RESEARCH ARTICLE


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ABSTRACT:
Present work describes a precise, accurate and reproducible Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method for simultaneous estimation of Ramipril and Amlodipine in tablet dosage form on Phenomenex C18 column (245 mm × 4.5 mm, 5 µm, particle size) using acetonitrile: phosphate buffer, (60:40 v/v) as mobile phase at flow rate of 1.0 ml/min and the detection wavelength was 222 nm. The retention time for Ramipril and Amlodipine was found to be 3.41 and 6.10 min respectively. Proposed method was validated for precision, accuracy, linearity range, robustness and ruggedness.

KEYWORDS: Amlodipine, Ramipril, RP-HPLC

INTRODUCTION:
Amlodipine chemically (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. Long-acting calcium channel blocker used as an anti-hypertensive and in the treatment of angina1. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance and hence reducing blood pressure, in angina it increases blood flow to the heart muscle. Literature survey reveals that few analytical methods were reported on Ramipril and in combination with other compounds viz. Spectroscopically2,3,4, HPTLC5 and HPLC6,7.

Ramipril (2S, 3aS, 6aS)-1-[(2S)-2-[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino] propanoyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid, is an angiotensin-converting enzyme (ACE) inhibitor. It acts on the renin-angiotensin aldosterone system. It inhibits the conversion of the inactive angiotensin I to the highly potent vasoconstrictor, angiotensin II, and also reduces the degradation of bradykinin8. Various analytical methods have been reported for estimation of Amlodipine in pure form and in combination with other compounds viz. Spectroscopically9,10 and HPLC11,12.

In this study the RP-HPLC method is developed for the determination of Amlodipine and Ramipril in tablet dosage form. Obtained results were statistically compared because this method is used for assay of Amlodipine and Ramipril in pharmaceutical sciences.

MATERIAL AND METHOD:
Apparatus:
Instrument used in present study was Shimadzu UFLC LC-20 AD Liquid chromatograph system having Shimadzu SPD-20 A UV/VIS Detector having SSQD (Slow suction quick delivery) Pumping method.

Materials:
Ramipril reference standard and Amlodipine reference standard were kindly provided by Lupin Pharmaceuticals Ltd, Pune. The pharmaceutical preparations of combination of Ramipril and Amlodipine that is CARDACE AM were obtained from local market.

Acetonitrile (RANKEM Ltd.), Water (RFCL Ltd New Delhi), Ortho-phosphoric acid (RANKEM Ltd.) were used of HPLC grade.

Chromatographic Condition:
The mobile phase containing acetonitrile: potassium dihydrogen phosphate buffer (0.05 M, pH 4.0) (60:40 v/v) was found to resolve Ramipril and Amlodipine. Orthophosphoric acid was used for pH adjustment of buffer. The mobile phase was filtered on a 0.45 micron membrane filter and then ultrasonicated for 15 min. The flow rate was set to 1.0 ml/min. Both drugs shows good absorbance at 222
nm, which was selected as wavelength for further analysis. All determinations were performed at ambient column temperature.

**Preparation of Standard Solutions:**
Standard stock solutions containing Ramipril and Amlodipine were prepared individually by dissolving 25 mg of Ramipril and quantity of AB equivalent to Amlodipine base 50 mg separately in 80 ml of methanol. It was then sonicated for 10 minutes and the final volumes of both the solutions were made up to 100 ml with methanol. Further dilutions were made to get the final concentration of 25µg/ml of Ramipril and 50µg/ml of Amlodipine.

**Preparation of Sample Solutions:**
A total of 20 tablets were accurately weighted and triturated with glass mortar and pestle. Weigh accurately powder sample equivalent to 2.5 mg Ramipril and 5.0 mg of Amlodipine into 100 ml volumetric flask. Add sufficient mobile phase, sonicate, cool and make up volume up to mark with mobile phase.

**Method Development:**
The Method for the estimation of ramipril and Amlodipine is developed using different mobile phases at different pH. The mobile phase acetonitrile: potassium dihydrogen phosphate buffer (0.05 M, pH 4.0) (60:40 v/v) was found to be ideal mobile phase for determination of Ramipril and Amlodipine at ambient temperature shown in fig 1 and fig 2.

**RESULT AND DISCUSSION:**
To develop a precise, accurate and suitable RP- HPLC method for the simultaneous estimation of Ramipril and Amlodipine, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The results obtained by the assay of marketed formulation are summarized in Table 1.

<table>
<thead>
<tr>
<th>Marketed Formulation</th>
<th>Drug</th>
<th>% Amount found</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARDACE AM</td>
<td>Ramipril</td>
<td>99.77</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Amlodipine</td>
<td>100.56</td>
<td>0.65</td>
</tr>
</tbody>
</table>

**Method Validation:**
The proposed HPLC method was validated as per ICHguidelines13.

**System suitability:**
The system suitability of Ramipril and Amlodipine were assessed by comparing the Theoretical plates, Asymmetry, % RSD for Area, % RSD for Retention time of standard Ramipril and Amlodipine. Results are shown in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ramipril</th>
<th>Amlodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates</td>
<td>4281.21</td>
<td>7627.16</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>1.34</td>
<td>1.25</td>
</tr>
<tr>
<td>% RSD for Area</td>
<td>0.77</td>
<td>0.65</td>
</tr>
<tr>
<td>% RSD for Retention time</td>
<td>0.40</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Linearity:**
Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Ramipril and Amlodipine 05-45 µg/ml and 10-90 µg/ml respectively. The regression equation for Ramipril and Amlodipine were found to be y = 37042x + 28835 and y = 40154x + 16156 with coefficient of correlation, (r) 0.999 and 0.999, respectively. The overlay of linearity peaks shown in fig no.3. Linearity curve for Ramipril and Amlodipine are shown in fig no. 4 and fig no. 5 respectively.

![Fig 1: Chromatogram of Working Standards](image1)

![Fig 2: Chromatogram of sample](image2)

![Fig 3: Overlay of linearity peaks](image3)
Intermediate Precision:
Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for analyst variations, which proves that method is precise. Results are shown in Table 3.

Recovery studies:
To check the degree of accuracy of the method, recovery studies were performed at 50%, 100% and 150% levels. Known amounts of standard Ramipril and Amlodipine were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table 4.

RESULT AND DISCUSSION:
The method describes procedure employing a Phenomenex C₁₈ column (245 mm × 4.5 mm, 5 µm, particle size) and mobile phase comprising of acetonitrile: Buffer (60:40) for separation of Ramipril and Amlodipine. Mobile phase containing acetonitrile-Buffer at various ratios gave tailing effect and Ortho- phosphoric acid was used as organic modifier to overcome this problem. Flow rate of 0.5 ml/min resulted in greater retention times and broader peaks while 1.2 ml/min resulted in very close retention times with poor resolution. A flow rate of 1 ml/min resulted in optimum retention times with good resolution, good peak shape and with all drug components eluting within 10 minutes.

The sampling wavelength was selected after scanning the drug solutions in the mobile phase and resulting UV spectra shows 222 nm was suitable wavelength of estimation. The concentration of Ramipril and Amlodipine is Linear over a range of 0.5-45 µg/ml and 10-90 µg/ml respectively, was found sufficient for obeying Beer’s law.

The limit of detection was found to be 4.45 µg/ml and 4.52 µg/ml for Ramipril and Amlodipine respectively, and limit of quantitation 13.51 µg/ml and 13.70 µg/ml for Ramipril and Amlodipine respectively.

The value of percent recovery and standard deviation shows that proposed method was reproducible, accurate and precise.

CONCLUSION:
The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous
determination of Ramipril and Amlodipine in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement.

REFERENCES:
1. The Merck Index, 14th ed., 2006, p. 493