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SIMPLE AND STABILITY INDICATING RP-HPLC ASSAY METHOD DEVELOPMENT AND VALIDATION FOR ENALAPRIL MELIATE BY RP-HPLC IN BULK AND DOSAGE FORM

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Abstract

A new simple, accurate, precise, sensitive and validated RP-HPLC method was developed for the estimation of Enalapril Meliate in bulk and pharmaceutical dosage form. The Chromatographic conditions used for the separation was X Bridge C18 (50 mm x4.6 mm, 3.5µm) and the mobile phase comprised of Ammonium dihydrogen ortho phosphate Buffer and Acetonitril(70:30 v/v). The flow rate was 0.6 ml/min The detection was carried out at 210 nm. The Assay method was validated as per ICH guidelines. The linearity was found to be in the range of 0.2 – 1.2 mg/ml (25% to 150%) with correlation coefficient(r) 0.9986. The proposed method is accurate with 99.76% - 99.98% recovery for Enalapril Meliate and precise. %RSD of repeatability, intraday and inter day variations were 0.074 - 0.372. The method can be used for the analysis of pharmaceutical formulation.

Keywords: Enalapril Meliate, Ammonium dihydrogen phosphate, 1-Octane Sulphonic acid sodium salt (1-OSS), TEA, H_3PO_4RP-HPLC, Method development and validation

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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 is an off-white, crystalline powder.[12, 13] This compound is a 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. The aim of present work is to develop and validate a simple RPHPLC method for assay of enalapril maleate in formulation to show specificity, linearity, precision, accuracy and stability in analytical solution.

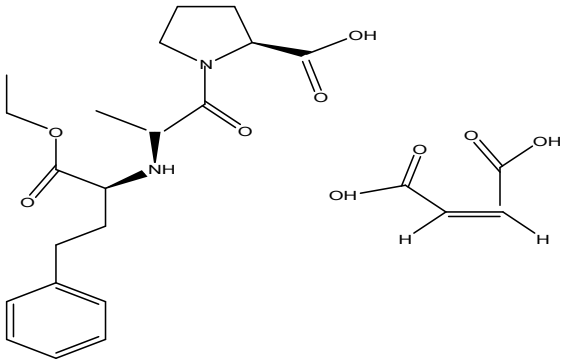


Fig.1Chemical Structure of Enalapril meliate

Figure-1: Chemical structure of Enalapril Meliate

Enalapril Meliate is available in strength of 5mg tablet dosage form. The strength of tablet is analyzed with the developed method. Enalapril Meliate has some published methods for estimation of assay and impurity profile by HPLC and UV/visible spectroscopy techniques. The objective of the research is to develop a simple RP-HPLC method. Method validation has performed as per the ICH and regulatory guidelines and review articles were revealed for method development and validation.

MATERIALS AND METHODS

Reagents and Materials

The reference sample of Enalapril Meliate was supplied as a gift sample from Hetero labs limited, Hyderabad, Telangana. The commercially available Enalapril Meliate (Enapri-5mg, INTAS, Pharmachutical) solid dosage forms were procured from the local market. Milli-Q-water was used throughout this research. HPLC grade Acetonitrile, analytical grade 1-Octane Sulfonic acid Sodium, H_3PO_4 and TEA were obtained from Merck Chemicals, India.

Chromatographic parameters:

The chromatography was performed on a LC 10 AT vp HPLC instrument (Shimadzu corporation, Japan) equipped with SPD-10A vp detector, SCL-HT A auto sampler and CTO-10A vp column oven. The data was monitored with LC solutions software. X Bridge C18 (50 mm x4.6 mm, 3.5µ Waters corporation, USA) was used as stationary phase. The flow rate was set at 0.6 µl/min. An injection volume of 10µL was used for the analysis. The detector was monitored at 210 nm. The column temperature was maintained at 60^0 C.

Preparation of buffer solution:

To 500 mL of 0.05M of Ammonium dihydrogen phosphate, added 2ml of TEA and then adjusted p^H to 2.6 with H_3PO_4. To the above solution added one gram of 1-Octane sulphonic acid sodium salt and mixed thoroughly.

Preparation of Mobile phase:

Mobile phase was prepared by mixing buffer and Acetonitril in the ratio of 70: 30 (v/v). The mixture was filtered and degassed through 0.45 µm membrane filter paper.

Preparation of diluent:

Diluent was prepared with buffer and acetonitrile with 70: 30 (v/v) ratio and degassed with 0.45µ filter.

Preparation of standard stock solutions

25 mg of Enalapril maleate reference standard is accurately weighed and transferred into a 25 ml of VF and was initially dissolved in 10 ml of (30:70 Acetonitrile: Buffer). The solution is then made up to a volume so as to obtain a stock solution of 1 mg/1 ml. From the stock suitable dilutions were prepared.

Preparation of calibration curve:

Aliquots of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml standard stock solution was transferred to the 10 ml of volumetric flasks and made up to the mark with mobile phase to get concentration of 20-120 µg/ml. The fixed standard solution was prepared by transferring 8 ml of Enalapril Meliate standard solution to 10 ml of volumetric flask and made up to the mark with mobile phase to get 80 µg/ml of Enalapril Meliate.

Sample preparation:

Two tablets of ENAPRI-5mg were taken and recorded the weight. Ground the tablets in Agate mortar and weighed a quantity equivalent to 10mg (about 460mg) of Enalapril meliate. Transferred to 10 ml standard flask and added 5ml of diluent. The flask was shaken for 15 min and diluted to the mark with diluent. The solution was then filtered through 0.45 micron membrane filter. Transferred 8ml into 10 ml volumetric flask and made up to the mark with mobile phase.

RESULTS AND DISCUSSIONS

Method development and optimization:

To develop simple and stability indicating RP-HPLC method for Enalapril meliate determination, several research experiments were performed with different salt buffers and mobile phase compositions. Finally, satisfactory separation with high peak symmetry were obtained with X Bridge C18 (50 mm x4.6 mm, 3.5µ, Waters corporation, USA) and the mobile phase comprised of Acetonitrile and Buffer (30:70 v/v) at a flow rate of 0.6 ml/min to get better reproducibility and repeatability. Quantification was achieved at 210 nm based on peak area. The retention time was found to be 4.11min. The optimized method was validated as per ICH and other regulatory guidelines. System suitability, specificity, linearity, accuracy, robustness and ruggedness were performed.

Table 1: Optimized Chromatographic Conditions

Mobile phase	Ammonium dihydrogen phosphate and 1-Octane sulphonic acid Sodium salt Buffer and Acetonitrile (70:30 v/v)
Stationary phase	X Bridge C18 (50 mm x4.6 mm, 3.5µm)
Wavelength	210nm
Run time	10 min
Flow rate	0.6 ml/min
Injection volume	10µL
Temperature	60^0C
Mode of operation	Isocratic elution
PH	2.6

METHOD VALIDATION:

The optimized Chromatographic method was validated as per the international guidelines.

System suitability test

10µL of the standard solution (0.8 mg/ml) was injected under optimized chromatographic conditions to evaluate the suitability of system. The values of system suitability parameters were shown in Table 2.

Table 2: System Suitability Test Parameters

System suitability parameters	Result
Retention time	4.11
Area	23535706 (0.8mg/ml)
Theoretical plate number	620
Tailing factor	1.039

Specificity

Specificity of the RP-HPLC method was demonstrated by the separation of the analysts from other potential components such as impurities and excipients. A volume of 10 µl of placebo sample solution was injected and the chromatogram was recorded. No peaks were found at retention time of 4.11min. Hence, the proposed method was specific for Enalapril meliate.

Linearity:

The linearity of calibration curve in pure solution was carried over the concentration range of 0.2-1.2 mg/ml through proposed RP-HPLC method. The data was represented in Table 3. The Correlation Coefficient is 0.9986 indicates that the method is Linear.

Table 3: Linearity Data

Linearity level	Concentration (mg/ml)	Peak area
1	0.2	6249790
2	0.4	12172199
3	0.6	18120092
4	0.8	23556800
5	1.0	28666373
6	1.2	33116238
Slope		270359.2
Intercept		1388435
Regression equation		Y=270359.2 X + 1388435
Correlation Coefficient®		0.9986
Coefficient of determination(r^2)		0.9972

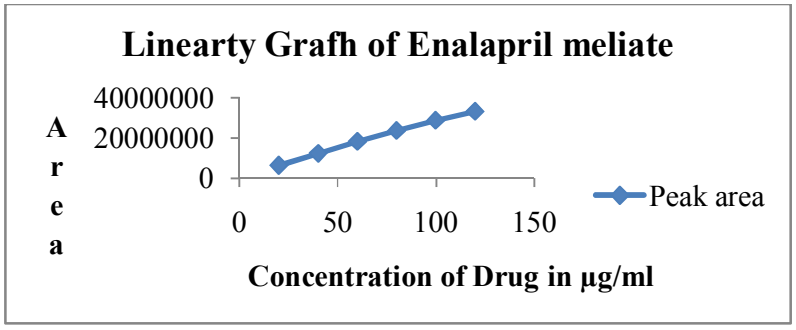


Figure-2: Enalapril Meliate Linearity Graph

Precision

The precision of the method was determined by injecting 0.8mg/ml concentration in 5 replicate .

Repeatability:

The Repeatability of the proposed method was ascertained by injecting five replicates of fixed concentration within the Beer's range and finding out the peak area by the proposed method. The method precision was carried out by intraday and inters day measurement. From this peak area % RSD was calculated. (Table 4) The calculated %RSD observed is well below 0.074 indicates that the method is Precise.

Table 3: Precision Data

Precision	
Repeatability (%RSD,n=5)	0.074
Intraday Precision(%RSD,n=5)	0.074-0.202
Interday Precision(%RSD,n=5)	0.202-0.372

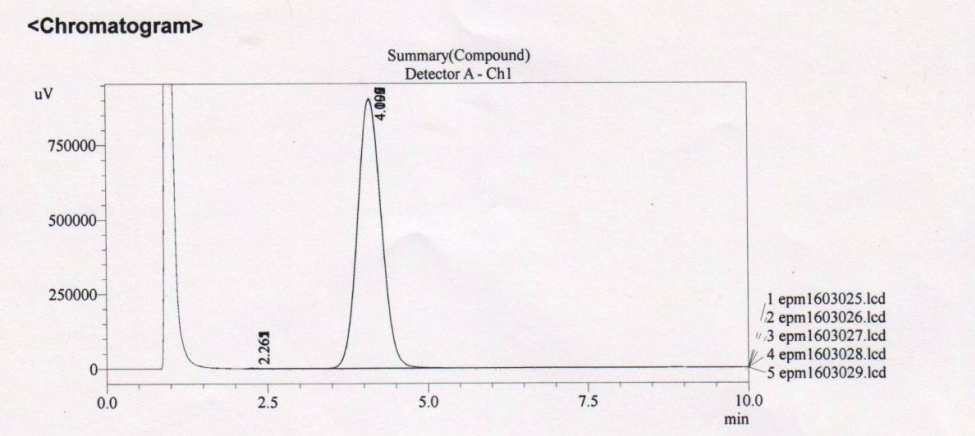


Figure-3: precision overlay chromatogram

Accuracy

For the accuracy of proposed method, recovery studies were performed by standard addition method at five different levels (25%, 50%, 75 %, 125% and 150% of final concentration). A known amount of standard pure drug was added to pre-analyzed tablet powder and the sample was then analyzed by proposed method. Results of recovery studies were found to be satisfactory and reported in Table 5 a and 5b. An overlay chromatogram of tablet and the standard solution were shown in the Fig.4

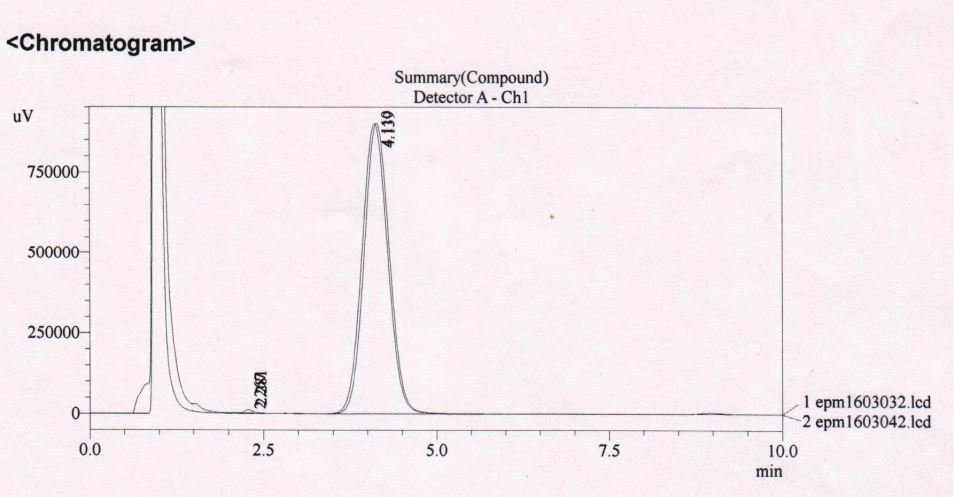
Table-5a: Accuracy Results

Drug name	Levels	Amount added(mg/ml)	Enalapril Meliate content(mg)	Percent recovery	Average of percentage recovery
Enalapril Meliate	25%	0.2	26.5	99.76	99.92
	50%	0.4	51.67	99.93	
	75%	0.6	76.9	99.97	
	125%	1.0	121.7	99.98	
	150%	1.2	140.6	99.96	

Table-6b: Accuracy Results for Tablet

Brand name	Drug name	Amount labeled	Amount found	%Recovery	Average area	Standard deviation	%RSD
(Enapri-5mg) Intas,Pharmaceuticals EastSikkim,India	Enalapril Meliate	10mg (2 Tab)	10.28	102.8	22919284.7	52821.81	0.23

Figure-4: Tablet and Drug solution overlay chromatogram



Stability of the analytical solutions:

The stability of the sample solution is determined by placing the sample solution for the short term stability by keeping at room temperature up to 24 hours and then comparing the obtained peak area with that of the similarly prepared fresh sample. Further, auto-sampler stability for up to 24 hours was studied and established. The result indicates that the sample solution is stable upto 24 hours.

Robustness:

Robustness was studied by deliberately changing the Flow rate and Temperature of the column. Analyzed the standard solution was changing the flow rate about ± 0.1 µl to the original flow rate 0.6 µl and also recorded the analysis data for changing the column oven temperature about ± 2^0C to the original 60^0C temarature. Method precision verified with different Flow rates and Temperatures. The % RSD for 0.5µl, 0.7µl and 58^0C, 620C is within the limits.

CONCLUSION

A rapid and reliable isocratic RP-HPLC-UV method for the determination of Lisinopril dehydrates has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method. It is highly simple, accurate, precise, sensitive, validated and analytical procedure and its retention time 4.72 min allows the analysis of large number of samples in a short period of time. So this can be used for routine analysis.

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