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, 5«m, 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 2.6and 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 linearity range for Losartan potassium and Ramipril were in the range of 0.04-100 µg/ml and 0.2-300 µg/ml, respectively. 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 potassi*um, Ramipril, validation, RP-HPLC**

I.INTRODUCTION

Losartan Potassium (2-Butyl-4-Chloro-1-{[2'-(1H-Tetrazole-5-Yl)Biphenyl]Methyl}1Himidazole-5-Yl) Methanol Monopotassium Salt.

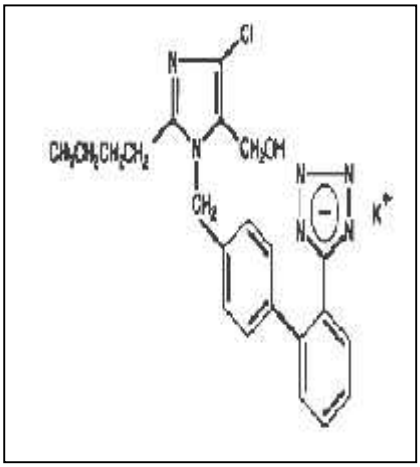

Fig.1.Losartan Potassium

Molecular Formula – $C_{22}H_{23}ClKN_6O$, 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 (AT_1) 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 ester[18] Molecular Formula -

$C_{23}H_{32}N_2O_5$, Molecular Weight – 416Freely 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.

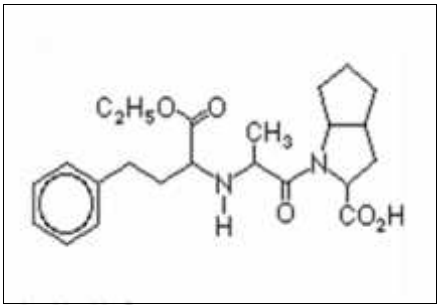

Fig.2 Ramipril

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: Acetonitrilein 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 Losartanworking 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 Losartanthe 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 LosartanTablet powder into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 4ml of Ramipril & Losartanthe 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:

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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 analyses time and acceptable resolution (Rs> 2).

In order to identify a suitable organic modifier, various compositions of acetonitrile andmethanol were tested. Methanol produced a high retention time for Ramipril and losartan highcolumn 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 pdparatio (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 no.1 Accuracy

DRUGS	%CONCENTRATION	% RECOVERY	MEAN RECOVERY
RAMIPRIL	50% 100% 150%	100.9% 99.3% 101.3%	100.5%
LOSARTAN	50% 100% 150%	101.5% 99.9% 101%	100.8%

Table no.2 Chromatographic parameters of Losartan and ramipril

PARAMETERS	RAMIPRIL	LOSARTAN
λmax	210	210
Correlation coefficient	0.9999	0.9999
%RSD	0.42	0.12
Standard Deviation	493.7	9864.6
%Assay	98	99.3
%Recovery	100.5	100.
Asymmentry factor	1.1.	1.7

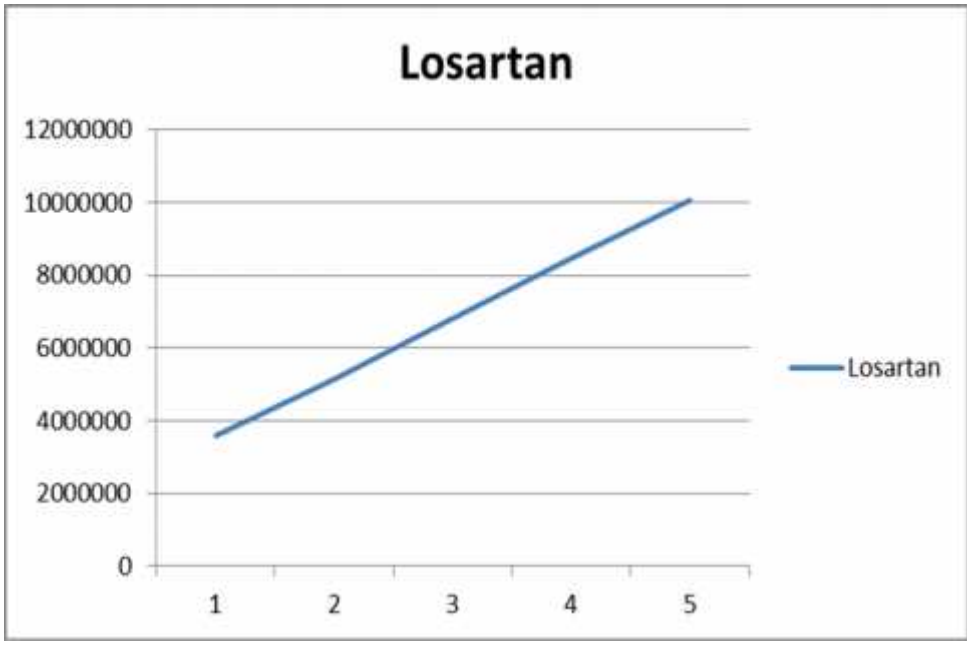

Fig.3Linearity of Losartan

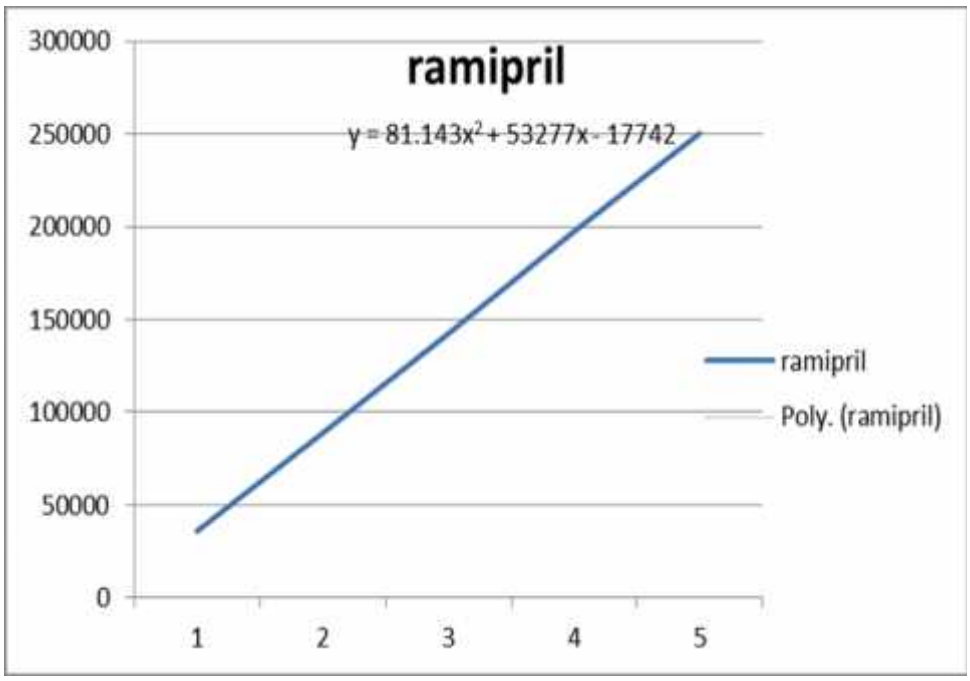

Fig.4.Linearity of Losartan

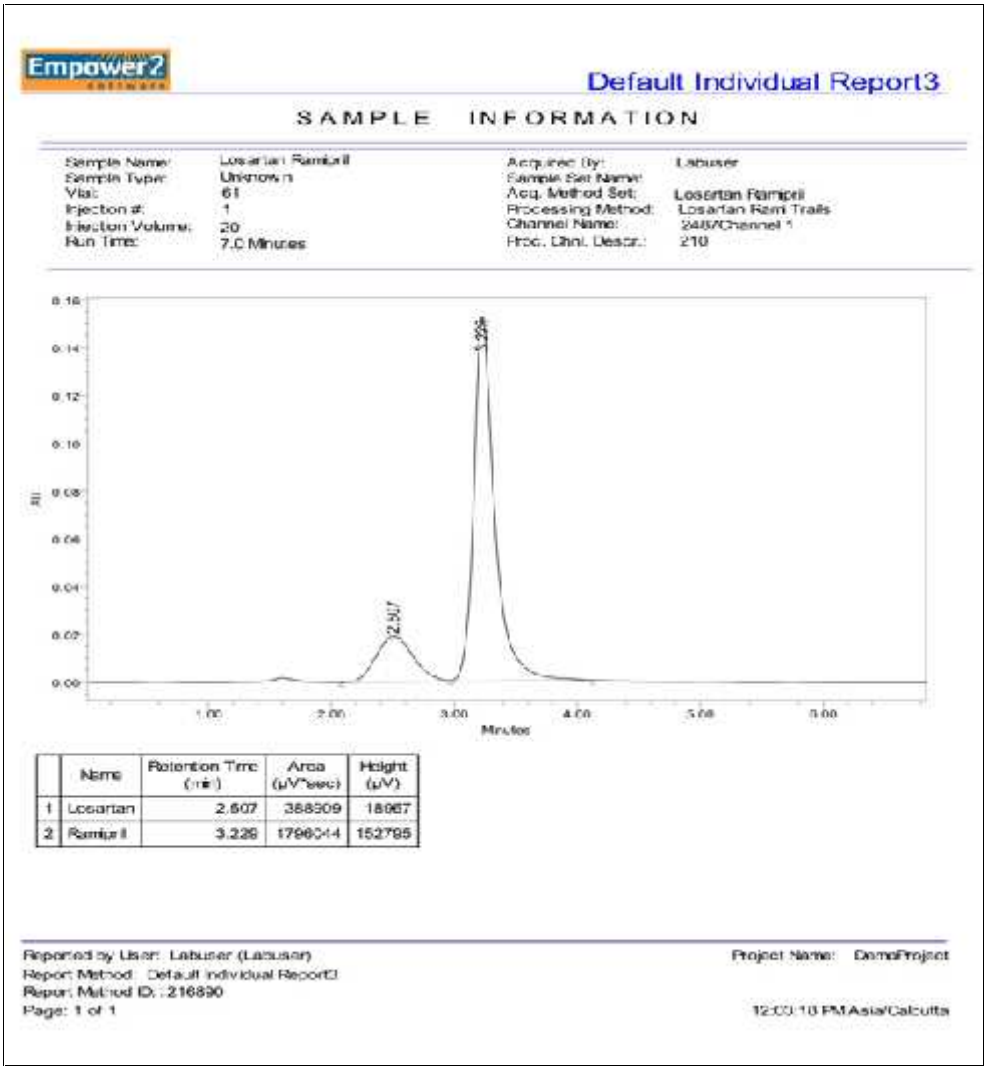

Fig.5.Chromatogram of losartan and Ramipril

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