Development and Validation of RP-HPLC Chromatographic Assay Method for the Estimation of Enalapril Maleate in Pharmaceutical Formulation

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Abstract: *A rapid, accurate, precise, simple, efficient and reproducible isocratic Reversed Phase-High Performance Liquid Chromatographic (RP-HPLC) method for the estimation of Enalapril Maleate in bulk and pharmaceutical dosage form. Enalapril Maleate was separated using Kromasil C18 column (250mm×4.6 mm, 5µm particle size), Shimadzu LC2030 HPLC system having UV detector and the mobile phase contained a mixture of Methanol and Phosphate buffer pH adjusted to 4.5 (70: 30 v/v). The flow rate was set to 1.1ml/min with the responses measured at 227nm. The retention time of Enalapril Maleate was found to be 5.102 min. Linearity was established for Enalapril Maleate in the range of 10-50 µg/ml with correlation coefficient (r2=0.9995). The accuracy values were found to be in the range of 98 –102% and every parameter found with in limit. Validation parameters such as specificity, linearity, precision, accuracy, and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. This method can be used for the estimation and analysis of Enalapril Maleate drug in active pharmaceutical ingredients and pharmaceuticals.*

Keywords: **Assay, Envas10, RP-HPLC, Method Development, Validation.**

1. Introduction

Enalapril is an ACE inhibitor. ACE stands for angiotensin converting enzyme. Enalapril is used to treat high blood pressure (hypertension) in adults and children who are at least 1 month old. Enalapril is also used to treat congestive heart failure (CHF). CHF is a disorder of the ventricles (the lower chambers of the heart) which decreases the heart's ability to pump blood to the body. Category of Enalapril maleate is Enalapril is an ACE inhibitor. ACE stands for angiotensin converting enzyme.

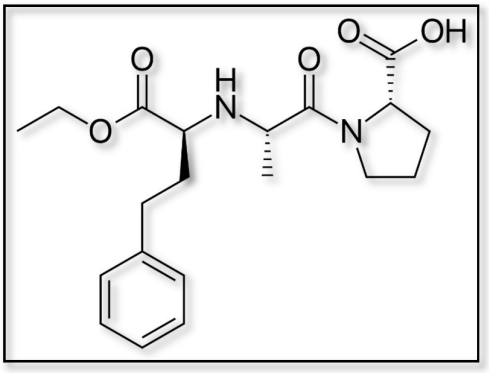

Figure.1 Structure of Enalapril Maleate

The renin-angiotensin-aldosterone system (RAAS) is a signalling pathway that works in synergism with the sympathetic nervous system to regulate blood pressure, and fluid and electrolyte homeostasis. Activation of this system upon stimulation by different factors, such as low blood pressure and nerve impulses, leads to increased release of norepinephrine (NE) from sympathetic nerve terminals and effects on the vascular growth, vasoconstriction, and salt retention in the kidneys. Renin is released from renin acts on the precursor protein angiotensinogen, which is a plasma globulin synthesized from the liver, to produce cleaved peptide hormone angiotensin I. Angiotensin I then can be further cleaved by ACE to produce angiotensin II, a vasoconstrictive peptide hormone. Label present in different isoforms, angiotensin converting enzyme (ACE) is peptidyl dipeptidase enzyme expressed in various tissues, including the vascular tissues, such as the heart, brain, and kidneys. ACE also plays a role in inactivation of bradykinin, a potent vasodepressor peptide., Label Angiotensin II mediates various actions on the body by working on its G-protein coupled receptors, AT1 and AT2. It causes direct vasoconstriction of precapillary arterioles and postcapillary venules, inhibits the reuptake of NE thereby increasing available levels, stimulates the release of catecholamines from the adrenal medulla, reduces urinary excretion of sodium ions and water by promoting proximal tubular reabsorption, stimulates synthesis and release of aldosterone from the adrenal cortex, and stimulates hypertrophy of both vascular smooth muscle cells and cardiac myocytes.

2. Material and Method

2.1. Material:

Table.1 Active Pharmaceutical Drug

Sr. No.	Name	Description
1.	Enalapril Maleate	• White powder, • uses on Hypertension
2.	Envas10	• 10 mg drug contain each tablet, • Manufactured by Cadila pharmaceutical LTD.

Table.2 List of Chemicals use in Research Work

Sr. No.	Name of Chemical	Molecular Formula	Properties	Manufacturer
1.	Acetonitrile	C_2H_3N	Solvent, BP 76-81.6°C	Merck Life science
2.	Methanol	CH_3OH	Flammable Solvent	Merck Life science
3.	Phosphate Buffer	KH_2PO_4	White Crystalline Powder	S D Fine Chem. Ltd, Mumbai
4.	Distilled Water	H_2O	Universal Solvent, BP 100°C	In-house

Table.3 List of Instruments

Sr. No.	Name of Equipment's/ Instruments	Model / Specification	Manufacturer
1	HPLC	Series LC2030	Shimadzu (I prominence plus)
	Pump	PU2080	
	Sample Injection Port	Autosampler	
	UV/Vis Detector	UV 2075 plus	
	Software	Chromolin	
2	pH Meter	101	Chemiline
3	Balance	AY-120	Shimadzu
4	Sonicator	UCB-40	Rolex
5	Deep Freezer	-	Blue Star
6	Refrigerator	-	Godrej

2.2. Methods:

2.2.1. Preliminary Analysis of Drug

a) Description: Color and texture of Enalapril Maleate was compared with reported characters mentioned in drug bank.

b) Solubility: Solubility of Enalapril Maleate was determined in various solvents like water, methanol, ethanol and Acetonitrile.

c) UV Analysis: UV analysis was carried out by scanning the solution of Enalapril Maleate at 200-400 nm.

2.2.2. Preparation of mobile phase:

70 ml of HPLC grade Methanol and 30 ml of Buffer pH was adjusted to 4.0 with orthophosphoric acid i.e. in 70: 30 v/v proportions. The. The solution was filtered through 0.45µ membrane filter and then sonicated in sonicator bath for 10 min.

2.2.3. Preparation of stock solutions of Enalapril Maleate:

Stock solution was prepared by dissolving 10 mg Enalapril Maleate in water and then diluted with Water in 10 ml of volumetric flask to get concentration of 1000 µg/ml. From the resulting solution 0.1 ml was diluted to 10 ml with water to obtain concentration of 10 µg/ml of Enalapril Maleate and labelled as standard stock Enalapril Maleate.

2.2.4. Selection of detection wavelength:

From the standard stock solution further dilutions were done using water and scanned over the range of 200-400 nm and the spectra were overlain. It was observed that drug showed considerable absorbance at 227 nm.

3. Results and Discussion

Validation

The proposed HPLC method was validated in terms of system suitability, specificity, precision, accuracy and robustness as per the International Conference on Harmonization (ICH) guidelines.

3.1. Range and Linearity:

The linearity of peak area response for Enalapril Maleate was determined from 10 % to 50 % level of working concentration of Enalapril Maleate. The stock solutions of standard Enalapril Maleate was diluted to five different known concentrations. Linearity graph of concentration (as x-value) versus area (as y-value) were plotted and correlation coefficient, y-intercept and slope of the regression were calculated and found results regression? coefficient r^2 value is 0.9995.

Table.4 Linearity Result of Enalapril Maleate

Sr.No.	Concentration (µg/ml)	Peak Area Enalapril Maleate
1	10	112747
2	20	225495
3	30	339243
4	40	460995
5	50	563738

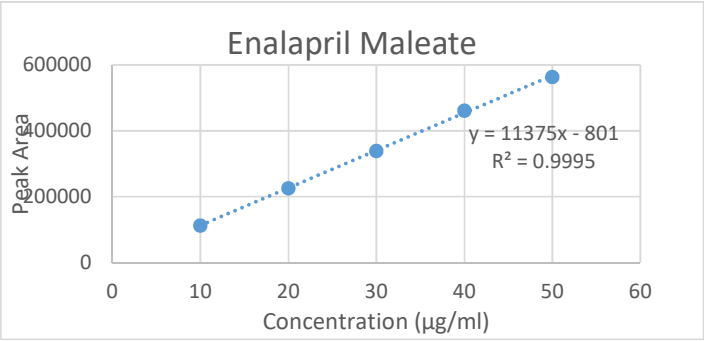

Figure.2 Calibration Curve of Enalapril Maleate

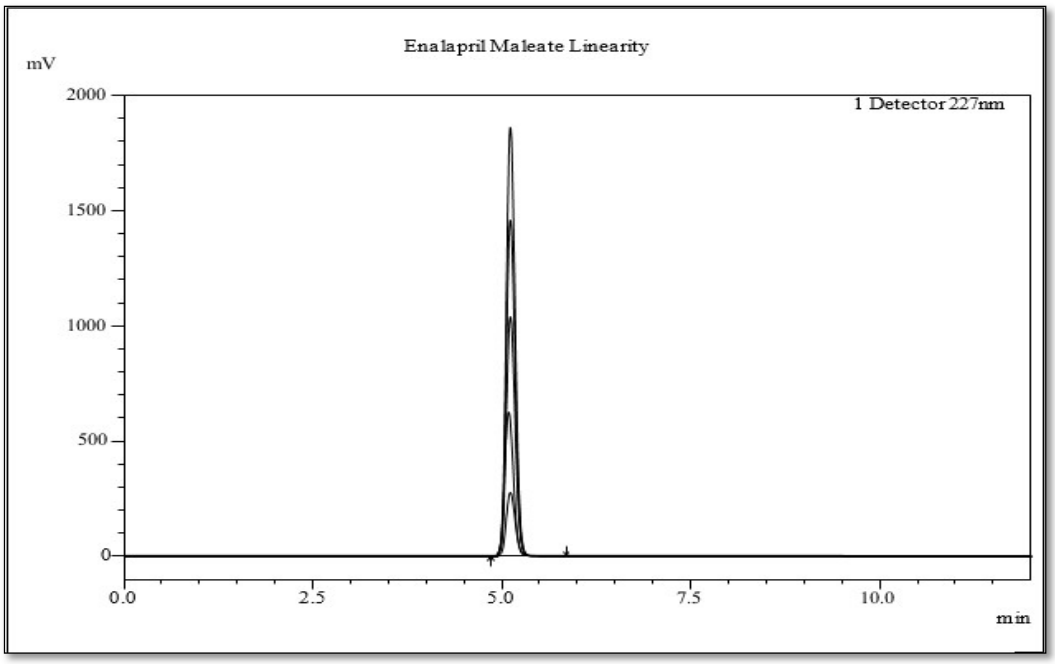

Figure.3 Overlain of Enalapril Maleate

Table.5 Characteristic parameters of Enalapril Maleate for the proposed HPLC method.

Parameter	Result
	Enalapril Maleate
Calibration range (µg/ml)	10-50
Detection wavelength (nm)	227
Solvent (Methanol: Buffer)	70:30
Regression equation (y*)	y = 11375x + 801
Slope (b)	11375
Intercept (a)	801
Correlation coefficient(r2)	0.9995
Limit of Detection (µg/ml)	0.0061
Limit of Quantitation (µg/ml)	0.0262

3.2. System Suitability:

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 40 µg/ml. The results which are given in Table No 6.15 were within acceptable limits.

Table.6 System suitability studies of Enalapril Maleate by HPLC method

Sr. No.	Properties	Values
1	Retention time	**5.1 ± 0.26**
2	Area	**8023 ± 760**
3	Asymmetry	**1.1 ± 0.37**

3.3. Specificity:

Chromatogram of blank was taken as shown in Fig No.4. Chromatogram of Enalapril Maleate showed peak at a retention time of 5.103 min. The mobile phase designed for the method resolved the drug very efficiently. The Retention time of Enalapril Maleate was 5.103 ± 0.0098min. The wavelength 227 nm was selected for detection because; it resulted in better detection sensitivity for the drug. The peak for Enalapril Maleate from the tablet formulation was Enalapril Maleate.

Table.7 Specificity of Enalapril Maleate by HPLC method

Concentration	API Area	Tablet Area
40	471020	462226
40	468990	456625
40	465995	461666
40	460991	466498
40	470990	453866
40	462990	454998
Mean	466829	459313
SD	4220.52	4922.62
40	471020	462226

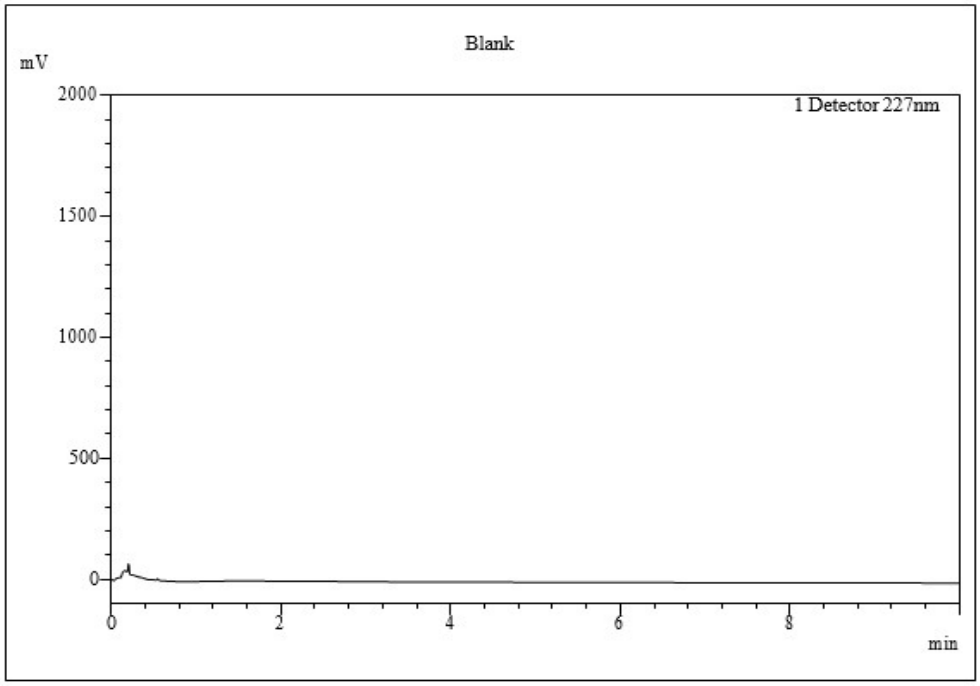

Figure.4 A typical chromatogram of Blank

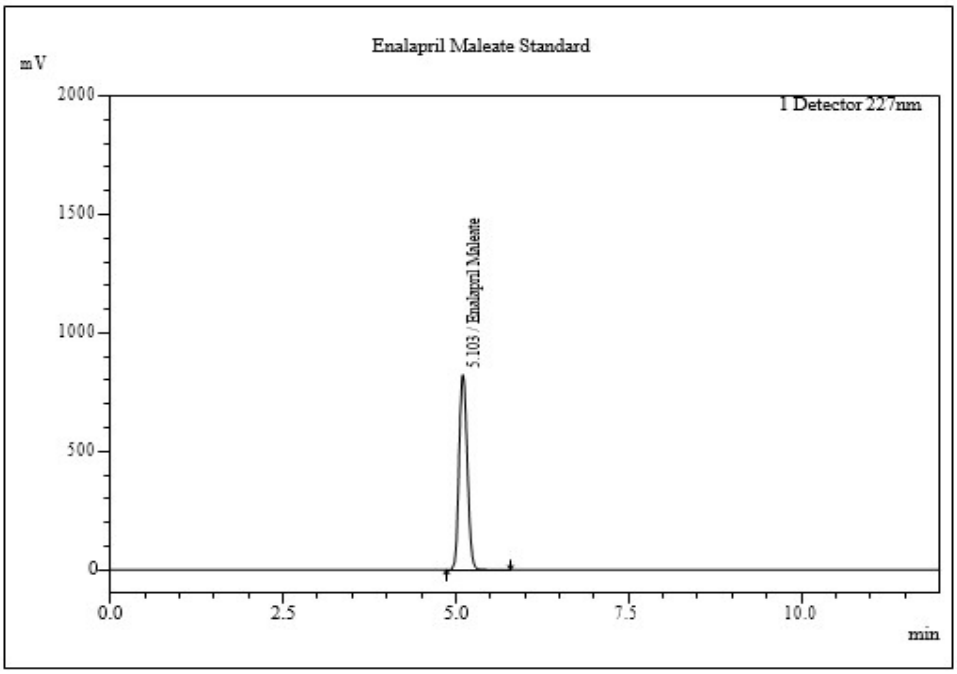

Figure.5 A typical chromatogram of Enalapril Maleate Standard

[Concentration 40ug/ml]

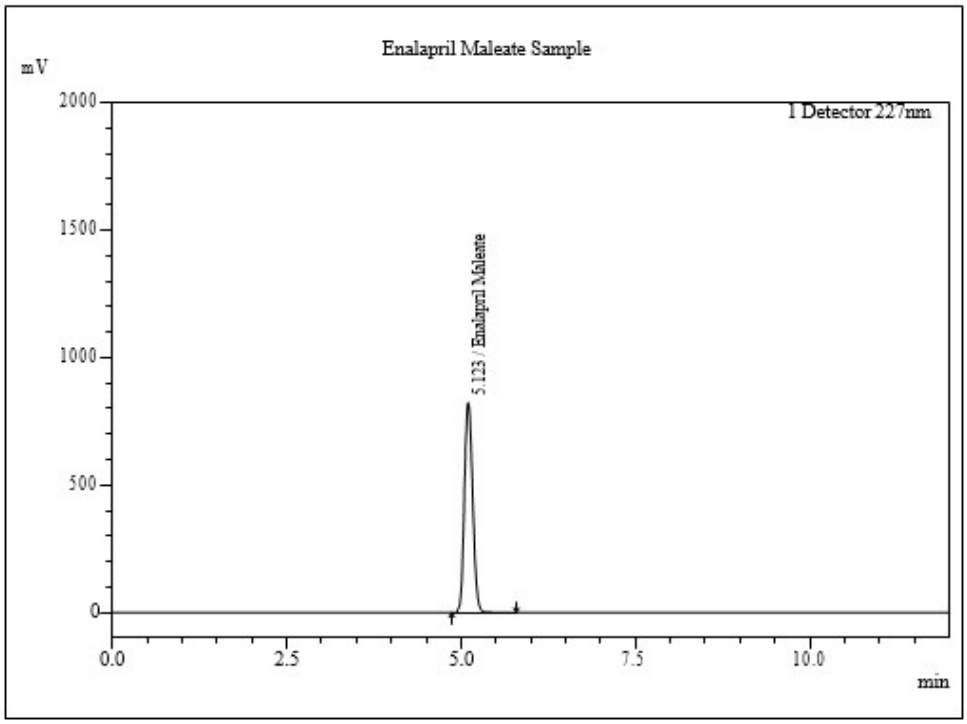

Figure.6 A typical chromatogram of Enalapril Maleate Sample

[Concentration 40ug/ml]

3.4. Sensitivity:

The sensitivity of measurement of Enalapril Maleate by use of the proposed method was estimated in terms of the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ were calculated by the use of signal to noise ratio. In order to estimate the LOD and LOQ values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level, while ten times the noise value gave the LOQ. LOD and LOQ were found to be 0.006147 and 0.02628 respectively.

3.5. Precision:

Demonstration of precision was done under two categories. The injection repeatability (System Precision) was assessed by using six injections of the standard solution of Enalapril Maleate and the % RSD of the replicate injections was calculated. In addition, to demonstrate the precision of method, six samples from the same batch of formulation were analysed individually and the assay content of each sample was estimated. The average for the six determinations was calculated along with the % RSD for the replicate determinations. Both the system precision and method precision were subjected for inter-day and intra-day variations as reported in Table 8 and 9 respectively.

Table.8 Intraday Precision of Enalapril Maleate

Concentration	Peak Area		
	0min	1 hr	2hr
40	471022	469028	461021
40	468991	465093	456992
40	465993	460991	465998
40	460900	460592	468994
40	470998	470099	459990
40	462990	459998	468991
Mean	466816	464300	463664
SD	4247.94	4468.05	5044.46
40	0.91	0.96	1.09

Table.9 Interday Precision of Enalapril Maleate

Concentration	Peak Area		
	1 day	2 day	3 day
40	471020	469593	468786
40	468990	467132	476954
40	465993	469018	475420
40	460991	469018	479358
40	470992	457293	467232
40	462990	469597	469846
Mean	466829	466942	472933
SD	42209.97	48133.00	49568.93
40	0.90	1.03	1.05

3.6 Accuracy:

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analysed samples of Enalapril Maleate (20 µg/ml) were spiked with 80, 100, and 120 % extra Enalapril Maleate standard and the mixtures were analysed by the proposed method.

Acceptance Criteria: % RSD should be less than 2.0

Table.10 Accuracy of Enalapril Maleate

Sr. No.	Concentration	Peak Area	recovery%
1	40	4609918	100.08
2	40	4669908	100.42
3	40	4609814	100.03
4	50	5634146	99.97
5	50	5637385	100.44
6	50	5636325	100.03
7	60	6764760	100.07
8	60	6764862	100.33
9	60	6759849	99.93

3.7. Robustness:

Effect of variation in mobile phase and flow rate:

This study was performed to determine the effect of variations in composition of mobile phase. The standard solution and test solution was prepared & injected in to HPLC system by changing the composition of mobile phase by ± 5, flow rate ± 0.1 mL/Minute and system suitability parameters were evaluated. The values were given in the following table no 11 and 12.

Table.11 Effect of flowrate

Flow Rate: - 0.9 ml/Min.		Flow Rate: - 1.1 ml/Min.	
Sr. No.	**Enalapril in %**	**Sr. No.**	**Enalapril in %**
Sample- A	97.5	Sample- A	97.6
Sample- B	98	Sample- B	98.1
Average	**97.8**	**Average**	**97.9**

Table.12 Effect of mobile phase

Mobile phase - 5%		Mobile phase + 5%	
Sr. No.	**Enalapril in %**	**Sr. No.**	**Enalapril in %**
Sample- A	99.3	Sample- A	99.2
Sample- B	99.1	Sample- B	98.6
Average	**99.2**	**Average**	**98.9**

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