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Research Article

ESTIMATION OF ATOSIBAN ACETATE IN PARENTERALS BY RP-HPLC

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ABSTRACT
A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Atosiban Acetate in dosage form. An XTerra MS C18 analytical column (250 x 4.6 mm, 5 µm partical size) with mobile phase consisting of mixture of buffer 0.03M Potassium Dihydrogen Orthophosphate in water and pH adjusted to 3.20 with ortho-phosphoric acid and acetonitrile in the gradient program was used. The flow rate was 1.0 mL/min and the effluents were monitored at 220 nm. The retention time was 11.3 min. The detector response was linear in the concentration of 15-225 mcg/mL. The respective linear regression equation being y = 1286.9 x -1290.8. The limit of detection (LOD) and limit of quantification (LOQ) for Atosiban Acetate were found to be 1.5 mcg/mL and 4.5 mcg/mL respectively. The percentage assay of Atosiban Acetate was 99.6 %. The method was validated by determining its accuracy, precision and linearity. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Atosiban Acetate in bulk drug and in its pharmaceutical parenterals.
Keywords: Atosiban Acetate, RP-HPLC and Parenterals.

INTRODUCTION

Atosiban is an inhibitor of the hormones oxytocin and vasopressin[1]. It is used as an intravenous medication as a labor repressant (tocolytic) to halt premature labor[2]. It is a modified form of oxytocin that inhibits the action of this hormone on the uterus, leading to a cessation of contractions[3]. Systematic IUPAC name is 1-(3-mercaptopropanoic acid)-2-(O-ethyl-D-tyrosine)-4-L-threonine-8-L-ornithine-oxytocin. The empirical formula is $C_{43}H_{67}N_{11}O_{12}S_2$; with a molecular weight of 994.2. Atosiban Acetate is a white to off-white crystalline powder. It is freely soluble in water. Atosiban Acetate injection is a sterile, clear, colorless, non-pyrogenic, isotonic, buffered solution for intravenous administration. One ml solution contains 7.5 mg Atosiban free-base in the form of Atosiban acetate in 5 mL vial. Formulation of Tractocile® contains the active substance. Atosiban is present as an acetate salt (the substance contains also water, acetic acid and ethanol) corresponding to 7.5 mg/ml of Atosiban free base, Mannitol as isotonicity agent- (50 mg/ml), pH is adjusted to 4.5 with 1 M hydrochloric acid, water for injections (to 1 ml). Tractocile® is indicated to delay imminent pre-term birth in pregnant women with regular uterine contractions of at least 30 seconds duration at a rate of 4 per 30 minutes. Literature survey[4-8] reveals no chromatographic methods for the estimation of Atosiban Acetate from pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Atosiban Acetate in pharmaceutical formulations.

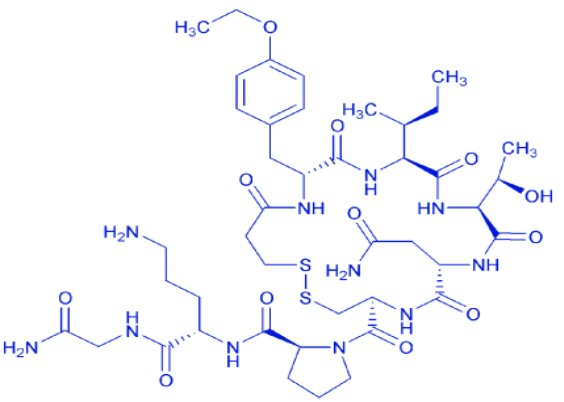

Figure 1: Structure of Atosiban

MATERIALS AND METHODS

Atosiban Acetate was obtained as a gift sample from M/s. Vishnu Chemicals Ltd, Hyderabad, India. Acetonitrile, Potassium Dihydrogen Orthophosphate and water used were of HPLC grade (Qualigens). Commercially available Atosiban Acetate Injectables (Tractocile®7.5 mg per mL) were procured from local market.

Instrument

Quantitative HPLC was performed on liquid Chromatograph, Shimadzu LC 2010 dual λ detector equipped with automatic injector with injection volume 20 µl. The HPLC system was equipped with LC solution Software.

HPLC Conditions

The contents of the mobile phase were mixture of buffer 0.03 M Potassium Dihydrogen Orthophosphate in water and pH adjusted to 3.20 with ortho-phosphoric acid and acetonitrile in the gradient program was used (shown in Table 4). They were filtered before use through a 0.45 µm membrane filter,

and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/min. The run time was set at 30.0 minutes and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 minutes with the mobile phase flowing through the system. The eluents were monitored at 220 nm.

Preparation of Standard Stock solution

A standard stock solution of the drug was prepared by dissolving 15 mg of Atosiban Acetate in 10 mL volumetric flask and dissolved in diluent (Acetonitrile and Water:50:50), sonicated for about 15 minutes and then made up to 10 mL with diluent get 1500 mcg/mL standard stock solution.

Working Standard solution

1.0 mL of the above stock solution was taken with micropipette in 10 mL volumetric flask and thereafter made up to 10 mL with diluent (Acetonitrile and Water: 50:50) to get a concentration of 150 mcg/mL.

Preparation of Sample solution

Twenty Injectables (Tractocile® 7.5 mg per mL vials) were taken and transfer the liquid into a 100 mL volumetric falsk. A sample of 2.0 mL equivalent to 15 mg of Atosiban Acetate of the active ingredient, add 10 mL of diluent to get working sample solution and further diluted to 150 mcg/mL and then filtered through a 0.45 µm membrane filter.

Linearity

Aliquots of standard Atosiban Acetate stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Atosiban Acetate are in the range of 15-225 mcg/mL. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 220 nm and a Calibration graph was obtained by plotting peak area versus concentration of Atosiban Acetate (Figure 3). The plot of peak area of each sample against respective concentration of Atosiban Acetate was found to be linear in the range of 15–225 mcg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table 1. The respective linear regression equation being y = 1286.9 x - 1290.8. The regression characteristics, such as slope, intercept, and % RSD were calculated for this method and given in Table 1.

Assay

20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 11.3 minutes. The amount of drug present per parentaral was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

Recovery Studies

Accuracy was determined by recovery studies of Atosiban Acetate, known amount of standard was added to the pre analysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

Table 1: Linear Regression Data for Calibration curves

Drug	Atosiban Acetate
Concentration range (mcg/mL)	15-225
Slope (m)	1286.9
Intercept (b)	-1290.8
Corrélation coefficient	0.9999
% RSD	0.68

Table 2: Results of HPLC Assay and Recovery studies

Sample	Amount claim (mg/Injectable)	% found by the proposed method	% Recovery*
1.	37.5	99.72	99.36
2.	37.5	99.50	99.21
3.	37.5	99.66	99.33

*Average of three different concentration levels.

Table 3: Validation Summary

Validation Parameter	Results
System Suitability	
Theoretical Plates (N)	85679
Tailing factor	1.18
Retention time in minutes	11.3
% Area	99.39
LOD (mcg/mL)	1.5
LOQ (mcg/mL)	4.5

Table 4: Gradient Program in HPLC method

Time in minutes	Buffer	Acetonotrile
0	80	20
5	80	20
12	30	70
20	30	70
25	80	20
30	80	20

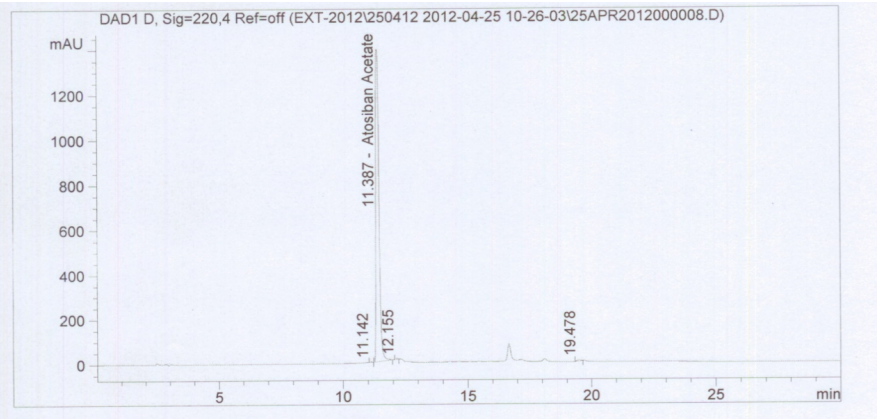

Figure 2: Typical Chromatogram of Atosiban Acetate by HPLC

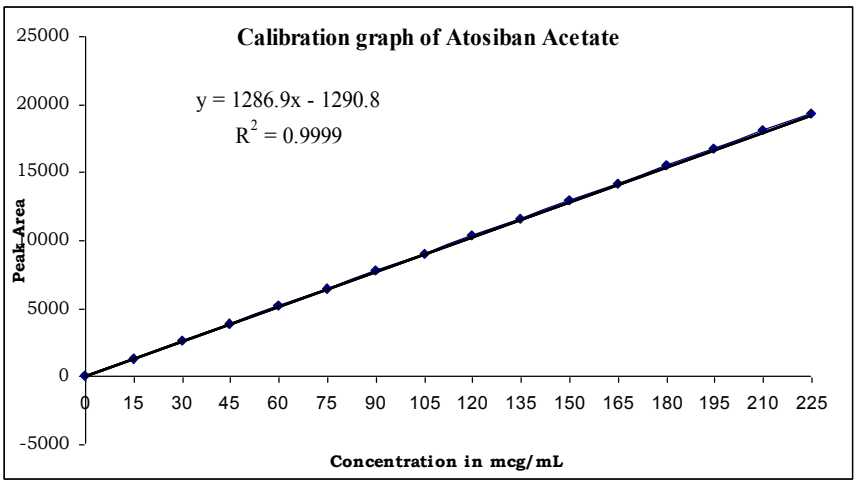

Figure 3: Calibration curve of the Atosiban Acetate by RP-HPLC

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of Atosiban Acetate. The parameters studied to evaluate the suitability of the system are given in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for Atosiban Acetate were found to be 1.5 mcg/mL and 4.5 mcg/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. From the typical chromatogram of Atosiban Acetate as shown in Figure 2, it was found that the retention time was 11.3 minutes. A mixture of buffer 0.03 M Potassium Dihydrogen Orthophosphate in water and pH adjusted to 3.20 with ortho-phosphoric acid and acetonitrile in the gradient program was used (shown in Table 4) was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r^2 = 0.9999) was observed between the concentration range of 15-225 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of Atosiban Acetate injectables was found to be 99.6 %. From the recovery studies it was found that about 99.3 % of Atosiban Acetate was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the injectables. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of parental dosage forms of Atosiban Acetate within a short analysis time.

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