World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Development and validation for the simultaneous estimation of olmesartan medoxomil and hydrochlorothiazide by using RP-HPLC methods

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World Journal of Advanced Research and Reviews, 2023, 17(02), 686–696

Publication history: Received on 30 December 2022; revised on 10 Februay 2023; accepted on 13 February 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.17.2.0247

Abstract

A simple, Accurate, precise method was developed for the simultaneous estimation of the Olmesartan Medoxomil and Hydrochlorothiazide in Tablet dosage form. Chromatogram was run through Inertsil -ODS C18,250 x 4.6 mm, 5µ. Mobile phase containing Methanoland Bufferin the ratio of 80:20 was pumped through column at a flow rate of 10ml/min. Optimized wavelength for Olmesartan Medoxomil and Hydrochlorothiazidewas 260nm. Retention time of Olmesartan Medoxomiland Hydrochlorothiazide were found to be 3.270min and 4.566 min. %assay was obtained as 99.91% and 99.95% for Olmesartan Medoxomiland Hydrochlorothiazide respectively. Regression equation of Olmesartan Medoxomilis y = 30712x - 31891, and y = 16499x + 8683. Of Hydrochlorothiazide.

Keywords: Olmesartan Medoxomil; Hydrochlorothiazide; RP-HPLC; Validation; Simultaneous Estimation.

1. Introduction

Analytical chemistry1 is a branch of chemistry that deals with the identification of compounds and mixtures (qualitative analysis) or the determination of the proportions of the constituents (quantitative analysis) [1]. The techniques commonly used are titration, precipitation, spectroscopy, chromatography, etc. Analytical Chemistry seeks ever-improved means of measuring the chemical composition of natural and artificial materials. The techniques of this science are used to identify the substances, which may be present in a material, and to determine the exact amounts of the identified substances. Analytical chemists work to improve the reliability of existing techniques to meet the demands for better chemical measurements, which arise constantly in our society [2].

Method validation can be defined as per ICH guidelines: "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" [3].

Method validation is an integral part of the method development. It is the process of demonstrating the analytical procedures that are suitable for their intended use and that they support the identity, quality, purity, and potency of the

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drug substances and drug products [4]. Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose. Method Validation, however, is generally a one-time process performed after the method has been developed to demonstrate that the method is scientifically sound and that itserves the intended analytical purpose [5]. All the variables of the method should be considered, including sampling procedure, sample preparation, chromatographic separation, and detection and data evaluation. For chromatographic methods used in analytical applications there is more consistency in validation practice [6].

2. Material and methods

2.1. Materials

Olmesartan Medoxomil and Hydrochlorothiazide, Combination Olmesartan Medoxomil and Hydrochlorothiazide tablets, 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.

2.2. Methods:[7-11]

2.2.1. Standard Preparation

Accurately Weighed and transferred 250mg of Olmesartan medoxomil and 100mgHydrochlorothiazide of 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.

2.2.2. Sample Preparation

For analysis of commercial formulation, 20 tablets of Olmesartan Medoxomil 20mg and Hydrochlorothiazide 12.5mg were weighed the average weight was calculated and powdered. A quantity equivalent to 20mg of Olmesartan Medoxomil and 12.5mg of Hydrochlorothiazide was weighed and transferred to a 100ml volumetric flask which contain mobile phase and then shake it for 10mins and sonicate it for 20mins.The solution was allowed to stand at a room temperature for 20-30mins and filterd it through a whatmann filter paper.

2.3. Pharmaceutical Formulation

BENITEC-H tab, manufactured by Glaxosmithline.

2.4. Linearity

Linearity solutions are prepared such that 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml,0.8ml from the Stock solutions Olmesartan Medoxomiland Hydrochlorothiazideare taken in to 7 different volumetric flasks and diluted to 10ml with diluents to get 20ppm, 30ppm, 40ppm, 50ppm, 60ppm, 70ppm,80ppm of Olmesartan Medoxomiland 10.25ppm, 15.25ppm, 20.25ppm, 25.25ppm, 30.25ppm, 35.25ppm,40.25ppm of Hydrochlorothiazide.

2.5. Accuracy

2.5.1. Standard Preparation: [12]

Accurately Weighed and transferred 250mg of Olmesartan medoxomil and 10mgHydrochlorothiazide of 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.

2.5.2. Preparation of 50% Spiked Solution

75mg of drug was taken into a 25ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 50% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

2.5.3. Preparation of 100% Spiked Solution:

150 mg of drug was taken into a 25ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 100% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

2.6. Method Development

2.6.1. Method Development

Many trials were done by changing columns and Mobile phases and were reported below [13].

Trial: 1

- Column Used: Inertsil - C18, BDS 250 x 4.6 mm, 5µ.
- Mobile phase: Methanol: Water (90:10)
- Flow rate: 1ml/min
- Wavelength: 260nm
- Temperature: Ambient
- Injection Volume: 20µl

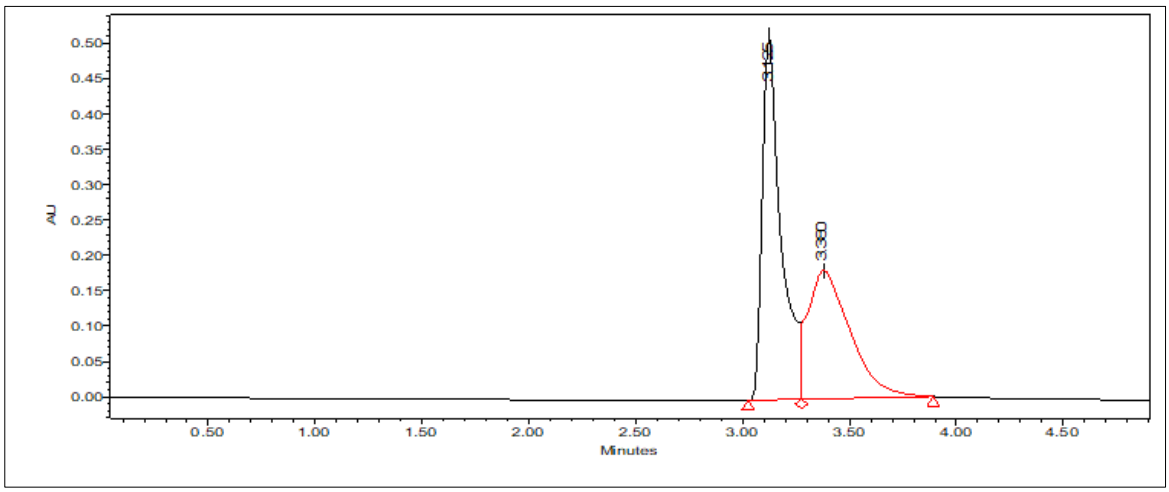

Figure 1 Trial chromatogram 1

Observation:peak shapes were not good.

Trial: 2

- **Column Used**:Kromasil 150 x 4.6 mm, 5µ.
- **Mobile phase**: Buffer (Kh2po4):Acetonitrile (30:70A)
- **Flow rate**: 1ml/min
- **Wavelength**: 260nm
- **Temperature**: Ambient
- **Injection Volume**: 20µl

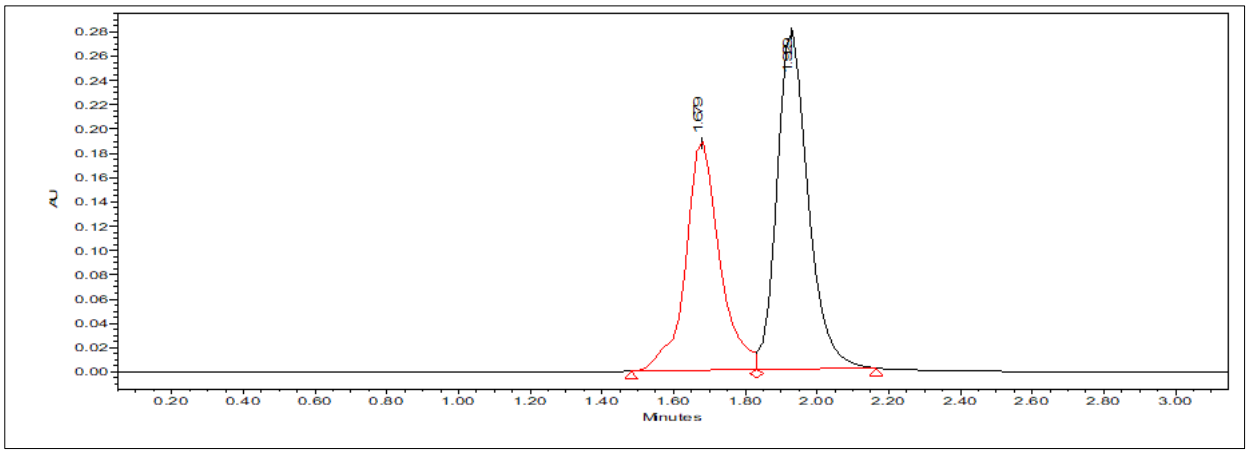

Figure 2 Trial chromatogram 2

Observation: The two peaks are not separated completely but peak shapes are not good.

3. Results and discussion

3.1. System suitability

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

Table 1 Systemsuitability studies ofOlmesartan and Hydrochlorothiazide method

Property	Olmesartan	Hydrochlorothiazide
Retention time (tR)	3.270 min	4.566min
Theoretical Plates (N)	9180 ± 63.48	6485 ±63.48
Tailing factor (T)	1.01 ±0.117	1.10 ±0.117

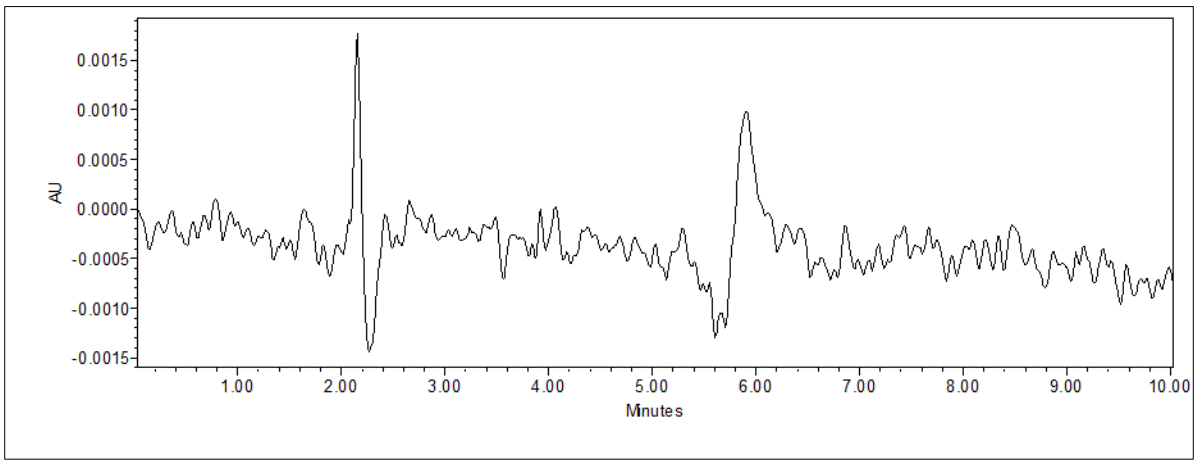

Figure 3 Chromatogram of blank

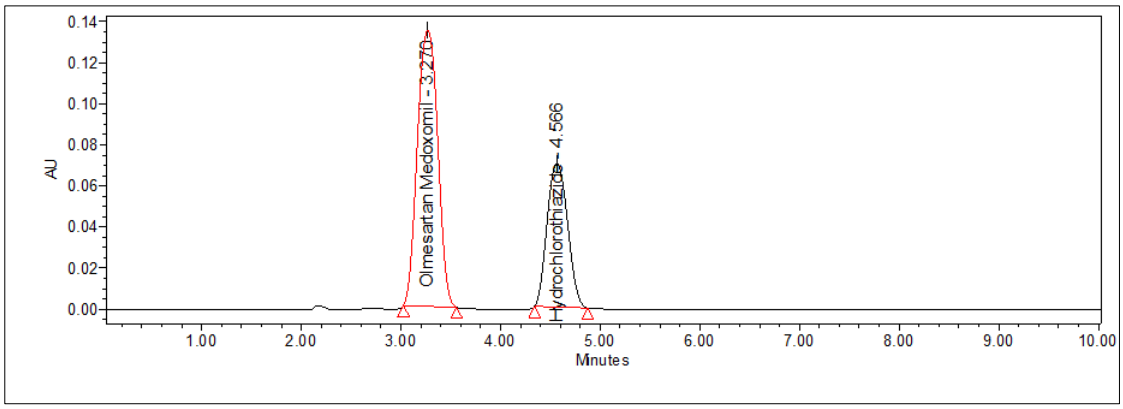

Figure 4 Typical chromatogram of Olmesartan Medoxomil and Hydrochlorothiazide

3.2. Linearity

Six Linear concentrations of Olmesartan Medoxomil (20-80ppm) and Hydrochlorothiazide (10.25-40.25ppm) are prepared and injected. Regression equation of the Olmesartan Medoxomiland Hydrochlorothiazide are found to be, y = 30712x - 31891, and y = 16499x + 8683. And regression co-efficient was 0.999.

Table 2 Calibrationdata of Olmesartan Medoxomil and Hydrochlorothiazide method

S.no	Concentration Olmesartan Medoxomil (µg/ml)	Response	Concentration Hydrochlorothiazide (µg/ml)	Response
1	0	0	0	0
2	20	588735	10.25	343650
3	30	885434	15.25	498630
4	40	1214943	20.25	674665
5	50	1489197	25.25	829406
6	60	1794937	30.25	992122
7	70	2101821	35.25	1160122
8	80	2450946	40.25	1336708

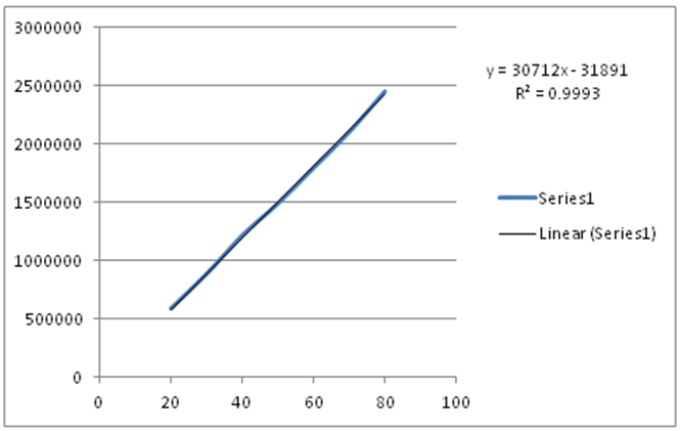

Figure 5 Calibration curve of Olmesartan Medoxomil

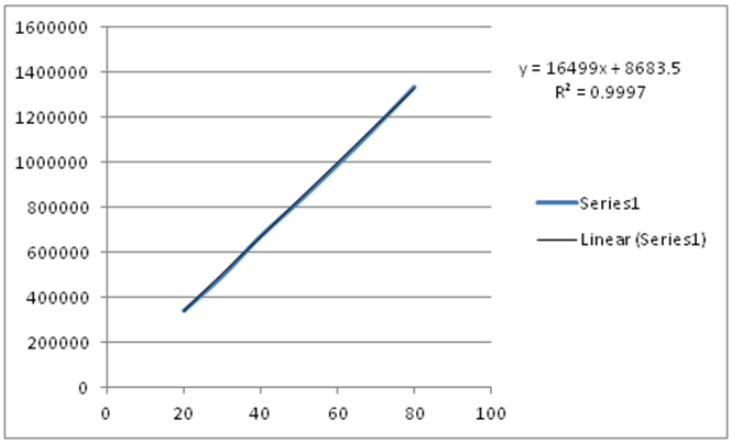

Figure 6 Calibration curve of Hydrochlorothiazide

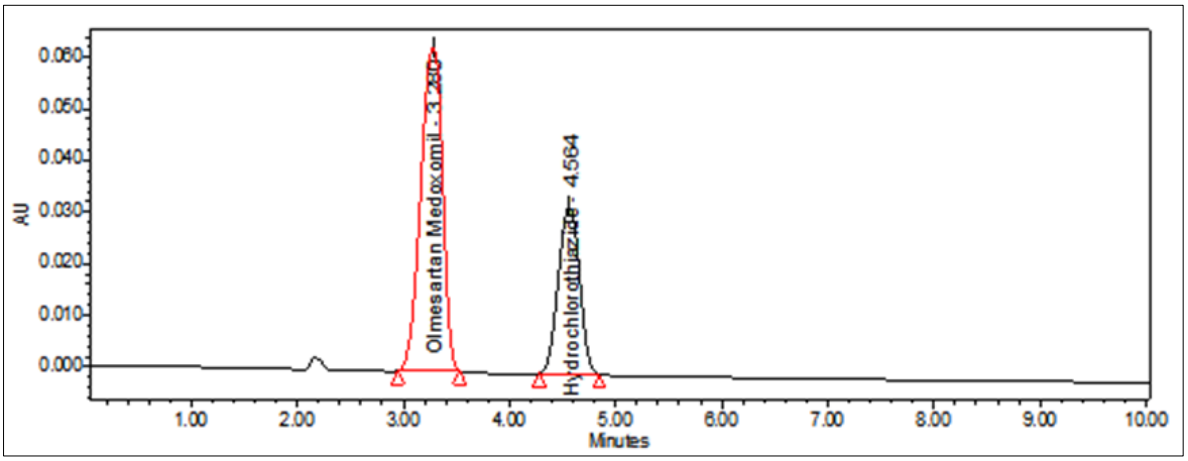

Figure 7 Linearity20%Chromatogram of Olmesartan medoxomil and Hydrochlorothiazide

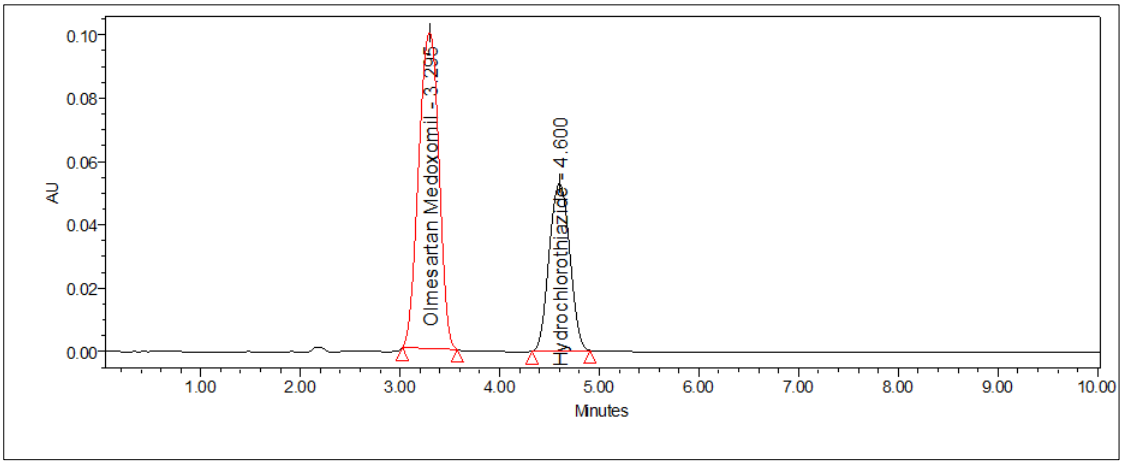

Figure 8 Linearity 30% Chromatogram of Olmesartan medoxomil and Hydrochlorothiazide

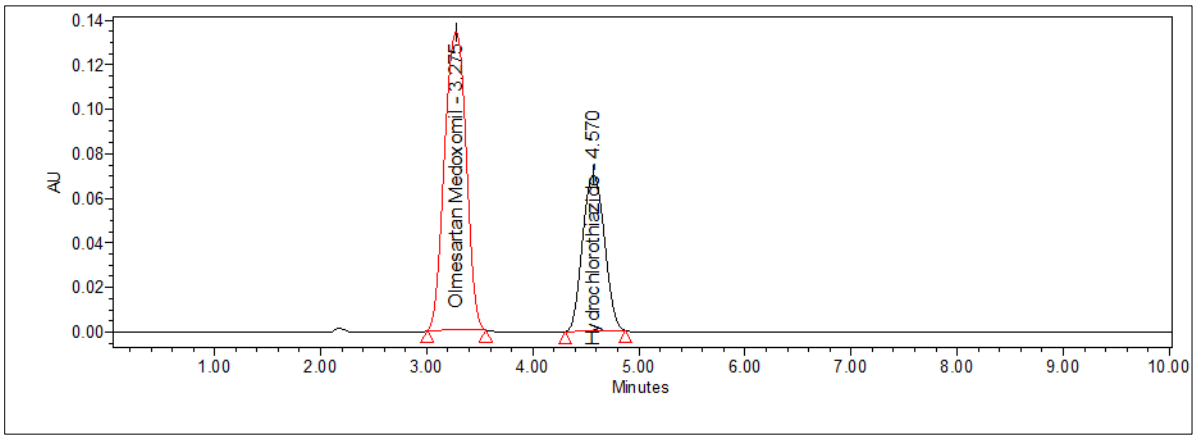

Figure 9 Linearity 40% Chromatogram of Olmesartan medoxomil and Hydrochlorothiazide

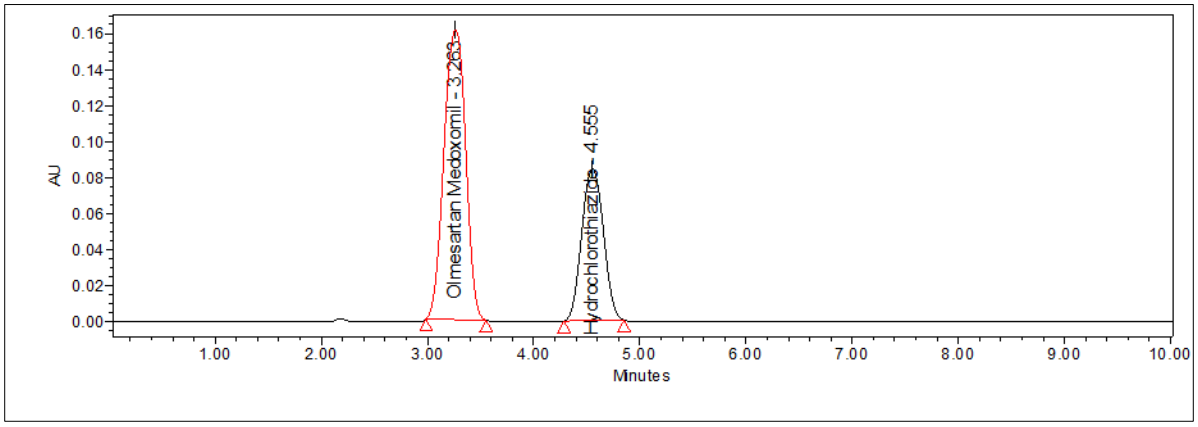

Figure 10 Linearity 50% Chromatogram of Olmesartan medoxomil and Hydrochlorothiazide

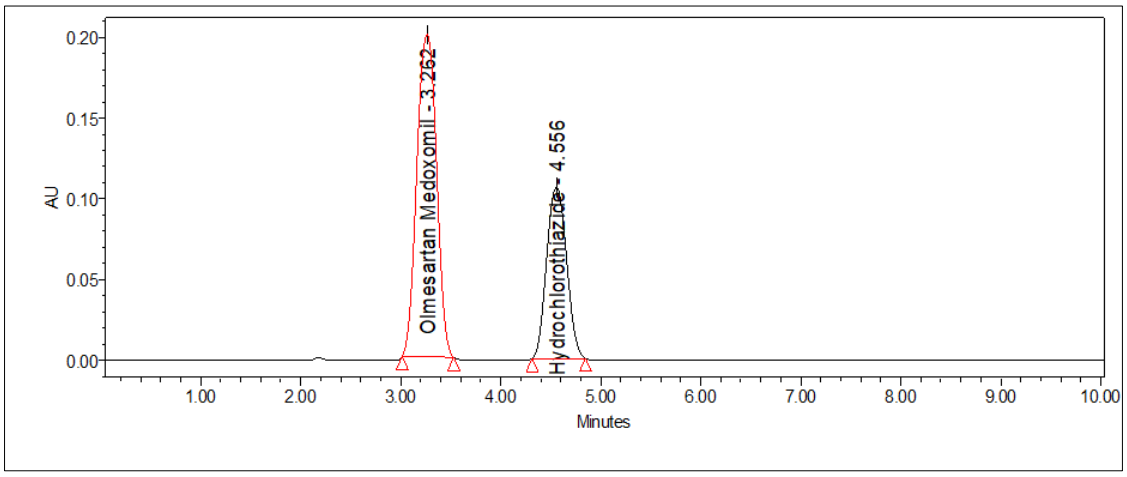

Figure 11 Linearity 60% Chromatogram of Olmesartan medoxomil and Hydrochlorothiazide

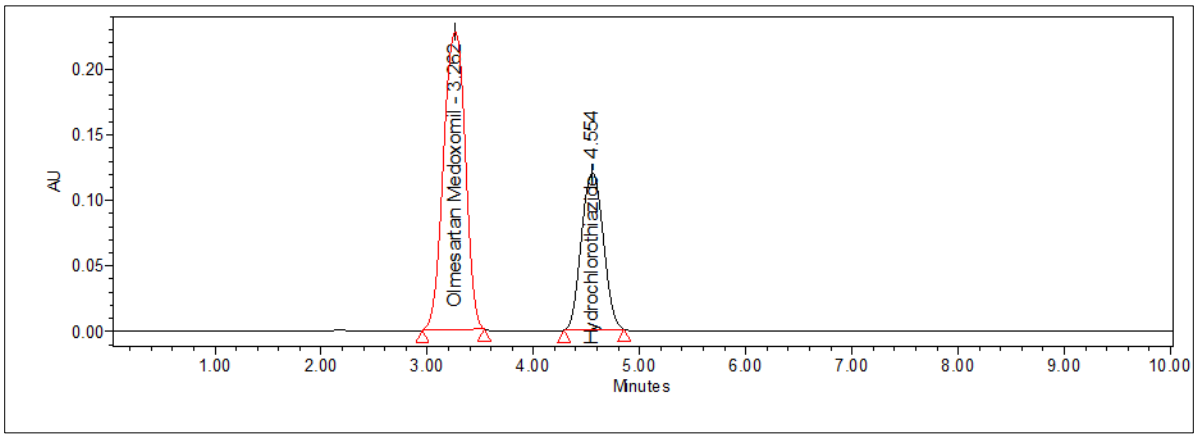

Figure 12 Linearity 70% Chromatogram of Olmesartan medoxomil & Hydrochlorothiazide

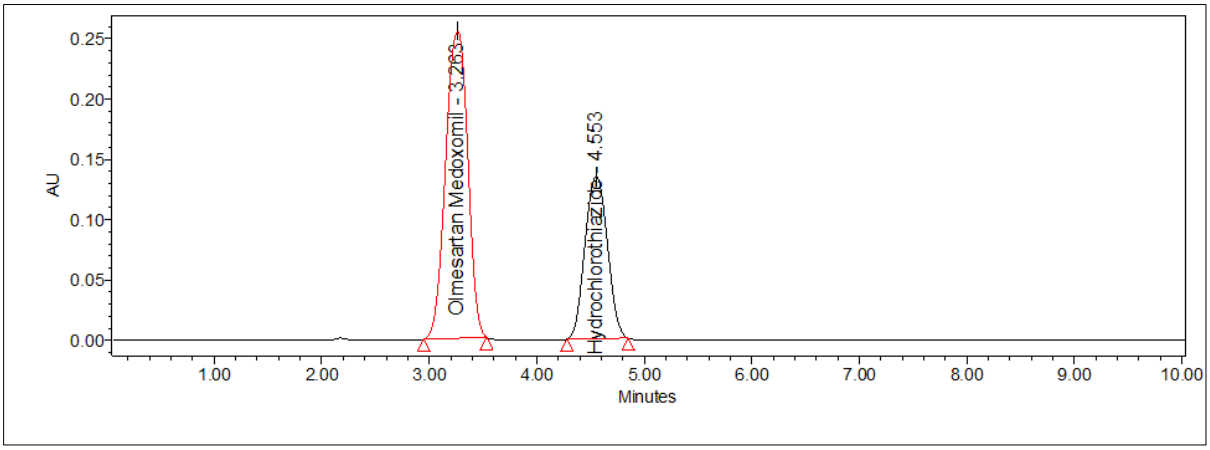

Figure 13 Linearity 80% Chromatogram of Olmesartan medoxomil & Hydrochlorothiazide

3.3. Accuracy

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 6

Table 3 Table of Accuracy

Sample	Concentration (%)	Amount Recovered	Recovery (%)	% RSD
Olmesartan Medoxomil	50	49.83	100.26	0.52
	100	100.03	100.31	0.22
	150	150.07	100.22	0.81
Hydrochlorothiazide	25.25	25.26	100.32	0.20
	50.25	50.23	100.02	0.06
	30.25	30.24	99.98	0.09

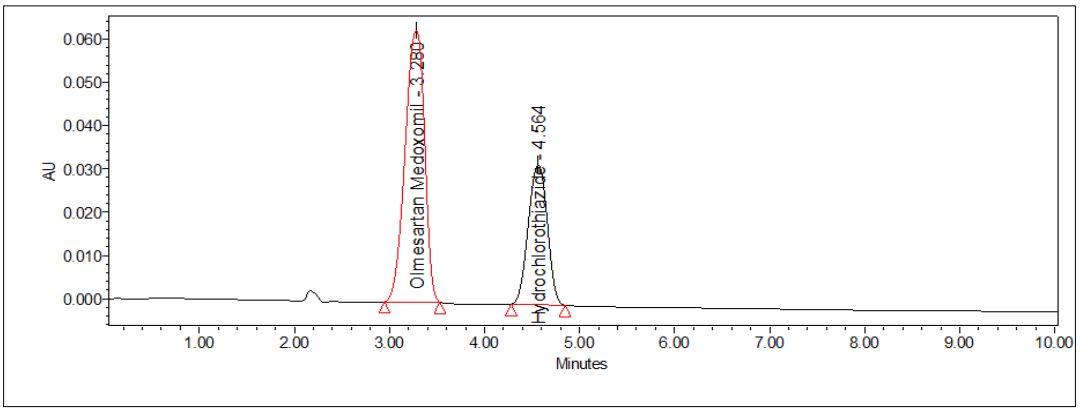

Figure 14 Accuracy 50% Chromatogram of Olmesartan Medoxomil & Hydrochlorothiazide

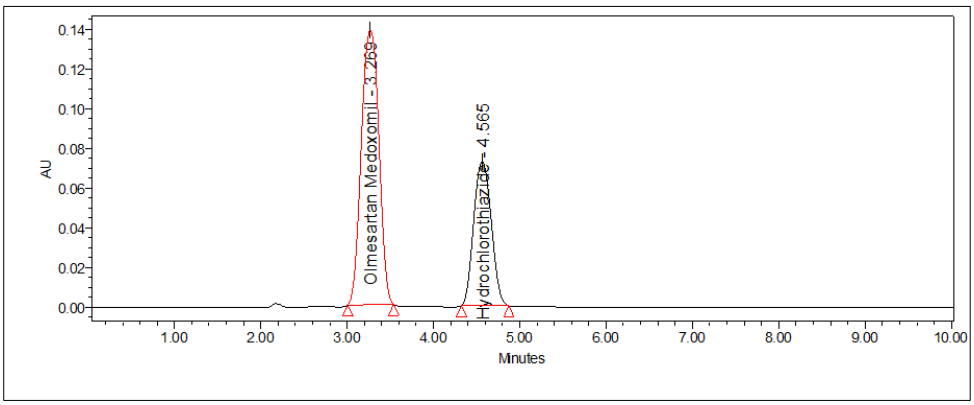

Figure 15 Accuracy 100% Chromatogram of Olmesartan and Hydrochlorothiazide

3.4. 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 99.24% and 99.82% for Olmesartan Medoxomil and Hydrochlorothiazide respectively.

Table 4 Assay of Tablet

S. No.	Olmesartan Medoxomil % Assay	Hydro chlorothiazide %A ssay
1	100.21	99.26
2	99.58	100.54
3	99.56	99.40
4	99.56	100.30
5	99.86	100.53
6	99.23	99.28
AVG	99.91333	99.95333
STDEV	0.503812	0.515754
%RSD	0.513321	0.536649

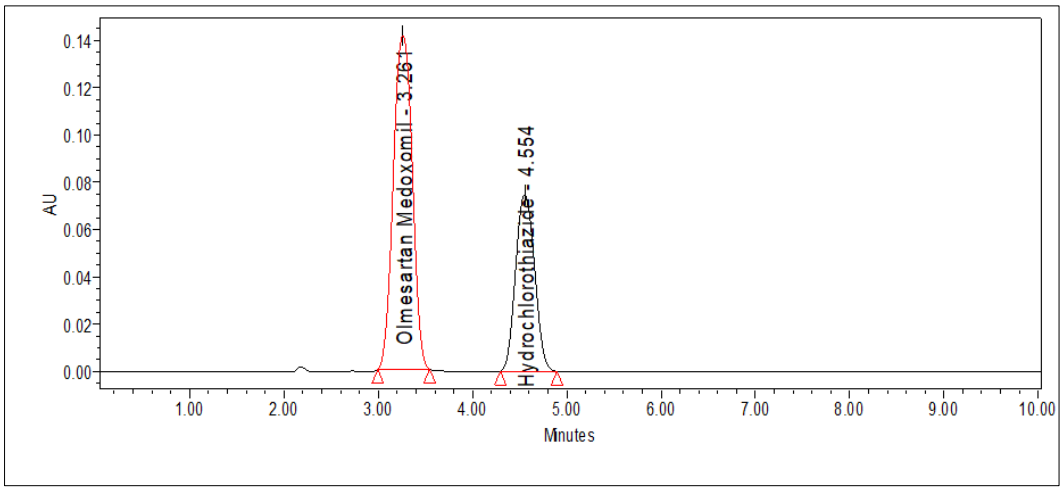

Figure 16 Assay of Tablet

4. Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Olmesartan Medoxomil and Hydrochlorothiazide in Tablet dosage form. Retention time of Olmesartan Medoxomil and Hydrochlorothiazide were found to be 3.270min and 4.566 min. %assay was obtained as 99.91% and 99.95% for Olmesartan Medoxomil and Hydrochlorothiazide respectively. Regression equation of Olmesartan Medoxomil is y = 30712x-31891 and y = 16499x + 8683 of Hydrochlorothiazide. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Compliance with ethical standards

Acknowledgments

I want to thank my esteemed Principal (Dr. M. *Pradeep* Kumar garu) at Vasavi institute of pharmaceutical sciences, Vasavi Nagar, Peddapalli (Village), Sidhout (Mandal), YSR Kadapa (Dist), Andhra Pradesh-516247, India. For encouragement and support for successfully completed these research work.

Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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