Journal of Pharmacreations
PharmaCreations
ISSN: 2348-6295
Pharmacreations/ Vol 10/ Issue 3/ Jul-Sept 2023
Journal Home Page: Pharmacreations.com
Research article Open Access

Simultaneous estimation of enalapril maleate and losartan potassium in tablet dosage form by using RP-HPLC

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Published on: September 30, 2023

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 (4.6 mm×150 mm, 5 µm) 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 276 nm. Retention time of Enalapril maleate and Losartan potassium were found to be 2.090 min and 5.289 min. 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.

INTRODUCTION

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 cm^3/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. [1-29]

Drug Profile of Enalapril Maleate

Table 1: Drug profile of Enalapril Maleate

Drug	Enalapril
Synonym	Enalaprila, Enalaprilum.
IUPAC Name	2S)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl] pyrrolidine-2-carboxylic acid.
Molecular formula	$C_{20}H_{28}N_2O_5$
Molecular Weight	376.4467g/mol
Solubility	Freely soluble in Methanol, DMSO
Melting point	143-144.5 ^{0}C
pKa	3.67
Log P	0.07[30]

Structure

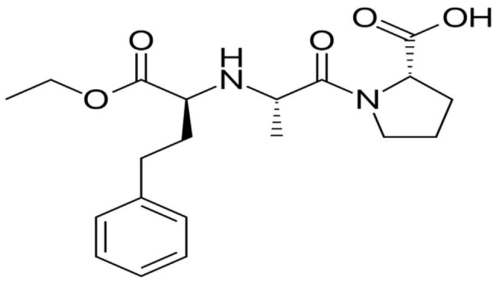

Fig 1: Structure of Enalapril Maleate

Drug Profile of Losartan Potassium

Table 2: Drug Profile of Losartan Potassium

Drug	Losartan Potassium
Drug Category	Antihypertensive Agents
IUPAC Name	[2-butyl-4-chloro-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazol-5-yl]methanol
Molecular formula	$C_{22}H_{23}ClKN_6O$
Molecular Weight	422.911 gm/mole.
Solubility	Water solubility
Melting point	184 °C
pKa	7.4
Log P	4.5[31]

Structure

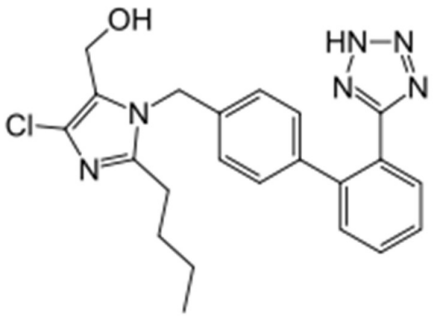

Fig. 2: Structure of Losartan Potassium

MATERIALS AND METHODS

Table 3: Instruments and Chemical used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module. 996 PDA detector, software: Empower 2
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Digital ultra sonicator	Labman
S. No	**Chemical**	**Brand names**
1	Enalapril maleate	Envas
2	Losartan potassium	Losar
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

Estimation of Enalapril maleate and Losartan potassium in pharmaceutical dosage form

Procedure

Preparation of mobile phase: Accurately measured 350 ml (350%) of HPLC Methanol and 650 ml of TEA (65%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation: The Mobile phase (Methanol: TEA Buffer (35:65 v/v) was used as the diluent.

METHOD VALIDATION PARAMETERS

SYSTEM SUITABILITY:

Accurately weigh and transfer 10 mg of Enalapril maleate and 10 mg of Losartan potassium working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 1 ml of Enalapril maleate and 3 ml of Losartan potassium from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Enalapril maleate and 10 mg of Losartan potassium working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 1 ml of Enalapril maleate and 3 ml of Losartan potassium from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Enalapril maleate and Losartan potassium sample into a 10 mL clean dry volumetric flask and add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1 ml of Enalapril maleate and 3 ml Losartan potassium above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent.

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY

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

Preparation of Level – I (60ppm of Enalapril maleate & 100ppm of Losartan potassium): Pipette out 0.6 ml of Enalapril maleate and 1 ml of Losartan potassium stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (80ppm of Enalapril maleate & 200ppm of Losartan potassium): Pipette out 0.8 ml of Enalapril maleate and 2 ml of Losartan potassium stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (100ppm of Enalapril maleate & 300ppm of Losartan potassium): Pipette out 1 ml of Enalapril maleate and 3 ml of Losartan potassium stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (120ppm of Enalapril maleate & 400ppm of Losartan potassium): Pipette out 1.2 ml of Enalapril maleate and 4 ml of Losartan potassium stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (140ppm of Enalapril maleate & 500ppm of Losartan potassium): Pipette out 1.4 ml of Enalapril maleate and 5 ml of Losartan potassium stock solutions was take in a 10 ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

Repeatability

Preparation of Enalapril maleate and Losartan potassium Product Solution for Precision

Accurately weigh and transfer 10 mg of Enalapril maleate and 10 mg of Losartan potassium working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 1 ml of Enalapril maleate and 3 ml of Losartan potassium from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision: To evaluate the intermediate precision of the method, Precision was performed on different days by maintaining same conditions.

ACCURACY

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Enalapril maleate and Losartan potassium and calculate the individual recovery and mean recovery values.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution: Accurately weigh and transfer 10 mg of Enalapril maleate and 10 mg of Losartan potassium working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1 ml of Enalapril maleate and 3 ml of Losartan potassium from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of flow conditions: The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1 ml/min, remaining conditions are same. 10 µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e., Methanol: TEA Buffer was taken in the ratio and 30:70, 40:60 instead of 35:65, remaining conditions are same. 10 µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

The developed method was validated as per ICH guidelines for parameters such as Linearity, Precision, Accuracy, LOD and LOQ, Robustness, Specificity.

Table 4: Optimized Chromatographic Conditions

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

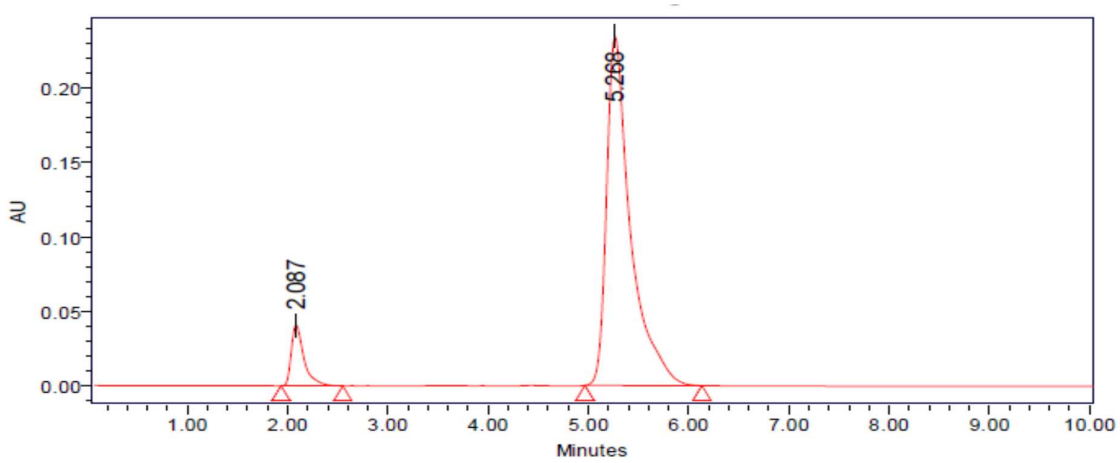

Fig 3: Optimized Chromatogram of Enalapril Maleate and Losartan Potassium

SYSTEM SUITABILITY

Table 5: Results of System Suitability for Enalapril Maleate

S.No.	Name	R_t	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**			

Table 6: 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**				

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.

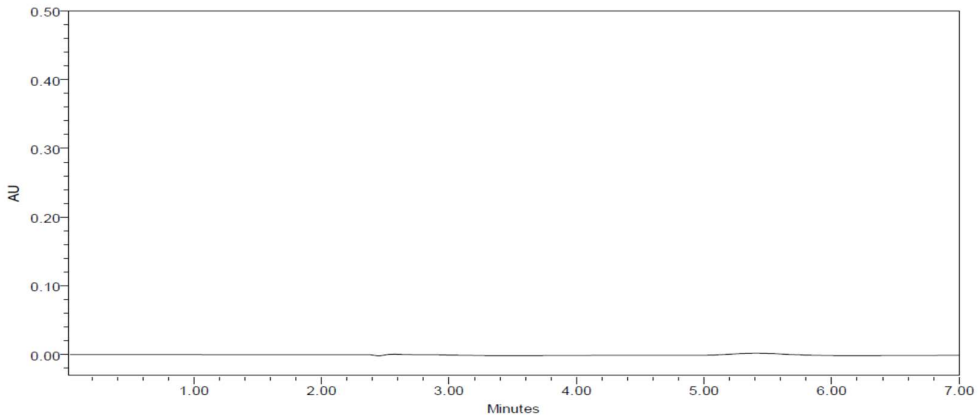

Fig 4: Chromatogram Showing Blank Solution

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

The % purity of Enalapril maleate and Losartan potassium in pharmaceutical dosage form was found to be 99.72%. The assay results were shown in table 6.

Table 7: Showing Assay Results

S. No.	Name of Compound	Label Claim	Amount taken	% Purity
1	Enalapril Maleate	10mg	9.98	99.72% ± 6

2	Losartan Potassium	50mg	48.6	99.72% ± 6

Linearity
Enalapril Maleate

Table 8: Linearity values of Enalapril Maleate

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

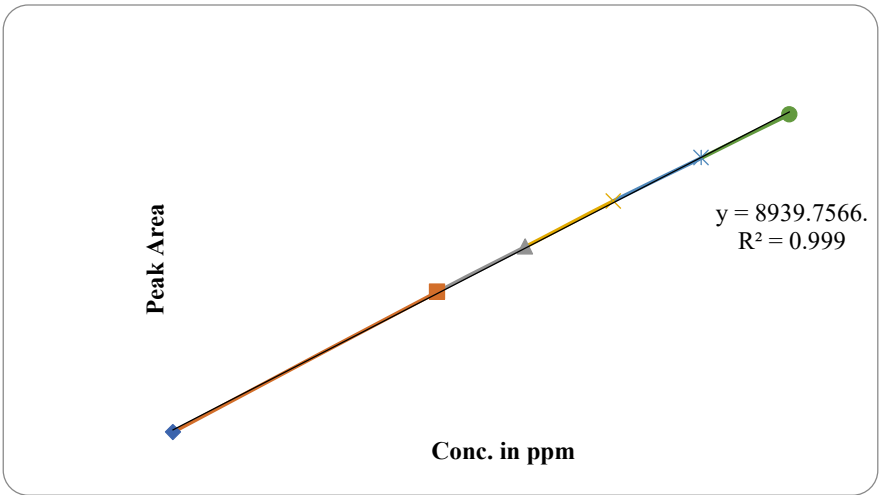

Fig. 5: Calibration Graph for Enalapril Maleate

Losartan Potassium

Table 9: Linearity values of Losartan Potassium

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

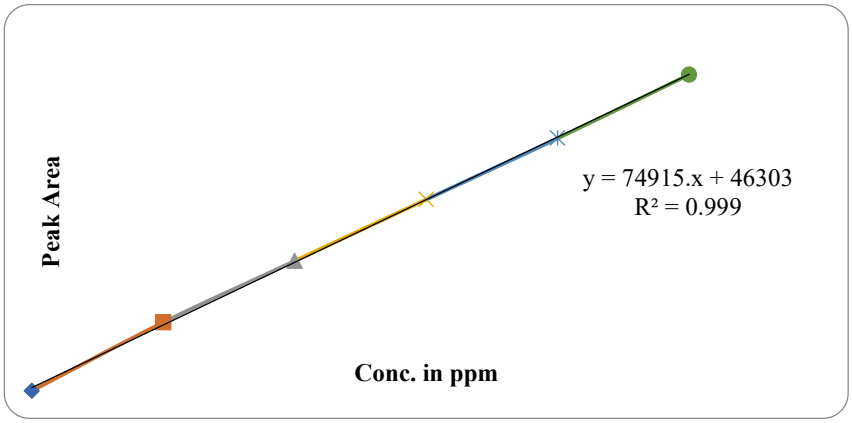

Fig 6: Calibration Graph for Losartan Potassium

PRECISION

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated %RDS. The results of repeatability were presented in table 9 & 10.

Table 10: Results of Repeatability for Enalapril Maleate

S.No.	Name	R_t	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**			

Table 11: Results of Method Precision for Losartan Potassium

S. No.	Name	R_t	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**				

Intermediate precision

Table 12: Results of Intermediate Precision for Enalapril Maleate

S. No.	Name	R_t	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**			

Table 13: Results of Intermediate Precision for Losartan Potassium

S.No.	Name	R_t	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**

Table 14: Results of Intermediate precision Day 2 for Enalapril Maleate

S.No.	Name	R_t	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

Table 15: Results of Intermediate precision for Losartan Potassium

S.No.	Name	R_t	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**				

ACCURACY

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated. The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate. The accuracy results was shown in table 15 & 16.

Table 16: 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 17: 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%	

LIMIT OF DETECTION & LIMIT OF QUANTIFICATION

The LOD & LOQ were determined using the formula based on standard deviation of the response and slope. The LOD & LOQ values data were presented in Table 17**.**

Table 18: LOD & LOQ data for Enalapril & Losartan

Drug	LOD (µg/ml)	LOQ (µg/ml)
Enalapril Maleate	0.7 µg/ml	0.9 µg/ml
Losartan Potassium	2.1 µg/ml	2.7 µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Enalapril Maleate and Losartan Potassium.

The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

The standard and samples of Enalapril maleate and Losartan potassium were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The robustness data was shown in table 18 & 19.

Variation in flow

Enalapril Maleate

Table 19: Variation for 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

Losartan Potassium

Table 20: Variation for Losartan Potassium

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/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

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 derivatization 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.

ACKNOWLEDGEMENT

We are thankful to CMR College of Pharmacy management for providing facility for analysis.

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