Estimation and Validation of the Combination of Ramipril and Losartan by RP-HPLC

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ABSTRACT:
A simple, specific and accurate reverse phase liquid chromatographic method was developed for the simultaneous determination of Ramipril and Losartan potassium in table dosage forms. A Symmetry C18 (4.6 x 150mm, 5µm, Make: ODS) or equivalent column in isocratic mode, with mobile phase Phosphate buffer: acetonitrile: 40:60% v/v was used. The flow rate was 0.8 ml/min and effluent was monitored at 210 nm. The retention times of Ramipril and Losartan potassium were 2.6 and 3.6 min, respectively. The method was validated for linearity, accuracy, precision, and limit of quantitation. Linearity, accuracy, and precision were acceptable in the ranges. The proposed method was also validated and successfully applied to the estimation of Ramipril and Losartan potassium in combined tablet formulations. High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture.

Keywords: Losartan potassium, Ramipril, validation, RP-HPLC

I. INTRODUCTION

Fig.1. Losartan Potassium
Molecular Formula – C₂₂H₂₃ClKN₂O, Molecular Weight – 461.91 Freely soluble in water. Its category is Angiotensin II receptor. Its mechanism of action is competitive angiotensin II receptor type 1 (AT₁) receptor antagonist. It is contraindicated in Concurrent use with NSAIDs may further worsen renal function. Pregnancy, lactation, children with CrCl <30 ml/min/1.73m². Ramipril (2S,3aS,6aS)-1[(S)-N-{(S)-1-Carboxy-3-phenylpropyl]leanly] octahydrocyclopenta [b]pyrrole-2-carboxylic acid, 1-ethyl ester¹⁸ Molecular Formula -

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C₂₃H₃₂N₂O₅, Molecular Weight – 416.Freely soluble in water. Its category is Angiotensin converting enzyme inhibitor. It act as Competitive ACE Inhibitor. It is contraindicated during Hypersensitivity, bilateral renal artery stenosis, a single kidney with unilateral renal artery stenosis, Aortic stenosis, Pregnancy and lactation.

**Fig.2 Ramipril**

Chromatographic Method: A simple and sensitive reverse phase HPLC method has been developed for the analysis of Ramipril & Losartan Tablets. The method utilizes sample preparation followed by separation on a Column Symmetry C18 (4.6 x 150mm, 5µm, Make: ODS) or equivalent. Analytes were monitored by UV detection at 210nm using an isocratic mode with Mixture of buffer: Acetonitrile in the ratio 40:60v/v as mobile phase. The flow rate was set at 0.8ml/min and effluent was monitored at 210nm. The retention time was 7 min. Calibration curves for Ramipril & Losartan was found respectively.

**Equipment and Apparatus used:**

- HPLC Waters Separation Module LC-20AT Prominence Liquid Chromatography
- UV Detector
- Chromatographic data Software : EMPOWER
- SymmetryColumn C18 (250*4.6* 5µ)
- Vacuum filter pump
- Mobile phase reservoir
- Ultra Sonicator, Membrane filter (0.45 and 0.2microns)

**Reagents:**

- Acetonitrile HPLC grade
- Water (HPLC)
- Potassium Dihydrogen Phosphate

**II. METHOD DEVELOPMENT**

The Objective of this study is to validate the method used for determination of Assay Ramipril and Losartanin the Ramipril & Losartan Tablet 5mg & 50mg by HPLC method is based on the literature survey.

**A. Preparation of the Ramipril & Losartan Standard & Sample Solution:**

**Standard Solution Preparation:**

Accurately weigh and transfer 10 mg of Ramipril and 10mg of Losartan working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.2ml of Ramipril & 2ml of Losartan the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Sample Solution Preparation:**

Accurately weigh and transfer 78.2 mg of Ramipril and Losartan Tablet powder into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 4ml of Ramipril & Losartan the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of mobile phase.**

Mix a mixture of Phosphate buffer 400 ml (40%) and 600 ml of Acetonitrile HPLC (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

**Chromatographic Parameters**

- Equipment : High performance liquid chromatography equipped with
Auto Sampler and DAD or UV detector

- **Column**: Symmetry C18 (4.6 x 150mm, 5µm, Make: ODS) or equivalent
- **Flow rate**: 0.8 ml per min
- **Wavelength**: 210 nm
- **Injection volume**: 20 µl
- **Column oven**: Ambient
- **Run time**: 7min

Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and percentage purity was found out by following formulae.

**B. Procedure**

**Preparation of the Ramipril & Losartan Standard & Sample Solution:**

**Standard stock Solution Preparation:**

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**III. RESULTS AND DISCUSSION**

Drug quality control, stability, metabolism, pharmacokinetics, and toxicity studies all necessitate the determination of drugs in pharmaceutical formulations and biological samples.

Correspondingly, efficient and validated analytical methods are very critical requirements for all these investigations. Chromatographic parameters were preliminary optimized to develop a LC method for validation report for assay of Ramipril & Losartan with short analysis time and acceptable resolution (Rs> 2).

In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested. Methanol produced a high retention time for Ramipril and losartan high column pressures due to the high viscosity. Acetonitrile was found to display advantageous separations. Change of percentage of acetonitrile in the mobile phase brought about a great influence on retention time.

The system suitability parameters prove that the proposed method is equally suitable for validation of Ramipril & Losartan, the chromatogram were found to be satisfactory on Symmetry C18 (4.6 x 150mm, 5µm, Make: ODS) or equivalent, using mobilephase composition of 4.5 PH p/paratio (400:600) with flow rate of 0.8 ml/min. The above method is suitable routine pharmaceutical applications involving the validation of Ramipril and Losartan.

Validation of analytical method for determination of assay of Ramipril 5 mg and losartan 25mg tablets was performed for the parameters including – Specificity (System precision), Method precision), Intermediate precision (Ruggedness), Accuracy and Robustness values are in table no 2.

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>%CONCENTRATION</th>
<th>% RECOVERY</th>
<th>MEAN RECOVERY</th>
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<tbody>
<tr>
<td>RAMIPRIL</td>
<td>50%</td>
<td>100.9%</td>
<td></td>
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<td></td>
<td>100%</td>
<td>99.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150%</td>
<td>101.3%</td>
<td>100.5%</td>
</tr>
<tr>
<td>LOSARTAN</td>
<td>50%</td>
<td>101.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>99.9%</td>
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<tr>
<td></td>
<td>150%</td>
<td>101%</td>
<td>100.8%</td>
</tr>
</tbody>
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Table no.2 Chromatographic parameters of Losartan and ramipril

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>RAMIPRIL</th>
<th>LOSARTAN</th>
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<td>$\lambda_{max}$</td>
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<td>Correlation coefficient</td>
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<td>%RSD</td>
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<td>Standard Deviation</td>
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<td>%Assay</td>
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<tr>
<td>%Recovery</td>
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<td>100.</td>
</tr>
<tr>
<td>Asymmetry factor</td>
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<td>1.7</td>
</tr>
</tbody>
</table>

Fig.3 Linearity of Losartan

Fig.4 Linearity of Losartan

\[
y = 81.143x^2 + 53277x - 17742
\]
Fig.5. Chromatogram of losartan and Ramipril

REFERENCES