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Research Article

DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE DETERMINATION OF TIROFIBAN IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, rapid and precise reverse phase high performance liquid chromatography method has been developed and validated for the determination of tirofiban in pharmaceutical formulation. Chromatography was performed by HPLC 2695 Waters Separation Module with Develosil C_8 column 4.6×250 mm, with 5 µ particle size and the column maintained at ambient temperature. The injection volume was 20 µl and the total run time was 10 min. The detection was carried out at 277 nm. The mobile phase consisted of 1-octane sulfonic acid buffer (pH 3.0) and acetonitrile (55:45 v/v). Prepared mobile phase was filtered through 0.45 µ membrane filter and sonicated. Sample solution was prepared by dissolving the contents of the vial in diluents. The mobile phase was delivered at a flow rate of 1 ml/min.

Keywords: Tirofiban, HPLC, Validation, Buffer.

INTRODUCTION

Tirofiban hydrochloride belongs to the category of antiplatelet agents. Chemically it is (*S*)-2-(butylsulfonamino)-3-(4-[4-(piperidin-4-yl)butoxy]phenyl)propanoic acid[1] (Fig. 1). It is a reversible antagonist of fibrinogen binding to the GP IIb/IIIa receptor, the major platelet surface receptor involved in platelet aggregation. When administered intravenously, it inhibits *ex vivo* platelet aggregation in a dose- and concentration-dependent manner. Literature survey revealed that few LC-MS[2-3] methods are available for determination of tirofiban. In the present investigation a simple, rapid, accurate and precise RP-HPLC method has been developed for the quantitative determination of tirofiban hydrochloride in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC 2695 separation module equipped with a reverse phase Develosil C_8 column (250mmx4.6mm; 5µm), a 20 µl injection loop and UV Visible detector.

Chemicals and solvents
The reference sample of tirofiban was supplied by M/s Natco Pharma Ltd., Hyderabad. HPLC grade water was prepared in the laboratory from triple distillation unit. Acetonitrile and 1-octane sulphonic acid are of HPLC grade, orthophosphoric acid of AR grade were obtained from E. Merck (India) Ltd., Mumbai.

Preparation of mobile phase solution
Mix 550 ml of pH 3.0 1-octane sulfonic acid buffer and 450 ml of acetonitrile into an appropriate flask. Filter it and sonicate it for 2 minutes.

Preparation of buffer
Accurately weigh and transfer 2 gm of 1-octane sulphonic acid into 1000 ml water. Adjust pH to 3.0 with orthophosphoric acid.

Preparation of standard solution
Weigh accurately 50 mg of tirofiban and transfer into a clean and dry 100 ml volumetric flask, add about 30 ml of diluent was added, sonicated for 20 minutes and diluted up to the mark with diluent. From this take 5 ml into a 50 ml volumetric flask and make up the volume with diluent.

Procedure
A mixture of buffer and acetonitrile in the ratio of 55:45 v/v was found to be the most suitable mobile phase for ideal separation of tirofiban hydrochloride. The solvent mixture was filtered through a membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1 ml/min. The column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 277 nm. The run time was set at 10 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 4.943 min. A typical chromatogram showing the separation of the drug is given in Fig. 2.

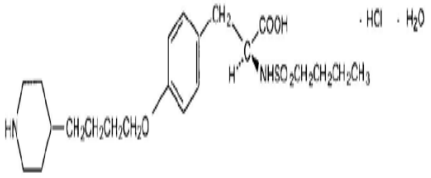

Fig. 1: Chemical structure of tirofiban

Linearity
The linearity of the method was demonstrated over the concentration range of 100-500 µg/ml. Aliquots of 100, 200, 300, 400 and 500 µg/ml were prepared from stock solution and labeled as solution 1, 2, 3, 4 and 5 respectively. The solutions were injected in to HPLC system as per test procedure.

Calibration plot
20 µl of each dilution was injected three times into the column at a flow rate of 1 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 100-500 µg/ml of the drug. The regression equation of this curve was computed. A calibration curve of tirofiban is given in Fig. 3. The relevant data are furnished in Table 1.

Validation of the proposed method
The specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of tirofiban hydrochloride. Tirofiban was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table 2 and Table 3. The accuracy of the HPLC method was assessed by analyzing the previously analyzed samples were spiked with known amounts of drug and

reanalyzed by the proposed method and the results are furnished in Table 4. The system suitability parameters are given in Table 5.

Estimation of tirofiban hydrochloride in injection

Transfer the contents of the vial into a clean and dry 100 ml volumetric flask and make up the volume with diluent. Separately inject equal volumes (20 µl) of diluents as blank, standard preparation and sample preparations into the chromatograph and record the chromatograms and measure the peak area responses for the analyte peaks and calculate the percentage content of tirofiban injection. The relevant results are furnished in Table 6.

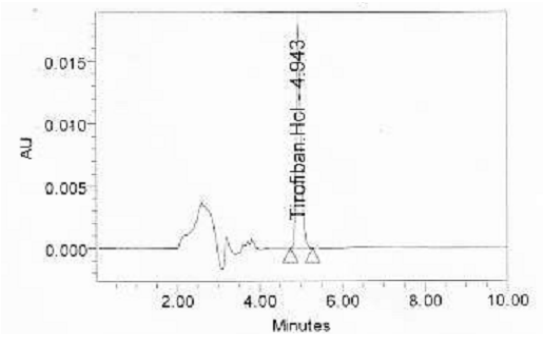

Fig. 2: Standard chromatogram of tirofiban hydrochloride

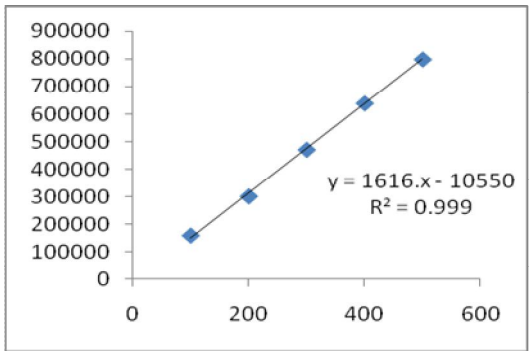

Fig. 3: Calibration curve of tirofiban

Table 1: Calibration data of tirofiban

Concentration	Area
100	159873
200	301846
300	471619
400	639492
500	799365

Table 2: Intra-day precision

Sample No.	Time intervals	Area
01	00	159998
02	02	159789
03	04	159897
04	06	159695
05	08	159967
06	10	159758
Mean	159850	
% RSD	0.07	

Table 3: Inter-day precision

Injection No.	Peak Area	% Recovery
1	159873	99.01
2	159648	98.87
3	159722	98.90
4	159980	99.07
5	159602	98.8
6	159945	99.05
Mean	159795	98.95
SD	159.37	0.36
% RSD	0.09	0.36

Table 4: Accuracy data of tirofiban

Sample No.	Concentration	Peak area	% Recovery	Mean % recovery	SD	% RSD
1	50%	76051	101.08			
2	50%	75982	101.03	101.05	0.025	0.024
3	50%	75994	101.05			
4	100%	151004	100.3			
5	100%	151252	100.5	100.5	0.2	0.199
6	100%	151468	100.7			
7	125%	228010	101.06			
8	125%	228246	101.17	101.15	0.09	0.088
9	125%	228405	101.24			

Table 5: System suitability parameters

Parameter	Results
Theoretical plates (N)	9065
Linearity range (µg/ml)	100-500
Retention time (min)	4.943
Tailing factor	1.3
Correlation coefficient	0.999
LOD (µg/ml)	0.010
LOQ (µg/ml)	0.032
% RSD	0.02

RESULTS AND DISCUSSION

In the proposed method, the retention time of tirofiban hydrochloride was found to be 4.943 min. Quantification was linear in the concentration range of 100-500 µg/ml. The regression equation of the linearity plot of concentration of tirofiban over its peak area was found to be Y=1616X-10550 (R^2=0.999), where X is the concentration of tirofiban (µg/ml) and Y is the corresponding peak area. The use of 1-octane sulfonic acid buffer and acetonitrile in the ratio of 55:45

v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed HPLC method.

Table 6: Estimation of tirofiban in pharmaceutical dosage form

Sample	Labeled amount (mg)	Amount found (mg)	% of drug present
Tirofiban injection	5 mg	4.95 mg	99%

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of tirofiban and can be reliably adopted for routine quality control analysis of tirofiban hydrochloride in bulk and in pharmaceutical formulation.

ACKNOWLEDGEMENTS

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