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METHOD DEVELOPMENT AND VALIDATION OF OLMESARTAN, CHLORTHALIDONE AND AMLODIPINE IN TABLETS DOSAGE FORMS BY USING RP-HPLC METHOD

Kundan Deore[1,2]*, Ujash Shah[2]

[1]*Department of Quality Assurance, Gangamai College of Pharmacy, Nagaon, Dhule-424005, Maharashtra, India*

[2]*Department of Pharmaceutical Science, Nootan Pharmacy College, Sankalchand Patel University, Kamana cross Road, Visnagar-384315, Gujarat, India*

*Corresponding Author: Kundan Deore, PhD Scholar, Nootan Pharmacy College, Sankalchand Patel University, Visanagar, Gujarat, India

Email Id- kundan2321994@gmail.com

Orchid ID- 0000-0002-4377-1863

ABSTRACT

A simple, accurate, precise and robust RP-HPLC method has been developed and validated for the simultaneous estimation of Olmesartan Medoxomil (OLM), Chlorthalidone (CHL) and Amlodipine (AML) in their combined tablet dosage form. The combination used for the Separation is Triolmesar CH 40. Separation was performed on a C18 column (4.6mm × 250 mm; 5 μm), with acetonitrile: 0.1% OPA pH 4.2 with TEA (60:40 V/V) flowing at 1 ml/min. Good sensitivity was found with UV detection at 254 nm. After method development the interference with the active compounds and excipients, repeatability and linearity were investigated. Retention times were found to be 2.30 min, 5.68 min, and 9.05 min. respectively, for OLM, CHL and AML. The method was validated over the analytical range from 40-200 μg/ml for OLM (r2=0.9996), 12.50-62.5 μg/ml CHL(r2=0.9995) and 5-25μg/ml for AML(r2=0.9999). This method showed good reproducibility and recovery with %RSD in the desired range. The proposed method can be successfully applied for the routine analysis of the OLM, CHL and AML drugs in their combine dosage form.

Keywords: Olmesartan Medoxomil (OLM), Chlorthalidone (CHL) and Amlodipine (AML), Reverse Phase High Performance Liquid Chromatography method, validation

INTRODUCTION

The most frequent co-occurring cardiovascular risk factor is hypertension. The primary cause of illness and death globally is coronary artery disease, or CAD. It puts a strain on the healthcare system and accounts for 24% of coronary heart disease and 57% of all stroke deaths in India[1-2]. In order to recover from these circumstances, knowledge and appropriate medical care are necessary. The development and validation of analytical methods are essential to the process of finding new drugs and creating dosage forms[3]. The creation of new analytical techniques is desperately needed to improve the quality of newly developed, developing medications. Because of its precision and reliable outcomes, HPLC is the most used analytical technique for estimating drugs both qualitatively and quantitatively[4]. Olmesartan Medoxomil (OLM) is (5-methyl-2-oxo-1, 3-dioxol-4-yl) methyl 5-(2- hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] imidazole-4-carboxylate. Functioning as an angiotensin II receptor blocker, Olmesartan Medoxomil serves as an antihypertensive agent [5-6]. Chlorthalidone (CHL) is a sulphamyl benzophenone derivative, chemically known as [2- chloro-5-(1-hydroxyl-3-oxo-2, 3-dihydro-1Hisoindol-1-yl) benzene-1-sulfonamide]. This compound is utilized as a thiazide diuretic with antihypertensive properties [7-9].Amlodipine, is chemically known as 3,5-Pyridinecarboxylic acid, 2-((aminoethoxy)methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl,3-ethyl-5-methylester. Amlodipine induces relaxation in peripheral and coronary vascular smooth muscle. It produces coronary vasodilation by inhibiting the entry of Ca ions into the voltage-sensitive channels of the vascular smooth muscle and myocardium during depolarization. Additionally, it increases myocardial O2 delivery in patients with vasospastic angina [10-12]. It is commercially available with fixed combination of OLM (40 mg), CHL (12.5 mg) and AML (5 mg) is available in the market as tablet formulation. The literature survey showed a very few chromatographic methods [13-20]. The present paper describes the development and validation of RP-HPLC analytical method for simultaneous estimation of OLM, CHL and AML in their combined pharmaceutical dosage form. The proposed method is optimized and validated as per ICH guidelines [21].

MATERIALS AND METHODS

Equipment

A Shimadzu 1800 series, was used for chromatographic separation. Chromatographic separation was carried out on an Agilent C18 column (4.6mm × 250 mm; 5 μm partical size).

Reagents and chemicals

API of Olmesartan, Chlorthalidone, and Amlodipine were obtained from Reliable's Shree Industrial Training Centre in Jalgaon. The table dosage form "Triolmesar CH 40" having combination of OLM (40 mg), CHL (12.5 mg) and AML (5mg) was procured commercially from the local market. All the chemicals used were of analytical grade.

Chromatographic conditions

Degradation experiments, validation, and method development were conducted using the Young Lin HPLC system. Data collection was done using the HPLC program YL 9100. Mobile phase consisting of acetonitrile: 0.1% OPA pH 4.2 with TEA (60:40 V/V) was used

in an isocratic mode. The flow rate was maintained at 1ml/min and the injection volume was 20μL. The UV detection was performed at 254 nm and the separation was achieved at room temperature[22-23].

EXPERIMENTAL

Preparation of Standard Solution

A 10 ml volumetric flask is filled with a 0.1 ml aliquot that has been transferred from a standard stock solution containing concentrations of 4000 μg/ml for Olmesartan, 500 μg/ml for Chlorthalidone, and 50 μg/ml for Amlodipine. The mobile phase is then used to adjust the volume to 10 ml[24]. After this dilution, the solution's final concentrations of amlodipine (5 μg/ml), chlorthalidone (12.5 μg/ml), and olmesartan (40 μg/ml) are obtained[25].

METHOD VALIDATION

The ICH Q2 B recommendations were followed in the validation of the analytical processes to assess the robustness, specificity, linearity, precision, accuracy, and system applicability[26-27].

System suitability

System suitability tests were performed to see whether the suggested method's resolution was sufficient and whether it could be repeated. To ensure system appropriateness, a 100% concentration sample containing 80 μg/ml of OLM, 25 μg/ml of CHL, and 10 μg/ml of AML was injected into an HPLC system. The asymmetry factor, number of theoretical plates, and retention duration were among the characteristics that were examined by six injections of conventional drug solutions.

Linearity

The ability of an analytical procedure to yield findings that are exactly proportionate to the analyte concentration in the sample is referred to as linearity. Standard calibration solutions were used to construct the standard calibration, with concentration ranges of 40-200 μg/ml for OLM, 12.5-62.5 μg/ml for CHL, and 5-25 μg/ml for AML. Under ideal chromatographic conditions, each solution was chromatographed for 12 minutes. Then, for all three medications, a calibration curve showing peak area responses versus concentration was plotted. The calibration curves' linear least square regression analysis proved the peak area responses' linearity with respect to concentration.

Precision

Separate tests of test samples were performed to assess the accuracy of the suggested approach. Assay values were collected, and the relative standard deviation (RSD %) was computed. The method's precision was measured by evaluating both intra-day (repeatability, measured by evaluating the drug solutions on the same day) and inter-day (measured by injecting the samples on three successive days) variations. Duplicates at 100% concentration of 40 μg/ml of OLM, 12.5 μg/ml of CHL, and 5 μg/ml of AML were injected during the study [28-29].

Accuracy

Recovery tests using the conventional addition procedure were carried out to verify the suggested method's correctness. This approach involved adding a known amount of a pure medication to pre-analyzed sample solutions at three distinct concentrations (i.e., 80%, 100%, and 120%), and then calculating the recovery of OLM, CHL, and AML for each concentration.

Selectivity/specificity

Under ideal circumstances for the suggested technique, the impact of a variety of excipients and other additives often used in the formulation of OLM, CHL and AML were examined. The analytical method's selectivity is its capacity to yield an analyte response even in the face of external interference. To demonstrate that the selected approach was both selective and specific, these tests were performed.

LOD and LOQ

The analytical method's limit of detection (LOD) is its capacity to identify the analyte's lowest concentration. Lowest amount of analyte that may be quantitatively examined with reasonable accuracy and precision is known as the limit of quantification, or LOQ. In accordance with ICH rules, it was computed using the calibration curve's slope and blank response. The S/N ratio (signal/noise), which is commonly employed for HPLC procedures, provides the basis for determining LOQ. For calculating LOD, a signal-to-noise ratio (S/N) of three was usually accepted, and for estimating LOQ, a signal-to-noise ratio (S/N) of ten. Based on the slope and response standard deviation, LOD and LOQ were computed[30].

Robustness

Robustness was assessed by making small, intentional adjustments to chromatographic parameters, such as the ratio of flow rate and the usage of various columns. The purpose of the robustness research was to assess the impact of a slight but intentional change in the chromatographic condition. By making minor adjustments to the settings, the robustness was verified. 1) Change in flow rate 2) Change in mobile phase 3) Change in wavelength was evaluated using system suitable parameters and the sample solution was injected after each adjustment[31].

Assay of marketed formulation

The tablet test was carried out by injecting a 20 μl volume of standard and sample solution of OLM, CHL, and AML in triplicates into an HPLC system. The assay's mean, standard deviation, and percent RSD of the sample peak area were computed and reported[32-33].

**RESULTS AND DISCUSSION**
System suitability

The current work aims to develop and verify an RP-HPLC technique that may be applied to routine pharmaceutical formulation analysis of OLM, CHL, and AML. Initially, the RP HPLC approach was used to optimize the chromatographic conditions for the

estimation of the medicines in a chosen multicomponent dosage form. The most appropriate combination of all was found to be a binary mixture of acetonitrile: 0.1% OPA pH 4.2 with TEA (60:40 V/V) as the mobile phase, as the chromatographic peaks produced were free from tailing and more well defined. The ICH criteria were utilized to validate the chromatographic technique that was developed. OLM, CHL, and AML provided retention durations of 2.30 min, 5.68 min, and 9.05 min, in that order. Figure 1 displays an optimized chromatogram that illustrates the separation of OLM, CHL, and AML at various retention times.

Six injections of OLM, CHL, and AML at 100% concentration were used to test the system's compatibility in the HPLC system. For the medications, which were within the parameters, the tailing factor was less than 2 and the theoretical plate number was higher than 2000 (Table 1).

Table 1. Results of System Suitability Parameters

Drug	Resolution	Retention Time	Area (mAU x min) (USP)	Asymmetry (USP)	Theoretical Plate
Olmesartan	0.0000	2.3167	6790.7065	1.60	3186.5
Chlorthalidone	12.6875	5.7000	808.2017	1.1875	8007.3
Amlodipine	8.4762	8.6667	466.0803	1.0833	10412.7
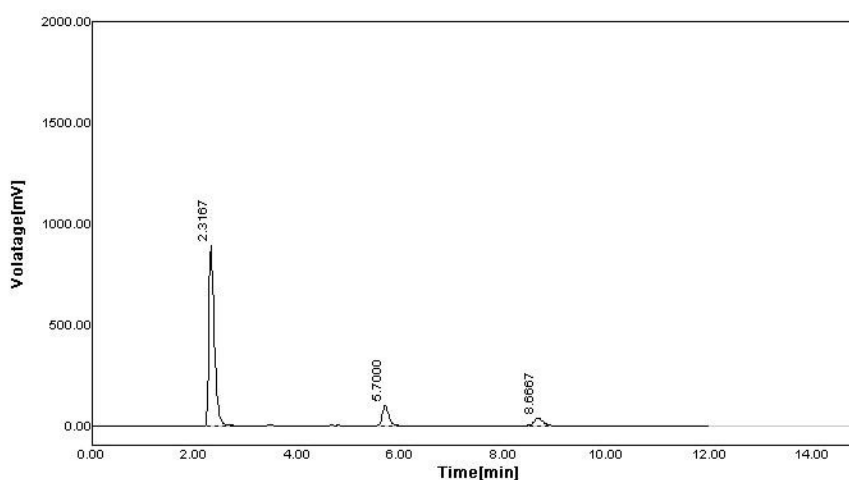
Figure 1. Optimized chromatogram of OLM, CHL and AML by RP-HPLC

Linearity

The linearity and range were found to be 40-200 μg/ml for OLM, 12.5-62.5 μg/ml for CHL and 5-25 μg/ml for AML. The correlation coefficients of OLM, CHL and AML were found to be 0.9996, 0.9995 and 0.9999, respectively (Figure 2) for OLM, CHL and AML thus indicate good linearity in the specified concentration range (Table 2A and 2B). The calibration curves for OLM, CHL and AML is shown in respectively, in Figure 3A, 3B and 3C.

Table 2 (A). Results of Linearity

Sr. No.	Olmesartan (µg/ml)	Peak Area± S.D.	Chlorthalidone (µg/ml)	Peak Area± S.D.	Amlodipine (µg/ml)	Peak Area± S.D.
1	40	3204.67± 6.43	12.5	370.39± 2.70	5	243.17± 1.73
2	80	6799.08± 26.25	25	801.54± 1.35	10	457.77± 6.51
3	120	10191.5± 5.41	37.5	1203.54± 6.62	15	679.62± 5.05
4	160	14097.78± 24.03	50	1672.91± 0.73	20	889.34± 7.07
5	200	17725.15± 78.69	62.5	2073.81± 0.17	25	1113.53± 14.16

Table 2 (B). Regression analysis data for OLM, CHL and AML

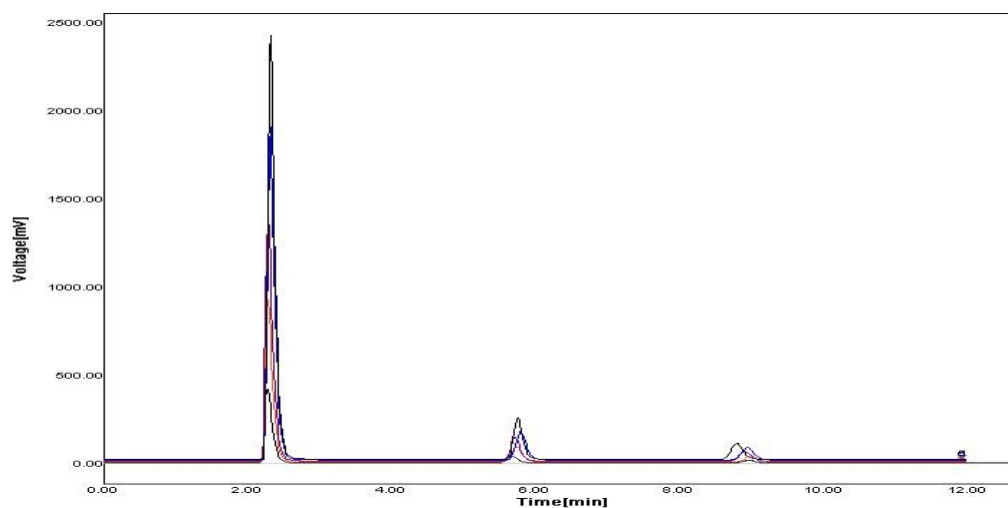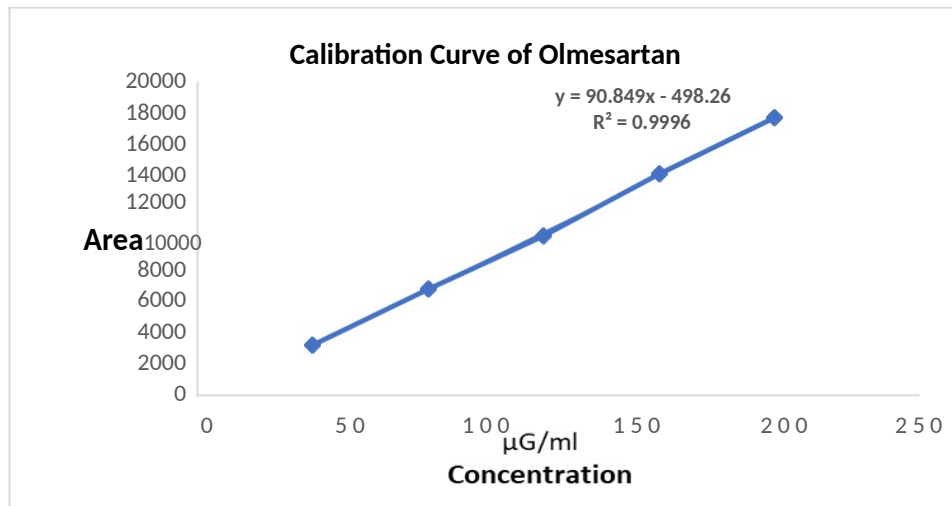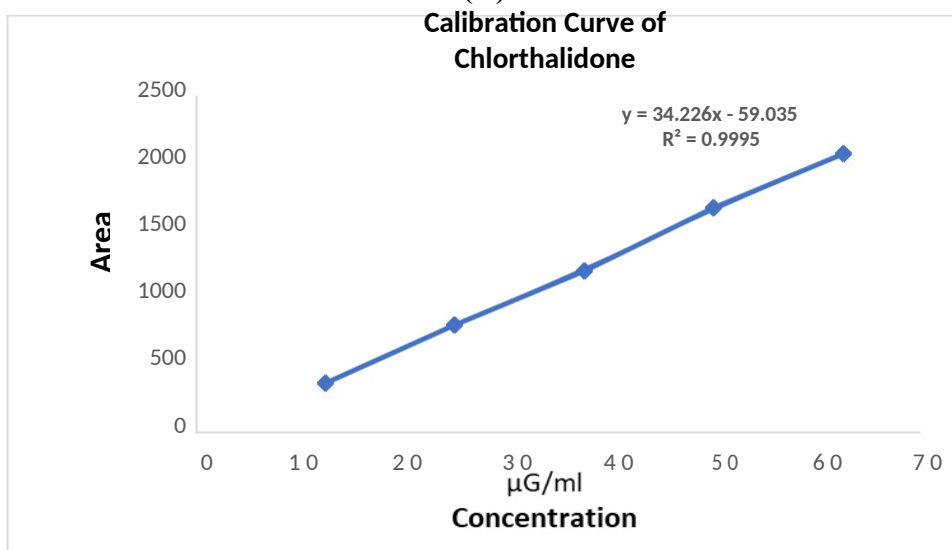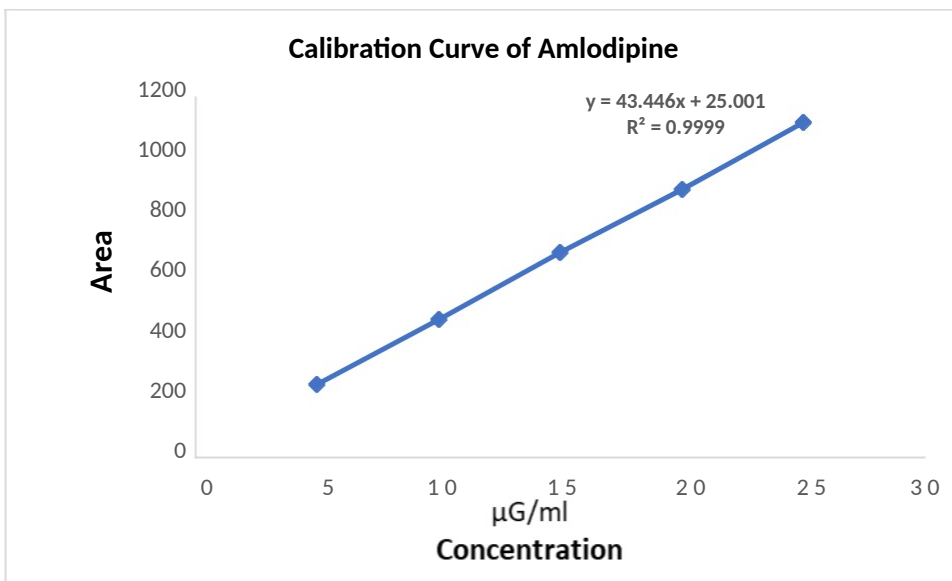| Regression Analysis	Olmesartan	Chlorthalidone	Amlodipine
Regression equation	y = 90.849x - 498.26	y = 34.226x - 59.027	y = 43.446x + 25.002
Correlation co-efficient (R^2)	0.9996	0.9995	0.9999
Slope(S)	90.849	34.226	43.446
Intercept (б)	498.26	59.027	25.002

Figure 2. Overlain linearity spectra of OLM (40-200 µg/ml), CHL (12.5-62.5 µg/ml) and AML (5-25 µg/ml)

(A)

(B)

(C)

Figure 3. Calibration curve of (A) Olmesartan (B) Chlorthalidone (C) Amlodipine

Precision

A detailed investigation was carried out to determine intraday and interday differences. The correspondence response was estimated three times on the same day and three different days for three different concentrations in the intraday and interday precision study of OLM, CHL, and AML. The results are reported in terms of percentage relative standard deviation (% RSD), but all of the results fall within acceptance limits (RSD < 2). The minimum variation in the % RSD values obtained indicated that the present method is precise (Table 3 and 4).

Table 3. Results of Intra-day Precision

OLM	CHL	AML	OLM	CHL	AML	OLM	CHL	AML	OLM	CHL	AML
40	12.5	5	3177.15±16.53	375.11±7.17	243.20±4.62	101.13±0.19	101.44±0.18	100.46±0.28	0.52	1.91	1.90
120	37.5	15	10191.79±8.01	1207.14±5.25	688.25±3.09	98.06±2.14	98.67±1.67	101.73±0.31	0.08	0.43	0.45
200	62.5	25	17733.99±10.66	2075.56±10.19	1117.04±6.25	100.35±1.37	99.79±1.21	100.52±.42	0.06	0.49	0.56
Conc. (µg/ml)			**Mean Peak Area*± S.D.**			**Mean %Assay* ± S.D.**			**%RSD**		

Table 4.Results of Inter-day Precision

OLM	CHL	AML	OLM	CHL	AML	OLM	CHL	AML	OLM	CHL	AML
40	12.5	5	3144.09±21.84	371.11±2.38	246.10±3.18	100.23±0.126	100.48±0.17	101.79±.02	0.69	0.64	1.29
120	37.5	15	10186.62±4.53	1201.64±7.21	692.75±4.97	98.02±0.19	98.21±1.10	102.47±0.03	0.04	0.60	0.72
200	62.5	25	17726.52±28.38	2076.56±6.97	1106.54±8.70	100.31±0.12	99.84±0.12	99.56±1.10	0.16	0.34	0.79
Conc. (µg/ml)			**Mean Peak Area*± S.D.**			**Mean %Assay* ± S.D.**			**%RSD**		

Accuracy

Recovery studies were conducted by adding a standard medication to pre-analysed samples in order to verify the accuracy of the procedure. The standard addition technique was used to test the accuracy of the suggested approach in triplicate at 80%, 100%, and 120% recovery levels to the pre-analyzed sample. After the additional standard was computed, the recovery percentages for OLM, CHL, and AML were determined to be 100.57-101.23%, 100.57-101.57 %, and 100.19-101.58 %, respectively. For three medications, the suggested

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RSD was less than 2, indicating that the method's accuracy compared favorably to the label claim (Table 5).

<div align="center">Table 5. Results of Recovery study (Accuracy)</div>

OLM	CHL	AML	OLM	CHL	AML	OLM	CHL	AML	OLM	CHL	AML
40	12.5	5	32	10	4	32.18±0.421	10.06±0.010	4.01±0.002	100.57	100.57	100.19
40	12.5	5	40	12.5	5	40.28±0.636	12.69±0.046	5.05±0.012	100.70	101.54	101.02
40	12.5	5	48	15	6	48.59±0.494	15.24±0.061	6.09±0.004	101.23	101.57	101.58
Amt. of sample (µg/ml)			**Amt. of drug added (µg/ml)**			**Amt. Recovered Mean ± S.D (µg/ml)**			**%Recovery**		

Selectivity/specificity

By examining the chromatograms that were taken from the sample solution, the specificity of the technique was evaluated. Since none of the excipients interacted with the target analyte, the approach was confirmed to be specific. Therefore, it was determined that the approach was appropriate for examining the commercial medication formulation (**Table 6**).

<div align="center">Table 6.   Results of Repeatability</div>

Sr. No.	Drug	Conc.(µg/ml)	Mean Peak Area	Mean % Assay	%RSD
1	Olmesartan	80	6783.36	100.19	0.15
2	Chlorthalidone	25	804.24	100.88	0.70
3	Amlodipine	10	455.40	99.00	0.47

LOD and LOQ

The average slope and standard deviation of the intercept on the y axis of the calibration curve were used to compute the parameters LOD and LOQ. The results showed that the LOQ for OLM, CHL, and AML was 3.099 µg/ml, 0.674 µg/ml, and 1.590 µg/ml, respectively, while the LOD was determined to be 1.022 µg/ml, 0.222 µg/ml, and 0.524 µg/ml, respectively. This demonstrated the method's excellent sensitivity (Table 7).

<div align="center">Table 7. Results of LOD and LOQ</div>

Regression Analysis	Olmesartan	Chlorthalidone	Amlodipine
LOD	1.022884126	0.222725413	0.524858445
LOQ	3.09964887	0.6749255	1.59048014

Robustness

By conducting the study under environments that changed the composition of the mobile phase, the detection time, and the impact on the drug area, the robustness of the

approach was assessed. The test for robustness involved varying the composition of the mobile phase (water + acetonitrile, 61+39 and 59+41 %v/v), wavelength (254±1) and the flow rate (± 0.1 ml/min). For each condition, a 100% concentration solution was made and injected in triplicate; the percentage RSD determined for each condition was found to be less than 2 (Table 8).

Table 8. Results of Robustness study

Condition	Variation	OLM			CHL			AML		
		Area Mean	SD	% RSD	Area Mean	SD	% RSD	Area Mean	SD	% RSD
Flow Rate (1±0.1ml/min)	0.9	7573.31	30.33	0.40	826.07	0.61	0.07	491.72	1.46	0.29
	1.1	6884.7	44.41	0.64	795.13	2.86	0.36	395.79	1.04	0.26
Change in Wavelength(254±1nm)	253	6891.5	14.15	0.20542	785.18	1.89	0.24	416.97	1.15	0.27
	255	6805.94	55.42	0.81	879.6	2.02	0.23	452.6	1.60	0.35
Change in Mobile Phase (60+40)	61+39	6979	55.92	0.80	880.4	1.65	0.18	444	1.90	0.42
	59+41	6778.8	70.81	1.04	872.98	1.03	0.11	453.2	0.74	0.16

Assay of marketed formulation

After injecting a total of 20 μl of sample solution into the chromatographic apparatus, the peak response was determined. Three injections of the solution were made into the column. By comparing the test and standard areas, the quantity in each tablet was determined, and the results for OLM, CHL, and AML were 100.92%, 101.49%, and 99.64%, respectively. The findings are displayed in a Table 9.

Table 9. Results of Assay (n=3) of marketed formulation

Tablet	Label Claim (in mg/ml)	Conc.	Area of Standard	Area of Sample	% Assay
Triolmesar CH 40	Olmesartan	160ppm	14723.487	14858.96	100.92
	Chlorthalidone	50ppm	1664.413	1689.245	101.49
	Amlodipine	20ppm	898.755	895.534	99.64

CONCLUSION

Olmesartan, chlorthalidone, and amlodipine dosage forms may all be determined simultaneously with the RP-HPLC test technique, which has been designed and validated for this purpose. The RP-HPLC method was created and validated, and it was discovered to be cost-effective because of the low solvent consumption (1 ml/min) and short run time (12 min), which resulted in an environmentally friendly chromatographic process that made it possible to analyze a large number of samples quickly. This method's wide range of linearity

and use of an easily accessible mobile phase have been discovered to make it superior to previously described approaches. Therefore, without causing any interference, the aforementioned analytical approach may be employed for routine quality control analysis of OLM, CHL, and AML finished goods concurrently.

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