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SIMULTANEOUS ESTIMATION OF ENALAPRIL MALEATE AND LOSARTAN POTASSIUM IN TABLET DOSAGE FORM BY USING RP-HPLC

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Abstract:

A simple, accurate, robust, specific and precise Reverse phase HPLC method was developed for the simultaneous estimation of the Enalapril maleate and Losartan potassium in pure and pharmaceutical dosage form as per ICH Guidelines. Chromatogram was run through Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size column and of Methanol: TEA Buffer pH-4.8 (35:65) v/v at a flow rate of 1.0 ml/min. Temperature was maintained at 38°C. Optimized wavelength selected was 276nm. Retention time of Enalapril maleate and Losartan potassium were found to be 2.090min and 5.289min. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification. The proposed method optimized and validated as per ICH guidelines. The method is validated as per ICH guideline by determining its specificity, accuracy, precision, linearity & range, ruggedness, robustness and system suitability. The results of the study show that the proposed method is simple, rapid, precise and accurate, which is useful for the routine determination of Enalapril maleate and Losartan potassium in bulk and tablet dosage forms. The method could be applied for determination of in its tablet dosage forms without any interference from excipients or endogenous substances. The proposed method is suitable for routine quality control analysis.

Keywords: *Enalapril maleate, Losartan potassium, RP-HPLC, Validation, ICH Guidelines.*

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INTRODUCTION:

1. Introduction to HPLC [1-15]

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good sensitivity.

High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

1.1.1 Types of Chromatography [1]

1. Normal-phase chromatography

Mechanism: Retention by interaction with the polar surface of the stationary phase with polar parts of the sample molecules.

Stationary phase: SiO2, Al2O3, -NH2, -CN, -Diol, -NO2, etc.

Mobile phase: Heptane, hexane, cyclohexane, CHCl3, CH2Cl2, dioxane, methanol, etc.

Application: Separation of non-ionic, non-polar to medium polar substances. Disadvantage: Lack of reproducibility of retention times as water or protic organic solvents change the hydration state of the silica or alumina chromatographic media.

2. Reversed-phase chromatography

Mechanism: Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.

Stationary phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, (CH2)n-CN, (CH2)n-diol, etc.

Mobile phase: Methanol, Acetonitrile, water, buffer (sometimes with additives of THF or Dioxane), etc.

Application: Separation of non-ionic and ion forming non-polar to medium polar substances (carboxylic acids, hydrocarbons). If ion forming substances (as carboxylic acids) are to be separated, a pH control by buffers is necessary.

3. Reversed-phase ion-pair chromatography

Mechanism: Ionic sample molecules are ionically bound to an ion-pair reagent. The ion- pair reagent contains an unpolar part suitable for interaction with the unpolar hydrocarbon chain of the stationary phase.

Stationary phase: Reversed phase materials (RP-18, RP-8, CN), etc.

Mobile phase: Methanol, Acetonitrile, buffer with added ion-pair reagent in the concentration range of 0.001 to 0.01 M, etc.

Application: Ionic substances often show very poor retention in reversed phase chromatography. To overcome this difficulty an ion-pair reagent is added to the eluent.

4. Ion-exchange chromatography

Mechanism: Retention of reversible ionic bonds on charged groups of the stationary phase

Stationary phase:

	Strong	Weak
Cation exchanger	SO_3^-	COO^-
Anion exchanger	NR_3^+	NHR_2^+

Mobile phase: Aqueous buffer systems.

Application: Separation of substances which can form ions such as inorganic ions, organic acids, organic bases, proteins, nucleic acids.

1.1.2 Advantages of HPLC [2]

1) It provides specific, sensitive and precise method for analysis of the different complicated sample.

2) There is ease of sample preparation and sample introduction.

3) There is speed of analysis.

4) The analysis by HPLC is specific, accurate and precise.

5) It offers advantage over gas chromatography in analysis of many polar, ionic substances, high molecular weight substances, metabolic products and thermo labile as well as nonvolatile substances.

1.1.3 Applications of HPLC [2]

a) Natural Products: HPLC is an ideal method for the estimation of various components in plant extracts which resemble in structure and thus demand a specific and very sensitive method e.g., analysis of digitalis, cinchona, liquorice, and ergot extracts.

b) Stability studies: HPLC is now used for ascertaining the stability of various pharmaceuticals. With HPLC the analysis of the various degradation products can be done and thus stability indicating HPLC systems have been developed.

c) Bioassays and its complementation: Complex molecules as antibiotics and peptide hormones are mainly analyzed by bioassay which suffers from

high cost, necessity replicates, poor precision and length of time required. Also bioassay gives an overall estimate of potency and gives no guidance about the composition. Thus HPLC can be used to complement bioassays and give an activity profile. It has been used for analysis of chloramphenicol, penicillins and clotrimoxazole, sulfas and peptides hormones.

d) HPLC has also been used in the cosmetic industry for quality control of various cosmetics.

The basic components of HPLC are: [4-8]

1. Pumping System
2. Sample Introduction Device
3. Chromatographic Column
4. Detector
5. Data handling Device

1. Pumping System: The HPLC pump is very important component of the system. It delivers the constant flow of the mobile phase or phases so that the separation of the components of the mixture occur in a reasonable time. Its performance directly affects retention time, reproducibility and detector sensitivity. Three main types of pumps are used in HPLC to propel the liquid mobile phase through the system are as under;

a. Displacement pump: It produces a flow that tends to independent of viscosity and backpressure and also output is pulse free. But it possesses limited capacity (250 ml).

b. Reciprocating pump: It has small internal volume (35 to 400 µl). It has high output pressure (up to 10,000 psi) and constant flow rates. But it produces a pulsed flow.

c. Pneumatic or constant pressure pump: They are pulse free, suffer from limited capacity as well as a dependence of flow rate on solvent viscosity and column back pressure. They are limited to pressure less than 2000 psi.

There are two type of elution process, i.e. isocratic and gradient

Isocratic: In this system, the things are kept constant throughout the run. In the case of pumping of mobile phase, the mobile phase composition is kept constant throughout the run. The nominal flow rate accuracy required is ±1% of the set flow

Gradient: There is some change purposely incorporated during the particular sample run to achieve a better or/and faster separation. In case of pumping mobile phase, the composition of mobile phase is continuously varied during the particular run. The gradient accuracy of ±1% of the step gradient composition is typical.

2. Sample Introducing Device

It is not possible to use direct syringe injection on column like GC, as the inlet pressure in LC is too high. Insertion of the sample onto the pressurized column must be as a narrow plug so that the peak broadening attributable to this step is negligible. The injection system itself should have no dead (void) volume. There are three important ways of introducing the sample into injection port.

a. Loop injection: In which, a fixed amount of volume is introduced by making use of fixed volume loop injector.

b. Valve injection: In which, a variable volume is introduced by making use of an injection valve.

c. On column injection: In which, a variable volume is introduced by means of a syringe through a septum.

3. Chromatographic Column

Column is a heart of chromatography. The column is usually made up of heavy glass or stainless steel tubing to withstand high pressure. The columns are usually 10-30 cm long and 4-10 mm inside diameter containing stationary phase at particle diameter of 25 µm or less. Columns with an internal diameter of 5 mm give good results because of compromise between efficiency, sample capacity, and the amount of packing and solvent required.

Column packing:

The packing used in modern HPLC consists of small, rigid particles having a narrow particle size distribution. There are three main types of column packing in HPLC.

a. Porous, polymeric beds: Porous, polymeric beds based on styrene divinyl benzene co-polymers used for ion exchange and size exclusion chromatography. For analytical purpose these have now been replaced by silica based, packing which are more efficient and more stable.

b. Porous layer beds: Consisting of a thin shell (1-3µm) of silica or modified silica on a spherical inert core (e.g. Glass). After the development of totally porous micro particulate packings, these have not been used in HPLC.

c. Totally Porous silica particles (dia. < 10µm): These packing have widely been used for analytical HPLC in recent years. Particles of diameter > 20µm are usually dry packed. While particles of diameter < 20µm are slurry packed in which particles are suspended on a suitable solvent and the slurry so obtained is driven into the column under pressure.

4. Detector

The function of the detector in HPLC is to monitor the mobile phase as it merges from the column. There are several detectors available in the market. However UV Visible detector, photo diode array detector, fluorescence detector, conductometric and coulometric detector are more commonly used. The new ELSD detector is proving to be important detector, while the MS detector is outstanding. Detectors are usually of two types:

a. Bulk property detectors: It compares overall changes in a physical property of the mobile phase with and without an eluting solute e.g. refractive index, dielectric constant or density.

b. Solute property detectors: It responds to a physical property of the solute, which is not

exhibited by the pure mobile phase e.g. UV absorbance, fluorescence or diffusion current.

5. Data handling Device

Computer-based system that controls all components of HPLC instrument (eluent composition (mixing of different solvents); temperature, injection sequence, etc.) and acquires data from the detector and monitors system performance (continuous monitoring of the mobile phase composition, temperature, back pressure, etc.)

EXPERIMENTAL METHODS

Instruments used

HPLC WATERS Alliance 2695 separation module. 996 PDA detector, software: Empower 2
pH meter Lab India
Weighing machine Sartorius
Digital ultra sonicator Labman

Chemicals used

Enalapril maleate Sura labs
Losartan potassium Sura labs
Water and Methanol for HPLC LICHROSOLV (MERCK)
Acetonitrile for HPLC Merck

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Tri Ethyl Amine Buffer (35:65% v/v)
Column : Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size
Flow rate : 1 ml/min
Wavelength : 261 nm
Column temp : 38ºC
Injection Volume : 10 µl
Run time : 10 minutes

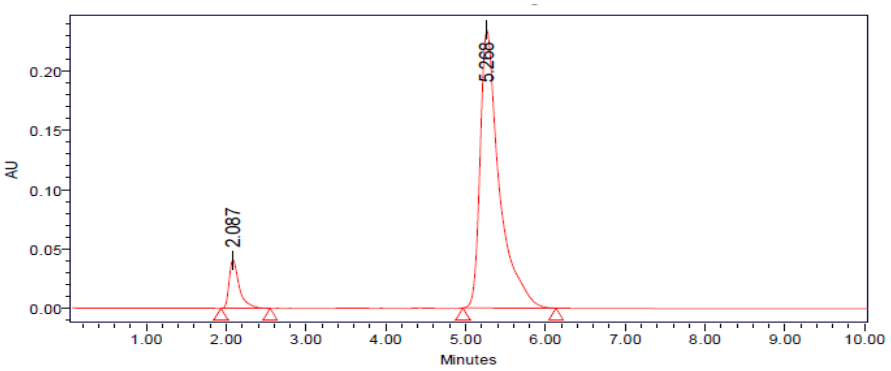

Fig-: Optimized Chromatogram

Table-: Peak Results for Optimized Chromatogram

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Enalapril maleate	2.087	3425413	567933		1.0	5565.5
2	Losartan potassium	5.268	1629854	517733	2.5	1.1	5355.2

Observation: From the above chromatogram it was observed that the Enalapril maleate and Losartan potassium peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

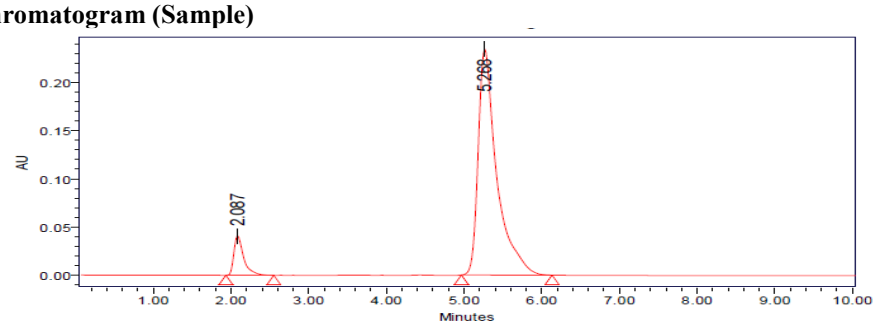

Figure: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Enalapril maleate	2.087	3468547	567933		1.0	5565.5
2	Losartan potassium	5.268	16289441	517733	2.5	1.1	5355.2

Acceptance Criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

Blank:

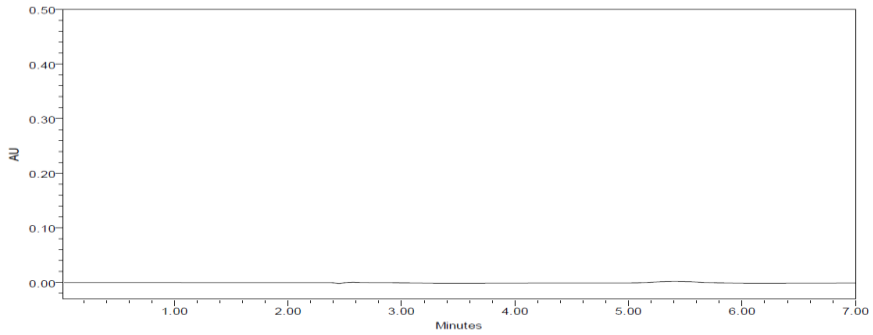

Fig: Chromatogram Showing Blank Solution (Mobile Phase Preparation)

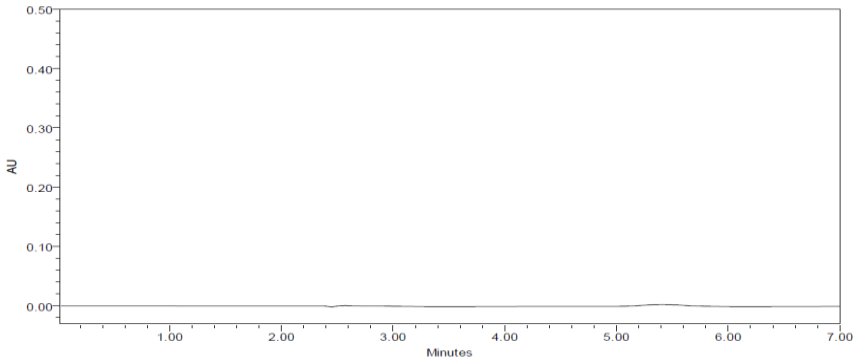

Fig: Chromatogram showing Placebo

System Suitability:

Table-: Results of System Suitability for Enalapril maleate

S.No.	Name	Rt	Peak Area	Height	USP plate Count	USP Tailing
1	Enalapril maleate	2.090	325896	39689	5653	1.42
2	Enalapril maleate	2.090	326989	39689	5695	1.42
3	Enalapril maleate	2.089	327985	39698	5598	1.44
4	Enalapril maleate	2.089	329477	40198	5569	1.43
5	Enalapril maleate	2.085	325858	40259	5612	1.47
Mean			**327241**			
Std. Dev			**1527.944**			
% RSD			**0.466917**			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table-: Results of System Suitability for Losartan potassium

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Losartan potassium	5.289	3576859	232352	5785	1.46	9.80
2	Losartan potassium	5.289	3585695	232365	5915	1.47	9.81
3	Losartan potassium	5.338	3596885	232451	5895	1.48	9.81
4	Losartan potassium	5.327	3565874	231653	5987	1.40	9.83
5	Losartan potassium	5.262	3598654	233658	5861	1.43	9.82
Mean			3588946				
Std. Dev			3585486				
% RSD			11360.78				

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Enalapril maleate and Losartan potassium in drug product.

Assay (Standard):

Table-: Peak Results for Assay Standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Enalapril maleate	2.090	328966	39586		1.70	5563	1
2	Losartan potassium	5.289	3574898	232356	9.80	1.77	5665	1
3	Enalapril maleate	2.089	327898	39568		1.66	5584	2
4	Losartan potassium	5.338	3569854	232548	9.93	1.83	5646	2
5	Enalapril maleate	2.089	328657	40526		1.68	5584	3
6	Losartan potassium	5.327	3565874	232547	9.91	1.86	5783	3

Assay (Sample):

Table-: Peak Results for Assay Sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Enalapril maleate	2.088	336589	40365		1.69	5569	1
2	Losartan potassium	5.276	3586985	232565	9.75	1.89	5658	1
3	Enalapril maleate	2.087	335684	41245		1.72	5548	2
4	Losartan potassium	5.268	3587896	235685	9.82	1.91	5864	2
5	Enalapril maleate	2.085	335876	40898		1.75	5496	3
6	Losartan potassium	5.262	3586848	234588	9.78	1.95	5754	3

Table: Showing Assay Results

S.No.	Name of Compound	Label Claim	Amount Taken (from Combination Tablet)	% Purity
1	Enalapril maleate	10mg	9.98	99.72%.
2	Losartan potassium	50mg	48.6	99.72%.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Enalapril maleate and Losartan potassium in pharmaceutical dosage form was found to be 99.72%.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Enalapril maleate:

Concentration µg/ml	Average Peak Area
20	164436
30	255571
40	348687
50	439024
60	534830

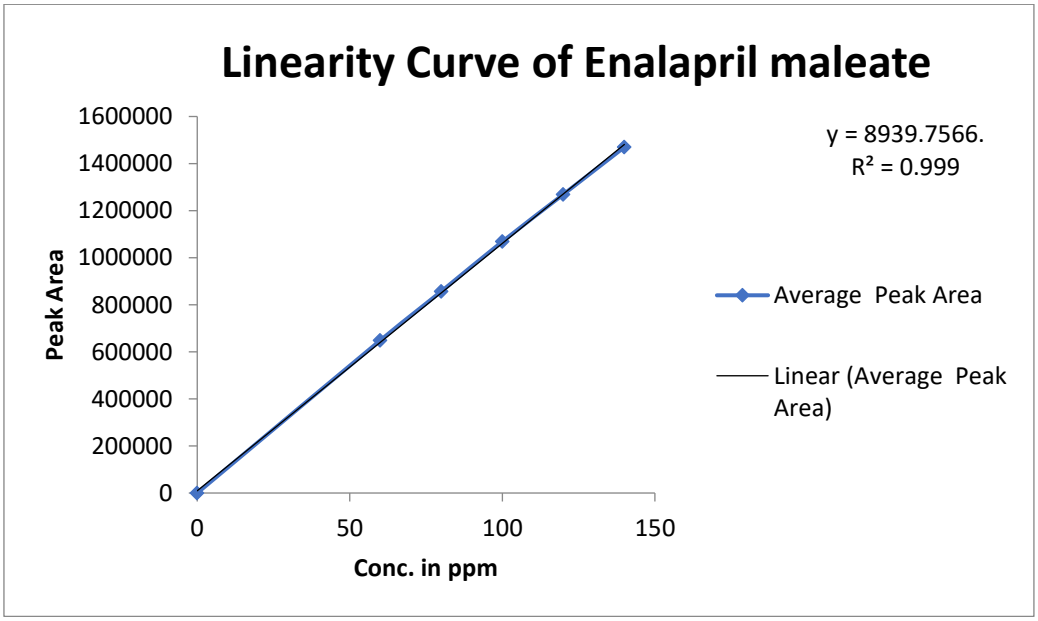

Figure 6.3.4 Calibration Graph for Enalapril maleate

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Enalapril maleate is a straight line.

Y = mx + c

Slope (m) = 8939

Intercept (c) = 9566

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 7566. These values meet the validation criteria.

Losartan potassium

Concentration µg/ml	Average Peak Area
25	1782454
37.5	2728974
50	3688678
62.5	4658022
75	5592695

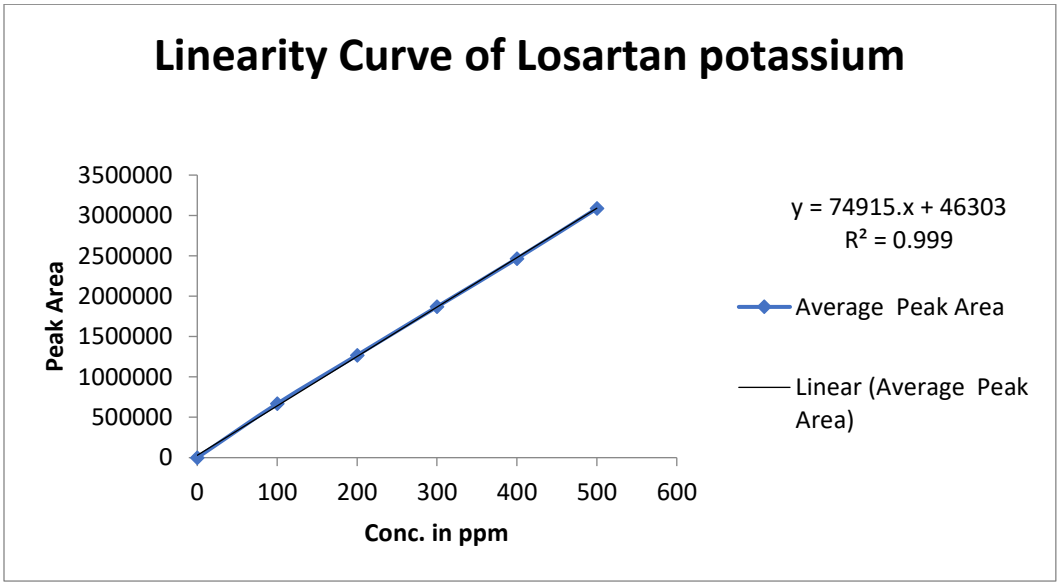

Figure 6.3.4 Calibration Graph for Losartan potassium

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Losartan potassium is a straight line.

Y = mx + c

Slope (m) = 74915

Intercept (c) = 46303

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 46303. These values meet the validation criteria.

PRECISION:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated %RDS.

Table-: Results of Repeatability for Enalapril maleate :

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Enalapril maleate	2.086	327689	41697	5081.3	1.8
2	Enalapril maleate	2.083	327978	41402	5144.1	1.8
3	Enalapril maleate	2.083	327879	41540	5118.1	1.8
4	Enalapril maleate	2.081	327868	42256	5147.3	1.8
5	Enalapril maleate	2.081	327859	42143	5101.8	1.8
Mean			**327854.6**			

Std. Dev			**104.2176**			
% RSD			**0.031788**			

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table-: Results of Method Precision for Losartan potassium

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Losartan potassium	5.178	3576985	241253	5969.5	2.0	9.8
2	Losartan potassium	5.199	3578989	2365824	5865.1	2.0	9.7
3	Losartan potassium	5.235	3576859	239568	5936.4	2.0	9.9
4	Losartan potassium	5.202	3578458	2386547	5964.4	2.0	9.8
5	Losartan potassium	5.206	3579864	241425	5045.6	2.0	9.5
Avg			**3578231**				
Std. Dev			**1296.889**				
% RSD			**0.036244**				

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Day 1:

Table-: Results of Intermediate Precision for Enalapril maleate

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Enalapril maleate	2.083	328986	42365	5556.2	1.6
2	Enalapril maleate	2.083	328898	42685	5524.6	1.6
3	Enalapril maleate	2.089	327789	42544	5465.2	1.6
4	Enalapril maleate	2.083	328758	42685	5464.5	1.6
5	Enalapril maleate	2.082	328869	42256	5589.4	1.8
6	Enalapril maleate	2.080	329687	42365	5565.5	1.8
Mean			**328831.2**			
Std. Dev			**608.8985**			
% RSD			**0.185171**			

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

Table-: Results of Intermediate Precision for Losartan potassium

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Losartan potassium	5.229	3578659	243659	5252.1	2.2	10.2

2	Losartan potassium	5.203	3578469	2436521	5256.4	2.1	10.0
3	Losartan potassium	5.133	3574865	245664	5356.8	2.1	10.0
4	Losartan potassium	5.229	3574824	243652	5265.6	2.2	10.2
5	Losartan potassium	5.151	3579861	244254	5235.7	1.5	9.9
6	Losartan potassium	5.112	3574898	236558	5986.2	1.6	9.9
Mean			**3576929**				
Std. Dev			**2112.55**				
% RSD			**0.05906**				

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

Day 2:

Table-: Results of Intermediate precision Day 2 for Enalapril maleate

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Enalapril maleate	2.078	370979	42978	7083.0	1.9
2	Enalapril maleate	2.082	371041	42568	8583.2	1.8
3	Enalapril maleate	2.080	371386	42211	7533.2	1.8
4	Enalapril maleate	2.089	369246	42277	6537.8	1.6
5	Enalapril maleate	2.083	370840	42065	5489.3	1.6
6	Enalapril maleate	2.089	369246	42277	6537.8	1.6
Mean			**370456.3**			
Std. Dev			**954.6004**			
% RSD		2.078	370979	42978	7083.0	1.9

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

Table-: Results of Intermediate precision for Losartan potassium

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Losartan potassium	5.077	3578985	246818	5208.0	1.5	10.1
2	Losartan potassium	5.151	3578415	242854	5127.6	1.3	10.0
3	Losartan potassium	5.112	3579864	242955	5269.7	1.5	10.2
4	Losartan potassium	5.133	3579862	242955	5269.7	1.6	10.2
5	Losartan potassium	5.203	3578948	242854	5127.6	1.5	10.0
6	Losartan potassium	5.133	3586775	242955	5269.7	1.6	10.2
Mean			**3580475**				
Std. Dev			**3137.978**				
% RSD			**0.087641**				

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:
Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table-: The Accuracy Results for Enalapril maleate

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	186584.7	20	20.026	100.13	100.435%
100%	367968.7	40	40.32	100.80	
150%	545922	60	60.225	100.375	

Table-: The accuracy results for Losartan potassium

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	949127	150	150.328	100.218%	100.15%
100%	1867824	300	300.441	100.147%	
150%	2785321	450	450.359	100.079%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result:

Enalapril maleate :
=0.7/ml

Losartan potassium:
=2.1µg/ml

LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result:

Enalapril maleate:
= 0.9µg/ml

Losartan potassium:
= 2.7µg/ml

Robustness

Table-: Results for Robustness

Enalapril maleate:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	327989	2.090	5698	1.70
Less Flow rate of 0.9 mL/min	302986	2.736	5569	1.82

More Flow rate of 1.1 mL/min	316989	1.673	5598	1.91
Less organic phase	315989	2.736	5651	1.82
More organic phase	308986	1.673	5452	1.91

Acceptance Criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Losartan potassium:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3576856	5.289	5689	1.77
Less Flow rate of 0.9 mL/min	3458978	6.746	5658	1.88
More Flow rate of 1.1 mL/min	3589871	4.032	5245	1.91
Less organic phase	3579124	6.746	5154	1.88
More organic phase	3578698	4.032	5652	1.91

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY

The analytical method was developed by studying different parameters.

First of all, maximum absorbance was found to be at 276 nm and the peak purity was excellent.

Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Symmetry ODS C18 (4.6×150mm, 5.0 µm) because it was giving good peak.

Ambient temperatures were found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol: TEA Buffer pH-4.8 (35:65) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Run time was selected to be 10min because analyze gave peak around 2.090, 5.289 ±0.02min respectively and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range 20-60µg/ml of Enalapril maleate and 25-75 µg/ml of Losartan potassium of the target concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Enalapril maleate and Losartan potassium in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Enalapril maleate is freely soluble in acetone, soluble in methanol and ethanol, and practically insoluble in water. Enalapril maleate is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide. Losartan potassium was found to be freely soluble in DMF, chloroform and ethyl acetate, soluble in dichloromethane, slightly soluble in ethanol and methanol, and insoluble in water.

Methanol: TEA Buffer pH-4.8 (35:65) was chosen as the mobile phase. The solvent system used in this method was economical.

The % RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Enalapril maleate and Losartan potassium in bulk drug and in pharmaceutical dosage forms.

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