Development and validation of assay method for estimation of Apixaban in bulk drug and its marketed formulation

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ABSTRACT

The paper involves the development of a simple, precise and sensitive method for estimation of Apixaban in bulk drug and its marketed formulation using the reverse-phase liquid chromatographic method. The separation was achieved on C18 INERTSIL ODS-2 Column (250 mm×4.6 mm×5 um) using mobile phase (Buffer: ACN) in the ratio of 55:45 (v/v) with a run time of 15 minutes and wavelength for estimation of Apixaban was taken as 280 nm. Literature survey reveals that there are very few HPLC, UV methods were available Hence an attempt has been made to develop an RP-HPLC method for estimation of Apixaban. The developed method was validated for Linearity, Accuracy (% Recovery), Precision, LOD, and LOQ etc. the linearity was found to be in the range of 1-3 μg/ml with correlation coefficient found for linearity is 0.999. The developed and validated RP-HPLC method is applied for the identification of eluted.

Keywords— Apixaban, RP-HPLC-PDA, Validation

1. INTRODUCTION

Apixaban belongs to the anti-coagulant category of drug which acts by directly inhibiting the Factor Xa involved in the conversion of prothrombin to thrombin in the coagulation cascade. Thus helps in inhibiting the clot formation. Apixaban is used in the treatment of venous thrombosis and reduce the risk of stroke and systemic embolism. Literature survey reveals that there are very few HPLC, UV methods were available. Hence an attempt has been made to develop an RP-HPLC method for estimation of Apixaban.

IUPAC NAME: 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperdin-1-yl) phenyl] 1H,4H,5H,6H,7H pyrazolo[3,4-c]pyridine 3-carboxamide

The molecular formula for Apixaban is C25H25N5O4 having good solubility in water, Acetonitrile and the developed method is validated for various parameters such as linearity, Accuracy (% recovery), precision, LOD, and LOQ in accordance with ICH Q2 (R1) guidelines.

2. EXPERIMENTAL WORK

2.1 Materials and reagents

Apixaban standard was procured from Macleods, India as gift sample and Eliquis tablets (marketed formulation) was purchased from local market. HPLC grade water was procured from J.K. Labs Mumbai and other reagents such as Acetonitrile, Methanol, IPA was procured from Merck chemicals cooperation Ltd. Mumbai India.
2.2 Instrumentation

The estimation of Apixaban was carried using JASCO HPLC system with Jasco MD-2018 plus Intelligent PDA Detector using ChromNAV software as an integrator, Analytical Balance (Mettler Toledo), pH meter (Lab India) and a sonicator (Spectralab). The column used for separation of Apixaban is INERTSIL ODS-2 (250 mm×4.6 mm×5 um).

2.3 Preparation of mobile phase

Weigh accurately 0.164 gm of sodium acetate and transfer in 200 mL beaker. Add 200 mL of water, sonicate and adjust the pH to 4.5 with Glacial acetic acid. Mix buffer and Acetonitrile in the ratio of 55: 45(V/V) and sonicated for 15 minutes.

2.4 Preparation of standard solution

10 mg of working standard of Apixaban was accurately weighed and transferred to 10 ml of volumetric flask, add about 4 ml of the mobile phase, sonicate to dissolve and make up with mobile phase to give a stock solution of 1000 μg/ml (solution A). Solution A is further diluted to get the concentration of 2 μg/ml.

2.5 Preparation of sample solution (Marketed formulation)

20 Eliquis tablets containing Apixaban (2.5mg) were accurately weighed and the average weight of a tablet was found out. Then the 10 tablets were finely powdered and powder equivalent to 10 mg of Apixaban was taken and transferred into a 10 ml volumetric flask. 10 ml of diluent was added and sonicated with occasional shaking for a few minutes. Solution A is further diluted to get the concentration of 2 μg/ml.

2.6 Selection of detection wavelength

10 mg of Apixaban was accurately weighed and dissolved in 10 mL of Mobile Phase to give a concentration of 1000 μg/mL (solution A). Solution A is further diluted to get the concentration of 10 μg/ml. UV spectrum of a solution having a concentration of 10 μg/ml was recorded using Mobile Phase as blank. It shows good absorbance at a wavelength of 280 nm, hence it was selected as λmax for Apixaban.

2.7 Method development

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>SPECIFICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC Pump</td>
<td>Jasco PU-2089 Plus Quaternary Gradient HPLC Pump</td>
</tr>
<tr>
<td>HPLC Detector</td>
<td>Jasco MD-2018 Plus Intelligent PDA Detector</td>
</tr>
<tr>
<td>Integrator</td>
<td>ChromNAV Chromatogram software</td>
</tr>
<tr>
<td>Column</td>
<td>INERTSIL ODS-2 C18 Column 250 mm×4.6 mm×5 um</td>
</tr>
<tr>
<td>Wavelength</td>
<td>280 nm</td>
</tr>
<tr>
<td>Injection loop</td>
<td>10 μl</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>0.01M Sodium Acetate pH 4.5 : ACN (55:45)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 ml/min</td>
</tr>
</tbody>
</table>

2.8 Method validation

2.8.1 Linearity: The linearity of the method was studied by the injecting the standard solutions over the concentration range of 1 ppm-3 ppm drug respectively and the correlation coefficient range was found. Drug levels of target concentrations were prepared and injected six times into the HPLC system keeping the constant injection volume. The peak areas were plotted against the concentrations to obtain the linearity graphs.

2.8.2 Precision: The precision of the developed analytical method was tested by injecting three replicate injections of concentration 1.00 μg/ml, 2.00 μg/ml and 3.00 μg/ml (50%, 100% and 150% of the working level). Intraday and Interday precision study were carried out by estimating the corresponding responses for the solutions of above 3 concentration levels on the same day and on a different day respectively.

2.8.3 Accuracy: Accuracy was carried out by applying the method to Apixaban sample to which known amounts of Apixaban standard was added. Concentration corresponding to 50, 100 and 150% of the working level was added mixed and the amount of drug recovered is determined by the system using optimized chromatographic conditions. The experiment was performed in triplicate and percentage recovery was calculated.

2.8.4 Sensitivity: The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the Standard solution of Apixaban using the developed HPLC method. This was done until a signal to noise ratio of NLT 3:1 and NLT 10:1 is maintained for LOD and LOQ respectively.

2.8.5 Specificity: Specificity of the method was determined by recording the chromatogram of a standard stock solution of Apixaban (2.0 μg/ml) and blank chromatogram (only diluent). Specificity signifies the identification of the analyte, interference from other peaks.

2.8.6 System suitability: System suitability was assessed by six replicate analyses of standard Apixaban in 2.0 μg/ml, followed by estimation of %RSD of Peak area, retention time and theoretical plates.
3. RESULT AND DISCUSSION

3.1 Linearity

The linearity was determined by a series of six injections whose concentration span 50% to 150% i.e. 2.0 μg/mL of working level in six replicates. Calibration curve plotted with the response (area) on the y-axis and the concentration (in μg/mL) on the x-axis (fig no 1) and Correlation coefficient ($r^2$), y-intercept was calculated and it was found to be 0.999.

![Figure 1: Calibration curve of Apixaban](image)

3.2 Precision

The precision of this analysis, as the intraday precision was evaluated by injecting three individual test samples prepared & calculated the % RSD. Interday precision of this method was analyzed by performing the same the procedure with on a different day. The % RSD values of the intra-day precision & interday precision study were found to be < 2.0% for Apixaban. This is confirmed that the method is precise.

3.3 Accuracy

The recovery of Apixaban was determined at 3 concentration levels (50%-150% of working level). % recovery was found out. The results are indicating that this method was accurate. % recovery obtained is shown in table no 2.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Amount spiked</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apixaban</td>
<td>50%</td>
<td>100.1</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100.18</td>
</tr>
<tr>
<td></td>
<td>150%</td>
<td>101.6</td>
</tr>
</tbody>
</table>

3.4 Sensitivity

Then the LOD value for Apixaban was found to be 0.30 μg /mL & the LOQ value 0.60 μg/mL respectively.

3.5 Specificity

The method was quite selective for Apixaban as there was no other interfering peak around the retention time of Apixaban (Rt-5.2). Even the baseline did not show any significant peak. Thus the method was found to be highly specific for apixaban as shown in figure 2 and figure 3.

![Figure 2: Chromatogram for blank](image)

![Figure 3: Chromatogram of Apixaban Standard](image)
3.6 System suitability
The standard solution of 2.0μg/mL was injected in six replicates and mean of system suitability parameters were estimated and summarized in table 3.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>System suitability parameters</th>
<th>Observations</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard solution</td>
<td>2.0 ug/ml</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Area</td>
<td>88124</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Retention time</td>
<td>5.20</td>
<td>NTP 2000</td>
</tr>
<tr>
<td>4</td>
<td>NTP</td>
<td>2627</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Tailing factor</td>
<td>1.6</td>
<td>NMT 2</td>
</tr>
</tbody>
</table>

3.7 Application of method to marketed formulation
The developed method is applied successfully for the determination of Apixaban in marketed formulation and % assay was found to be 98.63%.

![Chromatogram of Apixaban in marketed formulation](image)

4. CONCLUSION
It can be concluded that developed reverse phase liquid chromatographic method was found to be simple, accurate, and precise and provides a convenient and reproducible approach for estimation of apixaban. The method could be successful to the marketed formulation of apixaban and can be used for routine analysis.

5. ACKNOWLEDGMENT
The authors are grateful to the Macleods Pvt. Ltd, Mumbai, India for providing API of apixaban as a gift sample and to the Institution for providing the facilities.

6. REFERENCES