SIMULTANEOUS RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF CLOPIDOGREL AND RIVAROXABAN IN SYNTHETIC MIXTURE

Arun M. Prajapati, Harsh A. Patel*
Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India

*Corresponding author e-mail: patelharsh25892@gmail.com

ABSTRACT

Present work described a precise, accurate and reproducible RP-HPLC method for simultaneous estimation of Clopidogrel and Rivaroxaban. The separation was carried by Kromasil C₁₈ column (250 × 4.6 mm, 5µ) with UV detection at 240 nm. The mobile phase consisting of acetonitrile and water in a ratio of (90:10, v/v) and at a flow rate of 1 mL/min. The method was linear over the concentration range for Clopidogrel 1.0 - 20.0 μg/mL and for Rivaroxaban 1.0 - 20.0 μg/mL. The method was validated and was successfully employed for the analysis of Clopidogrel in combination with Rivaroxaban synthetic combination. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Rivaroxaban, Clopidogrel, RP-HPLC, Recovery, Validation, ICH guidelines.

INTRODUCTION

Clopidogrel chemically known as methyl (2S)-2-(2-chlorophenyl)-2-[4H, 5H, 6H, 7H-thieno [3, 2-c] pyridine-5-y1] acetate (Figure 1). Clopidogrel is an Anti-platelet drug. Rivaroxaban is chemically known as (S)-5-Chlor-N-(2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1, 3-oxazolidin-5-ylmethyl] thiophen-2 carboxamid (Figure 2). Rivaroxaban is comes under Anticoagulant category: Direct, selective inhibitor of blood-coagulation factor Xa. Clopidogrel is official in Indian Pharmacopoeia [1], British Pharmacopoeia [2] and United States Pharmacopoeia [3]. Literature survey reveals that HPLC methods have been reported for the quantification of Rivaroxaban in pharmaceutical dosage forms. Only HPLC [11, 12] methods available for estimation of Rivaroxaban alone. Present study involves development of a convenient, rapid user and friendly reversed-phase (RP)-HPLC method with a simple and easily available mobile phase for quantitative estimation of Clopidogrel and Rivaroxaban in synthetic mixture. The optimized method was developed and validated as per International Conference on Harmonization (ICH) guidelines [13].

Figure 1: Structure of Clopidogrel

![Figure 1: Structure of Clopidogrel](image1)

Figure 2: Structure of Rivaroxaban

![Figure 2: Structure of Rivaroxaban](image2)
MATERIALS AND METHODS

Apparatus:
- RP-HPLC instrument equipped with an UV-Visible detector and a photodiode array detector (LC-2010CHT, Shimadzu, Japan), an auto-sampler, an LC-solution software.
- Analytical balance (Sartorius CP224S, Germany)
- A hot air oven (Grover, New Delhi, India)
- An UV cabinet (CAMAG, Ancrom, Mumbai)
- Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad)
- Corning volumetric flasks
- Ultra sonic cleaner (Frontline FS 4, Mumbai)

Reagents and Materials:
- Clopidogrel and Rivaroxaban standard powder was kindly supplied as a gift sample from Torrent Research centre, Ahmedabad.
- Methanol (HPLC & Spectroscopy grade, Finar Chemicals Limited, Ahmedabad)
- Acetonitrile (HPLC & Spectroscopy grade, Finar Chemicals Limited, Ahmedabad)
- Water (HPLC & Spectroscopy grade, Finar Chemicals Limited, Ahmedabad)
- Nylon 0.45 μm 47 mm membrane filter (Gelman Laboratory, Mumbai)
- Whatman filter paper no. 41. (Whatman International Ltd., England)

Preparation of Solutions:
Preparation of standard stock solutions of Clopidogrel: Accurately weighed Clopidogrel (10 mg) was transferred to a 100 ml volumetric flask and dissolved and diluted up to 100 ml with methanol to obtain a standard stock solution (100 μg/ml).

Preparation of standard stock solutions of Rivaroxaban: Accurately weighed Rivaroxaban (10 mg) was transferred to a 100 ml volumetric flask and dissolved and diluted up to 100 ml with methanol to obtain a standard stock solution (100 μg/ml). The elution of Clopidogreal and Rivaroxaban was obtained by running HPLC in gradient mode using Acetonitrile and Water in a ratio of 90:10 v/v, flow rate was maintained at 1.0 mL/min with run time of 8 min. The retention time for Clopidogrel was obtained at 5.9 min and Rivaroxaban was obtained at 2.7 min detection was performed at 240 nm (figure 3). Mobile phase was previously filtered through Whatman filter paper no 41.

Figure 3: Chromatogram of Test solution at 240 nm

Method Validation: The method was validated in compliance with ICH guidelines.

Linearity: A stock solution of Clopidogrel and Rivaroxaban (100 μg/ml) was prepared individually with methanol. From it various working standard solutions were prepared in the range from 1-20 μg/ml and injected in to system. The calibration plot (peak area of Clopidogrel vs. concentration of Clopidogrel and same as for Rivaroxaban) was generated by replicate analysis (n=5).

Repeatability: The precision of the instrument was checked by repeatedly injecting six standard solutions of Clopidogrel (10 μg/ml) and Rivaroxaban (10 μg/ml) under the same chromatographic conditions. Peak area, retention time and tailing factor were measured. Percentage relative standard deviation (%RSD) should not be more than 2%.

Intermediate Precision: Intermediate precision of the method was determined by performing interday variation and intraday variation in terms of %RSD.
Intraday precision was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Interday precision was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

**Limit of Detection and Limit of Quantification:**
The limit of detection (LOD) and the limit of quantification (LOQ) for the proposed method was calculated using the following equations as per ICH guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S} \quad \text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, \(\sigma\) = the standard deviation of the response and \(S\) = slope of the calibration curve

**Accuracy:** To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (50%, 100% and 150%). A known amount of standard solution was added to pre-analyzed sample solution and percentage recoveries were calculated.

**Analysis of Clopidogrel and Rivaroxaban from synthetic mixture:** Clopidogrel (75 mg) and Rivaroxaban (15 mg) standard drug powder were accurately weighed and then mixed with commonly used formulation excipients like Micro crystalline cellulose, lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100 ml volumetric flask containing 50 ml methanol and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. The above solution was diluted with methanol to obtain our desired concentration. This desired concentration solution inject in HPLC instrument and check out the results at the detection wavelength 240 nm for both drug in synthetic mixture (Table 1).

<table>
<thead>
<tr>
<th>Synthetic mixture</th>
<th>Label Claim (mg)</th>
<th>Amount found (mg)</th>
<th>% Label claim ± %SD (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clopidogrel</td>
<td>75</td>
<td>75.92</td>
<td>101.4±0.46</td>
</tr>
<tr>
<td>Rivaroxaban</td>
<td>15</td>
<td>15.24</td>
<td>101.44±0.50</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. Satisfactory results for Clopidogrel and Rivaroxaban were obtained with a mobile phase comprising of Acetonitrile: water (90: 10, v/v) with a flow rate of 1.0 mL/min to get better reproducibility and repeatability. Quantification was achieved with help of Photo Diode Array detector and detection at 240 nm based on peak area. The retention time for Clopidogrel and Rivaroxaban was found to be 5.9 min and 2.7 min respectively (Figure 3). Linear correlation was obtained between peak areas of Clopidogrel vs. concentrations of Clopidogrel in the concentration ranges of 1-20 µg/ml and linear correlation was obtained between peak areas of Rivaroxaban vs. concentrations of Rivaroxaban in the concentration ranges of 1-20 µg/ml. The mean recovery obtained was 100.25±0.47% and 100.47±0.96% for Clopidogrel and Rivaroxaban, respectively which indicates accuracy of the proposed method. The proposed method was validated as per ICH guidelines. The %RSD value for Clopidogrel and Rivaroxaban was found to be <2%, which indicates that the proposed method is repeatable. The low %RSD values of interday and intraday variations for Clopidogrel and Rivaroxaban revealed that the proposed method is precise. The proposed method was successfully applied for the estimation of Clopidogrel and Rivaroxaban in synthetic mixture. The assay results were found 101.4±0.46 for Clopidogrel and 101.44±0.50 for Rivaroxaban (Table 2). LOD and LOQ values for Clopidogrel were found to be 0.32 µg/ml and 0.99 µg/ml, respectively and LOD and LOQ values for Rivaroxaban were found to be 0.07 µg/ml and 0.22 µg/ml, respectively (Table 2).These data show that the proposed method is sensitive for the determination of Clopidogrel and Rivaroxaban.
Table 2: Regression analysis data and summary of validation parameters for Clopidogrel and Rivaroxaban

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Clopidogrel</th>
<th>Rivaroxaban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range</td>
<td>1-20</td>
<td>1-20</td>
</tr>
<tr>
<td>Slope</td>
<td>23029.66</td>
<td>63541.66</td>
</tr>
<tr>
<td>Intercept</td>
<td>2289.66</td>
<td>23447.67</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.998</td>
<td>0.999</td>
</tr>
<tr>
<td>LOD</td>
<td>0.32</td>
<td>0.07</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.99</td>
<td>0.22</td>
</tr>
<tr>
<td>Repeatability (%RSD, n=6)</td>
<td>0.24</td>
<td>0.16</td>
</tr>
<tr>
<td>Precision (%RSD, n=3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraday</td>
<td>0.08-1.51</td>
<td>0.10-0.34</td>
</tr>
<tr>
<td>Inter-day</td>
<td>0.20-1.52</td>
<td>0.10-1.74</td>
</tr>
<tr>
<td>Accuracy± S.D</td>
<td>100.25±0.47</td>
<td>100.37±0.96</td>
</tr>
<tr>
<td>Assay± S.D</td>
<td>101.4±0.46</td>
<td>101.44±0.50</td>
</tr>
</tbody>
</table>

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