

## DEVELOPMENT AND VALIDATION OF A REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF TIROFIBAN IN PHARMACEUTICAL DOSAGE FORMS

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### ABSTRACT

Tirofiban in combination with heparin and aspirin is indicated in the management of patients with unstable angina or non-Q-wave myocardial infarction, including patients who may subsequently undergo percutaneous transluminal coronary angioplasty (PTCA)

Objective: To develop and validate a reverse phase high performance liquid chromatographic method for the estimation of tirofiban in pharmaceutical dosage forms.

Method: The method employed is reverse phase high performance liquid chromatography.

The method involved using of a mobile phase consisting of 1 – Octane sulfonic acid Buffer (pH 3.0): Acetonitrile (55:45, v/v) and Develosil C<sub>8</sub> (4.6×250 mm) column. The flow rate was maintained at 1.0 ml/min and the detection was done at 277 nm. The developed method was validated by performing studies related to linearity, accuracy, precision, specificity, robustness.

Results: The retention time was found to be 4.93 min. The method was found to be linear in the concentration range of 100-500 µg/ml. The method was found to be simple, precise and accurate.

Discussion: The method was found to be reliable for the determination of Tirofiban in pharmaceutical dosage forms.

**Keywords:** Tirofiban, Determination, RP-HPLC.

### INTRODUCTION

Tirofiban [1, 2] is an antiplatelet agent, which is chemically (S)-2-(butylsulfonamino)-3-(4-[4-(piperidin-4-yl) butoxy] phenyl) propanoic acid. It is useful in combination with heparin and aspirin in the management of patients with unstable angina or non-Q-wave myocardial infarction, including patients who may subsequently undergo percutaneous transluminal coronary angioplasty (PTCA)

Tirofiban 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, tirofiban inhibits *ex vivo* platelet aggregation in a dose- and concentration-dependent manner. The structure of tirofiban is shown in fig. 1.

When given according to the recommended regimen, >90% inhibition is attained by the end of the 30 minute infusion. Platelet aggregation inhibition is reversible following cessation of infusion of Tirofiban.

Literature survey reveals that few methods related to LC-MS [3, 4] for the determination of Tirofiban in biological fluids, study of compatibility [5-7] of Tirofiban with other drugs, metabolism and drug release behavior [8] are available. The aim of the present study is to develop simple, precise, rapid and accurate RP-HPLC[9, 10] method for the determination of Tirofiban in injections.

### MATERIALS AND METHODS

#### Chromatographic conditions

A Waters 2695 – separation module with a UV detector, Shimadzu UV-visible spectrophotometer – 1601 with UV – probe software and a Develosil C<sub>8</sub> Column (4.6 X 250 mm) were used. A 20 µL Hamilton injection syringe was used for sample injection. HPLC grade reagents were used for the preparation of buffer of pH 3. A freshly prepared 1 – Octane sulfonic acid Buffer (pH 3.0): Acetonitrile (55:45) was used as mobile phase. The solvents were filtered through 0.45 µm membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1.0ml/min. The column temperature was maintained at 25°C and the detection was carried out at 277 nm.

#### Selection of column

Column trials were performed using Unison C<sub>18</sub> 4.6×250mm, 5µ and Develosil C<sub>8</sub> 4.6×250 mm, 5µ. Better peak resolution with less tailing was observed with Develosil C<sub>8</sub> 4.6×250 mm, 5µ.

#### Selection of mobile phase

The method development for the determination of Tirofiban was tried with different solvent systems. Different mobile phases containing methanol, acetonitrile, water and 1 – Octane sulfonic acid Buffer (pH 3.0) in various compositions were tried and finally 1 – Octane sulfonic acid Buffer (pH 3.0): Acetonitrile (55:45) was selected as good chromatograms were obtained with that composition. 100% Purified Milli Q Water was selected as diluent.

#### Preparation of mobile phase

Buffer preparation: Accurately weighed and transferred 2g of 1 – octane sulphonic acid into 1000ml volumetric flask and added 500 ml of water, allowed it to dissolve and made upto the volume with water. pH was adjusted to 3.0 with ortho phosphoric acid.

550 ml of 1 – octane sulfonic acid buffer (pH 3.0) and 450 ml of acetonitrile were mixed (55:45, v/v). Mobile phase was filtered through a 0.45 µm membrane filter, degassed with a helium spurge for 20 min.

#### Selection of analytical wavelength

From the standard stock solution, different dilutions were prepared using mobile phase and scanned over the range of 200-400 nm and the spectrum was overlain. It was observed that 277 nm is the  $\lambda_{max}$  for Tirofiban and it was selected as a suitable wavelength for detection. Eluents were monitored at 277 nm.

#### Preparation of stock and standard solution

Accurately weighed 50 mg quantity of Tirofiban was transferred in to a 100 ml volumetric flask. 30 ml of diluent was added and sonicated for 15 min and made upto the volume with diluent. From the stock solution a working standard solution of 50µg/ml was prepared.

### Linearity and construction of calibration curve

Aliquots of 100, 200, 300, 400 and 500  $\mu\text{g/ml}$  were prepared from standard solution to determine the linearity range. Each of these drug solutions (20  $\mu\text{l}$ ) was injected 5 times into the column by maintaining a flow rate of 1.0 ml/min. The detection was carried out at 277 nm. Chromatograms were recorded and peak area was recorded for all injections. A calibration plot of concentration over the peak area was constructed. The regression was computed by least square regression method. This regression equation was used to estimate the amount of metoprolol tartarate in its pharmaceutical dosage forms.

### Estimation of metoprolol tartarate in injection

A Vial containing 5 mg of Tirofiban was chosen for estimation. A volume equivalent to 5 mg of Tirofiban was transferred in to a 100 ml volumetric flask and made upto the volume with the diluent to obtain a sample solution of 50  $\mu\text{g/ml}$ . Twenty micro liters of sample solution was then injected into the column. The sample was injected for five times. The mean peak area of the five determinations was calculated and the drug content in the sample was quantified using the regression equation obtained from the calibration curve.

## RESULTS AND DISCUSSION

### Method development

In the present investigation we have developed a simple precise and accurate reverse phase high performance liquid chromatographic method for the determination of Tirofiban. The method was developed using mobile phase, a mixture of 1 - Octane sulfonic acid Buffer (pH 3.0): Acetonitrile (55:45) and a column, Develosil C<sub>8</sub> Column (4.6 X 250 mm), 5 $\mu$ . The flow rate selected was 1.0 ml/min. The detection was carried out at 277 nm. The developed method was found to be appropriate for the determination of Tirofiban in an injection. A typical chromatogram of tirofiban obtained by using the above parameters was shown in fig. 2.

### Validation

#### Precision

Intra-day and inter-day precision studies were performed by injecting each concentration for six times and the percent RSD was calculated and it was found to be within limits. The results of intra-day and inter-day precision studies were shown in table 1

### Linearity

The linearity of the method was found to be in the range of 100-500 $\mu\text{g/ml}$ . The results of the linearity studies were shown in table 2. The regression coefficient was found to be 0.999 from the calibration curve. The Standard calibration curve of the proposed method was shown in fig. 3.

### Accuracy

The percentage recovery studies were carried out at three different concentration levels. The percentage recovery and the percentage RSD values were found within the limits. The results of accuracy studies were shown in table 3.

### Specificity

Chromatogram of blank did not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. No interference was observed from the excipients and degradation products of degradation studies. Hence the method was found to be specific and stable.

### Robustness

The robustness studies were performed by changing the organic phase proportion of the mobile phase and buffer pH. The results of robustness were shown in table 4.

### System suitability parameters:

System suitability parameters like retention time, peak area, theoretical plates and tailing factor were determined and the results were shown in table 5.

## CONCLUSION

The proposed HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of Tirofiban in pharmaceutical dosage forms. Hence this method can easily and conveniently adopt for routine analysis of Tirofiban in pure and its pharmaceutical formulations.

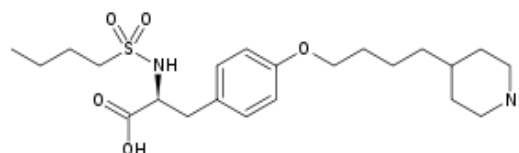


Fig. 1: Structure of Tirofiban

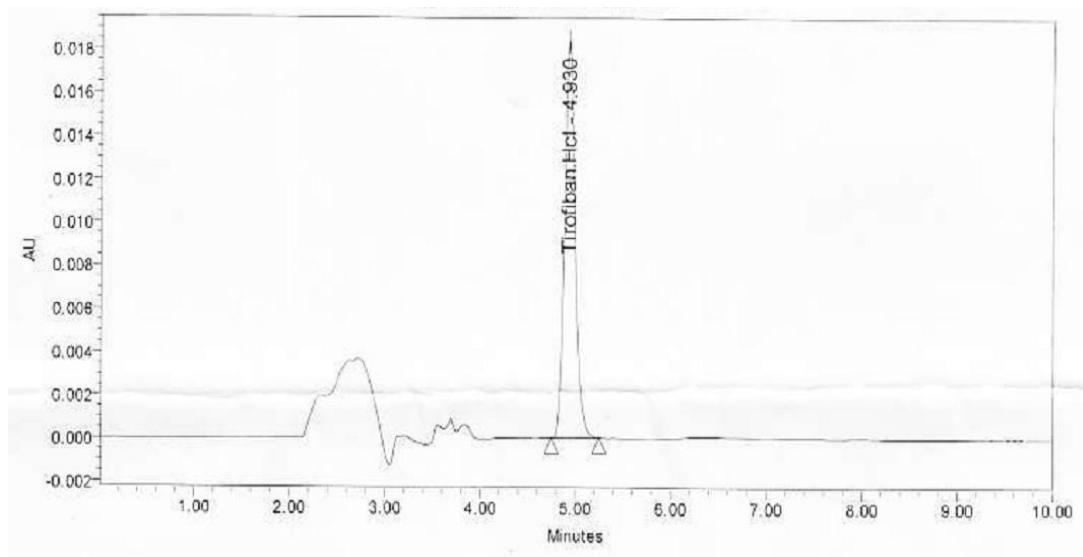


Fig. 2: A typical chromatogram of Tirofiban obtained from the proposed method

Table 1: Results of intra-day and inter-day precision studies

| Concentration of metoprolol (µg/ml) | Intra-day precision |                      |             | Inter-day precision |                      |             |
|-------------------------------------|---------------------|----------------------|-------------|---------------------|----------------------|-------------|
|                                     | Mean amount found   | Percent amount found | Percent RSD | Mean amount found   | Percent amount found | Percent RSD |
| 200                                 | 200.55              | 100.27               | 0.30        | 201.21              | 100.60               | 0.18        |
| 300                                 | 299.63              | 99.87                | 0.23        | 300.66              | 100.22               | 0.47        |
| 400                                 | 400.40              | 100.10               | 0.16        | 399.67              | 99.91                | 0.18        |

Table 2: Results of the linearity studies

| Concentration (µg/ml) | Area   |
|-----------------------|--------|
| 100                   | 159873 |
| 200                   | 301846 |
| 300                   | 471619 |
| 400                   | 639492 |
| 500                   | 799365 |

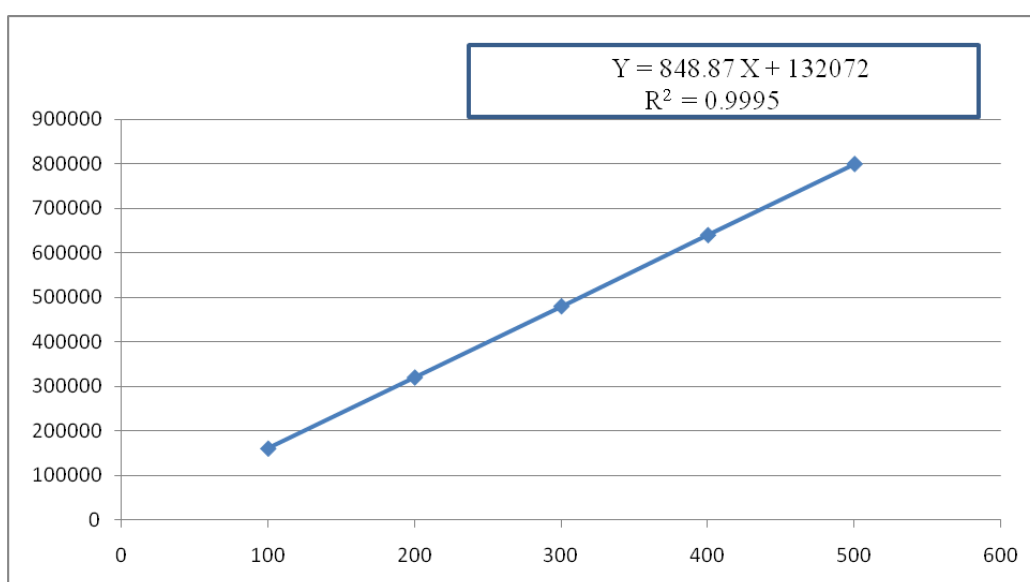


Fig. 3: Standard calibration curve of the proposed method

Table 3: Results of Accuracy studies

| Amount taken (µg) | Amount found (µg) | Percent recovery | % Mean recovery | % RSD |
|-------------------|-------------------|------------------|-----------------|-------|
| 100+25            | 124.62            | 99.69            |                 |       |
| 100+25            | 125.09            | 100.07           | 99.74           | 0.40  |
| 100+25            | 124.33            | 99.46            |                 |       |
| 100+50            | 151.26            | 100.84           |                 |       |
| 100+50            | 150.48            | 100.32           | 100.81          | 0.59  |
| 100+50            | 151.92            | 101.28           |                 |       |
| 100+75            | 174.96            | 99.97            |                 |       |
| 100+75            | 175.96            | 100.54           | 100.4           | 0.17  |
| 100+75            | 176.21            | 100.69           |                 |       |

Table 4: Results of robustness studies

| Result             | Flow rate (ml/min) |      |      | pH of the buffer |      |      | Organic proportion of the phase (%) |      |      |
|--------------------|--------------------|------|------|------------------|------|------|-------------------------------------|------|------|
|                    | 0.9                | 1.0  | 1.1  | 2.8              | 3.0  | 3.2  | 50                                  | 55   | 60   |
| Tailing factor     | 1.0                | 0.7  | 0.9  | 1.0              | 0.7  | 1.1  | 0.9                                 | 0.7  | 1.0  |
| Theoretical plates | 7856               | 8934 | 8125 | 7901             | 8934 | 8462 | 8211                                | 8934 | 7995 |

Table 5: System suitability parameters

| Drug      | Retention time (min) | Peak area | Theoretical plates | Tailing factor |
|-----------|----------------------|-----------|--------------------|----------------|
| Tirofiban | 4.93 min             | 150139    | 8934               | 0.7            |

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