REVIEW ARTICLE

Development and Validation of Stability-indicating Method for the Estimation of Cilnidipine, Olmesartan Medoxomil and Chlorthalidone by Reverse Phase High Performance Liquid Chromatography

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ABSTRACT

A new combination of Cilnidipine, Olmesartan medoxomil, and Chlorthalidoneis used to treat high blood pressure (hypertension), which helps in preventing strokes, heart attacks, and kidney problems. Reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the simultaneous estimation of CIL, OLM, and CHLin the pharmaceutical dosage form. Chromatography was carried out using Agilent C18 150x 4.6, 5.0 µm column with a flow rate of 0.9 mL/min. The mobile phase consisted of pH 3.0, 0.01 N Potassium dihydrogen phosphate buffer, and Acetonitrile in the ratio of 60:40. The retention times of CIL, OLM, and CHL were found to be 2.056, 2.434 and 2.926 minutes, respectively. The method obeys Beer's law in the concentration range of 2.5–15 µg/mL (R^2 = 0.999) for CIL, 10–60 µg/mL (R^2 = 0.999) for OLM, and 3.125–18.75 µg/mL (R^2 = 0.999) for CHL. The Limit of Detection (LoD) and Limit of Quantitation (LoQ) were found to be 0.7 µg/mL and 2.21 µg/mL for CIL, 0.84 µg/mL and 2.53 µg/mL for OLM and 0.9 µg/mL and 2.72 µg/mL for CHL, respectively. The method's accuracy was assessed by a recovery study in the dosage form at three concentration levels. The mean % recovery obtained was100.02% for CIL, 99.67% for OLM, and 104.25% for CHL. The content of CIL, OLM, and CHLper tablet was calculated. The method developed has been statistically validated according to ICH guidelines. The method showed good reproducibility and recovery with % RSD less than 2. Forced degradation studies established the stability-indicating capability of the method under stress conditions like acid, base, peroxide, UV, thermal, humidity. Hence, the chromatographic method developed for the estimation was rapid, simple, specific, sensitive, precise, accurate, robust, and reliable that can be effectively applied for routine analysis in research institutions and quality control departments in industries.

Keywords: Cilnidipine, Chlorthalidone, Method development, Olmesartan medoxomil, RP-HPLC, Stability indicating, Validation.

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INTRODUCTION

Olmesartan Medoxomil is an angiotensin receptor blocker (ARB) and Cilnidipine is a calcium channel blocker (CCB). Chlorthalidone is a diuretic,eliminatessuperfluous water content and electrolytes from the body by urine.

Commonly observed side effects of Olmesartan Medoxomil, Cilnidipine, Chlorthalidone combination are ankle swelling, headache, dizziness, tiredness, palpitations, sleepiness, increased blood uric acid, glucose intolerance, taste change, upset stomach.

Cilnidipine shows its effect on the L-type calcium channels of blood vessels by blocking the incoming calcium, suppressing blood vessels' contraction, and ultimately reducing the elevated blood pressure.[1]

Olmesartan medoxomil is an appropriate initial treatment for high blood pressure. It is a selective angiotensin II-type I receptor blocker. The blockage of olmesartan is done by the displacement of angiotensin II converting it hence, into a competitive antagonist.[2] The activity of olmesartan is mainly performed in vascular smooth muscle cells; hence, its activity prevents the vasoconstrictor effects of angiotensin II.

Chlorthalidone is a thiazide-like diuretic used to treat hypertension and manageedema caused by heart failure or renal impairment.[3] Chlorthalidone prevents reabsorption of sodium and chloride through inhibition of the Na+/Cl- symporter in the cortical diluting segment of the ascending limb of the loop of Henle. Reduction of sodium reabsorption subsequently reduces extracellular

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fluid and plasma volume via an osmotic, sodium-driven diuresis.[4] Olmesartan is an angiotensin receptor blocker (ARB), and Cilnidipine is a calcium channel blocker (CCB). Chlorthalidone is a thiazidediuretic used to manageedema caused by conditions such as heart failure or renal impairment. These tranquil and facilitatethe broadening of the blood vessels, thereby decreasing the elevated blood pressure. It also favors relaxing the blood vessels and increasing the blood flow.CDSCO approved the combination in September 2017.[5]

An extensive literature survey was conducted to find out the analytical methods reported for Olmesartan Medoxomil, Cilnidipine, Chlorthalidone.A literature survey discloses that few stability-indicating HPLC methods,[6-23] HPTLC, [24-25] Spectrophotometric methods [26-27] for the estimation of Cilnidipine, Olmesartan medoxomil, and Chlorthalidone individually and or along with drug combinations in pharmaceutical preparations.This study aims to develop and validate a stability-indicating, LC-MS compatible method with less runtime, which would separate and quantify a combination of CIL, OLM, and CHL in a single run. The developed LC-MS compatible method was validated as per ICH guidelines [28-29] and can be applied lucratively to quality control purposes.

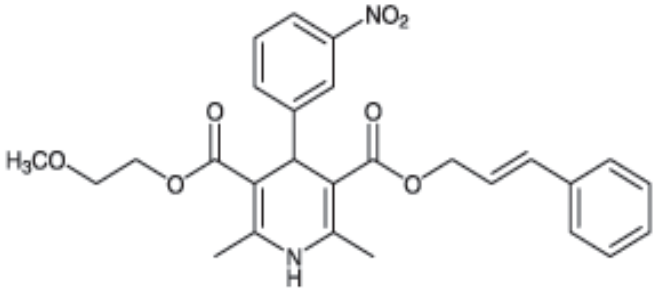

Figure 1: Structure of Cilnidipine

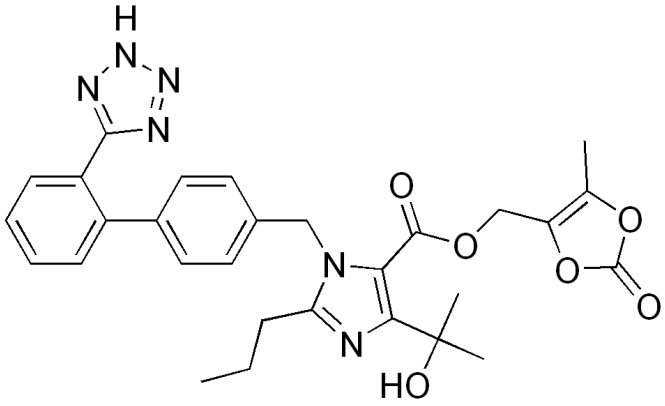

Figure 2: Structure of Olmesartan medoxomil

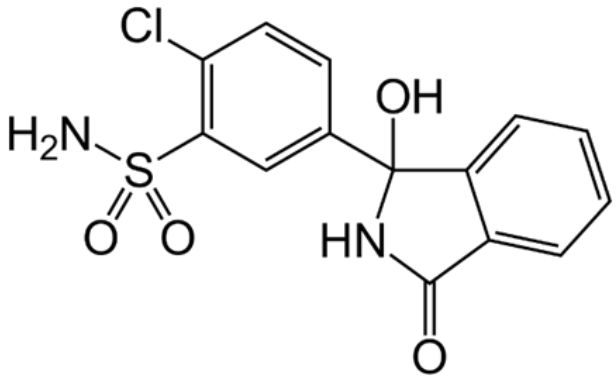

Figure 3: Structure of Chlorthalidone

MATERIALS AND METHODS

Equipment

The method development and validation was carried out using Waters Alliance-HPLC system equipped with 2695-separation module coupled to 2996-photo diode array detector and the data was acquired by Empower® version 2. The other equipment used were Mettler Toledo ME 204 weighing balance, Magnetic stirrer, Kemi Hot air oven, Eutech 700 pH meter, Double distillation apparatus.

Chemicals and Reagents

Cilnidipine, Olmesartan medoxomil, Chlorthalidone working standards, and Omten-Trio 40 tablets were kindly given as gift samples by Spectrum Pharma Limited, Hyderabad. HPLC grade solvents include acetonitrile, water, and methanol. Analytical grade chemicals include sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, Orthophosphoric acid, Triethylamine, and potassium dihydrogen phosphate were purchased from E. Merck Limited, Mumbai, India.

Chromatographic Conditions

HPLC analysis was carried out on Waters Alliance-HPLC system equipped with 2695-separation module coupled to 2996-photo diode array detector and the data was acquired by Empower® version 2. Separation was achieved using Agilent C18 150x 4.6, 5.0µm as a column with mobile phase 0.01 N Potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile is taken in the ratio 60:40. The samples were analyzed using 10mL injection volume, the Flow rate was maintained at 0.9mL/min with a runtime of 6 minutes, and the temperature was maintained at 30°C throughout the analysis. Detection and purity establishment of the drugs was achieved using PDA detector at 260 nm wavelength.

Preparation of Working Standard Solution

Accurately weighed and transferred 20mg of Olmesartan,5mg of Cilnidipine, and 6.25mg of Chlorthalidone working Standards into a clean, dry 50mLvolumetric flask, 5mL of diluent was added and sonicated for 10 minutes to dissolve. The volume was made up with the diluent and filtered through a 0.45 µ nylon filter to obtain a concentration of 400µg/mL Olmesartan, 100µg/mL Cilnidipine, 125µg/mL Chlorthalidone. From the above stock solution, 1 mL was pipetted out into a 10mL volumetric flask and then made up to the final volume with diluent. The obtained

final concentration of Olmesartan, Cilnidipine, Chlorthalidone were 40ppm, 10ppm, 12.5ppm, respectively.

Preparation of Sample Solution

Ten tablets were weighed, and the average weight was calculated. The tablets were crushed,and a portion of tablet powderequivalent to the weight of one tablet was accurately weighed and transferred to a clean, dry 100 mL volumetric flask.Approximately 50mL of diluent was added, and the mixture was sonicated for 15 minutes with intermittent shaking, then 20 mL of diluent was added and sonicated for another 25 minutes. The contents were allowed to reach room temperature and then diluted to the final volume with diluent,and the solution was filtered using a 0.45 µ nylon filter. The solution has a concentration of 400µg/mLof Olmesartan, 100µg/mL of Cilnidipine, 125µg/mLof Chlorthalidone 1.0 mL of the filtered solution was transferred to a 10 mL volumetric flask and made up to volume with diluent. The final concentration of Olmesartan, Cilnidipine, Chlorthalidone obtained were 40ppm, 10ppm, 12.5ppm, respectively.

METHOD VALIDATION

The developed and optimized RP-HPLC method was validated according to international conference on harmonization (ICH) guidelines Q2(R1) to determine the system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness, and robustness.

System Suitability

System suitability parameters were evaluated to verify system performance. 10 µL of the standard solution was injected five times,and the chromatograms were recorded.System suitability parameters were determined, and all the parameters were found to be within limits.

Specificit

The specificity of the developed analytical method was determined by injecting the solutions of 100µg/mL concentration each of blank, placebo, working standards, and sample solution individually to determine the interference from the representative peaks.

Precision

Repeatability/method precision was performed by injecting six replicates of CIL, OLM, and CHL with the same concentration (100 µg/mL) and calculated% assay and %RSD for each compound. Reproducibility/Ruggedness/Intermediate precision was performed using various analysts on another instrument and the other day in the same laboratory.

Accuracy

Accuracy was determined by performing a recovery study using the spiking method. It was carried out by adding known amounts (50%, 100%, and 150%) of the working standard solution to the pre-analyzed sample. The solutions were prepared in triplicates and injected to determine the accuracy of the proposed method.

Linearity

The linearity of the proposed method was determined by evaluating the standard solutions prepared of different concentrations of CIL, OLM, and CHL. 6 working standard solutions of different concentrations between 2.5–15 µg/mL for CIL, 10–60 µg/mL for OLM, and 3.125–18.75 µg/mL for CHL were prepared and injected. The results were evaluated by least-squares regression analysis,and the calibration equation and correlation coefficient were calculated.

Limit of Detection and Limit of Quantification

The limit of detection (LoD) and the limit of quantification (LoQ) of the present method was established using thecalibration curve method. Solutions of CIL, OLM, and CHL were prepared in triplicates in the range of linearity and injected.The results were calculated by plotting average peak areas against concentration.

ROBUSTNESS

To determine the robustness of the present developed method, a fewexperimental conditions were calculatedly changed, and the system suitability parameters of CIL, OLM, and CHL peaks were evaluated. To evaluate the effect of the flow rate on the developed method, it was altered by ± 0.2mL/min. The effect of column temperature on the developed method was evaluated by altering the temperature by± 5°C. The mobile phase composition was changed ±10% from the original composition of the organic phase, but the aqueous component of the mobile phase was maintained constant.

Forced Degradation Studies

To determine the stability-indicating property of the proposed developed method, stress studies were performed by considering OLM, CIL, CHL working standard solutions of concentrations 40ppm, 10ppm, 12.5ppm. Intentional degradation was attempted by the stress conditions of exposure to photolytic stress (1.2 million lux hours followed by 200 Watt-hours), heat (exposed at 105°C for 6 hours), acid (2 N HCl for 2 hours at 60°C), base (2 N NaOH for 2 hours at 60°C), oxidation (20% peroxide for 30 minutes at 60°C), water (refluxed for 12 hours at 60°C), and humidity (exposed to 85% RH for 72 hours). To evaluate the method's stability, the prepared solutionswereinjected,andthe chromatogramswere recorded and calculated.

RESULTS AND DISCUSSION

System Suitability

The column efficiency for CIL, OLM, and CHL peaks was identified from the theoretical plate count, whichwas more than 3000, and the tailing factor between 0.80 to 2.0 and the %RSD was less than 2.0%. The results of other system suitability parameters are summarized in Table 1. The results were satisfactory.

Specificity

The specificity results indicate the non-interference of the co-eluting peaks at the retention times of OLM, CIL, and CHL,

which determines that the analyte peak was pure and there is no interference with the excipients used in the formulation. The obtained chromatograms were summarized in figures 4 to 10. Figures 4 and 5 confirm that the blank and placebo peaks were not interfering at the retention time of CIL, OLM, and CHL.

Precision

% Assay for CIL, OLM, and CHL was found in 98–102% range, and the % RSD for them was within 2%. The results summarized in Table 2, confirm that the developed method was precise, rugged, and reproducible.

Accuracy

The % recovery for CIL, OLM, and CHLwas within the range of 98–102%. The % RSD for CIL, OLM, and CHL were found to be within 2%. From the results in table 3, it was evident that the developed method was accurate.

Linearity

Linearity was evaluated by analyzing different concentrations. The squared correlation coefficientobtained during linearity analysis was greater than 0.999 for CIL, OLM, and CHL. The results provided in table 4 confirm good linearity among the various concentrations of CIL, OLM, and CHL.

Table 1: System suitability data

Parameter	*CIL*	*OLM*	*CHL*	*Acceptance criteria*
USP Plate count	4280	5145	5828	NLT 3000
%RSD	1.3	0.6	1.8	NMT 2.0
Peak Tailing	1.15	1.14	1.11	NMT 2.0
Resolution	--	2.9	3.3	>1.5

*CIL: Cilnidipine, OLM: Olmesartan medoxomil, CHL: Chlorthalidone

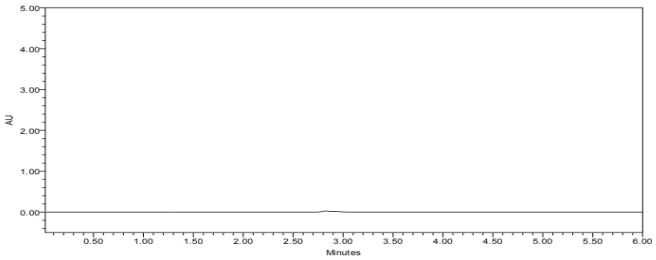

Figure 4: Chromatogram of blank

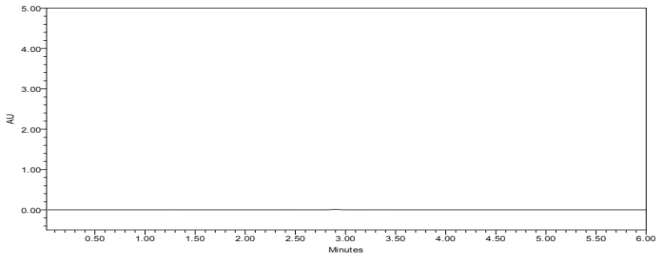

Figure 5: Typical chromatogram of placebo

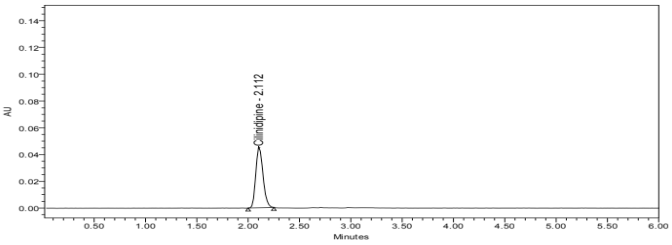

Figure 6: Typical chromatogramof Cilnidipine

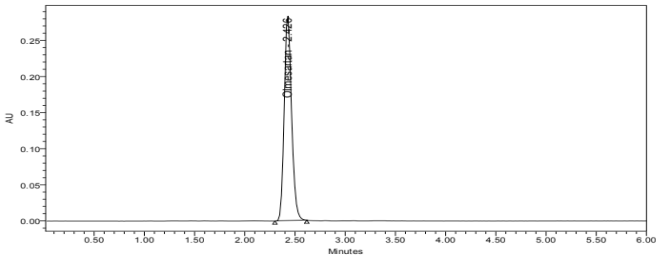

Figure 7: Typical chromatogram of Olmesartan medoxomil

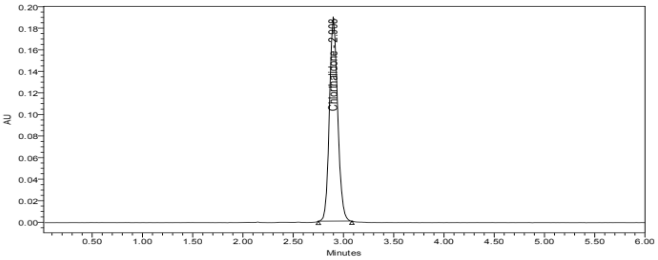

Figure 8: Typical chromatogram of Chlorthalidone

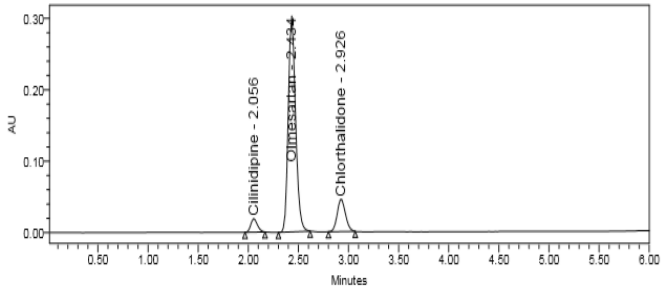

Figure 9: Typical chromatogramof mixture of Cilnidipine, Olmesartan medoxomil and Chlorthalidone standards

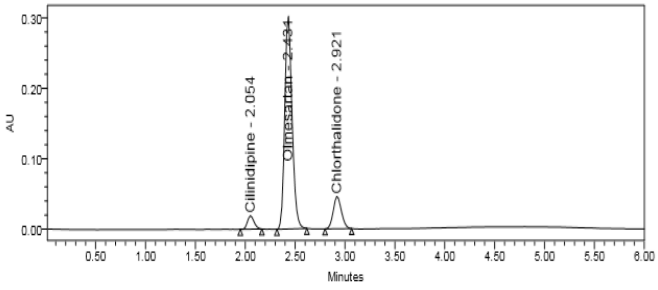

Figure 10: Typical chromatogramof Cilnidipine, Olmesartan medoxomil, and Chlorthalidone sample

Table 2: Precision data

	Peak Areas	*% Assay*	*Peak Areas*	*% Assay*	*Peak Areas*	*% Assay*
S.NO	*CIL*		*OLM*		*CHL*	
1	88661	99.93	1522482	99.15	262185	98.44
2	89506	100.89	1532574	99.81	261715	98.27
3	88631	99.90	1536938	100.09	264592	99.35
4	89295	100.65	1543537	100.52	264281	99.23
5	89576	100.96	1522868	99.18	266718	100.14
6	89125	100.46	1561797	101.71	264826	99.43
Mean	89132	100.46	1536699	100.08	264053	99.14
SD	409.0	0.461	14745.6	0.96	1843.8	0.692
% RSD	0.5	0.5	1.0	1.0	0.7	0.7

Table 3: Accuracy data

Drug name	*Conc. (%)*	*Amount spiked (µg/mL)*	*Amount recovered (µg/mL)*	*% recovery*	*Statistical parameters*
	50	5	5.01	100.35	
CIL	100	10	9.94	99.46	Mean %: 100.01
	150	15	15.03	100.22	
	50	25	74.8	99.7	
OLM	100	50	100.6	100.6	Mean %: 100.3
	150	75	125.9	100.7	
	50	10	30.2	100.6	
CHL	100	20	39.6	99	Mean %: 100.1
	150	30	50.4	100.8	

Table 4: Linearity data

	CIL		*OLM*		*CHL*	
S.NO	*Concentration (µg/mL)*	*Peak area**	*Concentration (µg/mL)*	*Peak area**	*Concentration (µg/mL)*	*Peak area**
1	2.5	22022	10	407704	3.125	63207
2	5	46035	20	777774	6.25	131515
3	7.5	69322	30	1193244	9.375	195424
4	10	90303	40	1540270	12.5	263146
5	12.5	114804	50	1951115	15.625	322136
6	15	134050	60	2282374	18.75	384481
	Regression equation $y = 8999.2x + 679.91$ $R^2 = 0.9991$		Regression equation $y = 37830x + 34707$ $R^2 = 0.9992$		Regression equation $y = 20534x + 2056.5$ $R^2 = 0.9995$	

* = Average peak area of 3 replicate injections for each concentration

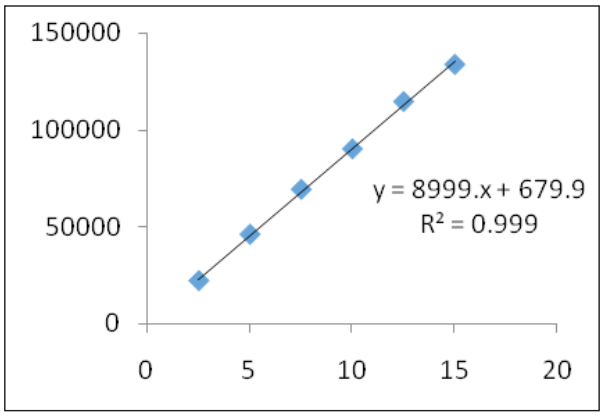

Figure 11a: Linearity of cilnidipine

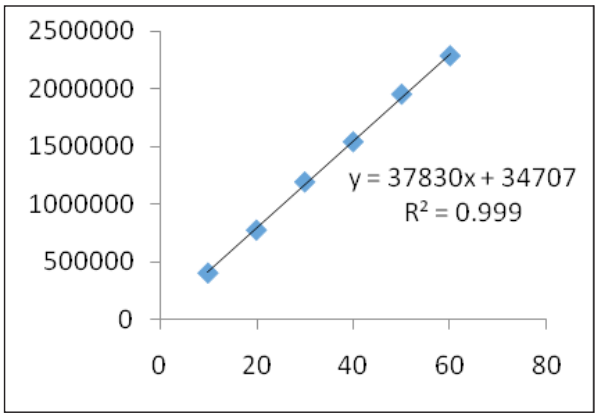

Figure 11b: Linearity of olmesartan medoxomil

The standard curvesof CIL, OLM, and CHL were shown in Figures 11a, 11b, and 11c, respectively.

LoD and LoQ

The LoD and LoQof CIL, OLM, and CHL were calculated from the calibration curve method using the following equations (ICH, Q2 (R1)) using standard deviation and the slope of the calibration curve. These LOD = 3.3 × σ/S and LOQ = 10 × σ/S

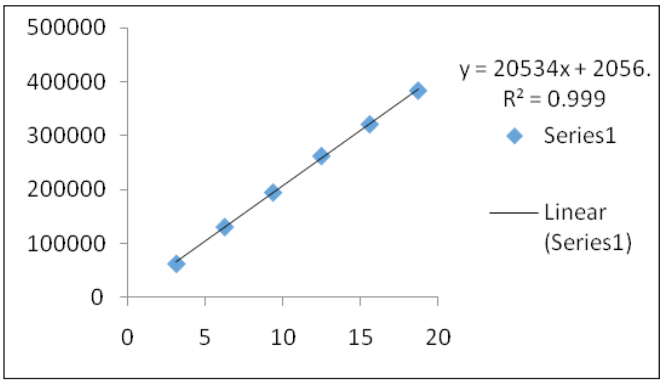

Fig. 11c: Linearity of Chlorthalidone

Table 5: LoD and LoQ data

Drug name	*LoD (µg/mL)*	*LoQ (µg/mL)*
CIL	0.7	2.1
OLM	0.8	2.53
CHL	0.9	2.7

Robustness

From the results presented in table 6, it was evident that the system suitability parameters of CIL, OLM, and CHL remained unaffected by deliberate changes. These results confirm that the present developed method was robust.

Forced Degradation Studies

To determine the purity of CIL, OLM, and CHL, samples subjected to intended degradation along with blank and placebo samples were analyzed with the optimized HPLC conditions. In all the intended degradation conditions, the peaks of CIL, OLM, and CHL were well resolved, indicating that the sample meant for stability analysis was not degraded. The peak purity of CIL, OLM, and CHL was determined based on purity angle and purity threshold. The results summarized in 7 confirm that the present developed method can be considered to "stability-indicating."

CONCLUSION

A simple, precise, accurate, linear, and rugged RP-HPLC method has been developed for the simultaneous determination of Cilnidipine, Olmesartan medoxomil, and Chlorthalidone in active pharmaceutical ingredients. The proposed method was validated following ICH guidelines by evaluating various parameters. The method was specific with no interference of the peaks of a blank, placebo, and the excipients used in the formulation. The results from the degradation studies where the purity angle is less than the purity threshold indicate peak purity of CIL, OLM, and CHL, and the method was found to be stability-indicating.

Table 6: Robustness data

		System Suitability Parameters				
Parameter		*RT (min)*	*Plate count*	*Peak tailing*	*Resolution*	*% RSD*
Optimized method	CIL	2.053	4280	1.14	-	1.3
	OLM	2.432	5145	1.14	2.8	0.6
	CHL	2.921	5828	1.1	3.3	1.2
Flow rate (0.8 mL/min)	CIL	2.132	4370	1.15	-	0.9
	OLM	2.531	5627	1.15	2.9	0.5
	CHL	2.302	5838	1.06	3.2	0.4
Flow rate (1.0 mL/min)	CIL	1.954	4367	1.15	-	0.4
	OLM	2.318	5079	1.17	2.9	0.8
	CHL	2.763	5641	1.1	3.2	0.7
Organic Phase (55:45)	CIL	1.989	4443	1.14	-	0.7
	OLM	2.328	5354	1.16	2.6	0.9
	CHL	2.63	5773	1.1	2.4	1.1
Organic Phase (65:35)	CIL	2.101	4133	1.14	-	1.1
	OLM	2.538	5030	1.15	3.2	0.9
	CHL	3.186	5955	1.05	4.1	1.3
Temperature (25°C)	CIL	2.078	4485	1.14	-	0.9
	OLM	2.496	5327	1.14	3.2	0.7
	CHL	3.047	6100	1.07	3.8	1.0
Temperature (35°C)	CIL	2.012	4439	1.16	-	0.8
	OLM	2.366	5369	1.17	2.8	0.9
	CHL	2.749	5870	1.1	2.8	1.3

Table 7: Forced degradation studies at various stress conditions

Stress Condition	*% Degradation*	*Purity Angle*	*Purity Threshold*
	Cilnidipine		
Acid stress	4.43	0.990	1.234
Base stress	4.15	0.631	0.749
Peroxide stress	2.71	0.122	0.212
Thermal stress	1.16	0.291	0.469
Fluorescent stress	1.68	0.245	0.414
Refluxed Water	0.68	0.219	0.388
	Olmesartan medoxomil		
Acid stress	5.04	0.236	0.405
Base stress	3.19	0.173	0.316
Peroxide stress	2.70	0.150	0.286
Thermal stress	1.80	0.135	0.279
Fluorescent stress	1.35	0.142	0.271
Refluxed Water	0.31	0.124	0.271
Chlorthalidone			
Acid stress	3.93	0.528	0.717
Base stress	3.61	0.384	0.512
Peroxide stress	2.94	0.231	0.386
Thermal stress	1.13	0.137	0.311
Fluorescent stress	1.95	0.135	0.319
Refluxed Water	0.86	0.129	0.309

Thus the stability-indicating RP-HPLC method developed to estimate CIL, OLM, and CHL can be implemented in the routine analysis in various pharmaceutical industries.

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