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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ASSAY FOR OLMESARTAN MEDOXOMIL IN FORMULATED PRODUCT BY REVERSE PHASE ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

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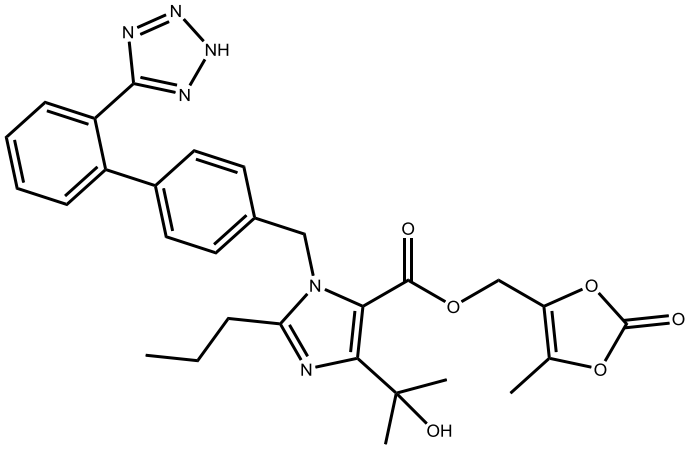
ABSTRACT
The novel approach was carried out to develop and validate a rapid and selective analytical method by using Reverse Phase Ultra performance Liquid Chromatography (RP-UPLC) technique for the analysis of Olmesartan Medoxomil in raw materials & their pharmaceutical dosage forms. The developed analytical UPLC method is superior in technology to conventional HPLC method with respect to speed, resolution, solvent consumption and cost of analysis. The compound was analyzed with a total run time of 3.5min. (in reverse phase) at 215nm wavelength. Optimum retention was achieved on Waters Acquity UPLC BEH C18 column (2.1 × 30mm, 1.7µm) using gradient elution with mobile phase i.e. 5mM Ammonium Acetate in water (volatile reagent) and Acetonitrile as organic solvent. The Olmesartan Medoxomil was first dissolved in Acetonitrile and the final dilution was done by using Water: Acetonitrile (30:70) v/v as diluents. The method showed excellent recoveries for all drugs in bulk. The developed UPLC method was validated with respect to specificity, linearity, precision, accuracy, ruggedness (reproducibility), robustness and stability. The test solution was found to be stable in diluents for 24hrs when stored at RT (i.e. 25°C). Recovery data was in the range of 98.82% to 99.76%. The method is economical in terms of the time taken and the amount of solvent used, thus promoting green chemistry concept. To the best of our knowledge, a work on method development and validation of Olmesartan Medoxomil by using RP-UPLC technique, disclosed in this investigation, was not published elsewhere.

KEY WORDS: Olmesartan Medoxomil, UPLC, new method development, validation.

INTRODUCTION
Olmesartan Medoxomil is a typical antihypertensive drug used in the treatment of high blood pressure like problems. Olmesartan Medoxomil has the chemical name (5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl] phenyl}methyl)-1H-imidazole-5-carboxylate (Figure 1). It has angiotensin II receptor antagonist like property.[1] The Olmesartan Medoxomil drug is mainly used for hypertension in countries like Japan and US. The Medoxomil moiety, which is enclosed with this drug, has endogenous ester moiety responsible for releasing metabolites in the body. Olmesartan Medoxomil has a favorable safety and efficacy profile, with blood pressure-lowering effects comparable to those of other angiotensin receptor blockers (i.e. Losartan, Valsartan, Irbesartan).[2]

This drug was developed by Sankyo in 1995, and is available in the market with the trade name of Benicar, Olmetec and Olmesar. High Performance Thin Layer Chromatography (HPTLC) has been used to quantify[3] Olmesartan Medoxomil in many cases. The earlier reported study of this drug was mainly performed by RP-HPLC methods[4-9] on long columns with higher particle size, which were more time consuming. Even though the method was using complex mobile phase mixture with high flow rates, the analysis was lacking sensitivity and peak symmetry. The purpose of the present study is to develop a simple, sensitive, accurate, precise, rugged, and time saving method for the determination of Olmesartan Medoxomil in formulated product. The target is attained by selecting more advance technique of Waters Acquity UPLC,[10] which gives more accurate result in shorter run time. The method development is done by optimizing the experimental conditions using mass compatible volatile buffer i.e. Ammonium Acetate, on a shorter column having 1.7µm particle size.[11-12] The developed method has been validated by following several parameters as mentioned in ICH guideline[13-15] i.e. linearity, specificity, accuracy, precision, robustness, ruggedness, stability. The pure Active Pharmaceutical Ingredient (API), used in this project, is manufactured by GLENMARK Company, and obtained from Jubilant Life

Science Limited with a COA (Certificate of Analysis). The Tablet used was OLMESAR, manufactured by Macleods Pharmaceutical Ltd.



Chemical Formula: $C_{29}H_{30}N_6O_6$
Exact Mass: 558.22

Figure 1. Structure of Olmesartan Medoxomil.

2. MATERIAL AND METHOD

Materials

Apparatus

Chemicals used in this study included gradient grade Acetonitrile (Sigma Aldrich, USA) and MS grade Ammonium acetate (Sigma Aldrich, USA). Water used for UPLC analysis was purified using Millipore Milli Q-Plus water purification system (Millipore SAS, France).

Reagents and Chemicals

A well-characterized working standard of Olmesartan Medoxomil was procured from Jubilant Life Sciences Limited, India. Commercially available OLMESAR (Olmesartan Medoxomil Tablet) purchased from local pharmacy (Noida, India) having batch number BOJ501A manufactured by Macleods Pharmaceuticals Ltd., India.

METHOD

Method Development and Optimization of Chromatographic Conditions.

Solubility

From the literature review, Olmesartan Medoxomil is freely soluble in Acetonitrile and slightly soluble in Water.

Selection of chromatographic method

Proper selection of the method depends upon the nature of the sample (ionic / ionisable / neutral molecule), its molecular weight and solubility. The drug selected in the present study is polar in nature and hence reversed phase or ion-pair or ion exchange chromatography method may be used. The reversed phase UPLC was selected for the separation because of its simplicity and suitability. This method can be directly used for LC-MS analysis also, on need basis.

Selection of wavelength

The sensitivity of the any LC method which uses UV detection depends upon the proper selection of wavelength. An ideal wavelength is one that gives good response for all the drugs to be detected. During conditions optimization we found that 215nm is the appropriate wavelength for this analysis.

Selection of mobile phase

Initially the mobile phase tried was Methanol and Water, Methanol and Acetonitrile, Acetonitrile and buffer with various combinations as well as varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Buffer (0.05mM Ammonium Acetate, as volatile buffer) in gradient proportion.

Optimization chromatographic operating conditions

Chromatography analysis was performed on Waters Acquity UPLC system (Waters, USA) equipped with a binary solvent delivery system, an auto-sampler and a photodiode array detector. Gradient separation method was applied in the analysis using a Waters Acquity UPLC BEH C18 column (30 × 2.1 mm, 1.7 µm) at 30°C, with a flow rate of 0.3 mL/min. The mobile phase consisted of (A) 5mM Ammonium acetate in water, and (B) Acetonitrile. A linear gradient elution method was applied as follows: 0 min: 5% B; 0.1min: 5% B; 1.80min: 50% B; 2.40 min: 95% B; 3.20min: 95% B; 3.50min: 5% B with curve 6. The auto sampler was maintained at 10°C, and the sample injection volume was 1µL.

Estimation of drugs in Tablet formulation by developed RP-UPLC method.

Mobile phase preparation
Buffer was prepared by dissolving 192.6 mg ammonium acetate in 500 mL Milli-Q water. It was dissolved well and degas it using vacuum filtration through 0.2µ (6,6-Nylon) membrane filter paper.Gradient grade Acetonitrile was used from different channel, for gradient elution purpose.

Standard stock solution Preparation
Accurately weighed and transferred 25 mg of Olmesartan Medoxomil standard in a 25 mL volumetric flask and added a little quantity of Acetonitrile. It was then sonicate to dissolve and dilute to volume with the diluents (Water: Acetonitrile; 30: 70).

Standard preparation
Transfer 2.00 mL of standard stock solution into 20mL volumetric flask and dilute to volume the same diluents, as above. The concentration of standard solution used in analysis is 100 µg/mL.

Sample Preparation
Finely grind pre weighed 20 Tablets. Transfer approx. 330 mg of grinded sample (equivalent to 20mg of API) to 20.0 mL volumetric flask added a little quantity of Acetonitrile. . It was then sonicate to dissolve, dilute to volume with the diluents (Water: Acetonitrile; 30: 70) and filtered through 0.2µm nylon filters. Then 2mL of this solution was further diluted into 20mL volumetric flask to get the final concentration ratio of 100 µg/mL.

Assay procedure
Inject 1µL of the standard and sample solutions into the UPLC system and the chromatograms were recorded and measured the areas for the Olmesartan Medoxomil peak and calculate the % Assay by using following formula.
% Assay = (At/As) x (Ws/Ds) x (Dt/Wt) x (P/100) x (Avg. weight/Label Claim) x 100
Where,
At = average area counts of sample preparation, As = average area counts of standard preparation
Ws= Weight of working standard taken in mg, Wt = Weight of sample taken in mg
Dt = sample dilution
DS= standard dilution
P= Purity of Standard
In RP-UPLC method, chromatographic conditions were optimized to obtain, an adequate separation of eluted compounds with shorter run time, less consumptions of solvent and mass compatible for further studies.

Method Validation
Linearity: A calibration curve was generated to confirm the linear relationship between the peak area and concentrations of six samples. The serial standard solution were prepared by accurately weighing and dissolving Olmesartan Medoxomil pure API in Acetonitrile and water (70:30) v/v to obtain the final concentration 25-150 µg/mL. These solutions were injected into chromatographic system in replicate. The Linear detector response of Olmesartan Medoxomil is demonstrated by concentration versus Area curve.

Precision and stability: The standard solution was analyzed for six replicates on the same day to assess intra-day variation, and once again on the next day for 6 consecutive samples to assess inter-day variation. The same sample was subjected to UPLC analysis immediately and after 2, 12 and 24hrs. to detect the peak area of the standard sample for the evaluation of stability.

Reproducibility: This was performed by analyzing an assay of six samples of Olmesartan Medoxomil against qualified reference standard. The %RSD for assay obtained from six precision samples & six standard samples were calculated to evaluate the reproducibility of the method.

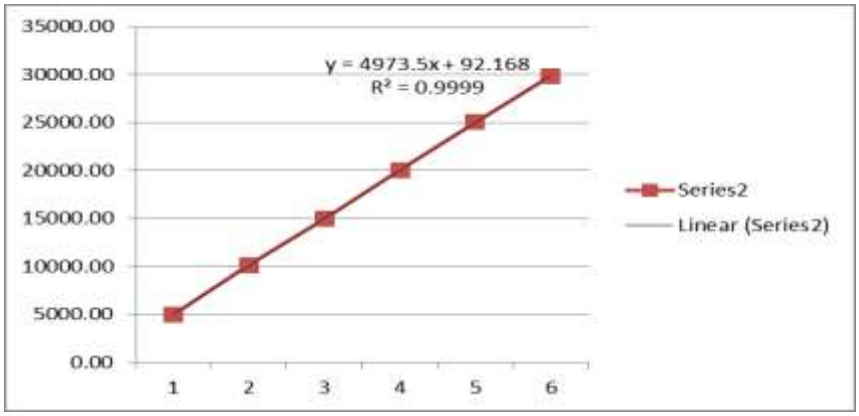
Recovery: Recovery of the assay method for Olmesartan Medoxomil was established by three determinations of test sample using Tablets at 50%, 100% and 150% of concentration. Each solution was injected twice (n=2) into UPLC system and the average peak area was calculated to obtain percentage recoveries after calculation.
Robustness: This was performed by analyzing standard solution & sample solution by changing the flow rate (±0.10 mL/min), buffer concentration (±10%), and temperature (±5 º C).

Validation of developed UPLC method
Different chromatographic conditions such as mobile phase, wavelength, column and column temperature were experimented to achieve efficiency of the chromatographic system. Different gradients of buffer and solvents were checked in order to attain optimum retention of the API. Minimizations of run time and cost were the major tasks while developing the method. Based on International Conference on Harmonization (ICH) guidelines, the method was validated with regard to precision, specificity, reproducibility, accuracy, linearity, stability of solution, robustness, limit of detection and quantification.

Linearity
Linearity was assessed in the range of 25%, 50%, 75%, 100%, 125% and 150% of working concentration. Injections of all concentrations were carried out in replicate. Calibration curve was constructed by plotting the mean peak area versus concentration which was observed to be linear. The Linearity co-efficient of mean response which was plotted against respective concentration, was calculated. The results are summarized in Table-1 and Figure 2.

Table-1: Linearity Data

Level	Concentration (ppm)	Area Count		Average Area
		Injection-1	Injection-1	
25%	25	4974.70	4965.46	4970.08
50%	50	10159.70	10145.63	10152.67
75%	75	14918.29	15001.75	14960.02
100%	100	20042.21	20045.87	20044.04
125%	125	25027.48	25023.45	25025.47
150%	150	29870.04	29818.01	29844.03



Peak Area

% Conc.

Figure-2 Calibration graph of Olmesartan Medoxomil

Accuracy

Recovery of the assay method for Olmesartan Medoxomil was established by three determinations of test sample using Tablets at 50%, 100% and 150% concentration. Each solution was injected twice (n=2) into UPLC system and the average peak area was calculated to obtain percentage recoveries. All the individual recoveries were found to be between 98.82 to 99.76%. All individual recovery levels were found to be within 0.17 to 0.45% (%RSD). The results are summarized in Table-2.

Table 2. Recovery studies for Olmesartan Medoxomil

Level	Sample area(per Injection)	Average area	Sample Wt. (mg)	Amount added (µg)	Amount recovered (µg)	% Recovery	Average % recovery	SD	% RSD
50%	10119.17	10087.19	16.5	165.00	165.13	100.08	99.76	0.45	**0.45**
	10055.21				164.08	99.44			
100%	20171.16	20143.25	33.0	330.00	329.16	99.74	99.61	0.20	**0.20**
	20115.33				328.24	99.47			
150%	29938.28	29975.14	49.5	495.00	488.54	98.69	98.82	0.17	**0.17**
	30012				489.74	98.94			

Precision

The precision of the method was evaluated by carrying out six independent injection of test sample against a qualified reference standard. The % RSD of peak area of the standard was found to be **0.29.** The results are summarized in Table 3 and Figure 3, Figure 4.

Table 3. Results of precision (System and Method)

S.No	Name	RT	Standard Area	Name	Test Area
1	S-Precision-1	1.78	20072.79	M-Precision-1	20177.75
2	S-Precision-2	1.77	19929.29	M-Precision-2	20299.59
3	S-Precision-3	1.78	20019.82	M-Precision-3	20089.34
4	S-Precision-4	1.78	19971.21	M-Precision-4	20195.42
5	S-Precision-5	1.77	19946.66	M-Precision-5	20251.56
6	S-Precision-6	1.78	20045.78	M-Precision-6	20188.35
	Average	1.78	19997.59		20200.34
	SD	0.01	57.32		71.38
	%RSD	**0.29**	**0.29**		**0.35**

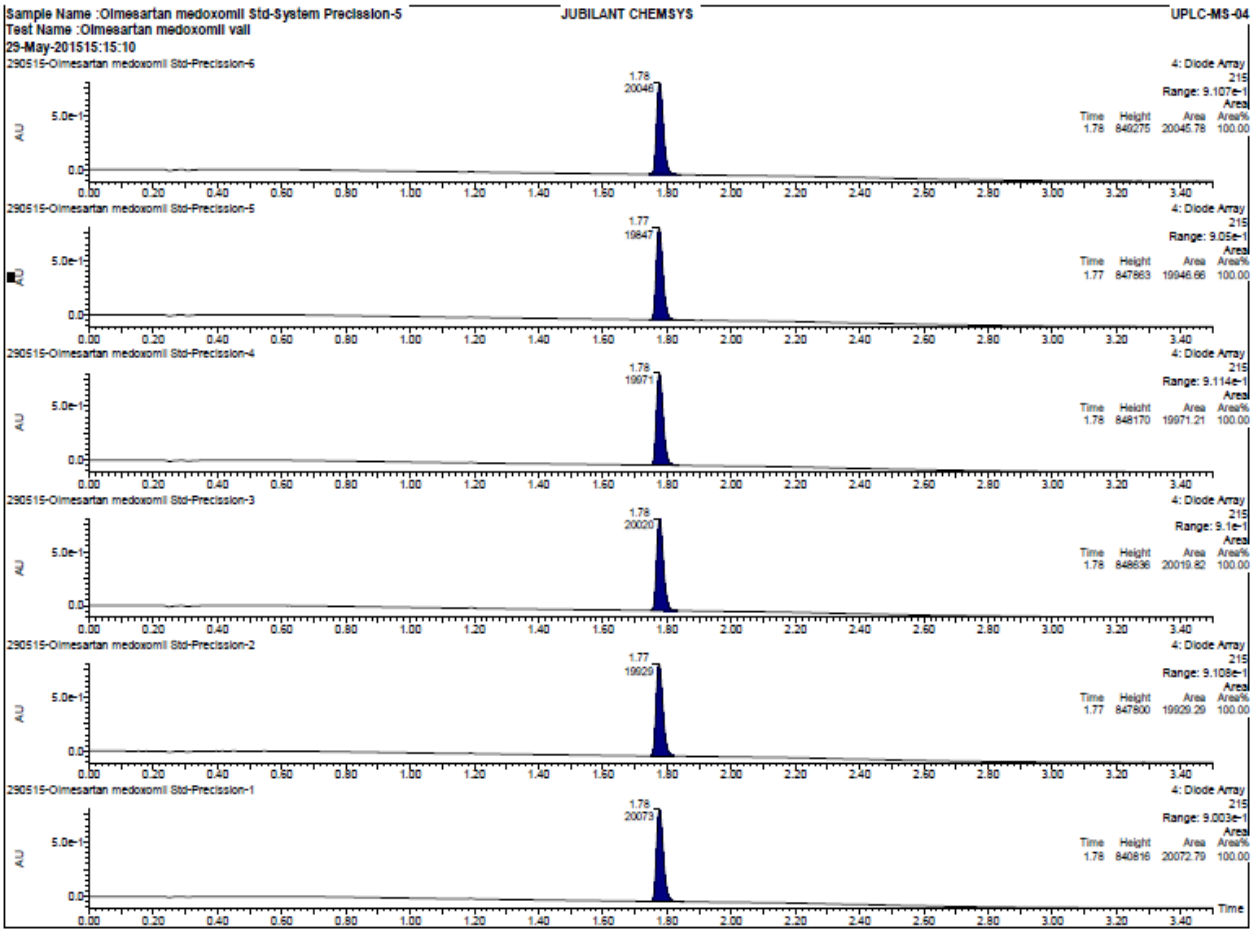


Figure 3. Chromatogram of Olmesartan Medoxomil Standard solution

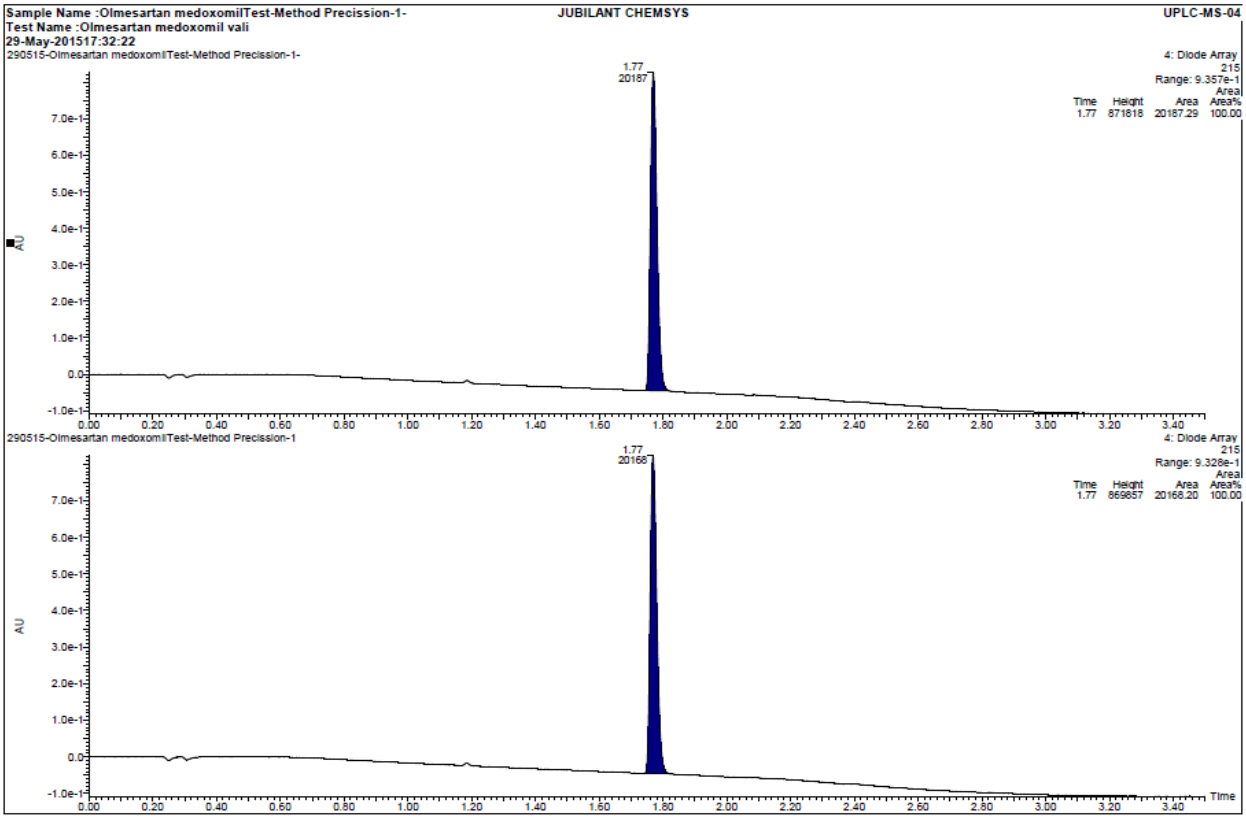


Figure 4. Chromatogram of Olmesartan Medoxomil Sample solution

Reproducibility (Intermediate Precision)

An assay was performed by analyzing six samples of Olmesartan Medoxomil against qualified reference standard. The %RSD obtained from these samples was observed as **0.24** and %RSD of peak area of reference standard was observed as **0.23**. The results are summarized in Table 4.

Table 4. Results of reproducibility (Intermediate precision)

S. No.	Name	Standard Area	Test Area		Average Test Area
			Injection-1	Injection-2	
1	Int. Precision-1	20200.27	20119.93	20189.87	20154.90
2	Int. Precision-2	20196.72	20161.7	20190.81	20176.26
3	Int. Precision-3	20131.54	20355.28	20203.69	20279.49
4	Int. Precision-4	20090.18	20218.25	20172.58	20195.42
5	Int. Precision-5	20145.6	20247.78	20255.33	20251.56
6	Int. Precision-6	20104.88	20165.13	20211.56	20188.35
	Average	20144.87			20207.66
	SD	45.88			47.71
	%RSD	**0.23**			**0.24**

Stability of Solution

The % Cumulative RSD for area and % assay of sample at initial hrs. and at predetermined time intervals (at room temperature) was found to be **0.03.** The Assay method is also reproducible and shows stability up to 24 hrs. The results are summarized in Table-5.

Table-5. Stability Data

Hours	Injection	RT	Area	Average Area	% Assay
0 Hr	Injection-1	1.75	20765.46	20745.04	98.33
	Injection-2	1.75	20724.61		
2.0 Hr	Injection-1	1.75	20775.29	20753.06	98.37
	Injection-2	1.74	20730.83		
12.0 Hr	Injection-1	1.74	20738.52	20747.40	98.34
	Injection-2	1.75	20756.28		
24.0 Hr	Injection-1	1.74	20725.00	20736.99	98.29
	Injection-2	1.74	20748.97		
Average		1.75		20745.62	98.33
SD		0.01		6.67	0.03
%RSD		**0.31**		**0.03**	**0.03**

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the sample containing Olmesartan Medoxomil standard stock with those of the test sample. The specificity study reveals the absence of interference of impurities with the drug, since no extra peak appeared at the same retention time. The RSD for six replicate measurements of peak area of standard preparation was found to be **0.23**. The results are summarized in Table 6.

Table 6. Results for specificity

S. No	Standard Sample	RT	Standard Area	Test Sample	Test Area
1	Replicate-1	1.77	20141.63	Test-1	20131.08
2	Replicate-2	1.78	20079.06	Test-2	20209.74
3	Replicate-3	1.77	20061.34		
4	Replicate-4	1.77	20177.89		
5	Replicate-5	1.77	20165.79		
6	Replicate-6	1.77	20134.32		
	Average	1.77	20126.70		20170.41
	SD	0.004	46.84		55.62
	%RSD	**0.23**	**0.23**		**0.28**

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. Such as change in flow rate (±0.10 mL/min), buffer concentration (±10%), column temperature (±5 °C). In all the above varied conditions, the component of mobile phase was held constant, but no marked changes were observed in the chromatograms, which confirmed that the developed UPLC method is robust.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ studies were carried out to evaluate the detection and quantization limits of the method to determine the presence of any impurities by using the following equation:

LOD = 3.3 σ/S and LOQ = 10 σ/S

Where σ is the standard deviation and S is the slope of the curve.

LOQ and LOD can be determined based on visual evaluation, signal-to-noise approach or standard deviation of the response and slope (calibration curve method). Limit of detection of Olmesartan Medoxomil was found to be **0.05 µg/mL** and the limit of quantification of Olmesartan Medoxomil was determined to be **0.15 µg/mL** using signal-to-noise approach for determination.

Table 7. Result data of Validation Summary

S. no	Parameter		Olmesartan Medoxomil	ICH acceptance limit
1	Accuracy		**99.39%**	98-102%
2	Precision		**0.29**	%RSD<2
3	Stability		**0.31**	%RSD<2
4	Specificity		**0.23**	%RSD<2
5	LOD		**0.05**	S/N=3
6	LOQ		**0.15**	S/N=3
7	Ruggedness (Method Precision)		**0.35**	%RSD<2
8	Robustness	Buffer Conc.	NO remarkable changes observed	%RSD<2
		Flow Rate		
		Column Temp.		

CONCLUSION

We have developed a Reverse phase UPLC-MS method to determine Olmesartan Medoxomil efficiently and accurately within a relatively short period. The new method showed a good precision (RSD<0.29%) and recovery (98.82% - 99.76%). The new gradient RP-UPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method is capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. Short run time allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. This method can be directly used for LC-MS analysis on need basis. It is suitable for rapid and accurate quality control of Olmesartan Medoxomil in Tablet formulations.

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