Original Article

Method Development and Its Validation for Simultaneous Estimation of Ramipril & Clopidogrel by RP-HPLC in Combination Tablet Dosage Form

Koduru Swathi *, Mitta Chaitanya, Kalepu Swathi

Department of Pharmaceutical Analysis, Bojjam Narsimhulu Pharmacy College for Women, Hyderabad- 500088, India

ARTICLE INFO

Received: 22 Mar 2015
Accepted: 18 Apr 2015

A selective and sensitive stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of Ramipril & Clopidogrel. The λmax of the two ingredients i.e. Ramipril & Clopidogrel, were found to be 210 nm and 225 nm respectively in methanol as solvent system. Accurately weighed 100 mg of Ramipril and 100 mg of Clopidogrel were transferred to 100 ml volumetric flask. About 40 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 10 ml of the above solution was diluted to 100 ml with the solvent system. Mobile phase was prepared by taking Potassium dihydrogen phosphate buffer + Dipotassium hydrogen phosphate (0.01 M, pH 3.0): acetonitrile (30:70). Mobile phase was filtered through 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min. The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Clopidogrel and Ramipril for 15 min. The retention time were found to be 2.03 min and 9.93 min respectively.

Keywords: Ramipril & Clopidogrel, RP-HPLC, Acetonitrile (30:70), Retention time.

1. INTRODUCTION

The oral drug delivery has been known for decades as the most widely utilized route of administration among all the routes that have been employed for the systemic delivery of drug via various pharmaceutical products of
different dosage forms. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration and the belief that oral administration of the drug is well absorbed. Ramipril (Altace) is an ACE inhibitor. ACE stands for angiotensin converting enzyme. Ramipril is used to treat high blood pressure (hypertension) or congestive heart failure, and to improve survival after a heart attack.

Ramipril (Altace) is an ACE inhibitor. ACE stands for angiotensin converting enzyme. Ramipril is used to treat high blood pressure (hypertension) or congestive heart failure, and to improve survival after a heart attack.

**Clopidogrel** (INN) is an oral, thienopyridine-class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, cerebrovascular disease, and to prevent myocardial infarction (heart attack). It is marketed by Bristol-Myers Squibb and Sanofi under the trade name Plavix. The drug works by irreversibly inhibiting a receptor called P2Y12, an adenosine diphosphate (ADP) chemoreceptor on platelet cell membranes.

### 2. EXPERIMENTAL WORK

#### 2.1 Method Development

*Standard & sample preparation for UV-spectrophotometer analysis:*

Selection of wavelength

The $\lambda_{max}$ of the two ingredients i.e. Ramipril & Clopidogrel, were found to be 210 nm and 225 nm respectively in methanol as solvent system. The isobestic point for the drugs were found at 225 nm.

Preparation of standard solution of Ramipril 10 mg of Ramipril was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100 g/ml of Ramipril. Preparation of standard solution of Clopidogrel by 10 mg of Clopidogrel was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100 g/ml of Clopidogrel. Preparation of mix. standard solution of Ramipril & Clopidogrel.

Accurately weighed 100 mg of Ramipril and 100 mg of Clopidogrel were transferred to 100 ml volumetric flask. About 40 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 10 ml of the above solution was diluted to 100 ml with the solvent system. The resultant solution was filtered through a 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. From the above standard solution several working standard solutions are prepared by serial dilution technique.
The HPLC instrument was switched on. The column was washed with HPLC water for 45 minutes. The column was then saturated with mobile phase for 45 minute. The mobile phase was run to find the peaks. After 20 minutes the standard drug solution was injected in HPLC.

2.3 Different chromatographic conditions used and their Optimizations

The different HPLC chromatographic conditions were used to find out the optimum chromatographic condition for best elution of drugs.

Table 1: Chromatographic condition

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Potassium dihydrogen phosphate +Dipotassium hydrogryn phosphate buffer (0.01 M, pH 3.0): acetonitrile (30:70)</th>
</tr>
</thead>
</table>

| Wavelength   | 225 nm                                                                                                   |
| Flow rate    | 1.0 ml/ min.                                                                                               |
| Run time     | 15 min.                                                                                                    |
| Column       | Develosil ODS HG-5 RP C18, 5μm, 15cmx4.6mm i.d.                                                        |

2.4 Preparation of mobile phase

Mobile phase was prepared by taking Potassium dihydrogen phosphate buffer+Dipotassium hydrogryn phosphate (0.01 M, pH 3.0): acetonitrile (30:70). Mobile phase was filtered through 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

2.5 Running the standard solution of Clopidogrel

2 ml of stock solution was pipetted out into a 10 ml volumetric flask. The volume was made up to the mark with methanol. The solution was filtered through the 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 27.

2.6 Running the standard solution of Ramipril

2 ml of stock solution was pipetted into a 10 ml volumetric flask. The volume was made up to the mark with methanol. The solution was filtered through the 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 27.

Fig 2: The chromatogram obtained after condition , typical chromatogram of Ramipril (rt=9.93 min) and Clopidogrel (rt=2.02 min).

Here resolution was good, theoretical plate count and symmetry was appropriate. Also no unwanted little peaks were seen between two peaks. Hence it was acceptable. The selected and optimized mobile phase was Potassium dihydrogen phosphate buffer+Dipotassium hydrogryn phosphate (0.01 M, pH 3.0): acetonitrile (30:70) Run time was 15 min. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs.

Fig 3: Chromatogram of Clopidogrel

Retention time was found to be 2.01 min.

Fig 4: Chromatogram of Ramipril

Retention time was found to be 9.83 min.
3. RESULT & DISCUSSION

The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Clopidogrel and Ramipril for 15 min. The retention time were found to be 2.03 min and 9.93 min respectively.

3.1 Method Validation

Preparation and running of synthetic mixture of Clopidogrel and Ramipril

For the specificity of the method the marketed formulations has been taken & the solution was injected into the HPLC system. The chromatograms obtained are shown in figure 5.

3.2 Linearity and Range

Method: for linearity various concentrations like 10, 20, 30, 40, 50 of clopidogrel & ramipril were prepared in mixture & then injected into HPLC.

Linearity range was found to be 0-50 µg/ml for Clopidogrel and 0-50 µg/ml for Ramipril. The correlation coefficients were found to be 0.999 & 0.997, the slopes were found to be 44623 & 13801 and intercept were found to be 10569 & 10378 for Clopidogrel and Ramipril respectively.

3.3 Accuracy: Clopidogrel

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Clopidogrel were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table-8.

Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Ramipril were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table-3.
Table 4: Percentage Recovery

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Concentration (µg/ml)</th>
<th>% Recovery of Pure drug</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>S&lt;sub&gt;1&lt;/sub&gt;</td>
<td>80 %</td>
<td>99.63</td>
<td>Mean = 99.67667%</td>
</tr>
<tr>
<td>S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>80 %</td>
<td>99.92</td>
<td>S.D. = 0.223681</td>
</tr>
<tr>
<td>S&lt;sub&gt;3&lt;/sub&gt;</td>
<td>80 %</td>
<td>99.48</td>
<td>% R.S.D. = 0.224407</td>
</tr>
<tr>
<td>S&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100 %</td>
<td>99.19</td>
<td>Mean = 99.19%</td>
</tr>
<tr>
<td>S&lt;sub&gt;5&lt;/sub&gt;</td>
<td>100 %</td>
<td>99.25</td>
<td>S.D. = 0.06</td>
</tr>
<tr>
<td>S&lt;sub&gt;6&lt;/sub&gt;</td>
<td>100 %</td>
<td>99.13</td>
<td>% R.S.D. = 0.06049</td>
</tr>
<tr>
<td>S&lt;sub&gt;7&lt;/sub&gt;</td>
<td>120 %</td>
<td>99.25</td>
<td>Mean = 99.49%</td>
</tr>
<tr>
<td>S&lt;sub&gt;8&lt;/sub&gt;</td>
<td>120 %</td>
<td>99.54</td>
<td>S.D. = 0.219317</td>
</tr>
<tr>
<td>S&lt;sub&gt;9&lt;/sub&gt;</td>
<td>120 %</td>
<td>99.68</td>
<td>% R.S.D. = 0.220441</td>
</tr>
</tbody>
</table>

The mean recoveries were found to be 99.67, 99.19, 99.49 % for Clopidogrel and 99.92, 100.72, 100.40% for Ramipril. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

3.4 Precision: Repeatability

The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug, Ramipril & Clopidogrel.

Table 5: The percent relative standard deviations

<table>
<thead>
<tr>
<th>HPLC Injection Replicates of Clopidogrel</th>
<th>Retention Time</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate – 1</td>
<td>2.02</td>
<td>1302869</td>
</tr>
<tr>
<td>Replicate – 2</td>
<td>2.02</td>
<td>1302586</td>
</tr>
<tr>
<td>Replicate – 3</td>
<td>2.02</td>
<td>1318521</td>
</tr>
<tr>
<td>Replicate – 4</td>
<td>2.01</td>
<td>1302569</td>
</tr>
<tr>
<td>Replicate – 5</td>
<td>2.02</td>
<td>1302896</td>
</tr>
<tr>
<td>Average</td>
<td>2.018</td>
<td>1305888</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.004472</td>
<td>7063.605</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.221612</td>
<td>0.540904</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The LOD was found to be 0.02 µg/ml and 0.06 µg/ml and LOQ was found to be 0.04 µg/ml and 1.2 µg/ml for Clopidogrel and Ramipril respectively which represents that sensitivity of the method is high.

5. REFERENCES

5. Validating Chromatographic Methods, David M.Bliesner, 1-4.

Conflict of Interest: None

Source of Funding: Nil