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Research Article

VALIDATION OF STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF ENALAPRIL MALEATE IN TABLET FORMULATIONS

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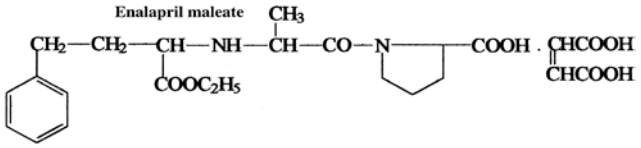
ABSTRACT

The aim of present work was to validate the high performance liquid chromatographic method for the analysis of enalapril maleate in pharmaceutical formulation. The method valdation of enalapril maleate was performed by using Hypersil MOS, 5µ (250 mm x 4.6 mm) as stationary phase with mobile phase consists of buffer solution and Acetonitrile (40:60) at flow rate of 1.5 ml/min. The column temperature and wavelength were monitored at 65^{0}C and 215 nm, respectively. The injection volume was 50 µl for the run time 25 min. The validated method found within limits in all validated parameters and is quick and reliable for quantitative analysis as well as quality control of enalapril maleate in pharmaceutical formulation.

Keywords: Stability indicating, HPLC, Enalapril maleate, Hypersil MOS, Specificity, Precision, Accuracy.

INTRODUCTION

Enalapril maleate (1-{*N*-[(s)-1-carboxyl-3-phenylpropyl]- L-alanyl-}- L-proline 1-ethyl ester maleate) is a potent angiotensinconverting (ACE) enzyme inhibitor[1,2,3]. It is a pro-drug without direct biological activity which is rapidly absorbed after oral administration and de-esterified *in vivo* to its active metabolite enalaprilat diketopiperazine derivative (DKP) and has little pharmacologic activity until hydrolyzed in the liver to enalaprilat[1,2,4-11]. Enalapril maleate an angiotensinconverting enzyme inhibitor is with the following structural formula[4].



Enalapril maleate off-white, crystalline powder[12,13] is derivative of 2 amino acids, L-alanine and L-proline, and is an antihypertensive and a vasodilator in congestive heart failure[14]. Enalapril maleate has been analyzed in pharmaceutical combinations containing 0.5-1% methylcellulose by extraction to acetonitrile and injecting the extracts to HPLC[15]. This agent is able to reduce cardiovascular mortality and morbidity in patients with heart failure[3].

High performance liquid chromatography (HPLC) has been the only practical technique for the determination of enalapril in pharmaceutical dosage forms without interference from degradation products. However, severe conditions that shorten column life, such as low pH of the solvent and high column temperature, are required for acceptable peak shape, because enalapril exists as two rotational isomers owing to the alanyl-proline moiety in its structure[16].

The aim of present work is to validate the method for assay of enalapril maleate in enalapril maleate formulation to show specificity, degradation studies, linearity response, precision, accuracy and stability in analytical solution.

MATERIALS AND METHODS

Material and reagents

The required materials and regents were Monobasic Potassium phosphate (AR grade), Water (Milli Q grade), Acetonitrile (HPLC grade), Orthophosphoric acid (85% w/w; AR grade), Enalapril maleate tablets of strength 5 mg/tablet, Placebo for enalapril tablets of strength 5 mg/tablet, Enalapril maleate working standard of percentage potency 99.55% w/w.

Instrumentation and software

A gradient HPLC (Waters 600 Controller) equipped with online degasser, Waters 600 pump, Manual injector system, Photodiode Array Detector (PAD, Waters 2996), C18 column (Hypersil MOS, size: 250 mm x 4.6 mm particle size 5µm) and Empower 2 software on computer (Window XP Professional 2002 professional); Vacuum filtration assembly (Pall corporation); Ultrasonic cleaner (Toshcon Model: SW 7); Analytical balance (Mettler Toledo, Model: XS 204)

Chromatographic conditions

The chromatographic column used was C18 Hypersil MOS, particle size 5µm (250 mm x 4.6 mm) of Thermo scientific make. The mobile phase consists of buffer solution and Acetonitrile (40: 60). The flow rate of the mobile phase was kept at 1.5 ml/min and the column temperature was maintained at 65^{0}C and the chromatograms were monitored at a wavelength of 215 nm. The injection volume was 50 µl for the run time 25 min.

Preparation of solvents and solutions

Buffer Solution preparation

136 mg of monobasic Potassium phosphate dissolved in 800 mL of water, adjusted with Phosphoric acid to a pH of 2.0, and then diluted with water to 1000 mL, and mixed.

Mobile phase preparation

A filtered and degassed mixture of buffer solution and Acetonitrile (40:60) was prepared.

Diluents preparation

Buffer solution was used as diluents.

Preparation of Placebo solution

A weighed portion of placebo powder, equivalent to 50 mg of Enalapril maleate in Enalapril maleate tablet was transferred into a 250 ml volumetric flask. About 150 ml buffer solution added and sonicated for 15 min., and maintained the volume upto the mark, mixed and filtered through 0.45µ or finer porosity membrane filter.

Standard solution preparation

50 mg of enalapril maleate working standard was transferred into a 250 ml volumetric flask, about 150 ml of buffer solution added and sonicated to dissolve. Maintained the volume upto the mark, mixed and filtered through 0.45µ or finer porosity membrane filter.

Sample solution preparation

20 tablets were weighed and finely powdered. An accurately weighted portion of powder, equivalent to about 50 mg of enalapril maleate was transferred into a 250 ml volumetric flask. About 150 ml of buffer solution was added and sonicated for 15 min. maintained the volume upto the mark with buffer solution, mixed properly and filtered through 0.45 µ or of finer porosity membrane filter, discarding the first few ml portion of the filtrate. The final solution contains about 200 µg/ml of enalapril maleate.

Procedure

Equal volumes of the standard solution and the sample solution were separately injected in duplicate. Record the chromatograms and measure the peak area count of the enalapril peak with the aid of an integrator.

Specificity

The placebo solution as well as sample solution of enalapril maleate tablets of strength 5 mg were analyzed as per proposed method and it was found that there was no interference of excipients with the enalapril peak. The peak purity of enalapril peak was checked in the sample solution spiked with known related substances of enalapril maleate (AL-1, Diketopiperazine and Enalaprilat). The peak purity data indicates that the peak is homogenous and it has no coeluting peaks indicating specificity of the method.

Degradation Studies

An accelerated degradation study was carried out on the enalapril maleate tablets according to the following conditions. The results are shown in Table 1.

a) Hydrolytic and Oxidative Degradation

Accurately weighed and finely powdered tablet blend, equivalent to 50 mg of enalapril maleate were separately transferred in three volumetric flasks of 250 ml. The samples were treated separately with 0.1 N HCl, 0.01 N NaOH and 1% v/v H_2O_2 and analyzed. Samples treated with 0.1N HCl, 0.01 N NaOH and 10% v/v H_2O_2 were analysed again after heating for 2 h on water bath maintained at 60^0C.

b) Thermal Degradation

The sample was subjected to accelerated thermal degradation by keeping at 80^0C for 24h. The sample was further analysed by the proposed method.

c) Photolytic Degradation

Photolytic degradation study was carried out by exposing the sample to light (1900 Lux) for 24 h and followed by analysis as per the proposed method.

Using the peak purity test, the purity of enalapril peak was checked at every stage of above study. The peak purity plots show that the enalapril peak is homogenous and there are no coeluting peaks indicating that method is stability indicating and specific.

Table 1: Degradation Studies

Mode of Degradation	Time (h)	Assay (mg/tablet)
Initial	-	4.94
0.1 N HCl (60^0C)	0 h	4.96
	2 h	5.03
0.1 N NaOH (60^0C)	0 h	5.19
	2 h	5.12
1 % v/v H_2O_2 (60^0C)	0 h	5.24
	2 h	4.26
Photolytic (1900 Lux)	24 h	4.54
Thermal (80^0C)	24 h	2.70

Linearity of response

The linearity of response for enalapril maleate was determined and found to be linear in the range of 150 to 250µg/ml. Results are shown in **Table 2.**

Table 2: Linearity of Response - Regression Output

Constant	100378.9
Std Err of Y Est	23325.93
R Squared	0.999201
No. of observations	7
Degree of Freedom	5
X Coefficient (s)	19660.63
Std Err of Coef.	248.6853

Precision

System Precision

Six replicate injections of enalapril maleate standard solution were made into the HPLC system as per proposed method. The results along with the percentage RSD of area counts for enalapril maleate indicated an acceptable level of precision (0.82) for the analytical system (Acceptance Criteria: RSD ≤2).

Method Precision

Six replicate samples of a single batch of enalapril maleate tablets were prepared and analysed by the proposed HPLC method. The calculated percentage RSD of assay indicated that the method has an acceptable level of precision (0.27) for the purposed method. (Acceptance Criteria % RSD ≤7.0).

Accuracy

A known amount of Enalapril maleate tablets placebo powder was taken and spiked with enalapril maleate working standard at three different levels in triplicate. The samples were analysed as per the proposed method. The results indicate that the method has an acceptable level recovery. (Acceptance criteria: % Recovery should be in the range 90% - 110%). Results are shown in **Table 3.**

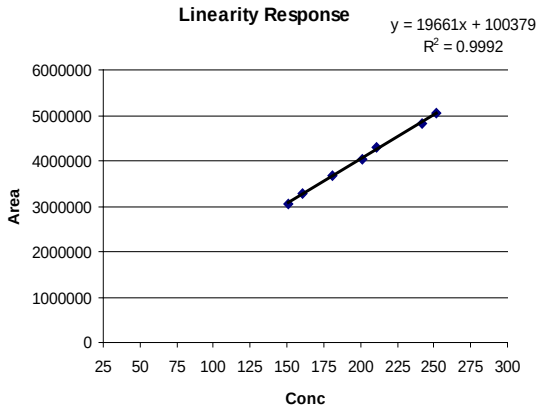


Table 3: Accuracy

Recovery Level	Enalapril Maleate		
	Amount added (mg)	Amount recovered (mg)	% Recovery
I – A	42.110	41.089	97.58
I – B	39.322	39.747	101.08
I – C	40.517	40.202	99.22
II – A	49.875	49.330	98.91
II – B	49.277	49.269	99.98
II – C	50.771	50.573	99.61
III – A	58.635	58.059	99.02
III – B	60.029	58.561	97.55
III – C	62.517	61.208	97.91
		Mean	**98.98**
		S.D.	**1.174**
		% RSD	**1.19**

Stability in analytical solution

A sample solution of Enalapril maleate tablet powder was prepared and kept at room temperature (25^{0}C). Sample solution was analysed at different time intervals. As the % RSD up to 1601 min. is 0.217 which is less than the % RSD for method precision (0.27), it was concluded that sample solution is stable in analytical solution for about 26 h.

System suitability data

Standard solution was injected during the validation studies and the column efficiency and tailing factor for enalapril peak was calculated. The results met within acceptance criteria of system suitability.

RESULTS AND DISCUSSION

The peak purity data indicates that the peak is homogenous and it has no co-eluting peaks with the main peak indicating specificity of the method as well as at the time of method validation studies the degradation study, linearity of response, precision, accuracy, stability in analytical solution and system suitability acceptance criteria were also found within limits.

Therefore, the proposed validated method is quick and reliable and can be used for routine quantitative analysis as well as quality control of Enalapril maleate in pharmaceutical formulation.

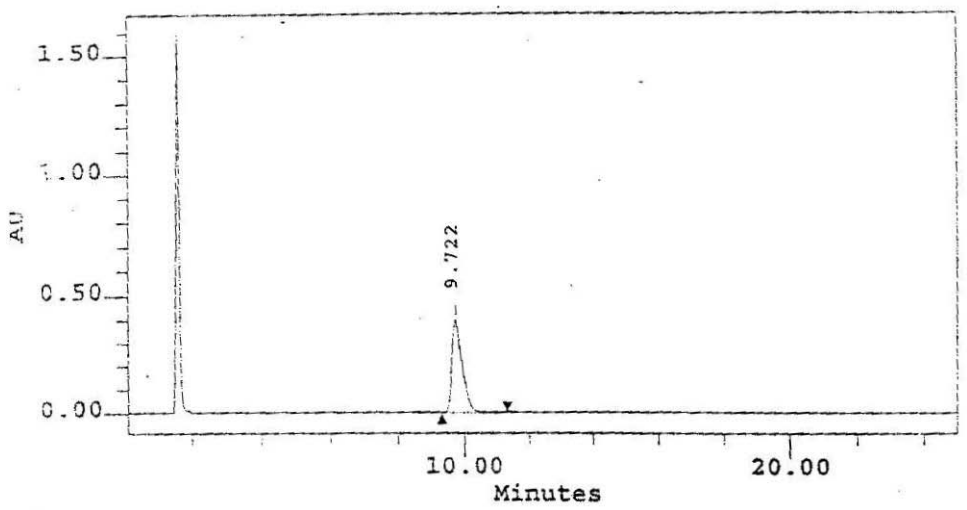


Fig. 1: A Typical HPLC chromatogram of Enalapril maleate standard

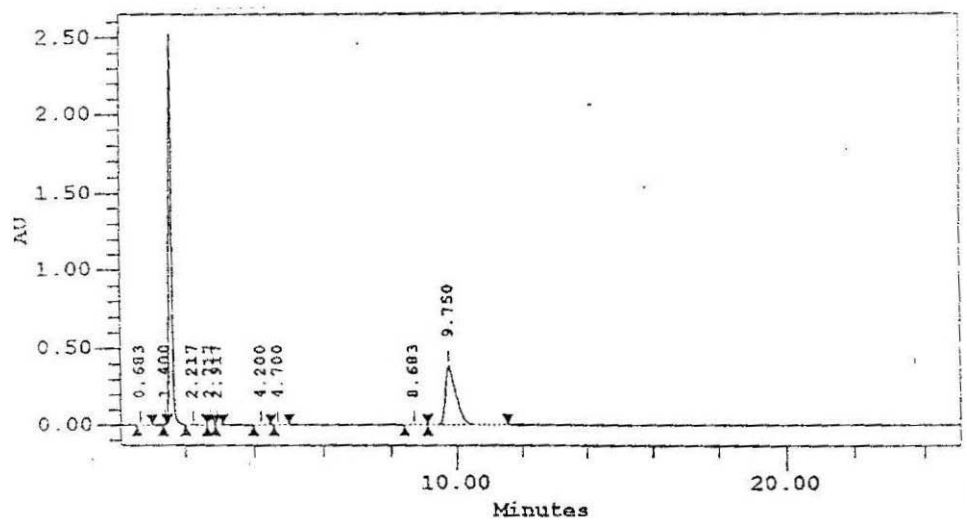


Fig. 2: A Typical HPLC chromatogram of Enalapril maleate in Sample

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