International Journal of Research in Pharmacy and Life Sciences

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Research Article **Open Access**

Method Development and Validation for the Simultaneus Estimation of Olmesartan & Hydrochlorothiazide by using RP-HPLC in Bulk and it's Pharmaceutical Dosage 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 Hydrochlorothiazide in Tablet dosage form. Chromatogram was run through Inertsil -ODS C18, 250 x 4.6 mm, 5µ. Mobile phase containing Methanol and Buffer in the ratio of 80:20 was pumped through column at a flow rate of 1ml/min. Optimized wavelength for Olmesartan Medoxomil and Hydrochlorothiazide was 260nm. Retention time of Olmesartan Medoxomil and Hydrochlorothiazide were found to be 3.270min and 4.566 min. %RSD of the Olmesartan Medoxomil and Hydrochlorothiazide were and found to be 1.56 and 1.01 respectively. %assay was obtained as 99.91% and 99.95% for Olmesartan Medoxomil and Hydrochlorothiazide respectively. LOD, LOQ values are obtained from regression equations of Olmesartan Medoxomil and Hydrochlorothiazide were 0.22ppm, 0.25ppm and 0.69ppm, 0.75ppm respectively. Regression equation of Olmesartan Medoxomil is y = 30712x - 31891, and y = 16499x + 8683 of Hydrochlorothiazide.

Keywords: Olmesartan Medoxomil, Hydrochlorothiazide, RP-HPL

A R T I C L E I N F O

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Article History: Received 29 July 2015, **Accepted** 19 September 2015, **Available Online** 24 November 2015

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Manuscript ID: IJRPLS2715

PAPER-QR CODE

Citation: Valluru Vijayamma, et al. Method Development and Validation for the Simultaneus Estimation of Olmesartan and Hydrochlorothiazide by using RP-HPLC in Bulk and it's Pharmaceutical Dosage Form. *Int. J. Res. Pharm, L. Sci*., 2015, 3(2): 336-341.

1. Introduction

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. 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]

High Performance Liquid Chromatography:

Russian botanist Tswett invented chromatography as a separation technique. He describes in detail the separation of pigments, the coloured substances by filtration through column, followed by developments with pure solvents. High-performance liquid chromatography (HPLC)is the fastest growing analytical technique for analysis of drugs [2]. Olmesertan medoxomile chemically 4-(2-hydroxy propan-2yl)-2-propyl-1-({4-[2-(1H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-imidazole-5 carboxylic acid. Hydrochlorothiazidechemically4-(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[3,4].

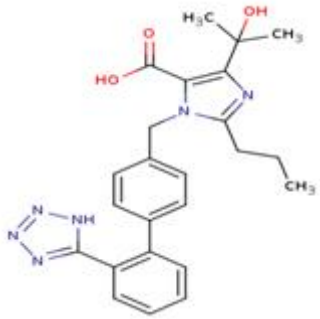

Figure 1: Strucure of Olmesartan Medoxomil

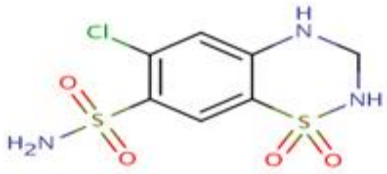

Figure 2: Strucure of Hydrochlorothiazide

2. Materials and Methods

Materials:

Olmesartan Medoxomil, 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.

Instrument:

HPLC instrument used was of WATERS HPLC 2695 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer Systrnonics Instruments and matched quartz was be used for measuring absorbance for Olmesartan Medoxomil and Hydrochlorothiazide solutions.

Methods:

Standard Preparation:

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

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.

Preparation of (KH2PO4 0.1M) buffer:

Weight 3.8954g of di-sodium hydrogen phosphate and 3.4023 of potassium dihydrogen phosphate in to a beaker containing 1000ml of distilled water and dissolve completely. Then ph is adjusted with orthophosphoric acid and then filtered through 0.45µm membrane filter.

Method Validation:

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".

System suitability test:

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

Precision:

The purpose of carrying out a determination is to obtain a valid estimate of a 'true' value. When one considers the criteria according to which an analytical procedure is selected, precision and accuracy are usually the first time to come to mind. Precision and accuracy together determine the error of an individual determination. They are among the most important criteria for judging analytical procedures by their results.

Accuracy:

Accuracy normally refers to the difference between the mean x of the set of results and the true or correct value for the quantity measured. According to IUPAC accuracy relates to the difference between results (or mean) and the true value. For analytical methods, there are two possible

ways of determining the accuracy, absolute method and comparative method.

Linearity:

The absorbance is proportional to the concentration of the absorbing species, if absorptivity and thickness of the medium are constant. When c is in moles per litre, the constant is called molar absorptivity. Beer's law limits values are expressed as µg ml^{-1} and $mole^{-1}$ cm^{-1} respectively.

Robustness:

The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, %organic solvent strength and buffer concentration etc. to determine the robustness of the method experimental conditions were purposely altered and chromatographic characters were evaluated.

Limit of Detection (LOD):

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b, by

Limit of Quantification (LOQ):

The LOQ is the concentration that can be quantitate reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

3. Results and Discussion

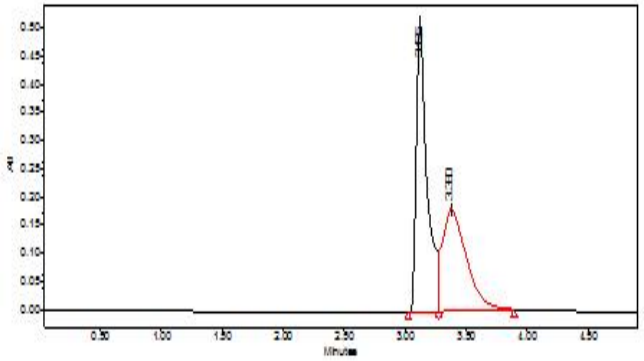

Figure 3: Trial chromatogram 1

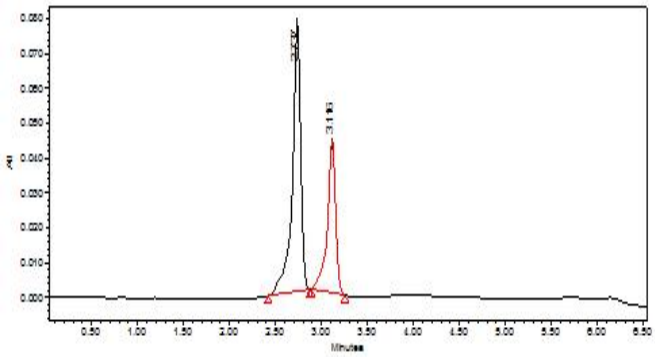

Figure 4: Trial chromatogram 2

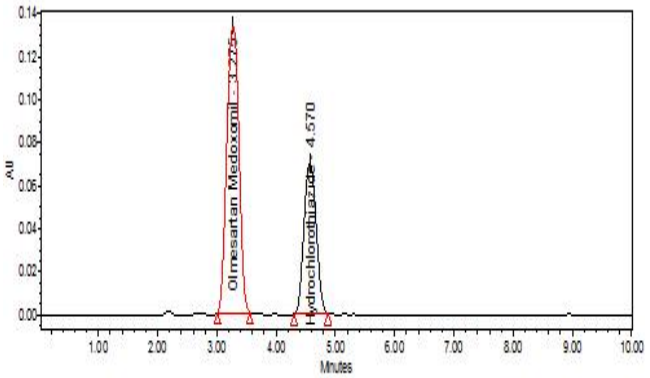

Figure 5: Chromatogram of Optimized method

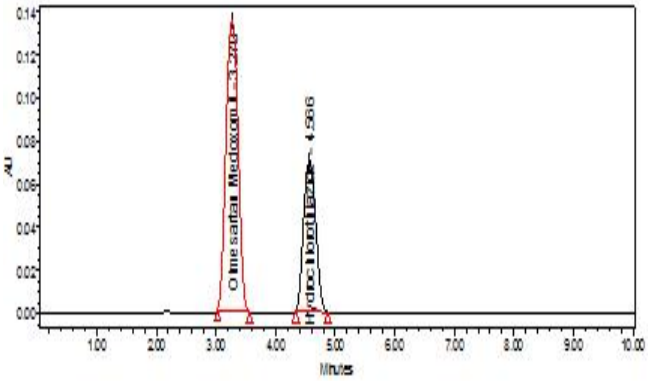

Figure 6: Chromatogram for system suitability

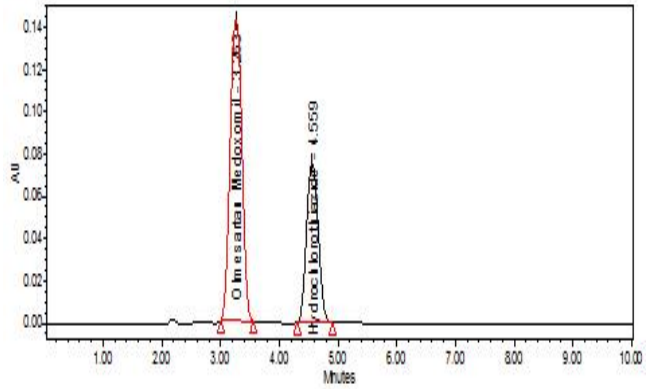

Figure 7: Inter day Chromatogram of Olmesartan Medoxomil and Hydrochlorothiazid

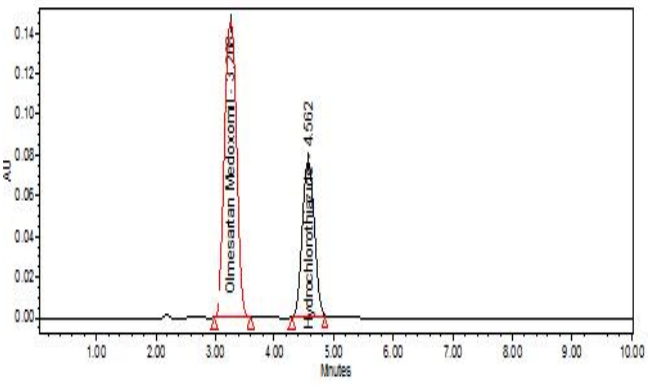

Figure 8: Inter day Chromatogram of Olmesartan Medoxomil and Hydrochlorothiazide

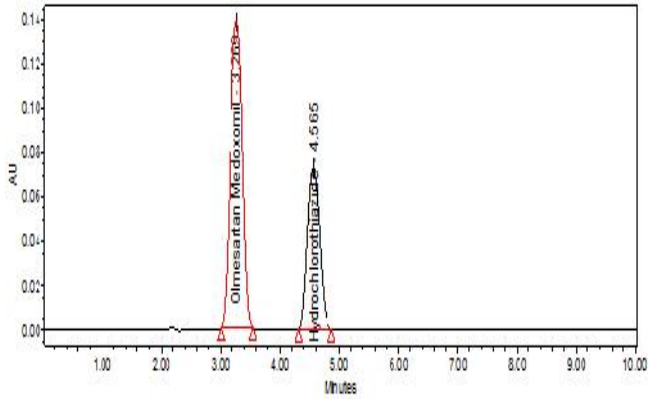

Figure 9: Chromatogram for Accuracy100%

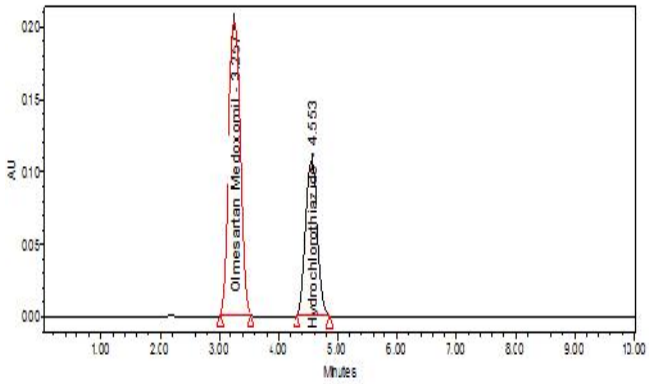

Figure 10: Chromatogram for Accuracy 150%

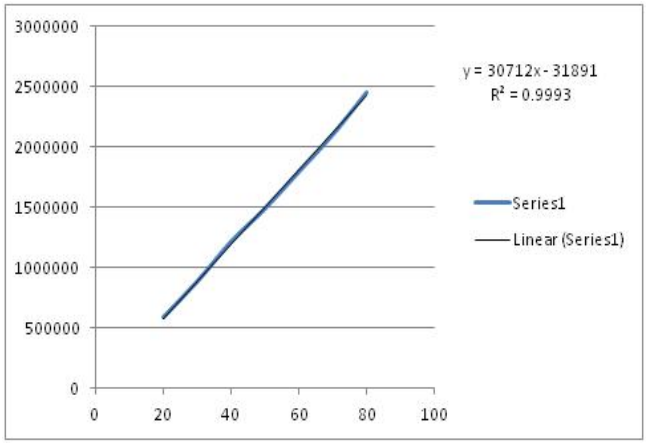

Figure 11: Linearity plot for Olmesartan Medoxomil

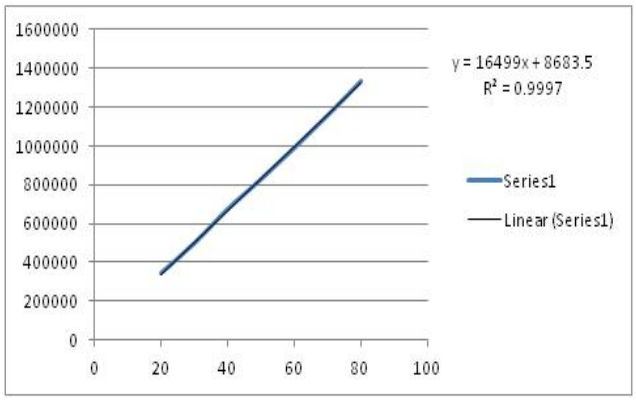

Figure 12: Linearity plot for Hydrochlorothiazide

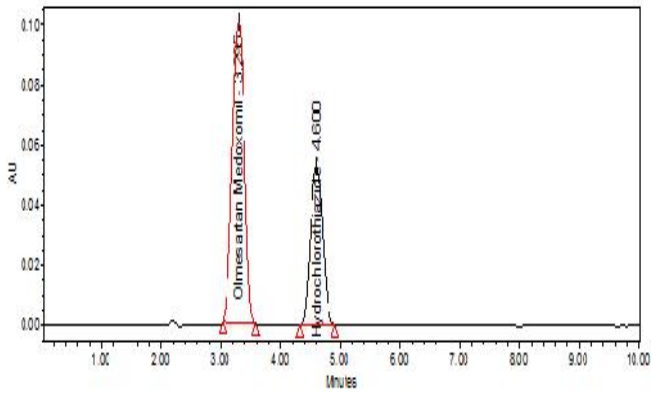

Figure 13: Chromatogram of Linearity for 30%

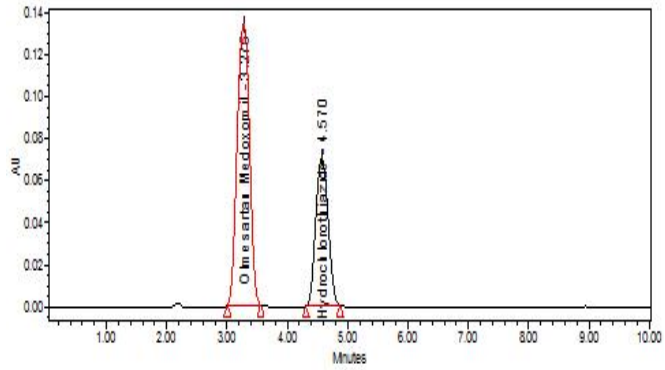

Figure 14: Chromatogram of Linearity for 40%

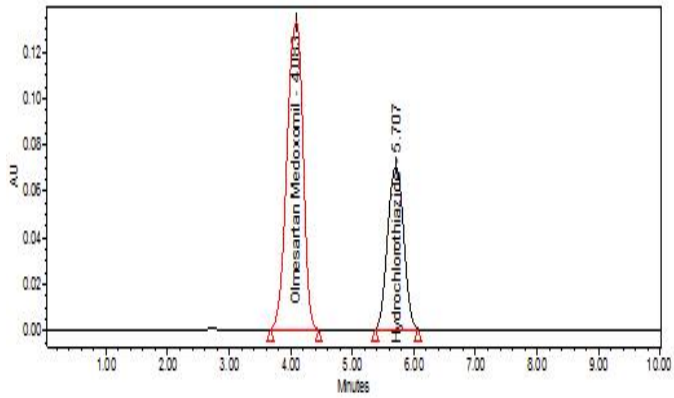

Figure 15: Chromatogram of low flow rate for Olmesartan Medoxomil and Hydrochlorothiazide

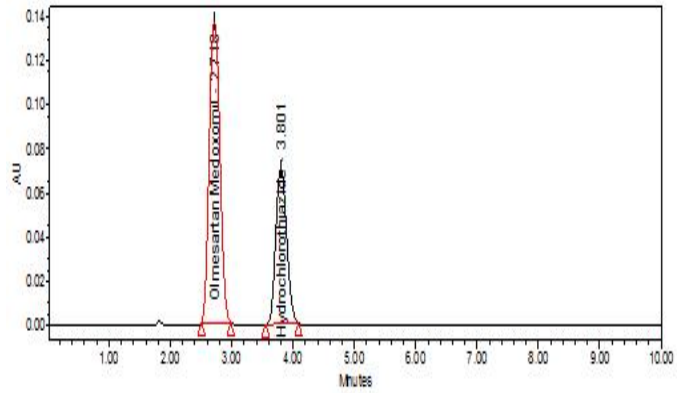

Figure 16: Chromatogram of high flow rate for Olmesartan Medoxomil and Hydrochlorothiazid

Limit of detection (LOD):

Limit of detection was calculated by STD deviation method Olmesartan Medoxomil and Hydrochlorothiazide and LOD for Olmesartan Medoxomil, Hydrochlorothiazide were found to be 0.22and 0.69respectively.

$$LOD = \frac{3.3\ \sigma}{S}$$

Limit of Quantification (LOQ):

Limit of Quantification was calculated by STD deviation method Olmesartan Medoxomil, Hydrochlorothiazide and LOQ for Olmesartan Medoxomil and Hydrochlorothiazide were found to be 0.25 and 0.75 respectively.

$$LOQ = \frac{10\ \sigma}{S}$$

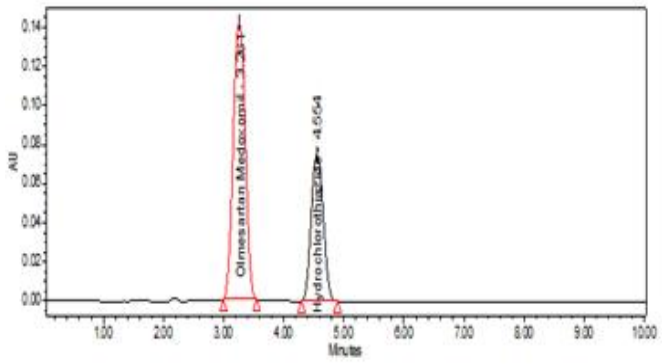

Figure 17: chromatogram for Assay

Table 1: System suitability parameters of Olmesartan Medoxomil and Hydrochlorothiazide

Property	Olmesartan Medoxomil	Hydrochlorothiazide
Retention time (Rt)	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

Table 2: T able of Accuracy

Sample	Concentration (%) (µg/ml)	Amount Recovered (µg/ml)	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

Table 3: Calibration data of Olmesartan Medoxomil and Hydrochlorothiazide method

S.No	Conc. 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

Table 4: Inter day precision results for Olmesartan Medoxomil and Hydrochlorothiazide

S. No.	Olmesartan Medoxomil	Hydrochlorothiazide %Assay
1	1239704	674665
2	1246846	672015
3	1252530	672211
4	1261073	677612
5	1266667	689531
6	1214943	694665
AVG	1246961	680116.5
STDEV	18419.11	9637.575
%RSD	1.477121	1.417

Table 5: Robustness data of Olmesartan Medoxomil and Hydrochlorothiazide

S.NO	Robustness condition	Olmesartan Medoxomil % RSD	Hydrochlorothiazide % RSD
1	Flow minus	0.08	0.7
2	Flow Plus	0.2	0.2
3	Mobile phase minus	0.21	0.41
4	Mobile phase Plus	0.32	0.33
5	Temperature minus	0.3	0.4
6	Temperature Plus	0.2	0.2

Table 6: Assay of Tablet

S. No	Olmesartan Medoxomil %Assay	Hydrochlorothiazide % Assay
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

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. %RSD of the Olmesartan Medoxomil and Hydrochlorothiazide were and found to be 1.56 and 1.01 respectively. %assay was obtained as 99.91% and 99.95% for Olmesartan Medoxomil and Hydrochlorothiazide respectively. LOD, LOQ values are obtained from regression equations of Olmesartan Medoxomil, Hydrochlorothiazide were 0.22ppm, 0.25ppm and 0.69ppm, 0.75ppm respectively.

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