ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF AMLODIPINE AND ATORVASTATIN BY RP-UPLC

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ABSTRACT

Objective: To develop and validate simple, sensitive, robust, rapid and specific isocratic RP-UPLC method for simultaneous estimation of Amlodipine and Atorvastatin in tablet dosage form. Methods: The developed method consisting the mobile phase of acetonitrile and 0.02 M Potassium dihydrogen phosphate (55:45) with isocratic programming, BEH C18 (100mmx2.1mm, 1.7μm) column as stationary phase with a flow rate of 0.3 mL/minute. Results and discussion: Proposed method was found to be linear for Amlodipine and Atorvastatin in the concentration range of 0.5 to 40.0 μg/mL with r² of 0.9999 for Amlodipine and 0.9997 for Atorvastatin respectively. Precision study showed that the percentage relative standard deviation was within the range of acceptable limits, and the mean recovery was found to be 100.79 % for assay of Amlodipine and 99.87% for Atorvastatin in tablet dosage form. The LOD and LOQ of Amlodipine and Atorvastatin were found to be 0.062 and 0.078µg/ml and 0.020 and 0.026 µg/mL.

Keywords: Amlodipine, Atorvastatin & UPLC method.

INTRODUCTION

Amlodipine (Fig. 2) is chemically [R-(R*, R’0)] [R-(R*, R’0)] -2-(4-Chlorophenyl)-β,γ-dehydroxy- 5- (1-methylethyl)-3- phenyl-4-[Sphenyl (9phenyl amino)-1,4-pyrrole-1-heptanoic acid. Amlodipine and Atorvastatin standards were obtained from Arene Life sciences, and DSM Sinochem Pharmaceutical India Pvt., Ltd., methanol, acetonitrile and potassium dihydrogen phosphate (HPLC grade) were obtained from RanChem. Amlodipine tablets were purchased commercially.

MATERIALS AND METHODS

Chromatographic Conditions

A chromatographic system Waters UPLC Acquity H-Class with photodiode array detector, BEHC18 (2.1*100) mm, 1.7 μm column was used. A flow of 0.30 mL/min, injection volume of0.80 μL, detection wavelength of 242 nm for both the analytes. The peak purity was checked with the photodiode array detector [3].

Mobile Phase

20 mM phosphate buffer and acetonitrile in the ratio of 45:55(v/v), the pH of the buffer was adjusted to 3.5 by (20%v/v) of ortho phosphoric acid in the milli-Q water. The mobile phase was mixed and filtered through a nylon filter and degassed.

Preparation of Amlodipine Besylate standard stock solution

Accurately weighed and transferred about 10.0 mg of Amlodipine Besylate working standard to 100 mL volumetric flasks, 30 mL of methanol was added to it and sonicated to dissolve. Volume was made up to the mark with methanol.

Preparation of Atorvastatin Calcium standard stock solution

Accurately weighed and transferred about 10 mg of Atorvastatin Calcium working standard to a 100 mL volumetric flasks, 30 mL of methanol was added to it and sonicated to dissolve for 5 min. Volume was made up to the mark with methanol.

Preparation of working standard solution

10 mL of the above prepared Atorvastatin Calcium stock solution and 5 mL from Amlodipine Besylate stock was transferred to 100 mL volumetric flask and the volume was made with mobile phase and filtered through 0.22 µm nylon filter.

Preparation of sample solution

Twenty tablets were weighed, average weight of the tablets was determined and finely powdered. A portion of powder equivalent to the weight of one tablet was accurately weighed into100 ml A-grade volumetric flask and 70 ml methanol was added. The volumetric flasks were sonicated for about 20min to effect complete dissolution of the Amlodipine besylate and Atorvastatin calcium, the solutions were then made up to volume with methanol. The solution was filtered through 0.22 μm nylon filter. Further dilute 10 ml of this solution to 100 mL with the mobile Phase.

Preparation of Atovastatin Calcium working standard to a 100 mL volumetric flasks, 30 mL of methanol was added to it and sonicated to dissolve for 5 min. Volume was made up to the mark with methanol.

Preparation of working standard solution

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Method Validation [4-8]

Linearity
The linearity of the calibration curves was determined for intra- and intraday precision on 3 different days.

Primary stock solution
Weighed accurately 10 mg of each Amlodipine and Atorvastatin in 100 mL of A-grade volumetric flask separately added 70 ml of methanol to dissolve and sonicated for 10 min and volume was made up to mark with methanol(100 μg/mL).

Intermediate stock solution
It was prepared in 100 mL of A-grade volumetric flask by diluting 50 mL primary stock solution of Amlodipine and Atorvastatin with mobile phase to 100 mL to get concentration of 50 μg/mL. Linearity standards of concentration 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 40 μg/mL were prepared in a separate set of 50 mL of A-grade volumetric flask by diluting 0.5, 1, 2, 5, 10, 20, 40 mL intermediate working stock solution with mobile phase.

The calibration curves were constructed by plotting the absolute peak area(y) versus the concentration (x), by using linear regression analysis.

Precision
Repeatability of sample preparation and measurement of sample area were carried out using six injection of tablet sample of Amlodipine and Atorvastatin. The intermediate precision were calculated using different column and different system. The mean area and % assay for those injections were calculated.

System precision was the result of the method operating over a short time interval under the same conditions. Six injections of standard preparation were injected and compliance for system suitability test was checked.

Limit of detection (LOD) and Limit of Quantification (LOQ)
The LOD (defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy) and the LOQ (defined as the lowest absolute concentration of analyte in a sample that can be detected but not necessarily quantified) were calculated according to ICH guideline.

Specificity
Specificity of the method was performed by analyzing drug and sample and interference from blank and placebo was checked using PDA detector and peak purity was confirmed.

Accuracy
The known amount of standard drug was spiked in triplicate to the placebo samples and the recovery of the drug was calculated. Accuracy was performed at 3 levels: 80%, 100%, 120% of sample concentration, in triplicate at each level, using the placebo spiked with drug. Samples were prepared by adding corresponding weight of Amlodipine and Atorvastatin in placebo and processes as per sample preparation.

Robustness
Robustness was tested by changing the following parameters of the method: a) Flow rate. b) Column temperature (c) Variation of pH of buffer (d) Wavelength of the detector and the results were compiled and % RSD of area was calculated.

Application of proposed method to tablet formulation
To determine the concentration of Amlodipine and Atorvastatin in tablets (label claim: 5 mg Amlodipine and 10 mg of Atorvastatin), the sample was prepared as per the earlier sample preparation procedure to get the concentration of 5µg/mL of Amlodipine and 10µg/mL of Atorvastatin. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied. Peak were found to be symmetric with good resolution as shown in figure 3.

RESULT

Calibration curve
The linear regression data for the calibration curve showed good linear relationship over the concentration range 0.5-40 μg/mL. Linear regression equation for Amlodipine and Atorvastatin were found to be y= 4670.8x-292.3, (r²=0.9999) and y=5901.2x+778.99 (r²=0.9997) respectively.

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Table 1: Precision study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Amlodipine %R.S.D. for area</th>
<th>Amlodipine %Assay (Area)</th>
<th>Amlodipine % Assay</th>
<th>Atorvastatin %R.S.D. for area</th>
<th>Atorvastatin %Assay (Area)</th>
<th>Atorvastatin % Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability</td>
<td>0.25</td>
<td>101.23</td>
<td>0.22</td>
<td>99.43</td>
<td>101.47</td>
<td>0.28</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.62</td>
<td>101.47</td>
<td>0.28</td>
<td>99.01</td>
<td>101.13</td>
<td>0.28</td>
</tr>
<tr>
<td>System</td>
<td>0.26</td>
<td>-</td>
<td>0.28</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

LOD and LOQ
Limit of Detection and Limit of Quantification for Amlodipine and Atorvastatin were found to be 0.26 and 0.78 µg/mL and 0.20 µg/mL and 0.62 µg/mL respectively based on signal to noise ratio method.

Accuracy
This parameter was evaluated by the recovery studies at concentration levels of 80, 100, and 120%, which consisted of adding known amounts of Amlodipine and Atorvastatin reference materials to the placebo. The amount of drug added and determined and the % recovery are listed in table 2.

Specificity
The peak purity of Amlodipine and Atorvastatin was assessed by PDA detector which revealed that both peak were pure. There was no interference from blank and placebo at the retention time of Amlodipine and Atorvastatin.

Robustness
Results of robustness were compiled as indicated in Table 3. % R.S.D was calculated for each parameter and was found to be less than 2%.

Table 2: Accuracy study.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim (mg/ tablets)</th>
<th>Amount of standard added (%)</th>
<th>%Drug recovered</th>
<th>% RSD</th>
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<tbody>
<tr>
<td>Amlodipine</td>
<td>5 mg</td>
<td>80</td>
<td>99.16</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>100.54</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>99.36</td>
<td>0.67</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>10 mg</td>
<td>80</td>
<td>99.95</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>99.46</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>101.13</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table 3: Robustness study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Amlodipine %R.S.D. for area</th>
<th>Amlodipine %</th>
<th>Atorvastatin %R.S.D. for area</th>
<th>Atorvastatin %</th>
<th>Flow rate</th>
<th>Variations</th>
<th>0.285 mL</th>
<th>0.315 mL</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0.59</td>
<td></td>
<td>0.08</td>
<td>0.285 mL</td>
<td>0.315 mL</td>
<td>1.33</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35</td>
<td></td>
<td>0.59</td>
<td>0.285 mL</td>
<td>0.315 mL</td>
<td>1.95</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.39</td>
<td></td>
<td>0.33</td>
<td>0.285 mL</td>
<td>0.315 mL</td>
<td>0.63</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.73</td>
<td></td>
<td>0.59</td>
<td>0.285 mL</td>
<td>0.315 mL</td>
<td>1.15</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Analysis of marketed formulation
The combination of Amlodipine and Atorvastatin was assayed in the tablet which contains Amlodipine 5 mg and Atorvastatin 10 mg was present. Mean assay of Amlodipine and Atorvastatin was found to be 100.05 and 100.63% w/w and % RSD of results of Amlodipine and Atorvastatin was found to be 0.413% and 0.65% w/w respectively.

CONCLUSION
A simple, sensitive and specific isocratic RP-UPLC method was developed for assay of Amlodipine and Atorvastatin in tablet dosage form. The proposed analytical method has been proved as rapid, simple, specific, accurate as well as cost effective and hence is considered to be reliable and suitable for the routine quality control analysis of Amlodipine and Atorvastatin.

REFERENCES
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