High Performance Liquid Chromatographic Method for Determination Ofamlodipine Besilate and Olmesartan Medoxomil in Tablet Dosage Form

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ABSTRACT

A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the estimation of Amlodipine besilate and Olmesartan medoxomil in pure and in pharmaceutical dosage forms. Phenomenex Luna C 18 column (250x4.6mm, 5μ) was used with a mobile phase containing a mixture of Phosphate buffer, Acetonitrile and Methanol in the ratio of 40: 30:30. The flow rate was 1.0ml/min and effluents were monitored at 238nm eluted at 3.50min (AML) and 2.80min (OLM). Calibration curve was plotted with a range from 27-42μg/ml for AML and 80-120μg/ml for OLM. The assay was validated for the parameters like accuracy, precision, robustness and system suitability parameters. The proposed method can be useful in the routine analysis for the determination on Amlodipine and Olmesartan in pharmaceutical dosage forms.

Keywords: Amlodipine besilate, Olmesartan medoxomil, Reverse phase HPLC, Pharmaceutical dosage form.

1. INTRODUCTION

Amlodipine besilate (AML), chemically, [3-ethyl-5-methyl (4RS)-2-{[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-methyl-1- dihydropyridine-3,5-dicarboxylate benzenesulfonate (1) , is a long acting calcium channel blocker used which is used as an antihypertensive agent (2,3,4). Olmesartan is an angiotensin II receptor antagonist used for hypertension and is chemically designated as 5-methyl-2-oxo-1,3-dioxolen-4-y1) methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-[2-(tetrazol-5-y1)-phenyllyphenyl]methylimidazol-5-carboxylate6-6). AML is official in BP (1), whereas OLM is not official in any pharmacopoeia. Both the drugs are marketed as combined dose tablet formulation in the ratio of AML: OLM 05:20 mg.

Literature survey revealed that a number of methods have been reported for estimation of AML and OLM individually or in combination with other drugs (6-12). However, there is no analytical method reported for the simultaneous estimation of AML and OLM in a combined dosage formulation. Present work describes simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of AML and OLM in tablet formulation.

2. MATERIALS AND METHODS

2.1. Reagents

Amlodipine and Olmesartan were obtained from Micro labs, Hosur, India. Acetonitrile and Methanol (HPLC grade, MERCK), water (Milli Q).Other reagents were of AR grade.

2.2. Instrumentation

The HPLC system consisted of a ShimadzuClass LC-10AT vp and Gelman science vacuum pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spincotech 1.7 software. Analysis was carried out at 238nm using a Phenomenex C18Reverse phase column of 250x 4.6 mm i.d., 5μm dimensions at ambient temperature. The mobile phase consisted of Phosphate buffer, Acetonitrile and Methanol in the ratio of 40: 30:30 v/v that was set at a flow rate of 1.0ml/min.

2.4. Preparation of standard stock solution

34.9 mg of each AML and 100.3 mg of OLM was taken in 100 ml volumetric flask separately and dissolved in50 ml mobile phase and volume was made up to 100ml with mobile phase. Above 5ml of the solution was transferred into 50 ml volumetric flask and made up with mobile phase to 50ml.

2.5. Preparation of mobile phase

2.5.1. Preparation of phosphate buffer
3.5 gm of K$_2$HPO$_4$ was dissolved in 1000ml of water and adjusted with triethylamine at pH 6. Mixed the above buffer and mobile phase in the ratio of 40:30:30 and degassed in sonicator for about 15 mins.

2.5.2. Mobile phase
Phosphate buffer: Acetonitrile: methanol (40:30:30)

2.5.3. Procedure for Sample Preparation
20 tablets were weighed and crushed, from the powdered tablets, weighed accurately about 1070.3 mg into a 100 ml volumetric flask, dissolve with 50 ml of mobile phase and made up to 100 ml with mobile phase. Shaken well and filtered the solution. From the above filtrate pipetted out 5.0 ml into a 50 ml standard flask and made up to 50 ml with mobile phase.

2.6. Validation parameters

2.6.1. Accuracy
To study accuracy of the method, recovery studies were carried out by addition of standard drug solution to sample at 3 different levels, 80%, 100% and 120% of the test concentration.

2.6.2. Precision
Precision of the method was checked by system precision and repeatability (Intra day and Inter day studies). In system precision 6 replicates of mixed standard (containing AML 125µg/ml and OLM 4µg/ml) were used. Repeatability was done by using 3 replicate readings at 3 concentration levels. For Intraday variability trials are taken in a day and for Interday variability studies were done on 3 consecutive days.

2.6.3. Robustness
Robustness of the method was determined by small, deliberate changes in flow rate, mobile phase ratio, Wavelength of detection and pH of mobile phase. Flow rate was changed to 1 + 0.05 ml/min. The mobile phase ratio was changed to 1% for methanol, pH of mobile phase was changed to 5 + 0.1.

2.6.4. LOD and LOQ Determination
Limit of detection can be calculated by using following formula,

$$\text{LOD} = 3.3 \sigma/S$$

Limit of quantitation can be calculated based on standard deviation of the response and the slope.

$$\text{LOQ} = 10 \sigma/S$$

Where $\sigma$ = Standard deviation of the response
$S$ = Slope of the calibration curve

2.6.5. System Suitability Testing
System suitability testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. Parameters such as theoretical plates, tailing factor, Resolution are determined and compared against the specifications and are presented in Table 1.

3. RESULTS AND DISCUSSION

The solutions of Amlodipine besilate (AML) and Olmesartan medoxomil (OLM) working standards were injected into the HPLC system and run in different solvent systems as mobile phases. Different mobile phases containing buffer, Acetonitrile, Methanol) in different proportions were tried and finally Acetonitrile, Methanol, Phosphate buffer adjusted to pH 6 with triethanolamine (40:30:30 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for both AML and OLM. Representative chromatogram of mixed standard of AML and OLM is shown in Figure 1.

![Fig.1: Chromatogram of working standard mixture of AML, OLM](image)

3.1. Method validation
The linear relationship was observed between the peak area and concentration over the range of 27-42 µg/ml for AML and 80-120 µg/ml for OLM. The linearity was expressed as correlation coefficient, which was 0.999 for AML and 0.999 for OLM. Correlation coefficient, y-intercept, slope of regression line are shown in Figure 2 and 3. Precision was carried out as system precision and repeatability as per ICH guidelines. It was determined at 3 concentration levels with 3 replicates at each level. For all three concentration levels % RSD obtained was less than 2% for both the drugs. The results of precision are given in Table no.3. Robustness studies were carried out after deliberate alterations of flow rate, mobile phase compositions, and mobile phase pH. It was observed that the small changes in these
operational parameters, did not lead to changes of retention times of peak of interest. The results are shown in Table 3.

Table 1: System suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Amlodipine</th>
<th>Olmesartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tailing factor</td>
<td>1.700</td>
<td>1.789</td>
</tr>
<tr>
<td>Theoretical plate</td>
<td>6569</td>
<td>4169</td>
</tr>
<tr>
<td>Resolution</td>
<td>4.119</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Assay result

<table>
<thead>
<tr>
<th>Drug</th>
<th>% Assay</th>
<th>Amount present (mg/tab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine</td>
<td>99.06</td>
<td>4.95</td>
</tr>
<tr>
<td>Olmesartan</td>
<td>99.95</td>
<td>20.29</td>
</tr>
</tbody>
</table>

Table 3: Validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Amlodipine</th>
<th>Olmesartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>% Recovery = 98.99</td>
<td>% Recovery = 98.60</td>
</tr>
<tr>
<td>System precision</td>
<td>%RSD = 0.35</td>
<td>%RSD = 0.28</td>
</tr>
<tr>
<td>Method precision</td>
<td>%RSD = 0.51</td>
<td>%RSD = 0.33</td>
</tr>
<tr>
<td>Linearity R²</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Range μg/ml</td>
<td>20-30</td>
<td>50-75</td>
</tr>
<tr>
<td>LOD 0.01μg/ml</td>
<td>0.03μg/ml</td>
<td></td>
</tr>
<tr>
<td>LOQ 0.08μg/ml</td>
<td>0.08μg/ml</td>
<td></td>
</tr>
</tbody>
</table>

The proposed method was evaluated in the assay of tablet formulation containing AML and OLM. Five replicate determinations were carried out on tablets. % assay found was 98.88-99.08 for AML and that for OLM was 99.85-100.1%. The results are shown in Table 2.

4. CONCLUSION

The method described enables the quantification of Amlodipine besilate and Olmesartan modoxomil in combined tablet dosage form. The validation data demonstrate good precision and accuracy, which prove the reliability of the proposed method. Hence this HPLC method can be used routinely for quantitative estimation of both components in solid oral dosage form.

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5. REFERENCE


