Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(3):2440-2448

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Optimization and validation of RP-HPLC stability indicating method for simultaneous determination of Olmesartan Medoxomil and Chlorthalidone in pure drug and pharmaceutical dosage form

Mohammad Yunoos*[1] and D. Gowri Sankar[2]

[1]*Department of Pharmaceutical Analysis, Bapatla College of Pharmacy, Bapatla, Guntur (Dist.), Andhra Pradesh, India*
[2]*College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India*

ABSTRACT

A simple and precise stability indicating RP-HPLC method was developed and validated for the simultaneous determination of Olmesartan Medoxomil and Chlorthalidonein pure drug and pharmaceutical dosage forms. Chromatography was carried out on STD HypersilC_{18}(150x4.6mm, 5µ particle size) analytical column using a mobile phase ofPhosphate buffer(KH_2PO_4) adjusted topH 5.0 with dilute orthophosphoric acidand methanol in the ratio of 40: 60 % v/v at a flow rate of 1.2 ml/min. The analyte was monitored using PDA detector at 240 nm. The retention time was found to be 2.240min and 3.042min for Olmesartan Medoxomil and Chlorthalidone respectively. Linearity was observed in the concentration range of 60-180 µg/mland 18.75-56.25 µg/mlfor both Olmesartan Medoxomil and Chlorthalidonewith correlation coefficient of 0.999 respectively. The mean % recoveries obtained for Olmesartan Medoxomil and Chlorthalidone were found to be 100.02% and 99.97% respectively.Stress testing which covered acid hydrolysis, base hydrolysis, peroxide, photolytic and thermal degradation was performed to prove the specificity of the proposed method and degradation was achieved. The developed method has been statistically validated according to ICH guide lines and found to be simple, precise and accurate with the prescribed values. Thus the proposed RP-HPLC method was successfully applied for the stability indicating simultaneous estimation of Olmesartan Medoxomil and Chlorthalidonein routine quality control analysis in bulk and marketed formulations.

KEYWORDS: Olmesartan Medoxomil, Chlorthalidone, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

Olmesartan Medoxomil:
Chemically, it is2, 3-dihydroxy-2-butenyl-4(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole5-carboxylate, cyclic 2, 3-carbonate. It has a molecular formula of $C_{29}H_{30}N_6O_6$ and molecular weight of 558.59 g/mol. Olmesartan Medoxomil belongs to class of Angiotensin II receptor antagonistsand used as an antihypertensive agent. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle. Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin converting enzyme (ACE, kininase II). Angiotensin II is the principal pressor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium. [1]

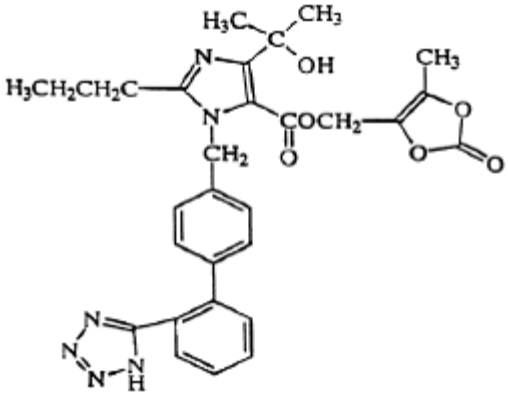

Fig.1:Chemical structure of Olmesartan Medoxomil

Chlorthalidone:
Chemically, it is 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide. It has a molecular formula of $C_{14}H_{11}ClN_2O_4S$ and molecular weight of 338.766 g/mol. It is used as an antihypertensive agent, diuretic and sodium chloride symporter inhibitor. Chlorthalidone inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, Chlorthalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism.

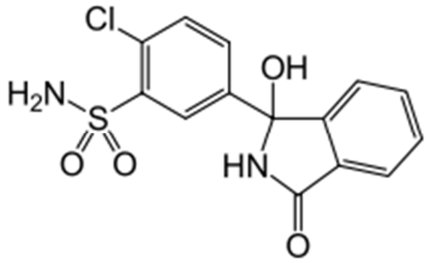

Fig.2:Chemical structure of Chlorthalidone

Literature survey reveals that few analytical methods were reported like LC-MS/MS methods in biological samples like human plasma [2-3], RP-HPLC methods[4-10]and spectrophotometric method [11]in single or in combination with other drugs in pharmaceutical dosage forms.However there was no stability indicating method reported for this drug combination and hence the present study was aimed to develop a simple, fast, economical, selective, accurate, precise and sensitive stability indicating RP-HPLC method for the simultaneousdeterminationof Olmesartan Medoxomil and Chlorthalidonein bulk and its Pharmaceutical dosage forms suitable for routine quality control analysis.

EXPERIMENTAL SECTION

Chemicals:
Olmesartan Medoxomil and Chlorthalidone were obtained as gift samples from Dr.Reddy's Laboratories, Hyderabad and IPCA Laboratories Ltd., Mumbai. HPLC grade water, methanol and acetonitrile were purchased from E.Merck. Chem.ltd., Mumbai. All the chemicals used were of analytical reagent grade (E.Merck). Fixed dose combination tablet formulation (Arbchek CT) containing 40 mg of Olmesartan Medoxomil and 12.5 mg of Chlorthalidone were procured from local market.

Instrumentation:
Quantitative HPLC was performed on Waters technologies2695 series, PDA detector module equipped with auto injector with empower software. A reverse phaseSTD HypersilC_{18}(150x 4.6mm, particle size 5µm) analytical column was used. Weighing was done on shimadzu balance (AX 200)and pH adjustments done using pH meter (Unichem AD102U) was used.

Chromatographic conditions:
Chromatographic separation and analysis was carried out on STD HypersilC_{18} column(150x4.6mm, 5 µ particle size) column. The optimized mobile phase consisting of phosphate buffer (potassium dihydogenortho phosphate pH adjusted to 5.0 with diluteorthophosphoric acid)and methanol in the ratio of 40:60 %v/v. Flow rate was maintained

at 1.2ml/min and run time for 7 min.Prior to sample injection, column was saturated with mobile phasefor 30 min and injection volume of10µl was injected using auto sampler mode. The detection response was measured at 240 nm at ambient temperature.

Preparation of Phosphate buffer:
Accurately weighed 2 gm of potassium dihydrogenortho phosphate in a 1000 ml of volumetric flask. Added about 900 ml of HPLC grade water and degas to sonicate for 5 min. and finally made up the volume with HPLC gradewater and then pH was adjusted to 5.0 using dil. OPA.

Preparation of mobile phase:
Prepared by mixing phosphate buffer and methanol in the ratio of 40: 60 % v/v, sonicated for 5 min, followed by degassing using vacuum filtration containing 0.45 µm membrane filter.

Preparation of Standard solutions:
Accurately weighed and transferred 40mg of Olmesartan medoxomil and 12.5mg of Chlorthalidone reference standards into a 50 ml clean dry volumetric flask and added 3/4th volume of methanol, sonicated for 5min and made up to the final volume with mobile phase. From the above stock solution, 1.5 ml was pipette out in to a 10 ml volumetric flask and then made up to the final volume with mobile phase to obtain concentration of 120µg/ml of Olmesartan medoxomil and 37.5µg/ml of Chlorthalidone respectively.

Preparation of sample solution:
20 tablets were weighed and determined the average weight and then crushed to a fine powder. The tablet powder equivalent to 40 mg of Olmesartan medoxomil and 12.5 mg of Chlorthalidone was weighed accurately and transferred into a 50 ml volumetric flask, 30 ml of mobile phase was added and sonicated for 5 min , then made up with mobile phase and filtered. From the filtered solution, 1.5ml was pipette out into a 10 ml volumetric flask and made up to mark with mobile phase. Injected 10 µl of filtered portion of the sample and standard preparation into the chromatograph. Record the responses for the major peaks. Calculated the content of Olmesartan medoxomil and Chlorthalidonepresent in each tablet.

Method validation:
System suitability:
System suitability test should be carried out to verify that the analytical system is working properly and can give accurate and precise results. Standard solutions were prepared as per the test method and injected five times into the chromatographic system. The system suitability parameters were evaluated for tailing factor, retention times and theoretical plates of standard chromatograms.The results obtained are shown in table no.1

Accuracy:
The accuracy of an analytical method is the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.The study was performed by making three different concentrations at 50%, 100% and 150% levels using standard addition method where sample preparations were spiked with known amount of standard and then each concentration was injected three times into the chromatographic system. The accuracy of an analytical method should be established across its range.

System Precision and Method precision:
The system precision was carried out to ensure that the analytical system is working properly by injectingstandard preparation six times into the HPLC and the retention time and peak areas were measured and % RSD was calculated**.** In method precision, a homogenous sample containing Olmesartan medoxomil and Chlorthalidoneof a single batch were analyzed six times and % RSD was calculated.

Specificity:
Specificity is the ability to assess unequivocally the analyte in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the Chromatograms obtained from the drug standards with that of obtained from the tablet preparations .The retention times of the drug standards and the drug from sample preparations were same, so the method was specific without interference from excipients in the tablets.

Linearity:
The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different concentrations of standard solutions were prepared by diluting aliquots (0.75- 2.25 ml) of standard

stock solution (800µg/ml for Olmesartan medoxomil and 250µg/ml for Chlorthalidone) in to10 ml volumetric flasks to obtained concentrations in the range of 60-180µg/ml for Olmesartan medoxomil and 18.75-56.25µg/ml for Chlorthalidoneand then injected each into the chromatographic system and the chromatograms were recorded.

Robustness:
The robustness of the proposed method was determined by analyzing aliquots from homogenous lots by differing physical parameters like mobile phase composition, flow rate and column temperature. The standard and sample solutions were injected into the chromatograph at varied conditions of flow rate ± 0.2 ml/min, mobile phase buffer pH ± 0.2 units and temperature by $\pm$ 5 ^{0}c.

Ruggedness (Intermediate precision):
It is carried out by injecting standard solution preparations six times into the chromatographic system on two different days. %RSD was determined for retention time and peak areas of standard and sample solutions ofOlmesartan medoxomil and Chlorthalidone.

Forced degradation:
Stress testing of the drug substance can help identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and to develop and validate the stability indicating power of the procedures used.

Acid degradation studies:
To 1.5 ml of stock solution of Olmesartan medoxomil and Chlorthalidone, 1.5 ml of 2N Hydrochloric acid was added and refluxed for 30 min at 60 ^{0}C and then neutralized the solution with 1.5 ml of 2N NaOH solution.The resultant solution was diluted with mobile phase in a 10 ml volumetric flask to obtain concentration of 120µg/ml & 37.5µg/ml respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample as shown in figure 9**.**

Base degradation studies:
To 1.5 ml of stock solution of Olmesartan medoxomil and Chlorthalidone, 1.5 ml of 2N sodium hydroxide was added and refluxed for 30min at 60 ^{0}C and then neutralized the solution with 1.5 ml of2N HCL solution. The resultant solution was diluted with mobile phase in a 10 ml volumetric flask to obtain concentration of 120µg/ml & 37.5µg/mlrespectively. Then 10 µl solutionswere injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample as shown in figure 10**.**

Oxidation (Peroxide) studies:
To 1.5 ml of stock solution of Olmesartan medoxomil and Chlorthalidone, 1.5ml of 20% Hydrogen peroxide (H_2O_2)was added and kept for 30 min at 60^{o}c. The resultant solution was diluted with mobile phase in a 10 ml volumetric flask to obtain concentration of 120µg/ml & 37.5µg/mlrespectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample as shown in figure 11**.**

Thermal studies:
Stress testing under neutral conditions was studied by refluxinginwaterfor6hrs at60ºc. For HPLC study, the resultant solution was then diluted with mobile phase in a 10 ml volumetric flask to obtain concentration of 120µg/ml & 37.5µg/mlrespectivelyand 10µl solutions were injected intothechromatographic systemandthechromatogramswererecorded toassessthestabilityofthesample as shown in figure 12.

Photolytic studies:
It is carried out by exposing 1.5 ml of stock solution of Olmesartan medoxomil and Chlorthalidone to UV light, by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber.The resultant solution was diluted with mobile phase in a 10 ml volumetric flask to obtain concentration of 120µg/ml & 37.5µg/mlrespectively and 10µl solutions were injected into the chromatographic system and the chromatograms were recordedtoassessthestabilityofsample as shown in figure 13.

RESULTS AND DISCUSSION

Olmesartan medoxomil is not official in any pharmacopoeia and there is no stability indicating RP-HPLC methodwas reported for simultaneous estimation with chlorthalidone in bulk and pharmaceutical dosage forms.

Hence author has planned to develop and validatestability indicating RP-HPLC method for simultaneous estimation of Olmesartan medoxomil and Chlorthalidonein pharmaceutical dosage form.

From this study, it was found that a simple, precise, accurate, sensitive and efficient stability indicating RP-HPLC method has been developed and validated for the estimation of Olmesartan medoxomil and Chlorthalidonein pharmaceutical dosage form. Chromatographic separation were carried out on STD HypersilC_{18}column (150x 4.6mm,5µ particle size) using mobile phase composed of Phosphate buffer (KH_2PO_4) adjusted topH 5.0 with dilute orthophosphoric acidand methanol in the ratio of 40: 60 % v/v at a flow rate of 1.2 ml/minusing PDA detection at 240 nm. The retention time of Olmesartan medoxomil and Chlorthalidonewere found to be 2.240min and 3.042minrespectively.

Linearity was evaluated in the concentration range of 60-180µg/ml for Olmesartan medoxomil and 18.75-56.25µg/ml for Chlorthalidone. The calibration curves of Olmesartan medoxomil and Chlorthalidonewere described by the equation y=36981x-2407.7 and y=177591x+2650.07 with correlation coefficient of 0.999 as shown in figure 3 and figure 4. The standard and sample chromatograms in the specifity studies are shown in figure 5 and figure 6. The Limit of detection (LOD) and limit of quantification (LOQ) have shown in figure 7 and figure 8. System suitability results as shown in table no.1. Accuracy data as shown in table no.2. Validation summary and assay resultsfrom marketed formulationsare given in table no.3.The results of robustness studies are shown in table no. 4 and 5. The stress testing results for both Olmesartan medoxomil and Chlorthalidoneare shown in table 6 and table 7.The %RSD in precision, accuracy and robustness studies were found to be less than 2.0%, indicating that the method is precise, accurate and robust. The blank and placebo injections were also identical without any interference from the excipients.

Table-1: System suitability studies

S.No	System suitability parameters	Olmesartan medoxomil	Chlorthalidone
1	Tailing factor(T_f)	1.41	1.27
2	Resolution (Rs)	6.50	
3	Retention time(Min)	2.240	3.042
4	Theoretical plates(N)	8138	7431

Table-2: Accuracy Results

Sample	Level	Peak area*	Mean % Recovery *± SD
Olmesartan medoxomil	50%	2213457	99.82±0.12
	100%	4432197	99.94±0.82
	150%	6680946	100.04± 0.53
Chlorthalidone	50%	3333808	99.84±0.24
	100%	6660827	99.76±0.33
	150%	9998688	99.88±0.62

**Mean of three determinations*

Linearity:

The calibration curve were found to be linear over the concentration range of 60-180 µg/mlfor Olmesartan Medoxomil and 18.75-56.25µg/ml for Chlorthalidone. The correlation coefficient was found to be 0.999for bothOlmesartan Medoxomil and Chlorthalidone respectively.

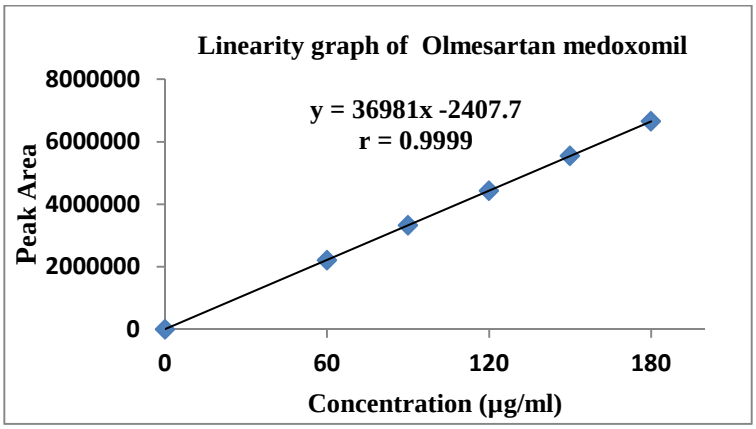

Fig.3: Linearity Graph of Olmesartan medoxomil (60-180 µg/ml)

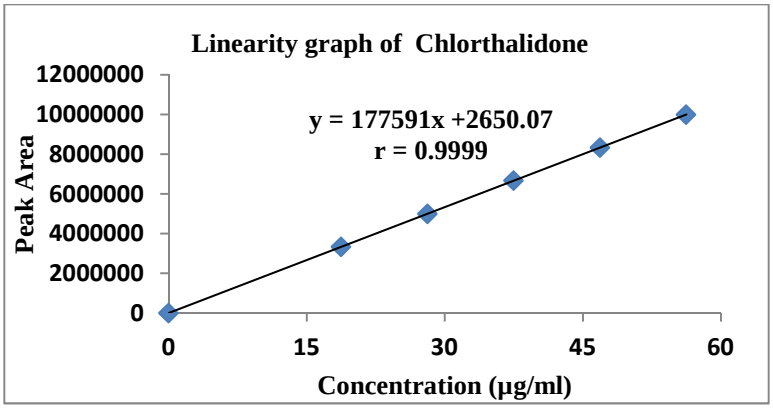

Fig.4: Linearity Graph ofChlorthalidone(18.75-56.25 µg/ml)

Table -3: Validation Parameters of the proposed RP-HPLC Method

Parameter	Olmesartan medoxomil	Chlorthalidone
Regression equation	y=36981x-2407.7	y=177591x+2650.07
Correlation coefficient	0.9999	0.9999
LOD (µg/ml)	0.50	0.36
LOQ (µg/ml)	1.52	1.12
System precision (% RSD)	0.52	0.37
Method precision(% RSD)	0.22	0.46
% Assay	99.6-99.8%	99.7-100.02%

Specificity studies:

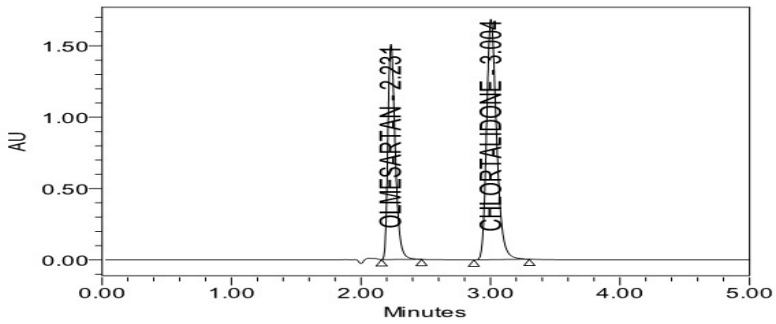

Fig.5: Typical chromatogram of standard

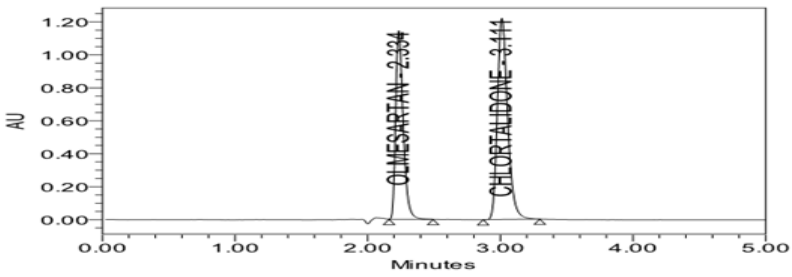

Fig.6: Typical chromatogram of sample

Limit of detection (LOD) and Limit of Quantification (LOQ):

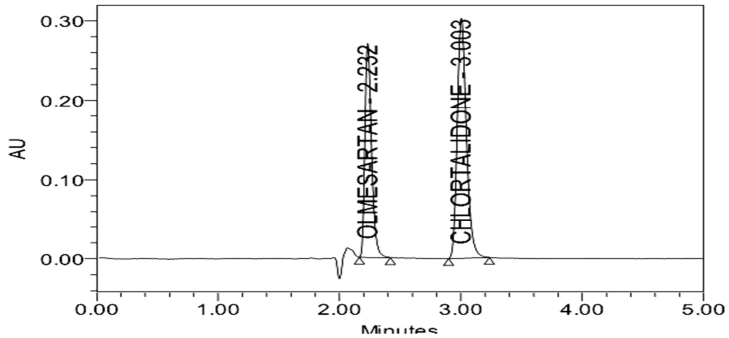

Fig.7: Typical chromatogram of Limit of detection (LOD)

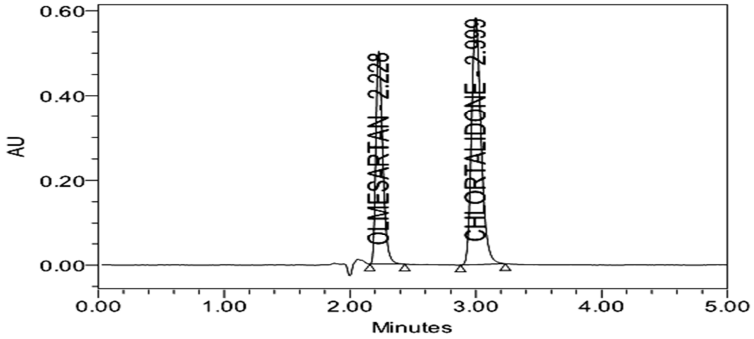

Fig.8: Typical chromatogram of Limit of Quantification (LOQ)

Robustness:

The developed method is robust with deliberate changes with variation of mobile organic phase composition, flow rate and temperature for both Olmesartan Medoxomil and Chlorthalidone**.**

Table-4: Results of robustness study of Olmesartan Medoxomil

S.No.	Parameter	Change Level	Olmesartan Medoxomil			
			Rt (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (±0.2ml/min)	1.0	2.651	5416993	1.45	8855
		1.4	1.899	3832931	1.40	7967
2.	Mobile organic phase composition (±10%v/v/v)	50:50	2.212	4666874	1.58	9723
		30:70	2.147	4856059	0.81	3371
3.	Temperature (±5°C)	25 ^{0}C	2.229	4477004	1.41	8276
		35 ^{0}C	2.220	4466892	1.42	8654

Table-5: Results of robustness study ofChlorthalidone

S.No.	Parameter	Change Level	Chlorthalidone			
			Rt (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (±0.2ml/min)	1.0	3.528	8053214	1.32	8731
		1.4	2.554	5735935	1.27	7095
2.	Mobile organic phase composition (±10%v/v/v)	50:50	2.650	7032668	1.35	10402
		30:70	3.313	6260982	1.28	4759
3.	Temperature (±5°C)	25 ^{0}C	3.001	6688374	1.28	7597
		35 ^{0}C	2.975	6702263	1.26	8119

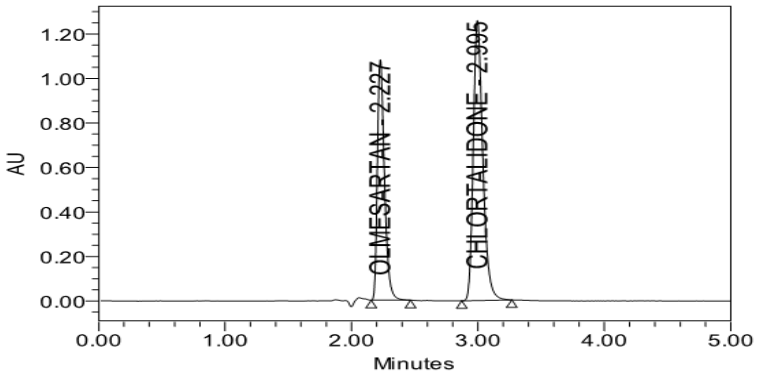

Fig.9: Chromatogram of Acid hydrolysis

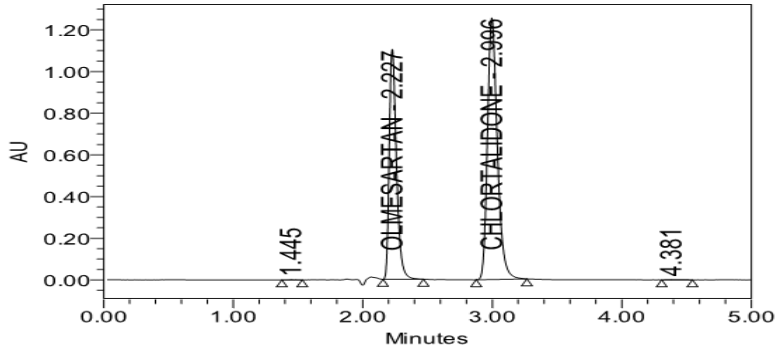

Fig.10: Chromatogram of Base hydrolysis

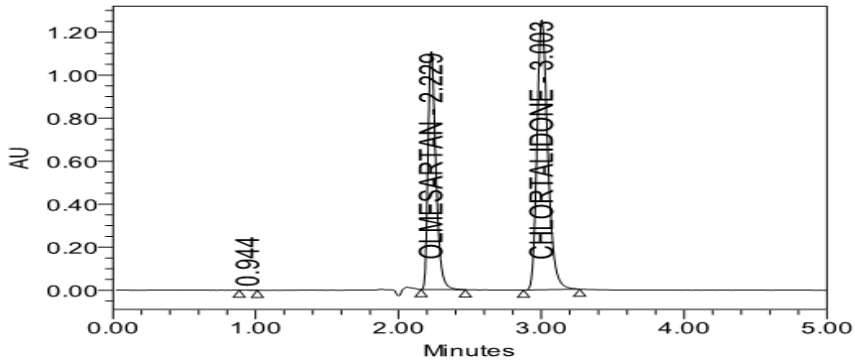

Fig.11: Chromatogram of Oxidation (peroxide)

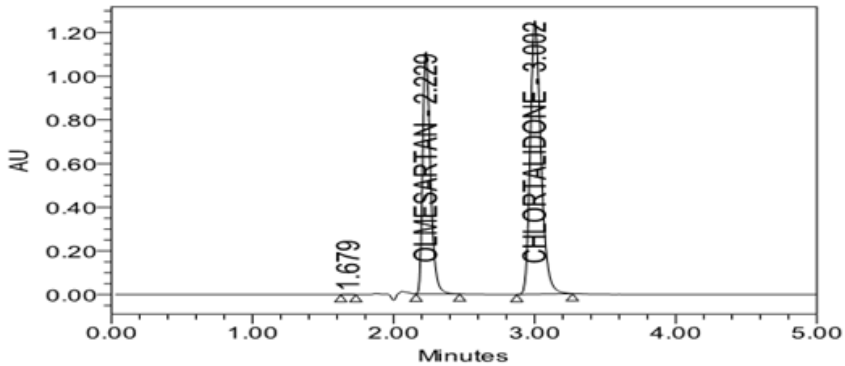

Fig.12:Chromatogram of Heat Exposure

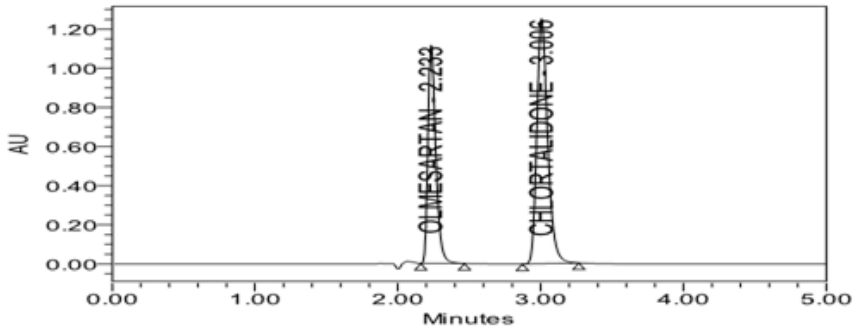

Fig.13:Chromatogram of UV Exposure

Table-6: Degradation Study of Olmesartan medoxomil

S.No.	Condition	Peak Area	Degradation % Assay	% Net Degradation
1	Acid degradation Hydrolysis	3229464	72.84	27.12
2	Base Hydrolysis	3198986	72.14	27.78
3	Oxidation (peroxide)	3237905	73.22	26.91
4	Heat Exposure	3311014	74.71	25.27
5	UV Exposure	3480429	78.45	21.47

Table-7: Degradation Study of Chlorthalidone

S.No.	Condition	Peak Area	Degradation % Assay	% Net Degradation
1	Acid degradation Hydrolysis	5107808	77.14	22.89
2	Base Hydrolysis	5227870	78.88	21.08
3	Oxidation (peroxide)	5290753	79.82	20.14
4	Heat Exposure	5380103	81.02	18.89
5	UV Exposure	5498028	82.84	17.02

CONCLUSION

From this study it is concluded that the proposed Stability Indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Olmesartan medoxomil and Chlorthalidone in bulk & Pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

Acknowledgement
The author is grateful to Bapatla College of Pharmacy, Guntur dist., Andhra Pradesh, India for providing research facilities.

REFERENCES

[1] SGChrysant; MA Weber;AC Wang and DJ Himman. *Am.J.Hypertens.***2004**, 17, 252-259.
[2]Vikas V. Vaidya;Shikha M. N. Roy; Santosh M. Y; Santosh S. Joshi. *Chromatographia*, **2008**, 67(1-2), 147-150.
[3]DLiu;P Hu ; N Matsushima; X Li . *J Chromatogr. B*, **2007**, 856(1-2), 190-197.
[4]Liu JF Wang.*J Pharm. Anal.*,**2006**, 26(5), 686-691.
[5]TMurakami;H Konno;F Kusu; et.al.*J Pharm. Biomed. Anal.*,**2008**, 47(3), 553-559.
[6]PVipulRane; R KiranPatil; et al. *Chromatographia*, **2009**, 69(1), 169-173.
[7]LisianeBajerski; Rochele C Rossi; Pedro E.*Chromatographia*, **2008**, 68(11), 991-996.
[8]JSidhdhaparaMital; Biraju Patel; Ashok Paramar. *International Journal of Pharmacy and Biological Sciences*, **2014**, 4 (1), 157-160.
[9]R. A. Mhaske; S. A Sahasrabudhe.*IJPSR*,**2012**, 3(4), 1116-1123.
[10]K MaheshBabu;J.Bikshalbabu.*journal of Atoms and Molecules*, **2012**, 2(1), 117-26.
[11]M Celebier; S Altinoz. *Pharmazie*,**2007**, 62(6), 419-422.