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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND OLMESARTAN MEDOXOMIL IN TABLET DOSAGE FORM BY UPLC USING DAD

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Keywords:

RP-UPLC, Cilnidipine, Olmesartan medoxmil, PDA detector

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ABSTRACT: Olmesartan Medoxomil and Cilnidipine combination lowers blood pressure effectively. Olmesartan Medoxomil is an angiotensin receptor blocker (ARB), and Cilnidipine is a calcium channel blocker (CCB). Cilnidipine and Olmesartan Medoxomil are widely used in hypertension. The scope of the study is to optimize the chromatographic conditions to develop a new RP-UPLC method for the simultaneous estimation of Olmesartan Medoxomil and Clinidipine, which is simple, accurate, precise, and rapid. A very few methods were developed by UPLC using DAD. The mobile phase used is 0.5% w/v Ammonium acetate buffer: methanol: Acetonitrile (40:50:10), and the detection was carried out at 240 nm by using PDA detector. The flow rate was optimized at 0.4ml/min. The retention time was found to be 0.587 and 0.992 for Olmesartan Medoxomil and Cilnidipine, respectively. All the parameters of the method development and validation meet the ICH guidelines criteria. Thus the work establishes that the reported method is more economical and can be regularly used in practical application for simultaneous analysis of the Cilnidipine (CIL) and Olmesartan medoxomil (OLME) in their combined dosage forms both in research and quality control laboratories.

INTRODUCTION: Analytical methods, which are a measure of the quality of the drugs, play a very comprehensive role in drug development and follow-up activities to assure that a drug product meets the established, is stable and continue to meet purported quality throughout its shelf life [1, 2]. Chromatography is a physicochemical method for the separation of complex mixtures was discovered at the very beginning of the twentieth century by Russian–Italian botanist M.S. Tswett [3, 4].

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Tswett gave a detailed description of the newly discovered phenomena of adsorption-based separation of complex mixtures, which he later called "chromatography" [5, 6].

UPLC refers to Ultra Performance Liquid Chromatography. This technique helps in significant increases in resolution, speed, and sensitivity in liquid chromatography. It uses fine particles and saves time, and reduces solvent consumption [22-24]. It improves in three areas: chromatographic resolution, speed, and sensitivity analysis [14-18].

It UPLC has come from HPLC. UPLC is higher sensitive compared to of HPLC. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus

resolution also increases. As particle size decreases to less than 2.5µm, there is a significant gain in efficiency and it's doesn't diminish at increased linear velocities or flow rates according to the common Van Deemter equation [19]. Using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits, known as Ultra Performance. In UPLC, particle size can be less than 2µm. The total run time in HPLC is 10mins, whereas in UPLC it is 1.5mins.

Cilnidipine **Fig. 1** is a Calcium channel blocker belonging to dihydropyridine class. It is also known as Atelec, Cinalong, Siscard. It is chemically known as 3-(2-methoxyethyl) 5-(2E)-3-phenyl-prop-2-en-1-yl 2, 6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine- 3, 5- dicarboxylate. Cilnidipine inhibits the cellular influx of calcium, thus causing vasodilation [55-56]. It has greater selectivity for vascular smooth muscle. It has little or no action at the SA or AV nodes, and –ve inotropic activity is rarely seen at therapeutic doses. Cilnidipine is freely absorbed by oral administration. It is rapidly metabolized in human liver microsomes in three metabolites. They are dehydrogenated metabolites of dihyropyridine ring of cilnidipine (M1), demethylation metabolite of lateral chain of dihyropyridine ring of cilnidipine (M2) and the dehydrogenation and demethylation metabolite of cilnidipine (M3). It is highly bound to plasma protein.

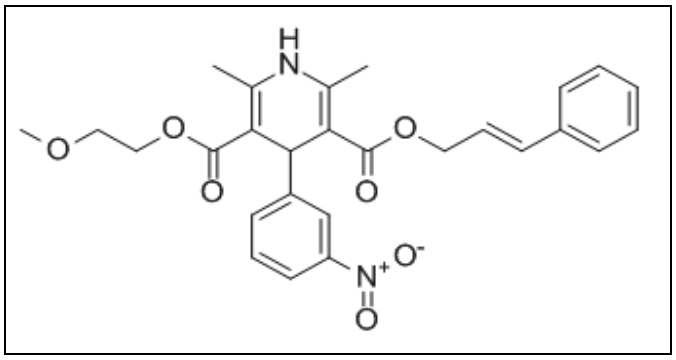

FIG. 1: CILNIDIPINE STRUCTURE

Olmesartan medoxomil **Fig. 2** is a specific angiotensin II type 1 (AT1) receptor antagonist. It is chemically called as 2,3 – dihydroxy-2-butenyl4(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole5-carboxylate,cyclic2,3 carbonate [57]. The prodrug is hydrolyzed to olmesartan during absorption from the gastrointestinal tract. Olmesartan is a selective AT1 subtype angiotensin II receptor antagonist. Angiotensin II is formed from angiotensin I in a reaction catalyzed by the angiotensin-converting enzyme (ACE, kininase II). Angiotensin II is the principal pressor agent of the rennin-angiotensin system, with effects include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorption of sodium. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle. Its action is, therefore, independent of the pathways for angiotensin II synthesis. An AT2 receptor is also found in many tissues, but this receptor is not known to be associated with cardiovascular homeostasis. Olmesartan has more than a 12,500-fold greater affinity for the AT1 receptor than for the AT2 receptor. Blockade of the rennin-angiotensin system with ACE inhibitors, which inhibit the biosynthesis of angiotensin II from angiotensin I, is a mechanism of many drugs used to treat hypertension. ACE inhibitors also inhibit the degradation of bradykinin, a reaction also catalyzed by ACE. Because olmesartan does not inhibit ACE (Kininase II), it does not affect the response to bradykinin. Whether this difference has clinical relevance is not yet known. Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II or rennin secretion, but the resulting increased plasma renin activity and circulating angiotensin II levels do not overcome the effect of olmesartan on blood pressure. It is highly bound to plasma proteins and does not penetrate red blood cells.

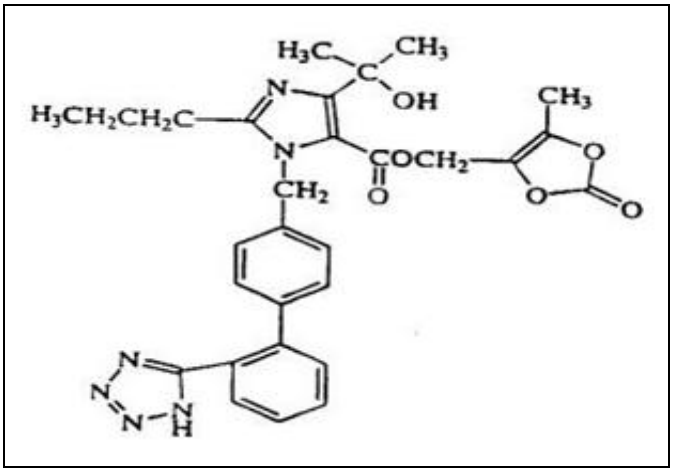

FIG. 2: OLMESARTAN MEDOXOMIL

Olmesartan Medoxomil and Cilnidipine combination lowers blood pressure effectively. Olmesartan Medoxomil is an angiotensin receptor blocker (ARB), and Cilnidipine is a calcium channel blocker (CCB). Cilnidipine and Olmesartan Medoxomil are widely used in hypertension. They work by relaxing the blood vessels and making the heart more efficient at pumping blood throughout the body. The proposed method is to develop a simple, precise, accurate, rapid, and reliable method for estimation of Cilnidipine and Olmesartan medoxomil in tablets dosage form using the following technique of UPLC.

MATERIALS AND METHODS: Shimadzu 1800 UV-Visible spectrophotometer, Thermo scientific UPLC with PDA detector, C18 Column (50×4.6mm,1.9µm) Thermo Scientific hypersil gold, Metler-toledo Analytical Balance, Sansel pH meter, Frontline Ultra sonicator, and Pippetes and burettes, Glass beakers, Measuring cylinder, Volumetric flask are from Borosil.

- Olmesartan medoxomil is obtained from S.R. Chemicals and Pharmaceuticals.
- Cilnidipine is obtained from Niksan Pharmaceuticals and manufactured by Ajanta Pharma Limited.
- Reagents used are:

1. Ammonium acetate – Loba Chemie Pvt. Ltd
2. Acetic acid – Chemspure
3. Acetonitrile – Merck specialties Pvt. Ltd
4. Methanol – Loba Chemie Pvt. Ltd
5. HPLC water – Merck Specialities Pvt. Ltd.

Chromatographic Conditions: The chromatographic separation was carried out using thermo scientific hypersil gold C_{18} Column (50×4.6mm, 1.9µm). Isocratic elution of mobile phase is made up of buffer: methanol: CAN (40:50:10v/v). Diluent used is methanol. The flow rate was adjusted to 0.4ml/min. Run time was set at 4min. 10µl volume of injection was given. The detector used is PDA detector, and it is detected at 240nm.

Preparation of 0.5% w/v Ammonium acetate Buffer Solution: Certain amount of Ammonium acetate is dissolved in acetic acid with the aid of ultra sonicator, and the volume is made up of water to 1000ml to get 0.5% w/v ammonium acetate buffer.

Preparation of Mobile Phase: Buffer: methanol: acetonitrile were mixed in the ratio of 40:50:10 and sonicate the solution using an ultra sonicator for 20 minutes, filter with 0.45µm membrane filter.

Preparation of Working Standard Stock Solution:

Cilnidipine Stock Solution: 25mg of CIL was precisely weighed and transferred into a 50ml volumetric flask, and a sufficient amount of methanol was added for dissolving. It is sonicated for 10mins and made up the volume up to the mark with methanol. Final concentration was 0.5mg/ml.

Olmesartan Medoxomil Stock Solution: 50mg of OLME was precisely weighed and transferred into a 50ml volumetric flask, and sufficient methanol was added to dissolve and sonicated for 10mins, and the volume was made upto the mark. Final concentration was 1mg/ml.

Preparation of Standard Solution: The standard solution containing 10µg/ml of Cilnidipine and 20µg/ml of Olmesartan was prepared and filtered with a 0.45µ membrane filter.

Preparation of Sample Solution: 20 tablets were accurately weighed and powdered. A quantity of powder weigh equivalent to 25mg of CIL and 50mg of OLME was weighed and transferred to a 50ml of volumetric flask, and sufficient diluent was added to dissolve it. Then the solution was sonicated for 10min. transfer 1ml from the above solution in 50ml dried and cleaned volumetric flask. This was then diluted with 30ml of diluent. The volume was made up to 50ml with the same solvent and filtered through 0.45µ membrane filter. Then the sample solution contains 10µg/ml of Cilnidipine and 20µg/ml of olmesartan. The resulting solution was then filtered through 0.45µ membrane filter.

The amount of Cilnidipine and Olmesartan medoxomil present in each tablet were calculated.

Analytical Method Validation:

Specificity:

Cilnidipine and Olmesartan Medoxomil Identification: The standard and sample solutions were prepared as per the test method and injected into the chromatographic system (**Fig. 3, 4, 5**.

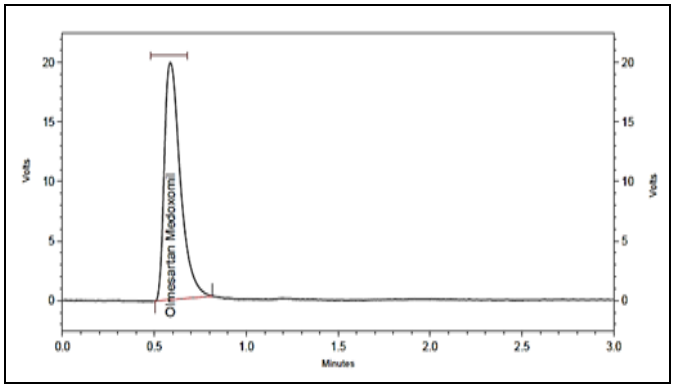

FIG. 3: CHROMATOGRAM OF OLMESARTAN MEDOXOMIL (WS)

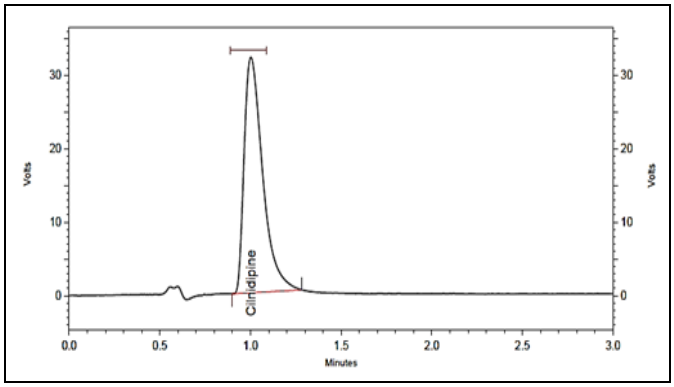

FIG. 4: CHROMATOGRAM OF CILNIDIPINE (WS)

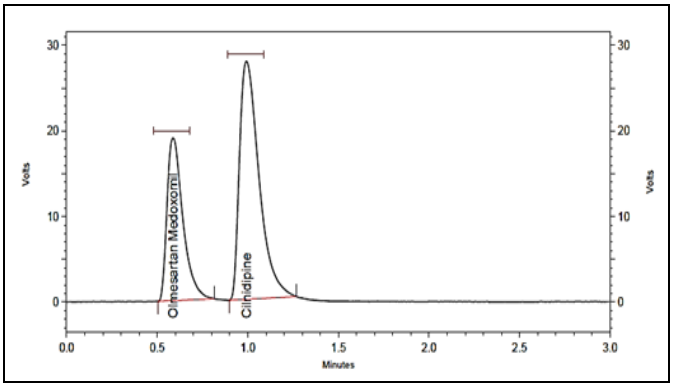

FIG. 5: CHROMATOGRAM OF SAMPLE SOLUTION

Name	Ret. Time	Area	Area %	Theoretical Plates (USP)	Resolution (USP)	Asymmetry
OLME	0.587	115528	35.47	3234	0.00	1.69
CIL	0.992	201293	64.53	3417	2.33	1.89
Total		326928	100.00			

System Suitability: Table 1, 2

Preparation of Standard Solution: The standard solution contains 10µg/ml of Cilnidipine, and 20µg/ml of Olmesartan was prepared and injected. The parameter was tested by giving five replicate injections of standard solution. All the system suitability parameters for CIL and OLME were found within the limits

TABLE 1: RESULTS OF SYSTEM SUITABILITY FOR CILNIDIPINE

Injection ID	Ret. Time	Peak Area	Theoretical Plates (USP)	Asymmetry
1	0.993	220248	3445	1.76
2	0.982	220217	3406	1.77
3	0.993	217274	3440	1.81
4	0.982	215530	3474	1.88
5	0.988	213265	3462	1.94
AVERAGE	-	217306.8	3445.4	1.832
STD DEV		3025		
% RSD		1.39		

TABLE 2: RESULTS OF SYSTEM SUITABILITY FOR OLMESARTAN MEDOXOMIL

Injection ID	Ret. Time	Peak Area	Theoretical Plates (USP)	Asymmetry
1	0.587	120245	3238	1.61
2	0.588	120059	3236	1.62
3	0.587	119767	3232	1.67
4	0.587	118265	3234	1.66
5	0.588	116670	3230	1.66
AVERAGE	-	119001.2	3234	1.644
STD DEV		1519		
% RSD		1.28		

Assay of Cilnidipine and Olmesartan medoxomil in Tablet dosage form: Table 3

Preparation of Standard Solution: The standard solution contains 10 µg/ml of Cilnidipine of 20µg/ml of Olmesartan. The resulting solution was then filtered with 0.45µ membrane filter.

Preparation of Sample Solution: The sample solution contains 10µg/ml of Cilnidipine and 20µg/ml of Olmesartan and is filtered through a 0.45µ membrane filter. Standard and sample solutions were injected into the chromatographic system with the help of an auto-injector separately.

TABLE 3: ASSAY OF CILNIDIPINE AND OLMESARTAN MEDOXOMIL

S. no.	Sample ID	Area		Amount present (mg)		% Assay	
		Cilnidipine	Olmesartan	Cilnidipine	Olmesartan	Cilnidipine	Olmesartan
1	Injection 1	201293	115528	9.997	20.03	99.97	100.15
2	Injection 2	200717	116750	9.968	20.24	99.68	101.20
3	Injection 3	201330	115672	9.998	20.06	99.98	100.30
Mean				9.987	20.11	99.87	100.55
% RSD				0.017	0.11	0.17	0.57

Linearity: The linearity of analytes over the range 80% to 120% of target concentration five different concentration solution of Dextrose (80%, 90%, 100%, 110%, and 120%) prepared and injected in UPLC by means of auto-injector **Table 4**.

Procedure: Cilnidipine Stock Solution: The concentration of 0.5 mg/ml was prepared.

Olmesartan Medoxomil Stock Solution: The concentration of 1mg/ml was prepared.

TABLE 4: LINEARITY RESULTS FOR OLMESARTAN MEDOXOMIL & CILNIDIPINE

S. no.	Linearity Level	Cilnidipine (CIL)		Olmesartan medoxomil (OLME)	
		Concentration in µg/ml	Area	Concentration in µg/ml	Area
1	80%	8	162049	16	89867
2	90%	9	185003	18	102792
3	100%	10	202292	20	112196
4	110%	11	223798	22	125343
5	120%	12	240239	24	134751
Correlation coefficient		0.9996		0.9997	
Slope		2018.7250		1129.1793	
Intercept		669.7500		59.8929	

Accuracy: The working standards of the drug were prepared at the level of 100%, 110%, 120%, and 130% **Table 5, 6**.

Preparation of Standard Stock Solution: The standard solution of 10 mcg/ml of Cilnidipine and 20 mcg/ml of Olmesartan was prepared.

TABLE 5: ACCURACY RESULTS OF CILNIDIPINE

Drug	% level	Area	Amount found (mg)	% Recovery	%Mean Recovery	%RSD
Cilnidipine (CIL)	100%	192195	9.99	99.87	99.51	0.31
		193136	9.93	99.32		
		193187	9.93	99.34		
	110%	213641	11.03	100.32	99.95	0.49
		213123	10.93	99.40		
		212490	11.01	100.14		
	120%	232658	11.91	99.25	99.92	0.60
		233344	12.05	100.41		
		231181	12.01	100.08		
	130%	252726	12.95	99.62	100.02	0.33
		251578	13.03	100.23		
		251578	13.02	100.15		
					99.85	0.23

TABLE 6: ACCURACY RESULTS OF OLMESARTAN MEDOXOMIL

Drug	% level	Area	Amount found (mg)	% Recovery	%Mean Recovery	%RSD
Olmesartan medoxomil (OLME)	100%	110512	19.68	98.40		
		110525	20.10	100.50	99.60	1.09
		110652	19.98	99.90		

	110%	122439	22.10	100.45		
		122164	22.01	100.04	100.04	0.40
		121858	21.92	99.64		
	120%	132863	24.12	100.48		
		132336	23.75	98.96	99.62	0.78
		132995	23.86	99.42		
	130%	144877	26.11	100.42		
		144451	26.07	100.27	100.08	0.47
		143879	25.88	99.54		
					99.84	0.26

Precision: The method precision by preparing six samples as per the test method of a single batch representing the 100% of test concentration was prepared **Table 7**.

TABLE 7: METHOD PRECISION RESULTS FOR CILNIDIPINE & OLMESARTAN MEDOXOMIL

Injection No.	Sample Weight in mg	Cilnidipine (CIL)		Olmesartan medoxomil (OLME)	
		Area	%Assay	Area	%Assay
1	172.17	189849	99.64	108968	99.84
2	173.45	190291	99.13	109856	99.91
3	175.06	192162	99.19	110049	99.16
4	174.04	189829	98.56	109247	99.02
5	172.38	191820	100.55	110211	100.85
6	171.22	189872	100.20	109027	100.44
Average		190637.16	99.54	109559.67	99.87
Standard Deviation		1068.07	0.74	544.62	0.71
% RSD		0.56	0.74	0.50	0.71

Limit of Detection (LOD) & Limit of Quantitation (LOQ): The LOD and LOQ of CIL and OLME shall be estimated from the standard deviation of the response and the slope of the calibration curve by using the following formula **Table 8**.

TABLE 8: LOD & LOQ RESULTS FOR CILNIDIPINE & OLMESARTAN MEDOXOMIL

Name	Slope	Standard deviation of response	LOD (µg/ml)	LOQ (µg/ml)
Cilnidipine (CIL)	2018.73	1899.59	3.11	9.41
Olmesartan medoxomil (OLME)	1129.18	1137.94	3.33	10.08

Range: The linearity study was done at the concentration range for Cilnidipine and Olmesartan medoxomil were 8-12µg/mL and 16-24µg/mL, respectively.

Robustness: Standard solution was prepared and injected into the chromatographic system as per the conditions specified in the method **Table 9, 10**.

TABLE 9: ROBUSTNESS OBSERVATION OF CILNIDIPINE

S. no.	Parameter	Condition	Retention time in min	Area	Theoretical plates (USP)	Asymmetry
1	Wavelength variation	238 nm	0.998	188105	3558	1.81
		240 nm	0.993	220248	3445	1.76
		242 nm	1.000	210430	3492	1.81
2	Flow Rate	0.375ml /min	1.113	250309	3503	1.84
		0.400ml /min	0.993	220248	3445	1.76
		0.425ml /min	0.912	202706	3514	1.75

TABLE 10: ROBUSTNESS OBSERVATION OF OLMESARTAN MEDOXOMIL

S. no.	Parameter	Condition	Retention time in min	Area	Theoretical plates (USP)	Asymmetry
1	Wavelength variation	238 nm	0.592	84299	3284	1.78
		240 nm	0.587	120245	3238	1.61
		242 nm	0.592	151591	3263	1.76
2	Flow Rate	0.375ml /min	0.660	138365	3267	1.81
		0.400ml /min	0.587	120245	3238	1.61
		0.425ml /min	0.533	109518	3268	1.64

RESULTS AND DISCUSSION: The analytical method for the Cilnidipine and Olmesartan medoxomil by UPLC was established, then optimized and applied on pharmaceutical dosage forms.

Various trials were performed in order to optimize the following analytical parameter like the choice of column, mobile phase composition, flow rate, and injection volume, and the results are shown.

TABLE 11: RESULTS OF ASSAY OF CIL & OLME IN TABLET DOSAGE FORM

S. no.	Drug Name	Label claim	Content present	Percentage Purity
1	Cilnidipine	10 mg	9.987 mg	99.87%
2	Olmesartan medoxomil	20 mg	20.11 mg	100.55%

TABLE 12: VALIDATION PARAMETERS

Validation Parameter	Acceptance Criteria	Observation		Remarks
		CIL	OLME	
Specificity	The peaks of diluent and excipients should not interfere with the main peak and peak of the standard and the sample should be identical with near retention time	Complies	Complies	Pass
System Suitability	Asymmetry NMT 2.0	1.832	1.644	Pass
	Theoretical plates NLT 2000	3445.4	3234	
	%RSD of area NMT 2.0	1.39	1.28	
Linearity	Correlation Coefficient NLT 0.99	0.9996	0.9997	Pass
Accuracy	The % recovery at each spike level shall be NLT 98.0% and NMT 102.0% of the added amount and % RSD of each level should not be more than 2.0	99-101% Avg %RSD 0.23%	99-101% Avg %RSD 0.26%	Pass
Precision	The % RSD of area and assay for the six determinations shall be NMT 2.0	0.56% 0.74%	0.50% 0.71%	Pass
LOD (µg/ml)	Not Specified	3.11	3.33	Pass
LOQ (µg/ml)	Not Specified	9.41	10.08	Pass
Range	Not Specified	8-12µg/mL	16-24µg/mL	Pass
Robustness	Theoretical plates (USP) ≥ 2000 Asymmetry ≤ 2	Complies	Complies	Pass

DISCUSSION: A new rapid RP-UPLC method was developed for the simultaneous estimation of Cilnidipine and Olmesartan medoxomil in the pharmaceutical dosage form. After performing different trials with change in runtime, concentration of mobile phase, and detection wavelengths, the chromatographic parameters were optimized as the mobile phase consisting of mixture of buffer: methanol: acetonitrile (40:50:10), thermo scientific hypersil gold C_{18} Column (50×4.6mm, 1.9µm) with a flow rate of 0.4ml/min, runtime 4mins and the detection at 240 nm using PDA detector gives the precise and accurate results.

The literature shows that the study conducted by Rashmi D.R. *et al*., gives the retention time at 3.35 and 1.833 for CIL and OLME, respectively using acetonitrile: methanol (60:40) mobile phase, which is more than compared to that of the present research work done where the retention time obtained for OLME & CIL was found to be 0.587 min & 0.993 min respectively.

The asymmetry factor was found to be 1.61 and 1.76, which indicates the symmetrical nature of the peak. Resolution (USP) between two peaks was 2.39, which satisfies the acceptance criteria. The specificity was performed with respect blank, and the chromatogram showed identical with near retention time with the standard.

The system suitability parameter such as the number of theoretical plates and asymmetry factor, % RSD of the area were recorded and found to be within limits mentioned in **Tables 1** & **2**. With the optimized conditions, the linearity range was fixed as (80-120)% *i.e.* (8-12) µg/ml of CIL and (16-24) µg/ml of OLME. Linearity range was evaluated by the visual inspection of a plot of peak area as a function of analyte concentration, and results are shown in **Table 4**. From the linearity studies, the specified concentration range was determined. It was observed that Cilnidipine and Olmesartan medoxomil was linear in the range of (8-12) µg/ml and (16-24) µg/ml respectively for the target concentrations.

The regression equation of Cilnidipine and Olmesartan medoxomil for concentration over its peak area ratio was found to be y=2018.73 + 669.75(R^2 = 0.9996) and y=1129.18x + 59.89 (R^2 = 0.9997) where y is the peak area ratio and x is the concentration of CIL & OLME in µg/ml.

Recovery (Spiking) studies verified the validation of the proposed method. The percentage recovery range was found between 99.0-100.5% for both CIL and OLME. This is a good index of accuracy, specificity, and repeatability of the method. The results were tabulated in **Tables 5** & **6**. All parameters, including flow rate, temperature, detection, wavelength, and sensitivity are maintained constant throughout the procedure.

The validation of the proposed method was verified by method precision, where a repeatability study was performed by preparing six samples as per the test method of a single batch representing 100% of test concentration. The % RSD of assay and area for method precision was calculated, and the data were tabulated and shown in **Table 3**.

LOD and LOQ were calculated from the linearity parameter data. The results were tabulated and shown in **Table 8**.

Robustness studies were made by varying wavelength (±2 nm), and flow rate (±0.025 ml/min) and the results were mentioned in **Table 9** & **10**.

CONCLUSION: From the results obtained it is concluded that the proposed method was simple, accurate, precise and rapid for simultaneous estimation of Cilnidipine and Olmesartan medoxomil in pharmaceutical tablet dosage form and could be used conveniently for routine analysis. All the parameters meet the criteria of ICH guidelines for method validation and are found to be simple, sensitive, accurate, and precise. It can therefore be concluded that the reported method is more economical and can find a practical application for simultaneous analysis of the Cilnidipine (CIL) and Olmesartan medoxomil (OLME) in their combined dosage forms both in research and quality control laboratories.

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