STABILITY INDICATING FORCED DEGRADATION RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF OLMESARTAN MEDOXOMIL

A. Srinivas* and Y. Sneha

SRR College of Pharmaceutical Sciences, Valbhapur (V), Elkathurthy (M), Karimnagar (Dist) - 505 476, Andhra Pradesh, India

Keywords: HPLC, method validation, ACN, recovery studies, precision, stability studies

ABSTRACT: A simple, precise, accurate, economical and reproducible HPLC method for estimation of Olmesartan in tablet dosage form has been developed. Quantitative HPLC was performed with HITACHI L2130 with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400), PUMP (LC-IOAT). C18 Develosil ODS HG-5 RP 150mm x 4.6mm 5µm particle size column receptor in vascular muscle. Its action is therefore independent of the pathway of angiotensin II synthesis was used in the study. The mobile phase of ACN: phosphate buffer (pH 3.1) was used in this study. The conditions optimized were: flow rate (1.0 ml/minute), wavelength (205nm) and run time was 10 min; column temperature was maintained at 40°C. Retention time was found to be 6.31 min. The linearity was found to be in the concentration range of 10-100 µg/ml. The developed method was evaluated in the assay of commercially available tablets OLMESE containing Olmesartan. The amount of drug in tablet was found to be 40mg. Results of analysis were validated statistically and by recovery studies. The recovery studies 99.27 % was indicative of the accuracy of proposed method. The precision was calculated as repeatability, inter and intraday variation (%RSD) for the drug. By using the method, stability of the drug has been studied.

INTRODUCTION: Hypertension is one of the most prevalent diseases with an estimated one billion cases worldwide.

The therapeutic standard was significantly improved in the 1980s by the introduction of losartan as the first nonpeptidic angiotensin II type-1 (AT1) receptor antagonist. An entire therapeutic class, the sartans, has since been developed ¹.

Olmesartan medoxomil, chemically 2,3-dihydroxy-2-butyl-4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-imidazole-5-carboxylate, cyclic 2,3-carbonate, is an angiotensin II re-ceptor blocker used as an antihypertensive agent (Figure 1) ². A thorough literature search has revealed that there are only a few HPLC methods reported for determination of olmesartan in human plasma ³.

Methods such as HPLC, HPTLC and simultaneous UV spectrophotometric have been reported for the estimation of olmesartan alone or in combination with other drugs ⁴⁻¹⁰. The present study compares LC determination of olmesartan by identification and characterizing the impurity of olmesartan medoxomil ¹¹⁻¹².
The impurity profile of Active Pharmaceutical ingredients (APIs) and evaluation of their toxicity effects are necessary steps in developing a safe and effective drug and are essential for medical safety reasons. It is mandatory that any new impurities present in the drug substance and drug product above the threshold limit are identified and characterized. The present manuscript describes the development and validation of stability-indicating RP-HPLC method and identification and characterization of alkaline degradation impurity of olmesartan medoxomile drug substance as well as drug product. Therefore, the objective of the reported research was to study the degradation of OLME under different International Conference of Harmonization (ICH) recommended stress conditions and to evaluate the degraded products by MS and to establish a stability-indicating RP-HPLC method for accurate quantification of OLME in pharmaceutical dosage forms.

MATERIALS AND METHOD:

**Instruments and Reagents:** The chromatographic separation was performed on HITACHI L2130 with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400), PUMP (LC-IOAT). C18 Develosil ODS HG-5 RP 150mm x 4.6mm 5µm particle size column was used as a stationary phase. pH Analyzer (ELICO), Electronic Balance, Ultra Sonicator has been used in the work. Olmesartan Active Pharmaceutical Ingredient (API) was provided by Hippo labs pvt ltd, kukatpally, Hyderabad. Orthophosphoric acid, Acetonitrile, & water of HPLC grade were from Standard reagents, Hyderabad. Commercial formulations of OLMESAR were used as sample.

**Optimized Chromatographic conditions:**

<table>
<thead>
<tr>
<th>Column</th>
<th>C18 Develosil ODS HG-5 RP150mm x 4.6mm 5µm Particle Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>Phosphate buffer: Acetonitrile (60; 40)</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1.0ml/minute</td>
</tr>
<tr>
<td>Wave length</td>
<td>205 nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 µl</td>
</tr>
<tr>
<td>Run time</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Column temperature</td>
<td>Ambient</td>
</tr>
<tr>
<td>Sampler cooler</td>
<td>Ambient</td>
</tr>
</tbody>
</table>

**Preparation of solutions:**

**Preparation of standard calibration curve:** 25 mg of Olmesartan standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Olmesartan, so that the same wave number can be utilized in HPLC UV detector for estimating the Olmesartan. While scanning the Olmesartan solution we observed the maxima at 205 nm as per Figure 1.

**FIG. 1: SPECTRA SHOWING THE CALIBRATION CURVE OF OLMESARTAN**
Analysis of Tablet Formulation:

**Preparation of Standard solution:** Working concentration should be around 10 µg/ml. Accurately weighed around 25mg of Olmesartan working standard, taken into a 25 ml volumetric flask, then dissolved and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000 mcg/ml. Further dilutions have been made to get the final concentration of 10 µg/ml (Figure 2).

**Preparation of Test solution:** Diluted quantitatively an accurately measured volume of label claim solution with diluents to obtain a solution containing about a linear range. The procedure for preparing the solution is as per the guideline. As per the label claim, the tablets contain 40mg of Olmesartan 10 tablets were taken & an equivalent powder weight has been taken which contains 10 mg of Olmesartan (each of these has been taken in separate volumetric flask), than the powders has been dissolved in mobile phase & made 25ml with the diluents. Further dilution was done by taking 1ml of this solution in 10ml volumetric flask, dissolve and make up with the diluents. To extract the drug in the solution, it has been sonicated for 5 minutes followed by cyclo-mixing for 5 minutes. Resulting solution was filtered by using Millipore syringe filter (0.42 micron). Resulting clear solution was injected in HPLC in duplicate as per the above mentioned HPLC method Figure 3.

**Method Validation:** As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantization.

**Preparation of Calibration Curves:** Calibration curve was prepared by taking appropriate aliquots of standard Olmesartan stock solution in different 10 ml volumetric flask and diluted up to the mark with diluents to obtain the final concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml of Olmesartan. Standard solutions (n=6) were injected, the sample volume was 20 µl with a flow rate of 1.0 ml/min.

**Forced degradation studies:** The protocol was strictly adhered to for forced degradation of Olmesartan Active Pharmaceutical Ingredient (API). The API (Olmesartan) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis and oxidative degradation.
Mother Sample: Before carry out the degradation studies the mother sample was prepared to find out the % degradation. It is prepared as the same method followed in sample or standard preparation.

Acid Hydrolysis: An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 1 M HCl was added & make up to the mark & kept for 8 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with diluent, then injected for HPLC analysis Figure 4.

![FIG. 4: CHROMATOGRAM SHOWING DEGRADATION IN 0.1 M HCL](image)

Basic Hydrolysis: An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 1 M NaOH was added & make up to the mark & kept for 8 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with diluent, then injected for HPLC analysis Figure 5.

![FIG. 5: CHROMATOGRAM SHOWING DEGRADATION IN 0.1 M NAOH](image)
Oxidation with (3%) H$_2$O$_2$: An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 3% H$_2$O$_2$ was added & make up to the mark & kept for 8 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with diluent then injected for HPLC analysis Figure 6.

FIG. 6: CHROMATOGRAM SHOWING DEGRADATION IN 3% H$_2$O$_2$

RESULTS & DISCUSSION:

Results of degradation studies: The results of the stress studies indicated the specificity of the method that has been developed.

Olmesartan was degraded only in 3% H$_2$O$_2$ & 1N HCl, 1N NaOH conditions. The result of forced degradation studies are given in the following Table 1.

TABLE 1: RESULTS OF FORCE DEGRADATION STUDIES OF OLMESARTAN API

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Time</th>
<th>Assay of active substance</th>
<th>Assay of degraded products</th>
<th>Mass Balance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Hydrolysis (0.1 M HCl)</td>
<td>24Hrs.</td>
<td>78.36</td>
<td>20.23</td>
<td>98.86</td>
</tr>
<tr>
<td>Basic Hydrolysis (0.1 M NaOH)</td>
<td>24Hrs.</td>
<td>98.32</td>
<td></td>
<td>98.32</td>
</tr>
<tr>
<td>3 % hydrogen peroxide</td>
<td>24Hrs.</td>
<td>93.31</td>
<td></td>
<td>93.31</td>
</tr>
</tbody>
</table>

Method Validation:

Linearity and Range: The linearity of the method was determined at ten concentration levels ranging from 10-100 µg/ml for Olmesartan. The calibration curve showed good linearity in the range of 10 – 100 µg/ml, for Olmesartan (API) with correlation coefficient (r$^2$) of 0.994. A typical calibration curve has the regression equation of $y = 14694x + 67023$ for Olmesartan Figure 7.

FIG. 7: CALIBRATION CURVE OF OLMESARTAN API
**TABLE 2: LINEARITY READINGS**

<table>
<thead>
<tr>
<th>CONC.</th>
<th>AUC (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>307771</td>
</tr>
<tr>
<td>7.5</td>
<td>358580</td>
</tr>
<tr>
<td>10</td>
<td>489726</td>
</tr>
<tr>
<td>12.5</td>
<td>616383</td>
</tr>
<tr>
<td>15</td>
<td>735232</td>
</tr>
</tbody>
</table>

**Precision:** The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug Olmesartan (API). The percent relative standard deviations were calculated for Olmesartan. The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Olmesartan revealed that the proposed method is precise Table 4.

**Method Robustness:** Influence of small changes in chromatographic conditions such as change in flow rate (± 0.2 ml/min), Temperature (± 2°C), Wavelength of detection (± 2nm) and acetonitrile content in mobile phase (± 2%) studied to determine the robustness of the method are also in favour of (% RSD < 2%) the developed RP-HPLC method for the analysis of Olmesartan Table 5.
CONCLUSION: To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Olmesartan. Different chromatographic conditions were applied & the results observed are presented. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here waters C18, 5µm, 15cm x 4.5 mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluents for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 1M NaOH, IM HCl).

The drug was found to be soluble in methanol. Drug was highly soluble in acetonitrile. Using these solvents with appropriate composition newer methods can be developed and validated. The result shows the developed method is yet another suitable method for assay and stability studies which can help in the analysis of simvastatin in different formulations. The precision of the method was demonstrated by intra-day and inter-day variation studies. For intra-day studies the drug having concentration value 80%, 100 % & 120% of the target concentration (n = 3), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days.

Data were subjected to statistical treatment for the calculation of SD and %RSD. The value of %RSD for Olmesartan was found to be 0.97, 0.42, and 0.14 for intra-day studies. The values for inter-day studies were 0.96, 0.43, and 0.33 respectively. This shows that values are not more than 2%, indicates that the developed method is precise. The proposed method is simple, sensitive and reproducible and hence can be used in routine analysis.

ACKNOWLEDGEMENT: The authors are thankful to the Director of Hippo pharmaceuticals, Hyderabad for providing the necessary facilities to carry out this work.

REFERENCES:

12. V.P. Rane, K.R. Patil, J.N. Sangshetti. Stability-Indicating LC Method for the Determination of...


How to cite this article: