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RP-HPLC method development and validation for the simultaneous estimation of ramipril and losartan in tablet and pharmaceutical dosage form

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Ramipril and Losartan in pharmaceutical tablet dosage form. Chromatographic analysis was performed on a Inertsil ODS (250×4.6× 5µ)column at ambient temperature with a mixture of 10mM KH2PO4:ACN (30:70) v/v (1.36gm of potassium di hydrogen phosphate (KH2PO4) was weighed and dissolved in 1000ml of water and volume was made up to 1000ml with water. Adjust the pH to 3.0 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases) as mobile phase, at a flow rate of 1.0 ml/min^{-1}. UV detection was performed at 211 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Ramipril and Losartan were 2.667 and 3.887 min, respectively. Calibration plots were linear over the concentration ranges 3-7 µg mL^{-1} and 60-140 µg mL^{-1} for Ramipril and Losartan, respectively. The Limit of detection was 0.24 and 1.09 µg mL^{-1} and the quantification limit was 0.72 µg mL^{-1} and 3.30 µg mL^{-1} for Ramipril and Losartan, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 100.12% to 100.43%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of Ramipril and Losartan in pharmaceutical tablet dosage form.

Keywords: Ramipril, Losartan, RP-HPLC, Validation.

INTRODUCTION

RAMIPRIL IS CHEMICALLY DESCRIBED AS (2S,3aS,6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[b]pyrrole-2carboxylic acid.

Uses: It is used mainly in treatment of several diseases of the cardiovascular system, especially hypertension Antihypertensive drug, Angiotensin-converting Enzyme Inhibitor

Mechanism of action: The principle active metabolite of ramipril, competes with ATI for binding to ACE and inhibits and enzymatic proteolysis of ATI to ATII. Decreasing ATII levels in the body decreases blood pressure by inhibiting the pressure effects of ATII Ramipril also causes an increase in plasma renin activity likely due to a loss of feedback inhibition mediated by ATII on the release of renin and/or stimulation of reflex mechanisms via baroreceptors.

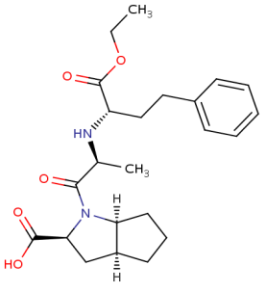
LOSARTAN IS CHEMICALLY DESCRIBED AS

[2-butyl-4-chloro-1-({4-[2-(2H-1, 2, 3, 4- tetrazol-5-yl) phenyl] phenyl} methyl)-1H-imidazol-5-yl] methanol

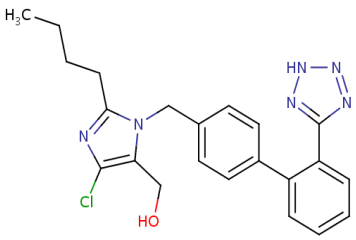
Uses: It is used as Antihypertensive Agent, Anti-Arrhythmia Agent, Angiotensin II Type 1 Receptor Blocker.

Mechanisam of action: Losartan is an angiotensin-receptor blocker (ARB Losartan competitively inhibits the binding of angiotensin II to AT1 in many tissues including vascular smooth muscle and the adrenal glands. Losartan is metabolized to its active metabolite, E-3174, which is 10 to 40 times more potent than losartan and acts as a non-competitive AT1 antagonist. Inhibition of angiotensin II binding to AT1 inhibits its AT1-mediated vasoconstrictive and aldosterone-secreting effects and results in decreased vascular resistance and blood pressure. Losartan is 1,000 times more selective for AT1 than AT2. Inhibition of aldosterone secretion may increase sodium and water excretion while decreasing potassium excretion. Losartan is effective for reducing blood pressure and may be used to treat essential hypertension, left ventricular hypertrophy and diabetic nephropathy. Fix dosage combination containing Ramipril (2.5mg) and Losartan (50 mg) available in market. A new combination formulation of Ramipril and Losartan seems to be beneficial in the treatment and management of essential hypertension in terms of its convenience and patient compliance. Literature survey revealed Developed for the simultaneous determination of losartan potassium and ramipril in table dosage forms by Reversed-Phase HPLC.LOSARTAN alone or in combination with other drugs like Amlodipine besylate, Perindpril erbumine, Ramipril with telmisartan, Metolazone etc. However, HPLC Method, has been developed for the simultaneous determination of both the drugs in tablets. The present research work describes the rapid, accurate, sensitive and reproducible RP-HPLC method for simultaneous estimation of RAMIPRIL and LOSARTAN from the tablet formulation.

RAMIPRIL



LOSARTAN



MATERIALS AND METHODS

CHEMICALS/ REAGENTS AND SOLVENTS

Ramipril -2.5mg and Losartan-50mg were obtained from, Carsyon (Micro Labs Ltd) Mumbai. Double Distilled Water (HPLC grade), Methanol (HPLC grade), Acetonitrile (HPLC grade), Sodium acetate, Potassium phosphate, Ammonium acetate, and Triethylamine were of AR grade. The pharmaceutical preparations of combination of Ramipril and Losartan that is SARTACE tablet (Carsyon (Micro Labs Ltd, Mumbai.)

INSTRUMENTATION AND EQUIPMENTS

The HPLC analysis was accomplished on Shimadzu,high pressure liquid chromatography outfitted with 515 reciprocating dual column HPLC pump, a manually operating Hamilton injector with 20µL sample loop, Inertsil ODS 3V(250x4.6mm) 5µm, C18 analytical column reversed-phase material of 5µ size and a photodiode array UV-Visible detector. All the parameters of HPLC were controlled by Spinchrome software. Other instruments used were Nicolet evolution UV-Vis spectrophotometer of model 100, Shimadzu electronic balance, global digital pH meter and Citizen, Digital Ultrasonic Cleaner ultrasonic bath sonicator.

ANALYTICAL METHOD DEVELOPMENT

OPTIMIZATION OF UV CONDITIONS

A Intersil ODS,C-18,250×4.6mm ID,5 µm Particle size Column was used for chromatographic separation. Ther mobile phase composed of Pottasium di hydrogen Phosphate buffer (3.0 pH): Acetonitrile (30:70), at flow rate 1.0mL/min with run time 6mins. Mobile phase and sample solution were filtered through a 0.45µm membrane filter and degassed. The detection of both drugs was carried out at 211nm.

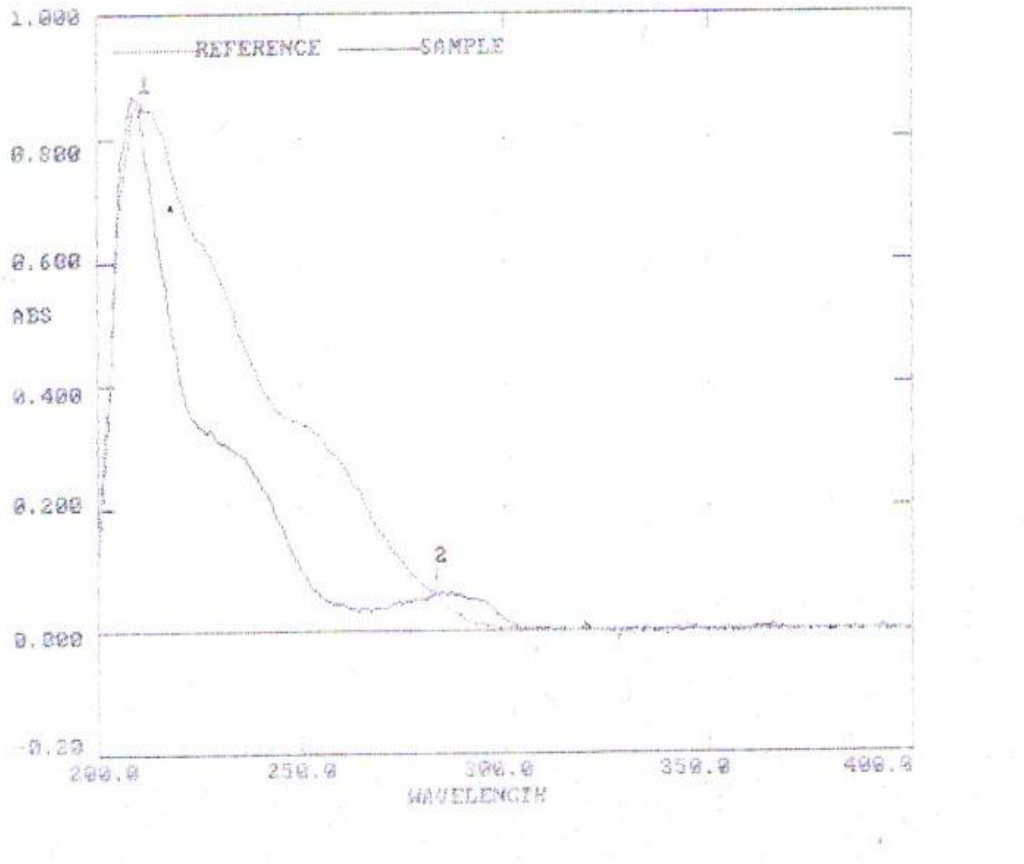


Figure-1. Isobestic point of Ramipril And Losartan.

OPTIMIZED METHOD PARAMETERS

Mobile Phase :Pottasium di hydrogen Phosphate buffer (3.0 pH): Acetonitrile(30:70)

Column (Stationary Phase): Intersil ODS,C-18,250×4.6mm ID,5 µm Particle size

Flow rate (ml/min): 1.0

Column temperature (°C): Ambient

Volume of injection loop (µl): 20

Detection wavelength (nm):211

Drug RT (min**):** Ramipril- 2.667, Losartan- 3.887.

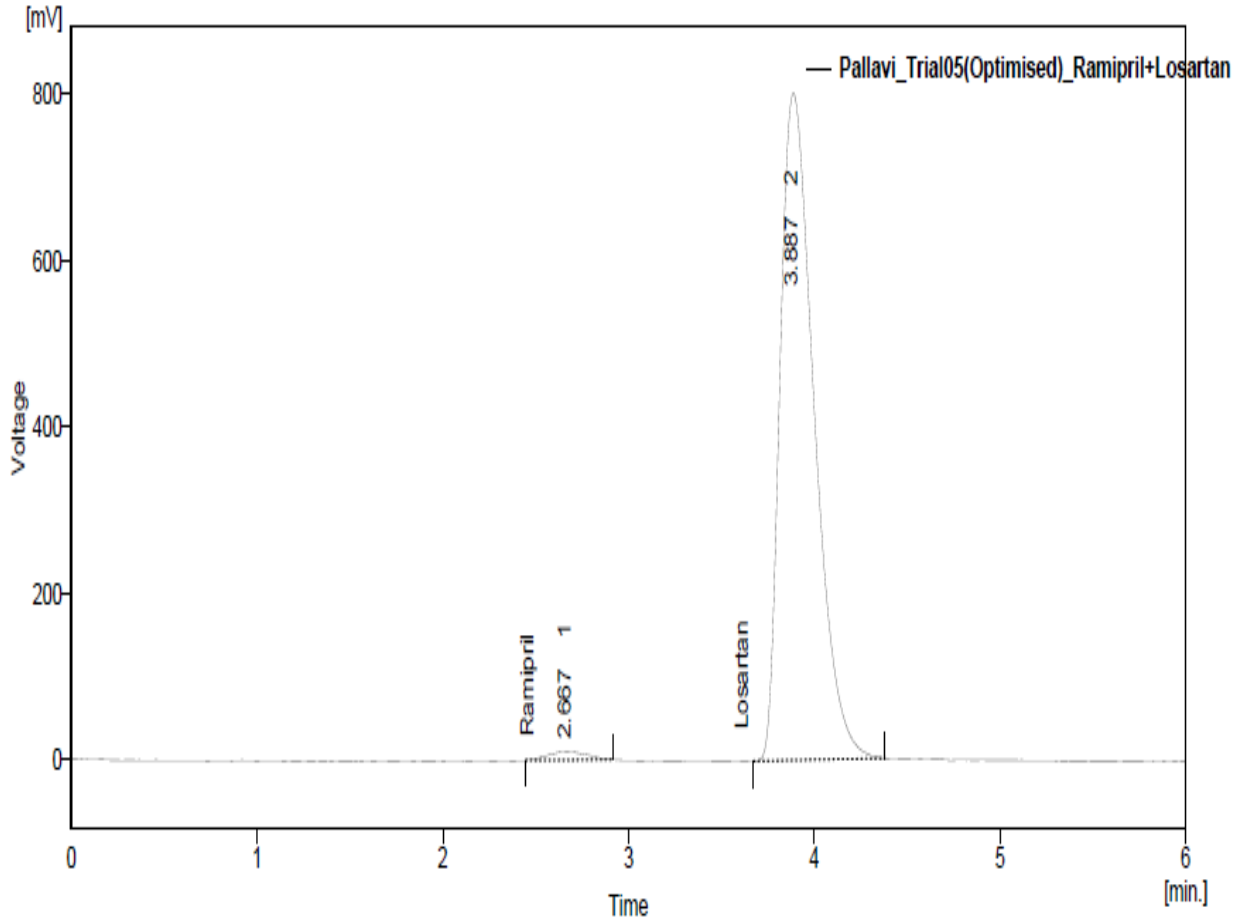


Figure 1.1.Optimized chromatogram

PROCEDURE FOR PREPARATION OF SOLUTION

PREPARATION OF BUFFER

1.36gm of potassium di hydrogen phosphate (KH2PO4) was weighed and dissolved in 1000ml of water and volume was made up to 1000ml with water. Adjust the pH to 3.0 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

PREPARATION OF MOBILE PHASE

A mixture of 30 volumes of 10mM Phosphate Buffer pH 3.0, 70volumes of Acetonitrile was prepared. The mobile phase was sonicated for 10min to remove gases.

Diluent Preparation:

Use Mobile phase Diluent Phase

ASSAY

PREPARATION OF THE RAMIPRIL AND LOSARTAN STANDARD & SAMPLE SOLUTION

PREPARATION OF MIXED STANDARD SOLUTION

Weigh accurately 2.5 mg of RAMIPRIL and 50 mg of LOSARTAN and transfer to 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. Transfer 1.0 ml from the above stock solutions of RAMIPRIL and LOSARTAN into a 10mL volumetric flask and make up the volume

with mobile phase. Above stock solution contains 2.5 µg/ml of RAMIPRIL and 50 µg/ml of LOSARTAN. This solution is used for recording the chromatogram.

SAMPLE SOLUTION PREPARATION

20 tablets (each tablet contains 2.5 mg of RAMIPRIL and50 mg of LOSARTAN) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of RAMIPRIL and LOSARTAN (µg/ml) were prepared by dissolving weight equivalent to 2.5 mg of RAMIPRIL and 50 mg of LOSARTAN and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 5 µg/ml of RAMIPRIL and 100µg/ml of LOSARTAN was made by adding 1 ml of stock solution to 10 ml of mobile phase.

PROCEDURE

20 µL of the standard and sample solutions were injected into the chromatographic system and areas for the Ramipril And Losartan peaks were measured. %Assay was calculated by using the formulae.

CALCULATION

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

Where:

AT = Average area counts of sample preparation

AS = Average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = LABEL CLAIM mg/ml.

ANALYTICAL METHOD VALIDATION

The HPLC method was validated in accordance with ICH guidelines.

ACCURACY

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of RAM and LOS a known amount of standard drug powder of RAM and LOS were added at 80, 100 and 120 % level.

PRECISION

The system precision of the method was verified by five replicate injections of standard solution containing RAM and LOS. The method precision was carried out the analyte five times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of RAM and LOS.

LINEARITY

The linearity was determined separately for RAM and LOS Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

ROBUSTNESS

Robustness was evaluated by making deliberate variations in method parameters such as variation of wave length; flow rate and change in mobile phase composition. The robustness of the method was studied for RAM and LOS

RESULTS

SELECTION OF CHROMATOGRAPHIC CONDITIONS AND OPTIMIZATION OF MOBILE PHASE

Mobile phase was optimized to separate RAM and LOS using C18 column (Stationary Phase): Intersil ODS,C-18,(250×4.6mm ID),5 µm Particle size. Initially, Pottassium di hydrogen phosphate buffer and ACN in the Equal proportions were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of mixed phosphate buffer to 3.0 with ortho-phosphoric acid, and mobile phase composition (Pottassium di hydrogen phosphate buffer, and ACN and in 30:70 % v/v*)* was tried for resolution of both drugs. Good resolution and

symmetric peaks were obtained for both drugs when the pH of the mobile phase (buffer) was adjusted to 3.0. The flow rate of the mobile phase was 1.0 mL min-1. Under optimum chromatographic conditions, the retention time for RAM and LOS was found to be 2.667 and 3.887 min, respectively when the detection was carried out at 211nm. A typical chromatogram of two drugs is shown in (**Figure 1**).

Table-1: ACCURACY DATA

Recovery level	Accuracy RAMIPRIL					Average %Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	5	119.895	119.923	5.02	100.46	100.12%
	5	119.884				
	5	119.990				
100%	6	140.200	140.573	5.89	98.19	
	6	140.110				
	6	141.410				
120%	7	161.224	163.290	7.12	101.72	
	7	161.907				
	7	166.740				

Recovery level	Accuracy LOSARTAN					Average %Recovery
	ount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	100	9868.786	9922.316	101.62	101.62	100.43%
	100	9917.121				
	100	9981.041				
100%	120	11790.173	11872.569	118.19	98.49	
	120	11971.355				
	120	11856.178				
120%	140	13716.886	13729.251	141.65	101.18	
	140	13735.434				
	140	13735.434				

Table-2: PRECISION

RAMIPRIL			LOSARTAN		
S.No.	Rt	Area	S.No.	Rt	Area
1	2.563	139.547	1	3.893	10085.919
2	2.547	138.050	2	3.897	10241.241
3	2.520	138.093	3	3.890	10135.153
4	2.547	139.825	4	3.890	10250.418
5	2.563	139.547	5	3.893	10085.919
6	2.547	139.050	6	3.897	10241.241
avg	2.5478	139.019	avg	3.893	10173.315
stdev	0.0157	0.775	stdev	0.003	79.881
%RSD	0.62	0.56	%RSD	0.08	0.79

Table-3: LINEARITY RESULTS OF RAMIPRIL AND LOSARTAN

Linearity observation of Ramipril

S.No	Linearity Level	Concentrationμμ(μg/ml)	Area
1	I	3 ppm	76.174
2	II	4 ppm	95.497
3	III	5 ppm	119.304
4	IV	6 ppm	137.593
5	V	7 ppm	164.621

Correlation Coefficient	**0.999**

Linearity observation of Losartan

S.No	Linearity Level	Concentration	Area
1	**I**	**60 ppm**	**6025.881**
2	**II**	**80 ppm**	**7811.081**
3	**III**	**100 ppm**	**10045.494**
4	**IV**	**120 ppm**	**11630.470**
5	**V**	**140 ppm**	**13699.397**
Correlation Coefficient			**0.999**

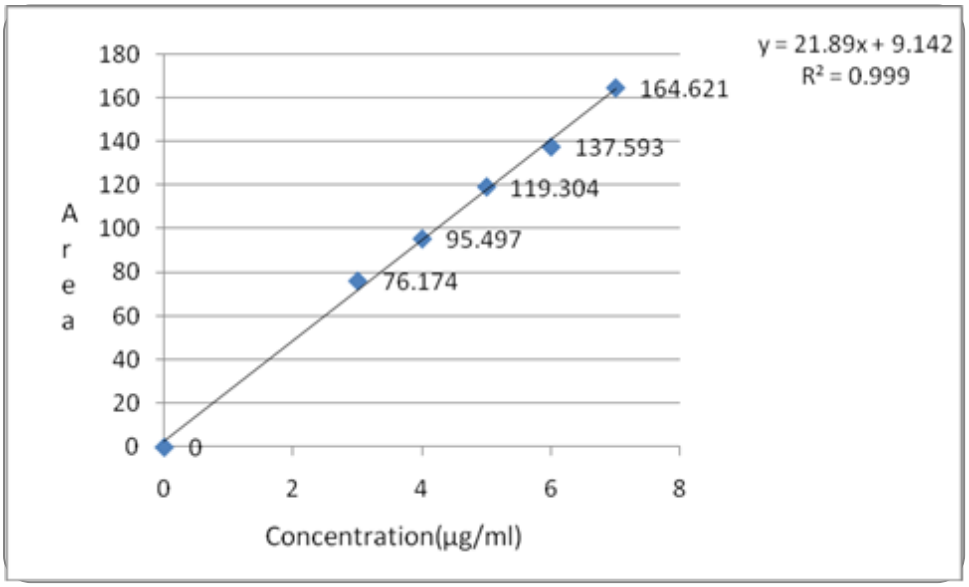


Fig 2: LINEARITY GRAPH OF RAMIPRIL

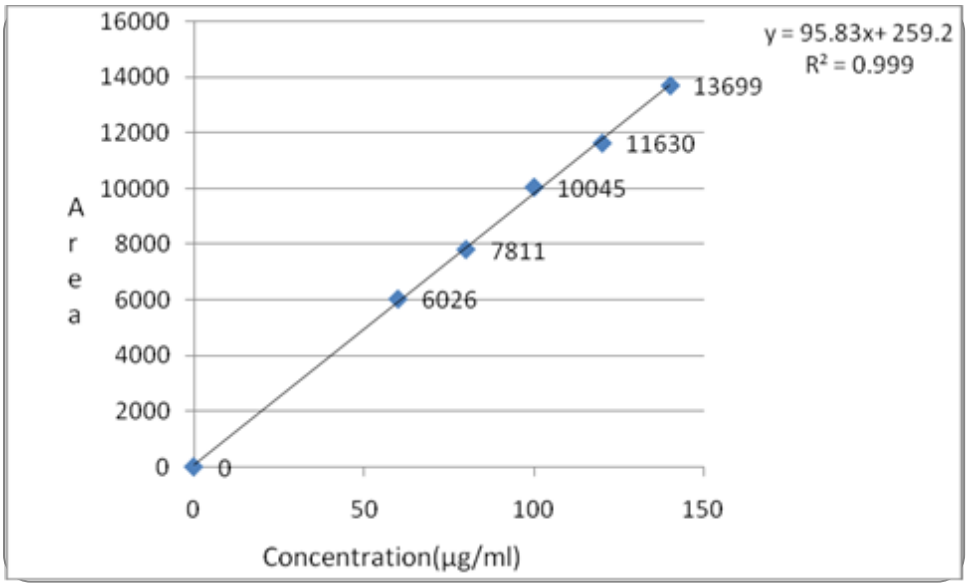


Fig 3: LINEARITY GRAPH OF LOSARTAN

Table-4 LOD AND LOQ RESULTS

S.No	Drug name	Standard deviation	Slope	LOD	LOQ
1	Ramipril	1.6	21.89	0.24	0.72
2	Losartan	31.62	95.83	1.09	3.30

Table-5: ROBUSTNESS RESULTS

Parameter	**RAMIPRIL**		**LOSARTAN**	
	Retention time(min)	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow Rate	3.143	1.230	4.827	1.644
0.8 ml/min	2.563	1.297	3.893	1.681
1.0 ml/min	2.133	1.219	3.267	1.609
1.2 ml/min				
Wavelength	2.523	1.225	3.887	1.626
209nm	2.563	1.297	3.893	1.681
211nm	2.550	1.234	3.887	1.608
213nm				

RESULTS AND DISCUSSION

ACCURACY

The accuracy of the method studied at three different concentration levels i.e. 80%, 100 % and 120 % showed acceptable % recoveries in the range of 100.12%% for RAM and 100.43% for LOS . The results are shown in **Table 1.**

PRECISION

The precision study was evaluated on the basis of % RSD value was found to be in the range 0f 0.62% for Ramipril and 0.08 for Losartan %, respectively. As the RSD values were < 2% therefore developed method was precise. Results of precision study are shown in **Table 2.**

LINEARITY

The linearity was determined separately for RAM and LOS .Linearity of the method was studied by injecting 5 concentrations of both drugs prepared in methanol and calibration curves were constructed by plotting peak area against the respective concentrations. The RAM and LOS followed linearity in the concentration range of 3– 7µg mL-1 and 60-140µg mL-1; respectively. The results are shown in **Table 3**.and **F**ig no 2.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The LOD for RAM and LOS was found to be 0.24 and 1.09 µg, respectively. The LOQ for RAM and LOS was found to be 0.72 and 3.30 µg, respectively. The low values of LOD and LOQ indicates high sensitivity of the method. The results are shown in Table 4.

ROBUSTNESS STUDY

Robustness of the method was studied by making deliberate changes in the chromatographic conditions and the effects on the results were examined. The low value changes of theoretical plates, tailing factor indicating robustness of the method. The results are shown in Table 5.

ANALYSIS OF MARKETED TABLET FORMULATION

3 replicates of the samples solutions (20 µL) were injected for quantitative analysis. The amounts of RAM and LOS estimated were found to 101.84 % and 100.26 %, respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical formulations. The results are shown in **Table 6**.

SYSTEM SUITABILITY TEST

The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied and were summarized in **Table 7.**

Table 6: ASSAY RESULTS

Assay Results Drug	Amount present/tablet	% of Assay
RAMIPRIL	2.5mg	101.84%
LOSARTAN	50 mg	100.26%

Table 7: SYSTEM SUITABILITY PARAMETERS

System suitability Parameters	RAMIPRIL	LOSARTAN
Tailing Factor	1.26	1.63
Theoritical plates	3426	2187
Resolution	--	3.203

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of Ramipril and Loartan in tablet formulation. The method was validated as per ICH guidelines

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