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Development and validation of a stability-indicating RP-HPLC method for the simultaneous quantification of Olmesartan Medoxomil and Chlorthalidone in solid dosage form

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ABSTRACT

A simple, rapid, accurate, precise and economical reverse phase high performance liquid chromatographic method was developed for simultaneous quantification of two anti-hypertensive drugs, viz., Olmesartan medoxomil and Chlorthalidone. The separation of both the drugs was achieved on ODS C 18 column (250 × 4.6 mm id, 5 µm particle size) using a mobile phase of Potassium dihydrogen ortho phosphate buffer solution (at pH 4): Acetonitrile (25:75 v/v). The flow rate was 1.2 ml/min and detection was done at 240 nm. The retention time of Chlorthalidone and for Olmesartan medoxomil was 2.3 mins and 3.7 mins respectively. The proposed method was validated as per ICH guidelines. The linearity of the method was evaluated at a range of 50 to 300µg/ml and 31.25 to 187.5µg/ml for Olmesartan medoxomil and Chlorthalidone respectively. The Correlation Coefficient of Olmesartan Medoxomil and Chlorthalidone were 0.999 each. Precision studies were carried out and % RSD of peak areas of Olmesartan Medoxomil and Chlorthalidone was about 0.69 and 0.64 respectively. The percentage recoveries of both the drugs Olmesartan Medoxomil and Chlorthalidone from the tablet formulation were 100.12% and 100.10% respectively. Results obtained for LOQ, LOD and Robustness were well within the acceptance criteria. Validation results indicated that the method is linear, accurate, precise, and robust. The simple mobile phase composition makes this method cost effective, rapid, and non-tedious and can also be successfully employed for simultaneous estimation of both drugs in commercial products.

Keywords: RP-HPLC, Chlorthalidone, Olmesartan medoxomil.

INTRODUCTION

The Olmesartan medoxomil and Chlorthalidone fixed-dose combination is found to show superior antihypertensive efficacy in blood pressure reduction in patients with hypertension when compared with the maximum approved dose of Olmesartan / hydrochlorothiazide.

Olmesartan Medoxomil is an Angiotensin II receptor antagonist with the chemical name (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. It is a white to light yellowish-white powder or crystalline powder which is sparingly soluble in aqueous buffers & soluble in organic solvents [1-3].

Chlorthalidone is a diuretic agent employed in the treatment of hypertension. It is a sulfonamide derivative with different chemical structure from thiazide but the same pharmacological actions as that of thiazide diuretic. The chemical name is 2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H- isoindol-1 yl)benzene-1-sulfonamide. . It is a white or yellowish-white, odorless crystalline powder which is soluble in organic solvents and slightly soluble in water [4].

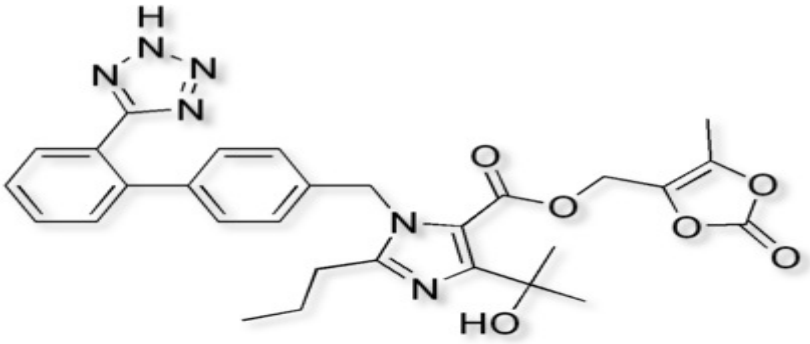

Figure 1: Structure of Olmesartan medoxomil

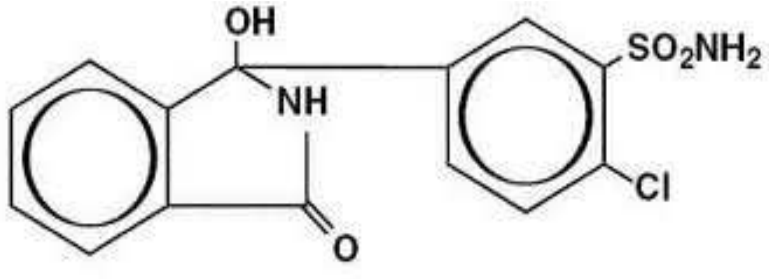

Figure 2: Structure of Chlorthalidone

The combination is US FDA approved as Olmezest-CH 20 tablets on 2013, to treat hypertension in adults. It is available in 20mg and 12.5mg dosages. It is an active ARB (AT2) type and is more effective in lowering blood pressure within 24 hours as compared to other ARBs. Olmesartan Medoxomil an ARB is combined with Chlorthalidone, a thiazide type diuretic in treating hypertension significantly when compared to other fixed dose antihypertensive combination without the difference in safety measurements. Chlorthalidone acts at the proximal portion of the distal convoluted tubule of the nephron and shows longest duration of action when compared to other thiazide diuretics.

The literature survey shows that spectroscopic [5, 6] and chromatographic methods for individual drugs but there is only two methods available for simultaneous quantitation of Olmesartan Medoxomil and Chlorthalidone in solid dosage forms [7-11]. Thus it is inevitable to develop a sensitive, accurate, precise and rapid method for routine analysis of this combination in pharmaceutical dosage form successfully.

MATERIALS AND METHODS

Instrumentation

A Waters 2695 HPLC system with Photodiode Array detector 2996 with data handling system Empower 2 solutions was utilized for the study. Chemicals were weighed using electronic balance Denver; all pH measurements were done on Thermo scientific pH meter.

Reagents and Chemicals

HPLC grade solvents methanol, orthophosphoric acid and Acetonitrile were obtained from Merck Specialties Pvt Ltd, India. AR grade Potassium dihydrogen Orthophosphate and HPLC grade milli-Q water were obtained from Rankem Pharmaceuticals India Ltd. Olmesartan Medoxomil and Chlorthalidone were obtained as pure standards from Divis Labs Pvt Ltd, Hyderabad, India and samples were obtained as [tablets of Olmesartan Medoxomil (20mg) and Chlorthalidone (12.5mg)].

Preparation of buffer (pH 4)

Accurately weighed and transferred 1.36gm of Potassium dihydrogen Orthophosphate in a 1000ml of volumetric flask, about 900ml of HPLC water was added and sonicated to degas and finally made up the volume with water. Then pH was adjusted to 4 with dil. ortho phosphoric acid solution. The solution was filtered through 0.45 µm membrane filter.

Standard preparation

Accurately weighed and transferred 20mg of Olmesartan Medoxomil and 12.5mg of Chlorthalidone working standards into a 10 ml clean dry volumetric flask, 7ml of diluent was added and sonicated to dissolve and the final volume made up with diluent. The solution was filtered through 0.45um filter. From the filtered solution 0.1ml was pipetted out into a 10 ml volumetric flask and made upto 10.0ml with diluent.

Sample preparation

A quantity of powder equivalent to 20mg of Olmesartan Medoxomil and 12.5mg of Chlorthalidone was accurately weighed and transferred into 10ml volumetric flask. About 7ml of diluent was added and sonicated for 15 minutes with intermediate shaking. Cooled to room temperature and diluted to volume with diluent. The solution was filtered through 0.45um PVDF filter. From the filtered solution 0.1ml was pipetted out into a 10 ml volumetric flask and made upto 10.0ml with diluent.

Selection of wavelength maxima

Olmesartan Medoxomil showed absorption maxima at 274.4 nm and Chlorthalidone showed at 256.6 nm. For simultaneous estimation a common wavelength for detection was selected at 240nm.

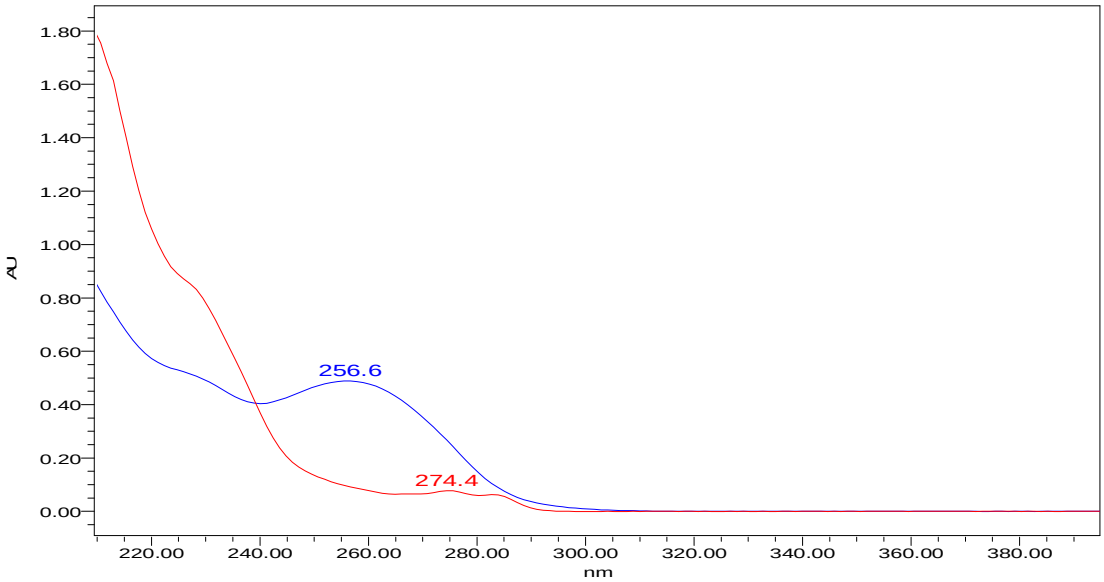

Fig. 3: UV Spectrum of Olmesartan Medoxomil and Chlorthalidone

Method Development

By using the chromatographic conditions that were used for assay of Angiotensin- II blocker as reference, various trials were made. At each trial mixture of known components were injected and observed for resolution and tailing factor. Various proportions of buffer and Acetonitrile were tried as mobile phase and a ratio of buffer to Acetonitrile as 25:75 gave improved peak symmetry and resolution. Different flow rates of the mobile phase were tried for good resolution. Both the drugs Olmesartan Medoxomil and Chlorthalidone were found to be soluble and stable in a mixture of buffer pH4 and Acetonitrile. Finally the chromatographic conditions were optimized at flow rate 1.2ml/min, injection volume of 10 µL, run time of 8 minutes, at column oven temp 30°C with methanol: water (50:50) sonicated and degassed used as diluent with a STD ODS, C18, (250mm x 4.6mm), 5 µm columns.

Table 1: Standards of Olmesartan Medoxomil and Chlorthalidone

Serial. no	Peak name	Retention time	Area	Plate count	Tailing
1	Chlorthalidone	2.317	4321255	7799	1.18
2	Olmesartan	3.763	2668035	7604	1.17

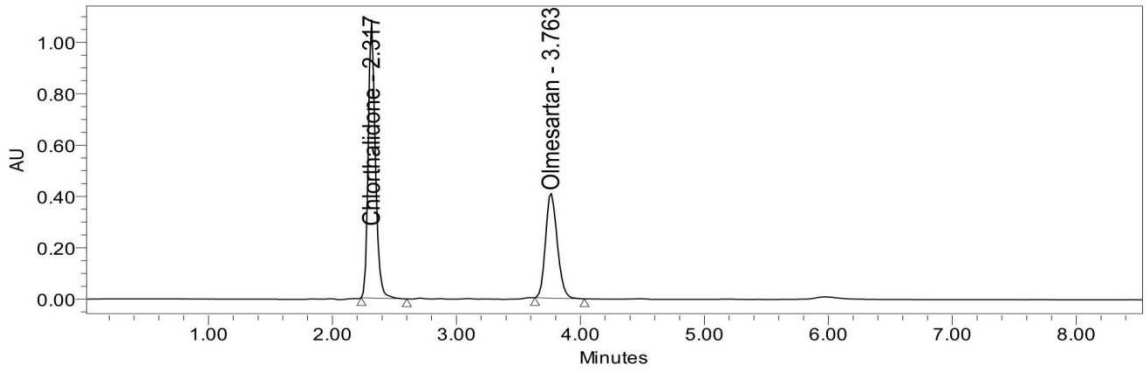

Fig. 4: Representative chromatogram of Olmesartan Medoxomil and Chlorthalidone

The retention time for Olmesartan Medoxomil and Chlorthalidone was found to be 2.3 minutes and 3.7 minutes respectively.

For both the drugs Olmesartan Medoxomil and Chlorthalidone the tailing factor was found to be < 2.
Further the method was validated under the proposed chromatographic conditions.

Method Validation
Once chromatographic conditions were established, the method was validated in compliance with ICH guidelines. The following parameters like system suitability along with specificity, linearity, precision, and accuracy, limits of detection and limit of quantification were performed.

Forced degradation studies
Oxidation
To 1 ml of stock solution of Olmesartan Medoxomil and Chlorthalidone, 1ml of 3% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 60°C for 8 hours. For HPLC study, the resultant solution was diluted to obtain 200µg/ml & 125µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The chromatograms were represented as **Fig no: 9**.

Acid Degradation Studies
To 1ml of stock solution of Olmesartan Medoxomil and Chlorthalidone, 1ml of 0.1N Hydrochloric acid was added and refluxed for 60°C for 8 hours. The resultant solution was diluted to obtain 200µg/ml & 125µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. The chromatograms were represented as **Fig no: 10**.

Alkali Degradation Studies
To 1 ml of stock solution Olmesartan Medoxomil and Chlorthalidone, 1 ml of 0.1N sodium hydroxide was added and refluxed for 60°C for 8 hours. The resultant solution was diluted to obtain 200µg/ml & 125µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The chromatograms were represented as **Fig no: 11**.

Dry Heat Degradation Studies
The standard drug solution was placed in oven at 105°C for 8 hours to study dry heat degradation. For HPLC study, the resultant solution was diluted to 200µg/ml & 125µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.** The chromatograms were represented as **Fig no: 12**.

Photo Stability studies
The photochemical stability of the drug was also studied by exposing the 1ml solution to UV Light by keeping the beaker in UV Chamber for 24 hours. For HPLC study, the resultant solution was diluted to obtain 200µg/ml & 125µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. The chromatograms were represented as **Fig no: 13**.

RESULTS AND DISCUSSION

System Suitability

The standard solution was prepared by using working standard as per the method. For six replicate injections system suitability parameters like number of theoretical plates, USP Tailing was found to be within specified limits. The results are given in **Table 2**.

Table 2: Retention time of Olmesartan Medoxomil and Chlorthalidone

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Chlorthalidone	2.314	3770559	7582	1.19
2	Chlorthalidone	2.323	3856012	7221	1.26
3	Chlorthalidone	2.323	3820957	7109	1.26
4	Chlorthalidone	2.324	3802654	7099	1.27
5	Chlorthalidone	2.328	3864979	7195	1.19
6	Chlorthalidone	2.329	3809284	7342	1.19
Mean			3820741		
Std.Dev.			35150		
%RSD			0.9		

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Olmesartan	3.761	2303645	7503	1.18
2	Olmesartan	3.767	2317112	7693	1.17
3	Olmesartan	3.769	2315630	7647	1.17
4	Olmesartan	3.770	2320573	7586	1.16
5	Olmesartan	3.770	2295842	7560	1.17
6	Olmesartan	3.774	2322107	7417	1.17
Mean			2312485		
Std.Dev.			10435		
%RSD			0.5		

Specificity

It is the ability of the method to measure the analyte of interest specifically in presence of matrix and other components. Samples of blank and placebo were injected as per the test procedure. The chromatograms of placebo were represented as **Fig no: 5 & 6**.

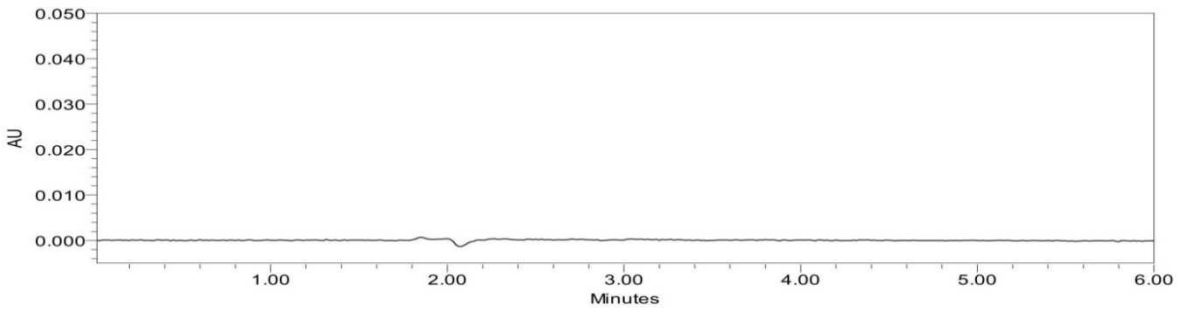

Fig-5 chromatogram of Placebo

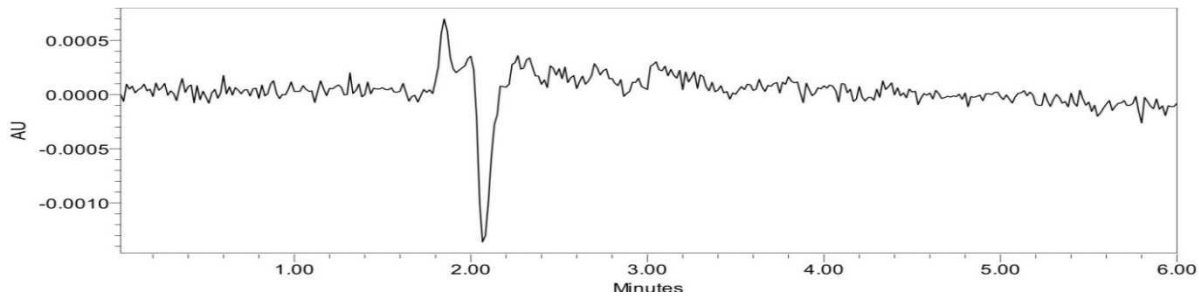

Fig: 6: Chromatogram of blank

Linearity

Linearity of detector response was established by plotting graph between concentrations versus peak areas of the analytes. Data is shown in **Table 3** and represented graphically in Graph **Fig 7 and Fig 8.**

Table 3: Results of Linearity

Levels	Concentration (µg/ml)		Average area count	
	Olmesartan	Chlorthalidone	Olmesartan	Chlorthalidone
Level-25%	50	31.25	655654	1049938
Level-50%	100	62.5	1202192	1959197
Level-75%	150	93.75	1849116	2987350
Level-100%	200	125	2423296	3859159
Level-125%	250	156.25	3124963	4935006
Level-150%	300	187.5	3683575	6002943
Correlation Coefficient	-	-	0.999	0.999

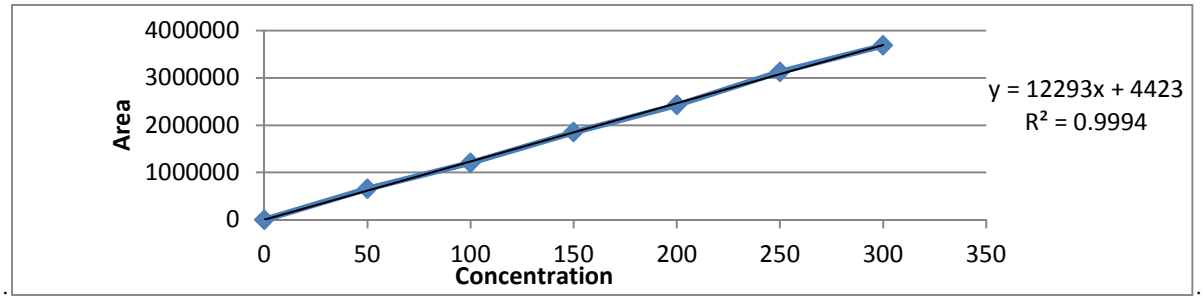

Fig. 7: Linearity Curve of Olmesartan Medoxomil

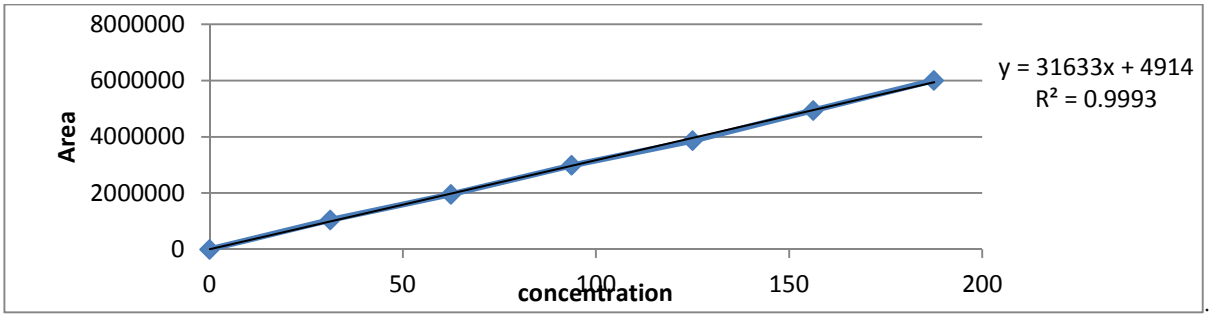

Fig. 8: Linearity Curve of Chlorthalidone

Accuracy

Accuracy was determined by recovery studies at three different levels equivalent to 50%, 100%, 150%. Sample at each level is injected in triplicate. The concentration of the drug product in the solution was determined using assay method. The % RSD, mean recoveries was calculated, which shows that method is accurate. Data was shown in **Table 4.**

Table 4: Results of Accuracy (%Recovery Studies)

Sample No.	% Level	%assay of OLM	Mean % assay of OLM	%assay of CTD	Mean %assay of CTD
1	**50%**	99.20	99.90	99.55	99.79
2		99.74		100.21	
3		100.77		99.61	
1	**100%**	99.27	100.14	99.50	100.17
2		100.61		100.45	
3		100.54		100.56	
1	**150%**	100.33	100.34	99.33	100.05
2		100.66		100.65	
3		100.03		100.18	

Precision

System precision:

Six replicate injections of standard solution were injected into the HPLC system. The %RSD of peak areas for six replicate injections was found to be in the limits. Data was shown in **Table 5.**

Method precision:
The precision of test method was evaluated by analyzing assay for six individual samples prepared from same batch by the proposed method. The average % Assay and the relative standard deviation for the six sample preparation were found to be in the specified limits. Data was shown in **Table 6.**

Table 5: Results of System precision

System Precision	Olmesartan Areas	Chlorthalidone Areas
1	2303645	3770559
2	2317112	3856012
3	2315630	3820957
4	2320573	3802654
5	2295842	3864979
6	2322107	3809284
AVG	2312485	3820741
SD	10435	35150
%RSD	0.5	0.9

Table 6*:* Results of method precision

Sample ID	% Assay Olmesartan	% Assay Chlorthalidone
1	99.22	100.60
2	100.23	100.74
3	100.76	99.65
4	99.61	99.70
5	99.29	99.05
6	98.94	100.18
Mean	99.67	99.99
SD	0.6945	0.6418
% RSD	0.69	0.64

Limit of Detection (LOD) and Limit of Quantification (LOQ):
The LOD and LOQ were determined and reported based on the calibration curve of standard solution.

LOD = 3.3 × D/S and LOQ = 10 × D/S, where, D is the standard deviation of Y-intercepts of regression line and S is the slope of the calibration curve.

The LOD is the lowest concentration of the analyte that gives a measurable response. LOD of Olmesartan medoxomil and Chlorthalidone were found to be 1.19µg/mL & 0.5119µg/mL. The LOQ is the lowest concentration of the analyte, which gives response that can be accurately quantified. The LOQ of Olmesartan medoxomil and Chlorthalidone were found to be 3.60µg/mL & 1.5560µg/mL.

Robustness
Robustness of the method was investigated by varying the instrumental conditions such as flow rate (±10%) & organic content in mobile phase (±2%). Standard solution was prepared and analyzed as per the test procedure and the system suitability parameters were monitored.

Table 7: Results of Robustness

System suitability Parameters		USP Tailing		USP Plate count		% RSD	
		OLM	CTD	OLM	CTD	OLM	CTD
Flow Rate	1.11ml/min	1.16	1.12	7824	7765	0.1	0.9
	1.21ml/min	1.23	1.17	7376	7697	0.5	0.8
	1.31ml/min	1.18	1.18	7277	7904	0.1	0.2
Mobile Phase	15:85	1.18	1.17	7473	7451	0.2	0.1
	25:75	1.2	1.17	7376	7697	0.6	0.8
	35:65	1.16	1.20	7450	7973	0.1	1.3
Temperature	25ºC	1.17	1.18	7277	7451	0.1	1.0
	30 ºC	1.34	1.18	7376	7697	0.5	0.8
	35 ºC	1.17	1.26	7765	7450	1.3	1.7

OLM- Olmesartan, CTD- Chlorthalidone

Forced Degradation Studies:
The Data for Forced degradation are tabulated in **Table 8**. There was no interference of any peak at the retention time of analyte peaks from blank and placebo, Peak purity of all the treated samples was well within the limits.

From this it has been concluded that the proposed method is specific and stability indicating for the estimation of Olmesartan Medoxomil and Chlorthalidone, in the tablet dosage form.

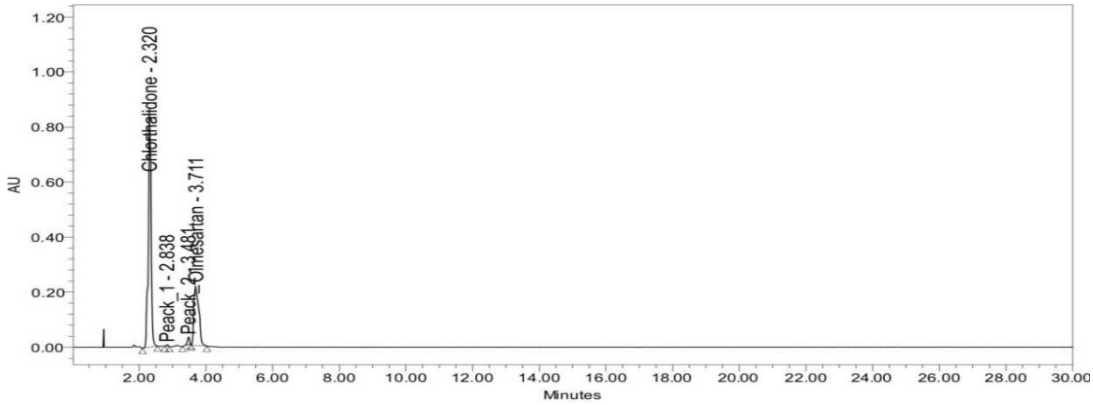

Figure 9: Typical chromatogram of Oxidation

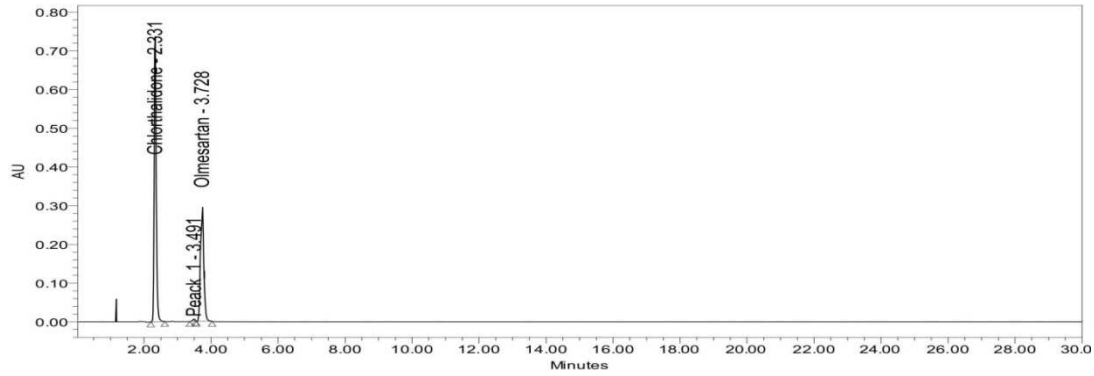

Figure 10: Typical chromatogram of Acid Hydrolysis

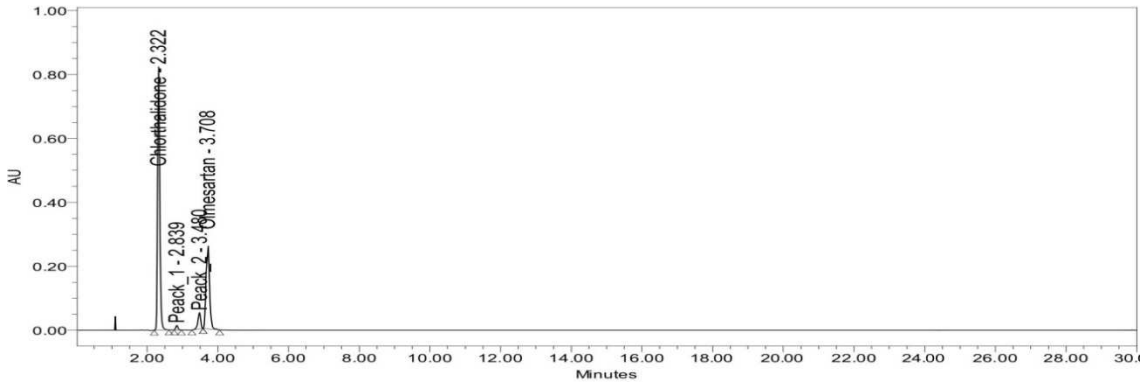

Figure 11: Typical chromatogram of Alkali Hydrolysis

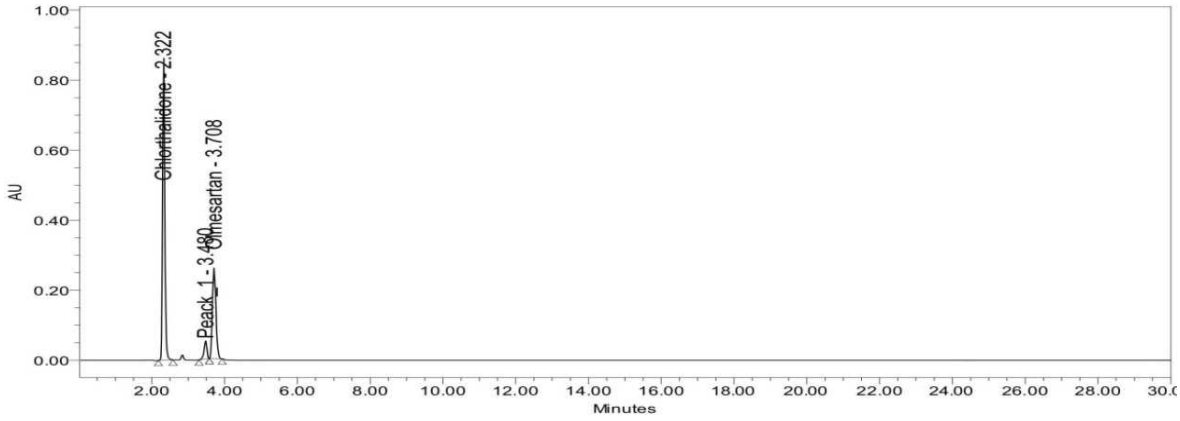

Figure 12: Typical Chromatogram of Thermal Degradation

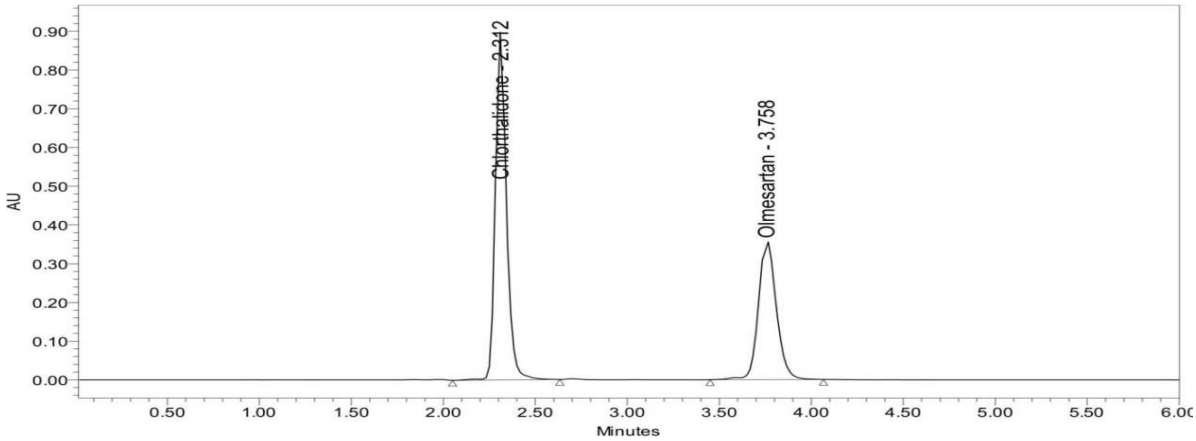

Figure 13: Typical chromatogram of UV

Table 8: Data of forced degradation

S.No	Sample condition	Analytes	% ASSAY	% Degradation	Purity angle	Purity threshold
1	Untreated sample	OLM	99.24	-	0.264	0.578
		CTD	99.21	-	0.448	0.428
2	Peroxide treated	OLM	94.44	4.8	0.321	0.541
		CTD	91.3	7.91	0.122	0.306
3	Acid treated	OLM	92.23	7.01	0.199	0.422
		CTD	92.52	6.69	0.130	0.412
4	Alkali treated	OLM	93.40	5.84	0.156	0.568
		CTD	92.91	6.3	0.494	0.692
5	Thermal /Dry heat exposed	OLM	95.94	3.30	0.282	0.392
		CTD	94.66	4.55	0.494	0.691
6	Photolytic degradation	OLM	98.61	0.6	0.206	0.417
		CTD	97.99	1.22	0.493	0.603

CONCLUSION

An attempt was made to develop a stability indicating RP-HPLC method for the simultaneous estimation of Olmesartan and Chlorthalidone. The method was optimized and the accountability of the newly developed method was established by validation as per ICH guidelines. Further the method was subjected to forced degradation studies and the percentage degradation at each degradation study was within the limits. The results of each validation parameter were in good agreement with acceptance criteria. Therefore the method has been proven to be linear, precise, accurate, specific, robust and stable. Hence we recommend that this method can be a good approach for the quantification of Olmesartan and Chlorthalidone in combination dosage form and can be adopted for the routine quality control analysis of these drugs.

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