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A NOVEL VALIDATED STABILITY INDACATING METHOD OF OLMESARTAN BY USING REVERSE PHASE CHROMATOGRAPHIC TECHNIQUES

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Abstract: The objective of the current study was to develop and validate a rapid, precise, specific and stability-indicating reverse phase HPLC method for the quantitative determination of olmesartan in its dosage form. The determination is done for the active pharmaceutical ingredient in its pharmaceutical dosage form in the presence of degradation products. The drug was subjected to stress conditions of acid, alkali, thermal, photolytic, humidity and peroxide. As per international conference on harmonization (ICH) prescribed stress conditions to show the stability-indicating power of the method. It was found olmesartan is very sensitive to various stress conditions. The chromatographic conditions were optimized using the samples generated from forced degradation studies. Regression analysis shows an r value (correlation coefficient) 0.999 for olmesartan. The chromatographic separation was achieved on a symmetry C18 stationary phase. The method employed an isocratic elution and the detection wave-length was set at 379 nm. The mobile phases consists of water and methanol delivered at a flow rate of 1.5 mL/min. The developed RP-HPLC method was validated with respect to linearity, accuracy, precision and robustness.

Keywords: Olmesartan, Forced degradation, Assay, Method Validation, HPLC

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INTRODUCTION

OLMAT (Olmesartan)[1] is a tablet dosage form belonging to ANTI-HYPERTENSIVE which is used as an anti-hypertensive, These oral administrative dosage forms are always convenient and lead to better compliance. This drug is beneficial and better compliance in terms of cost and therapeutic categorization, CAS Number:144689-63-4, chemically: (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl) methyl 4-(2-hydroxypropan-2-yl)- 2- propyl- 1 - ({4-[2-(2*H*- 1, 2, 3, 4- tetrazol - 5 - yl) phenyl] phenyl} methyl) - 1 *H* - imidazole - 5 - carboxylate[2]. There are several research publications for determination of olmesartan. A Reversed phase high performance liquid chromatographic method and validated for the estimation of Olmesartan medoxomil in bulk and formulation[3], another such method was developed and validated a simple, sensitive and precise RP-HPLC-DAD method for the determination of olmesartan medoxomil (AT-II receptor blocker) in the presence of its degradation products [4], another accurate, precise, specific, and reproducible and stability indicating HPLC method for the estimation of Olmesartan medoxomil (OLM) in presence of its degradation products and related impurities for assessment of purity of bulk drug and stability of its dosage forms.[5]. Further a simple reversed phase HPLC method for the simultaneous determination of olmesartanmedoxomil in combination with hydrochlorothiazide.[6] another such method has described a simple, precise, rapid, efficient and reproducible reverse Phase high performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of AT and OLM present in its tablet dosage forms[7], another such method described reversed phase liquid chromatography (RP-HPLC) and thin layer chromatography (HPTLC) densitometry methods as a stability indicating assays of olmesartan medoxomil in presence of its acid or alkaline induced degradation products[8.] A simple, sensitive and specific liquid chromatography (RP-HPLC) method and validated for the quantification of hydrochlorothiazide and losartan potassium in tablet dosage form.[9] Another simple and accurate RP-HPLC method for the simultaneous estimation of Hydrochlorothiazide, Amlodipine besylate and Valsartan.[10] such, another simple, sensitive, and inexpensive high-performance liquid-chromatographic method for simultaneous determination of hydrochlorothiazide and candesartan cilexetil in pharmaceutical formulations.[11] development of a simple, selective, rapid, precise and economical reverse phase high pressure liquid chromatographic method for the simultaneous estimation of nebivolol and hydrochlorthiazide from pharmaceutical formulation.[12] A simple, precise and rapid HPLC method and validated for the estimation of quinapril and hydrochlorothiazide simultaneously in combined dosage form.[13] A HPLC method on C18 column using methanol-0.1% phosphoric acid (20: 80) as mobile phase,

and the detection wavelength was 327 nm, the flow rate was 1.0 ml x min (-1) and the temperature of column was 40 degrees C.[14]. Another method has been developed and validated a simple, specific, accurate and precise stability-indicating reversed-phase high-performance liquid chromatographic method for simultaneous estimation of olmesartanmedoxomile (olme), amlodipine besylate (amlo) and hydrochlorothiazide (hctz) in tablet dosage form.[15] development of a simple, fast and precise reverse phase, isocratic HPLC method for the separation and quantification of telmisartan and hydrochlorothiazide in pharmaceutical dosage form.[16]. All the methods discussed are lacking in one or more areas with some essential data so, simple method which is simple, accurate, precise and reproducible was discussed.

MATERIALS AND METHODS

INSTRUMENTION

Waters LC system equipped with 2695 pump and 2996 photodiode array detector was used. The output signals were monitored and integrated using waters Empower 2.0software.Analytical balance (Model:AB 204S, Make: Mettle Toledo) and Micro balance(Model: XP 6, Make: Mettle Toledo) were used for weighing. Systronics digital pH meter 361 was used to adjust the pH of the buffer.Degassing of the mobile phase was done by sonication using spinco Biotech ultra sonicator)Filtration was done by using millipore vaccum filter.

Drugs and chemicals:

Pure working standard of olmesartan was kindly gifted from Hetero drugs Ltd., Hyderabad, India. The HPLC grade methanol, was purchased from Merck.

PREPARATION OF SOLUTIONS

Preparation of Mobile phase

A mixture of HPLC Water 400ml (40%) and 600 ml of Methanol HPLC (60%) were taken and degased in ultrasonicator for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of diluent: The mobile phase itself is used as a diluent

Preparation of standard solution

Accurately weighed and transferred 10 mg of olmesartan working standard into a 25ml clean dry volumetric flask, added about 15ml of diluent and sonicated to dissolve it completely and made volume up to the mark with the same diluent. further diluted 1ml of the above solution to 10ml .

Preparation of placebo solution

Weighed accurately 88mg of placebo powder into 25mL volumetric flask, added 15ml of the diluent and sonicated for 20min and diluted to the volume with diluent. Further 1ml of the above solution is diluted to 10 ml.

Test preparation

Accurately weighed and finely powdered 10 tablets of **OLMAT** and transferred an amount of the powder equivalent to 10mg of olmesartan into a 25ml of volumetric flask, added 15ml of the diluent and sonicated for 20min and diluted to the volume with diluent. Further 1ml of the above solution is diluted to 10ml

Optimized chromatographic conditions:

After systematic and detailed study of the various parameters involved in the method, the following conditions were employed.

Mobile phase: water : methanol (40:60 v/v)

Column : Symmetry C18 (4.6 x 150mm, 3.5 μm, Make: YMC) or equivalent

Flow rate : 1.5 ml per min

Wavelength :379 nm

Injection volume : 20 μL

Column oven Temperature : Ambient

Run time : 8min.

PROCEDURE

Column was equilibrated for at least 60 minutes with the mobile phase flowing through the system at a rate of 1.5mL/min. Detector was set at a wavelength of 379nm. Separately injected 20μL of diluent, placebo, standard solution, test solutions into the chromatograph and the chromatograms were recorded. The percent assay values of the olmesartan were calculated by using the following formulae.

% Assay of the olmesartan:

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Peak Area of olmesartan obtained with test preparation

AS = Peak Area of olmesartan obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

ANALYTICAL METHOD VALIDATION

System suitability:

According to the USP 33 System suitability is the integral part of the chromatographic method. This test was conducted to verify that the reproducibility and effectiveness of the system is adequate for the analysis.

To ascertain its effectiveness 20µL of freshly prepared standard solution containing 40µg/mL of olmesartan was injected 6 times into the the HPLC system by using optimized chromatographic conditions and System suitability results were calculated.

The %RSD for the peak area and retention time of the drug was found to be less than 2.0%. The theoretical plates were more Than 2000 for the drug. Tailing factor was found to be less than 2.0. All the results were tabulated in the table no:1

Specificity:

Blank and placebo interference:

A study to establish the interference of blank and placebo was conducted. Analysis was performed on placebo preparation described previously in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of Blank and placebo solutions shown no peaks at the retention time of olmesartan. This indicates that the excipients used in the formulation did not interfere in the estimation. The chromatograms of blank and placebo using the proposed method were shown in figure 2 and 3

Interference from degradation products

Preparation of degradation samples

Preparation of sample for Acid degradation

OLMAT sample was refluxed with the 1M HCl at 60°C for 1hour and then neutralized with 1N NaOH. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

Preparation of sample for Alkaline degradation

OLMAT sample was refluxed with the 1M NaOH. at 60°C for 1hour and then neutralized with 1M HCl. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

Preparation of sample for Oxidative degradation

OLMAT sample was refluxed with the 10%H_2O_2 by heating on water bath at 60°C for 1 hour. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

Preparation of sample for photolytic degradation

OLMAT sample was exposed to UV (200watt-hr/m^2) and visible (1.2 million lux hrs) The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

Preparation of sample for thermal degradation

OLMAT sample was exposed to temperature at 105°c for 24hrs . The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

Preparation of sample for humidity degradation

OLMAT sample was exposed to 85% humidity for 24hrs. The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

All the stressed samples were injected into the HPLC system by using optimized chromatographic conditions and the chromatographs were recorded. The chromatograms of the stressed samples were evaluated for peak purity of the drug using PDA detector and Empower software. In all forced degradation samples all the three drugs passed the peak purity (purity angle is less than purity threshold). All the degradant peaks were observed for the drug. Thus the method can be used for estimation of olmesartan in bulk and pharmaceutical formulations and also the method is stability indicating. The results are given in the table no. 2

Method precision

Precision of the method was conducted by performing the assay of OLMAT tablets 6 times. The samples were prepared six times according to the test preparation mentioned earlier and analyzed by using the test method. The % Assay values were calculated for the drug and found to be in between 98.0% - 102.0%. The %RSD values were found to be less than 2.0%. The results were given in the table no.3

Limit of Detection and Limit of Quantification

A study to establish the Limit of Detection and Limit of Quantification of olmesartan was conducted. Limit of detection and Limit and quantification were established based on signal to noise ratio. A series of dilutions of the test solution were injected. Limit of detection was established by identifying the concentration which give signal to noise ratio of about 3. Limit of Quantification was established by identifying the concentration which give signal to noise ratio of about 10.The results of the LOQ and LOD are given in the table no 4

Accuracy

Accuracy for olmesartan was conducted by spiking the drug to the placebo powder at five different levels of the target concentration (i.e. 50%, 75%, 100% , 125% and 150%) and each level three times. The mean %Recovery and %RSD values were calculated. The %Recovery values for the drug was found to be between 98.0% to 102.0% and %RSD values were found to be less than 2.0% . The accuracy results were tabulated in the table No.5

Linearity and range

Linearity of the detector response was established by plotting a graph of concentration versus peak area. A series of solutions of standard were prepared by appropriate dilutions of Linearity standard stock solution.

Preparation of Linearity stock solution:

Weighed accurately and transferred 25.0 mg olmesartan WS into 50mL volumetric flask, added 30 mL diluent of the diluent and sonicated for 20min and diluted to the volume with diluent, filtered through 0.45µm filter.

Preparation of Linearity solutions

Series of solutions in the range of 25% to 150% of target concentration were prepared by transferring 0.5mL, 1.0mL, 1.5mL, 2.0mL, 2.5mL, 3.0mL of Linearity stock solution into separate 25.0mL volumetric flasks and making the volume up to the mark with the diluent. The detector response was found to be linear in the range of 10.0 to 60.0µg/mL for olmesartan .The correlation coefficient values were found to be within the limits. The linearity and the regression data was tabulated in Tables No's 6 &7

Ruggedness

A study to establish ruggedness of the method was conducted by preparing and analyzing the standard and test preparation on two different days by two different analysts on two different columns and two different HPLC systems. The system suitability parameters and the % Assay values of all the three drugs were calculated and the differences between the two analysts were evaluated and the method was found to rugged. The results were tabulated in the table no.8

Robustness

A study to establish the effect of variation in flow rate, column temperature, pH of the buffer in the mobile phase was conducted. Standard and test solutions prepared as per the proposed method and were injected into the HPLC system. The system suitability parameters, and the %Assay values were evaluated and the method was found to be robust. All the results were tabulated in the table no.9

RESULTS AND DISCUSSIONS

The drug solution was scanned from 200-400 nm, it was observed that the drug show appreciable absorbance at 379nm., hence detection was set at 379nm for method development purpose. Attempts were made to get good separation of the drug by varying parameters like, flow rate, pH, buffer molarity, buffer components, type of organic modifier, gradient times, and buffer: organic modifier ratio and could get good elution time in isocratic mode. To achieve this, experiments were conducted by changing the columns and mobile shares but unsuccessful in getting good peaks with less run time. Then method was optimized to separate the main peak. The satisfactory chromatographic separation,

with good peak shapes were achieved on Symmetry C18 (4.6 x 150mm, 3.5 μm, Make: YMC) or equivalent with mobile phase water : methanol (40:60) with a flow rate of 1.5 ml/min. All the System Suitability parameters are within the acceptance limits. The calibration curve for olmesartan was obtained by plotting the respective peak areas against their concentration. The graph was found to be linear over the range 10-60 μg/ml for olmesartan with the correlation coefficient 0.999 respectively. The drug which shows that the good correlation exists between peak area and concentration of the drug. The ruggedness was performed and the % rsd was less than 2, hence, method was rugged. The high % recovery values obtained for the drug show that the method is accurate. The LOD value of olmesartan was found to be 0.01 μg/ml, The LOQ was 0.05μg/ml respectively. The low values of LOD and LOQ show that the method is sensitive and can estimate at micro gram level. The absence of additional peaks indicates the method is specific and the drugs were stable in the diluents for 8 hours which is sufficient to complete the work. The stability indicating studies were performed for the above mentioned drug viz…. acid, alkali, Thermal, Humidity, Photolytic, Peroxid and the percentage degradation was 5.2%, 7.4%, 6.0%, 9.2%, 8.7%, 4.8%, respectively.

CONCLUSION

The proposed R.P. high-performance liquid chromatographic method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the linear concentration range. In this method, there was no interference from matrix sources. Moreover, the lower solvent consumption along with the short analytical run time of 10 minutes that allows the analysis of a large number of samples in a short period of time. Therefore, this HPLC method can Be used for routine analysis of these drug in bulk, pharmaceutical formulations and also for stability studies.

Figures and Tables

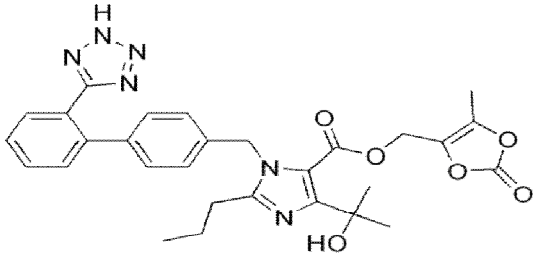

Fig no.1 Stucture of Olmesartan

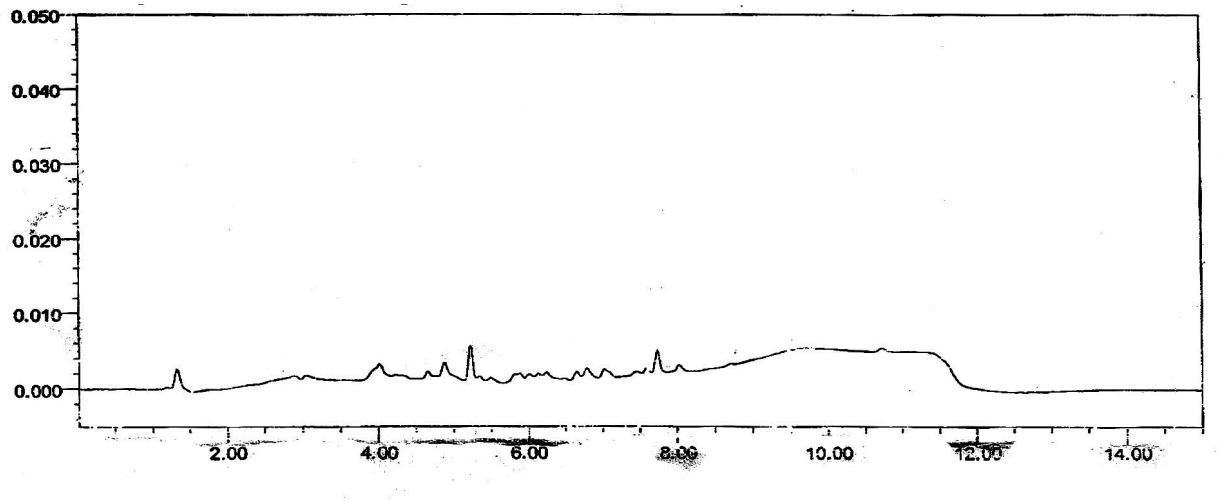

Fig no.2 Representative chromatogram of the Blank solution

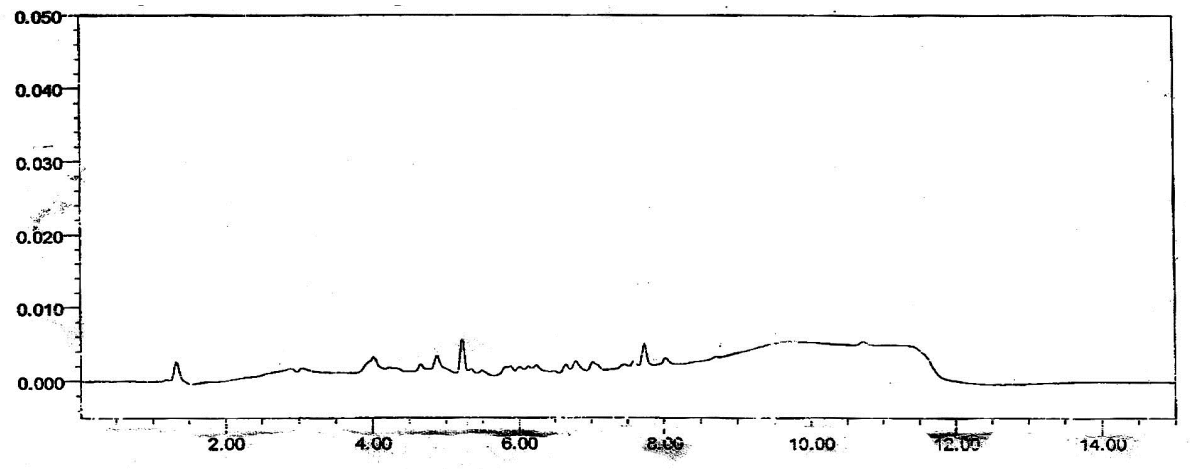

Fig no.3 Representative chromatogram of the placebo

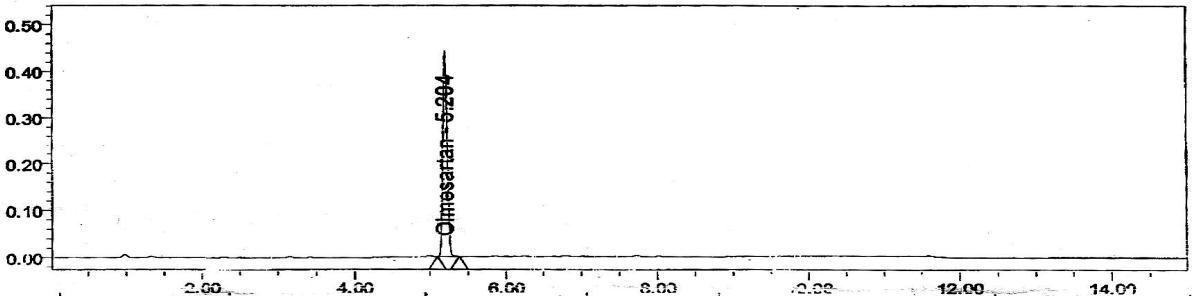

Fig no.4 Representative chromatogram of the standard solution

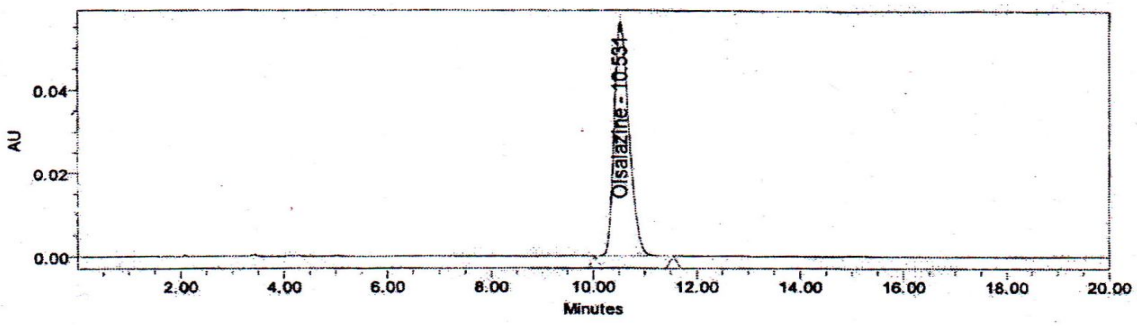

Fig no.5 Representative chromatogram of the sample solution

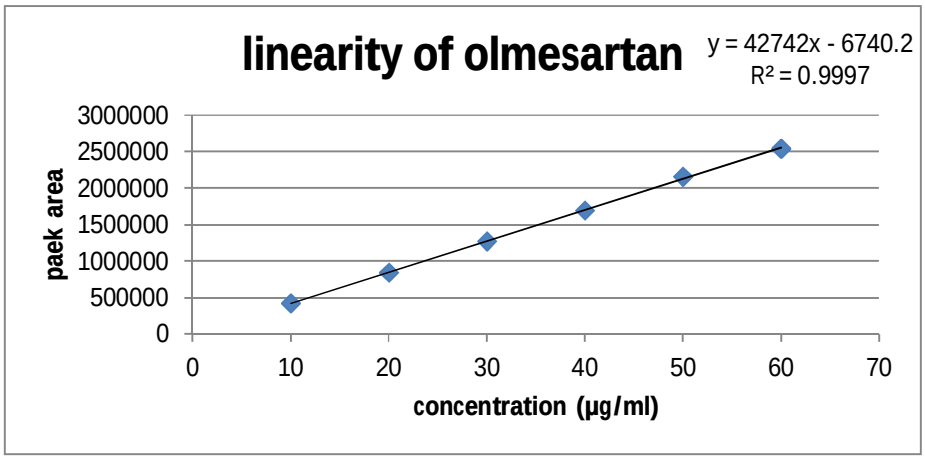

Fig no 6. Graphical Representation of linearity of olmesartan

Table 1 System suitability for Olmesartan

S.no	Retention time	Peak area	Theoretical plates	Tailing
1	5.184	1694718	6594	105
2	5.194	1698725	6542	1.04
3	5.204	1694029	6854	1.06
4	5.298	1699085	6589	1.06
5	5.192	1694975	6523	1.06
6	5.201	1699914	6854	1.05
7	5.204	1694934	6548	1.06
8	5.199	1698975	6587	1.05
9	5.204	1695749	6598	1.07
10	5.196	1697941	6547	1.09
AVARAGE	5.208	1696905		
SD	0.032	2223.0		
%RSD	0.6	0.1		

Table No. 2 Degradation results of Olmesartan

STRESS CONDITION	PURITY ANGLE	PURITY THRESHOLD	% ASSAY	%DEGRADATION
Acid degradation	0.17	0.21	97.5	5.2
Alkali degradation	0.14	0.19	99.7	7.4
Thermal degradation	0.21	0.24	94.9	6.0
Humidity degradation	0.15	0.18	98.3	9.2
Photolytic degradation	0.19	0.25	99.1	8.7
Peroxide degradation	0.23	0.29	93.5	4.8

Table no 3 Method precision for Olmesartan

S.No.	%Assay
	OLMESARTAN
1	100.7
2	99.4
3	98.7
4	98.9
5	99.5
6	99.5
AVERAGE	99.5
SD	0.7
% RSD	0.7

Table No. 4 LOD and LOQ data

Component name	Limit of Detection	Limit of Quantification		
	Concentration(µg/ml)	Concentration(µg/ml)	%Mean recovery	Concentration(µg/ml)
Olmesartan	0.01	0.05	100.9	1.1

Table No. 5 Accuracy for Olmesartan

S.No.	%Spike level	Amount added(mg)	Amount found(mg)	%Recovery	Statistical parameters
1	50%	19.51	19.49	99.9	Mean=99.8
2		19.79	19.71	99.6	
3		19.81	19.85	100.2	SD=0.25
4		19.62	19.58	99.8	
5		19.59	19.49	99.5	%RSD=0.25
6		19.92	19.9	99.9	
7	75%	30.12	30.05	99.8	Mean=99.8
8		30.18	30.1	99.7	SD=0.09
9		29.95	29.92	99.9	%RSD=0.09
10	100%	40.15	40.05	99.8	Mean=99.7
11		40.45	40.35	99.8	SD=0.07
12		41.55	41.4	99.6	%RSD=0.07
13	125%	50.87	50.79	99.8	Mean=99.89
14		50.12	50.009	99.8	SD=0.14
15		50.18	50.2	100.0	%RSD=0.14
16	150%	60.59	60.55	99.9	Mean=99.9

17		60.41	60.35	99.9	
18		60.48	60.45	100.0	**SD=0.25**
19		60.73	60.7	99.9	
20		60.97	60.95	100.0	**%RSD=0.25**
21		60.81	60.79	100.0	

TABLE NO 6. LINEARITY FOR OLMESARTAN

S.NO.	Linearity level	Concentration(µg/ml)	Peak area
1	25	10	422858
2	50	20	845458
3	75	30	1271454
4	100	40	1695018
5	125	50	2058144
6	150	60	2542487

TABLE NO 7. REGRESSION DATA OF THE PROPOSED METHOD

Sno.	PARAMETERS	OLMESARTAN
1	Leniarity (µg/ml)	10.0 - 60.0
2	Regression(mx+c)	41885x+6593
3	Slope(m)	41885
4	Intercept(c)	6593
5	Correlation coefficient (r^2)	0.999

Table no: 8. Ruggedness of Olmesartan

S.No	OLMESARTAN		
	ANALYST-1	ANALYST-2	OVERALL RESULTS
1	99.8	98.4	Mean 99
2	99.1	99.7	SD 0.7
3	99.2	98.1	%RSD 0.7
4	99.7	99.5	
5	98.5	99.4	
6	100.7	99.4	
AVARAGE	99.5	99.1	
SD	0.8	0.7	
% RSD	0.8	0.7	

Table no 9. Robustness for proposed method

Optimum conditions	Modifications	Retention time	Asymmetric factor	Theoretical plates
		OLM	OLM	OLM
Mobile phase composition (Water :Methanol) (40:60 v/v)	40:60	5.012	0.96	5421
	60:40	5.412	1.218	5312
pH (2.5)	2.4	5.318	1.21	5681
Column temperature (40°C)	2.6	5.108	1.31	5512
	35°C	5.812	1.21	7061
Flow rate (1.5 mL/min)	45°C	5.312	0.98	5816
	1.4	2.798	1.21	6012
Wave length (379nm)	1.6	3.572	0.98	7568

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