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 (250x4.6x 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. 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
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-(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.

Mechanism 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 tablet 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 5μm 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-18,250x4.6mm 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: Potassium 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

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, 70 volumes 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
and 50 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 % =

\[
\frac{AT}{Wt} \times \frac{WS}{DT} \times \frac{DT}{P} \times \frac{Avg.}{\text{Label Claim}}
\]

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,
Pottakium 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

<table>
<thead>
<tr>
<th>Recovery level</th>
<th>Accuracy RAMIPRIL</th>
<th>Average % Recovery</th>
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<tbody>
<tr>
<td></td>
<td>Amount taken(mcg/ml)</td>
<td>Area</td>
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<tr>
<td>80%</td>
<td>5</td>
<td>119.895</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>119.884</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>119.990</td>
</tr>
<tr>
<td>100%</td>
<td>6</td>
<td>140.200</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>140.110</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>141.410</td>
</tr>
<tr>
<td>120%</td>
<td>7</td>
<td>161.224</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>161.907</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>166.740</td>
</tr>
<tr>
<td>Recovery level</td>
<td>Accuracy</td>
<td>LOSARTAN</td>
</tr>
<tr>
<td>----------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>Amount</td>
<td>Area</td>
</tr>
<tr>
<td></td>
<td>taken(mcg/ml)</td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td>100</td>
<td>9868.786</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9917.121</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9981.041</td>
</tr>
<tr>
<td>100%</td>
<td>120</td>
<td>11790.173</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>11971.355</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>11856.178</td>
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<tr>
<td>120%</td>
<td>140</td>
<td>13716.886</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>13735.434</td>
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<td></td>
<td>140</td>
<td>13735.434</td>
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</table>

Table-2: PRECISION

<table>
<thead>
<tr>
<th>RAMIPRIL</th>
<th>LOSARTAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.No.</td>
<td>Rt</td>
</tr>
<tr>
<td>1</td>
<td>2.563</td>
</tr>
<tr>
<td>2</td>
<td>2.547</td>
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<tr>
<td>3</td>
<td>2.520</td>
</tr>
<tr>
<td>4</td>
<td>2.547</td>
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<tr>
<td>5</td>
<td>2.563</td>
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<td>6</td>
<td>2.547</td>
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<tr>
<td>avg</td>
<td>2.5478</td>
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<tr>
<td>stdev</td>
<td>0.0157</td>
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<tr>
<td>% RSD</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Table-3: LINEARITY RESULTS OF RAMIPRIL AND LOSARTAN

Linearity observation of Ramipril

<table>
<thead>
<tr>
<th>S.No</th>
<th>Linearity Level</th>
<th>Concentration (µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>3 ppm</td>
<td>76.174</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>4 ppm</td>
<td>95.497</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>5 ppm</td>
<td>119.304</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>6 ppm</td>
<td>137.593</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>7 ppm</td>
<td>164.621</td>
</tr>
</tbody>
</table>
Linearity observation of Losartan

<table>
<thead>
<tr>
<th>S.No</th>
<th>Linearity Level</th>
<th>Concentration</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>60 ppm</td>
<td>6025.881</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>80 ppm</td>
<td>7811.081</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>100 ppm</td>
<td>10045.494</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>120 ppm</td>
<td>11630.470</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>140 ppm</td>
<td>13699.397</td>
</tr>
</tbody>
</table>

Correlation Coefficient 0.999

![Fig 2: LINEARITY GRAPH OF RAMIPRIL](image)
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 of 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 Fig 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>
<thead>
<tr>
<th>Table 6: ASSAY RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Results Drug</td>
</tr>
<tr>
<td>RAMIPRIL</td>
</tr>
<tr>
<td>LOSARTAN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 7: SYSTEM SUITABILITY PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>System suitability Parameters</td>
</tr>
<tr>
<td>Tailing Factor</td>
</tr>
<tr>
<td>Theoretical plates</td>
</tr>
<tr>
<td>Resolution</td>
</tr>
</tbody>
</table>

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 Losartan in tablet formulation. The method was validated as per ICH guidelines.

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