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A New Stability Indicating RP-HPLC Method for the Assay of Olmesartan In Market Formulations

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ABSTRACT

A new stability indicating RP-HPLC method was described for the assay of olmesartan in market formulations. This developed RP-HPLC method was based on high performance liquid chromatographic (HPLC) separation of olmesartan with the use of a reversed phase HPLC column [Kromasil BDS, C18, 100 x 4.6 mm, 5µ] with mobile phase consisting of .01M KH_2PO_4 buffer (pH-4.5) and acetonitrile in the ratio of 55:45 %v/v at ambient temperature. The flow rate of the mobile phase was adjusted to 1.0mL/min and the injection volume was 10µL. Detection was performed by photodiode array detector at a wavelength of 257nm and the chromatographic runtime was 8 minutes for the analysis. The reliability and analytical performance of the proposed method, including linearity, range, precision, accuracy, detection and quantitation limits, were statistically validated. The proposed method can be adopted apparently for routine quality control analysis of raw materials, formulations and testing

Keywords: Olmesartan, RP-HPLC, ICH guidelines.

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INTRODUCTION

Olmesartan[Figure 1] (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 [1-3] belongs to the class of angiotensin II receptor blockers (ARBs). It works by blocking angiotensin II in the body that causes the blood vessels to tighten. As a result, olmesartan relaxes the blood vessels there by lowers blood pressure and increases the supply of blood and oxygen to the heart. It is marketed by Unichem labs, India in the brand name of OLSAR (label claim 40mg of Olmesartan).

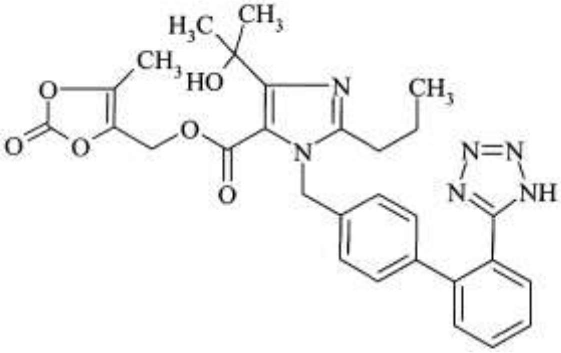


Figure 1: Structure Of Olmesartan

Literature survey revealed various publications [4-17] are available regarding determination of olmesartan in pharmaceutical dosage forms or in biological fluids On the basis of the above cited analytical survey till to date very few stability-indicating RP-HPLC methods [10 -17] were reported for the quantitative determination of olmesartan in pure and formulations that suffered from the drawbacks of long retention times and use of expensive reagents. The present paper describes the development and validation of a new stability indicating RP-HPLC method for olmesartan in pure and formulations using isocratic elution method as per ICH guidelines.

MATERIALS AND METHOD

Instrumentation:

The present analysis was carried out on HPLC Waters system equipped with Waters 2695 separation module equipped with Waters 2996 photodiode array and Kromasil C_{18} column, [250 mmx4.6 mm I.D; particle size 5 µm]. The volumes of standard and sample solutions were introduced to the column by means of a 10µL loop (Rheodyne) and the corresponding chromatographic data was processed using waters Empower 2 software.

Chemicals and solvents:

Olmesartan (99.9% Pure) used was supplied by Dr Reddys Labs, Hyderabad and formulation of olmesartan in the brand name of OLSAR (Strength: 40mg Olmesartan) manufactured by Unichem Laboratories Ltd, India were purchased from local pharmacy (Apollo). All chemicals and reagent used were of HPLC grade. Acetonitrile (HPLC Grade), Orthophosphoric acid (AR Grade), Potassium dihydrogen phosphate (AR Grade) and Triethylamine (AR Grade) were purchased from Qualigens Ltd., Mumbai, India. Hydrochloric acid, Hydrogen peroxide and sodium hydroxide was obtained from Rankem laboratories, India. Water purified with Millipore water system (Elix 10 C model) was used for the preparation of buffer.

Preparation of buffer:

Accurately weigh and transfer about 2.72gms of potassium dihydrogen phosphate and 1.0mL triethylamine in 1000mL of HPLC water. Adjust pH to 4.5 with 0.1%orthophosphoric acid solution. Filter the solution through 0.45µm membrane filter.

Preparation of mobile phase:

The mobile phase used in the present assay is prepared by mixing 0.01M KH_2PO_4 buffer pH-4.5 and acetonitrile in the ratio of 55:45%v/v respectively. Prior to use the mobile phase is filtered through 0.45µm nylon filter and degassed.

Diluent preparation:

Mobile phase is used as diluent in the present assay.

Preparation of standard drug solution (stock & working):

Standard stock solution of olmesartan (1.0mg/mL) was prepared by accurately weighing and transferring 100mg of olmesartan (99.9% pure) into a 100mL volumetric flask. To the above flask, add 30mL of diluent and sonicated to dissolve and later, diluted to volume with the same diluent. This stock solution was further diluted by transferring suitable aliquots into a separate 100mL volumetric flask and diluted to the volume with diluent to obtain final working standard solutions of olmesartan in the concentrations of range 5.0-30µg/mL, respectively.

Preparation of formulation sample solution:

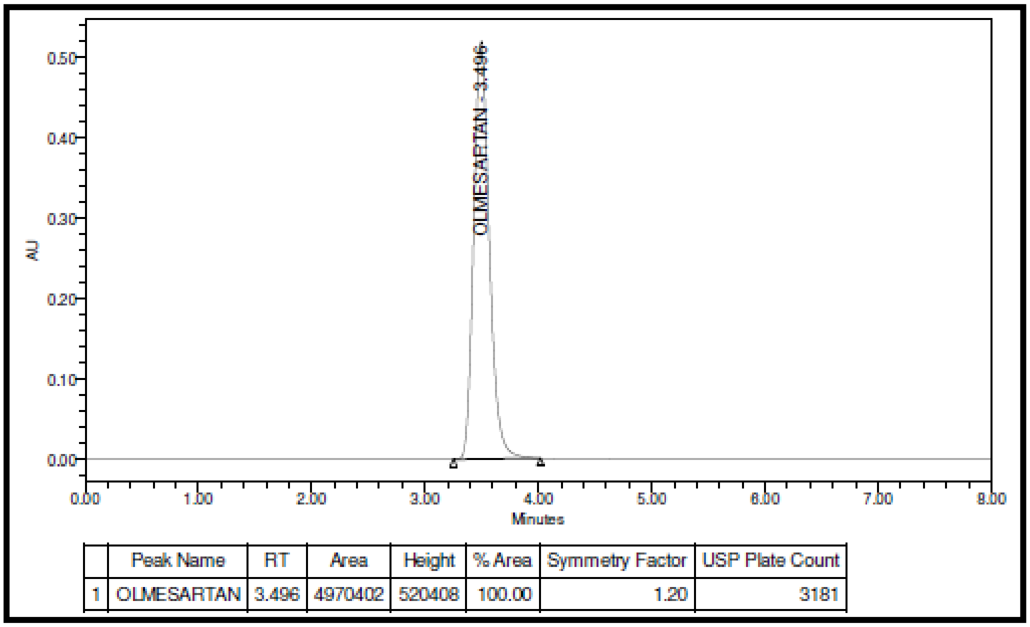
Ten tablets of OLSAR each containing 40 mg of olmesartan purchased from (local pharmacy, Hyderabad), were accurately weighed individually and finely powdered. Powdered sample containing 10 mg olmesartan was weighed and dissolved in methanol. The solution was filtered and the clear solution was transferred to a 100 ml volumetric flask. This solution was diluted with mobile phase to yield concentrations in the range of working standard solution and the solutions were filtered through a 0.45µm membrane then these solutions were injected into the HPLC system to analyze the olmesartan content by the proposed procedure.

RESULTS AND DISCUSSION

Method Development

Initially, efforts were made for the development of HPLC method quantification of olmesartan and for this purpose, Kromasil C18 (150 x 4.6) mm, 5 µ column, Water nova pack C18 (150 x 4.6) mm, 5 µ, Inertsil ODS 3V C18 (250 x 4.6) mm, 5 µ and Kromasil C18 (250 x 4.6) mm, 5 µ were tried. Out of these used HPLC columns, Kromasil C18 (250 x 4.6) mm, 5 µ, column found to be comparatively better and gave the graph with better Gaussian shape at retention time 4.096min. To improve the shape and width of the peak, for the above columns the mobile phase (0.01 M KH_2PO_4 and acetonitrile) in different combinations i.e, 40: 60, 50:50 and 55:45 (%v/v) at ambient temperature were tried. Out of these combinations, mobile phase in combination in the ratio 55:45 (%v/v) of exhibited good peak nature for olmesartan and the peak was found to be symmetrical at 257nm. And more over the tailing factor of the drug peak was less than 2% with good resolution

Finally, a flow rate of 1.0mL/min with an injection volume of 10µL with UV detection at 257nm was found to be best for the analysis of olmesartan. The validated chromatogram of olmesartan standard obtained with the proposed method is represented in (Figure 2) and the system suitability results of the proposed method are presented in Table 1.



	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	OLMESARTAN	3.496	4970402	520408	100.00	1.20	3181

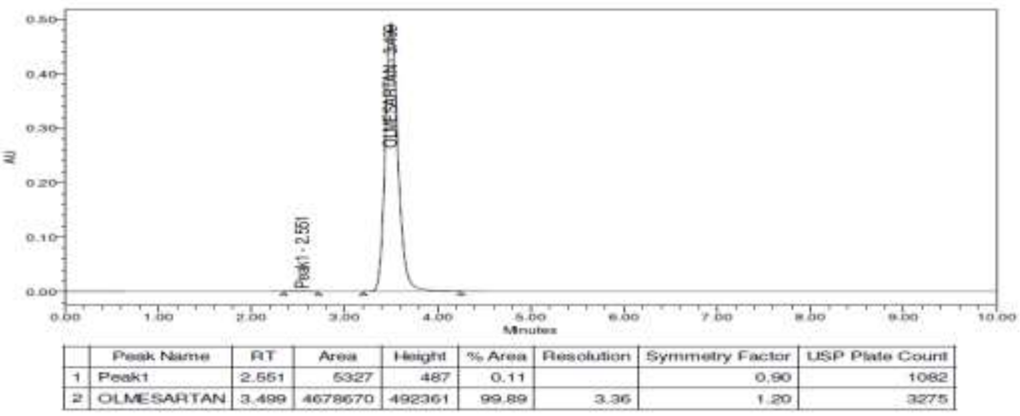
Figure: 2 - Typical HPLC Chromatogram Showing the Peak of Olmesartan

Chromatographic conditions:

The chromatographic separation of olmesartan was performed by injecting standard and sample volumes of 10µL into an kromasil C_{18} column, [250mm x 4.6mm I.D;particle size 5 µm] at ambient temperature at a flow rate of 1.0mL/min using the mobile phase containing potassium dihydrogen phosphate buffer [pH adjusted to 4.5 with 0.1% ortho phosphoric acid] and acetonitrile in the ratio of 55:45 %v/v at an wavelength of 257nm with an chromatographic runtime of 10 minutes respectively.

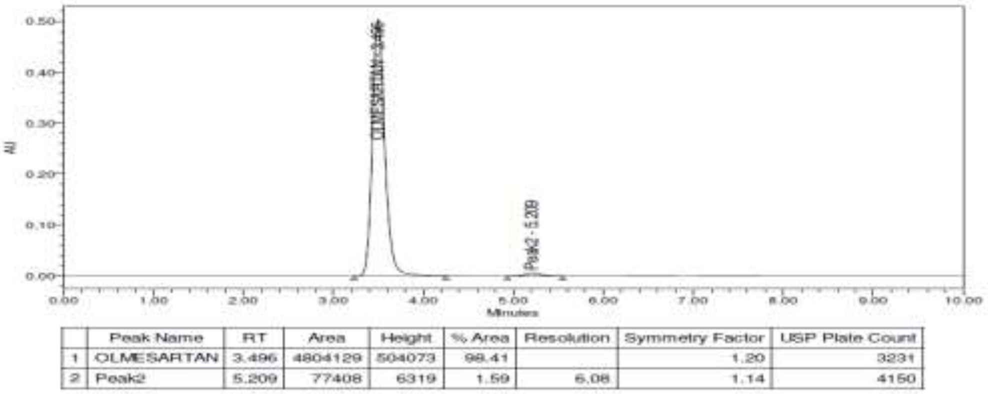
Forced degradation studies:

Stress studies were performed for olmesartan bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.1 N Hydrochloric acid), alkali (0.02 N NaOH, Hydrogen peroxide (30%), and heat (60^{o}C) to evaluate the ability of the proposed method to separate olmesartan from its degraded products. Peak purity tests were also performed by using of photo diode array detector to show the analyte chromatographic peak did not contain more than one substance. **Figure 3.a - d.**



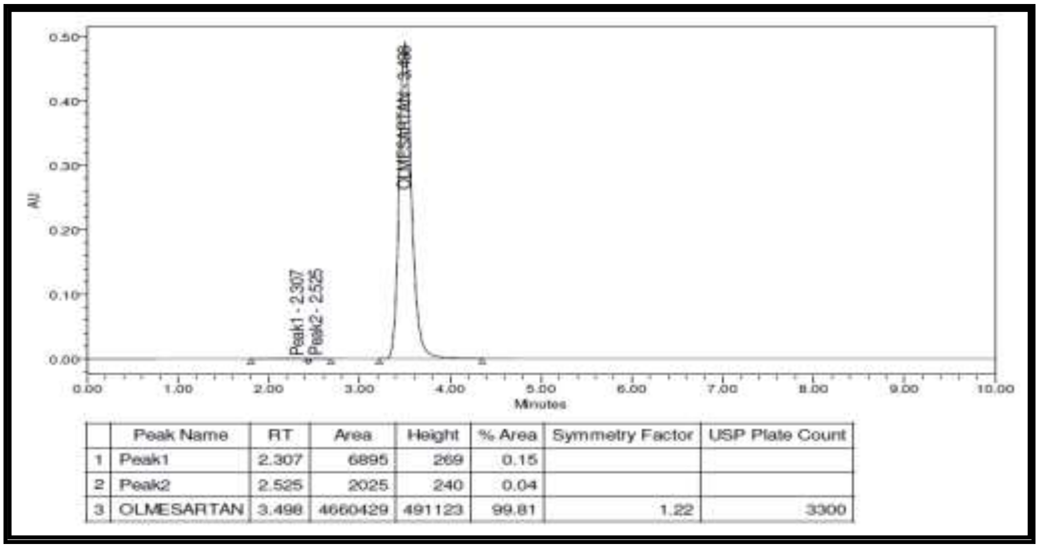
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	Peak1	2.551	5327	487	0.11		0.90	1082
2	OLMESARTAN	3.499	4678670	492361	99.89	3.36	1.20	3275

Figure: 3.A - HPLC Chromatogram of Olmesartan In Acidic Hydrolysis



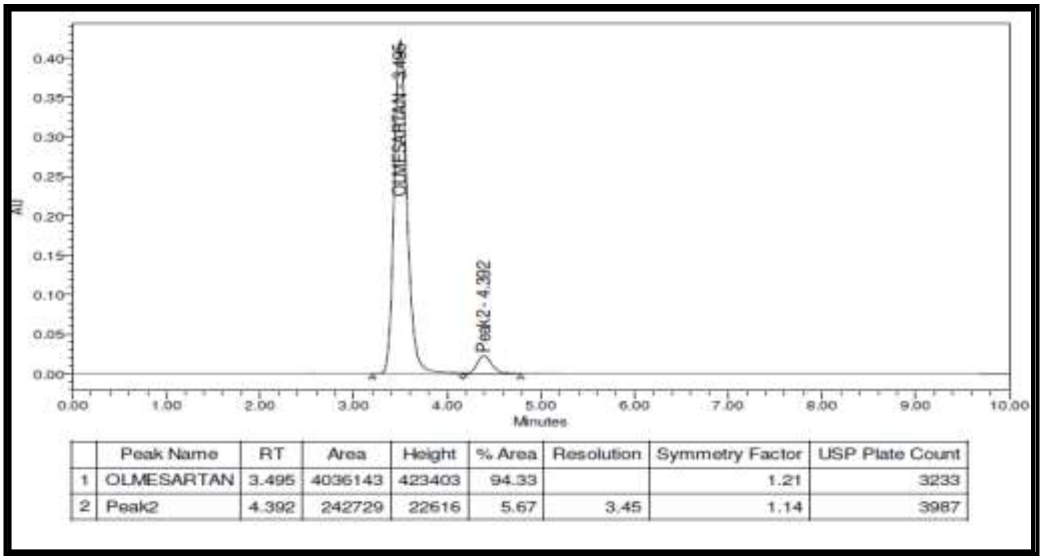
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	OLMESARTAN	3.496	4804129	504073	98.41		1.20	3231
2	Peak2	5.209	77408	6319	1.59	6.08	1.14	4150

Figure: 3.B - HPLC Chromatogram of Olmesartan In Basic Hydrolysis



	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	Peak1	2.307	6895	269	0.15		
2	Peak2	2.525	2025	240	0.04		
3	OLMESARTAN	3.498	4660429	491123	99.81	1.22	3300

Figure: 3.C - HPLC Chromatogram of Olmesartan In Peroxide Hydrolysis



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	OLMESARTAN	3.495	4036143	423403	94.33		1.21	3233
2	Peak2	4.392	242729	22616	5.67	3.45	1.14	3987

Figure: 3.D - HPLC Chromatogram Of Olmesartan In Thermal Degradation

METHOD VALIDATION:

After the development of suitable RP-HPLC method, it was subjected to validation as per ICH norms. All the validation studies were carried out with the injection volumes of 10µL of standard and sample solutions of olmesartan into the column respectively.

System suitability:

System suitability tests for the developed RP-HPLC were performed as per the ICH Guidelines. This test was carried at optimized chromatographic conditions by injecting 20µL of standard solution of olmesartan into the column at the concentration level of 20µg/ml and the results of system suitability parameters [Theoretical plates, resolution, tailing factor] were determined and are presented in Table 1 respectively.

Table: 1: System Suitability Parameters for Olmesartan by the Proposed Method

S.no	Parameters	Results
1.	Theoretical plates (n)	3181
2.	Asymmetry factor	1.2
3.	Retention time	3.496
4.	Peak area	4970402

Specificity:

The specificity of the developed RP-HPLC method for olmesartan was evaluated with the studies of diluent interference in the resolution of the present studied drug. From these studies it is revealed that the diluent has no interference on olmesartan resolution.

Linearity:

The linearity of the present RP-HPLC method was evaluated in the concentration range of 5.0-30µg/mL, corresponding to 25-150% of the nominal content of olmesartan. The peak area of each concentration was determined and recorded. A calibration plot was plotted with the peak areas recorded versus the concentration and was found to be linear in the investigation concentration range (Figure 4). The linear regression equation revealed y = 250703x - 12049 with correlation coefficient of 0.9999 respectively demonstrating its suitability for analysis. The linearity results for olmesartan were were tabulated in Table 2 From these studies it is revealed that a good functional linear relationship existed between the concentration of analyte and area under the peak.

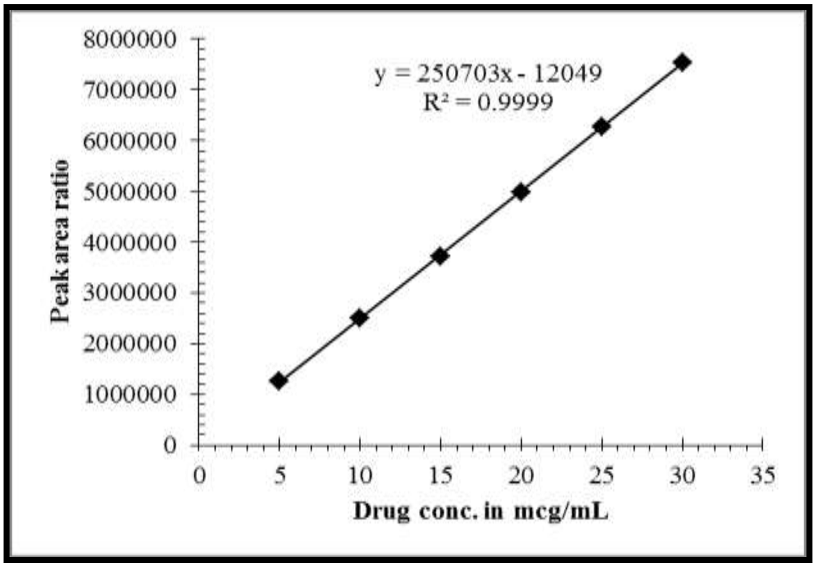


Figure:4 - Linearity Curve For Olmesartan

LOD & LOQ:

The LOD and LOQ of the proposed method was determined by the standard deviation method, as described above, were 0.309 and 1.30µg mL^{-1}, respectively, which indicated the proposed method can be used for detection and quantification of olmesartan over a very wide range of concentrations (Table.2).

Table:2: Results of Linearity Studies For Olmesartan by the Proposed Method

Linearity of Response For Olmesartan		
% Level (Approx.)	**Concentration (µg/mL)**	**Average area**
25	5	1265364
50	10	2501145
75	15	3720272
100	20	4970402
125	25	6259316
150	30	7535050
Slope; b		250703
Intercept; a		-12049
RSQ;R^2		0.9999
LOD (µg/mL)		0.309
LOQ (µg/mL)		1.30

Method precision:

Precision studies of the method were performed at one fixed concentrations of olmesartan in the linear range by using six independent series in the same day (intra-day precision) and the results were represented in **Table 3.** The measurement of the peak areas showed low value of % RSD (< 2), which suggested an excellent precision of the method.

Table 3: Results of Method Precision by the Proposed Method

S.No	RT	Area
1	3.489	4984879
2	3.491	4985044
3	3.496	4983265
4	3.492	4983540
5	3.495	4979057
6	3.49	4979545
Avg*	3.492	4982555
Std Dev*	0.003	2621.7
% RSD*	0.080	0.05

*Values are the averages of three determinations

Accuracy [recovery studies]:

The recovery experiments were performed by adding known amounts of the olmesartan in the placebo at three % levels, 50%, 100% and 150%. The recovery samples were prepared as aforementioned procedure and the solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for omlesartan ranged from

99.16% to 99.62% (Table 4) showed the best recoveries, indicating that the proposed method was accurate and suitable for intended use. The values are reported in Table 4 respectively.

Table: 4: Recovery Studies (Accuracy) Of Olmesartan

S. No	50% Area	100% Area	150% Area
Injection-1	2105021	4230213	6350535
Injection-2	2106045	4227856	6348546
Injection-3	2106656	4229989	6342033
Avg*	2105907	4229353	6347038
Amt recovered*	49.58	99.59	149.43
%Recovery*	99.16	99.59	99.62

***Values are the averages of three determinations

Robustness:

The evaluation of robustness of the developed RP-HPLC method was made by making slight changes in chromatographic conditions that include the change in flow rate and change in column temperature. On foregoing discussion the effect of flow rate on the resolution, it was changed by 0.2 units from 0.8 to 1.2 mL/min while the other mobile phase component was held as stated in chromatographic conditions. At the same time the effect of column temperature on resolution was studied at 33^{o}C and 37^{o}C instead of ambient temperature while the other mobile phase components were held constant stated in chromatographic condition and the results are represented in Table 5. The results of these studies reported negligible effect on the chromatographic parameters by slight variations in chromatographic conditions.

Table:5: Results of Robustness study

Robust conditions		Olmesartan		
		Theoretical plates	Rt	Peak area
Flow rate	0.8ml/min	3598	4.635	6690240
	1.2 ml/min	2831	2.812	4005720
Temperature	33^{o}C	3256	3.481	5021225
	37^{o}C	2836	2.785	5042120

Ruggedness:

The ruggedness of the present RP-HPLC method was evaluated by two different analysts (Analyst I & Analyst II) with different instrument in the same laboratory. The % RSD for peak areas were calculated and the experimental data were reported in **Table.6** and these results revealed the ruggedness of the developed method

Table:6: Results of Ruggedness Studies of Olmesartan

S NO	Analyst-1		Analyst -2	
	RT	Area	RT	Area
1	3.489	4984879	3.491	4967807
2	3.491	4985044	3.494	4967636
3	3.496	4983265	3.49	4969045
4	3.492	4983540	3.489	4968659
5	3.495	4979057	3.487	4968243
6	3.49	4979545	3.489	4981343
AVG*	3.492	4982555	3.490	4970456
STD DEV*	0.003	2621.7	0.002	5359.4
% RSD*	0.080	0.05	0.068	0.108

*Values are the averages of three determinations

Assay of olmesartan in formulations:

Analysis of marketed tablets (**OLSAR; Label claim-40mg**) was carried out using the above said optimized mobile phase and HPLC conditions. The % drug content of tablets obtained by the proposed method for olmesartan (**Table.7)** was found to be 99.99% respectively, indicated the suitability of this method for routine analysis of olmesartan in pharmaceutical dosage forms

Table.7: Results of Analysis of Olmesartan in Formulations

Pharmaceutical formulation	Amount of olmesartan		% recovery
	Labelled	Found*	
(OLSAR)	40 mg	39.99	99.99 %

*Values are the averages of three determinations

CONCLUSION

The present paper describes the development and validation of a new stability indicating RP-HPLC method for the assay of olmesartan in market formulations. Olmesartan was assayed on Kromasil BDS C-18 column (250mm×4.6mm, 5µ) column using buffer: Acetonitrile (55:45%v/v) as mobile phase and detection was carried out at 257nm. Results obtained in the validation studies indicated its suitability for routine tests. Under the conditions established there are no interference from vehicle. In addition, the good % recovery in tablet forms suggested that the excipients present in the dosage forms have no interference in their determination. The method is characterized by good precision and accuracy that enabled successful separation and quantitative determination of olmesartan in market formulations.. The %RSD was also less than 2.0% showing high degree of precision of the proposed method. The proposed method can be used as alternative method for routine analysis of olmesartan in market formulations respectively.

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REFERENCES

1. Chrysant SG, Chrysant GS.Expert Opinion on Pharmacotherapautics 2004;37: 657.
2. Unger T, McInnes GT, Neutel JM, Bohm M. Drugs 2004; 64:2731-2739.
3. Tomonori Murakami, Hidetoshi Konno, Naoto Fukutsu, Michinobu Onodera, Takao, Kawasaki, Fumiyo Kusu. Journal of Pharmaceutical and Biomedical Analysis 2008; 47:553-559.
4. Kun-Yan Li, Jian-Ping Liang, Bing-Qiang Hu,Yu Qiu, Chen-Hui Luo, Yun Jiang, Xiao-PingLin, Nong Yang.Clinical Therapeutics 2010; 32:1674-1680.
5. Dongyang Liu, Pei Hu, Nobuko Matsushima,Xiaoming Li, Li Li, Ji Jiang Celebier M,Altinoz S. Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography B 2007;856:190-197.
6. Chen SH. HPLC-MS for determining olmesartan in human plasmaNanFangVike DaXue Xue Bao 2008; 28: 1104.
7. Liud. Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry.J Chromatogr B 2007; 856:190.
8. Liu JF Wang. Determination of olmesartan in human plasma by HPLC with fluorescence detection.J Pharm Anal 2006; 26: 686.
9. Murakami T, Konno H, Fukutsu, Onodera M, Kawasaki T, Kusu F. Identification of a degradation product in stressed tablets of olmesartan medoxomil by the complementary use of HPLC hyphenated techniques. J Pharm Biomed Anal, 2008; 47: 553.
10. Vipul P Rane, Kiran R Patil, Jaiprakash N,Sangshetti, Ravindra D Yeole, Devanad B Shinde. Stability indicating LC method for the determination of olmesartan in bulk drug and in pharmaceutical dosage form. Chromatographia, 2009; 69:169.
11. Lisiane Bajerski, Rochele C Rossi, Carolina L Dias, Ana M Bergold, Pedro E Froehlich.
12. Stability indicating LC Determination of a new antihypertensive, olmesartan medoxomil in tablets. Chromatographia 2008;68; 991.
13. Celebier M, Altinoz S. Determination of olmesartan medoxomil in tablets by UV-Vis Spectrophotometry. Pharmazie, 2007;62(6):419.

14. Piyush Trivedi, Kartikeyan.C, Raman, Kachave, Rajendra Bhadane. stabilityindicating assay method for estimation of olmesartan medoxomil and its metabolite. Journal of Liquid Chromatography & Related Technologies 2009, 10, 1516.
15. Purnima D. Hamrapurkar, Kamalesh K. Gadapayale. Optimization and Validation of RP – HPLC: Stability Indicating Method for Determination of Olmesartan Medoxomil and Its Degraded Product Int. J. Appl. Sci. Eng 2013;11:2.
16. Chaitanya prasad MK, Vidyasagar G, Sambasiva Rao KRS, Ramanjeneyulu S. Development of RP-HPLC method for estimation of olmesartan medoxomil in tablet dosage forms. Der Pharma Chemica 2011; 3(6):208-212.
17. Selvadurai Muralidharan, Jaya Raj Kumar. Stability Indicating RP-HPLC method for the simultaneous determination of olmesartan medoxomil and atorvastatin calcium. Int J. of Pharm. & Life Sci 2012;3(11):2149-2152.
18. Chimalakonda Kameswara Rao, Kakumani Kishore Kumar, Maddala VijayaLaxmi, Polisetty Srinivasulu, Gutta Madhusudhan, Khagga Mukkanti1, Koduri Sai Venkata Srinivas. Development and Validation of Stability Indicating LC Method for Olmesartan Medoxomil. American Journal of Analytical Chemistry 2012;3:153-160.