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Determination of Olmesartan Medoxomil In Bulk And Pharmaceutical Formulations By Validated RP-HPLC Method

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Abstract--- *Analytical method was developed for the estimation of Olmesartan medoxomil drug substance by liquid chromatography. The chromatographic separation was achieved on Ascentris express C18 100*4.5um at ambient temperature. The separation was achieved by employing a mobile phase consisting of 0.1%v/v formic acid in water: Acetonitrile (50:50). The flow rate was 0.6 ml/ minute and UV detector was set at 230nm. The average retention time for Olmesartan medoxomil was found to be 1.9 min and the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear in the range of 50-150µg/ml for Olmesartan medoxomil .*

Keywords--- *Olmesartan medoxomil, Isocratic, HPLC, Trifluoro acetic acid, and Acetonitrile*

I INTRODUCTION

I.I. Drug Profile

Olmesartan (4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(1H-1,2,3,4-tetrazol-5-yl) phenyl] phe nyl} methyl)-1H-imidazole-5-carboxylic acid) is an antihypertensive agent, which belongs to the class of medications called angiotensin II receptor blockers (ARB)[1-13]. It is indicated for the treatment of high blood pressure and is marketed under the name Olmetec.

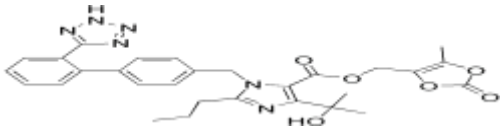

Fig 1: Olmesartan Structure

Several analytical methods[14-28] have been reported for the determination of Olmesartan medoxomil in pure drug, pharmaceutical dosage forms and in biological samples using spcetrophotometry, liquid chromatography, electro kinetic chromatography high performance thin layer chromatography either in single or in combined forms.

II MATERIALS AND METHODS

II.I. Instrumentation

Waters HPLC model 2695 with 2487detector containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a

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Ascentris express C18 100*4.5um. Degassing of the mobile phase was done using a Unichrome ultrasonic bath sonicator. A SCALETEC (model SAB224CL) Analytical balance was used for weighing the materials.

II.II. Chemicals and Solvents

The reference sample of Olmesartan(API) was obtained from Sun Pharma Pvt Ltd. The Formulation Olmesartan was procured from the local market. Acetonitrile, Trifluoro acetic acid, Methanol and formic acid used was of HPLC grade and purchased from Qualigens Limited, Mumbai, India.

II.III. The Mobile Phase

A mixture of of 0.1%v/v formic acid in water: Acetonitrile (50:50) was prepared and used as mobile phase.

II.IV. Preparation of Standard solution

A 25mg of pure Olmesartan medoxomil was weighed and transferred to 25 ml of volumetric flask and dissolved in Diluent. The flask was shaken and volume was made up to mark with Diluents to give a primary stock solution containing 1000mg/ml. From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with Diluent to give a solution containing 100µg/ml of Olmesartan medoxomil .

II.V. Preparation of Sample Solution

20 tablets (each tablet contains Olmesartan 40 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Olmesartan (µg/ml) were prepared by dissolving weight equivalent to 5 mg of Olmesartan dissolved in sufficient mobile phase. After that the solution is filtered using 0.45-micron syringe filter and sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 100 µg/ml of Olmesartan.

III METHOD DEVELOPMENT

A suitable method was developed[19-28] by carrying out systematic study of the effect of various factors by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

III.I. Detection wavelength

The spectrum of Olmesartansolution was recorded separately on UV spectrophotometer. The peak maximum of absorbance wavelength was observed. The spectra of Olmesartan were showed maximum absorbance at 230nm [Fig-2].

III.II. Choice of stationary phase and Mobile Phase

Finally the expected separation and peak shapes were obtained on Ascentris express C18 100*4.5um column. A mixture of 0.1v/v formic acid in water: Acetonitrile in the ratio of 50:50 was proved to be the most suitable for

all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

III.III. Flow rate:

Flow rates of the mobile phase were changed from 0.2 – 1.0 mL/min for optimum separation. It was found from the experiments that 0.6 mL/min flow rate was ideal for the successful elution of the analyte.

III.IV. Optimized chromatographic conditions

Chromatographic conditions optimized above were shown in Table 1. These optimized conditions were followed for the determination of Olmesartan in bulk samples and in its formulations. The chromatograms for Standard Drug and Placebo are identified. Among all these for the Placebo no significant peaks are detected.

IV VALIDATION OF PROPOSED METHOD AND REQUIREMENTS

The proposed method was validated as per ICH guidelines[29-42]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, and limit of quantification.

IV.I. Specificity

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in the above defined chromatographic conditions and the blank chromatogram was recorded. Chromatogram of Blank solution **(**Fig. no.-4**)** showed no peaks at the retention time of Olmesartan peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Olmesartan in Olsat - 40 tablets. Similarly typical representative chromatogram of standard was shown in figure -5

IV.II. System Suitability

Six replicate injections of API working standard solution were injected according to the method of analysis. The percentage relative standard deviations (% RSD) for the peak responses were determined. The % RSD of the peak responses due to Olmesartan for six injections must be less than or equal to 5.0 %. The analytical system complies with the requirements specified by the system suitability. The Results are tabulated in the Table 2

Linearity and range

In the concentration range of 50.0 – 150.0 µg/ml for Olmesartan standard curve was obtained. A statistical method known as linear regression analysis was used to evaluate the linearity of the curve. To assess the linearity of the proposed method slope, intercept and correlation coefficient [r^2] of standard curve was calculated and was given in Figure-3. The results were given in the Table- 3. From the data obtained (For Olmesartan), the method was found to be linear within the proposed range. The linearity chromatograms were given in figure- 6 -10. The LOD and LOQ results were given in Table – 7.

Accuracy

Accuracy is defined as the closeness of results obtained by that method to the true value for the sample. Accuracy is expressed in terms of percentage recovery. Recovery % is determined by the standard addition method. In the present study recovery studies were carried out at 50%, 100% and 150% spiked levels. The results of Recovery % were given in Table - 4 and the chromatograms were given in Figures 11-13.

Precision

The closeness of replicate results obtained from analysis of the same homogeneous sample is known as precision of the method. The precision of the method was assessed by six replicate injections of 100% test concentration. The precision was expressed in terms of standard deviation and %RSD. The results were given in Table- 5. The system precision was also analyzed and the results were given in the same table.

Robustness

The ability of the developed method to remain unaffected by the small changes in the parameters is known as Robustness. Robustness was assessed by varying the parameters such as percent organic content, pH of the mobile phase, buffer concentration, temperature, injection volume and flow rate.

In the present investigation, a variation of ± 0.1 mL/min in the flow rate, change in wavelength were adopted to study Robustness. The results were tabulated in Table -6.

V RESULTS AND DISCUSSION

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of 0.1v/v formic acid in water : Acetonitrie in the ratio of 50:50 and 0.6 mL/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 230nm at which much better detector responses for drug was obtained as shown in Fig 2. The retention time was 1.907 min for Olmesartan. Good number of theoretical plates was found, which indicates efficient performance of the column. A system suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits and are represented in Table 2. Thus, the system meets suitable criteria.

The calibration curve was obtained for a series of concentration in the range of 50-150µg/ml and it was found to be linear. Five points graphs was constructed covering a concentration range 50-150 µg/ml. The standard deviation of the slope and intercept were low. The data of regression analysis of the calibration curves are shown in Table 3.

Mean percentage recovery is found to be 99.5. The proposed method has been applied for the assay of the commercial tablets containing Olmesartan. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was

showing that the equipment used for the study was correctly calibrated and hence the developed analytical method is highly repetitive. For the intermediate precision analysis was carried out by different analysts working on the same day indicated a RSD of 0.1. This indicates good method precision. The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits. The statistical evaluation of the proposed method revealed good linearity, reproducibility and its validation for different parameters and can be concluded that it could be used for the rapid and reliable determination of Olmesartan in tablet formulation.

Table 1: Optimized chromatographic conditions for estimation Olmesartan

Column	Ascentris express C18 100*4.5um
Buffer preparation	0.1% Formic acid
Mobile phase	Buffer : ACN (50:50 v\v)
Flow rate	0.6 ml/min
Injection volume	10ul
Run time	4min
Wavelength	230nm
Diluents	Acetonitrile: (100%)

Table 2: System Suitability results

Parameter	Olmesartan medoxomil	Acceptance criteria
Retention time	1.907	+-10
Theoretical plates	3272	>2000
Tailing factor	1.05	<1.50
% RSD	1.15	<2.00

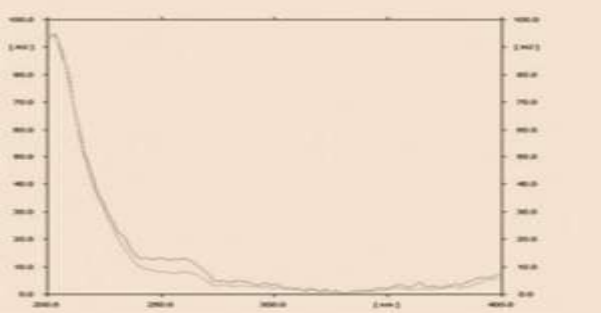

Fig 2: Olmesartan absorbance at 230nm

Table 3: Linearity of Detector Response for Olmesartan		
S.NO	**Level**	**Area**
1.	50	1401971
2.	75	2217414
3.	100	2857484
4.	125	3535604
5.	150	4304193
Correlation coefficient 0.9992		

Table 4: Accuracy data for Olmesartan medoxomil			
S.No	Accuracy level	injection	%Recovery
1	50%	1	100.6
		2	99.9
		3	101.0
2	100%	1	99.0
		2	99.2
		3	99.1
		4	99.6
		5	98.3
		6	98.2
3	150%	1	99.3
		2	99.8
		3	99.8

Linearity graph of Pantoprazole:

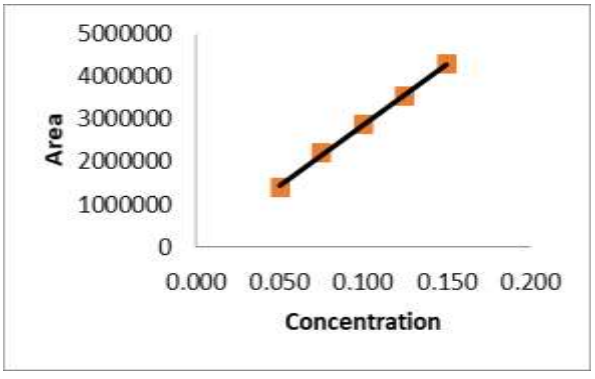

Fig 3: linearity of detector response graph for Olmesartan

Table 5: - Method precision data for Olmesartan			
S.NO	**RT**	**Area**	**%Assay**
Injection 1	1.912	2885532	99.0
Injection 2	1.910	2891022	99.2
Injection 3	1.910	2887943	99.1
Injection 4	1.913	2904597	99.6
Injection 5	1.913	2872538	98.3
Injection 6	1.910	2856288	98.2
Mean	1.911	2882987	98.9
Std. Dev.	0.002	16641	0.55
% RSD	**0.08**	**0.58**	**0.55**

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