Method Development and Validation for the Simultaneous Estimation of Rosuvastatin and Amlodipine in Bulk and its Formulation using Reverse-Phase High-Performance Liquid Chromatography

Narender Boggula, Gowri Manoja Mulagada, D. K. Shanthi Priya, Vasudha Bakshi, Himabindu Peddapalli

Department of Pharmaceutical Chemistry, School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar, Telangana, India

Abstract

Background: High-performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres which makes it much faster. All chromatographic separations including HPLC is based upon the resolution of the sample constituents as per the difference in their relative affinities towards stationary phase and mobile phase used. Aim: A simple, specific, accurate, and precise reverse-phase HPLC method was developed and validated for the estimation of rosuvastatin and amlodipine (Rosudapin) in pharmaceutical dosage form. Materials and Methods: An Aquasil column reversed phase C-18, 5 μm column having 4.6 mm × 250 mm i.d. in gradient mode, with mobile phase containing HPLC grade acetonitrile:phosphate buffer (pH 3.8):methanol in proportion 30:60:10 v/v, was used. The flow rate was 1 ml/min and effluents were monitored at 251 nm using PDA detector. Linearity was observed over a range of 5–25 μg/mL of rosuvastatin and 2.5–12.5 μg/mL of amlodipine, respectively. Results and Discussion: The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation, robustness, and ruggedness. The limit of detection and estimation of analytes was found to be 3.1 µg/ml and 2.98 µg/ml, and the limit of quantification of analytes was found to be 102 µg/ml and 9 µg/ml, respectively, for rosuvastatin and amlodipine. Conclusion: The proposed method was successfully applied for the quantitative determination of rosuvastatin and amlodipine in pharmaceutical dosage form.

Key words: Amlodipine, precision, reverse-phase high-performance liquid chromatography, rosudapin, rosuvastatin, simultaneous estimation

INTRODUCTION

Rosuvastatin belongs to the class of statins (antilipidemic). It is chemically (3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxy hept-6-enoic acid [Figure 1] and is available in its calcium form, commonly called as rosuvastatin calcium. Rosuvastatin is a hydroxymethylglutaryl-CoA reductase inhibitor or it reduces plasma concentrations of LDL-cholesterol, apolipoprotein B, and triglycerides and prevents cardiovascular disease. Rosuvastatin is commonly used as a statin with mild, asymptomatic, and

Address for correspondence:
Himabindu Peddapalli, Department of Pharmaceutics, School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar, Telangana – 500088, India.
Phone: +91-9989377400.
E-mail: bindu.sweety369@gmail.com

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self-limited serum aminotransferase elevations during therapy.

Amlodipine [Figure 2] is used to treat high blood pressure and coronary artery disease such as chronic stable angina. It is a long-acting 1,4-dihydropyridine calcium channel blocker. It is chemically (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. Amlodipine decreases arterial smooth muscle contractions and inhibits the influx of calcium ions through L-type calcium channels. Inhibition of the initial influx of calcium decreases the contractile activity of arterial smooth muscle results in vasodilation.

A combination of rosuvastatin and amlodipine (Rosudapin) is given in adults for the treatment of increased blood pressure and with high cholesterol level, when changing diet and doing more exercise were not enough to prevent cardiovascular events.

A detailed survey of the analytical literature for rosuvastatin and amlodipine revealed few methods based on a number of techniques such as ultraviolet-spectrometry and HPLC methods. Since a HPLC method has many advantages, it is often the first choice for developing an analytical. Conformation of the applicability of the developed method was validated according to the ICH guidelines.

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**MATERIALS AND METHODS**

**Instruments**

A water module equipped with autosampler and PDA detector (996 model) 2695 separation module for finding out the $\lambda_{max}$ values of the drug was used throughout this study. An Aquasil C18 (4.6 mm × 250 mm, 5 µm) (Make: Thermo Scientific) column was employed for the method development. The chromatographic system was monitored by EMPOWER2 software. The digital ultrasonicator and pH meter were from Enertech and Lab India, respectively.

**Chemicals**

HPLC grade acetonitrile and orthophosphoric acid were obtained from Merck India Ltd., Mumbai, India. Analytical grade methanol was obtained from Lichrosolv (Merck India Ltd.) and 0.45 µm membrane filter was obtained from Pall Life Sciences, Mumbai, India. High purity deionized water was obtained from a Milli-Q (Milipore, Milford, MA, USA) purification system. Anhydrous dihydrogen phosphate and citric acid were from Finar Chemicals.

**Preparation of phosphate buffer (pH - 3.8)**

About 0.9 g of anhydrous dihydrogen phosphate and 1.298 g of citric acid monohydrate were dissolved in sufficient water to produce 1000 mL with pH adjusted to 3.8 using orthophosphoric acid.

**Preparation of mobile phase**

Nearly 300 ml (30%) of HPLC acetonitrile, 600 ml of phosphate buffer (3.8) (60%), and 100 ml HPLC Methanol (10%) were mixed and degassed in a digital ultrasonicator for 10 min and then filtered through 0.45 µ filter under vacuum filtration.

**Diluent preparation**

The mobile phase was used as the diluent.

**Preparation of standard solution**

About 10 mg of rosuvastatin and 10 mg of amlodipine working standard wereee accurately weighed and transferred into 10 mL volumetric flask each. 10 ml of diluents were added, sonicated, and diluted with diluent up to the mark. Further pipette 0.15 ml and 0.075 ml of the above rosuvastatin and amlodipine stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents to form 15 µg/ml of rosuvastatin and 7.5 µg/ml of amlodipine solutions.
Preparation of Sample Solution

10 combination tablets were crushed and powder weight equivalent to 10 mg of Rosuvastatin and Amlodipine was weighed and added to 10 mL clean dry volumetric flask. Make up this with 10 mL of diluent and sonicate to dissolve it completely. Further pipette 0.15 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents to form 15 µg/ml of sample solution.

Optimized Chromatographic Conditions

Separation was achieved using a mobile phase consist of acetonitrile:phosphate buffer (pH 3.8):methanol in proportion 30:60:10 v/v, respectively, at a flow rate of 1 ml/min. The eluent was monitored using PDA detector at a wavelength of 251 nm. The column was maintained at ambient temperature, and an injection volume of 20 µl was used. The run time was 8 min. The mobile phase was filtered through 0.45 µm filter before use. Solubility of the compounds was enhanced by sonication on an ultrasonicator (Bandelin Sonorex).

RESULTS AND DISCUSSION

Validation

Before validation studies, blank solution was injected and chromatogram was noted [Figure 3]. Optimized conditions were maintained with good retention time and resolution which were shown in Figure 4.

Linearity

The linearity of the method was established by determining the absorbance of different concentrations over a range of 5-25 µg/mL of Rosuvastatin and 2.5-12.5 µg/mL of Amlodipine respectively. The calibration curve of Rosuvastatin and Amlodipine were given in Figures 5 and 6 respectively. The linearity data was given in Table 1.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out and percentage recovery and standard deviation are calculated and represented in Tables 2 and 3. Each sample was injected thrice each.

<table>
<thead>
<tr>
<th>Concentration of rosvastatin (µg/mL)</th>
<th>Peak area</th>
<th>Concentration of amlodipine (µg/mL)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1,692,344</td>
<td>2.5</td>
<td>927,035</td>
</tr>
<tr>
<td>10</td>
<td>3,214,138</td>
<td>5</td>
<td>1,706,996</td>
</tr>
<tr>
<td>15</td>
<td>479,1958</td>
<td>7.5</td>
<td>2,582,231</td>
</tr>
<tr>
<td>20</td>
<td>6,385,532</td>
<td>10</td>
<td>347,0152</td>
</tr>
<tr>
<td>25</td>
<td>7,730,420</td>
<td>12.5</td>
<td>4,180,508</td>
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</table>

<table>
<thead>
<tr>
<th>Accuracy level (%)</th>
<th>Peak area</th>
<th>Average peak area</th>
<th>Average % recovery</th>
<th>Standard deviation</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2,629,787</td>
<td>2,630,409</td>
<td>98.0%</td>
<td>10905.83</td>
<td>0.415</td>
</tr>
<tr>
<td>50</td>
<td>2,641,613</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>261,9828</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>5,283,037</td>
<td>5,277,055</td>
<td>99%</td>
<td>8566.092</td>
<td>0.162</td>
</tr>
<tr>
<td>100</td>
<td>5,267,242</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>5,280,886</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>7,524,348</td>
<td>7,514,836</td>
<td>100.6%</td>
<td>8276.242</td>
<td>0.110</td>
</tr>
<tr>
<td>150</td>
<td>7,509,284</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>7,510,875</td>
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</tr>
</tbody>
</table>

RSD: Relative standard deviation
Table 3: Results of accuracy data for amlodipine

<table>
<thead>
<tr>
<th>Accuracy level</th>
<th>Peak area</th>
<th>Average peak area</th>
<th>Average % recovery</th>
<th>Standard deviation</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>1,365,757</td>
<td>1,366,666</td>
<td>98%</td>
<td>6960.626</td>
<td>0.51</td>
</tr>
<tr>
<td>50%</td>
<td>1,374,036</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>1,360,204</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>2,782,810</td>
<td>2,777,487</td>
<td>100%</td>
<td>19844.78</td>
<td>0.714</td>
</tr>
<tr>
<td>100%</td>
<td>2,794,128</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>100%</td>
<td>2,755,524</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>4,156,891</td>
<td>4,151,220</td>
<td>99%</td>
<td>7650.882</td>
<td>0.184</td>
</tr>
<tr>
<td>150%</td>
<td>4,142,518</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>4,154,251</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RSD: Relative standard deviation

Table 4: Results of precision data

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Parameters</th>
<th>System precision</th>
<th>Method precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosuvastatin</td>
<td>Mean peak area</td>
<td>5,680,917</td>
<td>5,257,650</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>22699.72</td>
<td>45206.32</td>
</tr>
<tr>
<td></td>
<td>% RSD</td>
<td>0.39</td>
<td>0.85</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>Mean peak area</td>
<td>2,626,428</td>
<td>2,774,987</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>5215.789</td>
<td>22806.64</td>
</tr>
<tr>
<td></td>
<td>% RSD</td>
<td>0.198</td>
<td>0.82</td>
</tr>
</tbody>
</table>

RSD: Relative standard deviation

Table 5: Results of LOD and LOQ data

<table>
<thead>
<tr>
<th>Analytes</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosuvastatin</td>
<td>3.1</td>
<td>10.2</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>2.98</td>
<td>9.8</td>
</tr>
</tbody>
</table>

LOQ: Limit of quantification, LOD: Limit of detection

Figure 4: Optimized chromatogram of rosuvastatin and amlodipine

**Precision**

The precision of the method was demonstrated by method precision and system precision. Five replicate injections of the sample solutions were made, and the percentage relative standard deviation was calculated and is represented in Table 4.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

The values for LOD and LOQ are given in Table 5.

**Robustness**

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical
parameters like flow rate, mobile phase composition which may differ but the responses were still within the limits of the assay. The results of Robustness data are given in Table 6.

**ASSAY**

The commercial combination tablets (Rosudapin – 10 mg Amlodipine and 5 mg of Rosuvastatin) were analyzed by the proposed method. The assay procedure was performed and the assay percentage was calculated as per the given formula.[18] The value was found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of the analytes, Rosuvastatin and Amlodipine, in pharmaceutical dosage forms.

**Formulae:**

\[
\text{Assay } \% = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{AV}}{\text{LC}} \times 100
\]

Where

- \(\text{AT}\) = Average area counts of sample preparation.
- \(\text{AS}\) = Average area counts of standard preparation.
- \(\text{WS}\) = Weight of working standard taken in mg.
- \(\text{DS}\) = Weight of sample taken in mg.
- \(\text{DF}\) = Dilution factor.
- \(\text{WT}\) = Average weight.
- \(\text{P}\) = Percentage purity of working standard.

The assay results were given in Table 7 and the chromatogram of assay sample is given in Figure 7.

**CONCLUSION**

The proposed reverse-phase HPLC method is sensitive and accurate and can be used for routine quality control analysis for the determination of rosuvastatin and amlodipine in its tablet dosage form. It can be seen from the results presented that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations.

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CONFLICTS OF INTEREST

Authors declare that there are no conflicts of interest to disclose.

REFERENCES


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