown in Table.2

Results and Discussion

The developed RP-HPLC method for estimation of oxytocin in milk separated with mixture of acetonitrile and 0.03 M phosphate buffer, pH 3.5 in the ratio 21:79 with better resolution. On the other hand 1 ml triethyl amine helped in sharpening of the peaks. Linearity range of oxytocin was 0.5 to 2.5 µ g/ml (r=0.9985).The percentage recovery of the drug indicates that the method is highly accurate. Recovery studies are shown in Table 3.

Hence the proposed method is simple, precise, rapid and accurate for the estimation of oxytocin in milk.

Table 1: Optimized Chromatographic Conditions

S.No	Pararameters	Optimized conditions
1	HPLC Model	Schimadzu Prominence LC 20 AT
2	Column	Phenyl hexyl column (250mm x 4.6 mm id , 5µm)
3	Mobile phase	Acetonitrile: 0.03M Phosphate buffer(21:79), pH 3.5(Dil.Orthophosphoric acid)
4	Flow rate	1 ml/min.
5	Detection Wavwlength	At 197 nm
6	Injection volume	20µl
7	Retention time	4.78
8	Temperature	Room Temperature

Table.2: System Suitability Parameters

S.No	Parameters	Results
1	Theoretical plates (N)	3521
2	Resolution	6.7
3	Linearity range (µ g/ml)	0.5 -2.5
4	Percentage recovery %(Accuracy)	99.8
5	LOD (µg/ml)	3
6	LOQ (µg/ml)	9
7	Tailing factor	1.184
8	Capacity factor	0.86

Table.3: Recovery Studies

Name of the drug	Percentage Recovery	%RSD
Oxytocin	100%level	100% level
	99.8	0.002

***Mean of 6 observations**

FIG.1:STANDARD CHROMATOGRAM FOR OXYTOCIN

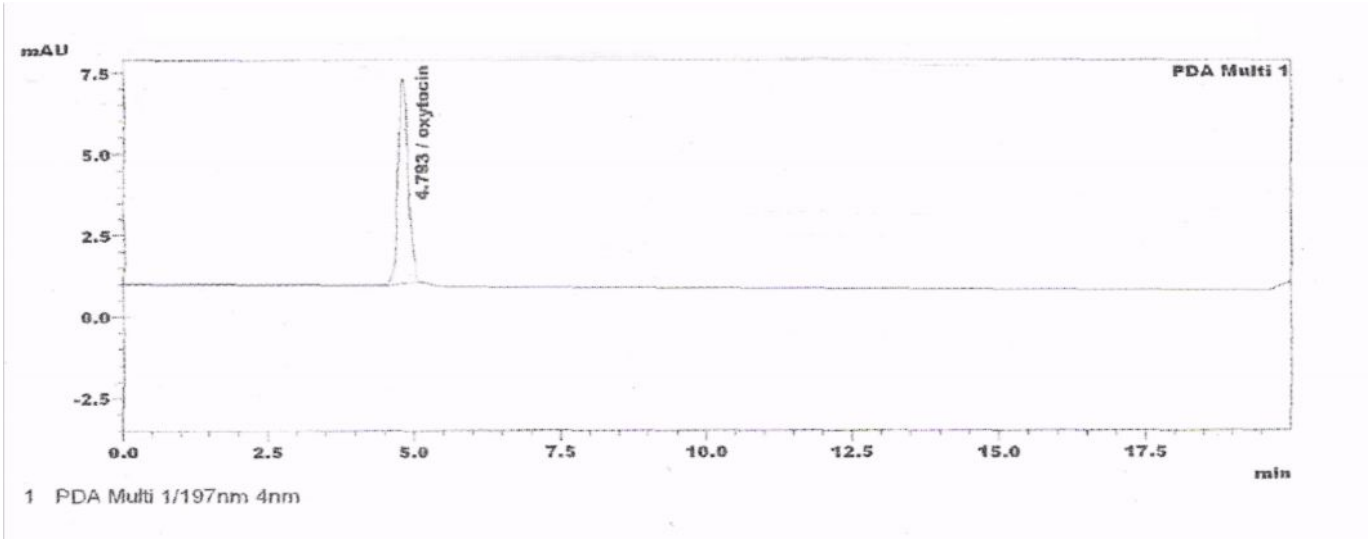
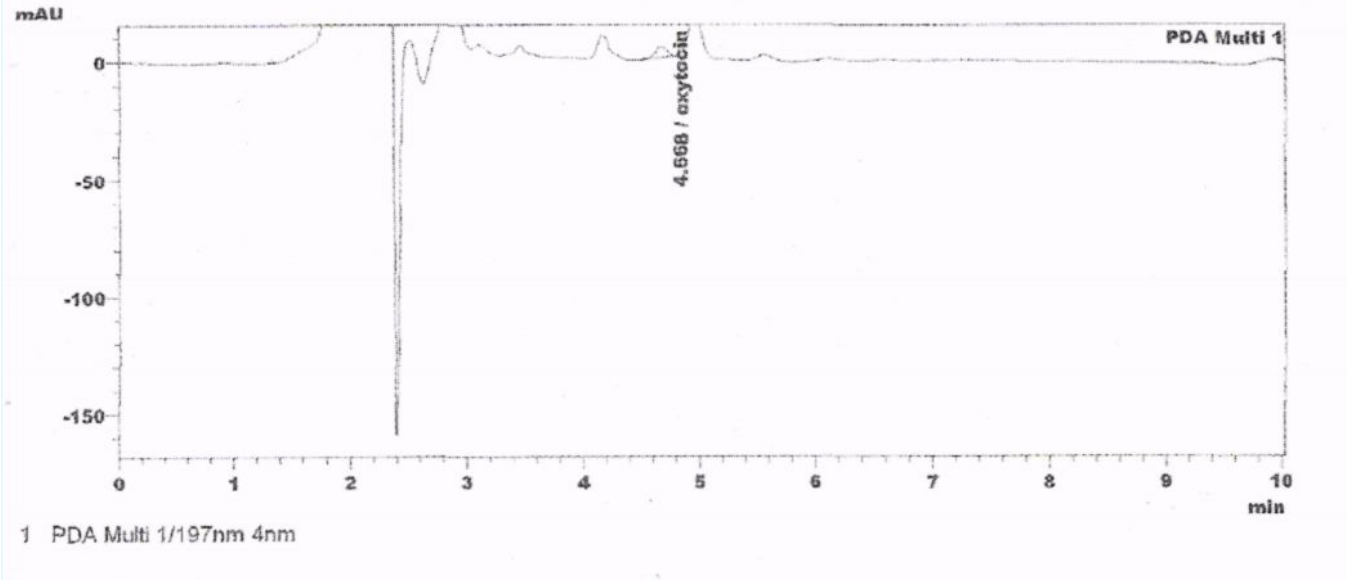


FIG -2 SAMPLE CHROMATOGRAM OF BRAND 1



References

1. Collin Dollery., Therapeutic drugs, Second edition, 1949, 3, 48.
2. American Hospital formulary Service (AHFS) Drugs Information, 2004, 3121.
3. Agarwal O. P., Chemistry of Natural products,1989, 1,210.
4. British pharmacopoeia., 1993, 1, 475.
5. Lawrence. A. Handbook of injectable drugs, 1922, 13, 1150.
6. Journal of Chromatography (USA) A, 536, 137-142.
7. United States Pharmacopoeia-National formulary, Page No1385
8. Klaus Florey, Analytical Profile of Drug Substances, 2005, 10, 564.
9. David R. Cool, David de Brose Department of Pharmacology and Toxicology, USA, Journal of Chromatography B, May 2003.
10. Larson .K, Hermann. W, Moller. P, Sanchez. D., Preparative High performance Liquid Chromatography of peptides on a new reverse phase packing material, Kromasil, C18, Ferring Pharmaceuticals, Sweden. Journal of chromatography October 1998, 450(1), 71-80.
11. Sandra Pereira FUKUDA., Salvador Massaguer ROIG., Louis Fransisco PRATA., Correlation between acidic ninhydrin and HPLC detection methods to evaluate addition of whey in milk. Veterinary University, Brazil, EDP sciences 2004.

*****
*****