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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CHLORTHALIDONE AND OLMESARTAN MEDOXOMIL IN TABLET DOSAGE FORM

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Abstract: A simple, accurate, precise and sensitive Reverse Phase Liquid Chromatography (RP-HPLC) methods was developed and validated for simultaneous estimation of Chlorthalidone (CLT) and Olmesartan Medoxomil (OLM) in tablet dosage form using a Enable C_{18} (250 mm -4.6 mm, 5 µm particle size) column and methanol:acetonitrile:water (60:20:20 v/v) as a mobile phase at a flow rate of 1.0 ml/min with detection wavelength 220nm. The retention time for OLM and CLT was obtained as 1.89±0.1 min and 2.98±0.2 min respectively. The linearity was found in the range of 6.25-37.50 µg/ml and 10-60 µg/ml for CLT and OLM respectively. The developed method was validated as per ICH guideline, for its accuracy, precision, limit of detection & limit of quantitation.

Keywords: Chlorthalidone (CLT), Olmesartan Medoxomil (OLM) and Reverse Phase Liquid Chromatography (RP-HPLC)

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INTRODUCTION

Olmesartan medoxamil (OLM) is a (5-methyl-2 oxo-1,3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1-methylethyl)-2-propyl-1{[2-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1H-imidazole-5-carboxylate[1,2] (fig. 1). It is an AT1 subtype angiotensin II receptor antagonist used in the treatment of hypertension. Chlorthalidone (CLT) is an oral diuretic used along with oral antihypertensive agent. Chemically it is (RS) 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide [3] (fig. 2). Many analytical methods are reported in the literature for the determination of OLM by UV-Spectrophotometry [4], RP-HPLC [5-7] and by HPTLC [8]. Several methods have been described for determination of CLT by UV-Spectrophotometry [9], RP-HPLC [10-11] and by HPTLC [12]. Although there are several chromatographic methods reported for determination of both these drugs. However, to the best of our knowledge, there is no LC analytical method reported for simultaneous determination of OLM and CLT in combined dosage form. The objective of the present work was to develop accurate and precise RP-HPLC method with UV-detection for the quantification of these drugs in pharmaceutical formulation.

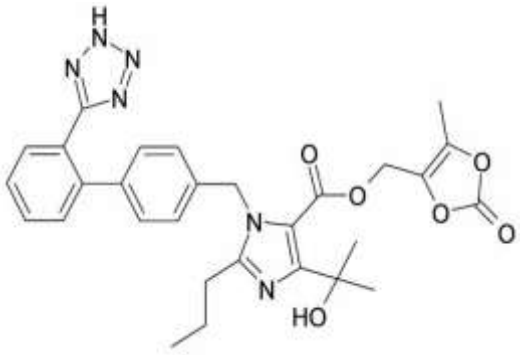

Figure 1: Olmesartan medoxomil

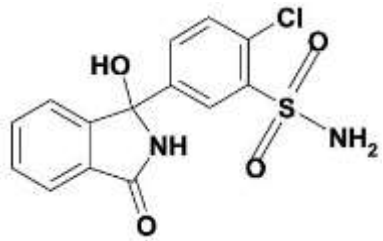

Figure 2: Chlorthalidone

METHOD AND MATERIALS

RP-HPLC Instrumentation and Conditions:

The chromatographic separation achieved by using Enable C18 (250 mm -4.6 mm, 5 µm particle size)column and methanol:acetonitrile:water (60:20:20 v/v) as a mobile phase at a flow rate of 1.0 ml/min with detection wavelength 220nm

Chemicals and Reagents

Olmesartan medoxomil was kind gift of Ajanta pharmaceutical Ltd. (Mumbai, India), Chlorthalidone was provided by Ipca laboratories (Mumbai, India). Pharmaceutical formulation of tablets containing olmesartan medoxomil 20 mg and chlorthalidone 12.5 mg was purchased

from local market of baroda. HPLC grade acetonitrile and methanol was procured from Merck, Mumbai. Double distilled de-ionized water was used throughout the study. All solutions were filtered through a Millipore vacuum filter system (0.22 µm) and degassed by sonicator.

Preparation of Stock and Standard Solution

Preparation of Standard stock solution of Chlorthalidone and Olmesartan Medoxomil:

The standard stock solutions of Chlorthalidone and Olmesartan Medoxomil were prepared by dissolving 62.5 mg and 100 mg of Chlorthalidone and Olmesartan Medoxomil respectively in Methanol:Acetonitrile:Water(60:20:20) separately and final volume was adjusted with same Mobile Phase in 100 ml of volumetric flask to get strength of 625 µg/ml and 1000 µg/ml respectively.

Preparation of working standard solution of Chlorthalidone and Olmesartan Medoxomil:

Working standards were prepared by diluting 10 ml of standard stock of each drug solutions in 100 ml volumetric flask with same Mobile Phase separately to get 62.5 µg/ml and 100 µg/ml strength of Chlorthalidone and Olmesartan Medoxomil respectively.

Preparation of combined standard stock solution of Chlorthalidone and

Olmesartan Medoxomil

Accurately weighed CLT (62.5mg) and OLM (100mg) were transferred in to 100ml volumetric flask and diluted up to the mark with mobile phase to give a stock solution (625µg/ml) of CLT and (1000µg/ml) of OLM. Stock solution (10ml) was transferred in 100 ml volumetric flask and diluted up to mark with mobile phase to obtain working standard solution (62.5µg/ml) of CLT and (100µg/ml) of OLM. This solution was used to prepare standard solution for linearity.

HPLC Method Development and Optimization

Selection of detecting wavelength:

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detecting wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. In the present study, drug solution of 25 µg/ml Chlorthalidone and 50 µg/ml of Olmesartan Medoxomil were prepared in methanol. The standard solutions were than scanned in UV region of 200-400 nm and the overlain spectrum were taken.

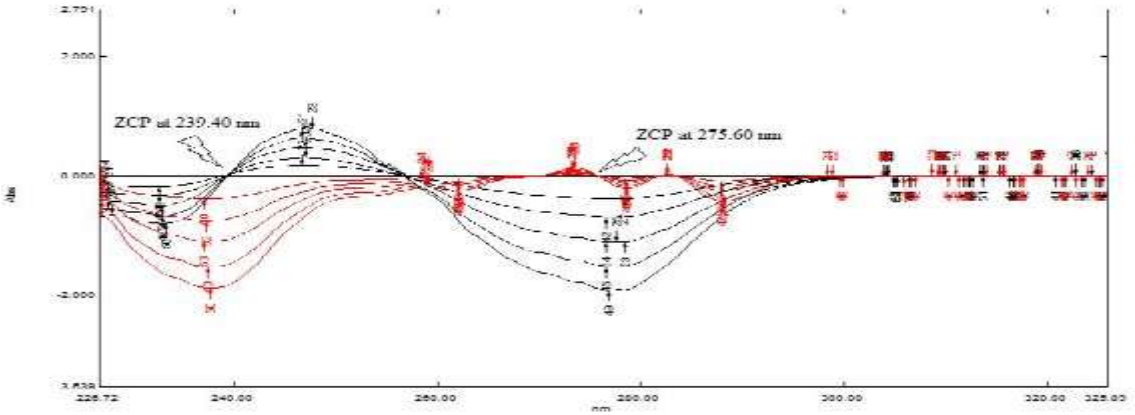

Figure 3: Overlain Spectra of Chlorthalidone and Olmesartan medoxomil

Optimization of RP-HPLC method:

The pure drug solution of Chlorthalidone (25 µg/ml) and Olmesartan Medoxomil (50 µg/ml) were injected individually in to HPLC system and allow to run in different mobile phase like Water: Methanol, Water: Acetonitrile, Methanol: Acetonitrile and Methanol: Acetonitrile:Water were tried in order to find the optimum condition for the separation of Chlorthalidone and Olmesartan Medoxomil. It was found that mobile phase containing Methanol:Acetonitrile:Water (60:20:20 v/v) at a flow rate of 1 ml/min with detecting wavelength 220nm gave satisfactory result with sharp, well defined and resolving peak with minimum tailing as compared to other mobile phases. Under these condition the retention time were typically 1.89 min. for Olmesartan Medoxomil and 2.98 min. for Chlorthalidone and optimized chromatographic condition.

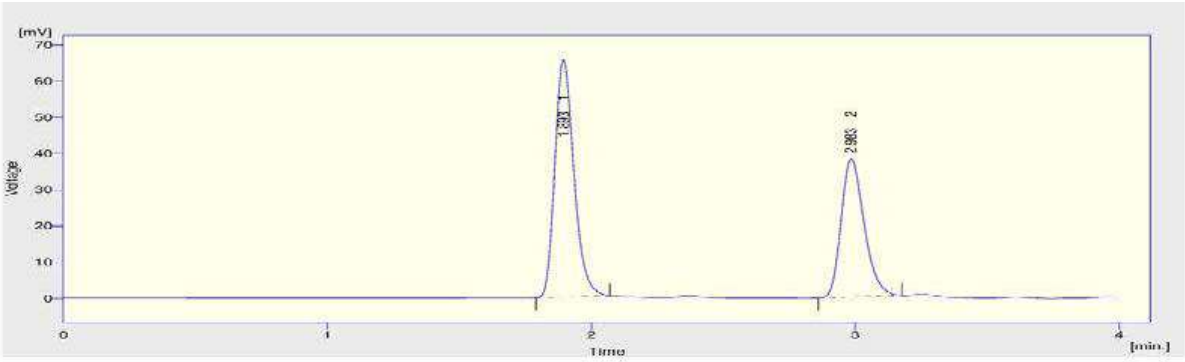

Figure 4: Chromatograph of CLT and OLM in Methanol : Acetonitrile : Water (60:20:20)

Table 1: Optimized chromatographic conditions for simultaneous estimation of OLM and CLT

Parameters	Condition
Stationary phase	Enable C18 (250mm X 4.6 mm i.d., 5 µm particle size)
Mobile phase	Methanol : Acetonitrile : Water (60:20:20)
Pump mode	Isocratic
Flow rate (ml/min)	1.0
Run time (min)	4.0
Volume of injection (µl)	20
Detection wavelength (nm)	220
Retention time (min)	OLM : 1.89 CLT : 2.98

Validation of RP-HPLC Method [13]:

Linearity

Linearity response for CLT and OLM were assessed in the concentration rang 6.25-37.5µg/ml and 10-60µg/ml of standard solution, respectively.

Sensitivity

The sensitivity measurement of CLT and OLM by the use of proposed method was estimated in term of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated using following equations.

LOD= 3.3*σ/S LOQ=10* σ/S Where

σ= the standard deviation of the response

s= slop of the calibration curve

Precision:

Intermediate precision (System precision): Intermediate precision of method was determined in the term of intra-day and inter-day variation (%RSD).

A) Intra-day precision: Intra-day precision (%RSD) was assessed by analyzing standard drug solution within the calibration range, three time on the same day.

B) Inter-day precision: Inter-day precision (%RSD) was assessed by analyzing drug solution within the range on three different days.

Accuracy

To the pre-analyzed sample, a known amount of standard solution of pure drug (CLT and OLM) were spiked at three different level. This study was carried out at 80%, 100% and 120% level.

System Suitability

To check system suitability Number of theoretical plate, Resolution, Retention time And Tailing factor were determined.

RESULTS AND DISCUSSION

The result of method development and validation study on simultaneous estimation of Chlorthalidone and Olmesartan medoxomil in current study involving Methanol:Acetonitrile:Water (60:20:20 v/v) as mobile phase for RP-HPLC are given below.

Method validation:

Linearity:

The linearity of analytical procedure is it ability (within given range) to obtain test result which are directly proportional to concentration of analyte in sample. The drug response was linear (R2 = 0.9995 for Chlorthalidone and 0.9996 for Olmesartan medoxomil) over the concentration range between 6.25-37.5 µg/ml for Chlorthalidone and 10-60 µg/ml for Olmesartan medoxomil. The linear equation for the calibration plots were y = 37.8x - 7.9395 for Chlorthalidone and y = 33.764x - 10.686 for Olmesartan medoxomil.

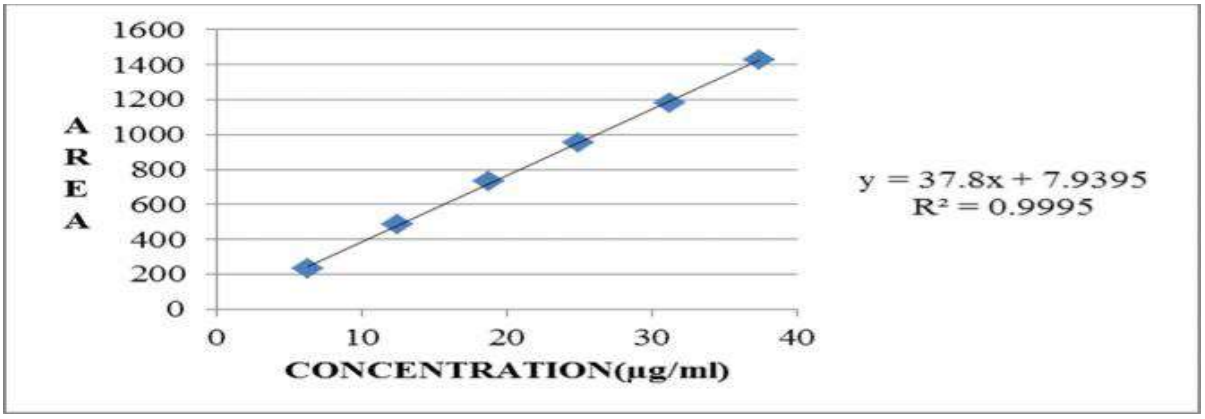

Figure 5: Calibration curve of Chlorthalidone

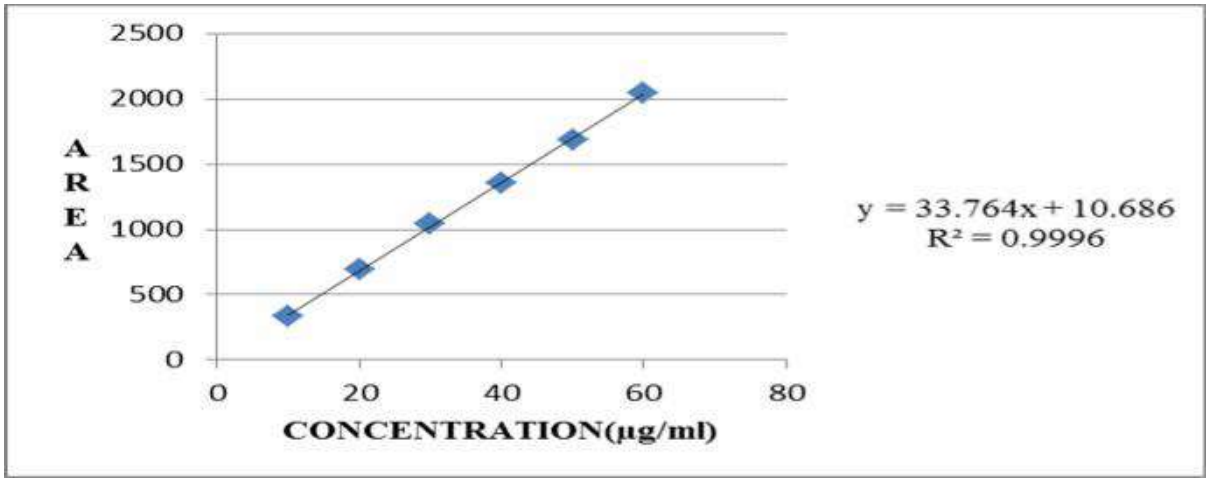

Figure 5: Calibration curve of Olmesartan medoxomil

Sensitivity:

The detection limit of an individual analytical procedure is the lowest amount of analyte in sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analyte procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were calculated by respective equations. The LOD value were found to be 0.694 µg/ml and 0.3031 µg/ml for Chlorthalidone and Olmesartan medoxomil respectively. The LOQ value were found to be 0.1052 µg/ml and 0.9185 µg/ml for Chlorthalidone and Olmesartan medoxomil respectively.

Precision:

The precision of analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of same homogenous sample under the reproducibility. Precision may be considered at three level: repeatability, intermediate precision and reproducibility . The results of the repeatability, intra-day precision and inter-day precision experiments are shown respectively as given in (Table: 3) and (Table: 4).the developed method was found to be precise as the RSD values for repeatability of intra-day and inter-day precision study were < 2%.

Table 2: Intraday and Interday Precision of Chlorthalidone

CONC µg/ml	AREA			CONC µg/ml			Mean	SD	RSD
	A	B	C	A	B	C			
Intraday									
6.25	242.54	243.03	241.97	6.21	6.21	6.19	6.20	0.01	**0.226**
12.5	477.82	478.24	480.17	12.4	12.44	12.49	12.46	0.03	**0.266**
18.75	713.94	714.03	713.05	18.7	18.68	18.65	18.67	0.01	**0.076**
Interday									
6.25	239.08	237.25	236.47	6.11	6.06	6.04	6.07	0.04	**0.583**
12.5	473.54	472.19	473.01	12.3	12.28	12.3	12.3	0.02	**0.146**
18.75	**707.94**	**708.91**	**709.14**	**18.5**	**18.54**	**18.55**	**18.54**	**0.02**	**0.090**

Table 3: Intraday and Interday Precision of Olmesartan medoxonil

CONC µg/ml	AREA			CONC µg/ml			Mean	SD	RSD
	A	B	C	A	B	C			
Intraday									
10	342.95	343.24	343.94	9.84	9.85	9.87	9.85	0.02	**0.153**
20	683.07	681.98	682.14	19.9	19.88	19.89	19.9	0.02	**0.087**
30	1015.27	1017.42	1018.49	29.8	29.82	29.85	29.81	0.05	**0.162**
Interday									
10	338.14	339.14	340.01	9.7	9.73	9.75	9.72	0.03	**0.284**
20	680.16	679.24	681.07	19.8	19.8	19.86	19.83	0.03	**0.136**
30	**1013.82**	**1014.32**	**1015.49**	**29.7**	**29.73**	**29.76**	**29.73**	**0.03**	**0.085**

Accuracy:

The accuracy of an analytical procedure express the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. As show in (table: 5) and (table: 6), good recovery of the Chlorthalidone and Olmesartan medoxomil in the range from 99.4 to 99.8 were obtained at various added concentrations.

For Chlorthalidone

6.25 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 220 nm. The amount of Chlorthalidone was calculated at each level and % recoveries were computed.

Table 4: Recovery data for Chlorthalidone

Conc µg/ml	Conc before spiking µg/ml	Ref std added µg/ml	Conc after spiking µg/ml	Recovered Conc µg/ml	% Recovery	SD	RSD
	6.21	5	11.18	4.97	99.44		
	6.19	5	11.15	4.96	99.25		
6.25	6.18	5	11.16	4.97	99.54	0.15	**0.15**
	6.21	6.25	12.38	6.16	98.59		
	6.16	6.25	12.32	6.16	98.61		
6.25	6.19	6.25	12.33	6.14	98.31	0.17	**0.17**
	6.19	7.5	13.57	7.38	98.40		
	6.19	7.5	13.59	7.39	98.64		
6.25	**6.22**	**7.5**	**13.58**	**7.36**	**98.18**	0.23	**0.23**

For Olmesartan medoxomil

10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 220 nm. The amount of Olmesartan medoxomil was calculated at each level and % recoveries were computed.

Table 5: Recovery data for Olmesartan medoxomil

Conc µg/ml	Conc before spiking µg/ml	Ref std added µg/ml	Conc after spiking µg/ml	Recovered Conc µg/ml	% Recovery	SD	RSD
	9.92	8	17.91	7.98	99.87		
	9.95	8	17.85	7.89	98.73		
10	9.90	8	17.80	7.89	98.65	0.68	**0.681**
	9.96	10	19.84	9.87	98.78		
	9.87	10	19.88	10.01	100.11		

10	9.88	10	19.83	9.95	99.57	0.67	**0.675**
	9.95	12	21.71	11.76	98.03		
	9.92	12	21.77	11.85	98.76		
10	**9.90**	**12**	**21.77**	**11.87**	**98.96**	0.49	**0.497**

System Suitability

Various system suitability parameter were calculated. The parameter were found within acceptance criteria (table: 6).

Table: 6 System suitability parameter

Parameter	Chlorthalidone	Olmesartan medoxomil	Acceptance criteria
Theoretical plate*	3103	5277	**>2000**
Retention time(min.)*	2.98	1.89	**-**
Tailing factor*	1.13	1.10	**<1.5**
resolution	**7.2**		**>2.0**

***Mean (n=3)**

Quantitative determination of pharmaceutical formulation:

When dosage form was analyzed, Chlorthalidone and Olmesartan medoxomil gave sharp and well defined peak at retention time 2.98 min. and 1.89 min. respectively, when scanned at 220nm. Assay result of marketed formulation is shown in (table: 9).

The assay results were comparable to labelled value (12.5mg Chlorthalidone and 20 mg Olmesartan medoxomil) of each drug in combined dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

Table 7: Quantitative determination of pharmaceutical formulation

Olmin 20-CH Tablet	Label claim mg	Concentration estimated µg/ml			% Assay ± SD
		A	B	C	
Chlorthalidone	12.5	12.37	12.29	12.46	**99.03±0.68**
Olmesartan Medoxomil	**20**	**19.94**	**19.72**	**19.88**	**99.26±0.58**

CONCLUSION

Development and validation of RP-HPLC method was found to be simple, accurate, precise and economical. This method can be applied for routine quantitative analysis of Chlorthalidone and Olmesartan Medoxomil in combined pharmaceutical dosage forms.

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