Method development and validation of Ifetroban by RP-UPLC

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ABSTRACT
The purpose of this work is to develop and validate reverse phase Ultra performance liquid chromatography (UPLC) method for the rapid and precise determination of ifetroban sodium in its pure form and in formulations. A simple, specific, accurate, precise isocratic UPLC method for analysis of Ifetroban sodium was developed and validated using a Phenomenex C18 column (50 mm x 3.0 mm, 3µ) as the stationary phase, in conjunction using Triethyl amine buffer: methanol in the proportion of 25:75 with a flow rate of 1.0 mL/min, run time is 3 min and UV detector is used at 235 nm wavelength. The developed UPLC technique was found to be rapid as the retention time was 0.56 minutes for Ifetroban peak to elute. The developed UPLC technique was validated as per the ICH guidelines for specificity, linearity, accuracy, precision, robustness and found to be satisfactory. Linearity was established in the concentration range 100-300 µg/mL with correlation coefficient of 0.998 and the equation obtained is y = 0.635x + 0.639. The percentage recovery is 100.41. The method is rugged and is trouble free and transferable. The study showed that the developed UPLC technique can be used for the estimation of drug purity, stability, solubility and with no interference of pharmaceutical excipients from the active pharmaceutical ingredient. The precision, accuracy, robustness results obtained enables rapid quantification of ifetroban for quantitative analysis.

INTRODUCTION
Ifetroban sodium is 2-[[1S,2R,3S,4R]-3-[4-[[Pentylamino]carbonyl]-2-oxazolyl]-7-oxabicyclo[2.2.1]hept-2-yl[methyl]-benzenepropanoic acid sodium salt (Figure 1). Ifetroban is thromboxane A2/prostaglandin H2 receptor antagonist (Drug Bank, 2020). In deviation to other cyclooxygenase products, thromboxane A2 helps in contraction of blood vessels and has been intertwined in platelet activation. Outcomes of case studies and experiments on animals suggest that hypertension is linked with hyper aggressiveness of platelets and excess thromboxane A2 amounts in body fluids (Rosenfeld et al., 2001). Not only thromoxane A2, prostaglandin G2, and prostaglandin H2 but their precursors also bind to the receptors, so a receptor antagonist was better than an inhibitor in switching the effects of thromboxane A2/prostaglandin H2.
An elaborate literature review uncovered that there is no published RP-UPLC technique for the quantification of ifetroban sodium. Several studies have been done for ifetroban like kinetic analysis by HPLC-electrospray mass spectrometry for the stability studies of aqueous solutions of ifetroban 1-O-acyl glucuronide a major metabolite (Khan et al., 1998), direct injection capillary GC-MS analysis for the assay of ifetroban (Jemal et al., 1995), FAIMS can be used to establish the location of the in-source CID in electron spray ionization mass (Xia and Jemal, 2009) spectrometer. Therefore, it was opportunistic to materialize a simple, specific, accurate, precise method procedure that can be available for the determination of ifetroban.

**EXPERIMENTAL**

**Chemicals and Reagents**

Ifetroban sodium was received from reputed pharmaceutical company as a development sample. Analytical grade Triethyl amine, ortho phosphoric acid, methanol and water of HPLC grade obtained from Rankem India Private Ltd.

**Preparation of buffer**

By mixing 1 mL of Triethyl amine in 990 mL of HPLC grade water and pH fixed at 3.0 using ortho phosphoric acid and made up to 1000 mL with water, Filter the buffer solution.

**Composition of mobile phase**

Buffer : methonal (25:75 %v/v), 250 mL of Buffer : methanol and 750 mL of pure HPLC methanol mixed, degassed prior to use.

**Chromatographic parameters and equipments**

Analytical separation was accomplished on Ultra Performance Liquid chromatography (Agilent 1220 Infinity with Open Lab CDS chemstation) in isocratic mode and was achieved with triethylamine buffer and methanol in the proportion of 25:75 on a Phenomenex column C18 (50 mm x 3.00 mm, 3μ) with a solvent delivery of 1.0 mL/min., injection capacity 2 μL, temperature of column at 25°C and wavelength at 235 nm.

**Preparation of standard solution**

Ifetroban standard was precisely weighed and solution of 1000 ppm was made with the mobile phase. Further diluted to get a solution of 200 ppm using mobile phase.

**Sample solution**

Sample weighed about 50 mg of Ifetroban sodium and transferred to a 50 mL volumetric flask, 20 mL of mobile phase was further added to dissolve and make up to the mark. Further diluted to get a solution of 200 ppm using mobile phase.

**Placebo solution preparation**

Formulation excipients such as mannitol, hydroxypropyl cellulose, microcrystalline cellulose, PEG 6000 and lactose (Signet Ltd., Mumbai, India) were mixed in an appropriate proportion for placebo and prepared similar to sample solution.

**Procedure for method validation**

Validation of the materialized UPLC technique was carried out in agreement with ICH guidelines Q2 (R1) (ICH Validation, 1988).

**Specificity**

Specificity is the capability of the technique to measure the analyte response in the presence of all its sample materials (Sajan and M, 2014). It was carried out by injecting diluent/placebo, standard and test solution.

**System suitability**

System suitability was verified by determining various parameters like tailing factor, column efficiency (N), selectivity and resolution factors, as shown in Table 1.

**Precision**

System precision was tested out by analyzing 6 injections of standard ifetroban solution and chromatogram evaluated for system suitability criteria. The intermediate and method precision studies were accomplished on alternate days with other analysts. % RSD method and inter-day precision were calculated.
Figure 2: Typical UPLC chromatogram of Ifetroban solution (200 µg/mL)

Figure 3: Typical UPLC chromatogram of placebo solution

Table 1: System Suitability studies

<table>
<thead>
<tr>
<th>Name</th>
<th>Mean Retention time</th>
<th>Mean Peak area ± SD</th>
<th>USP plate count ±SD</th>
<th>Asymmetry ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ifetroban</td>
<td>0.56 min.</td>
<td>125.301±0.21</td>
<td>1301.93±8.96</td>
<td>1.29±0.01</td>
</tr>
</tbody>
</table>

SD: Standard deviation, n = 6.
Figure 4: Linearity graph for Ifetroban

Table 2: Regression characteristics determined by the proposed method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ifetroban</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>2.5 ppm</td>
</tr>
<tr>
<td>LOQ</td>
<td>7.5 ppm</td>
</tr>
<tr>
<td>Linearity range (μg/mL)</td>
<td>100-300μg/mL</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.635</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.639</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.998</td>
</tr>
</tbody>
</table>

LOD is the minutest amount of compound that an analytical process can distinguish from other levels. LOQ is the minutest concentration of the compound in the sample which can be quantified with an accuracy and precision. The LOD and LOQ were projected from slope and the SD of the calibration curve, 

LOD = \(3.3 \times \sigma/S\) LOQ = \(10 \times \sigma/S\)

Where, \(\sigma\) is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Accuracy

Accuracy was studied by percent recovery, SD, and % RSD. Accuracy is the percentage of analyte reclaimed by an assay from the known added amount, was performed at 80, 100 and 120 % concentration. The recovery of the drug was studied.

Robustness

Robustness was studied by determining the influence of small but deliberate variations in the experimental condition like changing the wavelength (235± 2) flow rate (1±0.1 mL/min.), buffer pH (3.0±0.2), for the analytical performance. The system suitability criteria like peak area counts, theoretical plates and tailing factor should meet pre established criteria obtained under the nominal conditions.

Linearity

Five solutions of Ifetroban sodium standard solution prepared at concentration from 50% to 150% of the target concentration (100 to 300 μg/mL). Linearity chart of concentration as against to peak area was schemed and regression characteristics calculated.

RESULTS AND DISCUSSION

UPLC method development and optimization

The UPLC technique was outlined by freezing the chromatographic separation by developmental runs, changing the solvent, proportion of the mobile phase, pH, analytical column, analyte peak with very short run time. Methanol is selected as suitable solvent on the basis of stability and solubility of the drug in solvent system as well as extraction of the drug from its formulation. Columns from two or more brands used and Phenomenex C18 (50 mm x 3.0 mm, 3μ) is finalized, for better peak symmetry (about 1.25). The UPLC technique is then validated for linearity, robustness, ruggedness. Figure 2 shows a model chromatogram obtained by the UPLC method.

Method validation

Specificity

The diluent/placebo and test solution not showed any peak at the retention time of Ifetroban, Figure 3 represents representative chromatogram of placebo.

System suitability
Table 3: Accuracy Data for Ifetroban

<table>
<thead>
<tr>
<th>Drug</th>
<th>Recovery level</th>
<th>Amount taken (PPM)</th>
<th>Amount added (PPM)</th>
<th>Absolute Mean (n=3)</th>
<th>%Recovery ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ifetroban</td>
<td>80%</td>
<td>100</td>
<td>60</td>
<td>160.73</td>
<td>100.45±0.030</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100</td>
<td>100</td>
<td>201.15</td>
<td>100.57±0.188</td>
</tr>
<tr>
<td></td>
<td>120%</td>
<td>100</td>
<td>140</td>
<td>240.97</td>
<td>100.43±0.136</td>
</tr>
</tbody>
</table>

SD: Standard deviation, n = 3.

Table 4: Results from ruggedness study

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Analyst-1 (% Assay)</th>
<th>Analyst-2 (% Assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100.08</td>
<td>100.06</td>
</tr>
<tr>
<td>2</td>
<td>100.83</td>
<td>100.72</td>
</tr>
<tr>
<td>3</td>
<td>99.12</td>
<td>100.78</td>
</tr>
<tr>
<td>4</td>
<td>98.19</td>
<td>101.22</td>
</tr>
<tr>
<td>5</td>
<td>99.17</td>
<td>100.40</td>
</tr>
<tr>
<td>6</td>
<td>100.81</td>
<td>99.65</td>
</tr>
<tr>
<td>mean</td>
<td>99.70</td>
<td>100.47</td>
</tr>
<tr>
<td>SD</td>
<td>1.051</td>
<td>0.560</td>
</tr>
<tr>
<td>%RSD</td>
<td>1.054</td>
<td>0.565</td>
</tr>
</tbody>
</table>

Mean (n=12) 100.09
SD(n=12) 0.90
%RSD(n=12) 0.90

*%RSD: Percentage relative standard deviation; SD: Standard deviation, n = 6.

Table 5: Results of robustness study

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean Retention time (RSD ≤ 2.0)</th>
<th>Mean Tailing factor (RSD ≤ 2.0)</th>
<th>Mean Peak area (RSD ≤ 2.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9 mL/min</td>
<td>0.68</td>
<td>0.58</td>
<td>1.22</td>
</tr>
<tr>
<td>1.1 mL/min</td>
<td>1.86</td>
<td>0.74</td>
<td>0.26</td>
</tr>
<tr>
<td>Wavelength 233 nm</td>
<td>0.92</td>
<td>0.80</td>
<td>0.18</td>
</tr>
<tr>
<td>Wavelength 237 nm</td>
<td>1.49</td>
<td>0.58</td>
<td>0.61</td>
</tr>
<tr>
<td>At pH 2.8</td>
<td>1.76</td>
<td>0.91</td>
<td>0.19</td>
</tr>
<tr>
<td>At pH 3.2</td>
<td>1.34</td>
<td>1.07</td>
<td>0.96</td>
</tr>
</tbody>
</table>

*%RSD: Percentage relative standard deviation. n=6

The theoretical plate counts and peak asymmetry were projected from the standard solution. NMT 2.0 % RSD is the acceptance criteria for peak area counts and tailing factor NMT 2.0 for the analyte peak. The acceptance criterion for plate count was not less than 1200. The values captured substantiated the suitability of the chromatographic technique for the estimation of Ifetroban (Table 1).

**Linearity**

A linearity graph (Figure 4) of peak area at each level against concentration is plotted and the equation obtained is y = 0.635x + 0.639 with r²: 0.998. The range of the method is 100 to 300 ppm. Data are summarized in Table 2.

**Sensitivity**

The LOD is 2.5 and LOQ is 7.5 µg/mL determined from slope of linear regression curves, summarized in Table 2.

**Accuracy studies**

Solutions prepared at 3 levels (80%, 100% and 120%) of standard concentration level in triplex. The recoveries for Ifetroban were calculated and the results show the developed method has met the requirements of recovery and also indicates the absence of interference from pharmaceutical additives. The results are shown in Table 3.

**Method precision and Intermediate precision**
The precision of UPLC technique was appraised by carrying out six individual determinations the average % assay (n=6) of Ifetroban was 99.70 with RSD of 1.04%. Low % RSD values, indicates that the UPLC technique is precise (Trivedi et al., 2012). The reproducibility was checked by assaying the samples by second analyst using other chromatographic system and column on another day. Results are illustrated in Table 4.

**Robustness**

The system suitability criteria were found to meet the pre established acceptance values (Table 1) at all the modified conditions. %RSD should not be more than 2.0% with the results obtained with modified conditions against nominal conditions. The results and the range of the modified chromatographic variables appraised in the robustness are given in Table 5.

**Method Application**

The materialized UPLC technique was workable for estimation of Ifetroban in solid dosage forms and is also desirable for online monitoring for process control when lots of ten or more samples are to be assayed as the run time is 3 minutes.

**CONCLUSIONS**

A trial has been attempted to develop simple, accurate, precise, rapid and economical UPLC method for estimation of Ifetroban. The drug showed good linearity over concentrations ranging from 100 to 300 μg/ml with co-efficient of correlation, \( r^2 \) = 0.998. The mean recovery of the validation ranged between 100.43 - 100.57 %. The method is rugged and is trouble free and transferable (%RSD of NMT 2.0 from ruggedness studies).The method can be incorporated for the everyday analysis of the Ifetroban.

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**Conflict of Interest**

None.

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None.

**REFERENCES**