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RP-HPLC Method Development and Validation for the Estimation of Enalapril in Bulk and Pharmaceutical Dosage Form

Dr. M. Prasada Rao[1], Dr. B. V. Narasimha Rao[2], Dr. B. Ramarao[3], K. Gayathri Devi*, A. Udaya Krishna Veni[4], K. Deepika[5], K. Trikoteswar Reddy[6], T. Gnaneswar Babu[7], Soleman Dewan[8], KH Matinur Rahman[9]

*Assistant Professor, Department of Pharmaceutical Analysis, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India.
[1,2&3] Professor, Department of Pharmaceutical Analysis, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India.
[4]Assistant Professor, Department of Pharmaceutical Analysis, SS and N College of Pharmacy, Narasaraopet, Andhra Pradesh, India.
[5-9] IV/IV B. Pharm Student, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India.

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ABSTRACT

In the present study, Enalapril was estimated in bulk and pharmaceutical formulations using a reverse phase high performance liquid chromatography method. Several factors, including buffer concentration, mobile phase ratio, and column type, were varied in statistically planned trials to evaluate the impact of these parameters on the chromatographic separation of Enalapril. Potassium Dihydrogen Orthophosphate buffer pH 3.0: Acetonitrile: in a ratio of 60:40 (v/v) was used to perform the separation on an Prontosil ODS C18 Column (250 x 4.6 mm and 5µm) at room temperature under isocratic conditions at a flow rate of 1.0 mL/min. A UV detector operating at 215 nm for a total of 6 minutes made the detection. In the concentration range of 2.5–15µg/mL, calibration curves were linear. The developed method's sensitivity is demonstrated by the observed LOD of 1.198µg/mL and the calculated LOQ of 3.059µg/mL. The method's robustness and ruggedness were confirmed by the %RSD being less than 2. In formulation analysis, the assay percentage was 99.20. As a result, Enalapril in bulk and pharmaceutical formulations was routinely analysed using this methodology.

Keywords: Enalapril, validation, accuracy, precision, ruggedness and robustness.

INTRODUCTION

Enalapril is a prescription medication primarily used to treat high blood pressure (hypertension) and heart failure. It acts as an Angiotensin-Converting Enzyme (ACE) inhibitors. Normally, angiotensin I is converted to angiotensin II by an angiotensin-converting enzyme (ACE). Angiotensin II constricts blood vessels, increasing blood pressure. Enalaprilat, the active metabolite of enalapril, inhibits ACE. Inhibition of ACE decreases levels of angiotensin II, leading to less vasoconstriction and decreased blood pressure [1,2]. Till now no RP-HPLC method was reported for the estimation of Enalapril alone in bulk and pharmaceutical formulation. The Chemical structure of Enalapril is shown as Fig 1.

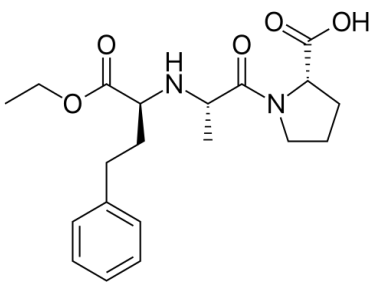

Fig 1. The Chemical structure of Enalapril

MATEIALS AND METHODS

Chemicals and Reagents

The working standard drug Enalapril (98.50% purity), were obtained from Dr. Reddy's Laboratories, Hyderabad, Telangana. The formulation dosage form having brand name Enalapril-10 containing 10 mg of Enalapril, was purchased from local Pharmacy. HPLC grade Methanol, Water and Acetonitrile were purchased form Merk chemicals private limited, Mumbai. Potassium Dihydrogen Phosphate and Orthophosphoric Acid used for the study were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of Phosphate Buffer (pH 3.0)

An accurately weighed 6.8 g of potassium dihydrogen phosphate was transferred into a 1000 mL volumetric flask and dissolved in about 900 mL of purified water. The pH of the solution was adjusted to 3.0 ± 0.05 using orthophosphoric acid. The volume was then made up to the mark with purified water and mixed well.

Preparation of Mobile Phase

The prepared phosphate buffer (pH 3.0) and acetonitrile were mixed in the ratio of 60:40 % v/v. The mobile phase was sonicated for 15 minutes to remove dissolved gases and then filtered through a 0.45 µm membrane filter before use.

Preparation of standard drug solution

An accurately weighed 10mg of Enalapril standard drug was taken in a 10ml volumetric flask. Then it was dissolved completely in 7ml of diluent (Potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile in the ratio of 60:40 % v/v) using ultrasonic Sonicator. The final volume in the volumetric flask was made up to the mark using same solvent and then the solution was filtered using 0.45µm membrane filter paper. Standard stock solution having 1000 µg/mL was obtained. The concentrations (10 µg/mL) required in method development and validation parameters were prepared from the stock (1000 µg/mL) solution of Enalapril.

Preparation of formulation solution

Twenty tablets of Enalapril tablets (label claim: 10 mg) were accurately weighed and finely powdered. A quantity of the tablet powder equivalent to 10 mg of Enalapril was accurately weighed and transferred into a 10 mL volumetric flask. About 7 mL of diluent (mobile phase) was added to the flask and the mixture was sonicated for 15 minutes to ensure complete extraction of the drug from the tablet matrix. The solution was allowed to cool to room temperature and the volume was made up to the mark with the same diluent. The resulting solution was mixed well to obtain a sample stock solution having a concentration of 1000 µg/mL. The sample stock solution was filtered through a 0.45 µm membrane filter, discarding the first few mL of the filtrate. From the filtered sample stock solution, 1.0 mL was accurately pipetted into a 10 mL volumetric flask and diluted to volume with the diluent to obtain a working sample solution of 100 µg/mL. Further dilutions were made from the working sample solution using the same diluent to obtain the required concentration for assay and validation studies.

METHOD DEVELOPMENT

Selection of Wavelength

To select a suitable wavelength, the standard solutions of 10 µg /mL was prepared and scanned in the UV-Vis spectrophotometer. The obtained wavelength maximum was selected as suitable wavelength for the detection.

Table 1. Optimized Chromatographic Conditions

Parameter	Condition
Mobile Phase	Phosphate buffer pH 3.0: acetonitrile and in the ratio of 60:40(v/v)
Column	Prontosil ODS C18 Column (250 x 4.6 mm and 5µm)
Flow Rate	1.0 ml/min
Wavelength	215nm
Injection Volume	20 µL
Temperature	Ambient
Run time	6min

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METHOD VALIDATION

The method was validated with respect to specificity, system suitability, LOD & LOQ, linearity, accuracy, precision, ruggedness and robustness according to the ICH guidelines. Validation studies were carried out by replicate injections of the sample and standard solutions into the column [3,4].

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

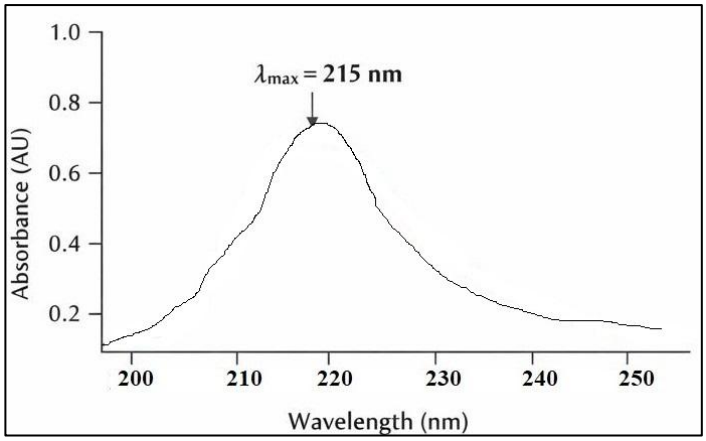

Fig 2. UV Spectra of Enalapril

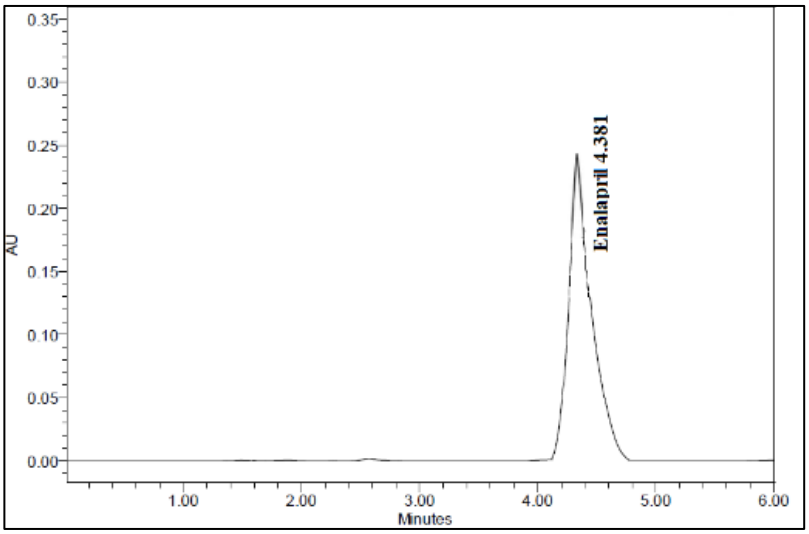

Fig 3. Optimized Chromatogram of Enalapril

Table 2. Results for Optimized Chromatogram

S.NO	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor
1	**Enalapril**	4.381	4582	1.09

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METHOD VALIDATION

Specificity

No inference of diluent & Placebo

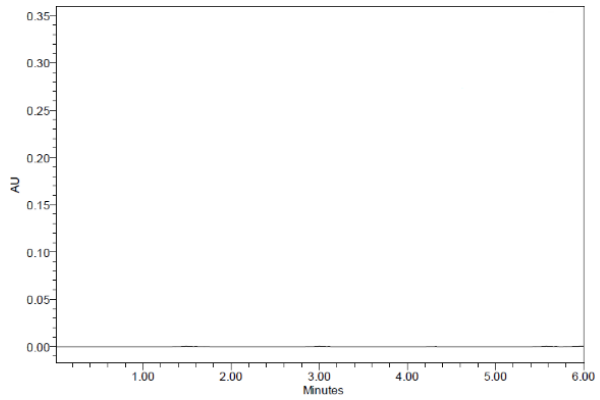

Fig 4. Chromatogram of Blank

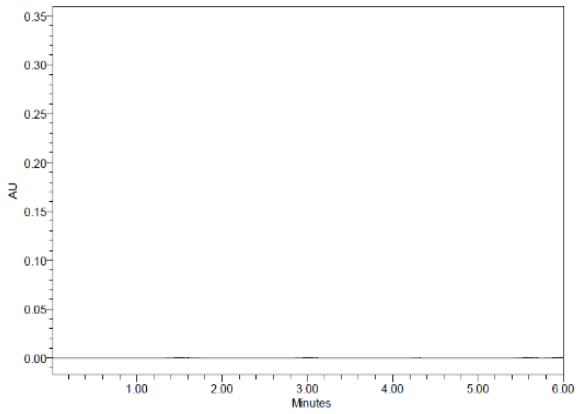

Fig 5. Chromatogram of Placebo

Linearity

Table 3. Results for Linearity

S. No	Level	Enalapril	
		Concentration in µg/mL	Peak Area
1	Level 1	2.5	76215
2	Level 2	5	152340
3	Level 3	7.5	228792
4	Level 4	10	304780
5	Level 5	12.5	380278
6	Level 6	15	448960

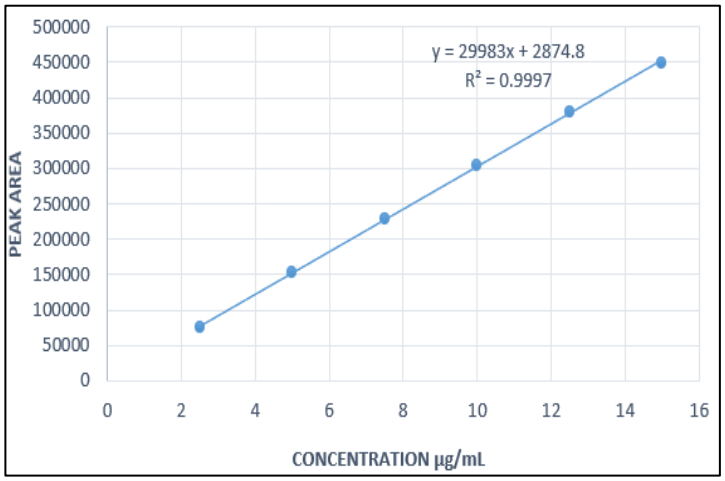

Fig 6. Linearity graph for Enalapril

LOD & LOQ

Enalapril 's LOD was found to be 1.198µg/mL, while its LOQ was determined to be 3.059µg/mL.

Precision

The % RSD was found to be 1.078 in System Precision and 0.045 in Method Precision respectively for Enalapril. The % RSD was found to be with in the acceptance limit of less than 2 for both in System Precision and Method Precision. Hence the developed method was found to be precise.

Table 4. Results for Precision

S.NO	Injection	System Precision		Method Precision	
		Retention Time	Peak Area	Retention Time	Peak Area
1	Injection-1	4.381	304493	4.385	304554
2	Injection-2	4.382	304563	4.379	304496
3	Injection-3	4.376	310924	4.381	304572
4	Injection-4	4.383	304399	4.376	304535
5	Injection-5	4.377	310849	4.378	304497
6	Injection-6	4.378	304485	4.381	304865
Mean		4.379	306618.8	4.38	304586.5
STD		0.002	3306.215	0.003098	139.7723
%RSD		0.04	1.078	0.06	0.045

Accuracy

The recovery percentage was found to be between 98.20 and 99.33%. At 50%, 100%, and 150% spiking levels, the percentage RSD was found to be within the permissible range for Enalapril. The results showed that the suggested approach was accurate, with a % RSD of less than two and an acceptability limit of 98-102.

Table 5. Results for Accuracy

Recovery Level	Concentration in µg/ml			Amount Found	% Recovery	% RSD
	Target	Spiked	Total			
50%	5	2.5	7.5	7.39	98.53	0.40
	5	2.5	7.5	7.42	98.93	
	5	2.5	7.5	7.45	99.33	
100%	5	5	10	9.84	98.40	0.20
	5	5	10	9.82	98.20	
	5	5	10	9.86	98.60	
150%	5	7.5	12.5	12.35	98.80	0.24
	5	7.5	12.5	12.31	98.48	
	5	7.5	12.5	12.37	98.96	

Ruggedness (Intermediate Precision)

The percentage RSD used to express ruggedness had to be less than 2. Enalapril 's percentage RSD in the developed technique was 0.047. The method's ruggedness is confirmed by results that fall within the acceptable range.

Table 6. Results for Ruggedness

S.NO	Injection	Retention Time	Peak Area
1	Injection-1	4.382	304672
2	Injection-2	4.380	304964
3	Injection-3	4.381	304595
4	Injection-4	4.378	304574
5	Injection-5	4.383	304620
6	Injection-6	4.386	304632
Mean		4.381	304676.2
STD		0.002733	144.9116
%RSD		0.045	0.047

Robustness

The percentage change for Enalapril in the developed technique was found to be within the permissible level of less than 2. As a consequence, the suggested approach was determined to be appropriate for the analysis of Enalapril when there was a slight alteration in the analytical conditions. This confirms that a slight change in the analytical conditions has no effect on the results.

Table 7. Results for Robustness

S. No	Condition	Enalapril		
		Retention Time	Peak Area	% Change
1	Standard	4.381	304780	--
2	+MP (65:35)	4.382	304681	0.18
3	-MP (55:45)	4.377	310849	0.17
4	Flow Rate 1.2ml/min	4.385	304646	0.25
5	Flow rate 0.8ml/min	4.376	310924	0.23
%RSD		0.09	1.16	

Assay

Enalapril 's assay percentage in formulation analysis was 99.20%. As a result, the technique was determined to be appropriate for the regular analysis of Enalapril in both formulations and bulk drug.

Table 8. Results for Formulation

S. No	Drug	Brand	Label Claim	Amount Found	% Assay
1	Enalapril	Enalapril-10	10 mg	9.92mg	99.20

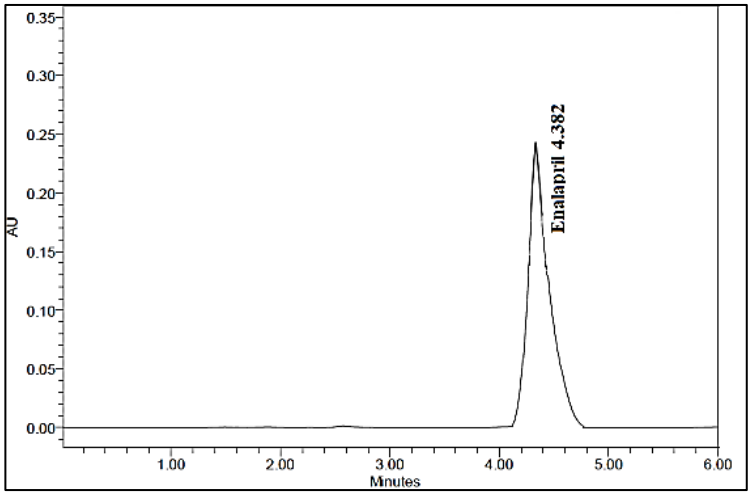

Fig 7. Chromatogram of Formulation

CONCLUSION

A sensitive, accurate, and precise RP-HPLC method has been developed by the author for the measurement of Enalapril in in bulk and pharmaceutical formulations. The recommended RP-HPLC technique for the Enalapril test was shown to be suitable for routine quantitative analysis following validation. The low standard deviation statistics illustrate the exceptionally high precision of the developed method. The linearity, accuracy, specificity, and precision values were found to be within the acceptable ranges. The chromatogram's lack of extra peaks showed that the common excipients employed in the tablet did not conflict with one another. Thus, it is shown that the devised RP-HPLC method is straightforward, linear, precise, sensitive, and repeatable. As a result, the devised approach is simple to use and has a fast analytical time for routine quality monitoring of Enalapril in bulk and pharmaceutical formulations. The reported findings demonstrate the good accuracy and precision of the suggested approach.

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Conflict of Interest Statement: All authors have nothing else to disclose.

	K. Gayathri Devi*, Assistant Professor, Department of Pharmaceutical Analysis, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India.
	Dr. M. Prasada Rao[1], Professor, Department of Pharmaceutical Analysis, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India.
	Dr. B. V. Narasimha Rao[2], Professor, Department of Pharmaceutical Analysis, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India.
	Dr. B. Ramarao[3], Professor, Department of Pharmaceutical Analysis, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India.
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	IV/IV B. Pharm Student[5-9], MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India.