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Development and Validation of RP-HPLC Method for the Estimation of Olmesartan Medoxomil and Chlorethalidone in Tablet Dossage Form

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A B S T R A C T

A simple, Accurate, precise method was developed for the simultaneous estimation of the olmesartan medoxomil and chlorethalidone in Tablet dosage form. Chromatogram was run through Kromasil (250mm 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrile in the ratio of 55:45A was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Olmesartan medoxomil and Chlorethalidone was 240nm. Retention time of Olmesartan medoxomil and Chlorethalidone were found to be 4.18 min and 3.30 min. %RSD of the Olmesartan medoxomil and Chlorethalidone were and found to be 0.3 and 1.6 respectively. %Recover was Obtained as 100.04% and 100.0% for Olmesartan medoxomil and Chlorethalidone. LOD, LOQ values were obtained from regression equations of Olmesartan medoxomil and Chlorethalidone were 0.03ppm, 0.09ppm and 0.04ppm, 0.11ppm respectively. Regression equation of Olmesartan medoxomil is y = 35709x + 752.6, and of Chlorethalidone is y = 27028x + 420.7.

Keywords: Olmesartan medoxomil, Chlorethalidone, RP-HPLC

A R T I C L E I N F O

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1. Introduction

Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter [1]. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation [2,3]. Pharmaceutical analysis techniques are applied mainly in two areas:

Traditionally, analytical chemistry has been split into two main types, Qualitative and Quantitative

1. Qualitative:

Qualitative analysis seeks to establish the presence of a given element or compound in a sample [4].

2. Quantitative:

Quantitative analysis seeks to establish the amount of a given element or compound in a sample [5].

High-Performance Liquid Chromatography (HPLC):

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector [6]. Compounds are separated by injecting a plug of the sample mixture into the column. The different components in the mixture pass through the column at different rates due to differences in their partition behavior between the mobile liquid phase and the stationary phase [7].

Method Validation:

It can be defined as (ICH) Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics [8]. Method validation is an integral part of the method development; it is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and the drug products. simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose[9]. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice [10,11].

Olmesartan Medoxomil

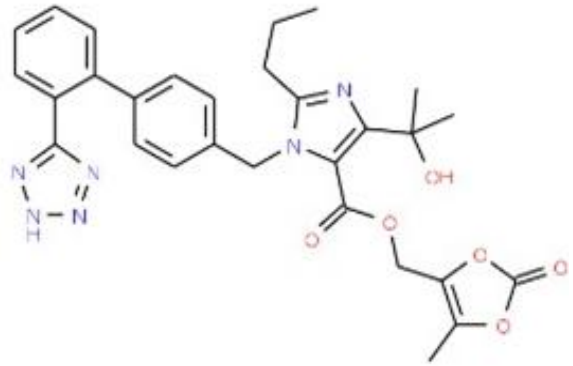

Figure 1: Strucure of Olmesartan Medoxomil

Table 1: Properties of Olmesartan Medoxomil

IUPAC Name	**4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(1H-1,2,3,4-tetrazol-5-yl)phenyl] phenyl} methyl)-1H-imidazole-5-carboxylic acid**
Application	An angiotensin II receptor antagonist
CAS Number	144689-63-4
Purity	≥99%
Molecular Weight	558.59
Molecular Formula	$C_{29}H_{30}N_6O_6$

Description

Olmesartan Medoxomil is belongs to angiotensin II receptor (AT1) antagonist, which inhibits the negative regulatory feedback on renin secretion, result of receptor inhibition is vasodilation and a reduction in peripheral resistance [12].

Chlorethalidone

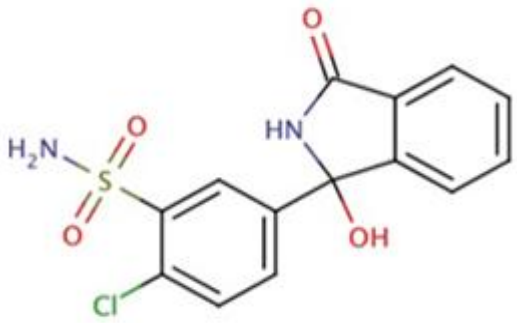

Figure 2: Strucure of Chlorethalidone

Table 2: Structural Properties of Chlorethalidone

IUPAC Name	**2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide**
CAS Number	77-36-1
Purity	≥95%
Molecular Weight	338.77
Molecular Formula	$C_{14}H_{11}ClN_2O_4S$

Description: A diuretic and anti-hypertensive [13].

2. Materials and Methods

Materials:

Olmesartan Medoxomil and Chlorethalidone, Combination of Olmesartan Medoxomil and Chlorethalidone tablet dosage forms, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instrument: [14]

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm

and matched quartz was be used for measuring absorbance for Olmesartan Medoxomil and Chlorethalidone solutions.

Methods:

Preparation of buffer:

Buffer: (0.01N Ammonium acetate)

Accurately weighed 0.77gm of Sodium Ammonium acetate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water pH adjusted to 3.5 with acetic acid[15].

Standard Preparation:

Accurately Weighed and transferred 16mg of olmesartan Medoxomil and 5 mg of chlorthalidone working Standards into a 25ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:

1tablet was weighed, powdered and then was transferred into a 25mL volumetric flask, 15mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.4ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Linearity:

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of Olmesartan Medoxomil and Chlorethalidone are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 16ppm, 32ppm, 48ppm, 64ppm, 80ppm, 96ppm of Olmesartan Medoxomil and 5ppm, 10ppm, 15ppm 20ppm, 25ppm, 30ppm of Chlorethalidone.

Accuracy: Accuracy solution are prepared such that 0.2ml, 0.4ml and 0.6ml from the standerd solution of Olmesartan Medoxomil and Chlorethalidone sample solution are taken in to 3 different volumetric flask and dilute 10ml with diluents to get 50%, 100% and 150% of Olmesartan Medoxomil and Chlorethalidone.

Method Development:

There are many trials were done by changing columns and Mobile phases and were reported below.

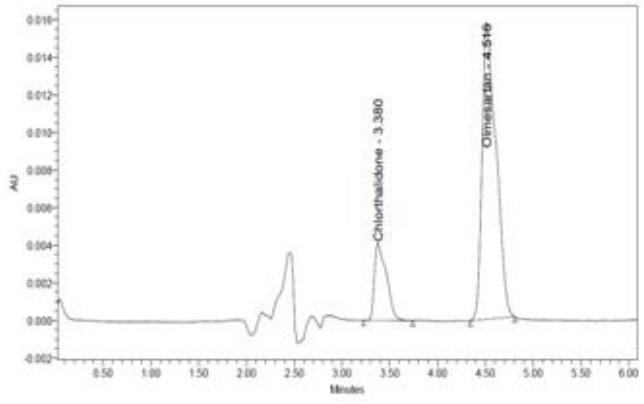

Figure 3: Trial chromatogram 1

Trial 1:

This trial was run through BDS 250 column with mobile phase composition of 60:30A Ammonium acetate Buffer and Acetonitrile, Flow rate set at 1ml/min.

Observation: Baseline noise was observed.

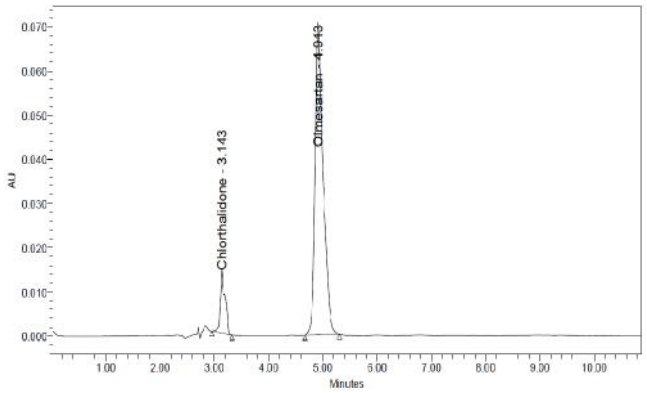

Figure 4: Trial chromatogram 2

Trial 2: This trial was run through BDS 250mm column with mobile phase composition of 70: 30 Ammonium acetate Buffer and Acetonitrile, Flow rate set at 1ml/min.

Observation:

Baseline noise was observed also Chlorthalidone peak splitting occurred.

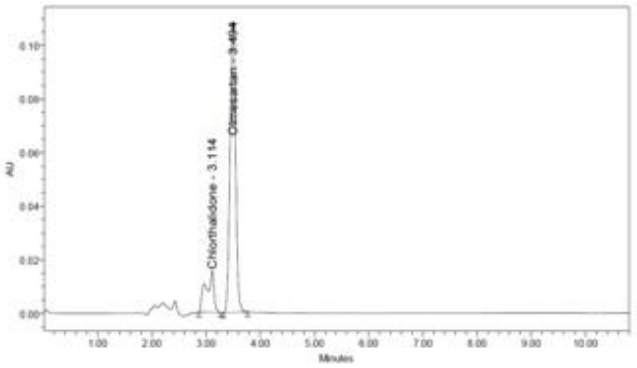

Figure 5: Trial chromatogram 3

Trial 3: This trial was run through Kromasil 250mm column with mobile phase composition of 50:50 Ammonium acetate Buffer and Acetonitrile, Flow rate set at 1ml/min.

Observation: In chlorthalidone peak fronting is observed.

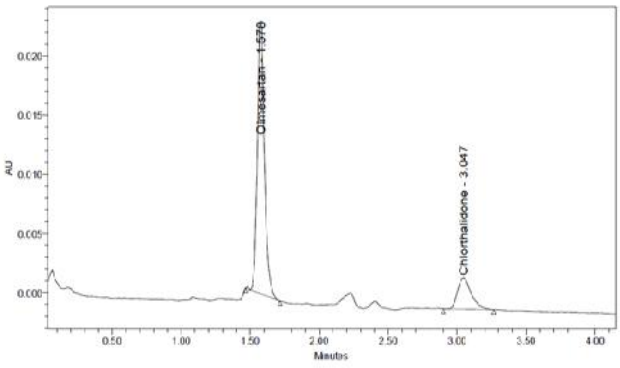

Figure 6: Trial chromatogram 4

Trial 4: This trial was run through Kromasil 250mm column with mobile phase composition of 50:50 water and Acetonitrile, Flow rate set at 1ml/min.

Observation: Olmesartan peak eluted in void volume.

Optimized Method: Drugs were eluted with good resolution, retention time all the parameters like Plate count and Tailing factor were within the limits.

Mobile phase : Buffer and Acetonitrile taken in the ratio 55:45A

Chromatographic conditions:

Flow rate	**:**	1 ml/min
Column	**:**	Kromasil 250 x 4.6 mm, 5µ.
Detector wave length	**:**	240nm
Column temperature	**:**	30°C
Injection volume	**:**	20µL
Run time	**:**	7 min
Diluent	**:**	water: acetonitrile 50:50

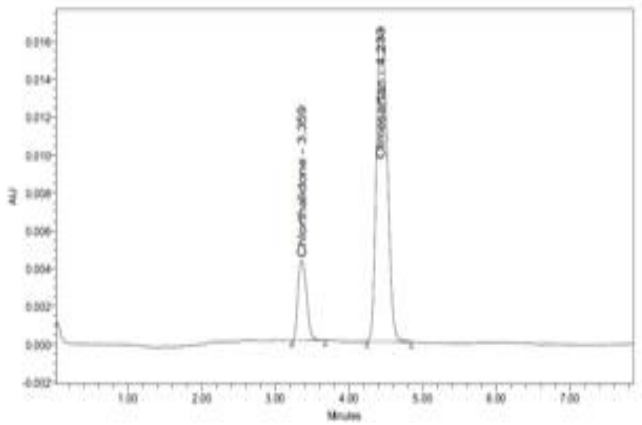

Figure 7: Optimized chromatogram of Olmesartan and Chlorethalidone

3. Results and Discussion

1. Systemsuitability:

All the system suitability parameters are within range and satisfactory as per ICH guidelines

Table 3: System suitability studies of Olmesartan and Chlorethalidone method

Property	Olmesartan	Chlorethalidone
Retention time (tR)	4.18± 0.3 min	3.30±0.3min
Theoretical plates (N)	5374 ± 163.48	8753± 163.48
Tailing factor (T)	1.65 ± 0.117	1.47± 0.117

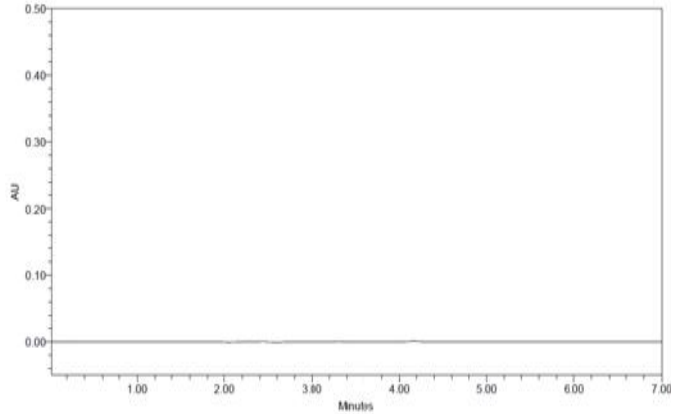

Figure 8: Chromatogram of blank

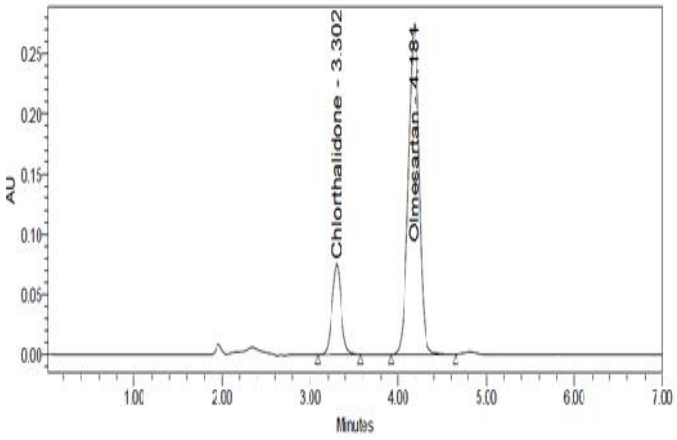

Figure 9: Typical chromatogram of Olmesartan and Chlorethalidone

2. Linearity:

Six Linear concentrations of Olmesartan (16-96ppm) and Chlorethalidone (5ppm to 30ppm) are prepared and injected. Regression equation of the the Olmesartan and Chlorethalidone are found to be, y = 35709x + 752.6, and y =27028x + 420.7 and the regression co-efficient was 0.999.

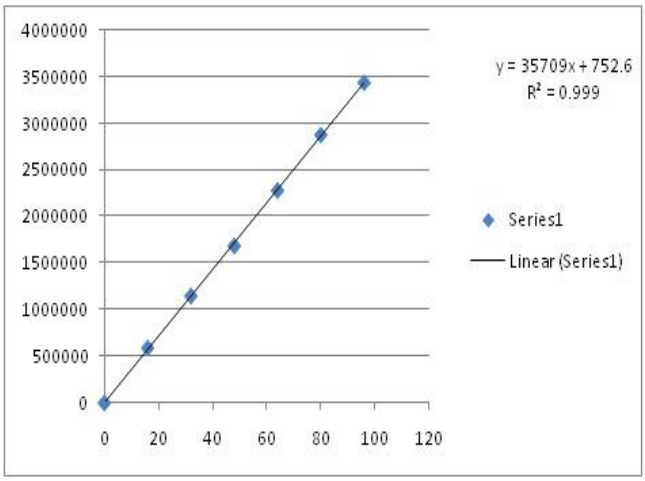

Figure 10: Calibration curve of Olmesartan

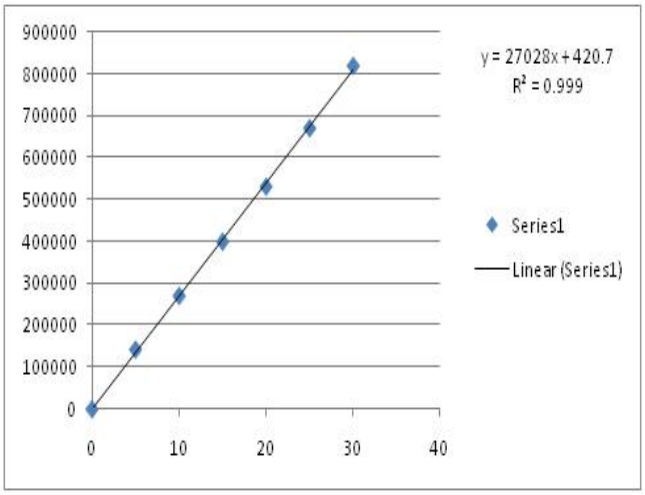

Figure 11: Calibration curve of Chlorethalidone

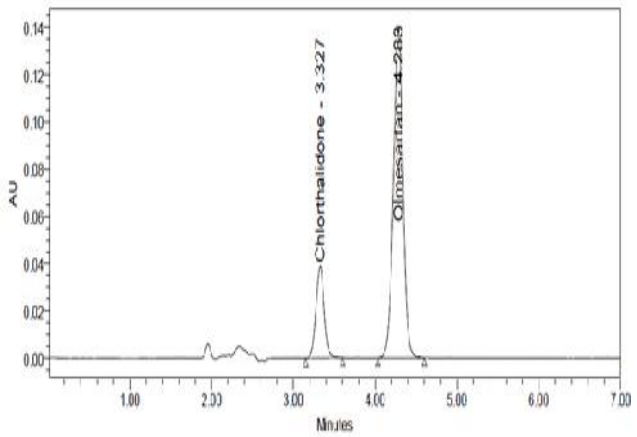

Figure 12: Linearity 50% Chromatogram of Olmesartan and Chlorethalidone method

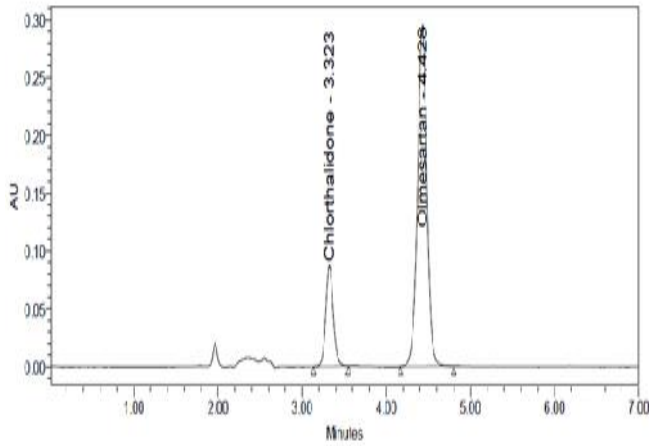

Figure 13: Linearity 100% Chromatogram of Olmesartan and Chlorethalidone method.

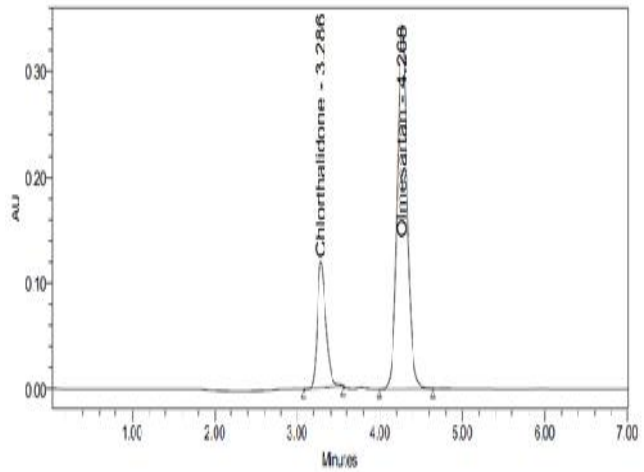

Figure 14: Linearity 150% Chromatogram of Olmesartan and Chlorethalidone method

3. Precision: Intraday precision (Repeatability):

Intraday Precision was performed and % RSD for Olmesartan and Chlorethalidone were found to be 0.97% and 1.00% respectively.

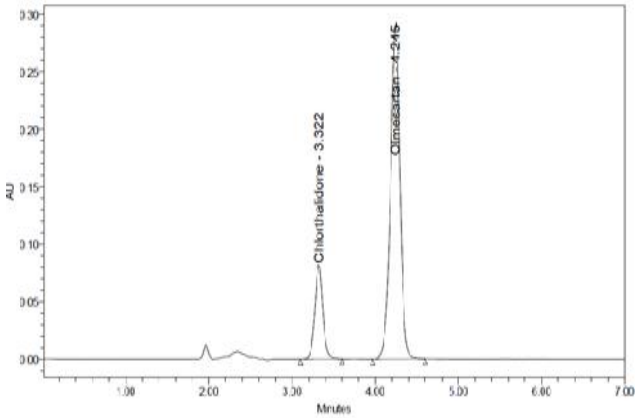

Figure 15: Repeatability Chromatogram of Olmesartan and Chlorethalidone method

Inter day precision:

Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Olmesartan and Chlorethalidone were 0.61% and 0.7%.

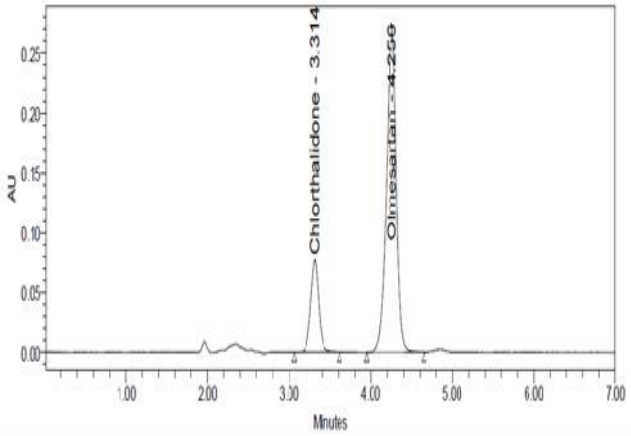

Figure 16: Inter Day precision Chromatogram of Olmesartan and Chlorethalidone method

4. Accuracy:

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 7.5.

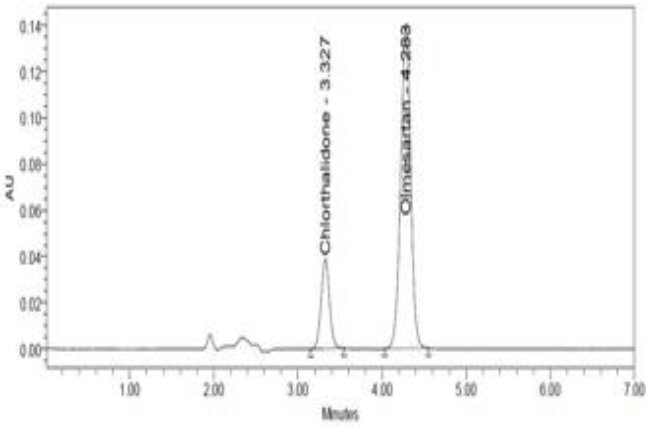

Figure 17: Accuracy 50% Chromatogram of Olmesartan and Chlorethalidone method

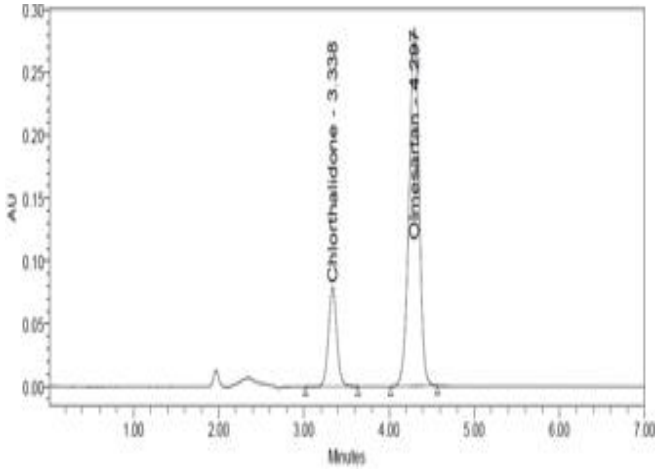

Figure 18: Accuracy 100% Chromatogram of Olmesartan and Chlorethalidone method

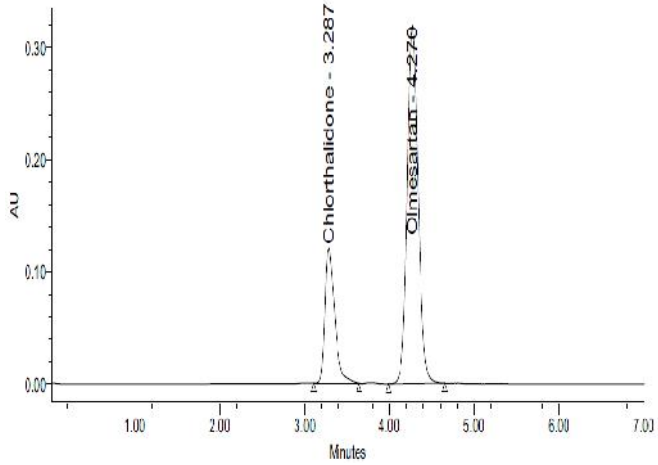

Figure 19: Accuracy 150% Chromatogram of Olmesartan and Chlorethalidone method

5. LOD:

Limit of detection for Olmesartan was found to be 0.03 and Chlorethalidone was 0.04 respectively.

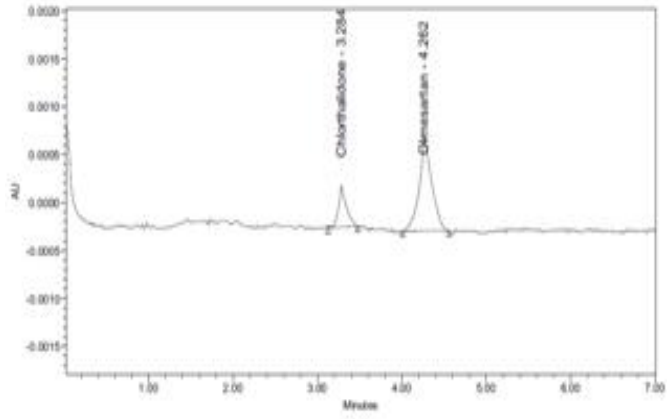

Figure 20: LOD Chromatogram of Olmesartan and Chlorethalidone method

6. LOQ: Limit of Quantification for Olmesartan and Chlorethalidone were found to be 0.09 & 0.11 respectively.

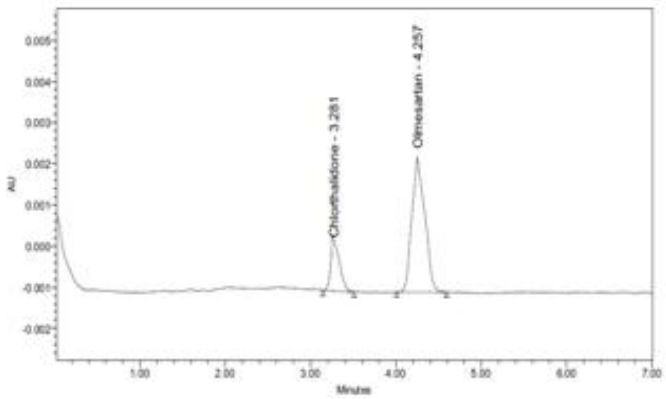

Figure 21: LOQ Chromatogram of of Olmesartan and Chlorethalidone method.

7. Robustness:

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Assay:

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.04 % and 100.00% for Olmesartan and Chlorethalidone respectively.

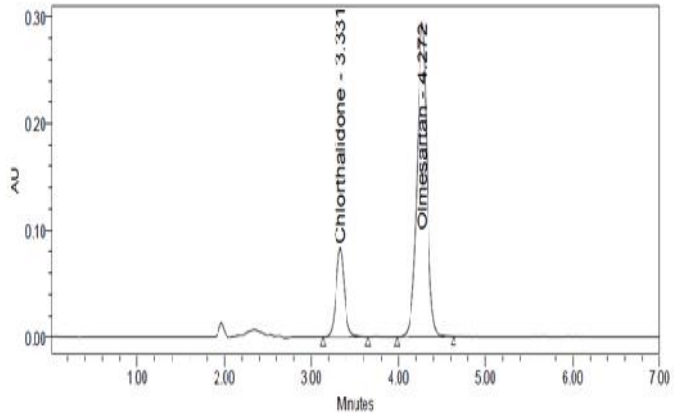

Figure 22: Assay Chromatogram

4. Conclusion

The method was found to be precise, accurate and linear over the linear concentration range. The method developed is unique in determining the impurities even at low levels than that of specifications. The analytical method validation of Olmesartan medoxomil and chlorethalidone by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Olmesartan medoxomil and chlorethalidone. Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, forced degradation studies, ruggedness, robustness and solution stability, Therefore, this HPLC method can be used as a routine analysis of these drugs in bulk, pharmaceutical formulations and also for stability studies.

Table 4: Calibration data of Olmesartan and Chlorethalidone method

S.No	Concentration Olmesartan (µg/ml)	Response	Concentration Chlorethalidone (µg/ml)	Response
1	0	0	0	0
2	16	589290	5	142818
3	32	1146923	10	271267
4	48	1683436	15	400810
5	64	2277919	20	532446
6	80	2872581	25	672272
7	96	3433357	30	821283

Table 5: Repeatability results for Olmesartan and Chlorethalidone

S. No.	Olmesartan %Assay	Chlorethalidone %Assay
1	2338723	534544
2	2277474	522180
3	2286388	527743
4	2291253	525579
5	2301626	532689
6	2293672	527393
AVG	2298189	528355
STDEV	21408.9	4565.6
%RSD	0.9	0.9

*Average of six determinations

Table 6: Inter day precision results for Olmesartan and Chlorethalidone

S. No.	Olmesartan %Assay	Chlorethalidone %Assay
1	2332262	523266
2	2274371	523318
3	2268227	541027
4	2309155	523145
5	2331552	531800
6	2334002	540886
AVG	2308262	530574
STDEV	30110.4	8698.9
%RSD	1.3	1.6

Table 7: Accuracy results of Olmesartan and Chlorethalidone

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Olmesartan	32	31.97	99.92	0.25
	64	63.53	99.27	0.98
	96	95.98	99.98	0.45
Chlorethalidone	10	9.99	99.94	0.84
	20	20.088	100.44	0.87
	30	29.88	99.60	0.84

Table 8: Robustness data of Olmesartan and Chlorethalidone method

S.No	Robustness condition	Olmesartan	Chlorethalidone
1	Flow minus	%RSD	%RSD
2	Flow Plus	0.4	1.2
3	Mobile phase minus	0.1	0.1
4	Mobile phase Plus	0.3	0.4
5	Temperature minus	0.7	0.4
6	Temperature Plus	2.3	2.0

Table 9: Assay of Olmesartan and Chlorethalidone

S. No.	Olmesartan %Assay	Chlorethalidone %Assay
1	101.08	98.62
2	98.57	98.63
3	98.30	101.97
4	100.08	98.60
5	101.05	100.23
6	101.15	101.94
AVG	100.04	100.00
STDEV	1.305	1.64
%RSD	1.30	1.64

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