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Novel and Validated Stability-Indicating HPLC Method for Simultaneous Estimation of Olmisartan and Chlorthalidone in Oral Solid 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 Olmesartan and Chlorthalidone. The separation of both the drugs was achieved on BDS C18 250mm x 4.6 mm, 5µ using a mobile phase of 10 mM orthophosphoric acid buffer and acetonitrile (45:55v/v) at a flow rate of 1.0 mL min^{-1} and detection was performed at 212 nm using photodiode array (PDA) detector. The drug was subjected to various ICH prescribed stress conditions including hydrolysis (neutral, acid and alkaline), oxidation, photolysis and thermal degradation. The proposed method was validated with respect to specificity, linearity, accuracy, and precision, limit of detection (LOD), limit of quantitation (LOQ), stability and robustness as per ICH guidelines. The proposed analytical method could effectively separate the drug from its degradation products employed as stability indicating studies.

Keywords: Stability Indicating, Validation, Reversed phase, Liquid Chromatography, Forced Degradation, Olmesartan and Chlorthalidone.

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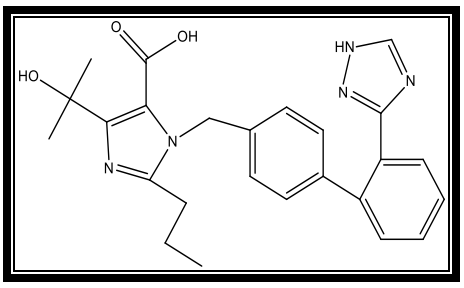
INTRODUCTION

Olmesartan is a 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5 yl) phenyl] phenyl] methyl] imidazole-4-carboxylic acid synthetic imidazole derivative[1-2] and angiotensin II receptor antagonist with antihypertensive activity (figure 1). Olmesartan selectively binds to the angiotensin type 1 (AT1) receptor subtype in vascular smooth muscle and adrenal gland, thereby competing with angiotensin II for binding to the AT1 receptor. This prevents angiotensin II-induced vasoconstriction and interferes with angiotensin II-mediated aldosterone secretion, thereby decreasing aldosterone production and preventing aldosterone-stimulated sodium retention and potassium excretion. Its molecular formula is $C_{24}H_{26}N_6O_3$ and molecular weight is 446.5 g/mol.

Chlorthalidone (CHL) is an oral diuretic used along with oral antihypertensive agent. Chemically it is (RS) 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1Hisoindol-1-yl) benzene-1-sulfonamide[3] (Figure: 2).

Many analytical methods are reported in the literature for the determination of Olmesartan by UV-Spectrophotometry [4], RP-HPLC [5-7] and OLM with hydrochlorothiazide by HPTLC [8]. Several methods have been described for determination of Chlorthalidone by UV-Spectrophotometry [9], RP-HPLC [10-11] and by HPTLC [12]. Although there are several chromatographic methods reported for determination of both these drugs. However, to the best of our knowledge, there is no LC analytical method reported for simultaneous determination of Olmesartan and Chlorthalidone in combined dosage form. The objective of the present work was to develop accurate and precise RP-HPLC method with UV-detection for the quantification of these drugs in pharmaceutical formulation.



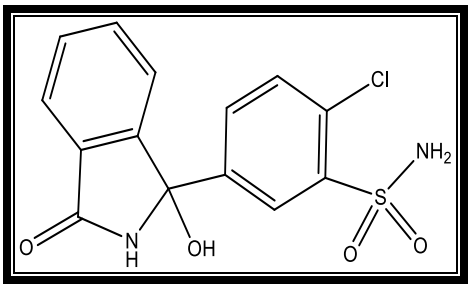


Figure 1: Chemical structure of Olmesartan Figure 2: Chemical structure of Chlorthalidone

MATERIALS AND METHOD

Chemicals and reagents

Pharmaceutical grade of Olmesartan and Chlorthalidone supplied as a gift sample by regional bulk drug company, Hyderabad, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. High purity Milli-Q water purification system was used.

Instrumentation and Chromatographic conditions

HPLC assay was performed on a Prominence Liquid Chromatograph waters HPLC PDA 2996 system which consists of a Quaternary solvent manager, a sample manager and a waters Empower2 software was used to control the equipment and to calculate data and responses from the LC system. The analysis was conducted using a reverse-phase technique and following the conditions are maintained: isocratic elution; flow rate of 1.0 mL min^{-1}; mobile phase consisting of 10 mm ortho phosphoric acid buffer, acetonitrile (45:55; v/v); detector at 212 nm. The mobile phase was prepared, filtered through a 0.45 µm membrane filter (Millipore) and sonicated before use. A Hypersil BDS C18(250 x4.6 mm i.d., 5 µm particle size) (Thermo Scientific) was used as the analytical column, and the HPLC system was operated at 25 ±1°C.Cintex digital water bath was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cintex, Mumbai, India).

Preparation of Stock and Standard Solutions

Accurately weighed and transferred 40mg & 12.5mg of Olmesartan and Chlorthalidone working Standards (80µg/ml Olmesartan & 25µg/ml Chlorthalidone) into a 50 ml clean dry volumetric flask, 30ml of diluent (acetonitrile: water (50:50, v/v))., sonicated for 30 minutes and made up to the final volume with diluents. From the above stock solution, 1 ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent in figure 3.

Preparation of Sample Solutions

Five tablets were weighed and calculated the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 50ml volumetric flask, 30ml of diluent was added and sonicated for 30 min, further the volume was made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent. The above solution was centrifuged at 4000rpm for 10 minutes in order to eliminate insoluble excipients and filtered through a 0.45 µm pore size Nylon 66 membrane filter and injected in HPLC system as per chromatographic conditions (Figure 4).

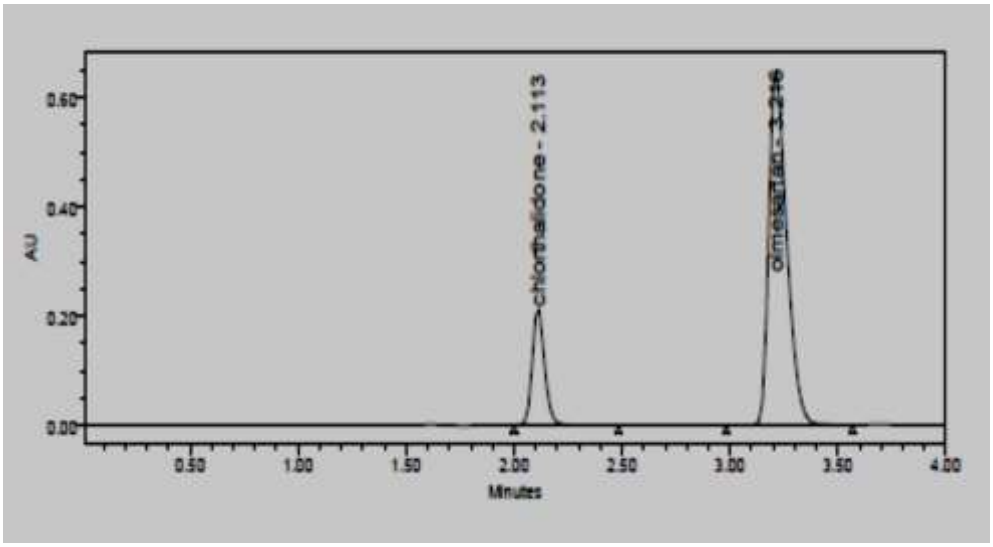


Figure 3: Chromatogram of standard sample

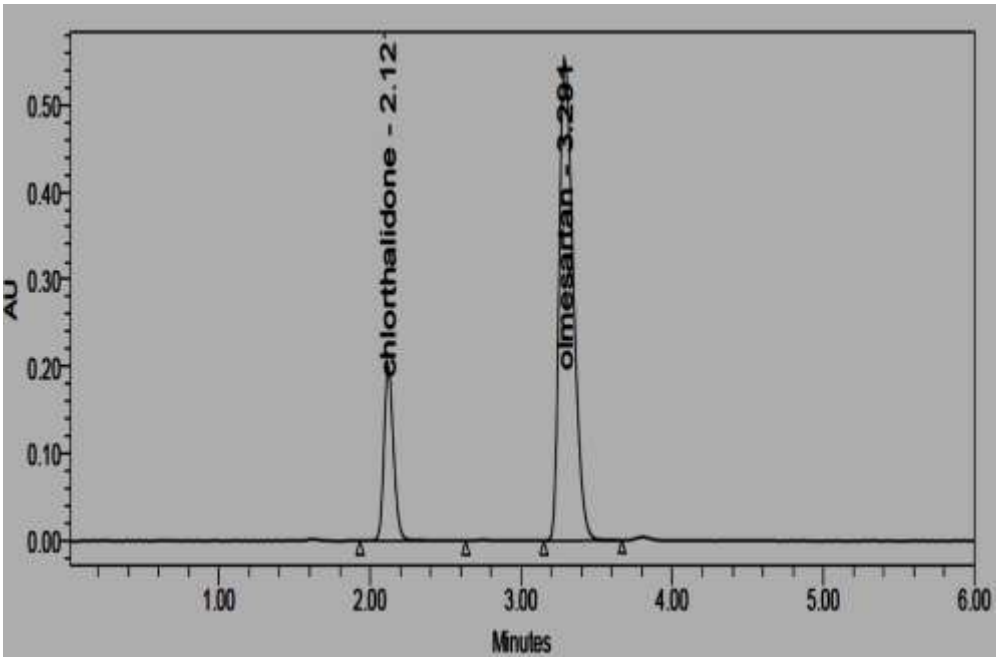


Figure 4: Chromatogram of sample

ANALYTICAL CHARACTERISTICS OF THE METHOD

The HPLC method was validated based on specificity, linearity, precision, accuracy as per ICH guidelines.

Specificity

The specificity experiments were assessed by testing analytical interferences from excipients. The influence of tablet composition was determined by analyzing a placebo solution, submitted to the same extraction procedure and comparing the spectrum to the reference analytical solution. The

excipients from original formulation were submitted for extraction and analysis when the drug was completely removed.

Linearity

Olmesartan and Chlorthalidone reference solutions were prepared using Diluent in triplicate, at concentrations of 20.0, 40.0, 60.0, 80.0, 100.0 and 120.0 µg mL^{-1} and 6.24, 12.5, 18.75, 25 and 31.25 are shown in table 1 and 2. Standard plots were constructed and linearity was evaluated statistically by linear regression analysis using least-squares regression and analysis of variance in figure 5 and 6.

Precision

All the six percentage assay values were within the acceptance limit of ± 3% (97% to 103and also with in a relative standard deviation of 2%. The mean assay value for six preparations of Olmesartan was 101.1 with a range of 99.9% to 102.6%. Corresponding values of Chlorthalidone were 98.9% with a range of 97.8% to 99.6%. Intermediate precision values were also within the acceptance values.

Accuracy

The accuracy was determined based on the recovery of known amounts of the Olmesartan and Chlorthalidone reference standard are added to samples at the levels of 50, 100 and 150% of the sample concentration (80µg/ml Olmesartan & 25µg/ml Chlorthalidone). For this assay, each tablet was previously weighed and transferred to 50 mL volumetric flasks containing the diluent and submitted to the extraction procedure for 20 min. The extractive solutions at 80 and 25 µg mL^{-1} was used to prepare the analytical samples in recovery test. This procedure was performed in triplicate. The results were expressed as the percentage of the Olmesartan and Chlorthalidone reference standard recovered from the sample. All solutions were prepared in triplicate.

RESULTS AND DISCUSSION

Method development and optimization HPLC Method

In the present work studies regarding the development and validation of analytical monitoring method for extraction/release of the drug are described. To develop a better way on quantitative method development, the formulation, composition and processing of the drug must be considered. Validated analytical methods for the qualitative or quantitative testing of drug molecules are assumed to be of greater importance when they are employed to generate quality and safety compliance data during the development and post approval of drug products. For accurate analysis, both qualitative and quantitative methods must be developed considering the complexity of the

tablet composition including the use of various excipients such as viscous polymers [30]. Besides the difficulty in accessing the drug due to its location in a polymeric compartment makes it difficult to assess which shows that the sample preparation should be given particular attention during analysis. Due to the complexity of excipients matrix in system, the extraction procedure by ultrasonic bath was reproduced, using: Water; Acetonitrile (50/50 v/v) as Diluent. Thus, the extraction was done to have a final concentration of 50 µg mL^{-1}, and the measurement was performed directly on the final solution. Although Diluent has been confirmed as effective by previous studies during extraction of Olmisartan and Chlorthalidone. During the method development, other solvents could be considered, but the results obtained on extraction procedure for HPLC analysis demonstrated that other organic solvents do not promote drug release from matrix. Thus, the main objective of the present work was to develop a stability-indicating assay method for Olmesartan and Chlorthalidone bulk drug and its pharmaceutical formulations. Number of mobile phases and stationary phases were investigated and performed system suitability during the development of assay method. Initially the method development was started with a stationary phase; BDS-C_{18} (250 mm × 4.6 mm, 5.0µ) and mobile phase; 1 mL of ortho-phosphoric acid in 1000/ml of water as buffer and acetonitrile in the ratio of 80:20 v/v respectively. Olmesartan and Chlorthalidone peaks shapes was unsymmetrical. For next trail the mobile phase ratio was modified slightly as 75:25 v/v respectively. In the above trail, the peak shape is broad, but column efficiency was reduced. After few logical attempts, we achieved the best separation; resolution and lower retention time using a BDS-C_{18} (250 mm × 4.6 mm, 5.0µ), Isocratic reversed phase LC method consist of buffer: acetonitrile in the ratio of 55:45 v/v respectively as mobile phase at flow rate 1.0 mL min^{-1}. The column temperature was maintained at 30 ºC and the detection was monitored at 212 nm. The peak shape of Olmesartan and Chlorthalidone was found symmetrical and retention time is 3.216 and 2.113 min. The chromatogram of Olmesartan and Chlorthalidone standards using the optimized method is shown in Figure 3 and 4. The proposed HPLC method was found to be specific for Olmesartan and Chlorthalidone and its stressed samples.

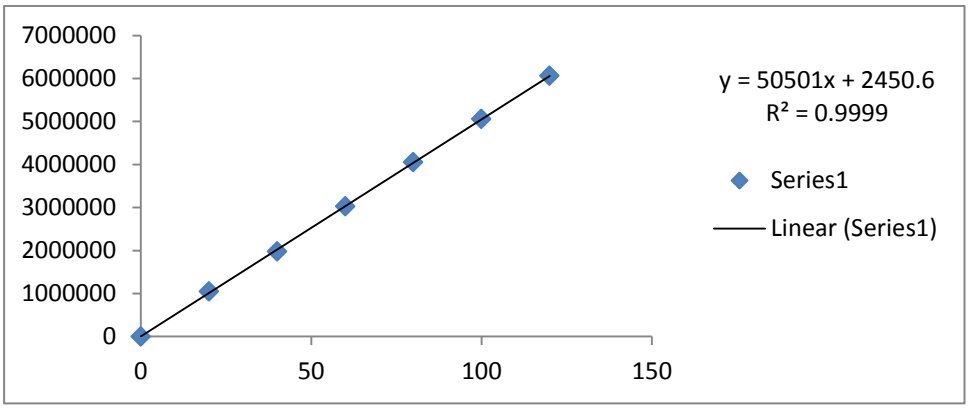


Figure 5: Linearity plot of Olmesartan

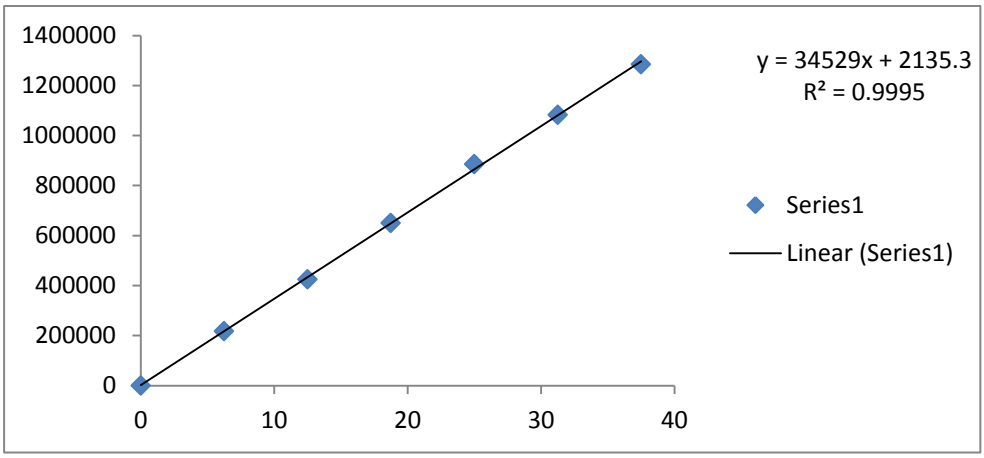


Figure 6: Linearity plot of Chlorthalidone

Table 1: Linearity of Olmesartan

S.No.	Concentration (ppm)	Peak Area
1	20	1048311
2	40	1978078
3	60	3026759
4	80	4055225
5	100	5057406
Slope	50501.543	
Y-Intercept	2450.456	
Correlation Coefficient	0.9999	

Table 2: Linearity of Chlorthalidone

S.No.	Concentration (ppm)	Peak Area
2	12.5	425556.3
3	18.75	650722.7
4	25	885707
5	31.25	1082435
Slope	34528.6667	
Y-Intercept	2135	

Correlation Coefficient	0.9994

Table 3: Results for Forced degradation of Olmesartan and Chlorthalidone

Stress Conditions	Purity angle	Purity threshold	Purity angle	Purity threshold
2N HCl solution for about 30 min at 60ºC	0.288	0.317	0.213	0.524
2 N NaOH solution for about 30min at 60ºC.	0.112	0.360	0.273	0.807
Exposed to 1% Hydrogen peroxide (H_2O_2) for about 1 hour at Bench top	0.114	0.315	0.520	63.958
Purified water for about 6 hrs at 60ºC.	0.127	0.346	0.105	0.412
Exposed to UV light for about 1.2 Million Lux hours.	0.113	0.319	0.046	0.415
Dry heating done at 105°C for about 6 hrs.	0.107	0.312	0.198	0.464

The significant achievements in the proposed method; 1) sensitive, rapid and isocratic reversed phase HPLC method; 2) lower retention time; 3) best separation and resolution.

Forced degradation studies were performed to prove non-interference of degradation products. The stress conditions employed for degradation study of Olmesartan and Chlorthalidone includes UV light and florescent light exposure, acid hydrolysis, base hydrolysis, water hydrolysis and oxidation. Acid hydrolysis stress was performed by refluxing for about 30 min at 60^0c (figure: 7). Base hydrolysis stress was achieved at 60^0c for about 30min with 2 N NaOH (figure: 8). Oxidation was carried out on bench top for 1 hour with 1% hydrogen peroxide (figure: 9). Another studies include UV, heating and water hydrolysis (figure:10 to 12). Peak purity of the principal peaks in the chromatogram of stressed samples were checked using photo diode array detector.

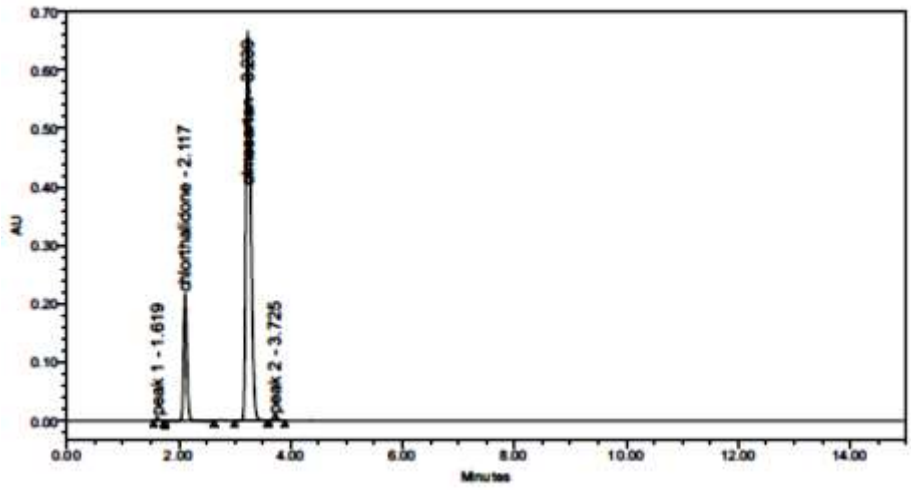


Figure: 7 Chromatogram of acid stress sample

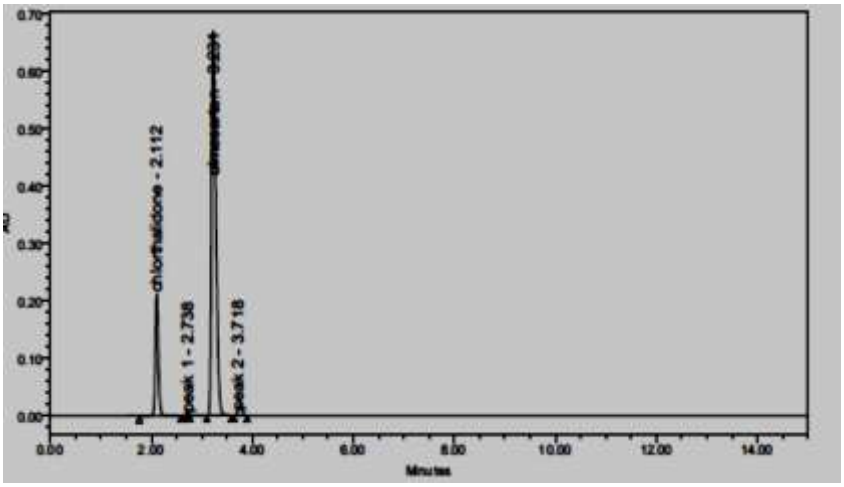


Figure 8: Chromatogram of base stress sample

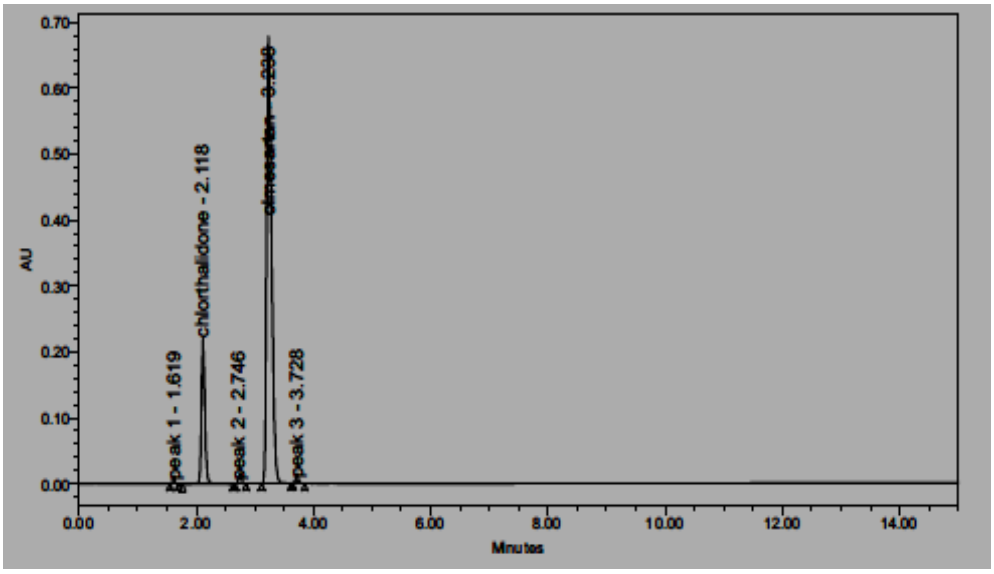


Figure 9 Chromatogram of Water Stress Sample

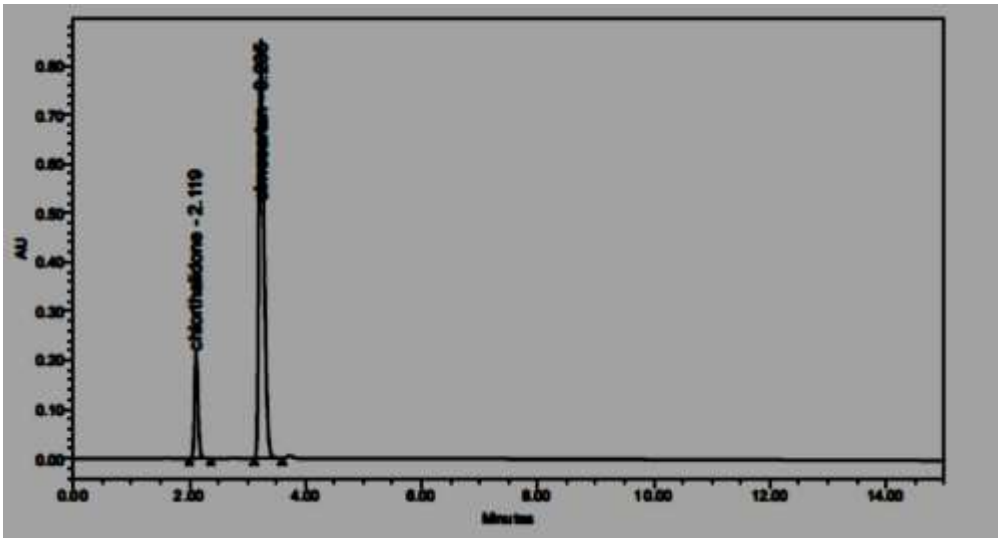


Figure 10: Chromatogram for as such sample

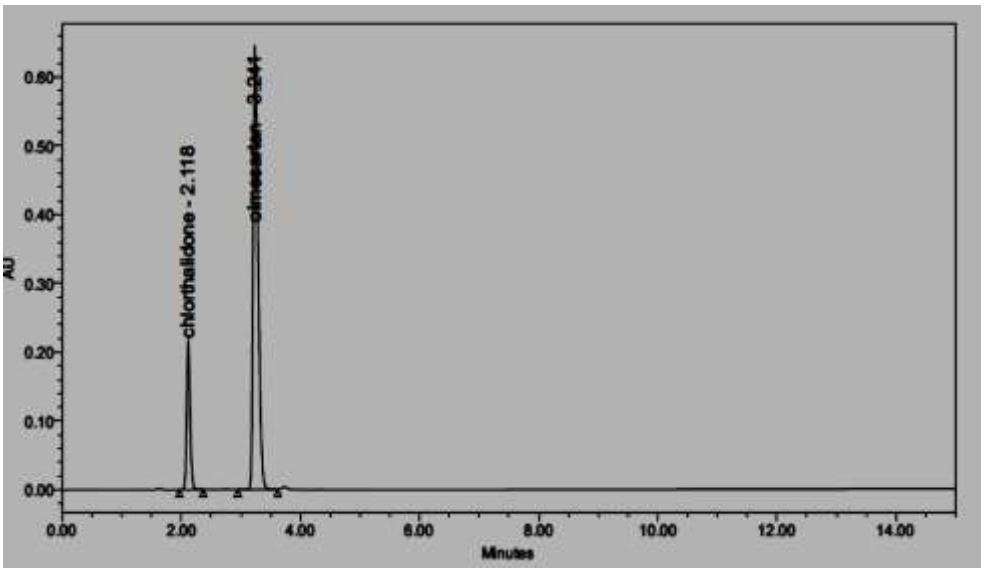


Figure 11: Chromatogram of UV Light Stress Sample

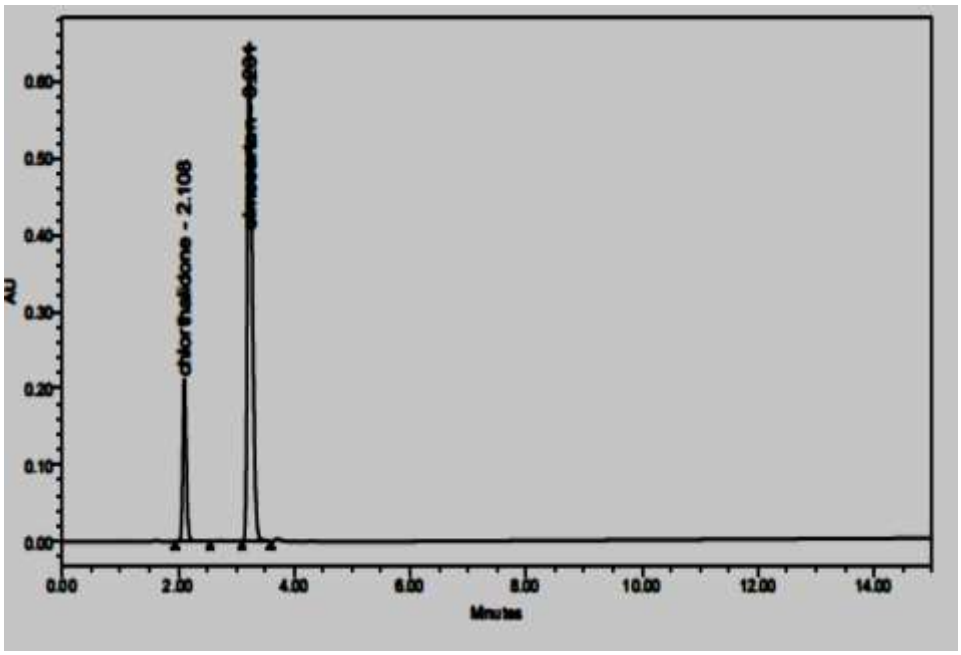


Figure 12: Chromatogram of Heat Stress Sample

CONCLUSION

The RP-HPLC method was developed, validated and made stability studies successfully in terms of accuracy, precision, linearity etc. The proposed method was found to be simple, rapid and accurate. Hence, this method can be used for easy and efficient routine analysis of Olmesartan and Chlorthalidone in the quality control laboratory

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