DEVELOPMENT AND VALIDATION OF RP- HPLC METHOD FOR DISSOLUTION STUDY OF OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE IN COMBINED DOSAGE FORM

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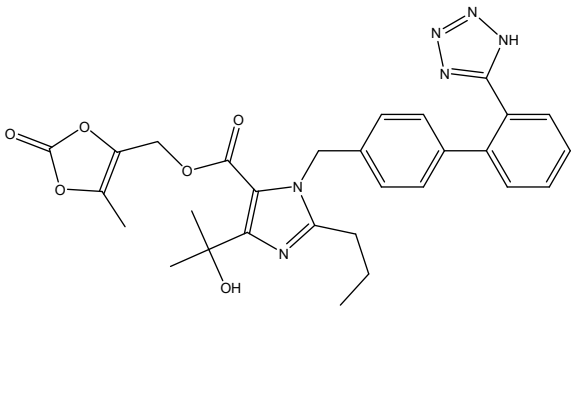
ABSTRACT

A simple, rapid, precise and accurate RP-HPLC method was developed for dissolution of Olmesartan Medoxomil and Hydrochlorothiazide in combined dosage form. RP HPLC method is carried out by Hypersil BDS column C_{18} (25 cm × 4.6 cm, 5 µm). Mobile phase used as combination of Buffer (pH 4.0 adjusted with o- phosphoric acid): Methanol (70:30 %v/v) and detection carried out at 276 nm. Retention time of Olmesartan Medoxomil and Hydrochlorothiazide were found to be 3.9 min and 5.3 min, respectively. Linearity range of Olmesartan Medoxomil is 20-60 µg/ml and for Hydrochlorothiazide 12.5-37.5 µg/ml. Correlation coefficients for both drugs were greater than 0.995 and mean recoveries for both drugs were between 99% - 100%.

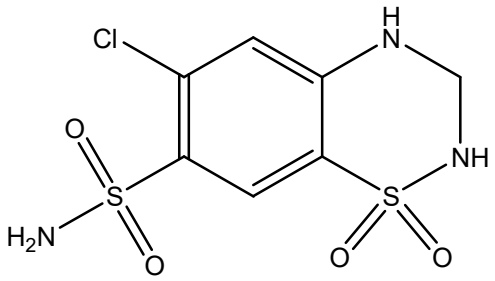
Keywords: Olmesartan Medoxomil, Hydrochlorothiazide, Hypertension, RP- HPLC.

1.0 INTRODUCTION[1-5]

Olmesartan Medoxomil (OLM) is an antihypertensive belong to class Angiotensin receptor blockers which is used in the treatment of hypertension. Angiotensin receptor blocker used as decrease in heart rate, cardiac output, blood pressure. Chemically known as (5- methyl-2-oxo-1,3-dioxol-4-yl) methyl 4-(2-hydroxypropan-2-yl)-2- propyl-1- ({4-[2-(1,2,3,4-tetrazol- 5-yl) phenyl] phenyl} methyl)- imidazole-5- carboxylate. It is selectively inhibit the binding site of Angiotensin II to AT1, which is found in many tissues such as vascular smooth muscles and different adrenal glands. These effectively inhibit the AT1- mediated vasoconstrictive and aldosterone- secretive effects of Angiotensin II and it results in a decrease in blood pressure and also vascular resistance. Hydrochlorothiazide (HCTZ) is an antihypertensive, belongs to class thiazide diuretic which us used in the treatment of hypertension. Chemically known as 6- chloro-1,1-dioxo-3,4- dihydro-1,2,4-benzothiadiazine-7- sulfonamide. Respective structure of OLM and HCTZ is shown in **Figure 1**.



Olmesartan Medoxomil



Hydrochlorothiazide

Figure 1: Structures of OLM and HCTZ

There is commercially available in market with combination of OLM (40 mg) and HCTZ (25 mg) as tablet formulation. The ratio of OLM and HCTZ in formulation is 1.6: 1, respectively.OLM and HCTZ both are official in USP. Various pharmacopoeial and reported methods like UV, RP- HPLC, RP-LC, HPTLC, methods has been developed for the estimation of OLM. While UV, LC, RP-HPLC, LC-MS are reported for estimation of

HCTZ. RP-HPLC method has been reported for the simultaneous estimation of OLM and HCTZ. This research paper demonstrates the development and validation of RP- HPLC method for simultaneous estimation of OLM and HCTZ into their combined pharmaceutical dosage forms. This analytical method is optimized, developed and validated as per ICH guidelines.

2.0 MATERIALS AND METHODS [6-18]

2.1 Equipment

A shimadzu HPLC instrument were used for chromatographic separation. Software is LC solution and equipped with UV detector having 20µl fixed loop. Chromatogram was recorded and peaks were qualified by software Delta Ace station. Chromatographic separation was carried out on a C_{18} column, Hypersil BDS having 25 cm X 4.6 cm, 5 µm diameters.

2.2 Reagents and Chemicals

API of Olmesartan Medoxomil and Hydrochlorothiazide procured as gift samples from SHASHI Pharma, Chhtral. The tablet dosage form “Benicar HCT” having combination of OLM (40 mg) and HCTZ (25 mg) were procured from the local market. All reagents and chemicals used are of analytical grade.

2.3 Chromatographic conditions

Optimized mobile phase consisting of Phosphate Buffer (pH 4.0 adjusted with o-phosphoric acid): Methanol in ratio 70:30 % v/v, it was used in the isocratic mode. Initially, all reagents are filtered through 0.45 micron membrane filter and then sonicated for 15 minutes before used as mobile phase. Flow rate was maintained at 1.0 ml/min and injection volume was 20 µl. UV detection was carried out at 276 nm shown in **Figure 2** and separation was taken at room temperature.

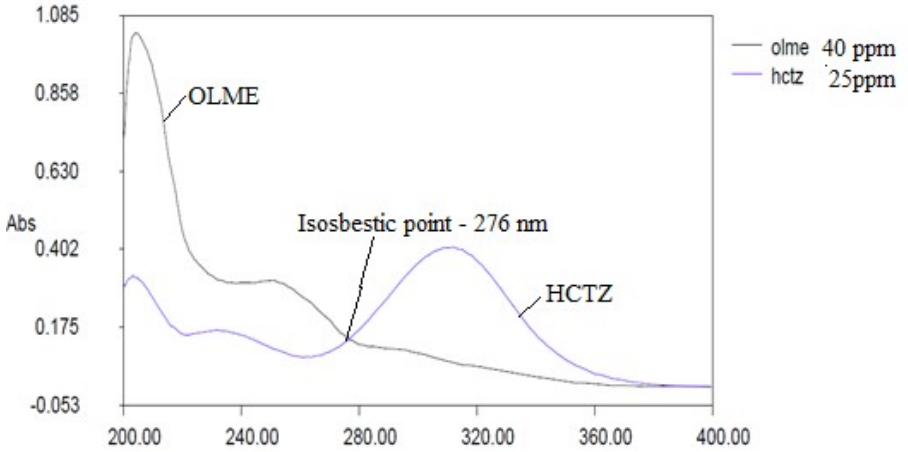


Figure 2: Selection of analytical wavelength (276 nm)

3.0 EXPERIMENTAL[6-18]

3.1 Preparation of Stock solutions for OLM and HCTZ

Take accurately weighed 40 mg of OLM and 25 mg of HCTZ. It is separately transferred into 100 ml volumetric flasks. OLM (volumetric flask A) and HCTZ (volumetric flask B) was dissolved using methanol and make up the volume upto with methanol. This stock solution having strength of 400 µg/ml and 250 µg/ml for OLM and HCTZ, respectively. From the volumetric flask A, 1 ml was transferred into 10 ml vol. flask and volume was made with methanol to get final dilution of 40 µg/ml of OLM. Than from volumetric flask B, 1 ml was transferred into 10 ml vol. flask and volume was made with methanol to get final dilution of 25 µg/ml of HCTZ.

3.2 Preparation of Test solutions

Accurately measure 1 ml of solution and then transfer it into 10 ml volumetric flask and make up volume with methanol. Final stock solutions containing 40 µg/ml of OLM and 25 µg/ml of HCTZ were prepared by further dilution with the mobile phase.

4.0 METHOD VALIDATION [19, 20]

This analytical procedure was validated according to ICH Q2 B guidelines in order to determine the system suitability, linearity, precision, accuracy, specificity and robustness of analysis.

4.1 System suitability

System suitability was carried out by injecting 100% concentration (sample having 40 µg/ml of OLM and 25 µg/ml of HCTZ) into chromatographic system. These are repeated six times under similar condition. The tailing factor (T), Numbers of theoretical plate (N) and Resolution obtained are given in **Table 1**. Optimized Chromatogram of Olmesartan Medoxomil and Hydrochlorothiazide is shown in **Figure 3.**

Table 1: Results of various system suitability parameters

Parameters	Data observed	
	Olmesartan Medoxomil	Hydrochlorothiazide
Retention Time (Min)	5.3	3.9
Theoretical plates per column (N)	4566	7074
Symmetry factor/Tailing factor (S)	3.923	5.360
Resolution (Rs)	5.898	

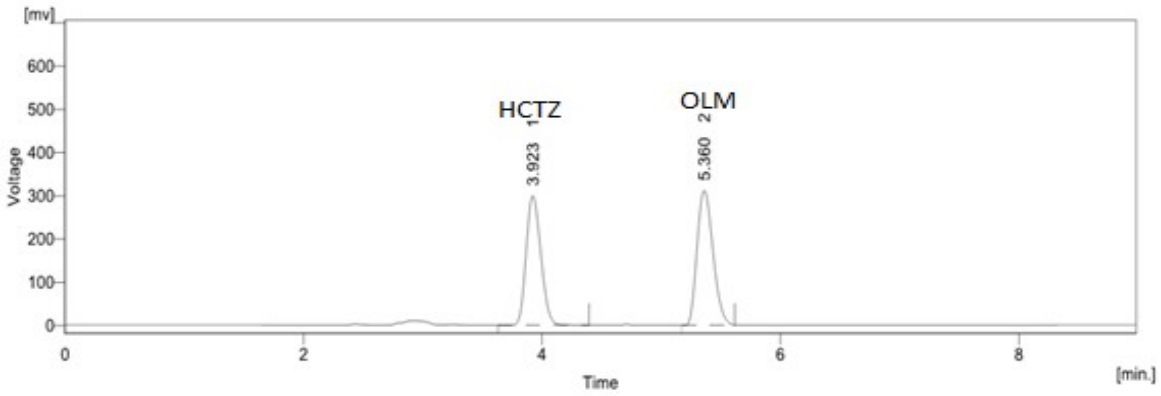


Figure 3: Optimized final chromatogram of OLM and HCTZ

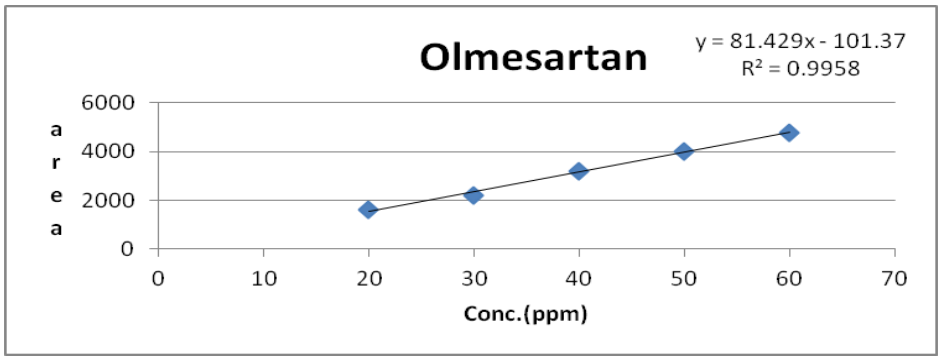
4.2 Linearity

Standard calibration was prepared using five mixed standard calibration solution in a concentration range of 20-60 µg/mL µg/ml for OLM and 12.5-37.5 µg/ml for HCTZ. Each solution was chromatographed for 8 min under the mentioned optimized chromatographic conditions. The peak area was found out by post run analysis of the chromatogram using

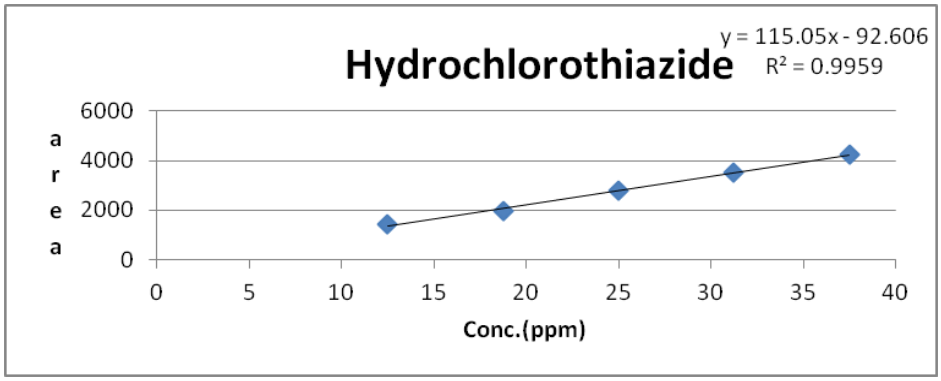
LC Delta Ace station Software. Calibration curve of peak area Vs concentration was demonstrated by linear square regression analysis of the calibration curves. The calibration curves for OLM and HCTZ are shown in **Figure 4a and 4b** and their corresponding linearity parameters are given in **Table 2**. The graphs for linearity study are shown in **Figure 4a and 4b**. Overlain Chromatogram for OLM and HCTZ is also shown in **Figure 4c.**

Table 2: Linearity Results (n=6)

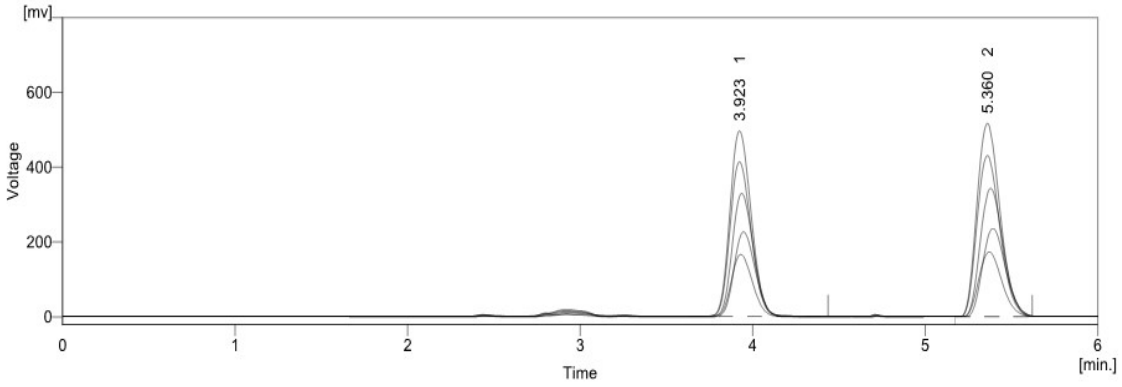
Regression Analysis	OLM	HCTZ
Regression equation	Y= 81.429x -101.37	Y= 115.05x -92.606
Correlation co-efficient	0.9958	0.9959
Slope	81.429	115.05
LOQ	11.886 µg/ml	7.736 µg/ml
LOD	3.922 µg/ml	2.421 µg/ml



(a)



(b)



(c)

Figure 4: (a) Calibration curve of OLM; (b) Calibration curve of HCTZ; (c) Overlain chromatogram for OLM and HCTZ

4.3 Precision

Precision was carried out by injecting six replicates of 100% concentration (40 µg/ml of OLM and 25 µg/ml of HCTZ). The % RSD values of peak area are given in **Table 3.**

Table 3: Precision results (n=6)

Drugs	Inter day Precision (% RSD)	Intraday Precision (%RSD)
OLMESARTAN MEDOXOMIL	1.418-1.295	1.041-0.409
HYDROCHLOROTHIAZIDE	1.205- 0.346	0.327- 0.283

4.4 Accuracy

To study the accuracy of proposed analytical method, recovery experiment was performed by the standard addition technique. In this method, a known quantity of a pure drug was added at three different various levels, i.e. 80%, 100% and 120% to pre-analyzed sample solutions and the recovery of OLM and HCTZ was calculated for each concentration. Results are shown in **Table 4.**

Table 4: Results of Recovery study (Accuracy) n=3

Recovery Level	Sample Amount (µg/mL)		Amt of drug Added (µg/mL)		Amount Recovered Mean (µg/mL)		% Recovery	
	OLM	HCTZ	OLM	HCTZ	OLM	HCTZ	OLM	HCTZ
80 %	20	12.5	16	10	99.21± 0.59	100.25 ± 1.47	99.85	101.73
100 %	20	12.5	20	12.5	99.35 ± 0.83	99.32 ± 0.32	100.30	99.18
120 %	20	12.5	24	15	99.16 ± 0.95	99.35 ± 0.49	99.13	99.13

4.5 Selectivity/ Specificity

Selectivity is an ability of analytical method to procedure a response for the analyte in presence of other interference. In order to prove that the method chosen was specific and selective. The parameters retention time (Rt), resolution (Rs) and tailing factor were calculated and given in **Table 1**.

4.6 LOD and LOQ

LOD and LOQ of a drug were calculated using equations which are given below according to ICH guideline.

LOD= 3.3 X σ/S and LOQ= 10 X σ/S

Where σ is the SD of response and

S is the slope of calibration curve

Results of LOD and LOQ were given in **Table 2.**

4.7 Robustness

The Robustness was performed to evaluate the influence of smallest but deliberate variation in different chromatographic condition. The Robustness was evaluated by changing small variation in parameters.

1. Flow rate (±0.2 ml/min),
2. Mobile phase (±2 ml),
3. pH (±0.2).

After each change, sample solution was injected and % assay with system suitability parameters are checked. Values obtained of % RSD are given in **Table 5.**

Table 5: Robustness results

Conditions	Variation	Olmesartan Medoxomil			Hydrochlorothiazide		
		Area	S.D. (n=3)	% RSD	Area	S.D. (n=3)	% RSD
Flow Rate (1.0 ± 0.2 ml/min)	-0.2 ml/min	2431.07	19.38	0.797	2356.24	37.84	1.606
	+0.2 ml/min	2306.84	15.54	0.674	2199.78	27.23	1.238
pH of mobile phase (4.0)	-0.2	2420.04	12.33	0.510	2311.89	31.00	1.341
	+0.2	2247.18	20.78	0.925	2162.25	27.85	1.288
Mobile phase Phosphate Buffer (pH 4.0 adjusted with o-phosphoric acid): Methanol (70:30 %v/v)	-2.0	2414.77	14.51	0.601	2314.64	31.79	1.373
	+2.0	2284.20	33.89	1.484	2215.38	38.88	1.755

5.0 ASSAY OF MARKETED PRODUCT

Total 10 µL sample solution was injected into the chromatographic system and response of peak was measured. The solution is injected three times into column. The amount present in each and every tablet was calculated by comparison of area of test with the standard and it found to be 101.02 ± 0.65 and 99.87 ± 0.39 respectively, for OLM and HCTZ. The results are shown in **Table 6.**

Table 6: Results of Assay (n=3) of Marketed Formulation

Tablet	mg/Tablet powder		Assay (% of label claim) Mean ± S.D (n=3)	
	Olmesartan Medoxomil	Hydrochlorothiazide	% Olmesartan Medoxomil	% Hydrochlorothiazide
Benicar HCT	40	25	101.02 ± 0.65	99.87 ± 0.39

6.0 RESULTS AND DISCUSSION

An objective of present work is to develop and validate RP- HPLC method which can be used for routine analysis of OLM and HCTZ in Pharmaceutical Marketed dosage forms. First of all chromatographic conditions were optimized for the estimation of both selected drugs by RP- HPLC method. A mixture of Phosphate buffer (pH 4 adjusted with o-phosphoric acid): Methanol in ratio of 70:30 %v/v was selected as mobile phase that proved to the most suitable for all combinations in which the chromatographic peaks obtained were better defined as well as resolved and also free from tailing. The detection was carried out at 276 nm at which both the drugs show measurable absorbance **(Figure 2).**

The developed chromatographic method was also be validated by ICH guidelines. The retention times obtained for OLM and HCTZ were 5.3 and 3.9 min, respectively. Various optimized chromatogram show the separation of OLM and HCTZ at different retention time is shown in **Figure 3**.

System suitability was carried out by the injecting 100% concentration of OLM and HCTZ, six times into HPLC system. The tailing factor was less than 6 and theoretical plates were more than 2000 for both the selected drugs which comply with the standard limits. **(Table 1)**

The linearity and range were found to be Concentration range of 20-60 µg/mL µg/ml for OLM and 12.5-37.5 µg/ml for HCTZ. The correlation coefficients of OLM and HCTZ were found to be 0.9958 and 0.9959, respectively for OLM and HCTZ thus indicate good linearity in the specified concentration range **(Table 2)**. The calibration curves for OLM and HCTZ is shown in respectively in **Figure 4a and 4b**. Overlain Chromatogram of OLM and HCTZ is described in **Figure 4c**.

The minimum variation in the % RSD values obtained indicated that the present method is precise **(Table 3)**.

The results show that the % recoveries for drug OLM and HCTZ were found to be 99.21-99.16 % and 100.25-99.35% respectively which is well within the acceptance criteria. Hence method is can be termed accurate.

The method specificity was determined by study the various chromatograms obtained from the test solutions. The method was found to be specific as none of the excipients interfered with analyte of interest. Hence the method was found to be suitable for analyzing the marketed formulation.

LOD were found to be 3.922 µg/mL for OLM and 2.421 µg/mL for HCTZ and LOQ were found to be 11.886 µg/mL for OLM and 7.736 µg/mL for HCTZ (**Table 2**).

Robustness was studied by taking % variation for ideal system suitability parameter like flow rate and wavelength. Solution of 100% concentration was prepared and to inject in triplicate for each conditions and the % RSD calculated was found to be less than 2 for every conditions. **(Table 5)**

7.0 CONCLUSION

A Simple, economic, rapid and selective RP- HPLC method has been optimized, developed and validated for an estimation of Olmesartan Medoxomil and Hydrochlorothiazide in the pharmaceutical dosage form. All method validation parameters are complies to its acceptance criteria as per ICH Q2 (R1) guideline. So we concluded that method is selective, accurate, linear and precise also. Hence, it can also successfully used for the routine analysis of Pharmaceutical dosage form.

8.0 ACKNOWLEDGEMENT

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