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ANALYTICAL METHOD DEVELOPMENT FOR THE ESTIMATION OF ANTIHYPERTENSIVE DRUG (OLMESARTAN) BY RP-HPLC METHOD

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ABSTRACT

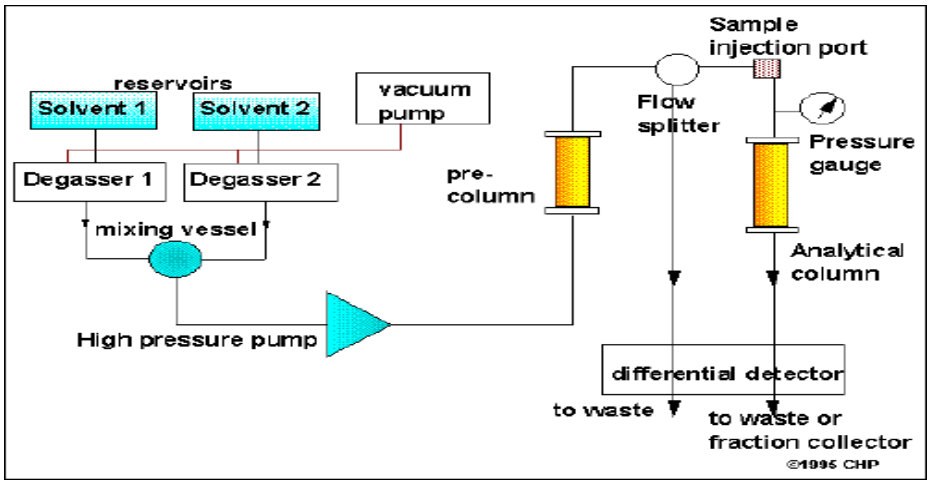
A sensitive and selective RP-HPLC method has been developed and validated for the determination of Olmesartan in pure form and Pharmaceutical dosage form. The Retention time of Olmesartan was found to be 3.544minutes. The Percentage Standard Deviation (%RSD) of the Olmesartan was and found to be 1.63%. The detector response was linear in the concentration range of 0-16µg/ml. The respective linear regression equation being Y= 58945.x + 9634 with R2 = 0.999. The percentage recovery values of Olmesartan in pharmaceutical dosage form were found to be within the limits. The limit of detection and the limit of quantification for Olmesartan were found to be 0.90µg/ml and 2.90µg/ml respectively. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility, accuracy. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Olmesartan in different marketed formulations. The developed method was validated as per ICH guidelines for accuracy calculated as % recovery was in the range of 98.0% to 102.0%. The statistical analysis of the data showed that the method is reproducible and selective for the estimation of Olmesartan in pure form and marketed Pharmaceutical dosage form during routine analysis. The results of the study showed that, the proposed RP-HPLC method was simple, rapid, precise, accurate which can be used for the routine determination of Olmesartan in pure form and marketed pharmaceutical dosage forms.

Keywords: RP-HPLC, Olmesartan, Method development, Method validation parameters

INTRODUCTION [1 2 3]

Quality is important and essential in every product or service but it is more vital in medicines as it is related to life. A Compromise in pharmaceutical quality is nothing but playing with the life of consumer. Pharmaceutical analysis plays a key role here. The terms quality, quality control, quality assuarance, total quality management are correlated. The ultimate goal of all these is to provide a product with good quality, safety, efficiency, purity, strength and identity. Analytical chemistry is divided into two areas called as qualitative analysis and quantitative analysis.

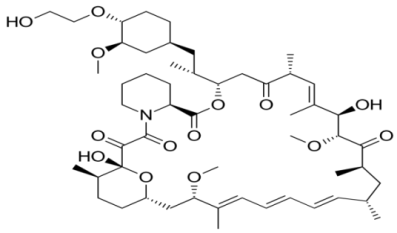
High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids.



DRUG PROFILE

Name[1] : Olmesartan

Structure[2] :



Molecular Weight : 446.5016

Chemical Formula : $C_{24}H_{26}N_6O_3$

Indication : Olmesartan is indicated for the treatment of hypertension either alone or associated with other antihypertensive agents. Hypertension is a sustained elevation of resting blood pressure. The hypertensive effect can affect the systolic blood pressure, diastolic blood pressure or both. This condition tends to be asymptomatic until it reaches a severe or long-standing state.

Mechanism of Action[3] : Olmesartan is a selective angiotensin II-type I receptor blocker with a large affinity. The activity of olmesartan is mainly performed in vascular smooth muscle cells and hence its activity prevents the vasoconstrictor effects of angiotensin.

METHOD DEVELOPMENT

Table 1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	T60-LAB INDIA UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

Table 2: List of Chemicals used

		Specifications		
S.N.	Name	Purity	Grade	Manufacturer/Supplier
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai

SOLUBILITY STUDY

Table 3: Lists of solvents

SOLVENT	SOLUBILITY
Ethanol	Soluble
DMSO	Soluble
Dimethyl formamide	Soluble
Aqueous Buffers	Sparingly soluble

Optimization Of Chromatographic Conditions

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table 4: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.	ACN : Water = 50 : 50	0.8 ml/min	268nm	Low response	Method rejected
Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.	Methanol : Water = 70 : 30	1.0 ml/min	268nm	Very low response	Method rejected
Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.	ACN: Methanol = 60 : 40	1.0 ml/min	268nm	Tailing peak	Method rejected
Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.	ACN: Phosphate buffer = 55:45	1.0 ml/ min	268nm	Broad Peak	Method rejected
Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.	ACN : Phosphate buffer (pH=3.2) =65:35	1.0 ml/ min	268nm	Tailing peak	Method rejected
Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.	ACN : Water = 50 : 50	1.0 ml/min	268nm	Good Peak	Method accepted

Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table 5: Summary of optimised Chromatographic conditions

Mobile phase	ACN : Water =50:50
Column	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
Flow rate	1.0 ml/ min.
Wavelength	268nm
Sampling System	Automatic
Temp. of Auto sampler	Ambient
Volume of injection	10µl
Run time	07 mins
Mode of Separation	Isocratic

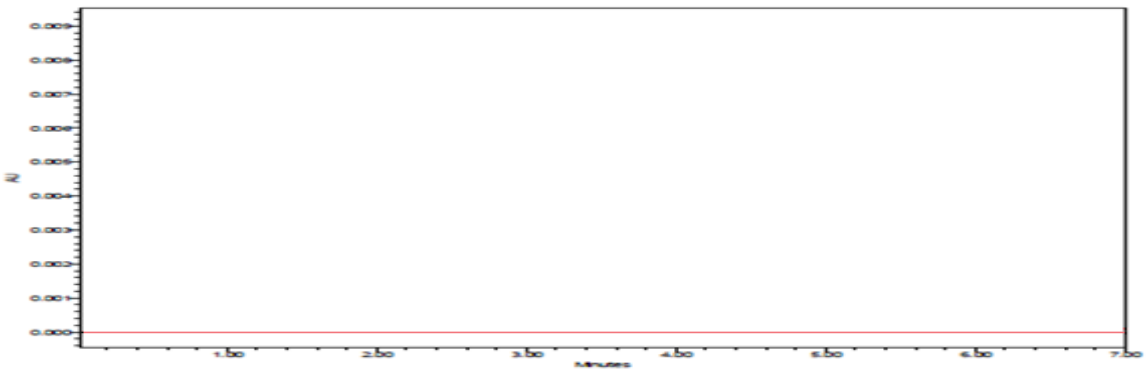


Fig 1: Chromatogram for Blank Solution

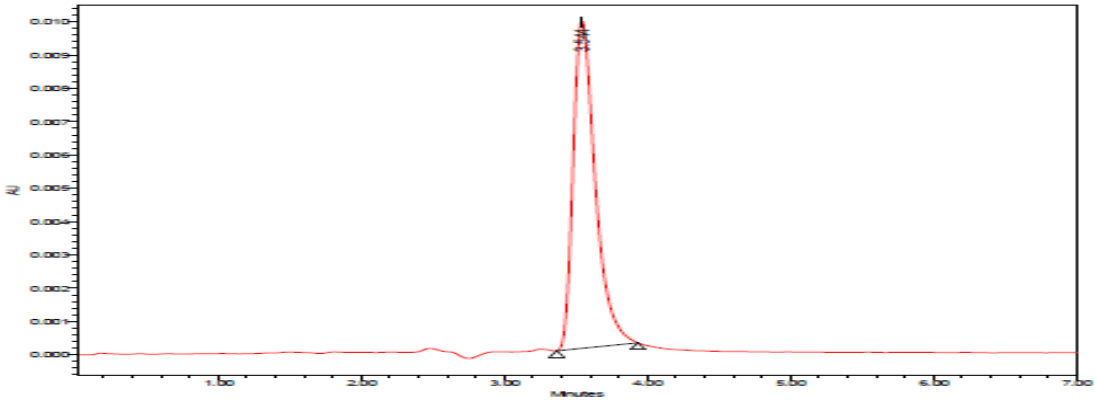


Fig 2: Chromatogram of Olmesartan in Optimized Condition

Table 6: Peak results of Optimized Condition

Drug Name	Rt	Peak Area	Tailing Factor	Theoretical Plates
Olmesartan	3.544	321458	0.99	3324

Preparation of mobile phase

300ml of Acetonitrile and 700mL of HPLC Grade Water were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

Final Result & Discussion

The selected and optimized mobile phase was Acetonitrile and Water in the ratio of 50:50 and conditions optimized were flow rate (1.0 ml/minute), wavelength (268nm), Run time was 07 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

METHOD VALIDATION

Accuracy: Recovery study

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of OLMESARTAN were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in Table-7.

Table 7: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.069	485317	100.862	Mean= 100.5413%
S_2 : 80 %	8	7.958	478751	99.475	S.D. = 0.947606
S_3 : 80 %	8	8.103	487312	101.287	% R.S.D.= 0.942503
S_4 : 100 %	10	10.048	601947	100.48	Mean= 100.2367%
S_5 : 100 %	10	10.073	603395	100.73	S.D. = 0.650103
S_6 : 100 %	10	9.950	596176	99.50	% R.S.D.= 0.648568
S_7 : 120 %	12	11.985	716127	99.875	Mean= 100.3607%
S_8 : 120 %	12	12.116	723840	100.966	S.D. = 0.555257
S_9 : 120 %	12	12.029	718706	100.241	% R.S.D. = 0.553262

Precision

Repeatability

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 deviation was calculated for Olmesartan are presented in the Table-8

Table 8: Repeatability readings

HPLC Injection Replicates of Olmesartan	Retention Time	Peak Area
Replicate – 1	3.545	661022
Replicate – 2	3.537	683137

Replicate – 3	3.543	671941
Replicate – 4	3.538	682245
Replicate – 5	3.542	671941
Replicate – 6	3.550	692444
Average	**3.5425**	**677121.7**
Standard Deviation	**0.004764**	**11046.13**
% RSD	**0.134494**	**1.631336**

Intermediate precision: (Intra-assay & inter-assay)

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 9: Results of intra-assay & inter-assay

Conc. Of Olmesartan (API) (µg/ml)	Observed Conc. Of Olmesartan (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.76	0.82	8.28	0.98
10	10.16	0.42	9.59	0.23
12	11.68	0.13	12.19	0.33

Linearity & Range

The calibration curve showed good linearity in the range of 0 – 16 µg/ml, for Olmesartan (API) with correlation coefficient (r^2) of 0.999 (Fig-30). A typical calibration curve has the regression equation of y = 58945x + 9634 for Olmesartan.

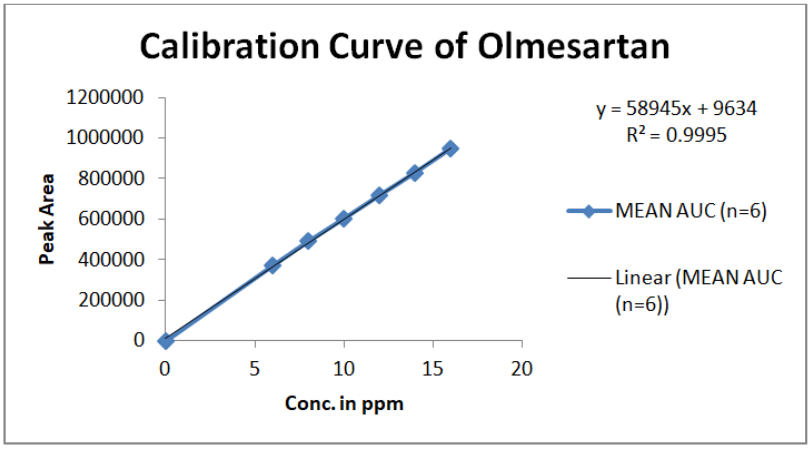


Fig 3: Calibration curve of Olmesartan (API)

Table 10: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
6	370200
8	490231
10	602707
12	717538
14	829248
16	947852

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-38, % RSD < 2%) the developed RP-HPLC method for the analysis of Olmesartan (API).

Table 11: Result of method robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.52
Flow (0.9 ml/min)	0.56
Temperature (27^0C)	0.52
Temperature (23^0C)	0.49
Wavelength of Detection (270 nm)	0.97
Wavelength of detection (266 nm)	0.98

LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.90 & 2.90 µg/ml respectively.

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-12.

Table 12: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.45
2	Asymmetry	T ≤ 2	Olmesartan=0.25
3	Theoretical plate	N > 2000	Olmesartan=5248

Table 13: Recovery Data for estimation Olmesartan medoxomil in Ritemed tablets

Brand name of Tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Assay % (±SD)
Olmesartan	20	19.96 (±0.498)	99.8 (±0.343)

The amount of drug found in Olmesartan Tablet was found to be 19.96 (±0.498) mg/tab for Olmesartan & % assay was 99.8 (±0.343).

RESULTS AND DISCUSSION

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 in previous chapters.

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 Symmetry C18 Column, 250 mm x 4.6 mm and 5µm column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl).

The drug was found to be Soluble in ethanol, DMSO, Dimethyl formamide, sparingly soluble in aqueous buffers. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Olmesartan it is evident that most of the HPLC work can be accomplished in the wavelength range of 210-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay and stability related impurity studies which can help in the analysis of Olmesartan in different formulations.

CONCLUSION

A sensitive and selective RP-HPLC method has been developed and validated for the determination of Olmesartan in pure form and Pharmaceutical dosage form. The UV detection was performed at 268nm. The Retention time of Olmesartan was found to be 3.544minutes. The Percentage Standard Deviation (%RSD) of the Olmesartan was and found to be 1.63%. The detector response was linear in the concentration range of 0-16µg/ml. The respective linear regression equation being Y= 58945.x + 9634 with R2 = 0.999. The percentage recovery values of Olmesartan in pharmaceutical dosage form were found to be within the limits. The limit of detection and the limit of quantification for Olmesartan were found to be 0.90µg/ml and 2.90µg/ml respectively. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility, accuracy. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Olmesartan in different marketed formulations. The developed method was validated as per ICH guidelines for accuracy calculated as % recovery was in the range of 98.0% to 102.0%. The statistical analysis of the data showed that the method is reproducible and selective for the estimation of Olmesartan in pure form and marketed Pharmaceutical dosage form during routine analysis. The results of the study showed

that, the proposed RP-HPLC method was simple, rapid, precise, accurate which can be used for the routine determination of Olmesartan in pure form and marketed pharmaceutical dosage forms.

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