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ORIGINAL ARTICLE

Development and Validation of a Reverse Phase-High Performance Liquid Chromatography – Mass Spectroscopy (LC-MS) Method for Simultaneous Estimating Chlorthalidone and Olmesartan in Tablet Formulation and Bulk Product

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ABSTRACT
A new, accurate, precise, and durable RP-HPLC method with sensitive features has been developed for the simultaneous assessment of Chlorthalidone (CHL) and Olmesartan (OLM) in both bulk and tablet format. An Agilent C_{18} column with a size of 100 mm × 2.1 mm, 3 µm was used to estimate the solutes. CHL and OLM were eluted in a 15-minute gradient trial at a flow rate of 1 ml/min with an ambient column temperature of 25°C and monitored at a wavelength of 256 nm using Water: Methanol in a 95:5 v/v ratio. The retention times of CHL and OLM were found to be 8.162 minutes and 10.106 minutes, respectively. The Q2A and Q2B validations of the analytical method demonstrated good linearity throughout the concentration ranges of 0-10 µg/mL for CHL and 0-10 µg/mL for OLM, with r^2 of 0.994 in both cases. High accuracy, excellent precision (inter-day and intra-day), and remarkable resilience values were also shown by the technique. The suggested analytical method proved precise, accurate, and robust for frequent analysis of the drug combination in bulk and tablet forms.
Keywords: *Chlorthalidone, Olmesartan, RP-HPLC, Mass spectroscopy, Simultaneous estimation, Validation.*

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INTRODUCTION

Olmesartan medoxomil (OLM), chemically is [(5-methyl-2-oxo-1,3-dioxol-4-yl)-methyl-5-(2-hydroxypropan-2-yl)-2-propyl][(4-(2(-2-4-tetrazole-5-yl)phenyl]phenyl]methyl]-imidazole-4-carboxylate (Figure 1A). It is an anti-hypertensive drug that blocks the vasoconstrictor effect of angiotensin-II by selectively blocking the binding of angiotensin–II to the angiotensin-1 (AT1) receptor in vascular smooth muscle [1]. Chlorthalidone (CHL), chemically is 2-chloro-5-[(1-*R*,*S*)-1-hydroxy-3-oxo-2-dihydro-1H-isoindol-1-yl] benzenesulfonamide (Figure 1B). It is a diuretic molecule that inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of the henle [2].

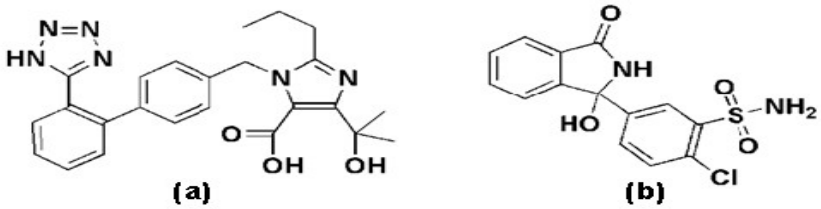

Figure 1. Structure of drugs(A) Chlorthalidone and (B) Olmesartan.

The mixture of the two drugs is recommended in case of hypertension with renal failure conditions. OLM and CHL are official in IP and BP and both describes a method for their assay. Literature survey has revealed that many analytical methods are specified for the determination of OLM and CHL as individual and combined dosage form with other combination of drugs, UV-Vis [3], RP-HPLC [4], HPTLC [5], etc. Abdullah *et al*. [6] reported spectrophotometric determination of chlorthalidone in pharmaceutical formulation using different order methods. A new method was developed for the estimation of telmisartan and CHL using first order derivative spectrophotometry [7]. Similarly, method was developed for the simultaneous estimation of atenolol and CHL in bulk and in combined tablet dosage form. Interestingly, few novel spectrophotometric methods for simultaneous determination of Chlorthalidoneand OLM in tablet dosage form have also been reported [8-9].

However, no works have been reported so far for a Reverse Phase-High Performance Liquid Chromatography (RP-HPLC)-coupled Mass Spectroscopy method for simultaneous estimation of OLM and CHL in bulk as well as pharmaceutical dosage form.

MATERIAL AND METHODS

Materials

Ankaleshwar, Gujarat-based Purechem Pvt. Ltd. provided a sample of CHL and OLM as a present. The OLSAR CH® Tablet (containing 40 mg of OLM and 12.5 CHL) was supplied by Torrent Pharmaceuticals Ltd., Mumbai. HiMedia Ltd., Mumbai provided analytical quality chemicals and HPLC grade solvents for the study.

Instruments

A Shimadzu® AUW220D balance was used for the weighing (Kyoto, Japan). The pH was measured using a VSI® VSI-1B digital pH meter (Mohali, India). The sonication was done using a Transonic Digital S sonicator (Mumbai, India). The method was developed using a reverse-phase HypersilGold C_{18} column with a particle size of 3 µm and a dimension of 100 mm × 2.1 mm, which was connected to an Agilent®6200 Gradient UPLC system TOF-6500 Series with a QTOP detector 2996 and a manual rheodyne injector (05µL loop), all of which were controlled by Chemstation v.2 software.

Selection of the mobile phase

The mobile phase must be carefully selected for the elution of the solutes. The mobile phase was selected based on theoretical plates, peak purity index, and peak symmetry. The experiment started with buffer systems and an eluent like methanol, acetonitrile, or other solvents. Low-intensity peaks with a lot of tailing were produced by elution with an equal combination of buffer KH_2PO_4 and methanol. Although this was an improvement over the previous experiment, the combination of KH_2PO_4 buffer (pH 4.8) with acetonitrile resulted in the formation of a broad peak with tailing. When employed in an equal ratio with methanol, the peak symmetry improved considerably and tailing was reduced when the buffer was replaced with orthophosphoric acid (OPA) (0.05%), but it was still inadequate to elute the solutes. Water was combined with Methanol to produce a crisp peak with a good Gaussian peak. The 95:5 v/v ratio generated the most theoretical plates as well as the greatest peak purity index. The mobile phase was degassed under vacuum before being filtered using a 0.45 µm membrane filter. Allowing the mobile phase to equilibrate until it achieved a stable baseline was permitted.

Chromatographic conditions

CHL and OLM were eluted at a flow rate of 1 ml/min with an ambient column temperature of 25°C in a 10-minute gradient trial and monitored at a wavelength of 256 nm using a 95:5 v/v Water: Methanol.

Preparation of analytical solutions

Preparation of mobile phase

Water was thoroughly mixed with methanol in a 95:5 v/v ratio. After that, the solution was degassed for 5 minutes with sonication before being filtered under vacuum through a 0.45 µm membrane filter.

Standard preparation

In a 10 mL dry volumetric flask, a precise quantity of 40 mg CHL and 10 mg OLM were introduced. Sufficient amount of mobile phase was added to dissolve the drug to get a standard stock solution of 400 µg/mL and 100 µg/mL concentrations. The aforementioned content was sonicated for 10 minutes and the volume was made up to 10 mL.

Sample preparation

The average weight of 20 tablets was determined after they were properly weighed. A weight equal to a tablet was transferred to a 100 mL volumetric flask and half-filled with the diluent. The contents were sonicated for 20 minutes and then filtered to produce 10 mg/mL of OLM.

Method validation

The technique was verified using the Q2A and Q2B guidelines from the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), as well as guidance from the USFDA.

Linearity and Range

The linearity of the technique was tested using five different solute concentrations, ranging from 0 to 10 µg/mL for CHL and 0 to 10 µg/mL for OLM. The solutions were prepared with the diluent and an equal quantity was injected into the HPLC equipment to determine the peak area. On a linearity graph, the concentration and average area of each solute were plotted. The r^2 value of the regression coefficient was computed as well[10].

Accuracy

The accuracy of the HPLC system was tested by spiking the reference drug solutions at concentrations of 80%, 100%, and 120% (recovery). The experiment was repeated three times, with the results given as % recovery % relative error based on the concentrations used[11].

Precision

The precision of the suggested method was tested in terms of inter-day and intra-day variability by spiking concentrations of 40%, 60%, and 80% six times in a single day (intra-day) and on three different days (inter-day). % relative error precision was used to describe the data[12].

Robustness

The method's robustness was evaluated by varying the mobile phase composition by 1% v/v (i.e. 71:29 % v/v and 69.31 % v/v), flow rate by 0.1 mL/min (i.e. 0.6 mL/min and 0.7 mL/min), and wavelength by 1 nm (i.e. 256 nm and 258 nm), while keeping all the other chromatographic parameters fixed [13].

Systems suitability parameters

The analytical method's repeatability profile was determined by injecting five times the standard solution and monitoring data such as retention length, peak area, theoretical plates, and tailing factor [14].

Limit of detection

Although it is not necessary to define the exact amount, the limit of detection (LOD) is the lowest concentration that any analytical method can detect [15].

The limit of detection (LOD) was determined by the formula:

$$\mathbf{LOD = 3.3\ (\sigma\ /\ S)}$$

Where, $\boldsymbol{\sigma}$ = standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

Limit of quantification

The limit of quantification is the smallest amount that can be measured with a given degree of accuracy and precision using any analytical method (LOQ) [16].

The limit of quantification (LOQ) is determined by the formula:

$$\mathbf{LOQ = 10\ (\sigma\ /\ S)}$$

Where, $\boldsymbol{\sigma}$ = standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

Because there were no previous similar methods, the new methodology was entirely based on trial and error. However, considerable influence was drawn from earlier reports when deciding on the stationary phase. The reverse phase C_{18} stationary phase from Agilent® was utilized, with a particle size of 3 µm and a diameter of 100 mm × 2.1 mm i.d. The mobile phase Water: Methanol in the ratio 95:5 v/v was utilized for the elution after several continuous trials. Peak tailing was minimized and the analytical method's robustness was significantly enhanced by keeping the mobile phase at a low pH. The use of acidic pH was justified to a greater extent because high basic pH caused dissolution in silica-based reverse-phase columns. The pH of the mobile phase and the pKa of the solute were also found to be in close agreement, enabling them to remain in the unionized state. As a consequence, the pH value was chosen based on two units.

The elution was place on an Agilent® C_{18} column in isocratic mode for 12 minutes with a mobile phase of 95:5 v/v Water: Methanol. The flow rate was maintained at 1 mL/min, the column temperature at 25°C, and the detection wavelength at 256 nm. The retention times for CHL and OLM were 8.162 minutes (**Figure 2A**)and 10.106 minutes(**Figure 2B**), respectively. The mass spectra of both the drugs; CHL (**Figure 2C**) and OLM (**Figure 2D**) demonstrated base peaks corresponding to their molecular masses (337 and 557, respectively).

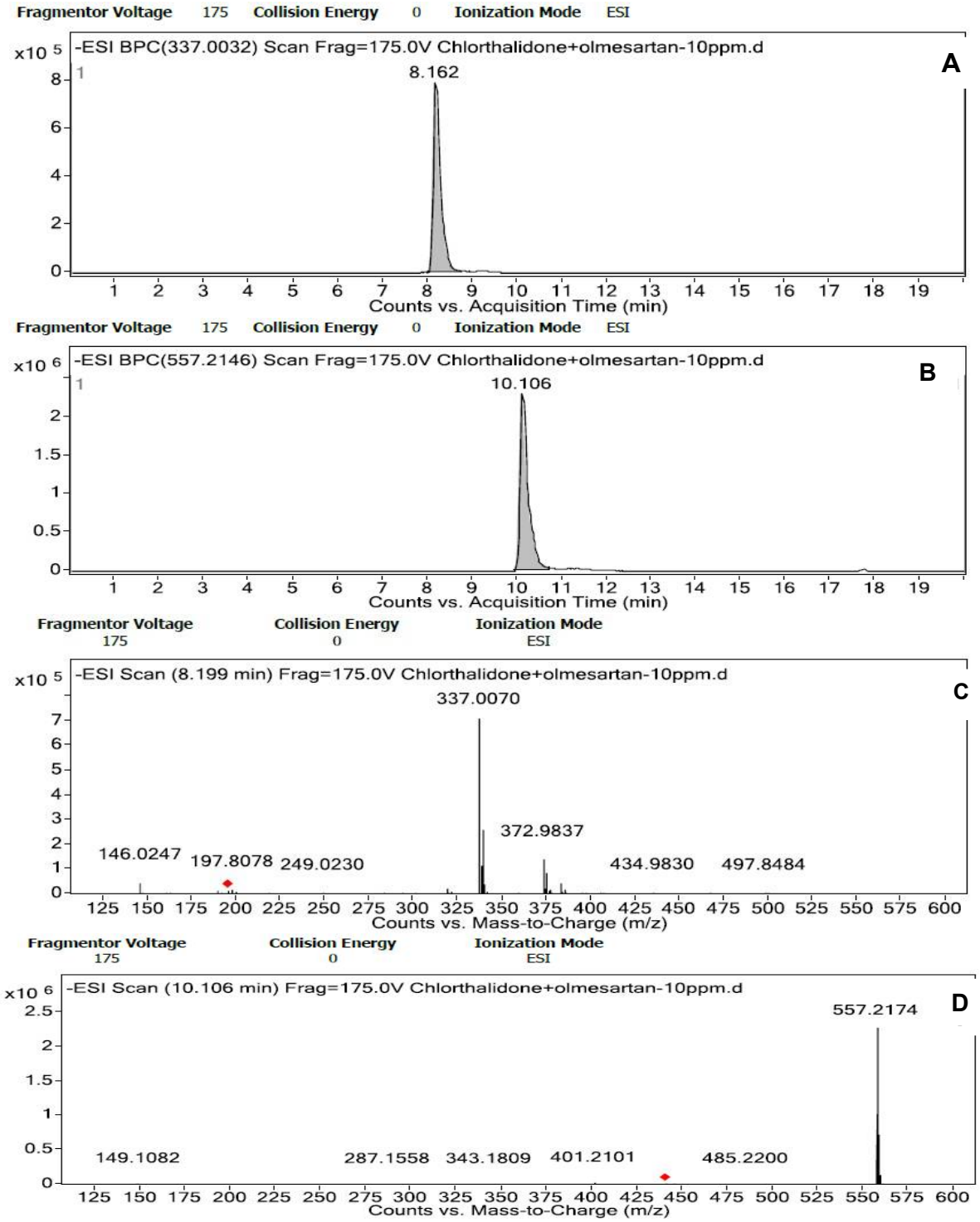

Figure 2. Chromatogram of (A)Chlorthalidone and (B)Olmesartan and mass spectral data of (C)Chlorthalidone and (D)Olmesartan, after method optimization.

Analysis of sample

In the tablet sample solution, CHL had a retention time of 8.173 minutes while OLM had a retention time of 10.119 minutes (**Figure 3A**). The mass spectra of both the drugs; CHL(**Figure 3B**) and OLM(**Figure 3C**) demonstrated base peaks corresponding to their molecular masses (337 and 557, respectively). This clearly showed that the suggested analytical method for routine medicine combination analysis in bulk and tablet forms was exact, accurate, and robust.

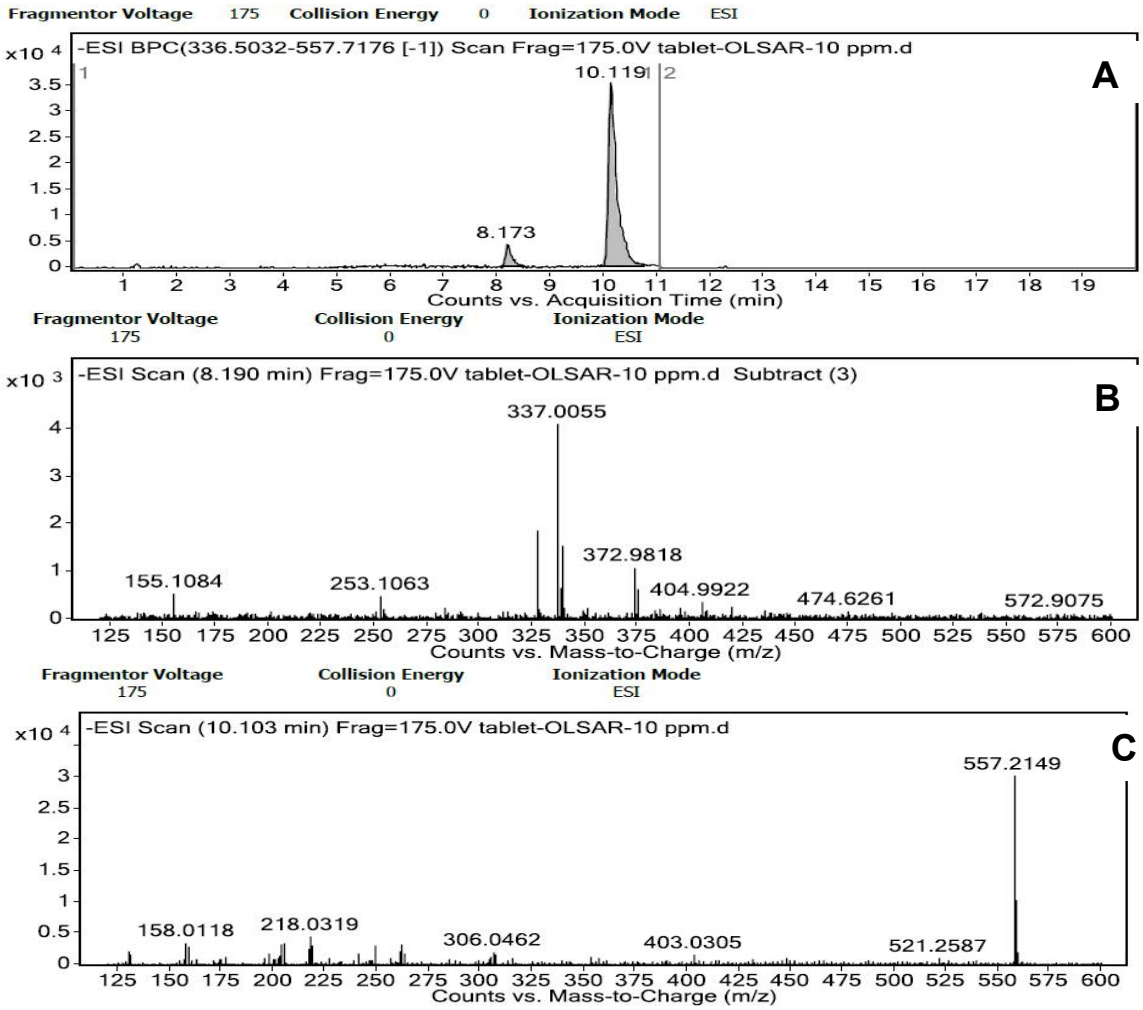

Figure 3. Chromatogram of (A)Chlorthalidone and Olmesartan and mass spectral data of (B)Chlorthalidone and (C)Olmesartan, after sample analysis.

Method validation

Linearity and range

Throughout the dose and peak area ranges of 0-10 g/mL for CHL and 0-10 g/mL for OLM, there was very high linearity, with linear regression equations of y = *88956x + 276.4*and y = *59742x + 143.7*, respectively. The regression coefficient values were 0.994 in both cases, suggesting that there was a high level of linearity (**Figure 4**).

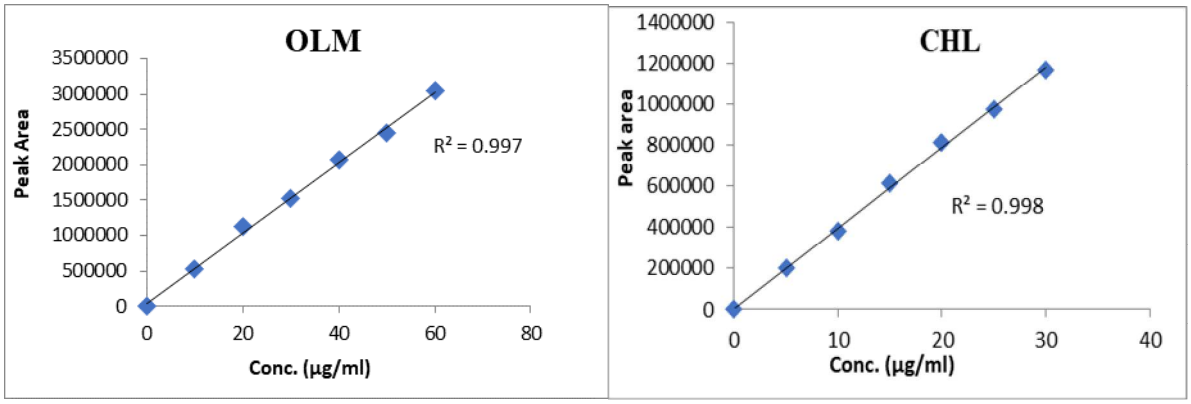

Figure 4. Linearity plot of (A) Chlorthalidone and (B) Olmesartan.

Accuracy

The % recovery characteristic of the proposed method for simultaneous estimation by utilizing the calibration curve was determined in part by the Y-intercept and slope of the graph. CHL's % RSD values

were 0.91, 0.22, and 0.53, respectively, while OLM's were 0.44, 0.28, and 0.29, all of which were less than the US Pharmacopeia's acceptance threshold of 2% (**Table 1**). Overall, the method revealed that the data retrieved was correct.

Table 1. Recovery for accuracy studies for the combination.

Spiked level %	Conc. of drug added (µg/mL)	Conc. of drug found (µg/mL)	Recovery %	Mean %	% RSD
CHLORTHALIDONE					
80	16	16.09386	100.30	100.88	0.91
	16	16.18407	101.44		
100	20	20.24659	99.28	99.33	0.22
	20	20.0717	99.43		
120	24	24.3347	99.82	100.89	0.53
	24	24.31274	99.98		
OLMESARTAN					
80	8	8.117429	102.83	102.59	0.44
	8	8.208364	103.32		
100	10	10.960449	99.65	99.73	0.28
	10	10.979085	99.85		
120	12	11.972432	99.82	99.94	0.29
	12	11.937307	99.98		

Conc., Concentration; RSD, relative standard deviation

Precision

In both intra-day and inter-day variability testing for precision data, the method was proven to be highly accurate across the tested ranges of 20-100 µg/mL for CHL and OLM. The peak area of the sample solution matched that of the standard solution in both cases, with a % RSD of less than 2%. CHL and OLM had % RSDs of 0.13 % - 1.37 % and 0.27 % - 0.57 % in intra-day studies (**Table 2**), respectively, whereas CHL and OLM had % RSDs of 0.19 % - 0.94 % and 0.07 % - 1.42 % in inter-day studies, indicating high precision and minimal variation (**Table 3**).

Table 2. Recovery studies for the combination.

Spiked level %	Conc. of drug added (µg/mL)	Conc. of drug found (µg/mL)	Recovery %	Mean %	% RSD
CHLORTHALIDONE					
80	16	16.09386	100.30	100.77	1.37
	16	16.18407	101.44		
100	20	20.24659	99.28	99.24	0.13
	20	20.0717	99.43		
120	24	24.3347	99.82	100.88	0.53
	24	24.31274	99.98		
OLMESARTAN					
80	8	8.117429	102.83	102.08	0.57
	8	8.208364	103.32		
100	10	10.960449	99.65	99.62	0.27
	10	10.979085	99.85		
120	12	11.972432	99.82	99.84	0.29
	12	11.937307	99.98		

Conc., Concentration; RSD, relative standard deviation

Table 3. Precision data of intra-day OLM and CHL.

Drug	Conc. (µg/mL)	Peak area of standard (mV)	Peak area of sample (mV)	% label claim	%RSD
CHL	40	4719.767	4691.41	98.30	0.85
	60	7001.133	7000.07	100.39	0.02
	80	9467.191	9386.05	102.31	1.22
OLM	8	622.0565	621.86	98.70	0.04
	12	931.2762	931.50	100.00	0.03
	16	1240.078	1244.28	100.91	0.48

Conc., Concentration; RSD, relative standard deviation

Robustness

The intentional change of several critical chromatographic parameters such as mobile phase composition, flow rate, and wavelength by 1%, 0.1 mL/min, and 1 nm, respectively, resulted in a substantial shift in the chromatogram for both medicines. When the mobile phase combination was adjusted to 66:34 v/v, the % RSD was determined to be 2% (0.22 for CHL and 0.34 for OLM). Similarly, the % RSD was found to be less than 2% when the composition was altered by 64:36 v/v where CHL has a value of 0.26, whereas OLM has a value of 0.49. When the flow rate was raised by 0.1 ml/min, the % RSD was determined to be 2% (0.12 for CHL and 0.31 for OLM). A similar reduction in flow rate, on the other hand, resulted in a % RSD of < 2 (specifically, CHL showed 0.16 while OLM showed 0.38). A variation of 1 nm in wavelength resulted in a RSD value of less than 2% where CHL demonstrated 0.21 and 0.18, respectively and OLM demonstrated 0.26 and 0.36, respectively. All of the tests indicated that the suggested method has robust characteristics due to the deliberate change of the parameters.

Systems suitability parameters

The system suitability features of the suggested approach demonstrated a high degree of repeatability and may be utilized for routine drug combination analyses. The suggested CHL method yielded an average retention time (Rt) of 8.162 minutes and a mean theoretical plate (TP) of 7148. The Rt and TP for OLM were 10.106 minutes and 6897, respectively (Table 5). A tailing value of less than 2% showed no specific tailing in any cases. Both symmetric and asymmetric components are of similar magnitude in an ideal Gaussian peak with excellent peak symmetry (asymmetric factor = 1). Because the suggested method met the minimum requirements of US Pharmacopoeia monographs (minimum theoretical plates of 2000 and tailing factor of less than 2%), it has a high resolution, significant separation, high column effectiveness, and enhanced repeatability. The separation factor (α) and resolution factor (Rs) were found to be significantly higher than the ICH limits and required recommendations of 1 and 1.5, respectively, indicating that the suggested analytical technique produces a greater separation of both peaks with less tailing and greater resolution. The method may be utilized for routine analysis because of its high precision, reproducibility, and accuracy.

Table 4. Systems suitability parameters.

CHLORTHALIDONE						OLMESARTAN					
Rt (min)	Area (mV)	Theoretical Plates (TP)	Separation Factor	Resolution Factor	Tailing Factor	Rt (min)	Area (mV)	Theoretical Plates (TP)	Separation Factor	Resolution Factor	Tailing Factor
3.587	368856	6019	1.642	1.847	1.22	5.633	2432754	5760	1.645	1.989	1.60
3.589	364903	6049	1.648	1.843	1.41	5.632	2428072	5762	1.638	1.998	1.69
3.583	367942	6055	1.647	1.832	1.36	5.637	2427628	5759	1.648	1.981	1.76
3.581	367493	6021	1.643	1.841	1.29	5.638	2428903	5753	1.643	1.987	1.56
3.582	366224	6027	1.652	1.837	1.33	5.630	2423582	5776	1.657	1.992	1.64
3.585	**366288**	**6033**	**1.648**	**1.834**	**1.34**	**5.635**	**2628386**	**5755**	**1.642**	**1.994**	**1.68**
% RSD		**0.57**				**0.88**					

Limit of detection and Limit of quantification

CHL had a LOD of 0.312867 µg/mL and a LOQ of 0.722524 µg/mL, while OLM had a LOD of 0.214769 µg/mL and a LOQ of 1.8156 µg/mL, showing the method's remarkable detection capacity for the lowest possible concentration of the solute concurrently from the combination or formulation.

CONCLUSION

The suggested analytical method may be utilized to estimate CHL and OLM in bulk and tablet formulations at the same time. According to the ICH validation criteria, the method exhibits linearity throughout the range, accuracy, precision, and resilience. The % RSD, theoretical plates, and tailing values all met the minimum requirements of the US Pharmacopoeia. The validated stress degradation tests under thermal, oxidative, alkali, and acid conditions showed the possibly damaged components, which

chemists would find very helpful for quality control and assurance. The method may be utilized for routine analysis because of its high precision, reproducibility, and accuracy.

CONFLICT OF INTEREST

No conflict of interest is declared.

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None

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