Development and Validation of Stability Indicating Ultra Performance Liquid Chromatographic method for Olmesartan Medoxomil

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ABSTRACT

A novel rapid, sensitive and reproducible, ultra performance liquid chromatographic method was developed for quantitative determination of olmesartan medoxomil in active pharmaceutical ingredients and its dosage forms. Olmesartan medoxomil is a medicine used for the treatment of hypertension. It belongs to a group of angiotensin II receptor blocker. The method is applicable to the quantification of related compounds and assay of olmesartan medoxomil drug. Chromatographic separation of drug from the possible impurities and the degradation products was achieved on a Poroshell 120 EC-C18 4.6 x 50mm, 2.7µm column; the gradient elution achieved with in 7.0 min. 0.1% orthophosphoric acid in water and acetonitrile was used as mobile phase. The flow rate was 2.0mL/min, column temperature 25°C and the detection was done at 210 nm. The above developed UPLC method was further subjected to hydrolytic, oxidative, photolytic and thermal stress conditions. The method was validated for specificity, precision, linearity, accuracy and robustness and can be used for quality control during manufacture and for assessment of the stability of samples of olmesartan medoximil. To the best of our knowledge, a validated stability indicating UPLC method which separates all the four impurities reported in USP and EP monograph along with two more impurities has not been published elsewhere. Total elution time was about 7 min which allowed quantification of more than 100 samples per day.

KEYWORDS: Olmesartan medoxomil, Forced degradation, Validation, Angiotensin II

INTRODUCTION

Olmesartan medoxomil is a type of medicine called an angiotensin II antagonist. It works by preventing the action of a hormone in the body called angiotensin II. Angiotensin II is a very potent chemical that causes muscles surrounding blood vessels to contract, thereby narrowing blood vessels, this narrowing increases the pressure within the vessels and can cause hypertension1-2.

Figure-1. Structures of Olmesartan medoximil and its impurities.

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A comprehensive literature survey revealed several methods reported for the estimation of olmesartan in biological fluids, the techniques of determinations include HPLC coupled with mass spectrometry, elmisartan determination by UV-Vis spectrophotometry in tablets has been reported. Shah NJ, Suhagia BN developed HPTLC method for the simultaneous determination of olmesartan & Hydrochlorothiazide in tablets. A capillary electrophoresis, many LC methods has been reported for the simultaneous determination of olmesartan with other drugs. Also there are several stability indicating HPLC methods reported and olmesartan is listed in pending monographs of United States Pharmacopeia and European Pharmacopoeia. However there are no UPLC methods reported in the literature for the quantification of olmesartan medoxomil and its related compounds. Hence the objective of the work is to develop an economic, time-efficient, RP-UPLC method and demonstrate its stability-indicating capability by forced degradation followed with method validation of the developed method for the accurate quantification of impurities and assay of olmesartan medoxomil in bulk drug samples.

EXPERIMENTAL

Materials and Reagents:
Active pharmaceutical ingredient and its related impurities (Fig.1) were procured from ALSACHIM and LGC Promochem. Commercially available Benicar in 40 mg tablets was used for the dosage form analysis. Acetonitrile purchased from Merck, Darmstadt, Germany. Orthophosphoric acid, purchased from spectrochem. HPLC grade water was prepared from Millipore and Milli-Q purification system.

Equipment:
UPLC system was equipped with binary gradient pumps with auto sampler and auto injector (Model Acquity UPLC from Waters, USA) connected with a photo diode array detector (PDA) controlled with Empower software (Waters).

Preparation of Standard and sample solutions:
A standard preparation consisting of 0.001mg mL-1 concentration of all impurities along with 0.001mg mL-1 concentration of olmesartan medoxomil was prepared for related substances. A sample solution consisting of olmesartan medoximil 1.0mg mL-1 spiked with all impurities at 0.1% level (0.001 mg mL-1) was prepared for related substances method. The standard and sample concentration for the assay method was 0.10mg/mL.

Chromatographic conditions:
Mobile phase-A consisted of 0.1% orthophosphoric acid in water. Mobile phase-B consisted of acetonitrile. Before use the mobile phase was filtered through a 0.2μm PTFE filter and degassed by ultrasonication. The system was equilibrated for 15 min and the analysis was carried out under linear gradient condition using a flow rate of 2.0mL min-1 at 25°C and Poroshell 120 EC-C18 4.6 x 50mm, 2.7μm column was used for separation. Chromatograms were recorded at 210nm. The injection volume was 3.0μL and the following linear gradient programme was used for the separation:

**Time (min)** | 0.0 | 5.0 | 5.5 | 7.0  
--- | --- | --- | --- | ---  
**Mobile phase-A** | 75 | 25 | 75 | 75  
**Mobile phase-B** | 25 | 85 | 25 | 25  

**Forced degradation:**
Olmesartan medoximil was deliberately subjected to stress to establish the stability-indicating nature of the method. The compound was exposed to fluorescent light (1.2x10^6 LUX hours), UV light for a total exposure of 200 W·hr·m², heat (60°C for 7 days), acid (0.1N HCl, 80°C for 24 Hrs), alkali (0.1N NaOH, 60°C for 24 Hrs), oxidation (3.0 % H₂O₂ for 4 Hrs), and humidity (40°C & 70% RH for 7 days) to evaluate the ability of the method to separate olmesartan medoximil from its degradation products. Peak purity was determined using PDA detector.

**Precision System precision:**
The system precision was examined by analyzing standard solution in six replicates. For assay method, standard solution-1 was injected in five replicates prepared in system suitability preparation.

**Method precision:**
For related substances method, method precision was examined by analyzing olmesartan medoximil six preparations of sample solution spiked with mixture of impurities at specification limit, against standard preparation containing olmesartan medoximil RSD was calculated for the individual impurity and total impurity values. For assay method, method precision was examined by analyzing six preparations of sample solution against olmesartan medoximil standard solution. RSD was calculated on the assay values.

**Intermediate precision – Ruggedness:**
Precision was repeated using different analyst, on different day, on different instrument and using column of different lot. Overall RSD was calculated for the individual impurity and total impurities for related substances method and overall RSD for assay was calculated.

**Linearity:**
The Linearity of the method was determined by using different concentration of mixture of olmesartan medoximil and impurities prepared and analyzed in triplicate from QL to 160% of the specification limit concentration (0.001 mg mL-1). The peak response versus concentration data was treated by linear regression analysis for each ingredient was performed.

**Quantification limit (QL) and Detection Limit (DL):**
The lower end of the linear range was considered to be the QL for the method. The QL concentrations were determined by injecting diluted standard solution to a level such that %RSD was not more than 10%, precision study was also carried at the QL level by injecting six individual preparations of olmesartan medoximil and its impurities and calculating the %RSD of the area. The DL was theoretically calculated from QL using the following expression. DL = QL/3

**Accuracy:**
The accuracy study was carried out in triplicate sample preparation of olmesartan medoximil spiked with impurities at QL 100% and 120% levels. The percentages of recoveries were calculated from the respective known concentrations.

**Robustness**
To determine the robustness of the analytical method, experimental conditions were deliberately altered in order to determine the robustness of the method. To study the effect of flow rate, flow was changed by 0.1 units from 1.9 to 2.1 mL min-1. The effect of the column temperature was studied at 20 and 30°C. For related substances method, the RSD for the individual impurity and total impurities were calculated. For assay method, RSD for the assay value was calculated.

**Solution Stability:**
The stability of the analyte was established for standard and sample solutions under conditions as prescribed in the method. The purpose of this procedure was to determine the time during which the standard and sample solutions remain stable. In this validation, three solutions were studied: Stock standard solution, working standard solution and sample solution.
RESULTS AND DISCUSSIONS

Method development and optimization:
Olmesartan medoxomil drug substance has a reported pKa of 13.72 and logP value of 5.9. The aim of the chromatographic method was to achieve the separation of precursors, intermediates, degradents and the main component. From the UV profiling it was found that the suitable wavelength for the olmesartan medoxomil drug and its related impurities were 210nm. Several fast LC methods aiming for shorter run time and high throughput were tried for the separation of six impurities and olmesartan medoximil from each other. These includes different stationary phase, column dimension and buffers. Anticipating the possible base line interferences at lower wavelength high pure orthophosphoric acid was selected as the buffering reagent for the quantification of olmesartan medoxomil. From various trials, 0.1% orthophosphoric acid in water as mobile phase-A and acetonitrile as mobile phase-B was tried and gradient was optimized so that all the impurities and olmesartan peak were well separated from each other. No blank peak interference at the retention time of known peak was obtained as shown in Fig-2.
Figure 2. Chromatograms of forced degradation studies.
System suitability:
For the related substances method, the resolution between Impurity-C and Impurity-A from the system suitability preparation was greater than 3.0, RSD for the area of olmesartan medoximil peak and all the impurities from the replicate injections of standard preparation was less than 1.2% and USP plate count for olmesartan medoximil peak in the standard preparation was 20506. The above three system suitability parameters were met during the course of entire validation. For the assay method, the tailing factor for the olmesartan medoximil standard peak from the first injection of the standard preparation-1 was 1.1. The relative standard deviation for the mean area calculated for olmesartan medoximil peak from the five replicate injections of standard preparation-1 was 0.66% and the % recovery calculated between standard preparation -1 and standard preparation-2 was within 100 ±1 %. The above three system suitability parameters were met during the course of entire validation.

Specificity:
Olmesartan medoximil peak was well separated from all other impurities; also no blank peak interference at the retention time of known peaks, the purity angle is less than purity threshold for the olmesartan medoximil peak in the spiked sample, the method is selective and specific. Fig 2 demonstrates the specificity of the method.

Forced degradation:
Degradation was mild when olmesartan medoximil exposed to heat & humidity (40°C & 70% RH for 7 days), thermal (60°C for 7 days) and photolytic conditions of fluorescent light (1.2x10^2 LUX hours), UV light for a total exposure of 200 W•hr/m^2. However the drug was more susceptible during acid hydrolysis (0.1N HCl 80°C for 24 Hrs), base hydrolysis (0.1N NaOH, 60°C for 24 Hrs) and oxidative stress, leading to the formation of IMP D (Fig. 2). Results from peak purity testing Table-3 confirmed the main compound peak obtained by analysis of all the stress samples was homogenous and pure and unaffected by the presence of its degradation products, confirming the stability indicating nature of the method. The results from forced degradation studies are summarized in Table 2.

Precision:
The precision and intermediate precision were successfully demonstrated and RSD for the individual and total impurity values were found to be below the acceptance value. For related substance method the RSD of individual impurity and total impurities were calculated and found less than 5%. The overall RSD between method precision and intermediate precision values are of less than 5% demonstrates good precision of the method. The assay of olmesartan medoximil was determined as per the method of analysis using two columns of different lots, different UPLC instrument on two different days and two different analysts. Results were summarized in Table 1.

Linearity:
Linear regression analysis for each ingredient showed that the calibration curves were linear over the concentration range of QL to 0.16 %. The analytical data and linearity results for olmesartan medoximil and its impurities are shown in Table 1.

Quantification limit (QL) and Detection limit (DL):
The quantification limit (QL) and detection limit (DL) of olmesartan medoximil and its related impurities are shown in Table 1.

Accuracy:
The recovery of three sample preparation at each level was examined and ranged from 99.2% to 105.4%. Results are summarized in Table 1.

Robustness:
In all the deliberate varied chromatographic conditions (flow rate and column temperature) the results obtained were well within the limit for related substance method (RSD NMT 5%) and assay method. (RSD NMT 2%).

Stability:
The stock standard solution, working standard solution and sample solution were prepared as per the method, after dispensing an amount for the testing of initial time, the solutions were stored in volumetric flasks and kept in refrigerator (5 ±3 °C) and also at room temperature, prior to the testing at each time interval of 1 hour for stock standard, working standard solution and sample solution, the flasks were taken out of the refrigerator, allowed to equilibrate to room temperature before use.

The % recovery of each analyte meets the re-

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**Table 1. Summary of Method Validation**

<table>
<thead>
<tr>
<th>Validation Parameter</th>
<th>IMP D</th>
<th>OLME</th>
<th>IMP E</th>
<th>IMP F</th>
<th>IMP C</th>
<th>IMP A</th>
<th>IMP B</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>System Precision</td>
<td>0.6</td>
<td>0.6</td>
<td>1.2</td>
<td>0.4</td>
<td>0.7</td>
<td>0.7</td>
<td>1</td>
<td>1</td>
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<tr>
<td>% RSD of peak area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Difference of Retention time (last two std)</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>% Difference of Retention time (last std and check std)</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Resolution</td>
<td>NA</td>
<td>11.2</td>
<td>7.9</td>
<td>6.6</td>
<td>39.1</td>
<td>3</td>
<td>20.6</td>
<td>1</td>
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<td>Tailing Factor</td>
<td>0.9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.9</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>Column efficiency</td>
<td>5361</td>
<td>20506</td>
<td>33074</td>
<td>43377</td>
<td>88937</td>
<td>132131</td>
<td>163948</td>
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</tr>
<tr>
<td>Linearity (µg/mL)</td>
<td>0.15-9.91</td>
<td>0.15-2.04</td>
<td>0.15-11.9</td>
<td>0.11-2.18</td>
<td>0.05-2.19</td>
<td>0.24-1.59</td>
<td>0.24-1.92</td>
<td>2</td>
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<tr>
<td>RSD</td>
<td>0.9995</td>
<td>0.9959</td>
<td>1</td>
<td>0.9999</td>
<td>0.9996</td>
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<tr>
<td>RRF</td>
<td>1.0703</td>
<td>1</td>
<td>1.0007</td>
<td>0.6388</td>
<td>0.4134</td>
<td>1.3958</td>
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<tr>
<td>Quantification limit(µg/mL)</td>
<td>0.1486</td>
<td>0.1529</td>
<td>0.1488</td>
<td>0.1089</td>
<td>0.0547</td>
<td>0.2379</td>
<td>0.2397</td>
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<tr>
<td>Detection limit(µg/mL)</td>
<td>0.0495</td>
<td>0.0501</td>
<td>0.0496</td>
<td>0.03063</td>
<td>0.0312</td>
<td>0.0793</td>
<td>0.0799</td>
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</tr>
<tr>
<td>Accuracy Mean % Recovery at QL</td>
<td>105.4</td>
<td>100.6</td>
<td>103.5</td>
<td>103.4</td>
<td>105</td>
<td>102</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>103.3</td>
<td>NA</td>
<td>101.4</td>
<td>102.6</td>
<td>100.8</td>
<td>100</td>
<td>102.6</td>
<td>1</td>
</tr>
<tr>
<td>120 % of target</td>
<td>102.9</td>
<td>101.4</td>
<td>101.8</td>
<td>101.8</td>
<td>101</td>
<td>100</td>
<td>99.2</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate Method Precision</td>
<td>100.6</td>
<td>NA</td>
<td>100.5</td>
<td>99.2</td>
<td>108.8</td>
<td>94</td>
<td>99.6</td>
<td></td>
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<tr>
<td>% RSD</td>
<td>0.5</td>
<td>0</td>
<td>0.2</td>
<td>0.8</td>
<td>1.8</td>
<td>2.9</td>
<td>2</td>
<td>9</td>
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<tr>
<td>Stability of Solutions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stock Standard Solution (5±3°C)</td>
<td>28 hours</td>
<td>28 hours</td>
<td>28 hours</td>
<td>28 hours</td>
<td>28 hours</td>
<td>28 hours</td>
<td>28 hours</td>
<td></td>
</tr>
<tr>
<td>Working Standard Solution (5±3°C)</td>
<td>24 hours</td>
<td>24 hours</td>
<td>24 hours</td>
<td>24 hours</td>
<td>24 hours</td>
<td>24 hours</td>
<td>24 hours</td>
<td></td>
</tr>
<tr>
<td>Working Standard Solution (Room temp)</td>
<td>2 hours</td>
<td>2 hours</td>
<td>2 hours</td>
<td>2 hours</td>
<td>2 hours</td>
<td>2 hours</td>
<td>2 hours</td>
<td></td>
</tr>
<tr>
<td>Sample Solution (5±3°C)</td>
<td>9 hours</td>
<td>9 hours</td>
<td>9 hours</td>
<td>9 hours</td>
<td>9 hours</td>
<td>9 hours</td>
<td>9 hours</td>
<td>9 hours</td>
</tr>
<tr>
<td>Sample Solution (Room temp)</td>
<td>1 hours</td>
<td>1 hours</td>
<td>1 hours</td>
<td>1 hours</td>
<td>1 hours</td>
<td>1 hours</td>
<td>1 hours</td>
<td>1 hours</td>
</tr>
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</table>

**Table 2. Forced degradation results**

<table>
<thead>
<tr>
<th>Stress Condition</th>
<th>IMP D</th>
<th>IMP E</th>
<th>IMP F</th>
<th>IMP C</th>
<th>IMP A</th>
<th>IMP B</th>
<th>Total Impurities Assay</th>
<th>% Mass Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Hydrolysis</td>
<td>2.20%</td>
<td>0.20%</td>
<td>0.01%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.1</td>
<td>96.1</td>
</tr>
<tr>
<td>Base Hydrolysis</td>
<td>2.50%</td>
<td>0.30%</td>
<td>0.01%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>5</td>
<td>94.3</td>
</tr>
<tr>
<td>Oxidative Degradation</td>
<td>5.10%</td>
<td>0.10%</td>
<td>0.01%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>7</td>
<td>92.1</td>
</tr>
<tr>
<td>Thermal degradation</td>
<td>0.60%</td>
<td>0.08%</td>
<td>0.01%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1</td>
<td>98.2</td>
</tr>
<tr>
<td>Heat &amp; Humidity</td>
<td>0.40%</td>
<td>0.09%</td>
<td>0.01%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.8</td>
<td>98.3</td>
</tr>
<tr>
<td>Photolytic degradation</td>
<td>0.10%</td>
<td>0.08%</td>
<td>0.01%</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.3</td>
<td>98.7</td>
</tr>
</tbody>
</table>

**Table 3. Peak Purity results**

<table>
<thead>
<tr>
<th>Sample Condition</th>
<th>Condition</th>
<th>Peak Purity angle</th>
<th>Purity threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Hydrolysis</td>
<td>0.1N HCl 24 hours at 80°C</td>
<td>0.248</td>
<td>0.499</td>
</tr>
<tr>
<td>Base Hydrolysis</td>
<td>0.1N NaOH 24 hours at 60°C</td>
<td>0.138</td>
<td>0.601</td>
</tr>
<tr>
<td>Oxidative Degradation</td>
<td>3% H₂O₂, RT for 4 Hrs</td>
<td>0.231</td>
<td>0.522</td>
</tr>
<tr>
<td>Thermal degradation</td>
<td>60°C for 7 days</td>
<td>0.141</td>
<td>0.488</td>
</tr>
<tr>
<td>Heat &amp; Humidity</td>
<td>40°C &amp; 70% RH for 7 days</td>
<td>0.155</td>
<td>0.456</td>
</tr>
<tr>
<td>Photolytic degradation</td>
<td>Fluorescent light (1.2x10^2 LUX hours), UV light of 200 W/m²</td>
<td>0.161</td>
<td>0.483</td>
</tr>
</tbody>
</table>
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