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A New RP-HPLC Method for the Assay of Chlorthalidone and Olmesartan in Combined Dosage Forms

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ABSTRACT

A sensitive and reproducible RP-HPLC method was described for the quantitative determination of chlorthalidone and olmesartan in combined dosage form. This method was based on high performance liquid chromatographic (HPLC) separation of chlorthalidone and olmesartan with the use of a reversed phase HPLC column [Hypersil BDS, C18, 100 x 4.6 mm, 5µ] at mobile phase consisting of phosphate buffer (pH-3.3) and acetonitrile in the ratio of 55:45 v/v ambient temperature. The flow rate of the mobile phase was adjusted to 1.0mL/min and the injection volume was 10µL. Detection was performed by photodiode array detector at a wavelength of 210nm and the chromatographic runtime was 6 minutes for the analysis. The reliability and analytical performance of the proposed method, including linearity, range, precision, accuracy, detection and quantitation limits, were statistically validated. The proposed method can be useful in the quality control of combination drug products

Keywords: Chlorthalidone, Olmesartan, ICH guidelines.

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INTRODUCTION

Chlorthalidone[Figure 1]. [1, 2] 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl) benzenesulfonamide is used to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems It is also used to reduce extra salt and water in the body caused by conditions such as heart failure, liver disease, and kidney disease. Chlortalidone increases the excretion of sodium, chloride, and water into the renal lumen by inhibiting sodium ion transport across the renal tubular epithelium. By increasing the delivery of sodium to the distal renal tubule, chlortalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism (i.e. apical ROMK/Na channels coupled with basolateral NKATPases). Olmesartan [Figure 2][3,4], 3-dihydroxy-2-butenyl 4(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole5-carboxylate, cyclic 2,3-carbonate belongs to a class of drugs called angiotensin receptor blockers used to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. It blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle there by relaxing blood vessels so that blood can flow more easily. These two drugs are being used either alone or in combination for the treatment of hypertension. Literature survey revealed that several papers [6-20] have been reported for have been reported for estimation of the above selected drugs in single or in combination forms. However, only one stability indicating method[21] has been yet reported for the simultaneous determination of chlorthalidone and olmesartan in combined dosage form. In the present paper an attempt have been made to develop a RP-HPLC method for assay of chlorthalidone and olmesartan in combined dosage form and was validated following ICH guidelines.

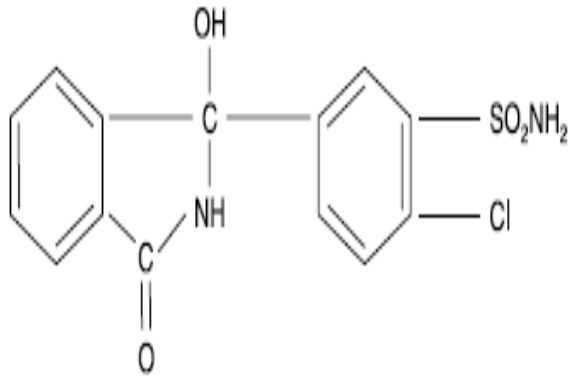

Figure 1: Structure of Chlorthalidone

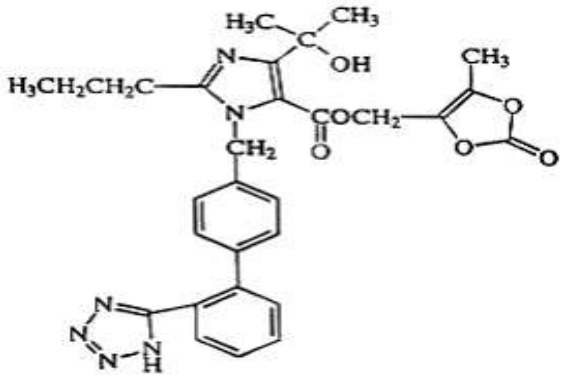

Figure 2: Structure of Olmesartan

MATERIALS AND METHOD

Instrumentation:

The chromatographic system used to perform development and validation of this assay method was Water's 2695 HPLC system provided with Hamilton Syringe, auto sampler and 2996 Photodiode array detector. The HPLC system was equipped with a column compartment with temperature control and an on-line degasser. Data acquisition, analysis and reporting were performed by Waters Empower 2 software. A reverse phase HPLC column [Hypersil BDS, C18, 100 x 4.6 mm, 5µ] was used in the present assay. Pharmaceutically pure samples of chlorthalidone and olmesartan were obtained as gift samples from Spectrum Pharma Research Solutions, Hyderabad along with their analytical reports The commercial tablets, OLMEZEST-CH 40 mg [label claim 40mg of Olmesartan and 12.5mg of Chlorthalidone] were procured from the local pharmacy. Potassium di-hydrogen ortho-phosphate, HPLC grade Water, Acetonitrile and Methanol were obtained from Ranchem.

Buffer Preparation (pH-3.3):

Accurately weighed and transferred 2.72gm of Potassium dihydrogen Orthophosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added add 1ml of triethylamine and degass to sonicate and finally make up the volume with water, then pH adjusted to 3.3 with dil. ortho phosphoric acid solution.

Mobile Phase Preparation:

Isocratic mobile phase consisting of phosphate buffer (pH-3.3) and acetonitrile in the ratio of 55:45 v/v was used in the present assay. Prior to use this mobile phase was filtered through a 0.45µm membrane filter (Millipore Pvt. Ltd. Bangalore, India) and degassed using an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai).

Diluent Preparation:

Mobile phase was used as a diluent.

Standard Preparation:

Weigh accurately 40mg of Olmesartan and 12.5mg of Chlorthalidone working Standards and transfer into a 10 ml clean dry volumetric flask. Add 7ml of diluent and sonicate for 30 minutes to aid dissolution and make up to the final volume. Aliquots of this stock solution were pipetted and transferred into a series of 10ml volumetric flask separately and volume in each flask was made up to the mark with the same diluent to obtain working standard solutions of concentrations 12.5µg/ml, 31.25µg/ml, 43.75µg/ml, 62.5µg/ml, 75µg/ml and 93.75µg/ml for

chlorthalidone and 40µg/ml, 100µg/ml, 140µg/ml, 200µg/ml, 240µg/ml and 300µg/ml for olmesartan respectively.

Preparation of Formulated Sample:

Commercially available tablets of chlorthalidone and olmesartan with the brand name OLMEZEST-CH 40 mg [label claim of 12.5mg of Chlorthalidone and 40mg of Olmesartan] were procured from the local pharmacy and the average weight was determined. From this formulation, 10 tablets were weighed and grinded to fine powder. 100mg of this fine sample powder was transferred into a 100mL volumetric flask. To this 50mL of diluent was added and sonicated for 30 minutes with intermittent shaking Then, content was brought back to room temperature and make up to the final volume with same diluent. Later, this solution was filtered through 0.45µm membrane filter. Transfer the aliquots of this filtered sample solution into a series of 10ml volumetric flasks and make up to mark with the diluent to obtain concentrations 12.5-93.75µg/mL of chlorthalidone and 40-300µg/ml for olmesartan respectively.

RESULTS AND DISCUSSION

Development and Optimization of the HPLC Method

Chromatographic parameters were optimized based on the chemical nature of the Chlorthalidone and olmesartan. Initially chromatographic separation of chlorthalidone and olmesartan was tried using various ratios of acetonitrile and water as mobile phase, in which both the drugs did not respond properly, and the resolution was also poor. Then water was replaced with phosphate buffer the acidic pH range from 3.0 to 5.0 along with acetonitrile resulted in elution of the two cited drugs. Among the pH ranges from 3.0 to 5.0, the mobile phase with pH-3.3 showed better resolution and sharp peak shapes for both the drugs. Hence phosphate buffer (pH-3.3) was finalized as one of the component in the mobile phase. [Buffer and acetonitrile in the ratio of 50:50 v/v, 55:45 v/v and 65:40 v/v] were carried to obtain best separation for chlorthalidone and olmesartan. From these studies it was inferred that phosphate buffer: Acetonitrile (55:45v/v) was selected as a mobile phase, at a flow rate of 1.0ml/min. The stationary phase was Hypersil BDS C-18 column (100mm×4.6mm, 5µ). At the same time wavelength detection studies were also made for chlorthalidone and olmesartan at which these two drugs showed maximum absorption at the wave length of 210nm and this was selected as the detection wave length in the present assay. The retention times were found to be 2.7±0.5 min and 5.0±0.5 min for chlorthalidone and olmesartan respectively. The column selection in the present assay has been done on the basis of back pressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the

retention time and resolution between chlorthalidone and olmesartan peaks. After evaluating all these factors, Hypersil BDS C-18 column (250mm×4.6mm, 5µ) was found to be suitable as it gave satisfactory results. The chromatogram of trails carried out by the author for simultaneous estimation of chlorthalidone and olmesartan by using the aforementioned mobile phase from 10µL of standard and sample solutions are represented in Figure 5 and the results of the developed method are presented in Table.1.

Chromatographic Conditions:

The chromatographic analysis was performed by using the HPLC column [Hypersil BDS, C18, 100 x 4.6 mm, 5µ] at ambient temperature. The flow rate of the mobile phase was adjusted to 1.0mL/min and the injection volume was fixed at 10µL Detection was performed by photodiode array detector at a wavelength of 210nm and the chromatographic runtime was 6 minutes for the present analysis.

Method Validation:

The developed HPLC method for the simultaneous determination of chlorthalidone and olmesartan was validated as per the ICH guidelines.

System Suitability:

The system suitability parameters established for the developed method include number of Theoretical plates (efficiency), Resolution and Tailing factor. The HPLC system was equilibrated using the initial mobile phase composition, followed by six injections of the standard solution of 100% concentration containing 7.5µg/mL chlorthalidone and 30µg/mL of olmesartan respectively. These six consecutive injections were used to evaluate the system suitability on each day of method validation. The results were given in the Table.1.

Table 1: System Suitability of Chlorthalidone and Olmesartan

Parameters	OMS	CLT
No. of theoretical plates	5252	6411
Tailing factor	1.22	1.02

Blank and Placebo Interference:

For this study the diluent and placebo solutions were injected as per defined chromatographic conditions. The Chromatograms of blank and placebo solutions showed no peaks at the retention time of chlorthalidone and olmesartan which indicates that there is no interference at the retention time of both the drugs (Figure 3& Figure 4).

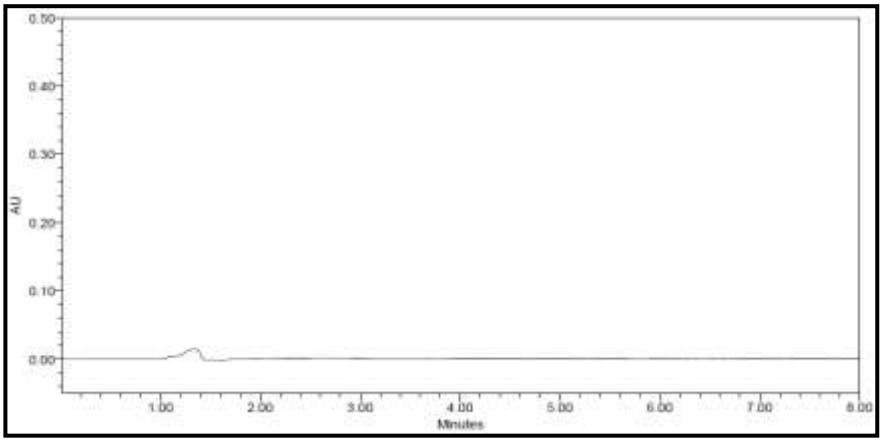

Figure 3: Blank chromatogram

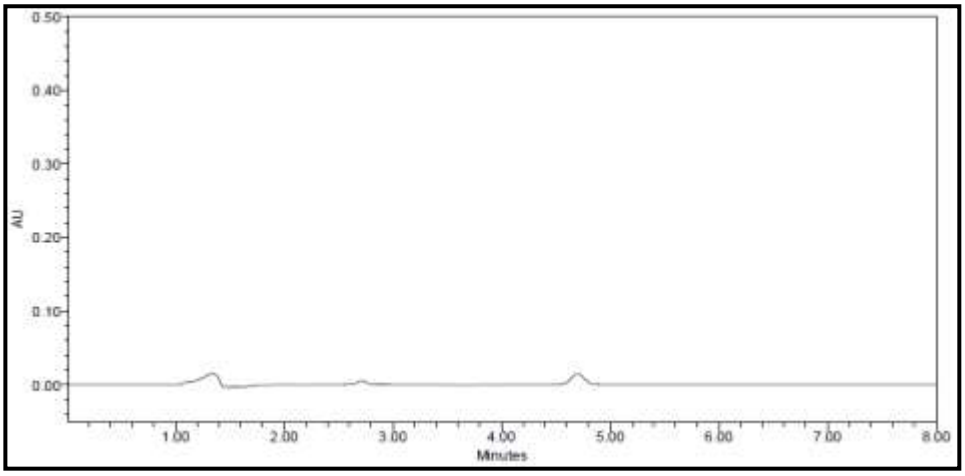

Figure 4: Placebo chromatogram

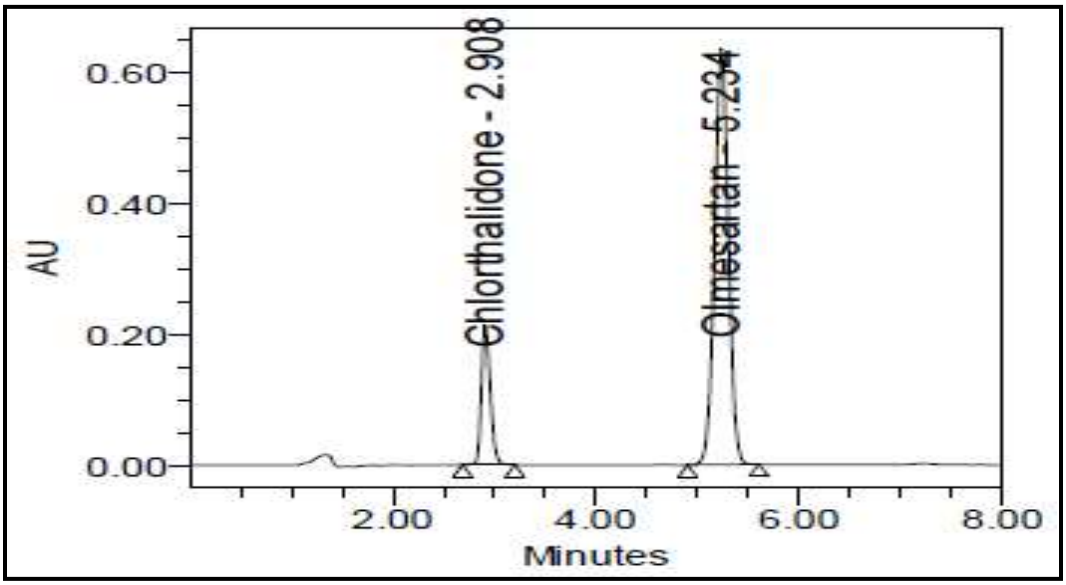

Figure 5: System suitability chromatogram of Chlorthalidone and olmesartan

Linearity:

The linearity was evaluated at seven calibration levels with concentration range from 12.5-93.75µg/mL for chlorthalidone and 40-300µg/ml for olmesartan respectively. The linear regression equations for Chlorthalidone and olmesartan were y = 20946x + 26149 and y = 31175x + 6852.8 with correlation coefficient of 0.9997and 0.9998 respectively. The regression results of chlorthalidone and olmesartan are reported in Table.2 and 3. From the obtained data it was confirmed that an excellent correlation exists between response factor and concentration of cited drugs within the concentration range. The LOD and LOQ values were found to be 2.9218µg/mL and 9.737µg/mL for Chlorthalidone, 2.809µg/mL and 9.36µg/mL for olmesartan respectively.

Table 2: Results of Linearity of Chlorthalidone

PPM	Set-1	Set-2	Set-3	AVERAGE
12.5	262410	262784	262307	262500
31.25	648239	648426	648306	648324
43.75	925524	925731	925525	925593
62.5	1324115	1322199	1324016	1323443
75	1588946	1589196	1583757	1587300
93.75	1947884	1948274	1946533	1947564
Slope, b		20946		
Intercept, a		2614		
Correlation, r^2		0.9997		

Table 3: Results of Linearity of Olmesartan

PPM	Set-1	Set-2	Set-3	AVERAGE
40	1196643	1190437	1197344	1194808
100	3158279	3163280	3165313	3162291
140	4429101	4418017	4438037	4428385
200	6284942	6288932	6292021	6288632
240	7427395	7431383	7410398	7423059
300	9348221	9353528	9347383	9349711
Slope, b		31175		
Intercept, a		6852.8		
Correlation, r^2		0.9998		

Precision:

The precision of the developed method was evaluated by carrying out inter-day and intraday analysis. Inter- day analysis was carried out for two consecutive days and the precision of the method was assessed by six replicate injections of 100% test concentration. The precision was expressed in terms of standard deviation and %RSD. The results were given in Table 4. The developed method was found to be precise for chlorthalidone and olmesartan as the %RSD

values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

Table 4: Precision Table of Chlorthalidone and Olmesartan

Validation Parameter	%Mean		SD		%RSD	
	OMS	CLT	OMS	CLT	OMS	CLT
Repeatability	99.96	99.55	0.5817	0.6127	0.58	0.62
Day-Day	99.99999	100	0.142922	0.150064	0.142922	0.150064

Accuracy:

In the present study the accuracy was checked by using standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution at three different concentration levels (50%, 100% and 150%) within the linearity range in triplicate. The results of recovery % were given in Table.5.

Table 5: Accuracy Table for Olmesartan and Chorthalidone

	Spiked amount (ppm)		Standard drug solution (ppm)		% Recovered	
	OMS	CLT	OMS	CLT	OMS	CLT
50%	100	31.25	200	62.5	99.75	100.39
	100	31.25	200	62.5	99.53	99.02
	100	31.25	200	62.5	99.99	99.83
100%	200	62.5	200	62.5	99.11	100.34
	200	62.5	200	62.5	99.38	99.53
	200	62.5	200	62.5	100.10	99.13
150%	300	93.75	200	62.5	99.44	100.97
	300	93.75	200	62.5	99.98	100.90
	300	93.75	200	62.5	99.40	100.11
MEAN					99.63	100.02
SD					0.3406	0.70
%RSD					0.34	0.70

Robustness:

A variation in the ratio of mobile phase by ±10%, column temperature ±5°c and the flow rate ±0.1mL were adopted to study the robustness of the developed method. The results were tabulated in Table.6. The data shows no significant changes in the chromatography pattern when the above modifications were made in the experimental conditions, showing that the method is robust.

Table 6: Robustness Data of Chlorthalidone and Olmesartan

	Changed value	Retention time		Tailing factor		% assay	
		OMS	CLT	OMS	CLT	OMS	CLT
Column Temperature	25	4.6	2.6	1.02	1.26	99.2	98.9
	35	4.4	2.6	1.02	1.25	98.6	99.5
Flow Rate	0.9	5.1	2.8	1.02	1.24	100.2	100.6
	1.1	4.4	2.4	1.01	1.23	100.5	101
Mobile Phase Composition	45:65	4.3	2.5	1.04	1.24	101.8	100.9
	65:45	5.3	2.9	1.01	1.25	100.6	100.9
Mean						100.15	100.3
Std						1.127	0.883
RSD						1.125	0.88

Assay in Marketed Formulations:

This proposed method was applied to the determination of chlorthalidone and olmesartan in commercially available OLMEZEST-CH 40 mg tablets [label claim of 12.5mg of Chlorthalidone and 40mg of Olmesartan]. The results of the assays (n = 6) yielded 99.55% (%RSD = 0.62%) and 99.96% (%RSD = 0.58%) for chlorthalidone and olmesartan respectively revealing that the developed RP-HPLC method is selective for the analysis of both chlorthalidone and olmesartan without interference from the excipients (Table.8).

Table 7: Stability Data of Chlorthalidone and Olmesartan

Drug	% Assay at 0 hr	% Assay at 24 hr
OMS	99.4	98.2
CLT	99.8	98.3

Table 8: Assay of the Formulation

Sample No.	Peak area		% Assay	
	OMS	CLT	OMS	CLT
1	6063120	1318696	100.02	99.84
2	6019762	1325985	99.30	100.39
3	6082743	1302750	100.34	98.63
4	6107522	1308635	100.75	99.08
5	6017517	1316054	99.26	99.64
6	6063661	1316337	100.03	99.66
Average			99.96	99.55
STDEV			0.5817	0.6127
%RSD			0.58	0.62

CONCLUSION

The present paper describes the development and validation of a simple, accurate and precise RP-HPLC method for the assay of chlorthalidone and olmesartan in combined dosage forms. Chlorthalidone and olmesartan were estimated on Hypersil BDS C-18 column (250mm×4.6mm,

5µ) column using buffer: Acetonitrile (55:45v/v) as mobile phase and detection was carried out at 210nm. The method for chlorthalidone and olmesartan exhibited linear response in stated range and is accurate and precise. The obtained percentage of recovery in tablet forms indicates the accuracy of the method and absence of interference of the excipients present in the formulation. The %RSD was also less than 2.0% showing high degree of precision of the proposed method. The proposed method can be used as alternative method for routine analysis of chlorthalidone and olmesartan respectively.

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