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STABILITY INDICATING FORCED DEGRADATION RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF OLMESARTAN MEDOXOMIL

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ABSTRACT: A simple, precise, accurate, economical and reproducible HPLC method for estimation of Olmesartan in tablet dosage form has been developed. Quantitative HPLC was performed with HITACHI L2130 with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400), PUMP (LC-IOAT). C18 Develosil ODS HG-5 RP 150mm x 4.6mm 5µm particle size column receptor in vascular muscle. Its action is therefore independent of the pathway of angiotensin II synthesis was used in the study. The mobile phase of ACN: phosphate buffer (pH 3.1) was used in this study. The conditions optimized were: flow rate (1.0 ml/minute), wavelength (205nm) and run time was 10 min; column temperature was maintained at 40^0C. Retention time was found to be 6.31 min. The linearity was found to be in the concentration range of 10-100 µg/ml. The developed method was evaluated in the assay of commercially available tablets OLMESAR containing Olmesartan. The amount of drug in tablet was found to be 40mg. Results of analysis were validated statistically and by recovery studies. The recovery studies 99.27 % was indicative of the accuracy of proposed method. The precision was calculated as repeatability, inter and intraday variation (%RSD) for the drug. By using the method, stability of the drug has been studied.

INTRODUCTION: Hypertension is one of the most prevalent diseases with an estimated one billion cases worldwide.

The therapeutic standard was significantly improved in the 1980s by the introduction of losartan as the first nonpeptidic angiotensin II type-1 (AT1) receptor anta gonist. An entire therapeutic class, the sartans, has since been developed [1].

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Olmesartan medoxomil, chemically 2,3-dihyd-roxy-2-butenyl-4-(1-hydroxy-1-methylethyl)-2-propyl--1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-imidazole-5 carboxylate, cyclic 2,3-carbonate, is an angiotensin II re-ceptor blocker used as an antihypertensive agent (**Figure** 1) [2] . A thorough literature search has revealed that there are only a few HPLC methods reported for determination of olmesartan in human plasma [3].

Methods such as HPLC, HPTLC and simultaneous UV spectrophotometric have been reported for the estimation of olmesartan alone or in combination with other drugs [4-10]. The present study compares LC determination of olmesartan by identification and characterizing the impurity of olmesartan medoxomil [11-12].

The impurity profile of Active Pharmaceutical ingredients (APIs) and evaluation of their toxicity effects are necessary steps in developing a safe and effective drug and are essential for medical safety reasons [13]. It is mandatory that any new impurities present in the drug substance and drug product above the threshold limit are identified and characterized. The present manuscript describes the development and validation of stability-indicating RP-HPLC method and identification and characterization of alkaline degradation impurity of olmesartan medoxomile drug substance as well as drug product. Therefore, the objective of the reported research was to study the degradation of OLME under different International Conference of Harmonization (ICH) recommended stress conditions [14] and to evaluate the degraded products by MS and to establish a stability-indicating RP-HPLC method for accurate quantification of OLME in pharmaceutical dosage forms.

MATERIALS AND METHOD:

Instruments and Reagents: The chromatographic separation was performed on HITACHI L2130 with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400), PUMP (LC-IOAT). C18 Develosil ODS HG-5 RP 150mm x 4.6mm 5µm particle size column was used as a stationary phase. P^H Analyzer (ELICO), Electronic Balance, Ultra Sonicator has been used in the work. Olmesartan Active Pharmaceutical Ingredient (API) was provided by Hippo labs pvt ltd, kukatpally, Hyderabad. Orthophosporic acid, Acetonitrile, & water of HPLC grade were from Standard reagents, Hyderabad. Commercial formulations of OLMESAR were used as sample.

Optimized Chromatographic conditions:

Column	: C_{18} Develosil ODS HG-5 RP150mm x 4.6mm 5µm Particle Size
Mobile Phase	: Phosphate buffer: Acetonitrile (60; 40)
Flow Rate	: 1.0ml/minute
Wave length	: 205 nm
Injection volume	: 20 µl
Run time	: 10 minutes
Column temperature	: Ambient
Sampler cooler	: Ambient

Preparation of solutions:

Preparation of standard calibration curve: 25 mg of Olmesartan standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Olmesartan, so that the same wave number can be utilized in HPLC UV detector for estimating the Olmesartan. While scanning the Olmesartan solution we observed the maxima at 205 nm as per **Figure 1.**

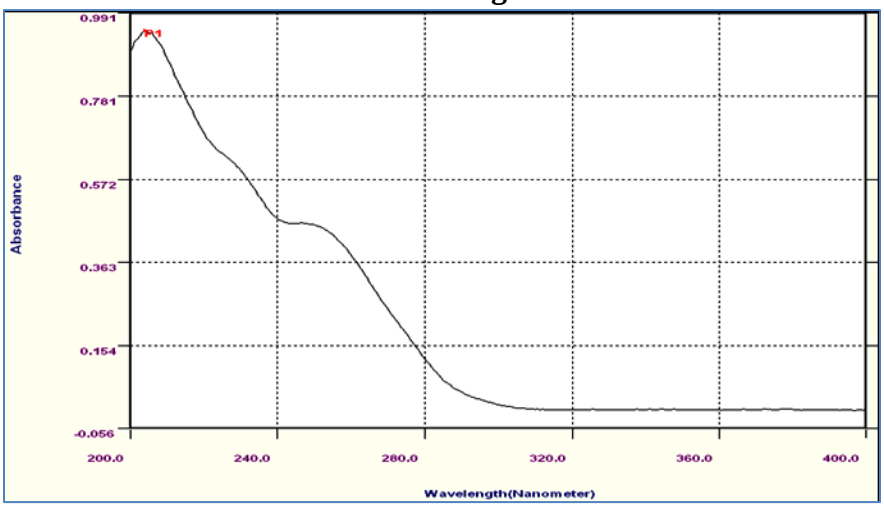

FIG. 1: SPECTRA SHOWING THE CALIBRATION CURVE OF OLMESARTAN

Analysis of Tablet Formulation:

Preparation of Standard solution: Working concentration should be around 10 µg/ml. Accurately weighed around 25mg of Olmesartan working standard, taken into a 25 ml volumetric flask, then dissolved and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000 mcg/ml. Further dilutions have been made to get the final concentration of 10 µg/ml **(Figure 2).**

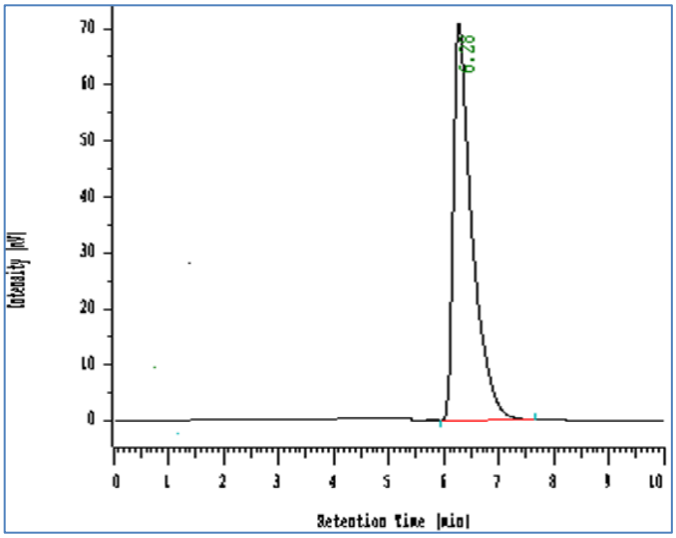

FIG. 2: CHROMATOGRAM OF OLMESARTAN (10 PPM) IN OPTIMIZED CONDITIONS

Preparation of Test solution: Diluted quantitatively an accurately measured volume of label claim solution with diluents to obtain a solution containing about a linear range. The procedure for preparing the solution is as per the guideline. As per the label claim, the tablets contain 40mg of Olmesartan 10 tablets were taken & an equivalent powder weight has been taken which contains 10 mg of Olmesartan (each of these has been taken in separate volumetric flask), than the powders has been dissolved in mobile phase & made 25ml with the diluents. Further dilution was done by taking 1ml of this solution in 10ml volumetric flask, dissolve and make up with the diluents. To extract the drug in the solution, it has been sonicated for 5 minutes followed by cyclo-mixing for 5 minutes. Resulting solution was filtered by using Millipore syringe filter (0.42 micron). Resulting clear solution was injected in HPLC in duplicate as per the above mentioned HPLC method **Figure 3.**

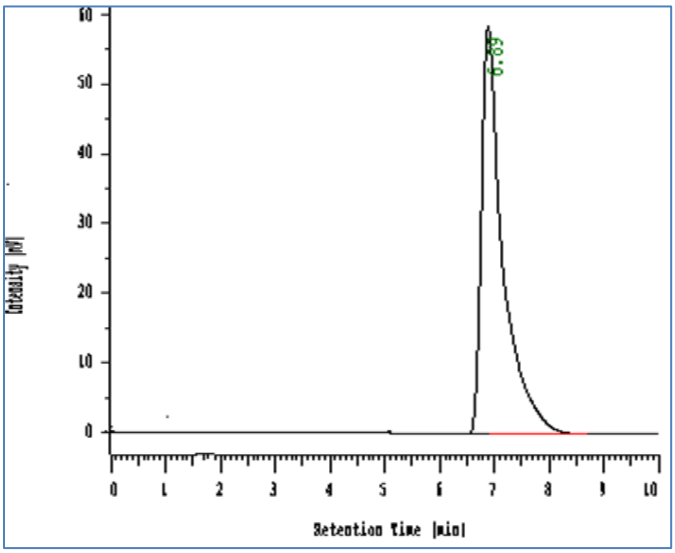

FIG. 3: CHROMATOGRAM OF OLMESARTAN MARKETED SAMPLE

Method Validation: As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantization.

Preparation of Calibration Curves: Calibration curve was prepared by taking appropriate aliquots of standard Olmesartan stock solution in different 10 ml volumetric flask and diluted up to the mark with diluents to obtain the final concentrations of 10, 20, 30, 40, 50, 60, 70 ,80, 90, 100 µg/ml of Olmesartan. Standard solutions (n=6) were injected, the sample volume was 20 µl with a flow rate of 1.0 ml/min.

Forced degradation studies: The protocol was strictly adhered to for forced degradation of Olmesartan Active Pharmaceutical Ingredient (API). The API (Olmesartan) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis and oxidative degradation.

Mother Sample: Before carry out the degradation studies the mother sample was prepared to find out the % degradation. It is prepared as the same method followed in sample or standard preparation.

Acid Hydrolysis: An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 1 M HCl was added & make up to the mark & kept for 8 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with diluent, then injected for HPLC analysis **Figure 4.**

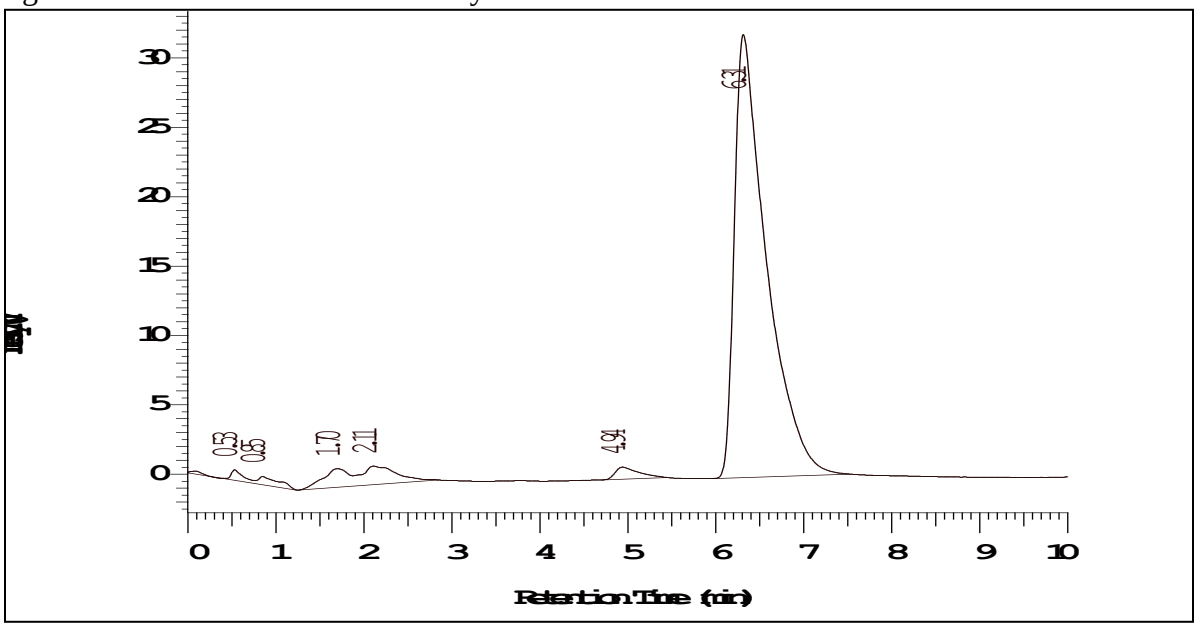

FIG. 4: CHROMATOGRAM SHOWING DEGRADATION IN 0.1 M HCL

Basic Hydrolysis: An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 1 M NaOH was added & make up to the mark & kept for 8 hrs. from that 1ml was taken in to a 10 ml volumetric flask & make up to the mark with diluent, then injected for HPLC analysis **Figure 5.**

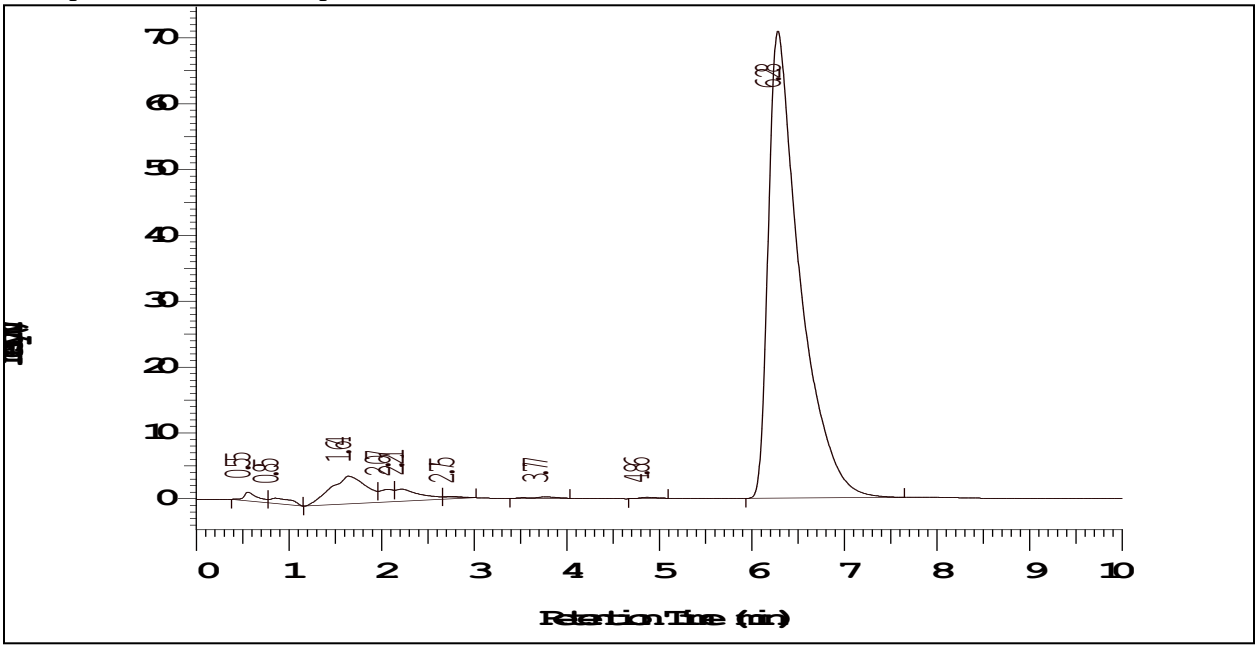

FIG. 5: CHROMATOGRAM SHOWING DEGRADATION IN 0.1 M NAOH

Oxidation with (3%) H_2O_2: An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 3% H_2O_2 was added & make up to the mark & kept for 8 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with diluent then injected for HPLC analysis **Figure 6.**

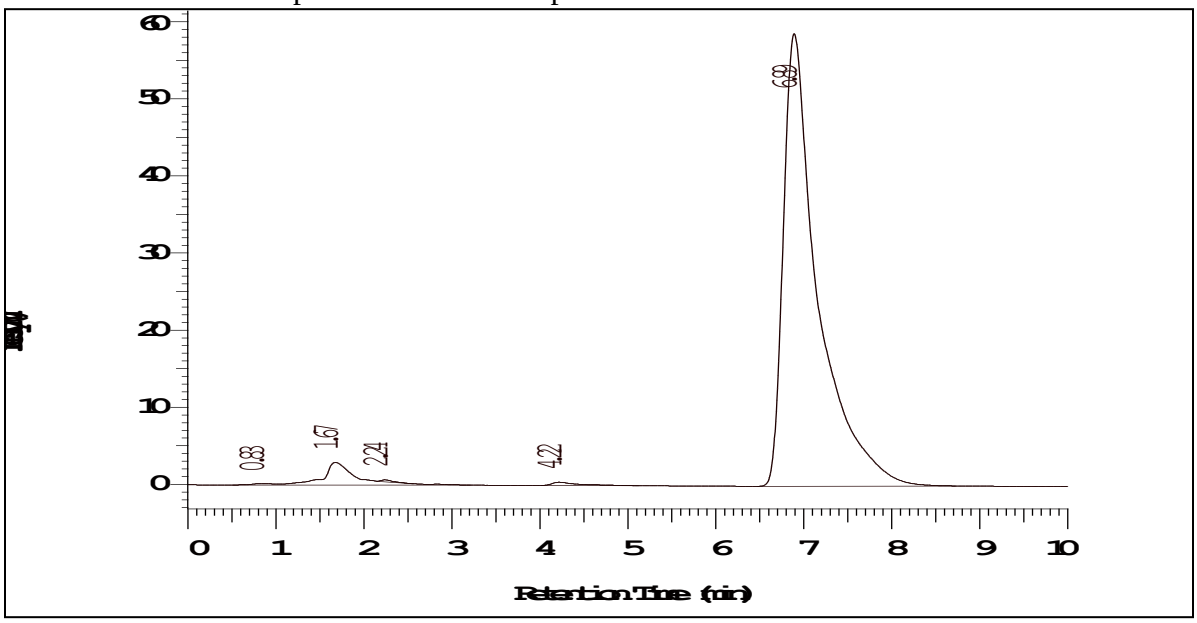

FIG. 6: CHROMATOGRAM SHOWING DEGRADATION IN 3% H_2O_2

RESULTS & DISCUSSION:

Results of degradation studies: The results of the stress studies indicated the **specificity** of the method that has been developed.

Olmesartan was degraded only in 3% H_2O_2 & 1N HCl, 1N NaOH conditions. The result of forced degradation studies are given in the following **Table 1.**

TABLE 1: RESULTS OF FORCE DEGRADATION STUDIES OF OLMESARTAN API

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	78.36	20.23	98.86
Basic Hydrolysis (0.I M NaOH)	24Hrs.	98.32	----------	98.32
3 % hydrogen peroxide	24 Hrs	93.31	----------	93.31

Method Validation:

Linearity and Range: The linearity of the method was determined at ten concentration levels ranging from 10-100 µg/ml for Omesartan. The calibration curve showed good linearity in the range of 10 – 100 µg/ml, for Olmesartan (API) with correlation coefficient (r^2) of 0.994. A typical calibration curve has the regression equation of y = 14694x + 67023 for Olmesartan **Figure 7.**

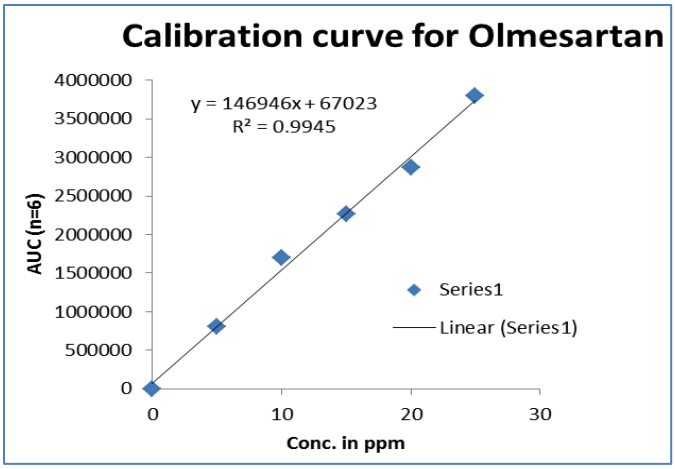

FIG. 7: CALIBRATION CURVE OF OLMESARTAN API

TABLE 2: LINEARITY READINGS

CONC.	AUC (n=6)
0	0
5	307771
7.5	358580
10	489726
12.5	616383
15	735232

Accuracy: The accuracy of the method was determined by recovery experiments. The recovery study was carried out by the standard addition method at three levels of 80, 100 and 120%. Each solution was injected in triplicate and the percentage recovery was calculated. Recovery was within the range of 100 ± 5% which indicates accuracy of the method presented in **Table 3.**

TABLE 3: RESULT OF RECOVERY STUDIES

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	99.18	Mean= 98.97667%
S_2 : 80 %	8	10	98.78	S.D. = 0.200083
S_3 : 80 %	8	10	98.97	% R.S.D.= 0.202152
S_4 : 100 %	10	10	99.87	Mean= 99.54%
S_5 : 100 %	10	10	99.54	S.D. = 0.33
S_6 : 100 %	10	10	99.21	% R.S.D.= 0.331525
S_7 : 120 %	12	10	99.32	Mean= 99.567%
S_8 : 120 %	12	10	99.65	S.D. = 0.33
S_9 : 120 %	12	10	99.98	% R.S.D. = 0.331159

Precision: 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 deviations were calculated for Olmesartan. 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.

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 4.**

TABLE 4: RESULTS OF INTRA-ASSAY & INTER-ASSAY

Conc. Of Doxofylline (API) (µg/ml)	Observed Conc. of Doxofylline (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.08	0.96	10.03	0.97
20	20.04	0.40	30.03	0.42S
40	39.97	0.33	39.95	0.14

Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.2ml/min), Temperature (± 2^{o}C), Wavelength of detection (± 2nm) and acetonitrile content in mobile phase (± 2%) studied to determine the robustness of the method are also in favour of (% RSD < 2%) the developed RP-HPLC method for the analysis of Olmesartan **Table 5.**

TABLE 5: RESULT OF METHOD ROBUSTNESS TEST

Change in parameter	% RSD
Flow (1.1 ml/min)	0.02
Flow (0.9 ml/min)	0.08
Temperature (27^{0}C)	0.04
Temperature (23^{0}C)	0.16
Wavelength of Detection (206 nm)	0.05
Wavelength of detection (204 nm)	0.07

CONCLUSION: 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. 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 waters C_{18}, 5µm, 15cm x 4.5 mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluents for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 1M NaOH, IM HCl).

The drug was found to be soluble in methanol. Drug was highly soluble in acetonitrile. Using these solvents with appropriate composition newer methods can be developed and validated. The result shows the developed method is yet another suitable method for assay and stability studies which can help in the analysis of simvastatin in different formulations. The precision of the method was demonstrated by intra-day and inter-day variation studies. For intra-day studies the drug having concentration value 80%, 100 % & 120% of the target concentration (n = 3), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days.

Data were subjected to statistical treatment for the calculation of SD and %RSD. The value of %RSD for Olmesartan was found to be 0.97, 0.42, and 0.14 for intra-day studies. The values for inter-day studies were 0.96, 0.43, and 0.33 respectively. This shows that values are not more than 2%, indicates that the developed method is precise. The proposed method is simple, sensitive and reproducible and hence can be used in routine analysis.

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REFERENCES:

1. A. Sampath, R. Reddy, B. Yakambaram, A. Thirupathi, M. Prabhakar, P.P. Reddy, P.V. Reddy. Journal of Pharmaceutical and Biomedical Analysis, 2009; 50: 405–412.
2. Chimalakonda Kameswara Rao, Kakumani Kishore Kumar, Maddala VijayaLaxmi, Polisetty Srinivasulu, Gutta Madhusudhan, Khagga Mukkanti, Koduri Sai Venkata Srinivas, Development and Validation of Stability Indicating LC Method for Olmesartan Medoxomil, American Journal of Analytical Chemistry, 2012: 3: 153-160
3. R. N. Sharma and S. S. Pnacholi, "RP-HPLC-DAD Method for Determination of Olmesartan Medoxomil in Bulk Drug and Tablets Exposed to Forced Conditions," Acta Pharmaceutica, 2010; 61: 13-24.
4. Gaurang P. Pandya and Hitendra S. Joshi, Development and validation of stability indicating HPLC assay method for simultaneous determination of amlodipine besylate, olmesartan medoxomil and hydrochlorothiazide in tablet formulation , Der Pharmacia Sinica, 2013; 4(2):145-152.
5. A.Y. Kamblea, M.V. Mahadika, L.D. Khatala, S.R. Dhaneshwara, Validated HPLC and HPTLC Method for Simultaneous Quantitation of Amlodipine Besylate and Olmesartan Medoxomil in Bulk Drug and Formulation, Analytical Letters, 2010; 43:251–258.
6. Nandini R Pai and Seema S. Sawant,Development and validation of new RP-HPLC method for determining impurity profiling in olmesartan medoxomil drug as well as in tablet dosage form, Der Pharma Chemica, 2013, 5(4):274-281
7. T.D.G. Parambi, M. Mathew, A. Jose, K.G. Ravikumar. A Validated Hptlc Determination Of An Angiotensin Receptor Blocker Olmesartan Medoxomil From Tablet Dosage Form, International Journal of Pharmaceutical Sciences Review and Research, 2010; 4:36-39.
8. P. Sengupta, A. Das and T. K. Pal, "Stability Indicating RP-HPLC Method for Simultaneous Determination of Olmesartan Medoxamil and Pioglitazone in Fixed Dose Combination Tablet Dosage Form," *Asian Journal of Chemistry*, 2010; 22: 6471-6479.
9. B. Raveendra, R.A. Gandurib, S.P. Lanka, R.P. Jayachandra, M. Mustafa, Stress Induced Method Development and Validation of Olmesartan in Bulk and Pharmaceutical Dosage Form by UV Spectrometric Method, European Journal of Analytical Chemistry, 2010; 5(2):145-151.
10. P. Sengupta, A. Das, T.K. Pal, Stability-Indicating RP-HPLC Method for Simultaneous Determination of Olmesartan Medoxamil and Pioglitazone in Fixed Dose Combination Tablet Dosage Form , Asian Journal of Chemistry, 2010,; 22(8): 6471-6479.
11. L. Bajerski, R.C. Rossi, CL. Dias, Stability-indicating LC determination of a new antihypertensive, olmesartan medoxomil in tabletsChromatographia 2008; 68 :991-996
12. V.P. Rane, K.R. Patil, J.N. Sangshetti. Stability-Indicating LC Method for the Determination of

Olmesartan in Bulk Drug and in Pharmaceutical Dosage Form, Chromatographia 2009; 69: 169-173.

13. B. Raman, B.A. Sharma, G. Mahale, D. Singh A. Kumar, investigation and structural elucidation of a process related impurity in candesartan cilexetil by LC/ESI-ITMS and NMR Journal of Pharmaceutical and Biomedical Analysis, 2011; 56:256– 263.
14. ICH (Q2, R1).Note for guidance on validation of analytical methods: Definitions and terminology. International conference on harmonization, 1994; 9-13.

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