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STABILITY INDICATING RP-HPLC-PDA METHOD FOR SIMULTAMEOUS QUANTIFICATION OF OLMESARTAN, CILINIDIPINE AND CHLORTHALIDONE TABLETS

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ABSTRACT

A simple, fast, accurate and specific RP-HPLC-PDA method has been developed for the simultaneous quantification of Olmesartan, Cilinidipine and Chlorthalidone in bulk and tablet dosage form. The chromatographic separation was performed on a reverse phase Azilent C18 Column (150×4.6mm, 5µm particle size) consisting mobile phase of Buffer and Acetonitrie (60:40 v/v), with a flow rate 1ml/min, temperature 30˚C and UV detection wavelength 260nm. The retention times of Olmesartan, Cilinidipine and Chlorthalidone were observed as 2.165 min, 3.504min and 4.159 min respectively. The developed method was validated by validation parameters such as linearity, range, accuracy**,** precision and robustness. The results obtained for validation parameters are within the limits as per ICH guidelines. The linearity of the drugs was obtained in the range of 5ppm-30ppm for Olmesartan, 2.5ppm-15ppm Cilinidipine and 3.125ppm-18.75ppm for Chlorthalidone. %RSD from precision studies were 0.2, 0.4 and 0.3, mean percentage recovery from accuracy studies were found to be 99.01%, 99.42% and 99.18% for Olmesartan, Cilinidipine and Chlorthalidone, respectively. LOD, LOQ values obtained from regression equations of Olmesartan, Cilinidipine and Chlorthalidone were 0.02ppm, 0.07ppm, 0.13ppm and 0.38ppm, 0.04ppm, 0.13 ppm respectively. The method designed and validated can be successfully used for the regular quantification of Olmesartan, Cilinidipine and Chlorthalidone in tablet and bulk forms.

INTRODUCTION

Olmesartan (OLM) (Fig.1) is chemically (4-(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. Molecular formula of Olmesartan is $C_{24}H_{26}N_6O_3$. Molecular formula is 446.5 g/mol. Olmesartan is commonly used for the treatment of hypertension[1], it selectively inhibits the binding of angiotensin II to AT1 receptor, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. There are few methods reported for the estimation of Olmesartan

with other drugs by HPLC[2-3]. Cilnidipine (CIL) (Fig.2) is a calcium channel blocker. Cilnidipine is the novel calcium antagonist accompanied with L-type and N-type calcium channel blocking function with molecular formula $C_{27}H_{28}N_2O_7$ and molecular weight 492.5 g/mol[4]. There are many methods for the estimation of Cilnidipine with other drugs by HPLC[5-7]. Chlorthalidone (CHL) (Fig.3) is chemically 2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide. Molecular formula and molecular weight of Chlorthalidone are $C_{14}H_{11}ClN_2O_4S$ and 338.7 g/mol respectively. Chlorthalidone is thiazide diuretic. It acts by inhibiting sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle[8]. There are few methods reported for the estimation of Chlorthalidone with other drugs by HPLC[9-10]. Literature review reveals that only one method is developed so far for the simultaneous quantification of Olmesartan, Cilinidipine and Chlorthalidone in triple drug combination[11]. Hence the authors developed a simple, fast, precise, accurate, robust and stability indicating method for simultaneous assay of Olmesartan, Cilinidipine and Chlorthalidone, in tablet dosage form.

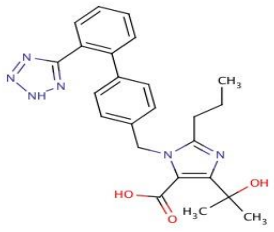

Fig.1. Structure of Olmesartan

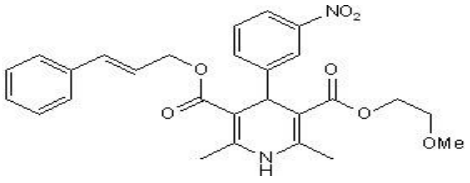

Fig.2. Sructure of Cilnidipine

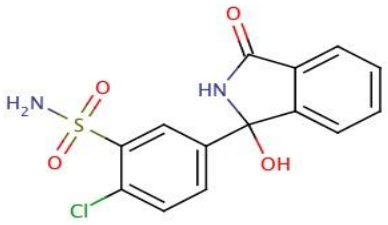

Fig.3. Sructure of Chlorthalidone

Experimental

Chemicals and reagents

Olmesartan, Cilinidipine and Chlorthalidone standards were obtained from Spectrum Pharma research solutions, Hyderabad. Olmesartan-20mg, Cilinidipine-10mg and Chlorthalidone-12.5mg, with brand name **OLKEM TRIP** manufactured by Alkem laboratories, Mumbai, India was purchased from local pharmacy. Acetonitrile, methanol, were procured from Thermo Fischer Scientific India Pvt. Ltd. Milli-Q water was used throughout the analysis for buffer preparation. WATERS HPLC system with Empower 2 software was used for chromatographic separation

Chromatographic Conditions: Chromatographic separation was carried out by emloying Agilent C18 Column(150mm×4.6µm particle size) using 0.1% OPA buffer and acetonitrile in the ratio 60:40%v/v as mobile phase. The flow rate was adjusted to 1ml/min with run time 8min. The coloumn temperature was maintained at 30˚C. The column effluents were detected at isobestic point 260nm and injection volume was 10µL.

Procedure for preparation of 0.1%OPA buffer solution: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water, filtered through 0.45µ membrane filter.

Procedure for mobile phase preparation: 600ml of **0.1%OPA** buffer solution and 400ml of acetonitrile mixture

was used as mobile phase. The mobile phase was filtered through 0.45µ membrane filter and sonicated by means of ultrasonication to remove dissolved gases.

Procedure for preparation of diluents: Water and methanol mixture in the ratio 50:50v/v was used as diluent for sample and standard solution preparation.

Standard solution preparation: Accurately weighed 5mg of Olmesartan, 2.5 mg of Cilinidipine and 3.125mg of Chlorthalidone and transferred to three 25ml volumetric flasks separately. 10ml of Diluent was added to flasks and sonicated for 20mins. The solutions were made up with water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3. 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and final volume made up with diluent. The final concentrations of Olmesartan, Cilinidipine and Chlorthalidone standard solution was found to be 20ppm, 10ppm and 12.5ppm of respectively.

Sample solution preparation: Sample solution was prepared by weighing and powdering 20 tablets. An amount equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25mL of diluent added and sonicated for 20 min, further the volume made up with diluent and filtered. 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. The final concentrations of sample solution of Olmesartan, Cilinidipine and Chlorthalidone was found to be 20ppm, 10ppm and 12.5ppm of respectively.

Method development and validation: The present method was developed as per ICH guidelines Q2(R1)[12]. Validation parameters employed for this study are selectivity, linearity, accuracy, precision, robustness, LOD and LOQ.

Specificity and Selectivity[13]: As shown in Fig.4 it was noticeable that there was no interference due to tablet excipients at the retention time of analyte peaks as well as stress degradation products

Linearity: Linearity of the analytes were determined by injecting 6 different concentrations of working standard solutions of Olmesartan (5 ppm -30 ppm), Cilinidipine (2.5 ppm -15 ppm), Chlorthalidone (3.125 ppm -18.75 ppm) into HPLC system. The calibration curves were shown in fig 5 to fig 7. Linearity data for Olmesartan, Cilinidipine and Chlorthalidone was shown in Table 1.

Accuracy: Standard addition method[14] was employed for the accuracy studies. Accuracy of the developed assay method was evaluated in triplicate at three concentration levels (50%, 150% and 150%) and the percentage recoveries were calculated. The percentage recovery was calculated and enlisted in Table 2.

Precision: System precision was established by injecting six different solutions of Olmesartan having concentration equal to 20ppm, Cilinidipine having concentration equal to 10ppm and Chlorthalidone having concentration equal to 12.5ppm. The mean and %RSD values of peak area and retention time were presented in Table-3 and Table-4.

Robustness: Robustness of the developed analytical method refers to its ability to remain unaffected due to small but deliberate variations in the method parameters (flow rate, mobile phase ratio and column temperature) which indicates reliability of the method for routine analysis. The flow rates selected for study were 0.8ml/min, 1ml/min and 1.2ml/min. The column temperature was changed from 25˚C to 30˚C and 35˚C. The sample solution was injected in to the HPLC system in triplicate and the peak areas were recorded and shown in Table-5.

RESULTS AND DISCUSSION

A new analytical method was developed and validated for simultaneous estimation of Olmesartan, Cilinidipine and Chlorthalidone, in tablet dosage form. Different coloumns were tried and the final column chosen was Agilent C18 Column (150mm×4.6µm) which gave satisfactory resolution and run time.

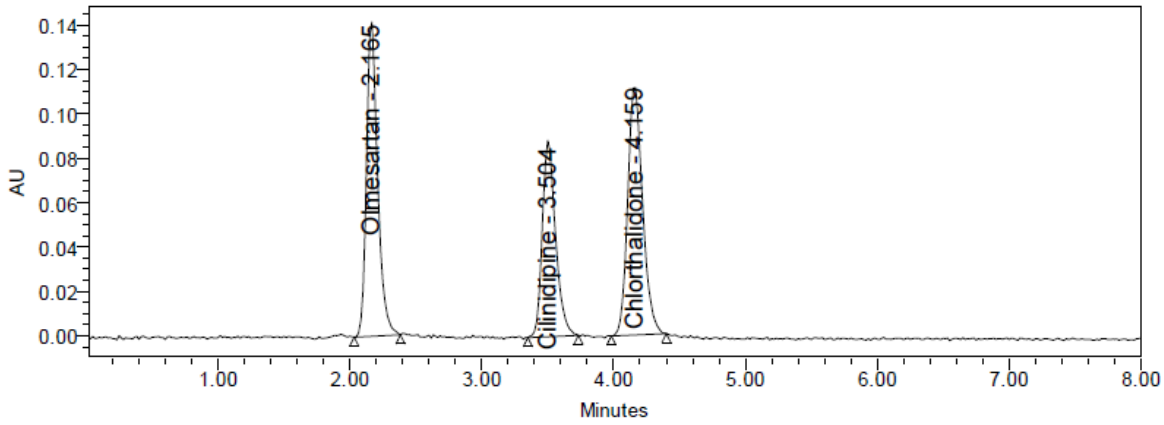

Fig.4.Standard Chromatogram of Olmesartan, Cilinidipine and Chlorthalidone

Table-1: Linearity Results for Olmesartan, Cilinidipine and Chlorthalidone

Olmesartan		Cilinidipine		Chlorthalidone	
Conc (ppm)	Peak area	Conc (ppm)	Peak area	Conc (ppm)	Peak area
5	214208	2.5	166633	3.125	244314
10	435422	5	323773	6.25	463109
15	657594	7.5	480662	9.375	716468
20	837646	10	627297	12.5	947033
25	1080598	12.5	789267	15.625	1178312
30	1292138	15	954398	18.75	1415818

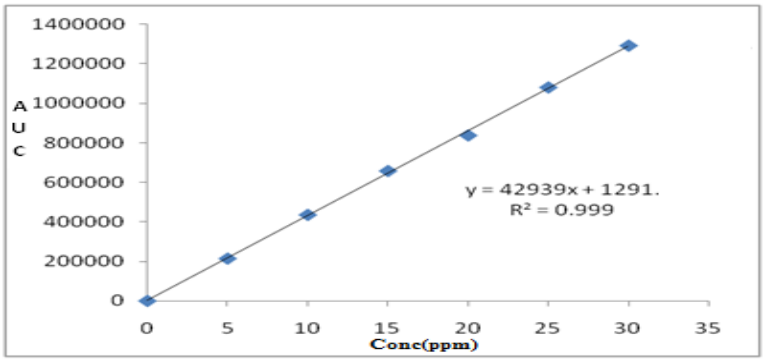

Fig.5 Linearity curve for Olmesartan

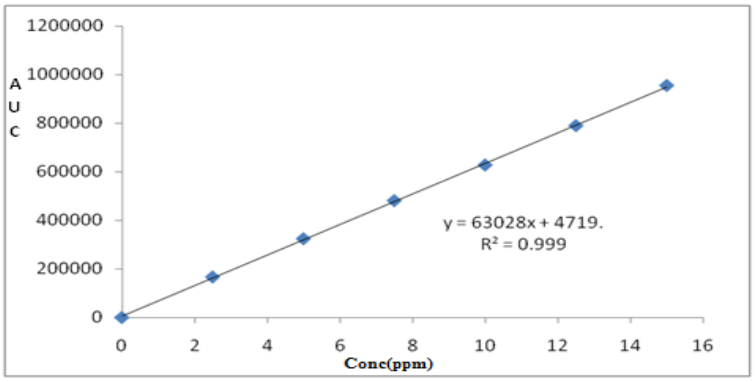

Fig.6 Linearity curve for Cilinidipine

Table 2-Accuracy results

Drugs	Spiked concentration (ppm)		Recovered concentration (ppm)	%Recovery
Olmesartan	10	50%	9.81	98.11
	20	100%	19.7	98.57
	30	150%	29.7	99.06
Cilinidipine	5	50%	5.00	100.01
	10	100%	9.97	99.78
	15	150%	14.8	98.88
Chlorthalidone	6.25	50%	6.18	98.93
	12.5	100%	12.3	98.56
	18.75	150%	18.5	98.94

Table.3: System precision values for OLM, CIL and CHL standard solutions

S.No	Average area*			Rt(min)*		
	OLM	CIL	CHL	OLM	CIL	CHL
1	836071	627909	942204	2.154	3.494	4.15
2	835061	624112	948665	2.159	3.503	4.158
3	831468	628450	947286	2.165	3.504	4.159
4	834705	626366	945417	2.17	3.515	4.171
5	831441	625325	948005	2.17	3.516	4.172
6	833526	621050	941256	2.171	3.51	4.174
Mean	833712	625535	945472	2	3.5	4
SD	1928.6	2720.6	3110.0	0.0	0.0	0.0
% RSD	0.2	0.4	0.3	0.3	0.2	0.2

***(n=6)**

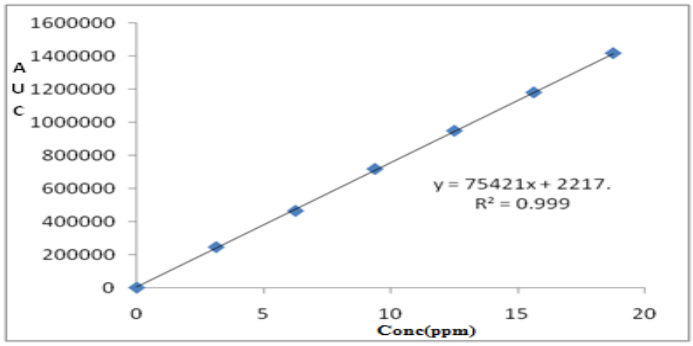

Fig 7: Linearity curve for Chlorthalidone

Table.4: Method precision values for OLM, CIL and CHL tablet sample solution

S.No	Average area*			Rt(min)*		
	OLM	CIL	CHL	OLM	CIL	CHL
1	830448	621933	944831	2.154	3.494	4.146
2	830713	626052	941354	2.154	3.499	4.153
3	833828	624919	945197	2.156	3.501	4.156
4	834469	622416	943607	2.158	3.503	4.158
5	830241	620675	940653	2.159	3.505	4.164
6	832144	623247	944962	2.162	3.506	4.166
Mean	831974	623207	943434	2.157	3.501	4.157
SD	1822.8	1984.3	1974.1	0.00	0.00	0.01
% RSD	0.2	0.3	0.2	0.1	0.1	0.2

***(n=6)**

Table-5: Results of robustness study

Chromatographic conditions	Rt(min)			Average area		
	OLM	CIL	CHL	OLM	CIL	CHL
Buffer: Acetonitrile 70:30(v/v)	2.181	3.546	4.235	868720	645475	954670
Buffer: Acetonitrile 60:40(v/v)	2.165	3.504	4.159	833712	625535	945472
Buffer: Acetonitrile 65:35(v/v)	2.182	3.558	4.248	877380	645476	944561
Flow rate (0.8 mL/min)	2.154	3.501	4.155	877120	650276	952658
Flow rate (1.0 mL/min)	2.165	3.504	4.159	833712	625535	945472
Flow rate (1.2 mL/min)	2.159	3.517	4.181	878076	649812	955148
Temperature 25°C	2.186	3.574	4.264	845101	620036	913646
Temperature 30˚C	2.165	3.504	4.159	833712	625535	945472
Temperature 35°C	2.183	3.562	4.255	848768	616834	926382

Table-6: Results of forced degradation study

Stress conditions	% Assay of active ingredients					
	OLM	% Degradation	CIL	% Degradation	CHL	% Degradation
Acid, 2N HCl,	95.35	4.65	95.44	4.56	95.03	4.97
Base, 2N NaOH	97	3.00	97.12	2.88	97.1	2.90
H_2O_2 (20%,v/v)	98.13	1.87	98.12	1.79	98.03	1.97
Dry heat (105 ºC)	99.32	0.68	99.04	0.96	99.07	0.93
UV	99.32	0.68	99.30	0.70	99.06	0.94
Water	99.21	0.79	99.44	0.56	99.11	0.89

Many mobile phase ratios were tried to resolve the three chromatographic peaks such as methanol water but broadness of the peaks were observed with methanol and water as mobile phase. To improve the sharpness of the peaks mobile phase was made slightly acidic, and produced symmetric peaks. The retention times of the Olmesartan, Cilinidipine and Chlorthalidone were 2.165min, 3.504min and 4.159min respectively (Fig 4). The best fit for the calibration curve could be achieved by separate linear regression equations, which were y = 42939.x + 1291 (OLM), y = 63028x +4719 (CIL), y = 75421.x + 2217 (CHL). The percentage recoveries of Olmesartan, Cilinidipine and Chlorthalidone were 99.01%, 99.42% and 99.18% respectively. LOD, LOQ values obtained from regression equations of Olmesartan, Cilinidipine and Chlorthalidone were 0.02ppm, 0.07ppm, 0.13ppm and 0.38ppm, 0.04ppm, 0.13 ppm respectively. The analytical method developed was validated according to ICH Q2R1 guidelines. Validation parameters according to ICH Q2R1 guidelines were Specificity, linearity, precision, accuracy, robustness, limit of detection and limit of quantification.

Forced degradation studies: Olmesartan, Cilinidipine and Chlorthalidone sample solution was subjected for stress degradation studies as per ICH guidelines entitled stability testing of new drug substances and products to elucidate the internal stability of the main analytes[15]. Standard test procedures were followed for the stress conditions like acid, base, peroxide, heat, water and UV light[16-17]. The three drugs were degraded in the selected stress conditions and showed 1.9-6% degradation in acidic, alkaline and peroxide conditions. Slight degradation was observed in heat, UV light and water degradation (Table 6). Though degradants peaks were observed in the chromatograms, no de degradants peas were interfering the retention times of Olmesartan, Cilinidipine and Chlorthalidone peaks, hence the method was proved to be stability indicating.

CONCLUSION

The developed RP-HPLC-PDA method was found to be suitable for the analysis of Olmesartan, Cilinidipine and Chlorthalidone in tablet dosage form and was found to be simple , reliable, economical and precise. Therefore, this RP-HPLC method for estimation of Olmesartan, Cilinidipine and Chlorthalidone can be used in various laboratories for its quantitative determination in bulk and pharmaceutical tablet dosage form.

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