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RP – HPLC METHOD DEVELOPMENT, METHOD VALIDATION AND SIMULTANEOUS ESTIMATION OF CLINIDIPINE AND OLMESARTAN MEDOXOMIL IN SOLID DOSAGE FORM

B. Sneha Reddy[1]*, Dr. K. Swathi Priya[2], Mrs. P. R. Sudha Rani[3], Dr. D. Nivedita[4], Mr. Ch. Bharath[5]

[1,2,3,4,5]Srinivasarao college of Pharmacy, P. M. Palem, Visakhapatnam-530041, Andhra Pradesh India.

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***Corresponding Author**
B. Sneha Reddy
Srinivasarao College of Pharmacy, P. M. Palem, Visakhapatnam-530041, Andhra Pradesh India.

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ABSTRACT

Validation is an essential process in the field of analytical chemistry, crucial for verifying that analytical methods are appropriate for their intended applications. This step ensures that the resulting data are trustworthy, consistent, and reproducible. The validation process assesses different parameters, each aimed at evaluating a specific dimension of the method's performance, including selectivity, precision, accuracy, linearity, range, stability, and the limits of detection and quantification. The peaks for Clinidipine and Olmesartan Medoxomil were well-separated, occurring at retention times of 6.381 and 5.070 minutes, respectively, with a flow rate of 1 ml/min in isocratic mode over a total run time of 15 minutes. The analysis utilized a Welchrom column measuring 250 mm x 4.6 mm with a particle size of 5 µm, employing a mobile phase consisting of ammonium acetate buffer, water, and methanol in a ratio of 20:10:70, with detection at 265 nm. The assay percentages were 99.50% for Clinidipine and 99.01% for Olmesartan Medoxomil. Linearity was established within the range of 40-120 ppm for Clinidipine and 32-96 ppm for Olmesartan Medoxomil, demonstrating correlation coefficients of 0.9947 and 0.9961, respectively. The newly proposed method was validated against ICH guidelines and was determined to be specific, sensitive, precise, reliable, and linear. This method is likely to effectively quantify Clinidipine and Olmesartan Medoxomil in commercially available formulations.

KEYWORDS: Clinidipine, Olmesartan Medoxomil, Method development and validation, ICH guidelines, and RP-HPLC.

1. INTRODUCTION

1.1 DRUG PROFILE OF CLINIDIPINE

Cilnidipine, a dihydropyridine calcium antagonist, was collaboratively developed by Fuji Viscera Pharmaceutical Company and Ajinomoto in Japan and received approval in 1995. Unlike many other calcium antagonists, cilnidipine targets both L-type calcium channels, which are common among drugs in this category, as well as N-type calcium channels located at sympathetic nerve endings. It is prescribed for heart-related issues such as angina (chest pain) and high blood pressure (hypertension). As a calcium channel blocker, its mechanism involves obstructing calcium ions from entering the muscle cells of the heart, which facilitates the relaxation and dilation of the heart's smooth muscles, consequently enhancing blood flow. It has been authorized for use in various countries including China, Japan, Korea, India, and several nations within the European Union. The medication's IUPAC name is 3-(2-methoxyethyl) 5-(2E)-3-phenylprop-2-en-1-yl 2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3,5-dicarboxylate, with the molecular formula $C_{27}H_{28}N_2O_7$ and a molecular mass of 492.52 g/mol.[1-12]

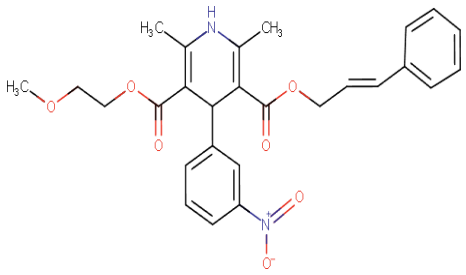


Figure 1: Chemical Structure of Clinidipine.

1.2 DRUG PROFILE OF OLMESARTAN MEDOXOMIL

Olmesartan is classified as an angiotensin II receptor blocker (ARB) and has received FDA approval for managing hypertension. It may be administered independently or alongside other antihypertensive drugs. Like other ARBs, Olmesartan proves effective as a standalone treatment for hypertension in patients who do not have complicating conditions such as chronic kidney disease, cerebrovascular events, heart failure, diabetes, or ischemic heart disease. For patients at elevated risk, such as those with a 10% or greater atherosclerotic cardiovascular disease (ASCVD) risk or stage-2 hypertension, ARBs can be paired with a

thiazide diuretic or calcium channel blocker to attain proper blood pressure regulation. The IUPAC nomenclature for Olmesartan is 4-(2-hydroxypropan-2-yl)-2-propyl-1-{[2'-(1H-1, 2, 3, 4-tetrazol-5-yl)-[1, 1'-biphenyl]-4-yl] methyl}-1H-imidazole-5-carboxylic acid, and its molecular formula is $C_{24}H_{26}N_6O_3$. Olmesartan has a molecular weight of 558.585 g/mol.[13-22] The structure is illustrated in the figure below.

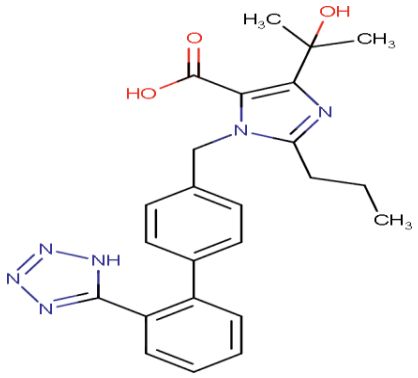


Figure 2: Chemical Structure of Olmesartan Medoxomil.

A comprehensive review of the literature[23-34] was conducted, and the authors found that there are only a limited number of spectroscopic and liquid chromatographic methods reported. Consequently, there is a necessity to develop a rapid, sensitive, and selective RP-HPLC method for quantifying Clinidipine and Olmesartan Medoxomil in both bulk and tablet dosage forms.

2. MATERIALS AND METHODS

2.1 APPARATUS & CHEMICALS

List of apparatus and chemicals used in this study were tabulated below.

Table 1: List of apparatus.

S.no	Name	Model	Manufacturer
1	HPLC	Waters 2690	ALLIANCE
2	pH meter	Model 152	RI
3	Weighing Balance	SAB 203 L	Scale tech
4	Pipettes, Beakers and Burettes	NA	Borosil Class-A
5	Ultra Sonicator	PSA-10A	DIGITAL PRO

Table 2: List of chemicals.

S.No	Name	Grade	Batch No
1	Water (Milli Q / HPLC Grade water)	HPLC	P24E100596
2	Ammonium acetate	HPLC	J058A24
3	Methanol	HPLC	R276G24

2.2 PREPARATION OF SOLUTIONS

Mobile phase: Ammonium acetate buffer, water & methanol were made in 20:10:70 ratios.

Preparation of buffer: A precise amount of 10 grams of ammonium acetate was measured and placed into a 2000 ml volumetric flask. The flask was filled with water up to the calibration mark and the solution was then filtered using a 0.45 µm membrane filter.

Diluent preparation: Water and methanol was mixed in 70:30 ratios.

Standard preparation: Measured 50 mg of Clinidipine and 40 mg of Olmesartan Medoxomil accurately and placed them into separate 100 ml volumetric flasks. Added 60 mL of diluent and sonicated the mixture for 5 minutes. The volume was then brought up to the mark with additional diluent. Subsequently, 4 mL from each solution was transferred into a 50 ml volumetric flask, and the volume was adjusted to the mark with the same diluent.

Preparation of Sample solution: A precise measurement of 50 mg of equivalent powder and 40 mg was taken from 20 tablets of OLMIN 40 LN, which contain Clinidipine and Olmesartan Medoxomil, and placed into a 100 ml volumetric flask. Following this, 60 mL of diluent was introduced, and the mixture was sonicated for 5 minutes. The volume was then brought up to the mark with additional diluent. Subsequently, 4 mL of this solution was transferred to a 50 ml volumetric flask, where the volume was adjusted to the mark using the same diluent.

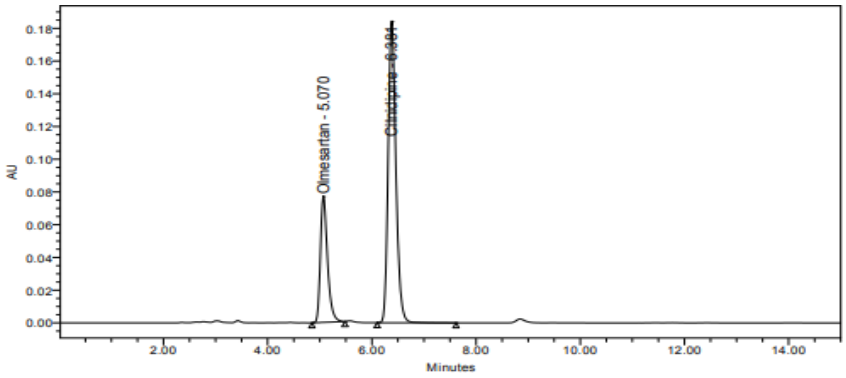
Optimized chromatographic conditions: A series of trials were conducted using RP-HPLC in isocratic mode, and the optimized chromatogram was achieved at 265nm with a flow rate of 1ml/min, utilizing a mixture of Ammonium acetate buffer, water, and methanol in a ratio of 20:10:70. The peaks were effectively separated using a Welchrom column measuring 250mm x 4.6mm with a particle size of 5µm, maintained at ambient temperature over a 15-minute run time. The sample temperature was kept at 20± 5°C.

3. RESULTS AND DISCUSSION

3.1 SYSTEM SUITABILITY

In chromatography, system suitability denotes a series of evaluations carried out to verify that the chromatographic system (which encompasses equipment, electronics, analytical procedures, and samples) is functioning properly before starting an analysis. These evaluations are critical to ensure that the system's performance meets the requirements for its

intended use and to guarantee the dependability of analytical results in line with ICH guidelines.[35]



	Peak Name	RT	Area	% Area	Height	USP Plate Count
1	Olmesartan	5.070	723725	27.54	77227	6845
2	Cilnidipine	6.381	1904035	72.46	184283	8830

	USP Tailing
1	1.4
2	1.2

Figure 3: System suitability for standard chromatogram.

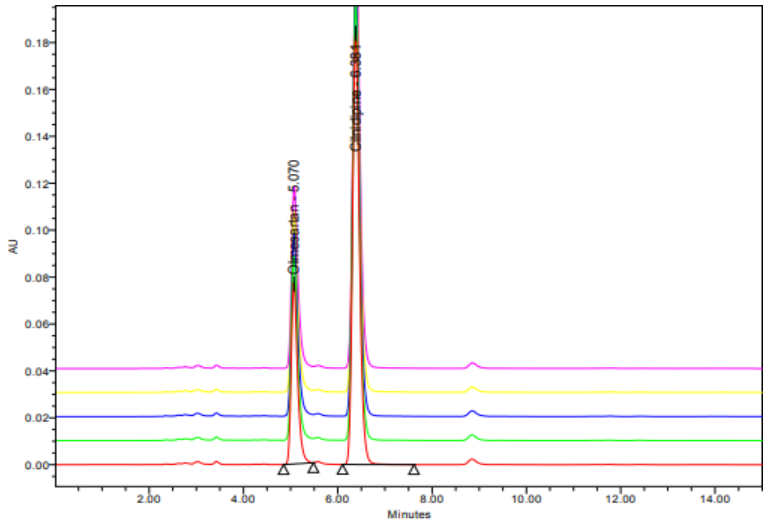


Figure 4: Overlay of System suitability for standard chromatograms.

Table 3: System suitability results.

		Clinidipine		Olmesartan Medoxomil	
		Retention Time	**Theoretical plates**	**Retention Time**	**Theoretical plates**
1	Mean*	6.384	8830	5.074	6845
2	Std. Dev	0.004		0.003	
3	% RSD	0.06		0.07	

***** Average of five replicate injections

Discussion: Since the theoretical plate count exceeds 2000, the tailing factor is below 2.0, and the % RSD is under 2.0%, the established method has successfully met the system suitability criteria.

3.2 SPECIFICITY

A strong degree of specificity guarantees that the chromatographic technique can effectively and accurately separate and recognize the target compound without disruption from other materials present in the sample.

Blank

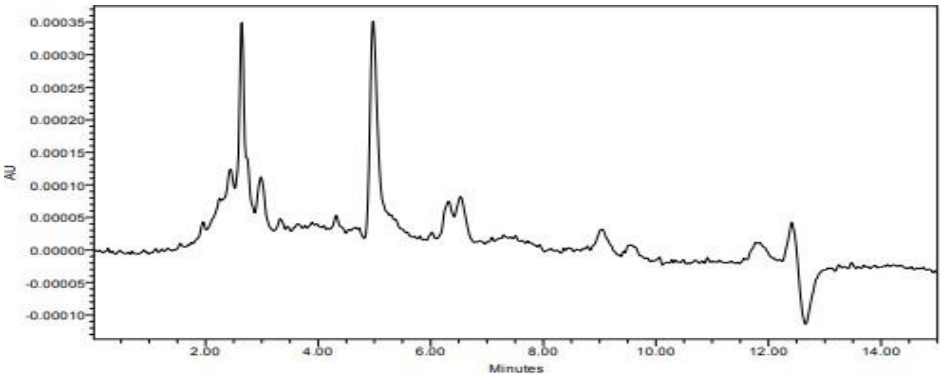


Figure 5: Blank chromatogram.

Discussion: The chromatogram for the blank showed no interference with the primary peak, indicating that the method is considered specific.

3.3 ACCURACY

The precision of an analytical method pertains to the degree to which the test results correspond with the actual value. To evaluate accuracy, solutions containing 50%, 100%, and 150% of the desired analyte concentration are introduced into the system, and the percentage recovery is calculated in relation to the anticipated values.

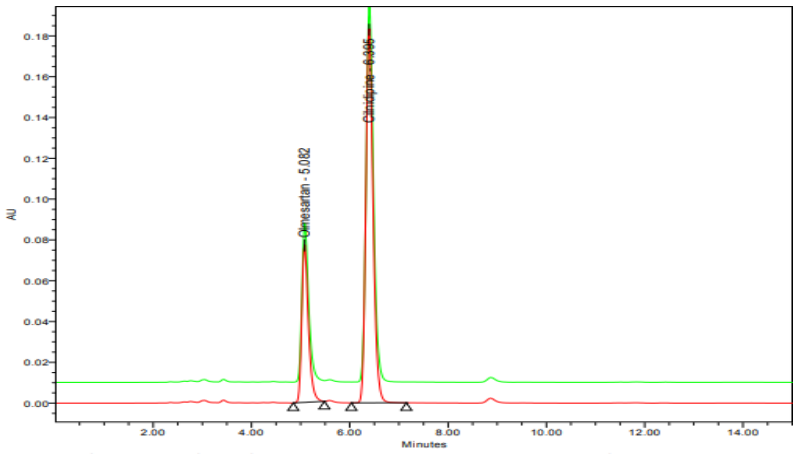


Figure 6: Sample solution chromatogram.

Table 4: Results for Accuracy.

S.No	Sample solution concentration*	Clinidipine		Olmesartan Medoxomil	
		% RSD	Recovery %	% RSD	Recovery %
1	50%	0.26	99.52%	0.36	100.41%
2	100%	0.26		0.70	
3	150%	0.02		0.18	

***** Average of three replicate injections

Discussion: The RSD percentage does not exceed 2.0%. Given that the acceptance criteria for percentage recovery fall between 98.0% and 102.0%, the method is considered accurate.

3.4 PRECISION

Consistency and reproducibility refer to obtaining the same results when analyzing the same sample multiple times under identical conditions. Precision quantifies the variability or scatter in these results and is expressed in statistical variables like Standard deviation (SD), Relative Standard Deviation (RSD) & Coefficient of variation (CV).

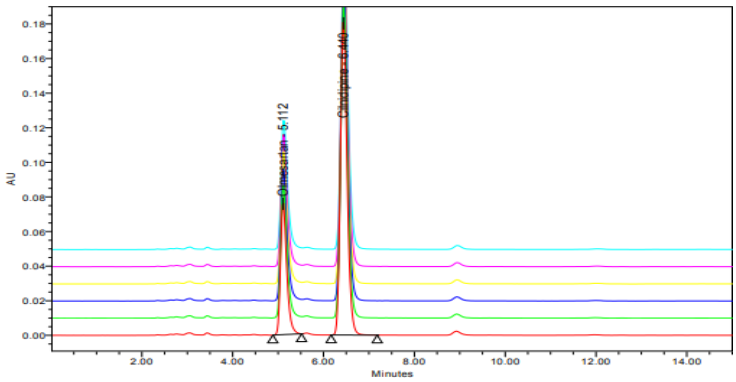


Figure 7: Overlay Precision chromatograms.

Table 5: Method precision results for Clinidipine and Olmesartan Medoxomil.

S. No	Peak Name*	Average	SD	% RSD
1	Clinidipine	1882461	0.32	0.3
2	Olmesartan Medoxomil	725288	0.53	0.5

***** Average of six replicate injections

Discussion: The percentage of RSD is not more than 2.0%. As the precision values are within the acceptance criteria for Clinidipine & Olmesartan Medoxomil, the method is said to be precise.

3.5 LINEARITY

Linearity in chromatography pertains to the correlation between the concentration or quantity of analyte injected and the response from the detector. A chromatogram that exhibits linearity would indicate that as the concentration of the analyte rises or falls, the detector response (measured by either peak area or height) alters in a proportional manner. This feature is crucial for the accurate and dependable quantification of substances in samples using chromatographic techniques. Five different concentrations of Clinidipine and Olmesartan Medoxomil were prepared, with each concentration being injected three times to evaluate linearity. A linearity graph was generated by plotting concentration on the x-axis and the average peak area on the y-axis.

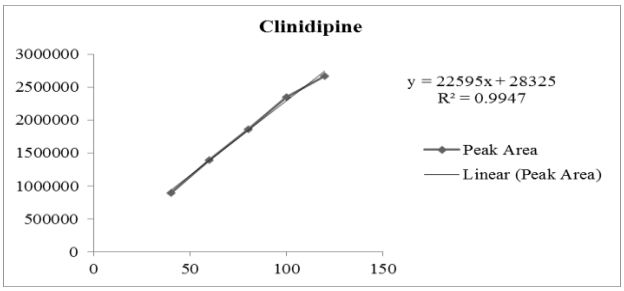


Figure 8: Linearity graph for Clinidipine.

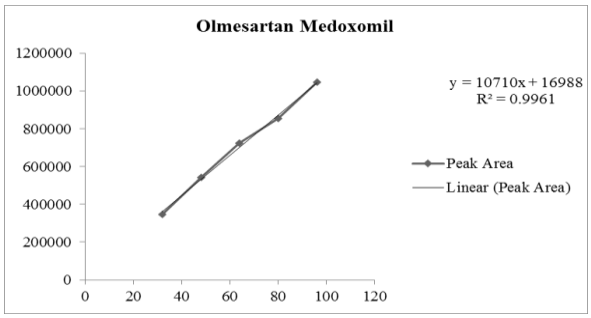


Figure 9: Linearity graph for Olmesartan Medoxomil.

Table 6: Results for linearity.

Clinidipine		Olmesartan Medoxomil	
Conc. in PPM*	**Peak Area**	**Conc. in PPM***	**Peak Area**
40	893556	32	345359
60	1396789	48	542225
80	1860729	64	723964
100	2354151	80	854754
120	2674367	96	1045914
Regression Equation	y = 22595x + 28325	Regression Equation	y = 10710x + 16988
Linearity Correlation Coefficient (R^2)	0.9947	Linearity Correlation Coefficient (R^2)	0.9961

***** Average of three replicate injections

Discussion: The R^2 values meet the acceptance criteria, which is no less than 0.99 for Clinidipine and Olmesartan Medoxomil, indicating that the method is linear.

3.6 RANGE

The range represents the span between the highest and lowest concentrations of the analyte in the sample where the technique has demonstrated precision, accuracy, and linearity.

Table 7: Range values for Clinidipine & Olmesartan Medoxomil.

Percentage of solution	% RSD for Clinidipine	% RSD for Olmesartan Medoxomil
50%	0.08%	0.11%
100%	0.45%	0.26%
150%	0.19%	0.33%

BRACKETING STANDARD

Bracketing is a technique used in analytical approaches where samples are evaluated at both the highest and lowest limits of a defined range to validate accuracy and precision throughout the entire spectrum.

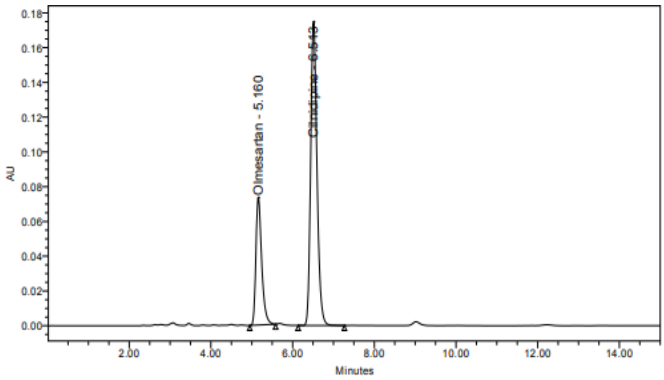


Figure 10: Sample solution Bracketing Standard chromatograms.

Method application to the analysis of Clinidipine & Olmesartan Medoxomil

Assay results are summarized in the below table with the marketed formulations.

Table 8: % Assay results data.

OLMIN 40 LN		
Name of the drug	**Labeled claim (mg)**	**%Assay***
Clinidipine	10	99.50
Olmesartan Medoxomil	40	99.01

***** Average of six replicate injections

SUMMARY AND CONCLUSION

Parameters	**Clinidipine**	**Olmesartan Medoxomil**
% Recovery in Accuracy	99.52%	100.41%
% RSD in Precision	0.3	0.5
Linearity Correlation coefficient	0.9947	0.9961
% Assay	99.50%	99.01%

The research successfully established and confirmed a reliable RP-HPLC method. This method employed a combination of ammonium acetate buffer, water, and methanol in a 20:10:70 ratio with isocratic flow, ensuring effective separation and quantification of both medications. Chromatographic analysis was conducted at a wavelength of 265 nm using a 250mm x 4.6mm, 5µm Welchrom column. System suitability tests, which evaluated parameters such as column efficiency, tailing factor, and percentage RSD, indicated adherence to the predefined criteria. The method was validated for specificity, accuracy, precision, linearity, and range, with all aspects meeting ICH approval standards. Recovery tests confirmed the method's precision and accuracy, yielding percentage recoveries between 98% and 102%. The primary goal of validation is to ensure that all processes and equipment in pharmaceutical manufacturing are utilized in a way that guarantees the product's safety, integrity, quality, and potency for public consumption. The technique demonstrated excellent

linearity for both drugs. The RP-HPLC method is dependable, precise, accurate, and reproducible, making it suitable for routine quality control of Clinidipine and Olmesartan Medoxomil in tablet formulations.

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