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Two Highly Sensitive Techniques (HPLC and HPTLC) for the Estimation of Anti-Platelet Drug Tirofiban from Infusion

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ABSTRACT

Two highly sensitive chromatographic methods were developed for the estimation of Tirofiban from infusion. The first method is HPLC, in which sodium dihydrogen phosphate (pH 5): Acetonitrile, 70:30% v/v was used as mobile phase and separation carried out on a C18 column. With 1 ml/min flow rate, tirofiban was eluted at 4.2min. The second method is HPTLC using a mobile phase consisting of hexane: methanol: acetic acid (5:3.5:0.5% v/v/v) on a precoated silica gel 60F254 plate. The drug was retained at a Rf of 0.46. Two analytical methods were validated for linearity, accuracy, precision, LOD, LOQ, ruggedness and robustness as per ICH guidelines. The linear concentration was 600-1400 ng/ml for HPLC and 3-18 ng/spot for HPTLC method. The LOD concentration was found to be 90 ng/ml and 1 ng/spot for HPLC and HPTLC, respectively The LOQ of Tirofiban is 400 ng/ml by HPLC and 3 ng/spot by HPTLC. The precision and accuracy values were found to be in acceptable limits. The method was successfully applied to Tirofiban infusion and the amount estimated was close to label claim. Further no interference from additives by both the methods.

Keywords: Antiplatelet agents, Tirofiban, HPLC, HPTLC, Tirofiban hydrochloride.

INTRODUCTION

Antiplatelet agents are widely prescribed to reduce the cardiovascular events that occurs due to acute coronary syndrome [1]. Tirofiban is N-(butylsulfonyl)-O-[4-(4-piperidinyl) butyl] L-tyrosine hydrochloride chemically [2], and it is an antiplatelet prescribed in various syndrome of cardiovascular events. It prevents blood from clotting during the episode of chest pain or heart attack. It is a non-peptide reversible antagonist of platelet glycoprotein IIb-IIIa receptor, inhibits platelet aggregation. It is available as Intravenous infusion with the strength of 5 mg/100 ml. Few HPLC methods were reported for the formulation [3-8] and serum [9]. A fluorometric method for the determination of Tirofiban in presence of Apixaban was reported for formulation [10]. The aim of current study was to develop highly sensitive HPLC and HPTLC methods for the determination of Tirofiban from infusion.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents used were of AR grade and all the solvents were of HPLC grade. Tirofiban hydrochloride was a gift sample from Gland pharma Ltd, Hyderabad, Telangana, with the sample of analysis.

Preparation of stock solution

A quantity of 10 mg of Tirofiban was weighed accurately and transferred to 10 ml volumetric flask and dissolved in methanol. It was diluted to 10 ml (1 mg/ml).

A concentration of 15 µg/ml of Tirofiban was prepared from the above solution and used for HPTLC. A working standard solution containing 50 µg/ml of Tirofiban was used in HPLC analysis.

Preparation of mobile phase

Sodium dihydrogen phosphate (10 mM, pH 5) was prepared in HPLC water and mixed with Acetonitrile (70:30%v/v) and used in isocratic elution. The solvent system consisted of Hexane: methanol: acetic acid (5:3.5:0.5%v/v/v) was premixed in a twin trough chamber and used for HPTLC method.

Validation of the method

The two chromatographic methods (HPLC and HPTLC) developed for Tirofiban were validated as per ICH guidelines [11] for the parameters like linearity, accuracy, precision, ruggedness, robustness, LOD and LOQ.

Analysis of infusion

Not less than 10 infusions were taken for analysis. They were carefully opened by a sterile needle and the contents were mixed together. A volume equivalent to 10 mg of Tirofiban hydrochloride was carefully transferred to a 10 ml volumetric flask and diluted with methanol. After thorough vortex, the solution was diluted and analyzed by the proposed HPLC and HPTLC methods.

RESULTS AND DISCUSSION

HPLC

HPLC method was developed for the estimation of Tirofiban from infusion by optimizing the chromatographic conditions such as selection of wavelength, solvent, buffer (strength, pH), ratio and flow rate. A symmetrical peak was obtained with the mobile phase system Sodium dihydrogen phosphate (10 mM, pH 5): Acetonitrile (70:30%v/v) running at 1 ml/min flow rate on a LUNA-5µ, C18 (250 × 4.6 mm) column. A wavelength of 226 nm was selected to quantify Tirofiban. The linearity was found to be between 600-1400 ng/ml and the slope, intercept and correlation coefficient values were 2381.7, 4044.5 and 0.9998 respectively. The calibration graph and the standard chromatogram of Tirofiban is shown in Figures 1 and 2.

Accuracy was confirmed by carrying out recovery studies. A known quantity of standard Tirofiban was spiked with preanalysed infusion and analysed by proposed method. The% recovery was found to be between 98.78-100.29 at 50,100, 150% levels. The repeated analysis on same and different days assures the method precision by resulting in percentage RSD less than 0.06. The LOD was 90 ng/ml and the LOQ was 400 ng/ml. Tirofiban solution was stable up to 12 hrs under room temperature. The system suitability parameters were found to be in acceptable limit. The tailing factor, retention time, number of theoretical plates, resolution values were found to be 0.977, 4.283, 2317 and 5.90, respectively.

Not less than ten infusions of Tirofiban hydrochloride were analyzed by the proposed method without interference of additives and the amount estimated is shown in Table 1.

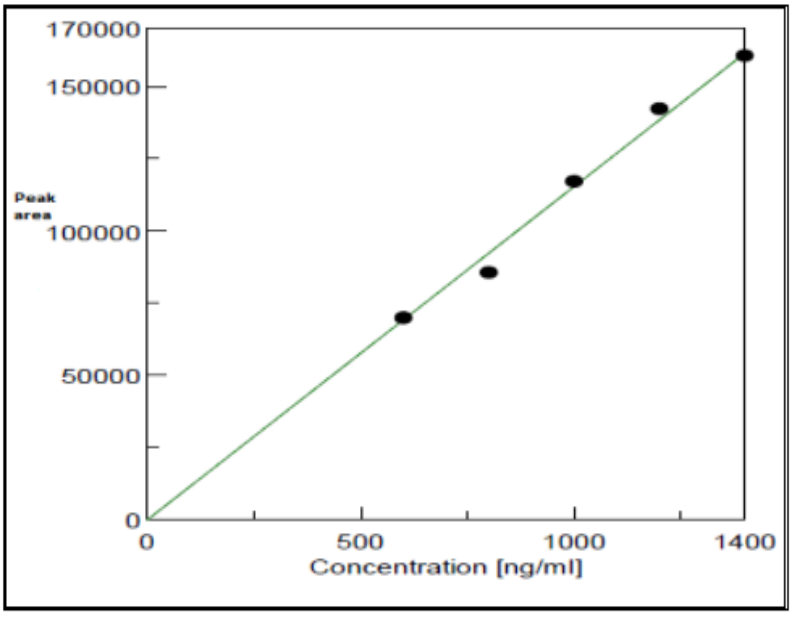

Figure 1: HPLC Calibration graph of Tirofiban (600-1400 ng/ml).

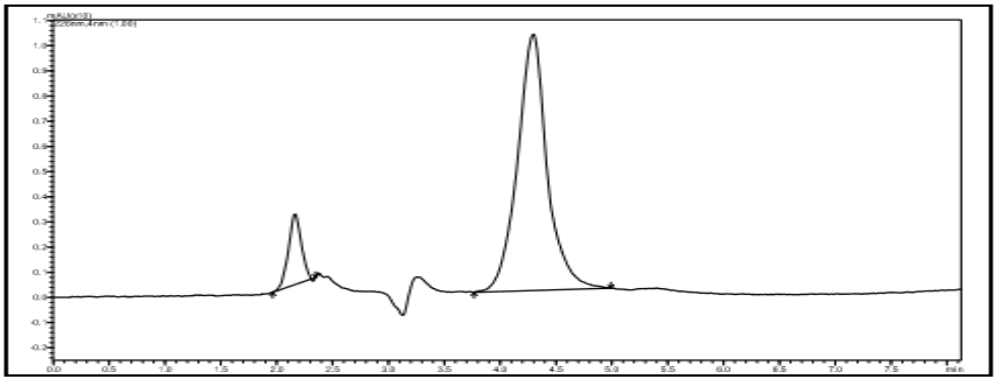

Figure 2: HPLC chromatogram of Standard Tirofiban (1400 ng/ml).

HPTLC

In the development of HPTLC method, various solvents were used in trial, such as cyclohexane, chloroform, toluene to develop a compact spot for Tirofiban hydrochloride. Among all hexane: Methanol: Acetic acid (5:3.5:0.5%v/v/v) gave a good compact symmetrical spot with an Rf value of 0.46. It was carried out on aluminium plates (20 × 20 cm) precoated with silica gel 60F254, the chamber saturation time was 15 min, the migration diatance was 80 mm and the deuterium lamp used detection at 226 nm.

A 15 µg/ml solution of Tirofiban was prepared in methanol using stock solution (1 mg/ml). Aliquots of 2, 4, 6, 8, 10 and 12 µL of Tirofiban hydrochloride were applied on the plate and developed. After scanning the calibration graph was plotted and shown in Figure 3. A densitogram of the drug is shown in Figure 4. The linear concentration of tirofiban was 3-18 ng/spot. The slope, intercept, correlation coefficient values were found to be 144.5, 1223.6 and 0.997 respectively. The accuracy was studied by standard addition technique at 50%, 100%, 150% level. The% recovery was between 98.75-100.15. The interday, intraday precision and repeatability values resulted in less than 1% RSD. The developed plate was found to be stable for 48 hours.

Not less than ten infusions were used for estimation. After dilution required volume in the linearity range was spotted and evaluated by developed method. The results are shown in Table 1.

Method	**Labelled amount (mg/100 ml)**	**Estimated amount (mg/100 ml)**	**% label claim***	**% RSD***
HPLC	5	4.97	99.4	0.431
HPTLC		4.98	99.6	0.403
*Average of 6 observations				

Table 1: Analysis of Tirofiban infusion.

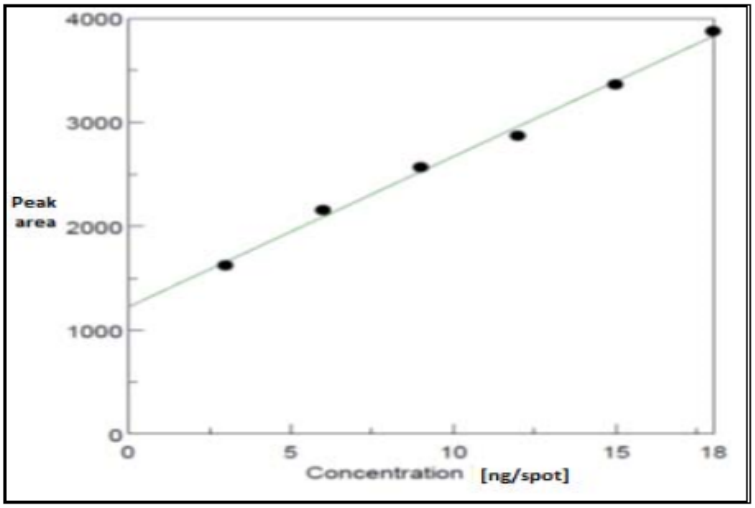

Figure 3: HPTLC Calibration graph of Tirofiban (3-18 ng/band).

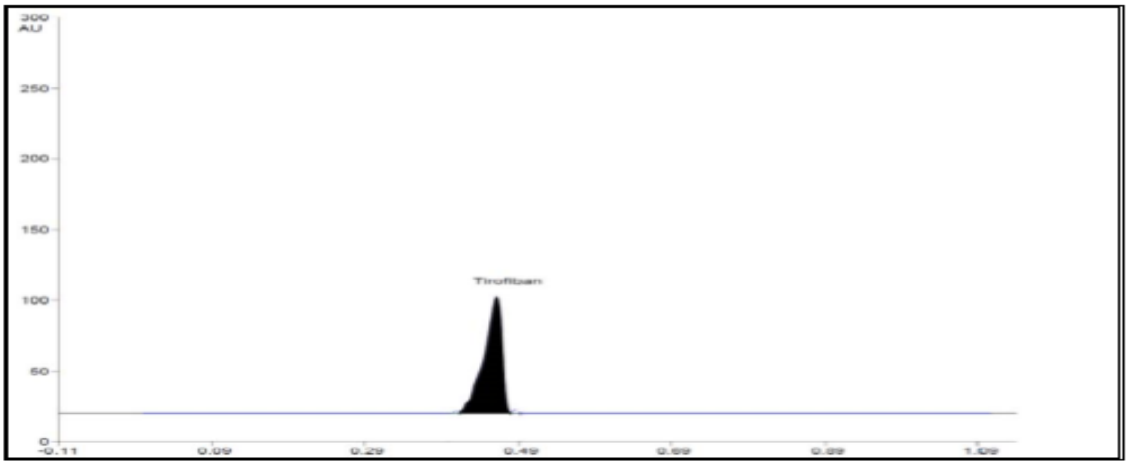

Figure 4: Densitogram of tirofiban (3 ng/spot).

CONCLUSION

Two chromatographic methods were developed to estimate Tirofiban hydrochloride from infusions. They were optimized, validated and applied successfully for infusion containing Tirofiban hydrochloride. HPTLC is cost effective and first one of the kinds as no such method reported so for. Both the methods are highly sensitive when compared to existing methods so that nanogram level of drug can be estimated.

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