WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 4, 975-987. Research Article ISSN 2277– 7105

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING STUDY OF OLMESARTAN MEDOXOMIL IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Sapana Jain*, Vaishali Jadhav, Ashish Jain, Harshada Khude, Shraddha Kachare, Priyanka Halse

Shri D D Vispute College of Pharmacy and Research Center, Department of Quality Assurance, Panvel, Navi Mumbai, Maharashtra.

Article Received on
28 January 2022,

Revised on 18 Feb. 2022,
Accepted on 08 March 2022

DOI: 10.20959/wjpr20224-23097

***Corresponding Author**
Sapana Jain
Shri D D Vispute College of Pharmacy and Research Center, Department of Quality Assurance, Panvel, Navi Mumbai, Maharashtra.

ABSTRACT

The purpose of stability testing is to provide evidence on how quality of drug substance varies under influence of temperature, humidity and light. Forced degradation is carried out to demonstrate as specificity which can help identify the likely degradation products. In the current study we have developed analytical method for Olmesartan Medoxomil in tablet dosage form. The studies were carried on Shimadzu HPLC system LC 2010, using Inertsil ODS, C18 Column, 150cm, 4.6mm, particle size 4µm. The wavelength selected was 257nm. The flow rate was 1ml/min. The mobile phase selected after optimization was ACN: Buffer (50:50). The regression coefficient of correlation was 0.9917. % RSD values reported were less than 2 all through validation which indicates variation in results are minimal. The forced degradation studies were conducted, and the least degradation was found in thermal condition. The method is quite simple, rapid and economical in terms of time taken and amount of solvents used. The stability indicating method was validated according to present ICH guidelines for specificity, linearity, precision and robustness.

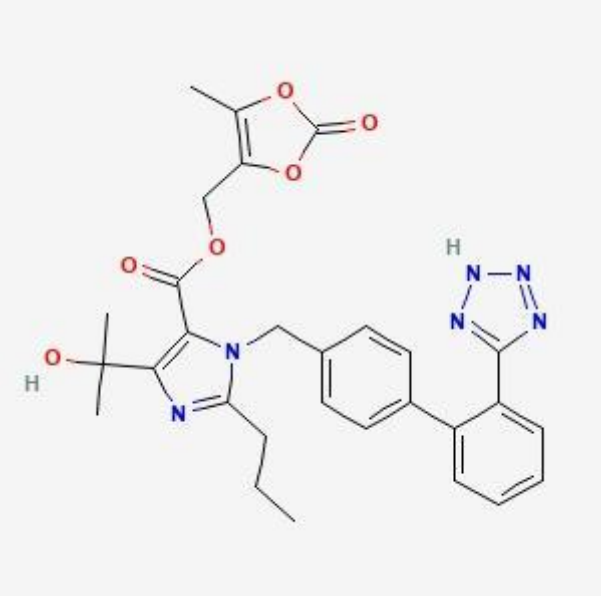
KEYWORDS: Olmesartan Medoxomil, HPLC, validation, stability, ICH.

INTRODUCTION

Olmesartan Medoxomil is a synthetic imidazole derivative prodrug.[1] It's used as antihypertensive agent which acts by inhibiting angiotensin II receptors. Orally available

Olmesartan is produced as the prodrug Olmesartan Medoxomil which is rapidly converted in vivo to pharmacologically active Olmesartan.[2] The drug Olmesartan binds to angiotensin (AT1) receptor of angiotensin II in smooth muscle of vascular lining and adrenal gland. Thus, it competes angiotensin II receptor binding. So, this prevents vasoconstriction which is induced by angiotensin II. It further decreases production of aldosterone. So, aldosterone stimulated sodium retention is prevented. Also, potassium excretion is prevented.[1] There are various methods reported for method development and validation of Olmesartan Medoxomil alone[3] and by UV method with drug like chlorthalidone.[4] Also in combination with other drugs like hydrochlorothiazide or amlodipine.[5-8] These chromatographic methods involve use of high-cost solvents, and some have used gradient flow. In some methods pH has to be maintained which makes it tedious. So, we have developed stability indicating analytical method using high performance liquid chromatography (HPLC). This is an analytical method which is capable of separating the degradant peaks from drug product peak.

Structure



IUPAC NAME: 5-methyl-2-oxo-1, 3-dioxol-4-yl)methyl 5-(2-hydroxypropane-2-yl)-2-propyl-3[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazole-4-carboxylate

Molecular weight: 558.9

CAS number: 144689-63-4

Molecular formula: $C_{29}H_{30}N_6O_6$

MATERIALS AND METHOD

Instruments

High performance liquid chromatography system equipped with diode array detector, analytical balance, Sonicator, pH meter, Volumetric flask, Beaker, pipettes.

Reagents, Solvents and Standard

Olmesartan Medoxomil standard,

Acetonitrile (grade: HPLC)

Monobasic potassium phosphate (AR grade)

Ortho phosphoric acid, water (HPLC grade)

Diluted phosphoric acid (0.2% phosphoric acid) transfer 0.24 ml of ortho phosphoric acid (85%) in 100 ml volumetric flask, dilute up to mark with water.

Reagents and Chemicals

A well characterized working standard of Olmesartan Medoxomil was procured from Amoli organics, Vapi, Gujrat. Olmesartan Medoxomil 40mg tablets (In-house) of batch No OLM/IRT/40/06-21/109 were used for analysis.

Preparation of buffer

Accurately weigh and transfer about 2.041g of Monobasic potassium phosphate in 1000ml beaker and add 900ml of water to dissolve. Adjust pH to 3.4 with diluted phosphoric acid. Make up the volume and filter through 0.45 µm membrane filter paper.

Preparation of standard solution

Accurately weigh and transfer 48mg of working standard in 100ml volumetric flask, add 25ml of diluent and sonicate to dissolve. Allow flask to col then make up the volume with diluent. Further dilute 5ml to 25 ml volumetric flask. This makes 96µg/ml of standard solution.

Preparation of sample solution

Weigh not less than 5 tablets of 40 mg strength. Crush them and weigh powder equivalent to 200 mg of powder triturate and dissolve in 250 ml volumetric flask and add 150 ml of diluent. Sonicate for 20 minutes and intermittent shaking. Allow to cool the flask and make up the volume with diluent Further dilute 6 ml of this solution in 50 ml of volumetric flask

and make up the volume. Filter through 4.5 µm nylon filter syringe. This makes 96 µg/ml of sample solution.

UV Method

A) Solubility studies: The solubility was checked in different solvents like water, Acetonitrile, Methanol, Methanol: water. Drug was found to be soluble in acetonitrile and methanol but less soluble in water. Solubility was also checked in Water: ACN in different combinations like 60:40,50:50,80:20 It was observed that the drug is more soluble in 60:40 ACN: water combination and hence this was taken as diluent.

B) Selection of wavelength: UV spectra of Olmesartan Medoxomil was run for 190-400 nm and lambda maximum of 257 nm 0f maximum absorbance was selected for detection.

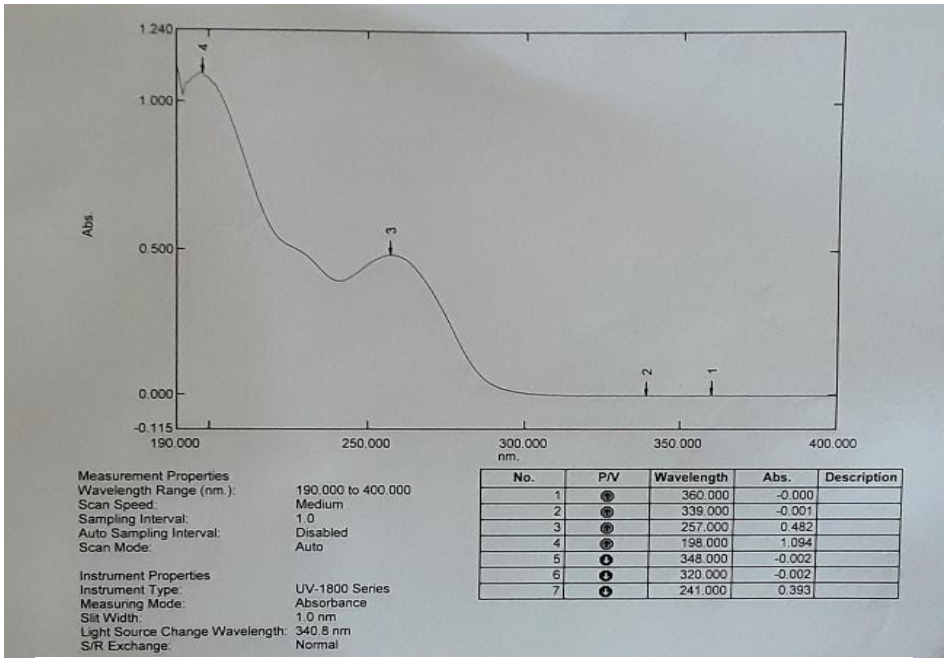


Fig. 1: UV absorption spectra for finding maximum wavelength.

Optimisation: Initially trials were conducted for mobile phase selection. It was observed

ACN: buffer in 40:60 ratio gave Rt of 11.6 min

ACN: buffer in 50:50 ratio gave Rt of 5.44 min

ACN: buffer in 60:40 ratio gave Rt of 3.38 min

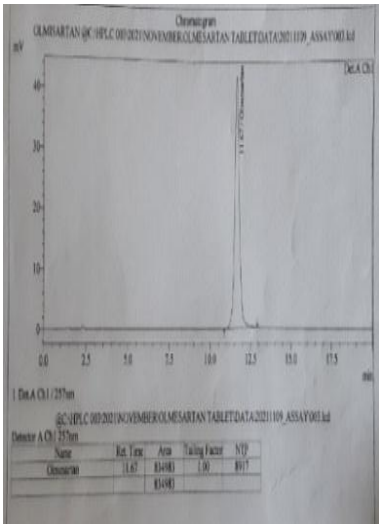
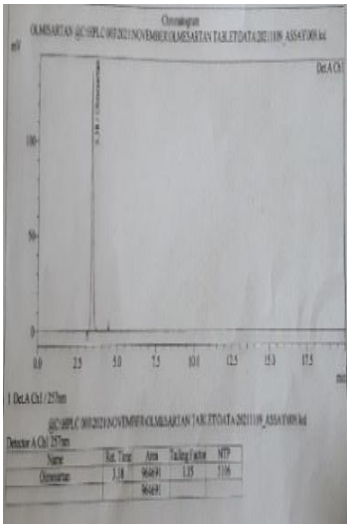
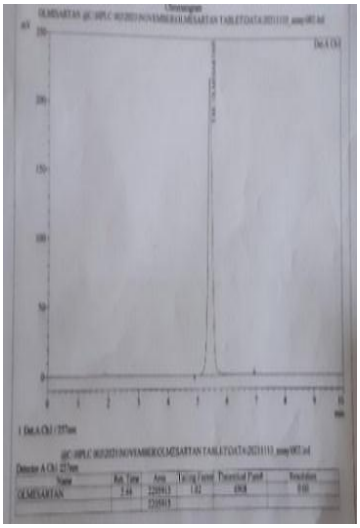




Fig. 2: 40: 60ACN: buffer. **Fig. 3: 60: 40 ACN: buffer.** **Fig. 4: 50: 50ACN: buffer.**

Looking at retention time, NTP finally ACN: Buffer 50:50 was selected as mobile phase.

Chromatographic condition

Column:	Inertsil ODS C18, 150 mm, 4.6mm, 4 µm
Flow rate:	1.0ml/min
Detection wavelength:	257nm
Injection volume:	10 µL
Run time:	10 min
Column temperature:	40^0C
Sample thermostat:	$10^{\ 0}$ C
Retention Time:	5.4min
Pump Mode:	Isocratic

Assay: Equilibrate the column with mobile phase for 30 min. Inject 1 u l of standard and sample solutions into HPLC system and measured the areas for Olmesartan medoxomil peak and calculate %assay by following formula

%Assay= (At/As) ×(Ws/Ds) ×(Dt/Wt) ×(P/100) ×Average weight /Label claim) ×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. The results are shown in Table 1.

Validation of HPLC method[9-10]

1) Specificity & Selectivity

The specificity study was the identification of main spectra and interference study. Spectra of standard solution, sample solution and placebo were analysed by direct comparison method to check that diluents or excipients are not interfering with the drug peak. This is seen from Fig5-Fig 8 The parameters like asymmetric factor, tailing factor and number of theorical plates were calculated. Good corelation was found between results of standard and sample solutions. Results are shown in table 2.

2) Linearity

Linear relationship should be evaluated across the range of the analytical procedure. Each linearity level preparation was analysed six times. Calibration curves were made by plotting graph of absorbance Vs concentration. Linearity response was determined over the range of 24-144 µg/ml for Olmesartan Medoxomil. Results are shown in Table 3 and Fig 9.

3) Accuracy

The accuracy of the method was determined by analysing with known amount of drug in order to result in sample solutions with following concentrations of drug (50%, 100%, 150%) in triplicate and analysed according to method of analysis. % Recovery was calculated. Results are shown in Table 4.

4) Precision

Interday precision of analytical method was determined by six set of standard solution for different days. Intraday precision or repeatability of the analytical method was determined by six set of standard solution in different times of a day. Intermediate precision was determined by analysing solution on different days and on different instruments by different analysts. % RSD of absorbance was calculated for six sets. . Results are shown in table 5.

5) Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were separately determined based on calibration curves. The

LOD = 3.3 σ S

The quantitation limit is calculated by

LOQ = 10 σ S

where σ is standard deviation of response and S is slope of calibration curve.Results are shown in table 5.

6) Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but, deliberate variations in method parameters. The wavelength, temperature and flow rates were changed, and effect was observed on system suitability.

Forced degradation studies[11]

Acid degradation: API, sample and placebo were weighed and taken in 100ml volumetric flask and 50% diluent was added. In each 5 ml of 0.5 N HCl was added. It was kept for 24 hours. Then it was neutralised and volume was made up with diluent. Further it was diluted to get concentration of 96 µg /ml These were injected into HPLC system and % degradation was calculated.

Alkaline degradation: API, sample and placebo were weighed and taken in 100ml volumetric flask and 50% diluent was added. In each 5 ml of 0.5 N NaOH was added. It was kept for 24 hours. Then it was neutralised and volume was made with diluent. Further it was diluted to get concentration of 96 µg /ml These were injected into HPLC system and % degradation was calculated.

Oxidation degradation: API, sample and placebo were weighed and taken into 100 ml volumetric flask and 50% diluent added and in each 5 ml of 10% H_2O_2 was added. It was kept for 24 hours. Then volume was made with diluent. Further it was diluted to get concentration of 96 µg /ml These were injected into HPLC system and % degradation was calculated.

Thermal degradation: API and sample and placebo were kept at 60 ^{0} C for 24 hours. Then required weight was taken in 100ml volumetric flask and 50% diluent added. Then volume was made with diluent. Further it was diluted to get concentration of 96 µg /ml These were injected into HPLC system and % degradation was calculated.

RESULTS

1) Specificity

No peak was obtained in blank solution and placebo of drug Olmesartan Medoxomil indicates the method is specific.

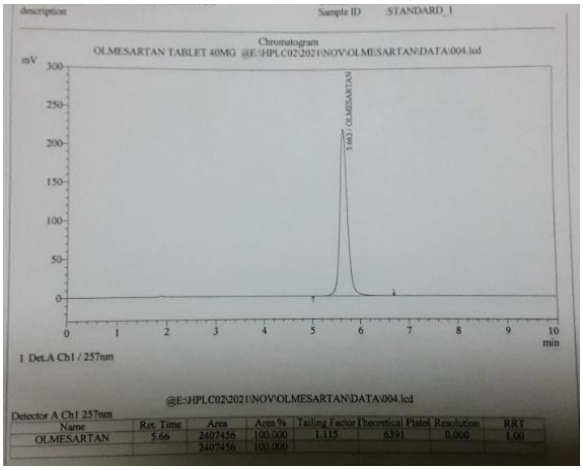


Fig. 5: Chromatogram of standard.

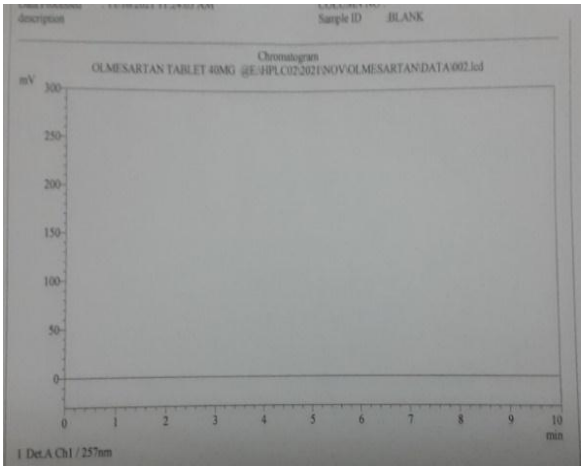


Fig. 6: Chromatogram of blank.

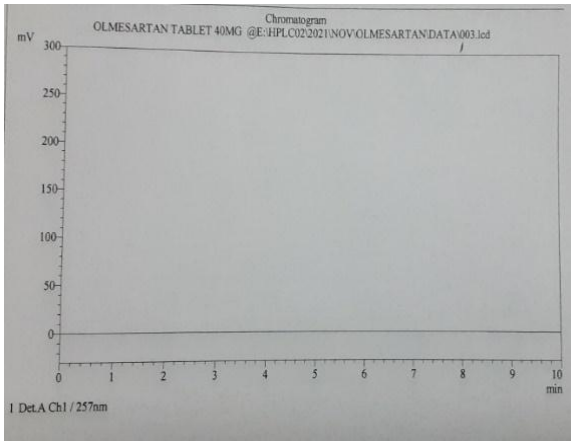


Fig. 7: Chromatogram of placebo.

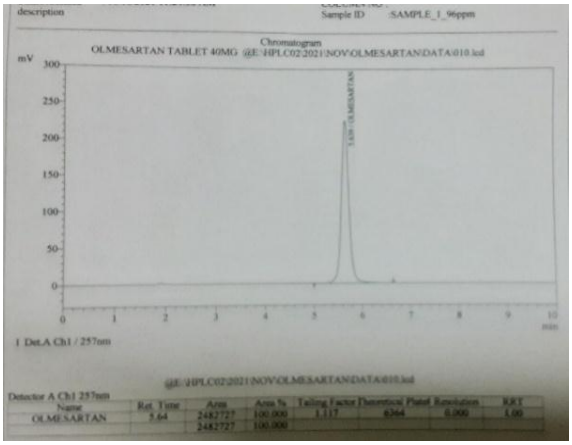


Fig. 8: Chromatogram of sample.

Table 1: Analysis of tablet formulation.

Olmesartan medoxomil	% Assay
1	98.03
2	101.83
3	102.77

Table 2: System suitability test.

Sr. no.	System suitability parameters	values
1	Retention time (min)	5.513
2	Number of Theoretical plates	8897
3	Asymmetric factor	1.03

Linearity: The Linearity studies of Olmesartan Medoxomil were performed using standard solution in range of 24-144µg /ml. Results are as shown in Table 3 and Fig 9

Table 3: Linearity studies.

Linearity level	Concentration in ppm	Area_1	Area_2	Area_3	Mean area
Level 25%	24.06	669547	670898	669510	669985
Level 50%	48.125	1264968	1266706	1266226	1265967
Level 75%	77.00	1944566	1951392	1953921	1949960
Level 100%	96.25	2367154	2367039	2367039	2367077
Level 125%	125.125	3548249	3554855	3552612	3551905
Level 150%	144.375	4236853	4234763	4239510	4237042

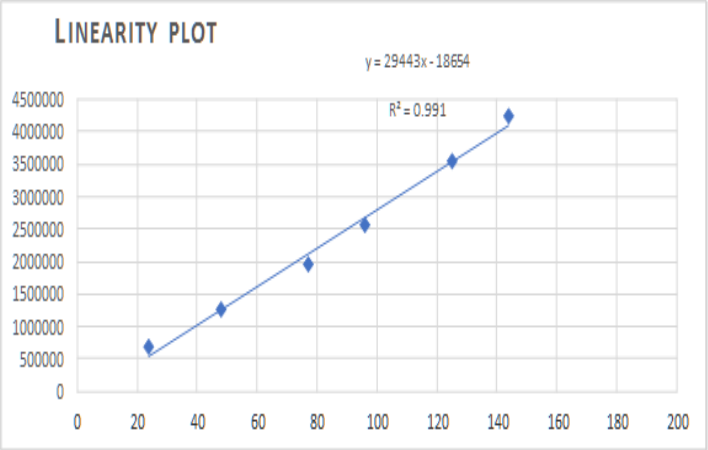


Fig. 9: Linearity graph.

Accuracy

Table 4: Results of recovery studies.

Accuracy Level	Weight of API	Weight of Placebo	Area	Amount added	Amount found	%Recovery
Recovery at 50 %	102.87	2000.15	1255552	102.56	103.37	100.8
Recovery at 50 %	102.91	2000.27	1255963	102.60	103.40	100.8
Recovery at 100 %	200.45	2000.45	2427622	199.87	199.87	100.0
Recovery at 100 %	200.78	2000.87	2430797	200.18	200.13	100.0
Recovery at 150 %	300.45	2000.56	3644613	299.55	300.06	100.2
Recovery at 150 %	300.63	2000.44	3649515	299.73	300.47	100.2

Precision: % RSD values for all types of precision were found to be within the specified limit. Also, LOD and LOQ were Results are shown in table 5

Table 5: Results of precision, LOD & LOQ.

Sr. no.	Precision	%RSD
1	Intraday	0.14
2	Interday	0.18
3	Intermediate	0.18
4	LOD	0.432
5	LOQ	1.309

Robustness: The method was found to be robust by changing parameters

a) By changing flow rate by +0.2 ml/min and – 0.2 ml/min b) By changing wavelength by +5nm and -5 nm c)By increasing column temperature by $+5^0$ C and -5^0 C. The % RSD was found within limits. Results are shown in table 6.

Table 6: Data showing robustness parameter.

Sr. no.	Flow(0.8ml /min		Flow (1.2ml/min)		Wavelength (259nm)		Wavelength (255nm)		Column temp(35^0C)		column temp(45^0C)	
no	RT	Area	Rt	Area	Rt	Area	Rt	Area	Rt	Area	Rt	Area
1	7.1	3069166	4.6	2049960	5.3	2408833	5.3	2460255	5.4	2465896	5.4	2463324
2	7.1	3069600	4.6	2052058	5.3	2410709	5.3	2457792	5.4	2465058	5.4	2462955
3	7.1	3070741	4.6	2049232	5.3	2412489	5.3	2454365	5.4	2463932	5.4	2463094
4	7.0	3075472	4.6	2052811	5.3	2414750	5.3	2451619	5.4	2464832	5.4	2462928
5	7.0	3074067	4.5	2054406	5.3	2417291	5.3	2451894	5.4	2464990	5.4	2461563
Avg	7.1	3071809	4.6	2051693	5.3	2412814	5.3	2455185	5.4	2464942	5.4	2462773
SD	0.1	2807	0.0	2110	0.01	3323	0.01	3766	0.01	700	0.01	694
%RSD	0.8	0.09	0.5	0.1	0.1	0.14	0.10	0.2	0.21	0.03	0.1	0.03
Tailing factor	1.07		1.1		1.09		1.09		1.09		0.05	
NTP	7978		6193		6917		6931		6755		7127	

Forced degradation Results: The chromatograms are shown in Fig 10- Fig 14. Optimisation was done for alkaline degradation. It was found from area that the drug base was degraded in 24 hours alkaline hydrolysis again API, placebo and sample were weighed and 1ml of 0.1 N NaOH was added and kept for 2 hours, neutralised made up the volume and then diluted to get 96 µg /ml and injected % degradation was calculated. The Results are shown in Table 7.

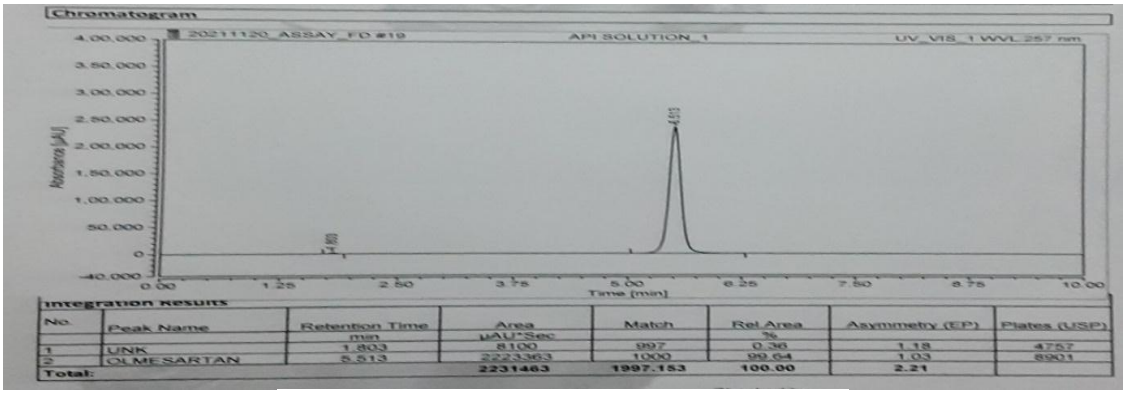


Fig. 10: Chromatogram of API untreated.

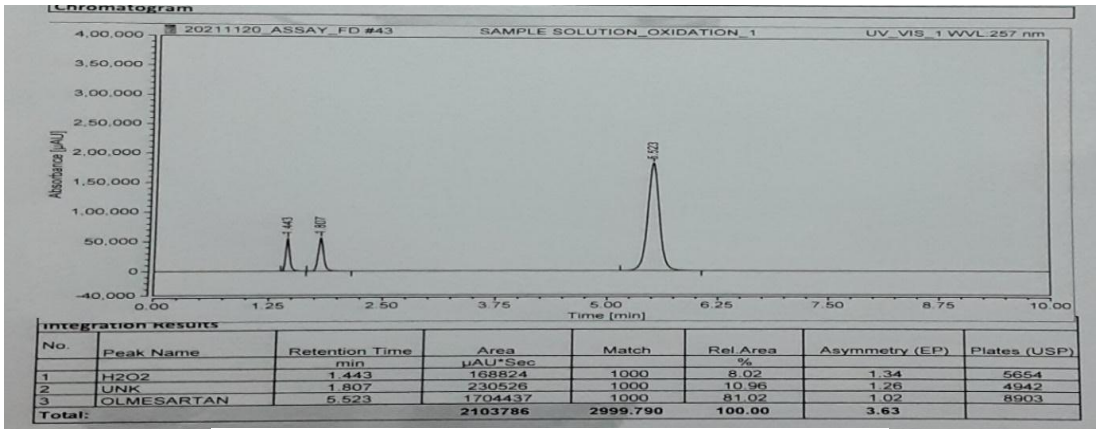


Fig. 11: Chromatogram of oxidation degradation.

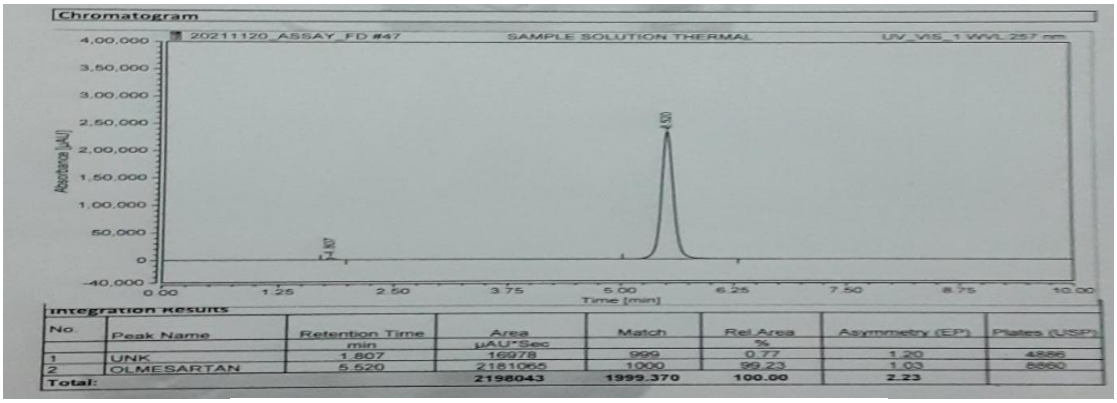


Fig. 12: Chromatogram of thermal degradation.

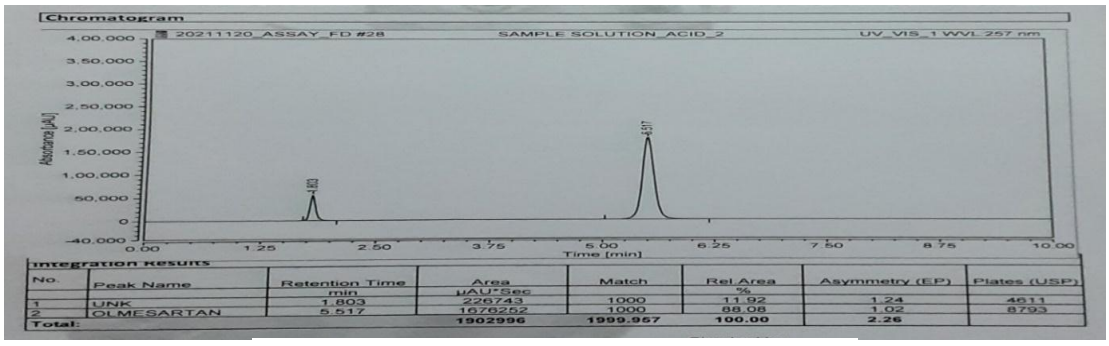


Fig. 13: Chromatogram of acid degradation.

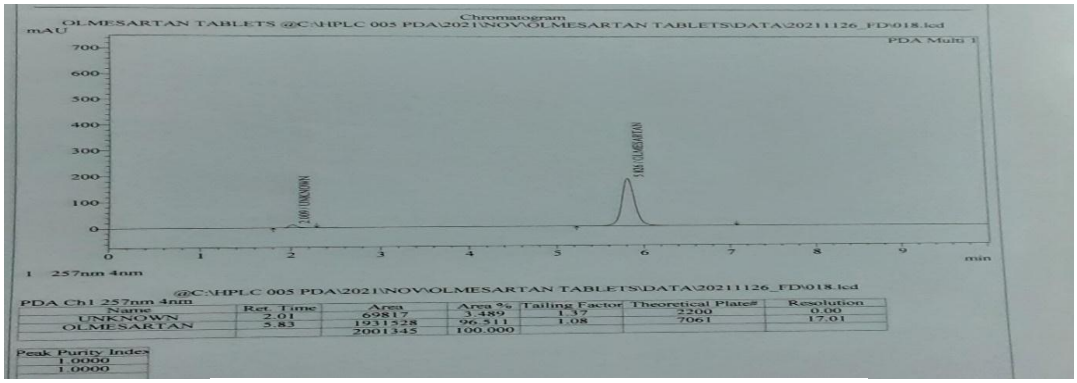


Fig. 14: Chromatogram of alkaline degradation.

Table 7: Forced degradation results.

Type of degradation	% Drug found	%degradation	Peak purity
Untreated	98.03	-	1
Acid	76.33	22.11	1
Alkali	88.82	9.62	1
Oxidation	77.18	21.26	1
Thermal	95.05	3.383	1

DISCUSSION

The present paper describes RP-HPLC method for Estimation of Olmesartan Medoxomil in tablet dosage form. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The stability studies have been performed and it showed the drug is degraded least in thermal condition, 10% in alkaline condition,21%&22 % in acid and oxidising condition respectively. The peak purity was found to be 1 which indicates specificity. The method is accurate, precise, linear, robust, simple and rapid. Acceptable regression values, RSD (%) and recovery data suggests it can be successfully used for estimation of Olmesartan Medoxomil in tablet formulation.

ACKNOWLEDGEMENT

The author is especially thankful to Umedica labs, Turbhe, Mr Mahadev Salunkhe Head of department, Mr. Shankar Karlekar, manager, R& D, analyst Mr. Swapnil Nikade for their immense support and laboratory instruments provided.

Conflict of interest

There is no conflict of interest.

REFERENCES

1. https://pubchem.ncbi.nlm.nih.gov/compound/Olmesartan-medoxomil, Accessed 21st Jan 2022.
2. Laeis P. Puchler K. Kirch W; The pharmacokinetic and metabolic profile of Olmesartan Medoxomil limits the risk of clinically relevant drug interaction; Journal of Hypertension Supplement, 2001; 19: S21–S32.
3. Bharti T., Mishra R., Rajput C., Singhal R., Analytical method deveopment and validation of assay for Olmesartan medoxomil in formulated product by reverse phase ultra performance liquid chromatography, European j. biomed. pharm. sci, 2016; 3(2): 215-222.
4. Sawale V., Dhabarde D., Mahapatra D.K., Development and validation of UV spectrophotometric method for simultaneous estimation of olmesartan medoxomil and Chlorthalidone in bulk and tablet, Eurasian J Anal Chem, Jan, 2017; 12(1): 55-66.
5. Patchala A., Nedendla R. Quantification and validation of Amlodipine Besylate, Olmesaartan Medoxomil and hydrochlorothiazide by RP-HPLC in marketed dosage form, IJPSR, 2020; 11(5): 2350-2355.
6. Chabukswar A, Kuchekar B, Jagdale S, Mehetre D, More A, Lokhande P, development and validation of a RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Amlodipine Besylate in tablet dosage form, Arch. Appl. Sci., Res, 2010; 2(4): 307-3127.
7. Suryadevara V, Reddyvalam L. S, Rao B.V, Koduri T, Marupudi R, Method development and validation for simultaneous estimation of Olmesartan Medoxomil and Hydrochlorothiazide by RP-HPLC, Orient. J Chem, 2014; 30(1): 195-201.
8. Napoleon A.A., Angajala G., raj A., HPLC method development and validation for simultaneous estimation of Olmesartan Medoxomil, Hydrochlorothiazide and Amlodipine besylate tablets, Scholars research library, der Pharmacia lettre, 2015; 7(5): 182-196.
9. Validation of analytical procedure: Text and methodology, November, 2005; 2(r1)-ICH
10. ICH Q2 (R1) Validation of Analytical Procedures https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf, Accessed 21st Jan, 2022.
11. https://database.ich.org › Q1A(R2) Guideline – ICH, Accessed 21st Jan, 2022.