Section A-Research paper
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Simultaneous Estimation of Olmesartan Medoxomil and Hydrochlorothiazide in Bulk Drug and its Tablet Formulation by RP-HPLC

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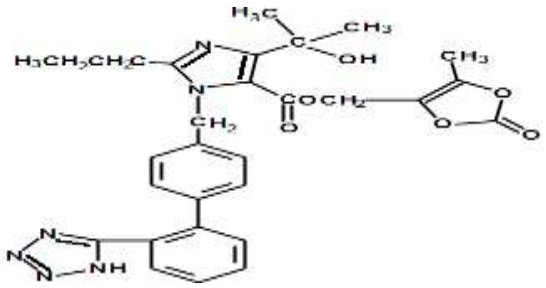
ABSTRACT: Olmesartan Medoxomil and Hydrochlorothiazide can be estimated simultaneously in commercial formulations using a straightforward, quick, precise, and accurate reverse phase high performance liquid chromatography approach. A Protocol C18 ENDURO Column (250 × 4.6 mm ×5µ), with a mobile phase composed of Acetonitrile and Water 50:50 v/v, was used to estimate the concentration of the medicines in this combination. At 270 nm, the separation was seen while the flow rate was 1 ml/min. OLM and HCTZ had retention times that were, respectively, 1.4 and 1.9 minutes. For OLM and HCTZ, it was discovered that the technique was linear over the range of 10–50 µg/ml. The technique was successfully used to estimate commercial formulations after receiving ICH guideline validation.

Key Words: Olmesartan Medoxomil, Hydrochlorothiazide, RP-HPLC.

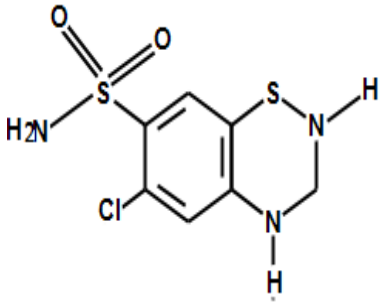
1. INTRDUCTION

A blocker of the angiotensin II receptor is called olmesartan medoxomil (OLM). It is utilised to control hypertension. Olmesartan Medoxomil is chemically known as 4-(1-Hydroxy-1-methylethyl).2, 1-propyl-1-[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl]methyl]methyl ester of 1-H-imidazole-5-carboxylic acid (5-methyl-2-oxo-1, 3-dioxol-4-yl). Olmesartan Medoxomil functions by preventing a chemical in the body that tightens blood vessels. Olmesartan causes blood vessels to relax as a result. As a result, the heart receives more blood and oxygen. Blood

pressure is also decreased. One of the first and most popular diuretics, hydrochlorothiazide (HCTZ), is also used to treat hypertension. Hydrochlorothiazide is chemically known as 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide 1, 1-dioxide. A diuretic called hydroclorothaizide is used to lower high blood pressure. As a first line of treatment, it is frequently suggested for high blood pressure.[1-5]A review of the literature revealed that several analytical methods, including reverse phase liquid chromatography (RP-LC), high performance thin layer chromatography (HPTLC), and estimation of OLM and HCTZ by reverse phase high performance liquid chromatography (RP-HPLC), have been reported for the determination of OLM and HCTZ.[6-8] It has been noted that OLM and HCTZ work well together or in conjunction with other antihypertensive drugs. [9-30]. In order to simultaneously estimate OLM and HCTZ, a simple, quick, precise, and accurate RP-HPLC method has been developed. When this method was compared to three previously published analytical methods, it was determined that the developed method was simpler and faster (RT for OLM was 1.4 minutes and RT for HCTZ was 1.9 minutes). So, create a technique you can utilise for regular analysis.



Olmesartan Medoxomil



Hydrochlorothiazide

2. EXPERIMENTAL

2.1 Equipment

The Agilent 1260 infinite Liquidchromatographic system, equipped with a variable wavelength programmable PDA detector and a 20 µL fixed loop injector, was used to carry out the chromatographic separation. a Protocol C18 ENDURO Column in reverse phase. (250× 4.6 mm× 5µ) (5µparticle size) was used.

2.2 Reagent and chemical

OLM and HCTZ were graciously provided by Glen Mark Pharma (Nashik) and Ajanta Pharmaceutical Pvt. (Paithan), respectively. 20 mg of the Marketed Formulation OLM dosage and 12.5 mg of HCTZ were purchased from a nearby market. Methanol, acetonitrile (Merck, Nashik), and water were all of HPLC grade.

2.3 Chromatographic Conditions

Chromatographic separation was performed using a reverse phase Protocol C18 ENDURO Column (250 x 4.6 mm) with a 270 nm detection wavelength.Acetonitrile and water in a 50:50 composition were chosen as the mobile phase, and the same mixture was also utilised to make

the standard and sample solutions. With the flow rate set to 1 ml/min, a 20 µl injection was made.

2.4 Preparation of mobile phase

A 0.45 µ nylon membrane filter was used to filter a 50:50 mixture of acetonitrile (HPLC grade) and water (triple distilled), which was then degassed.

2.5 Preparation of standard solution

Accurately weigh 10 mg of OLM and HCTZ each and transfer to two 10 ml volumetric flasks. Dissolve with 1 ml of acetonitrile from this solution and dilute to 10 ml to create a stock solution with 100 µg of OLM and HCTZ per ml.

2.6 Optimization of HPLC method

In order to simultaneously estimate Olmesartan Medoxomil and Hydrochlorothiazide in bulk medication and its tablet formulation by RP-HPLC, the HPLC method was optimised. The method was optimized with different mobile phase was used in Water: Methanol and Acetonitrile: Water elute the composition of Acetonitrile: Water as 50:50 v/v and they were observed the good system suitability parameter using Protocol C18 ENDURO Column (250×4.6mm) chromatogram shown in figure 1.

3. Validation of Developed Method

The optimised approach was validated in accordance with ICH Q2 (B) recommendations.

3.1 System Suitability

Inject the sample and check the system suitability for number of theoretical plates (N)obtained, calculated tailing factor, AUC, resolution, asymmetry etc. Were reported in Table No 1.

3.2 Linearity

For the purpose of determining linearity, the working stock solution A was pipetted into a succession of 10 ml volumetric flasks, and the volume was then filled with solvent to generate concentrations ranging from 10 to 50 µg/ml for OLM and 10 to 50 gµ/ml for HCTZ. The calibration curve was plotted with the measured peak areas against concentration after each solution was injected five times (five replicates), and the regression equation and correlation coefficient were then determined. Figure 3-4 displays the calibration curves for OLM and HCTZ. And Table No. 2 contained their linearity parameters.

3.3 Precision

By calculating the% RSD of three replicates on the same day, the repeatability of the approach was confirmed. For intermediate precision, the% RSD was obtained from repeated experiments on separate days, with a different analyst. Table No. 3 presented the results.

3.4 Accuracy

Recovery studies were conducted using the usual addition approach in order to guarantee the method's correctness and dependability. The Preanalysis sample had a known amount of pure drug added to it, and the content was then reanalyzed using the suggested procedure. The percent recovery was then reported. The outcomes are presented in Table No. 4.

3.5 Robustness

By altering the flow rate of the mobile phase and max, the robustness of the approach was confirmed. The %RSD were shown in Table No 5.

3.6 Limit of detection (LOD) and Limit of quantification (LOQ)

Using the formulas LOD = 3.3 σ /S and LOQ = 10 σ /S, the LOD and LOQ were determined from the slop (S) of the calibration plot and the standard deviation (SD) of the peak regions. the outcome was presented in Table No. 6.

3.7 Assay of marketed formulation

One tablet contains 20 mg of OLM and 12.5 mg of HCTZ was measured out and coarsely pulverised. was transferred into a volumetric flask of 100 ml.the 80 ml volumetric flask of acetonitrile. The solution shake vigorously for 5 min then make up the volume with Acetonitrile and then filtered with membrane filter paper.From the solution take 10 ml of solution and transfer into 100 ml volumetric flask and diluted with Acetonitrile up to 100 mlto get solution containing 20 µg/ml Of OLM and 12.5 µg/ml HCTZ. From the solution respective smaller dilution are prepared. The solution contained OLM and HCTZ in the proportion of 20:12.5. By extrapolating the calibration curve's area value, the amount of OLM and HCTZ in each tablet was determined. Table No. 2 displays the findings of the analysis of tablet formulation.

4. Result and discussion

Acetonitrile and Water 50:50 v/v was chosen as the mobile phase after a number of studies with mobile phase of various compositions due to good resolution and symmetrical peaks. When measured spectrophotometrically, OLM and HCTZ were found to exhibit noticeable absorbance at 270 nm, and this wavelength was chosen as the detection of OLM and HCTZ separation in an optimised chromatogram at various retention times (RT). Using five replicates, system suitability testing was done. Satisfactory results were obtained for the number of theoretical plates, tailing factor, resolution, asymmetry, etc. OLM and HCTZ concentration ranges of 10–50 µg/ml were discovered to be linear, with correlation coefficients of 0.9994 and 0.99999 for OLM and HCTZ, respectively. The proposed approach was found to be accurate and repeatable, with %RSD for OLM and HCTZ of 0.9744 and 0.7956, respectively. Recovery study was used to confirm the method's accuracy. Calculating the percent recovery of the standard added to the pre-analyzed sample, it was discovered to be 95.67-95.70% for OLM and HCTZ, and 97.35 and 98.24% for OLM. OLM and HCTZ were discovered to have LOD and LOQ values of 0.5745 µg/ml, 0.2238 µg/ml, and 0.241 µg/ml, 0.678 µg/ml, respectively. After adjusting the parameters, such as the detecting wavelength and flow rate %RSD, it was discovered that the approach was resilient.

5. Comparison of Develop Method

The develop method compared previously three reported a) S. Vidyadhra b) B. Jampala c) P.D Bari. Firstly, compared develop method with A) S. Vidyadhra et al. [42] linearity of develop method 20-100 µg/ml & 12.5-75.00 µg/ml for OLM and HCTZ. Linearity for reported method is 6-42 µg/ml & 10-70 µg/ml for OLM and HCTZ. Second Parameter is precision repeatability is 0.5635 & 0.9893 for OLM &HCTZ, day to day variation 0.9633 & 0.7956, analyst to analyst variation 0.3430 & 0.9569 for OLM & HCTZ, reported method was not perform intermediate precision only perform repeatability 0.57& 0.69. The % recovery for develop method 95.67-95.70% & 97.35-98.24% for OLM and HCTZ, recovery of reported method 99.02-101% & 98.39-100.94% for OLM & HCTZ.the LOQ for develop method 0.5745 µg/ml & 0.2238 µg/ml & LOD is 0.241 µg/ml & 0.678 µg/ml for OLM and HCTZ. Robustness for develop method change in wavelength a) 269nm % RSD is 0.2436 & 0.9419, b) 271nm %RSD is0.6322 & 0.9836. Change in flow rate a) 0.9 ml/min %RSD is 1.1202 & 0.9786 b) 1.1 ml/min % RSD is 0.8554 & 1.0918 for OLM & HCTZ. Robustness for reported method change in wavelength 0.43& 0.42, change in flow rate 0.48 & 0.66. % Assay for develop method is 99.85 & 99.36. assay for reported method 99.2& 100.34 for OLM and HCTZ.

B) B. Jampala et al.[30] linearity range is 4-22µg/ml& 2.5-15µg/ml and develop method linearity range is 20-100 µg/ml & 12.5-75.00 µg/ml. LOD &LOQ for reported method 0.44µg/ml &0.21µg/ml and 1.32 & 0.63 µg/ml for OLM and HCTZ etc. LOD &LOQ for develop method the 0.5745 µg/ml & 0.2238 µg/ml and 0.241 µg/ml & 0.678 µg/ml for OLM and HCTZ. % assay for reported method 100.24 & 100.1% and assay for develop method is 99.85% & 99.36% The reported method Precision, Accuracy and Robustness parameter was not perform.

C) P.D. Bari et al.[43] linearity range is 5-12µg/ml& 5-20µg/ml and develop method linearity range is 20-100 µg/ml & 12.5-75.00 µg/ml.% Assay for develop method is 99.85 & 99.36 .assay for reported method 100.97% & 100.78% for OLM and HCTZ. The reported method Precision, LOD, LOQ and Robustness parameter was not performed.

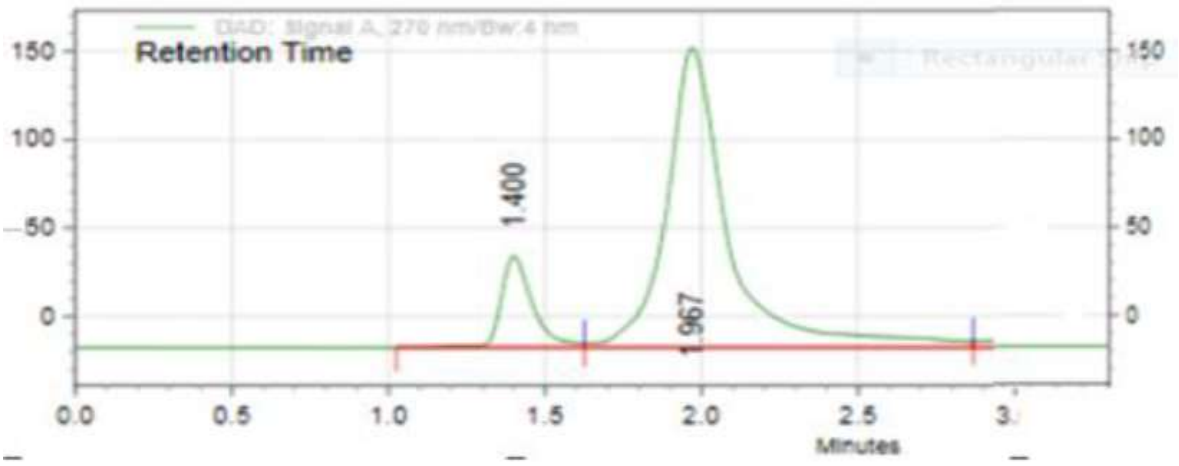


Fig. No 1. Chromatogram of OLM and HCTZ Bulk Drug.

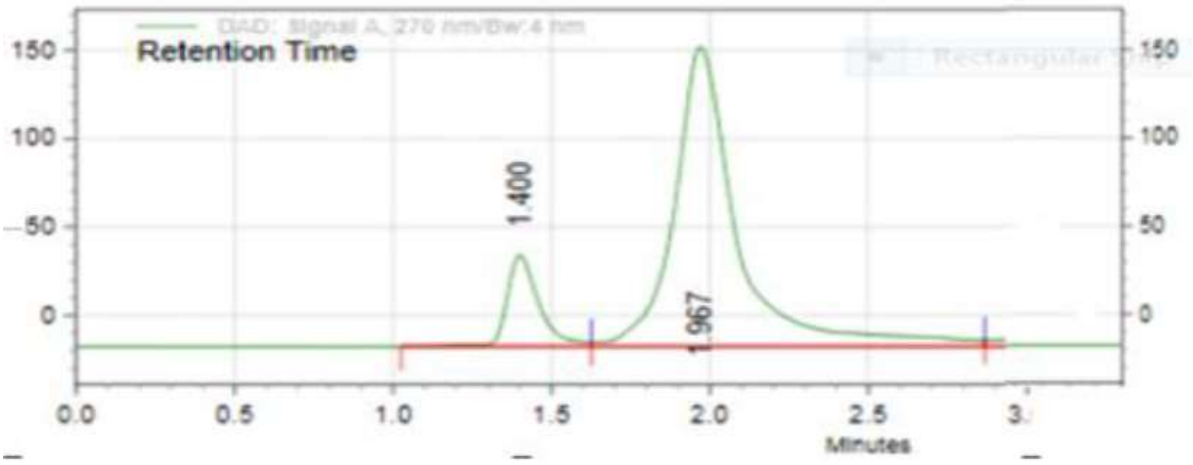


Fig. No 2. Chromatogram of OLM and HCTZ Tablet Formulation.

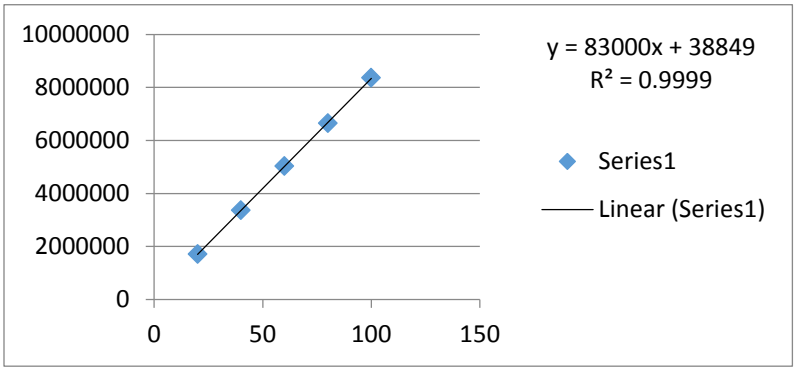


Fig. No 3. Calibration plot of OLM.

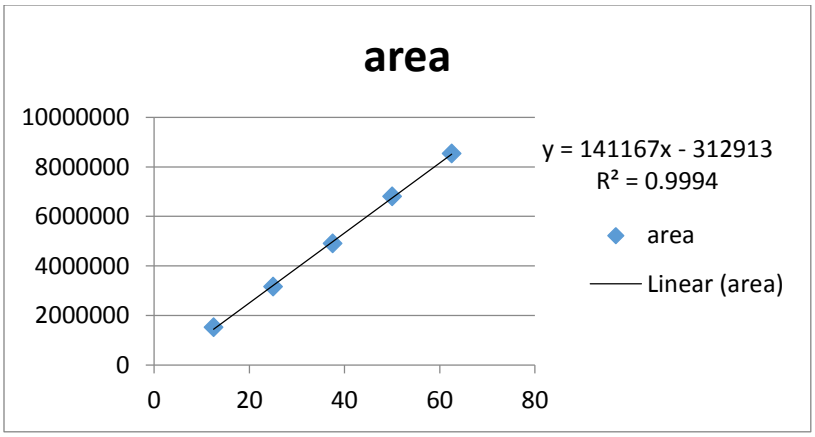


Fig. No 4. Calibration plot of HCTZ.

Table 1: system suitability parameter

Parameters	OLM	HCTZ
Retention time (min)	1.4	1.9
Area(AUC)	1701233	1522599
Tailing Factor	1.17	1.29
Theoretical plates(N)	1043	6981
Resolution	-	2.4022

Table 2: Results for Linearity

Data for Linearity	OLM	HCTZ
Linearity Range	10-50 (µg/ml)	10-50 (µg/ml)
Correlation Coefficient (r2)	0.9999	0.9994
Slope (m)	83000	141167
Y-intercept	38849	312913

Table 3: Result for precision

Data for Precision	OLM	HCTZ
Repeatability		
Mean	99.06 %	98.00 %
SD	0.5583	0.9695
%RSD	0.5635	0.9893
Intermediate precision		
Day to Day variation		
Mean	98.86%	98.66%
SD	0.9633	0.7849
%RSD	0.9744	0.7956
Analyst to Analyst variation		
Mean	98.67 %	97.96 %
SD	0.3385	0.9374
%RSD	0.3430	0.9569

Table 4: Result for Accuracy

Statistical Parameter	OLM	HCTZ
Mean	95.92	97.56
SD	0.4592	0.5879
%RSD	0.4812	0.6026

Table 5: Result for Robustness

Data for Robustness	OLM	HCTZ
Change in Wavelength		
269 nm		
Mean	98.49 %	98.68 %
SD	0.2401	0.9295
%RSD	0.2436	0.9419
270 nm		
Mean	99.06%	98.00%
SD	0.5583	0.9695
%RSD	0.5635	0.9893
271 nm		
Mean	97.61 %	98.32 %
SD	0.6171	0.9670
%RSD	0.6322	0.9836
Change in Flow Rate		
0.9 ml/min		
Mean	98.75 %	97.72 %
SD	1.187	0.9563
%RSD	1.2020	0.9786
1.0 ml/min		
Mean	99.06 %	98.00 %
SD	0.5580	0.9695
%RSD	0.5583	0.9893
1.2 ml/min		
Mean	98.47 %	98.17 %
SD	0.8423	1.0718
RSD	0.8554	1.0918

Table 6: Result for LOD and LOQ

Parameter	OLM	HCTZ
LOD (µg/ml)	0.5745	0.2238
LOQ (µg/ml)	0.241	0.678

Table 7: Result for Assay of marketed formulation

Statistical Parameters	OLM	HCTZ
% Conc Found	99.85	99.36
SD	0.6383	0.8551
%RSD	0.6393	0.8606

Table No 8. Comparative Study

Parameter	Develop Method	Reported Method						
			S. Vidyadhra. et al.**2014**[5]		J. Blaji et al.**2015**[6]		P.D.Bari et al.**2009**[7]	
	OLM	**HCTZ**	**OLM**	**HCTZ**	**OLM**	**HCTZ**	**OLM**	**HCTZ**
Linearity	10-50µg/ml	10-50µg/m	6-42µg/m	10-70µg/m	4-24µg/m	2.5-15µg/m	5-15µg/m	5-20µg/m
Precision-a)repeatability								
i)intraday-	0.5635	0.9893	0.57	0.69	-	-	-	-
ii)Interday-	0.3650	0.3125	0.72	1.13	-	-	-	-
b)intermediate								
i) Day to Day	0.9633	0.7956	-	-	-	-	-	-
ii) Analyst to Analyst	0.3430	0.9569	-	-	-	-	-	-
Accuracy (% recovery)	0.4812 95.67-95.70	0.6026 97.35-98.24	- 99.02-101.55	- 98.39-100.94	- - -	- - -	- 100.71-100.97	- 100.73-100.78
LOD	0.5745	0.2238	0.25	0.10	0.44	0.21	-	-
LOQ	0.241	0.678	0.78	0.32	1.32	0.63	-	-
Robustness a)change in wavelength	0.2436	0.9419	0.43	0.42	-	-	-	-
i) 269 nm	0.5635	0.9893	0.59	0.71	-	-	-	-
ii) 270 nm	0.6322	0.9836	-	-	-	-	-	-
iii) 271 nm								
b) change in flow rate								

i)0.9ml/min ii)1.0ml/min iii)1.1ml/min	1.1202 0.5583 0.8554	0.9786 0.9893 1.0918	0.48 - 0.78	0.66 - 0.72	- - -	- - -	- - -	- - -
System suitability								
a) R.T.	1.4	1.9	4.3	3.6	7.1	2.8	4.9	1.99
b) AUC	1701233	152259	-	-	-	-	-	-
c) HETP	1043	6981	11421	9897	-	-	-	-
d)Asymmetry	0.17106	1.39837	-	-	1.75	1.22	-	-
e) Tailing F.	1.17	1.28	1.9	-	-	-	-	-
% Assay	99.85	99.36	99.2	100.34	100.24	100.1	-	-

6. Conclusion

OLM and HCTZ are simultaneously estimated by RP-HPLC in both the bulk medication and its tablet formulation. According to the ICH guideline, all validation parameters have been found to be within the limit. This technique was discovered to be straightforward, precise, accurate, and robust and may be used in the routine study of the commercial formulation. Developed method is compared with three reported methods from above comparison develop method takes less time for separation of analyte than three reported method and they did not used any type of buffer. The developed method was the best in routine analysis as a result of all that. This approach has a number of important benefits, including simplicity, selectivity, accuracy, and precision, which make it appropriate for the 99.36% to 99.85% range of recovery experiments that have been conducted. It shows that there are no excipients interfering with the formulation.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors approved the manuscript for publication.

Availability of data and material

All required data is available.

Competing interests

All authors declare no competing interests.

Funding

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