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Dr. Vijaykumar Tirlapur
Department of Quality Assurance, Karnataka M.H. Goel College of Pharmacy, Bidar, Karnataka, India

Dr. Ashwini Rayaji
Department of Quality Assurance, Karnataka M.H. Goel College of Pharmacy, Bidar, Karnataka, India

Rajkumar Dhumansure
Department of Quality Assurance, Karnataka M.H. Goel College of Pharmacy, Bidar, Karnataka, India

Corresponding Author:
Dr. Vijaykumar Tirlapur
Department of Quality Assurance, Karnataka M.H. Goel College of Pharmacy, Bidar, Karnataka, India

Analytical method development and validation for simultaneous estimation of losartan and chlorthalidone by using RP-HPLC in bulk and dosage form

Vijaykumar Tirlapur, Ashwini Rayaji and Rajkumar Dhumansure

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Abstract

A high-performance liquid chromatography method was developed to estimate Losartan and Chlorthalidone together from the drug product they are combined. The method is simple, precise, accurate, and reproducible. The separation was done using a Shimadzu SPD-10A (250×4.6, 5µm) column with an inner diameter of 3.9 mm, C18 type. The mobile phase was a mix of acetonitrile and water in the ratio of 85:15 (v/v), and the flow rate was set at 1.2 ml/minute. The column was maintained at normal room temperature. The detector wavelength was 238 nm. The retention times for Losartan and Chlorthalidone were 1.76 minutes and 2.71 minutes, respectively, with a total run time of 10 minutes. The method was tested for different aspects such as system suitability, specificity, linearity, precision, accuracy, robustness, and ruggedness. Low percentages of RSD values showed the method is accurate and precise, making it suitable for the quantification of both drugs in drug products and biological samples. This method is suitable for use in routine quality control testing of final drug products without any interference from other components.

Keywords: Losartan, Chlorthalidone, RP-HPLC, Method validation, PDA Detection, Tablet dosage forms, ICH guidelines

Introduction

Method development and validation are important in the discovery, development, and production of pharmaceuticals, which is a broad process.

These processes include the analysis of small amounts of complex material in biology and the control of the quality of the final product. Analytical methods are created based on ICH guidelines. Some drug combinations are used to have a better effect, either to improve the treatment or make it longer lasting. The combination of Losartan and Chlorthalidone is used to lower blood pressure. Chlorthalidone is known as a thiazide-like diuretic with a known chemical structure. Losartan is the first non-peptide imidazole drug used as an oral angiotensin II receptor antagonist. Literature shows many HPLC methods focusing on estimating various anti-hypertensive drugs or combinations, but there is no method yet for the simultaneous quantification of both Losartan and Chlorthalidone in tablet form. This study aims to develop a quick, cost-effective, accurate, and reliable method for estimating both drugs in their tablet formulations. The proposed method is fast, simple, and reliable, making it useful for routine analysis of these drugs in tablet dosage forms.

Materials and Methods

Chemicals and Reagents

The drug samples were provided as gift samples by IPCA, Mumbai, India.

All chemicals and reagents were of HPLC grade, including methanol, acetonitrile, isopropyl alcohol, and water. The commercial drug product was bought from the local market, and it contained 50 mg of Losartan and 6.25 mg of Chlorthalidone per tablet.

Instrumentation

The chromatographic separation was performed on a Shimadzu liquid chromatography system fitted with an LC-10AD pump, SPD-10A photodiode array detector, and an injector with a 20 µl loop. The data was collected and processed using the LC solution data station (Shimadzu, Japan). A Phenomenex C18 column (250 mm × 4.6 mm, 5µm) was used for the analysis.

Method Development

Various mobile phases were tested to develop the RP-HPLC method for analyzing Losartan and Chlorthalidone. The most suitable mobile phase was chosen based on the selectivity and sensitivity.

Chromatographic Conditions

A mobile phase of acetonitrile and water in the ratio of 85:15 (v/v) was used to separate the two drugs.

The mobile phase was filtered through a 0.45-micron membrane filter and then sonicated for 30 minutes. The flow rate was set to 1.2 ml/min. Both drugs gave good absorbance at 238 nm, which was selected as the wavelength for the analysis. All measurements were carried out at room temperature. An individual drug solution at a concentration of 100µg/mL in methanol was scanned from 200 nm to 400 nm. UV overlay spectra of the two drug solutions indicated that Losartan and Chlorthalidone absorbed significantly at 238 nm and 284 nm, respectively.

Preparation of Standard Stock Solution (1mg/ml)

One hundred mg of each drug (Chlorthalidone and Losartan) was accurately weighed and dissolved in methanol using sonication in two separate 100 ml volumetric flasks and made up to 100 ml with methanol. (Stock solution-I, 1 mg/ml or 1000 µg/ml).

Preparation of Working Standard Solution (100 microgram/ml)

One ml of the above standard stock solution was transferred into a 10 ml volumetric flask and made up to the mark with methanol to get a final concentration of 100 µg/ml for both drugs.

Working standard mixtures were made by serial dilutions of these stock solutions (100 µg/ml) using the mobile phase over a concentration range of 10-100 µg/ml.

Calibration curve

A calibration curve was prepared by taking different volumes of standard stock solutions for Losartan and Chlorthalidone into 10 ml volumetric flasks and diluting to the mark with mobile phase.

This provided final concentrations of 20, 40, 60, 80, and 100 µg/ml for both Losartan and Chlorthalidone. The standard solutions (n=5) were injected through a loop injection system, and chromatograms were recorded. The effluent was analyzed at 238 nm. The calibration curve was made by plotting the average peak area against concentration, and a regression equation was calculated.

Method Validation

The method was validated for linearity, accuracy, specificity, limit of detection, limit of quantification, intra-day and inter-day precision, repeatability, robustness, ruggedness, and system suitability parameters.

Assay of market formulation

Twenty tablets were accurately weighed and crushed into a fine powder. The powder equivalent to 100 mg of the drug was transferred into a 100 ml volumetric flask. About 50 ml of methanol was added, and the mixture was sonicated for 8 minutes. The volume was then made up to the mark (100 ml) with methanol. The solution was filtered through a 0.45 micron Whatman filter paper. Then 1 mL of solution was further diluted to 10 mL with mobile phase to get the final sample concentration (100 microgram/mL). Standards and samples were injected to get the chromatograms.

Assay of market formulation

The findings from the assay of the marketed formulation are presented in Table 1 and Figure 5.

Results and Discussion

A reliable, accurate, and suitable RP-HPLC method was developed for the simultaneous estimation of Losartan and Chlorthalidone.

Different mobile phase combinations were tested, and the final chromatographic conditions were found to be appropriate for the accurate determination of both compounds. (Figures 1 & Figures; 2)

Linearity

To determine linearity, standard solutions were prepared at various concentration levels. The linearity range for both Losartan and Chlorthalidone was identified as 20-100 µg/ml. The regression equations for Losartan and Chlorthalidone were $y = 30965x + 32134$ and $y = 25618x + 9814.8$, with correlation coefficients (r) of 0.9989 and 0.9981, respectively. (Table 2 & Figures; 3 & Figures; 4)

Precision

Precision was assessed by preparing five separate samples from a single batch of the formulation. The sample preparation followed the method described. The percentage relative standard deviation (%RSD) was less than 2% for both intra-day and inter-day variations, confirming the method's precision. The results are detailed in Tables 3 & 4.

Accuracy (Recovery studies)

To evaluate the accuracy of the method, recovery studies were conducted in triplicate using the standard addition method at 80%, 100%, and 120% of the expected concentration. Standard quantities of Losartan and Chlorthalidone were added to pre-analyzed samples, which were then subjected to the proposed HPLC method. The results of the recovery studies are shown in Tables 5 & 6.

Specificity

The purity of the peaks for Losartan and Chlorthalidone was evaluated by comparing their retention times with those of the pure compounds. A strong correlation was found between the retention times of the standards and the samples.

System Suitability parameters

To confirm the validity of the analytical method, a system suitability test was performed. Six replicates of the standard solution (40 µg/ml) were injected. Parameters such as the number of theoretical plates (N), tailing factor, and retention time were calculated. (Table 7)

Robustness & Ruggedness of method

To assess the robustness of the developed RP-HPLC method, minor, intentional variations were introduced in the optimized parameters. The impact of changes in flow rate, wavelength, and mobile phase ratio on retention time and tailing factor was studied. The method remained consistent even with these changes, indicating its robustness. (Tables 8 & 9)

The results demonstrate that the method is robust.

LOQ and LOD

LOD refers to the lowest concentration of an analyte that can be detected, while LOQ is the lowest concentration that can be quantitatively determined with acceptable precision and accuracy. These values were calculated using the equations: LOQ = 10s/m and LOD = 3.3s/m, where s is the standard deviation of the response and m is the slope of the calibration curve.

For Losartan, the LOD was 1.97 µg/ml and the LOQ was 5.63 µg/ml.

For Chlorthalidone, the LOD was 1.67 µg/ml and the LOQ was 5.12 µg/ml.

Table 1: Assay of Losartan and Chlorthalidone Tablet Formulation

Estimation content of Losartan and Chlorthalidone			
Dosage form	**Labeled claim (mg)**	**Amount estimated (mg)**	**% Purity**
Losartan	50 (mg)	49.1 (mg)	98.2 %
Chlorthalidone	6.25 (mg)	6.11 (mg)	97.76 %

Table 2: Linearity studies of Losartan and Chlorthalidone

S.No	**Concentration ug/ml**	**Average Area *(n=5)**		**Concentration ug/ml found**	
		Losartan	**Chlorthalidone**	**Losartan**	**Chlorthalidone**
1.	20	655867	534256	20.1	20.47
2	40	1334622	1084115	42.1	41.94
3.	60	1887980	1490505	59.9	57.80
4.	80	2464718	2023954	78.6	78.62
5	100	3139120	2611392	100.3	101.6

Table 3: Precision Data of Losartan

Peak Area Intraday Precision							**Peak Area Interday Precision**		
S.No	**Conc. (ug/ml)**	**0 hrs**	**2 hrs**	**4 hrs**	**6 hrs**	**8 hrs**	**Day 1**	**Day 2**	**Day 3**
1.	40	1334626	1334526	1335626	1344926	1334526	1334528	1334426	1333526
2.	40	1334620	1334620	1336620	1334620	1334420	1334621	1334620	1336629
3.	40	1334621	1334721	1336621	1338632	1333621	1336722	1334711	1334725
4.	40	1334628	1334828	1334728	1335628	1333628	1334329	1334924	1334828
5.	40	1334632	1334632	1334632	1337612	1334622	1335632	1334631	1334630
6.	40	1335622	1334522	1335622	1329822	1334622	1334541	1334322	1335522
Mean		1334792	1334642	1335642	1336873	1334240	1335062	1334606	1334977
Standard D		406.88	117.71	868.46	499.67	440.41	934.8	212.6	1033
% RSD		0.03	0.01	0.07	0.09	0.03	0.07	0.02	0.08

Table 4: Precision Data of Chlorthalidone

Peak Area Intraday Precision							**Peak Area Interday Precision**		
S.No	**Conc. (ug/ml)**	**0 hrs**	**2 hrs**	**4 hrs**	**6 hrs**	**8 hrs**	**Day 1**	**Day 2**	**Day 3**
1.	40	1013215	1013315	1015215	1013211	1013213	1013216	1013313	1015315
2.	40	1014199	1014496	1015199	1014192	1012199	1014198	1014496	1015199
3.	40	1015122	1015322	1015122	1015124	1015123	1015122	1015322	1015122
4.	40	1014185	1014183	1014285	1014188	1014185	1014186	1014283	1014295
5.	40	1016119	1017129	1016119	1016119	1016119	1016119	1017129	1016119
6.	40	1017215	1017111	1017315	1017516	1016213	1017315	1018111	1017425
Mean		1015009	1015259	1015543	1015058	1014509	1015026	1015442	1015579
Standard D		1460.47	1577.96	1044.75	1555.00	1612.04	1490.68	1830.56	1074.33
% RSD		0.14	0.16	0.10	0.15	0.16	0.15	0.18	0.11

Table 5: Accuracy Data of Losartan

S.No	**Level of Recovery**	**Amount Added (ug/ml) Mean (n=3)**		**Total Amount Recovered (ug/ml)**	**% Recovery**
		Test	**Standard**		
1.	80 %	10	8.0	18.00	100.0
2.	100 %	10	10	19.84	99.20
3	120 %	10	12	21.17	96.22
Mean Recovery 96.22 - 100.00					

Table 6: Accuracy Data of Chlorthalidone

S.No	Level of Recovery	Amount Added (ug/ml) Mean (n=3)		Total Amount Recovered (ug/ml)	% Recovery
		Test	Standard		
1.	80 %	10	8.0	17.90	99.44
2.	100 %	10	10	19.84	99.20
3	120 %	10	12	21.19	96.31
Mean Recovery 96.31 - 99.44					

Table 7: System suitability & specificity Parameters of Losartan & Chlorthalidone

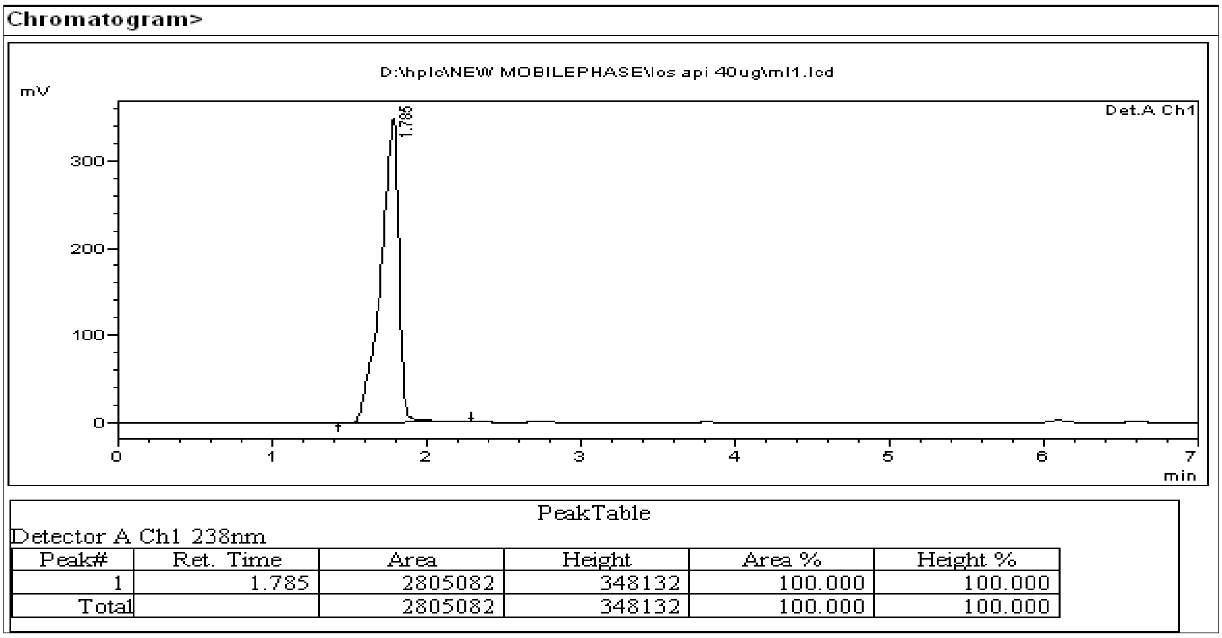
Parameters	Losartan	Chlorthalidone
Retention Time (min)	1.7262	2.6422
Resolution (Rs)	1.5	1.5
Tailing Factor (T)	0.0579	0.0378
Theoretical Plates (N)	525.32	2787.84
Range (ug/ml)	20 - 100	20 - 100

Table 8: Robustness studies of Losartan and Chlorthalidone

Parameter (n=5)	Condition	Level	Retention time		Tailing Factor	
			Losartan	Chlorthalidone	Losartan	Chlorthalidone
Flow rate(ml/min) (± 0.2mL)	1.0	-1	1.77	2.70	0.063	0.045
	1.2	0	1.72	2.64	0.057	0.037
	1.4	1	1.68	2.57	0.069	0.052
	Mean(n=3)		1.723	2.636	0.063	0.0446
Detection wavelength (nm) (± 1nm)	283	-1	1.67	3.71	0.056	0.046
	284	0	1.76	2.71	0.048	0.037
	285	1	1.69	3.38	0.061	0.048
	Mean(n=3)		1.693	3.243	0.055	0.043
Mobile phase composition (v/v) (± 10% of organic phase)	76.5:15	-1	2.20	3.56	0.071	0.057
	85:15	0	1.72	2.64	0.057	0.037
	93.5:15	1	1.58	2.29	0.063	0.047
	Mean(n=3)		1.83	2.83	0.063	0.047

Table 9: Ruggedness studies of Losartan and Chlorthalidone

S.No	Conditions	Conc. (ug/ml)	Peak Area Losartan	Peak Area Chlorthalidone
1.	Analyst-1	40	1334721	1015199
2.		40	1334721	1015124
3.		40	1335632	1016119
		Mean	1335025	1015481
		SD	525.966	554.08
		% RSD	0.04	0.05
4.	Analyst-2	40	1334631	1015122
5.		40	1336722	1015199
6.		40	1334728	1016213
		Mean	1335360	1015511
		SD	1180.23	608.88
		% RSD	0.09	0.06

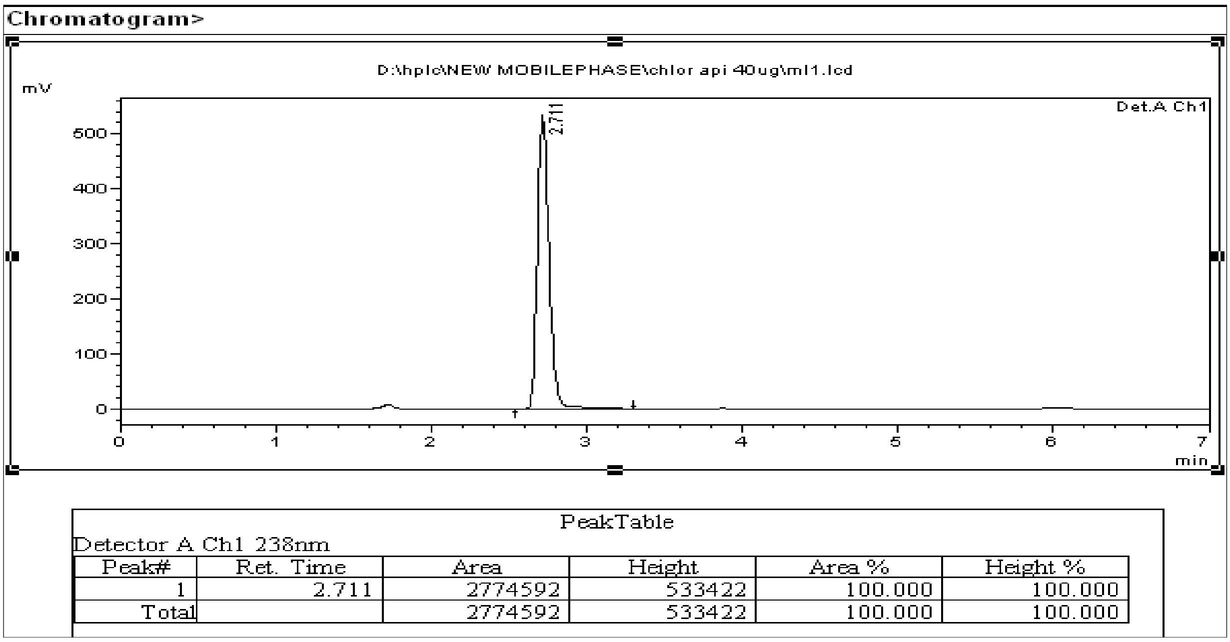


PeakTable

Detector A Ch1 238nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.785	2805082	348132	100.000	100.000
Total		2805082	348132	100.000	100.000

Fig 1: Chromatogram of Losartan Standard



PeakTable

Detector A Ch1 238nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.711	2774592	533422	100.000	100.000
Total		2774592	533422	100.000	100.000

Fig 2: Chromatogram of Chlorthalidone Standard

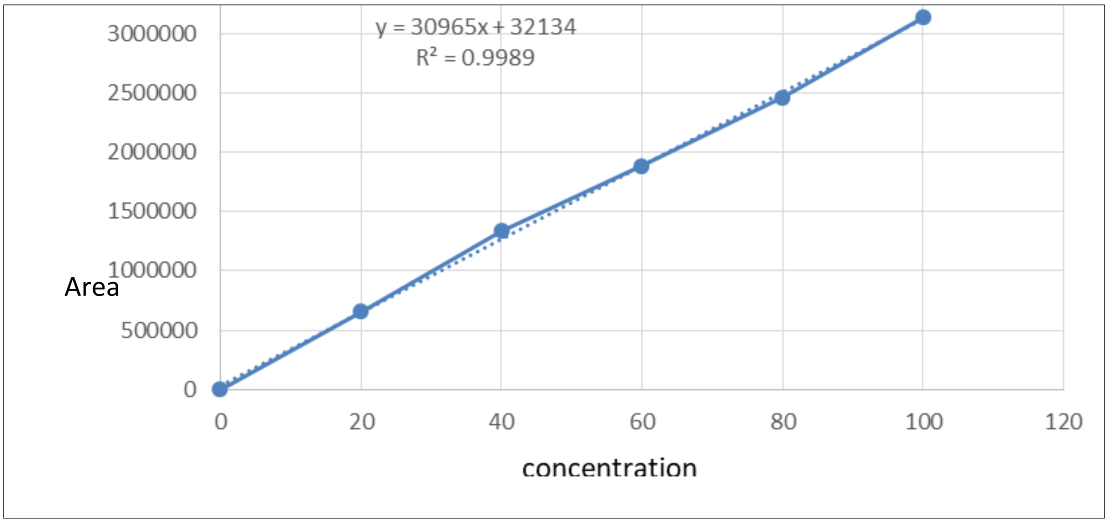


Fig 3: Calibration curve of Losartan

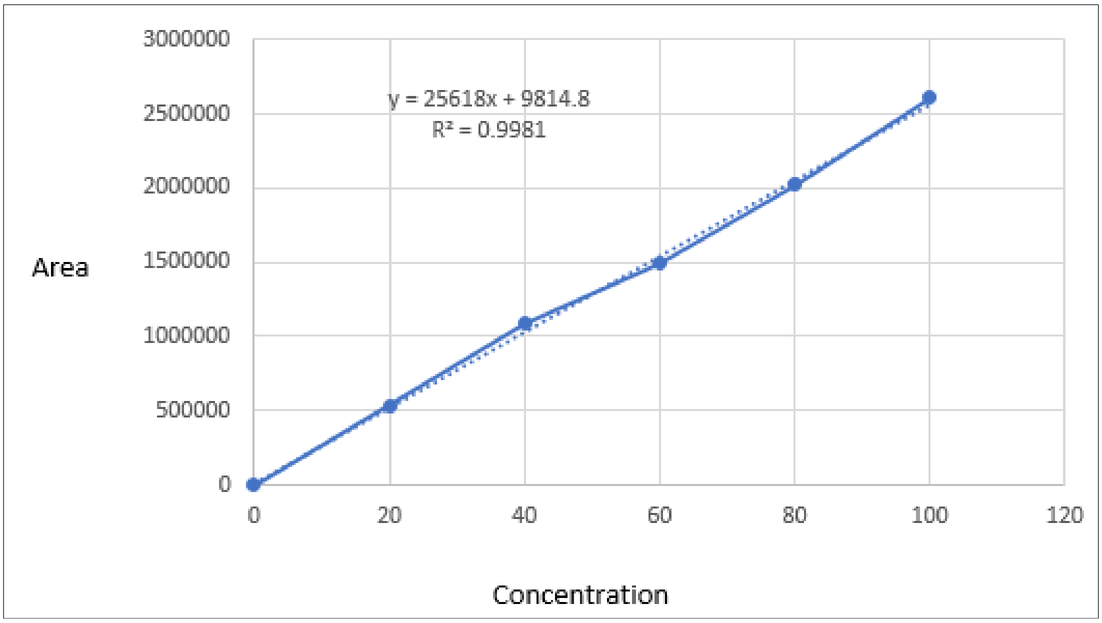
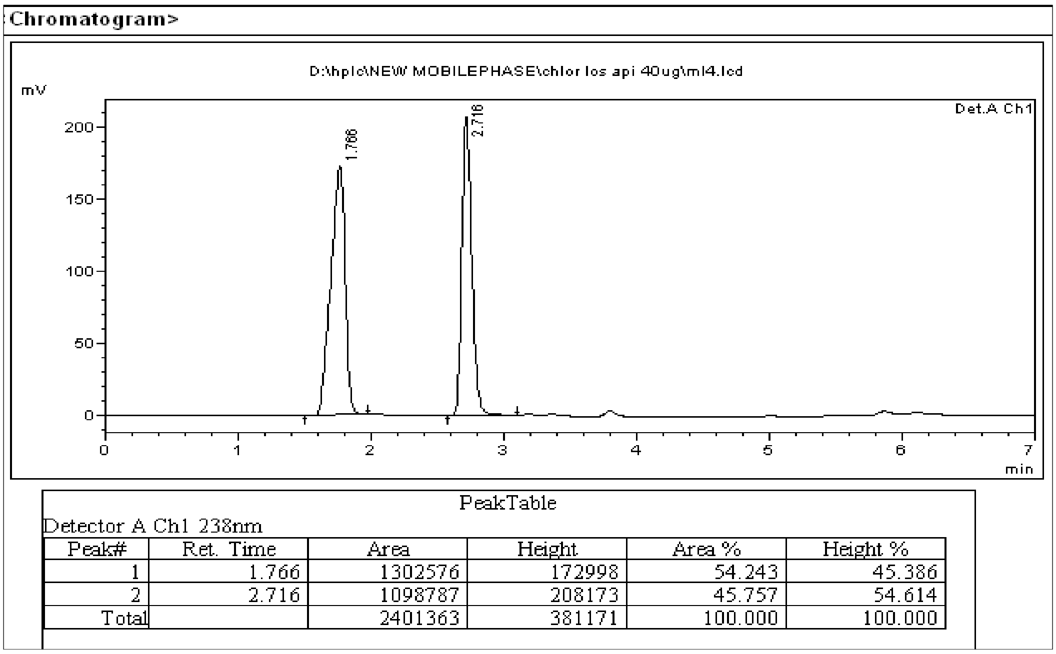


Fig 4: Calibration curve of Chlorthalidone



PeakTable

Detector A Ch1 238nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.766	1302576	172998	54.243	45.386
2	2.716	1098787	208173	45.757	54.614
Total		2401363	381171	100.000	100.000

Fig 5: Chromatogram of Dosage Form

Conclusion

The proposed method is simple, sensitive, and reproducible, making it suitable for routine analysis of Losartan and Chlorthalidone in bulk and pharmaceutical formulations.

Statistical analysis of the results showed high accuracy and good precision. The %RSD for all parameters was below 2, confirming the method's validity and the agreement of the assay results obtained. This method can be effectively used for the quantitative simultaneous determination of Losartan and Chlorthalidone in multi-component pharmaceutical preparations.

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