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METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD FOR THE SIMULTANEOUS ESTIMATION OF OLMESARTAN AND HYDROCHLOROTHIAZIDE IN BULK DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, rapid and accurate reversed phase HPLC method has been developed for the simultaneous estimation of olmesartan medoxomil and hydrochlorothiazide in bulk drug. The chromatographic method was based on reversed phase liquid chromatography using a shimpack GWS C18 column (250 x 4.6mm, 5µ) with UV detection at 274nm. The mobile phase consisting of acetonitrile and water in a ratio range (65:35) and at a flow rate of 1ml/min. Validation parameters such as system stability, linearity, precision accuracy, specificity, limit of detection (LOD), and limit of quantification (LOQ), stability of sample and standard stock solutions and robustness were studies to report in the ICH guidelines. The retention time of olmesartan and hydrochlorothiazide was 1.9 and 3.1min respectively. The method was linear over the concentration for olmesartan medoxomil 8-24µg/mL and for hydrochlorothiazide 5-15µg/ml and the R^2 is 0.999 & 0.999. The recoveries of olmesartan medoxomil and hydrochlorothiazide were found to be in the range of 98.7% and 99.6% respectively. The method was validated and was successfully employed for the analysis of pharmaceutical formulation containing olmesartan medoxomil and hydrochlorothiazide in combined tablet dosage form.

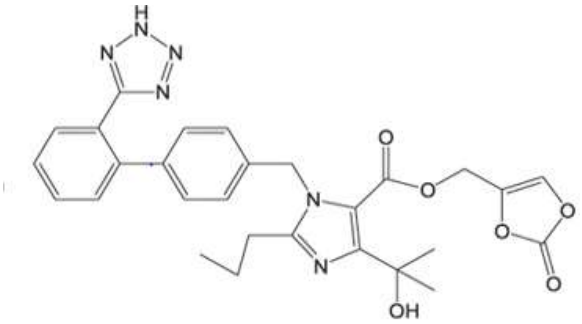
Keywords – *Olmesartan, Hydrochlorothiazide, RP-HPLC, Validation, Stressed degradation*

1. INTRODUCTION

Olmezest H 20 tablet act as an antihypertensive and diuretic. Olmesartan is an antihypertensive agent, class II angiotensin blocker. It is indicated to treatment of hypertension, diabetes. Hydrochlorothiazide is a thiazide diuretic and it has been used to increased excretion of water and electrolytes, edema, hypertension, diabetes.

Olmesartan medoxomil (OLM) is described chemically as the (5- methyl-2oxo-1, 3- dioxol-4-yl) methyl ester of 4-(1hydroxy-1-methylethyl)-2-propyl-1-{[2'-(1Htetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl}-1Himidazole-5-carboxylic acid molecular formula is

$C_{29}H_{30}N_6O_6$ having a molecular mass 558.58gm/mole. It is a selective AT1 subtype angiotensin II receptor blocker2, 3. Its action is therefore, independent of the pathways for angiotensin II synthesis.



Chemically hydrochlorothiazide (HCTZ) is 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine 7-sulphonamide 1, 1-dioxide. Molecular formula is $C_7H_8N_3O_4S_2Cl$ having molecular mass is 297.74gm/mole. One of the oldest and widely used thiazide diuretics. Several analytical methods have been reported for the determination of HCTZ in pharmaceutical formulations including polarography, LC, HPTLC and spectrofluorometry [1-19].

The main aim of this study was to develop simple and rapid precise method and it has to be validated for those two combined drugs of Olmesartan medoxomil and Hydrochlorthiazide. In several Literature authors detailed about the simple, accurate methods and with alone or combination with other drugs. But this method revealed with economically low cost and more precise.

2. MATERIALS AND METHODS

2.1 Instruments and chromatographic conditions

Apparatus and chromatographic condition the chromatographic separation was performed on WATERS HPLC with and UV detector. The analytical Shim pack GWS C18 column (250cm x 4.6 mm i.e., 5µm) was used for the separation. The mobile phase consisted of acetonitrile and water 65:35% (v/v) ratio. The mobile phase was freshly prepared, filtered, sonicated for 15mins. Flow rate at 1 ml/min, UV Detection 274 nm. The injection volume was 20µl.

2.2 Chemicals and reagents

The pharmaceutical grade pure samples of Olmesartan medoxomil and Hydrochlorothiazide were received as gift samples from Sai Moheshwar healthcare, Vadodara. The pharmaceutical formulation Olmezest H 20 is purchased from local pharmacy. HPLC grade Acetonitrile and water were purchased from Hi media laboratories Pvt. Ltd., Mumbai.

2.3 Preparation of standard

Weigh accurately 12.5mg of Hydrochlorothiazide and 20mg of Olmesartan in 10mL volumetric flask and make up to 10mL of Acetonitrile and sonicate for 15 min. 5ml from above stock solution were taken and transferred into 50ml of volumetric flask make up with 50ml diluents. 1ml from above stock solution was taken into a 10ml volumetric flask and made up to 10ml with diluents.

2.4 Preparation of sample solution

10 tablets are weighed and powdered. The weight 147 mg is equivalent to 12.5 mg of hydrochlorothiazide and 20mg of olmesartan was transferred into 1oml volumetric flask and make up to 10ml of acetonitrile and sonicate for 15 min. 5ml from above stock solution were taken and transferred into 50ml of volumetric flask make up with 50ml diluents. 1ml from above stock solution was taken into a 10ml volumetric flask and made up to 10ml with diluents.

3. RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines.

3.1 Method validation

The method was validated according to ICH guidelines. The different validation parameters are performed like linearity, precision, accuracy, limit of detection, limit of quantification, robustness.

3.1 .1 System suitability

To access system suitability of the proposed method, the repeatability, theoretical plates, tailing factor and retention time of six replicates of working standard solution of OLM and HCTZ were determined.

3.1.2 Linearity

Linearity was evaluated by analysis of preparing the solution range of OLM (8-24µg/ml) and HCTZ (5-15µg/ml). The linearity was plotted against peak area Vs concentration.

Table 1: Linearity of Olmesartan and Hydrochlorothiazide

Sample no.	Concentration(µg/ml)		Peak Area	
	OLME	HYDRO	OLME	HYDRO
1	8	5	126699	182859
2	12	7.5	317586	395509
3	16	10	532693	573926
4	20	12.5	754605	782838
5	24	15	936662	990714
Correlation coefficient			0.999	0.999

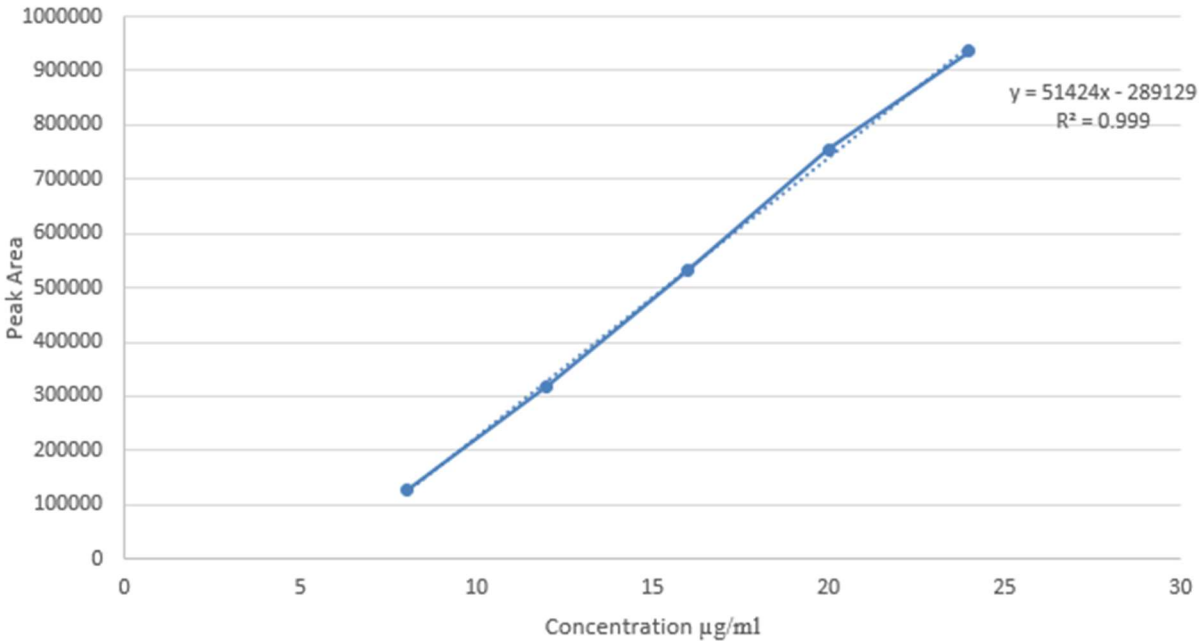


Figure 1: Linearity for olmesartan

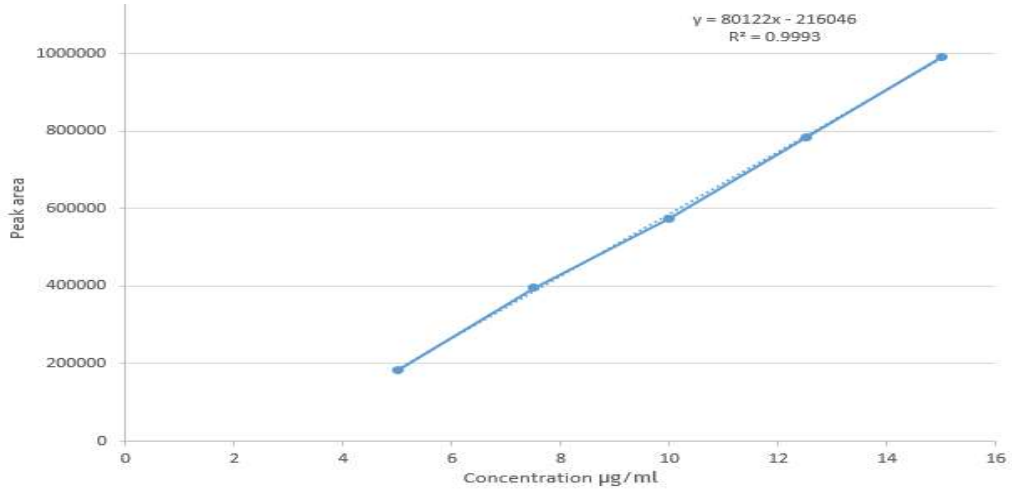


Figure 2: Linearity for Hydrochlorothiazide

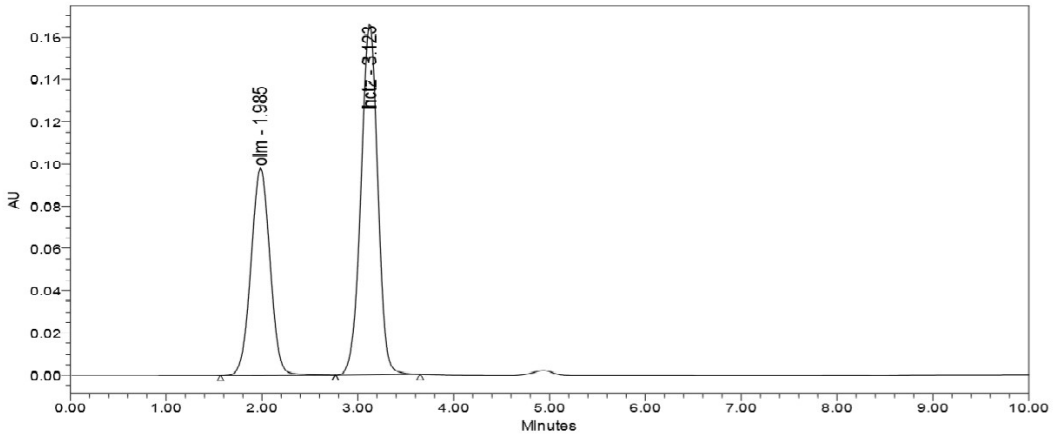


Figure 3: Standard chromatogram of mixtures

3.1.3 Accuracy

The accuracy study was performed for 50%, 100%, and 150% for OLM and HCTZ by three replicate analysis of the sample were injected. The method is used to determine the concentration of drug product in prepared solution. The %RSD, mean which are shows this method is accurate. The accuracy value ranged for Olmesartan and Hydrochlorothiazide was found to be 98.7% and 99.6%.

Table 2: Accuracy data for OLM & HCTZ

s.no	% concentration of sample	Nominal Concentration of the OLM (µg/ml)	% assay of OLM	% mean assay of OLM	Nominal concentration of the HCTZ (µg/ml)	% assay of HCTZ	% mean assay of HCTZ
1	50%	8	97.6	97.7	5	99.6	99.6
2			98.0			99.3	
3			97.6			100	
1	100%	16	99.4	99.2	10	99.9	99.8
2			99.2			99.9	
3			99.1			99.8	
1	150%	24	99.3	99.4	15	99.9	99.6
2			99.3			99.0	
3			99.7			99.9	

3.1.4 Precision

Repeatability was studied by carrying out system is precision. The results were reported in terms of the %RSD.

Table 3: Result of repeatability and intermediate precision for OLM & HCTZ

Drug name	Repeatability			Intermediate		
	Peak area	Std. dev	%RSD	Peak area	Std. dev	% RSD
Olmesartan	533235	695	0.130	532807	0.22	0.22
Hydrochlorothiazide	574442	856	0.149	574086	0.05	0.5

3.1.5 Limit of detection and limit of quantification

Limit of detection (LOD) and Limit if quantification (LOQ) of Olmesartan and Hydrochlorothiazide were determined by calibration curve method. Solutions of Olmesartan and Hydrochlorothiazide were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration.

LOD= 3.3 X SD/S

LOQ= 10X SD/S

Here,

SD= standard deviation of calibration curves

S= slope of intercepts of calibration curves

Table 4: LOD & LOQ data for OLM & HCTZ

Analyte	LOD(µg/ml)	LOQ(µg/ml)
Olmesartan	0.58	1.77
Hydrochlorothiazide	1.22	3.70

3.1.6 Robustness

The robustness of test method was determined by carrying out mobile phase variation ±2%, flow rate variation ±2% and wavelength variation ±2%.

Table 5: Robustness data for OLM & HCTZ

S.no	Condition	%RSD of Olmesartan	%RSD of Hydrochlorothiazide
1.	Flow rate (-)0.2ml/min	0.69	0.20
2.	Flow rate (+)0.2ml/min	0.12	0.03
3.	Mobile phase(63:37)	0.72	0.86
4.	Mobile phase(67:33)	0.55	0.22
5.	Wavelength (-) 272	0.16	0.24
6.	Wavelength (-) 276	0.11	0.16

3.1.7 Stressed degradation studies

a) Acid degradation studies

To 1ml of stock solution of Olmesartan and Hydrochlorothiazide, 1ml of 0.1M Hydrochloric acid was added refluxed for 3days at 40°C. The resultant solution was diluted to obtain 10µg/ml and 10µg/ml of all components

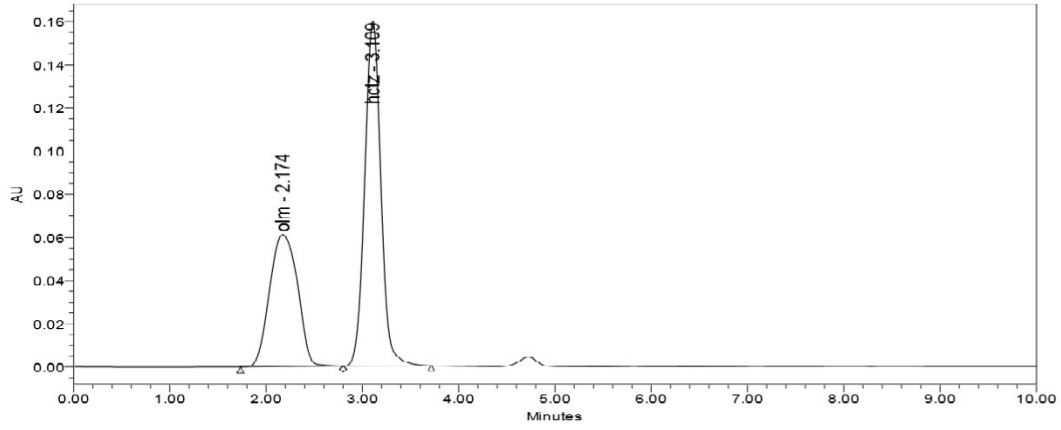


Figure 4: Chromatogram for Acid degradation

b) Alkaline degradation

To 1ml of stock solution of Olmesartan and Hydrochlorothiazide, 1ml of 0.1M Sodium hydroxide was added refluxed for 3days at 40°C. The resultant solution was diluted to obtain 10µg/ml and 10µg/ml of all components

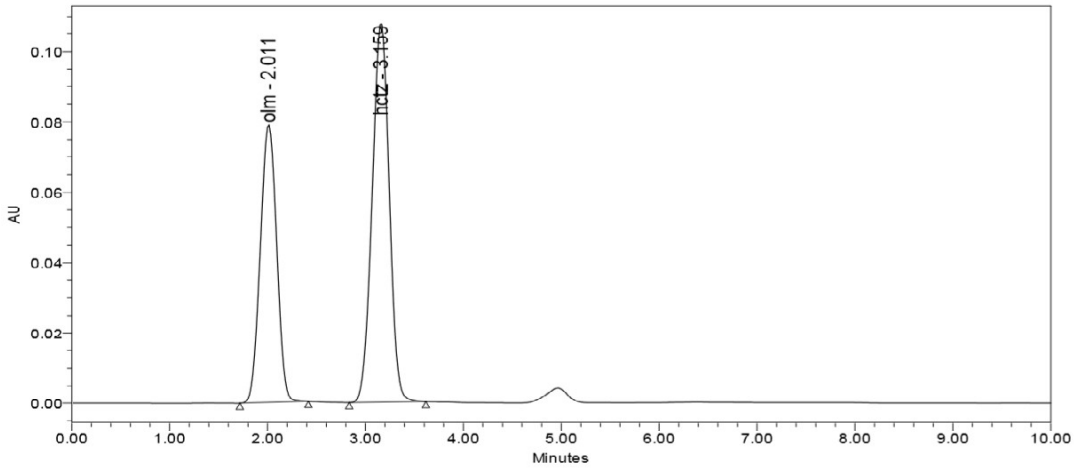


Figure 6: Chromatogram for Alkaline degradation

c) Oxidation

To 1ml of stock solution of Olmesartan and Hydrochlorothiazide, 1ml of 30% Hydrogen peroxide was added refluxed for 3days at 40°C. The resultant solution was diluted to obtain 10µg/ml and 10µg/ml of all components

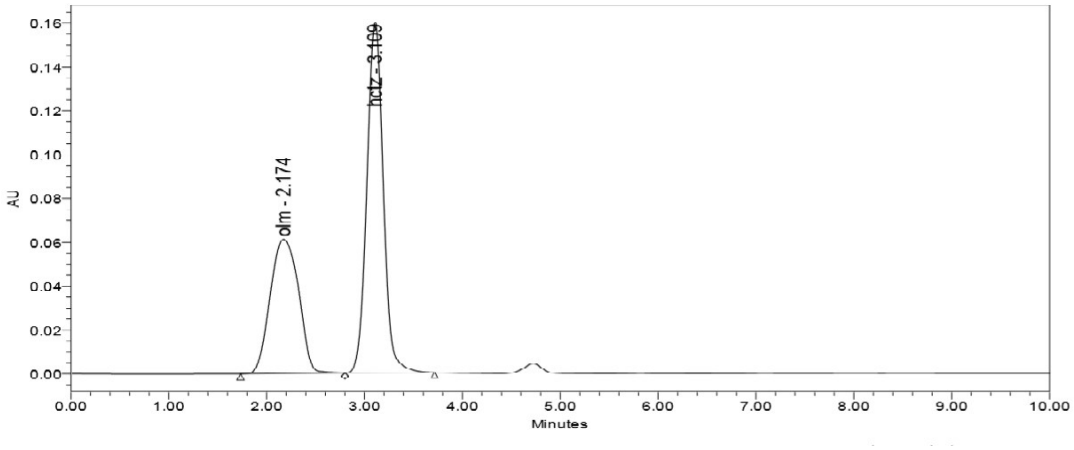


Figure 7: Chromatogram for Oxidation degradation

d) Thermal

To 1ml of stock solution of Olmesartan and Hydrochlorothiazide was kept at 60° for 3hrs. The resultant solution was diluted to obtain 10µg/ml and 10µg/ml of all components

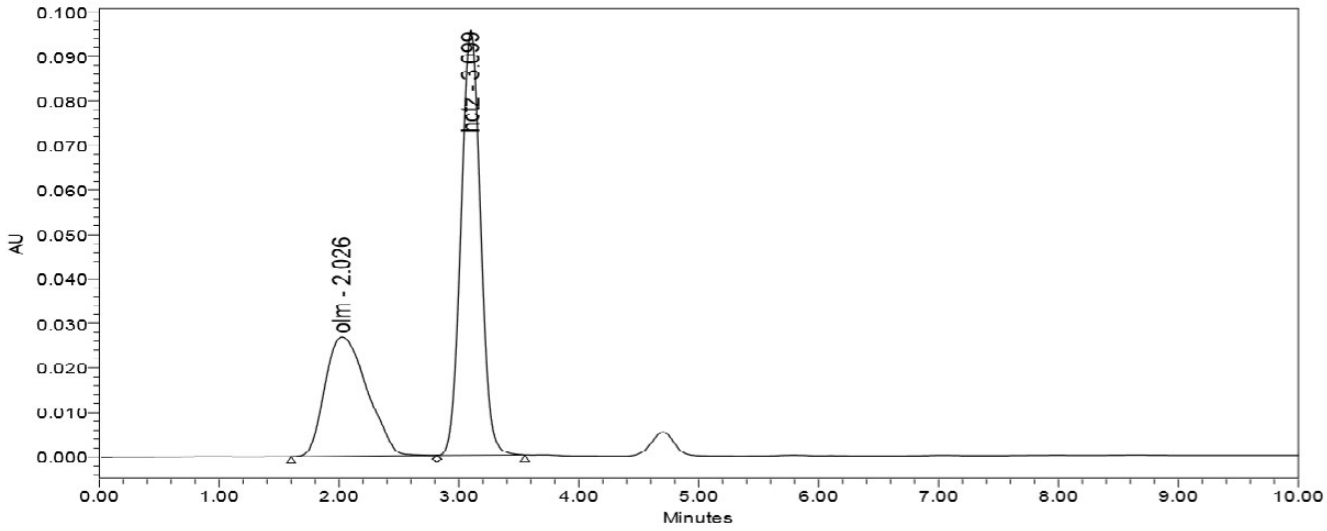


Figure 8: Chromatogram for Thermal degradation

e) Photolytic

To 1ml of stock solution of Olmesartan and Hydrochlorothiazide was kept under the sunlight for 3days. The resultant solution was diluted to obtain 10µg/ml and 10µg/ml of all components.

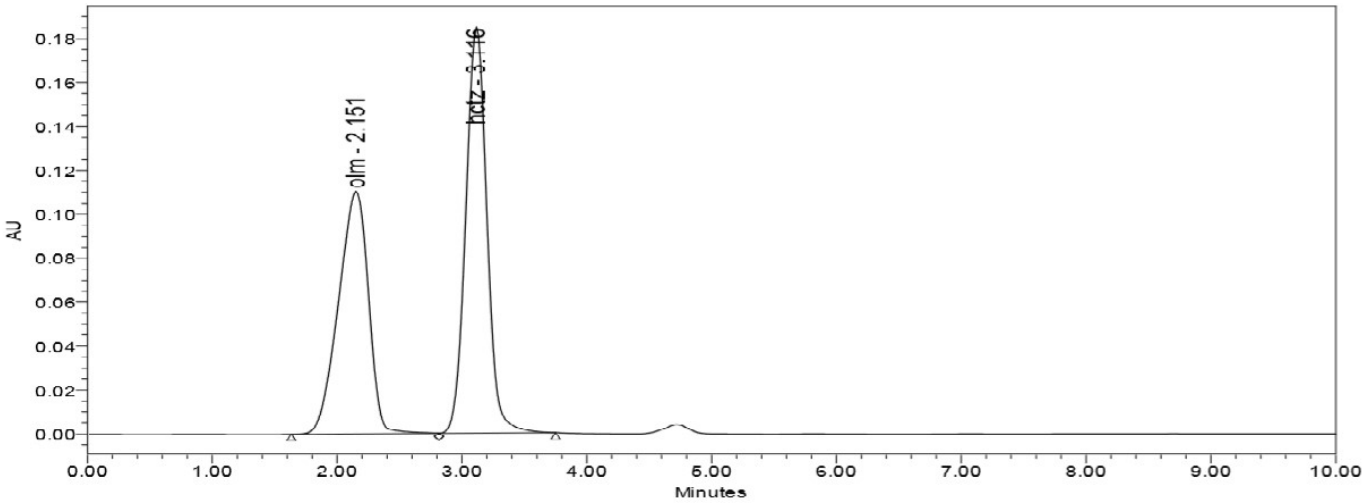


Figure 9: Chromatogram for Photolytic degradation

Table 5: Percentage Degradation dada for OLM & HCTZ

S.no	Stress Condition	Concentration	%assay		% degradation	
			Olme	Hctz	Olme	Hctz
1.	Acid	10µg/ml	94.9	92.7	5.1	7.3
2.	Alkaline	10µg/ml	93.6	94.7	6.37	94.7
3.	Oxidation	10µg/ml	94.4	94.9	5.6	5.1
4.	Photolytic	10µg/ml	93.2	93.3	6.8	6.7
5.	Thermal	10µg/ml	93.7	94.3	6.3	5.7

4. CONCLUSION

A simple, precise and accurate stability indicating HPLC method has been developed and validated as per ICH guidelines. An isocratic separation was achieved using C18 column shimpack (250 x 4.6mm, 5µm) with the flow rate of 1ml/min by using UV detector to monitor elute at 274nm. Different mobile phase was tried to select the ideal mobile phase. Among these Acetonitrile: Water (65: 35) was found to be ideal, since it gave good resolution and peak symmetry.

The method was validated for the specificity, linearity, LOD, LOQ, precision, accuracy, and Robustness. This method was linear over the concentration range of 8-24µg/ml and 5-15µg/ml for Olmesartan and Hydrochlorothiazide. The LOD was found to be 0.58µg/ml and 1.22µg/ml. The LOQ was found to be 1.77µg/ml and 3.70µg/ml for Olmesartan and Hydrochlorothiazide, the correlation coefficient was 0.999 and 0.999 respectively.

Precision and accuracy were determined between 98-100%. The degradation study results shows that the drugs were stable at acidic, alkaline and oxidation and also showed liability in dry heat at 60°C and photolytic condition.

The study concluded that developed method is statistically significant and the result of validation parameters it is evident that the proposed method is accurate, precise, selective, specific, robust and stability indicating. The good percentage of recovery indicates the reproducibility and accuracy of the method. Hence, this developed analytical method can be used in the industry for the routine analysis with more accurate results.

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