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Analytical method development and validation for simultaneous estimation of Enalapril Maleate and hydrochlorothiazide by RP-HPLC

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ABSTRACT

A simple, fast, precise reverse phase isocratic high performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous estimation of Enalapril Maleate and Hydrochlorothiazide in marketed formulations. Estimation of drugs in this combination was done with a C18 column [ODS UG column. 250mm × 4.5 mm] using mobile phase of composition Acetate buffer, Methanol and Acetonitrile (60:20:20 v/v, pH 5) The flow rate was 0.8 ml/min and the effluents were monitored at 232nm. The retention time of Enalapril Maleate and Hydrochlorothiazide were 2.8 min and 4.1 min respectively. The method was found to be linear over a range of 10-30 µg/ml for Enalapril Maleate and Hydrochlorothiazide. The method was validated according to the guidelines of International Conference on Harmonization (ICH) and was successfully employed in the estimation of commercial formulations.

Keywords: Enalapril Maleate, Hydrochlorothiazide, RP-HPLC, Method validation

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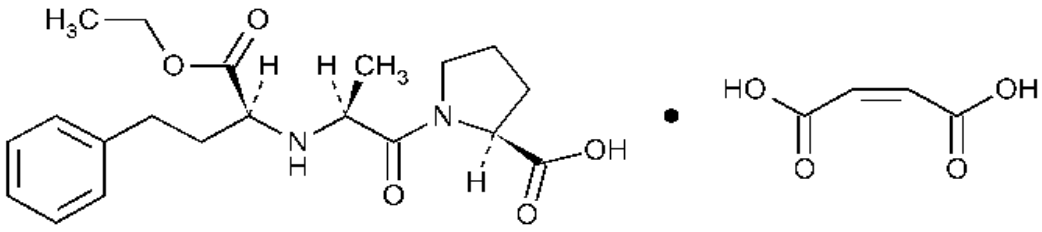
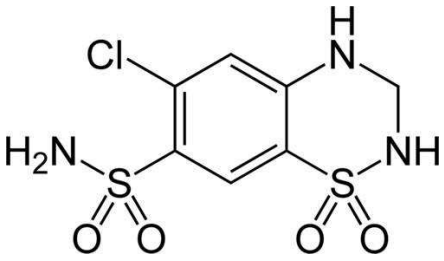
INTRODUCTION

Enalapril Maleate is the Angiotensin converting (ACE) enzyme inhibitor. Used for the treatment of essential or renovascular hypertension and symptomatic congestive heart failure. Chemically, Enalapril Maleate is described as *N*-[(1S)-1-(Ethoxy carbon-yl)-3-Phenylpropyl]-L- Proline [1]. Hydrochlorthiazide is one of the oldest and widely used diuretics. Which is also used in the treatement of Hypertension [2]. Chemically it is 6-chloro-3,4-dihydro-2h-1,2,4-benzothiadiazine-7-sulfonamide1,1-dioxide . The chemical structures of Enalapril Maleate and Hydrochlorothiazide were shown in the figure 1.

Extensive literature survey revealed that very few methods were reported for the simultaneous estimation of Enalapril maleate and Hydrochlorothiazide by RP-HPLC [3-10]. So, an attempt has been made to develop an accurate, precise and economically viable RP-HPLC method for the simultaneous estimation of combination of interest in the current research.



Enalapril Maleate



Hydrochlorothiazide

Fig 1: chemical structures of a) Enalapril Maleate and b) Hydrochlorothiazide

MATERIALS AND METHODS

Equipment used

The chromatographic separation was performed on Agilent 1120 compact liquid chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µl fixed loop. A reverse phase C18[Agilent ODS UG 5 column, 250mm × 4.5 mm] was used. Elico SL 218 double beam UV visible spectrophotometer and Axis AGN204-PO electronic balance were used for spectrophotometric determinations and weighing purposes respectively.

Reagents and chemicals

Pharmaceutical grade pure Enalapril Maleate and Hydrochlorothiazide gift samples were procured from Mylan Laboratories, Hyderabad. Marketed formulation Enapril Tablets with dose of 10mg of Enalapril maleate and 25mg of Hydrochlorothiazide were procured from local market. (Mfd.by Intas pharmaceuticals ltd). HPLC grade Acetonitrile, Methanol and Water were procured from Merck specialties private limited, Mumbai.

Chromatographic conditions

C18[Agilent ODS UG 5 column, 250mm × 4.5 mm] was used for the chromatographic separation at a detection wave length of 232 nm. Mobile phase of composition Acetate buffer pH 5, Acetonitrile, Methanol in a ratio of 60:20:20v/v was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 0.8ml/min and the injection volume was 20µl.

Preparation of Mobile phase

Acetate buffer pH5 was prepared by Dissolve 13.6gm of sodium acetate in 500ml of HPLC grade distilled water and add 6ml of glacial acetic acid, made up to 1000ml with distilled water. 600 ml of Buffer was added to 200ml of Acetonitrile and 200ml of Methanol, filtered through 0.45µ membrane filter and sonicated for 20 minutes.

Preparation of Standard solutions

25mg each of Enalapril maleate and Hydrochlorothiazide were accurately weighed and transferred into two 25ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solutions A (Enalapril maleate) B(Hydrochlorothiazide) of concentration 1000µg/ml of each drug . From the primary stock solutions , 0.3ml and 0.3ml were pipette out from A and B respectively, transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 30 µg/ml

and 30µg/ml of Enalapril Maleate and Hydrochlorothiazide respectively and this solution is (working stock solution A).

Preparation of Sample Solution

Twenty tablets of Enapril were weighed and crushed. Tablet powder equivalent to 10mg of Enalapril maleate and 25mg of Hydrochlorothiazide was weighed accurately and transferred to a 25ml volumetric flask. The content was dissolved with 10ml of mobile phase and then sonicated for 15min. the volume was made up with the mobile phase and filtered with Whatmann filter paper no.41. 0.5ml of this solution was pipetted out and transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain a concentration of 20µg/ml of Enalapril Maleate and 50µg/ml of Hydrochlorothiazide (working stock solution B).

Optimization of RP-HPLC method

The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of Enalapril Maleate and Hydrochlorothiazide. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Acetate buffer pH 5 , Acetonitrile and Methanol (60:20:20 v/v) using C_{18} column [Agilent ODS UG 5 column, 250mm × 4.5 mm]

Validation of the RP-HPLC method

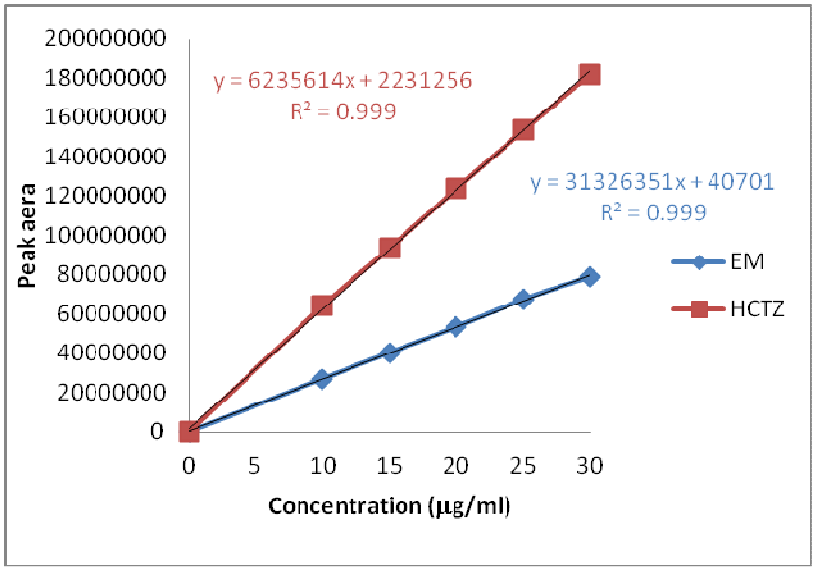
Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

System suitability

System suitability was carried out with five injections of solution of 100% concentration having 30 µg/ml of Enalapril Maleate and 30 µg/ml of Hydrochlorothiazide in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factors (T) were reported in table 1.

Table 1: system suitability parameters

Parameters	Enalapril Maleate	Hydrochlorothiazide
Retention time (min)	2.8	4.1
Theoretical plates (N)	11456	10366
Tailing factor (T)	1.1	1.3
Resolution (R_s)	2.89	



Graph 1: Calibration plot of Enalapril Maleate and Hydrochlorothiazide

Linearity

For the determination of linearity, appropriate aliquots were pipetted out from working stock solution A to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 10-

30µg/ml of Enalapril maleate and 10-30µg/ml of Hydrochlorothiazide. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for Enalapril Maleate and Hydrochlorothiazide were shown in Graph 1 and figure 2 and their corresponding linearity parameters were given in table 2.

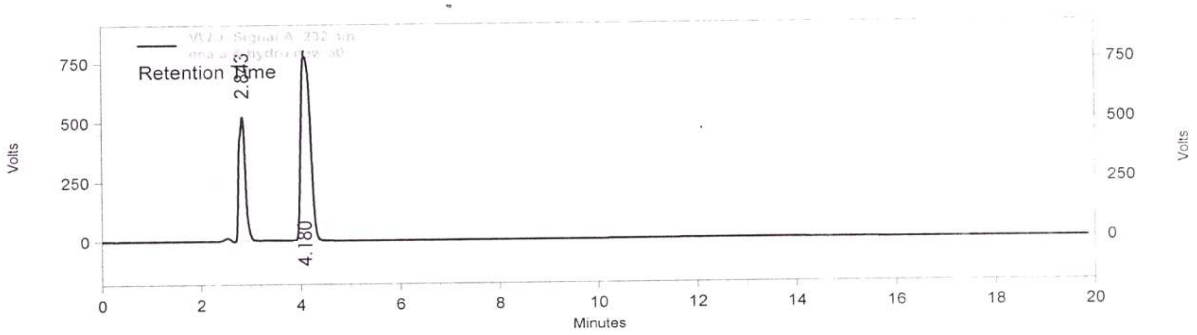


Fig 2: Optimized chromatogram of Enalapril Maleate and Hydrochlorothiazide

Table 2: Results for Linearity (n=3)

Parameters	Enalapril Maleate	Hydrochlorothiazide
Slope	31326351	6235614
y intercept	40701	2231256
Correlation coefficient r^2	0.999	0.999
Regression Equation	Y=31326351x+40701	Y=6235614x+2231256
Linearity range	10-30µg/ml	10-30µg/ml
LOD	0.16µg/ml	0.33µg/ml
LOQ	0.49µg/ml	1.01µg/ml

**n= No. of determinants*

Precision

The repeatability of the method was verified by calculating the %RSD of six replicate injections of 100% concentration (30 µg/ml of Enalapril Maleate and 30µg/ml of Hydrochlorothiazide) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days. The results were given in table 3.

Table 3: Results of precision (n=6)

Drug	Intraday Precision (%RSD)	Interday Precision (%RSD)
Enalapril Maleate	0.69	0.87
Hydrochlorothiazide	0.79	1.14

**n= No. of determinants*

Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the percent recovery was reported. The results were given in table 4.

Table 4: Results for Accuracy (n=3)

Recovery level	Enalapril maleate				Hydrochlorothiazide			
	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery	Amount Added (µg/ml)		Amount Found (µg/ml)	% Recovery
	std	test			std	Test		
50%	8	2	10.12	101.2	5	5	9.97	99.7
100%	18	2	19.98	99.90	15	5	20.15	100.75
150%	28	2	29.56	98.5	25	5	29.66	98.8
Mean recovery	98.2-101.2%w/w				98.8-100.75%w/w			

**n= No. of determinants*

Specificity
Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s. The results were given in table 2.

Robustness
Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wave length, etc. and the % RSD should be reported.

Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ±2nm in the detection wave length and ±0.2ml/min in the flow rate, were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions was injected to the instrument in triplicate. %RSD was reported in the table 5.

Table 5: Results for Robustness

Parameters (n=3)	%RSD	
	Enalapril Maleate	Hydrochlorothiazide
Detection wavelength at 230nm	0.39	0.76
Detection wavelength at 234m	0.61	0.38
Flow rate 0.6ml/min	0.36	0.56
Flow rate 1.0ml/min	0.31	0.38

**n= No. of determinants*

Assay of Marketed Formulations
20µl of sample solution of concentration 30 µg/ml of Enalapril Maleate and 30 µg/ml of Hydrochlorothiazide was injected into chromatographic system and the peak responses were measured and shown in the figure 3. The solution was injected three times in to the column. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of test samples.

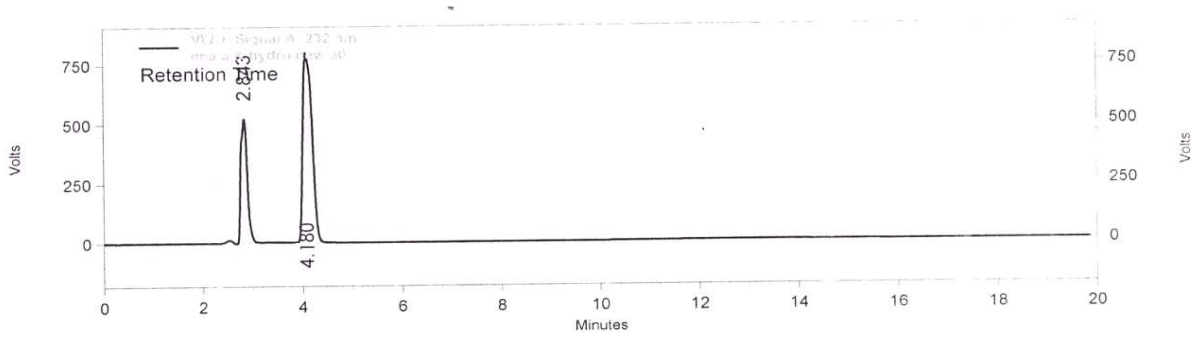


Figure 3: A typical chromatogram for assay of marketed formulation containing 30µg/ml of Enalapril Maleate and 30 µg/ml Hydrochlorothiazide

Table 6: Results for Assay (n=3) of Marketed formulation

Drug	Label claim (mg/tab)	Amount recovered	% Amount found in drug
Enalapril Maleate	5	5.06	101.20%w/w
Hydrochlorothiazide	25	24.95	99.8%w/w

**n= No. of determinants*

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, Acetate buffer pH 5, Acetonitrile, Methanol in the ratio 60:20:20v/v was selected as mobile phase because of better resolution and symmetric peaks. Enalapril Maleate and Hydrochlorothiazide were found to show appreciable absorbance at 232 nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of Enalapril Maleate and Hydrochlorothiazide at different R_Ts was shown in figure 2

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Enalapril Maleate and Hydrochlorothiazide at 2.8min and 4.1min respectively without any interferences. The parameters were given in table 1.

Concentration range of 10-30µg/ml for Enalapril Maleate and 10-30µg/ml for Hydrochlorothiazide were found to be linear with correlation coefficients 0.999 and 0.999 for Enalapril Maleate and Hydrochlorothiazide respectively. The results were given in table 2.

The proposed method was found to be precise and reproducible with %RSD of 0.87 and 1.14 for Enalapril Maleate and Hydrochlorothiazide respectively. %RSD was reported in table 3.

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 98.2 to 101.2% w/w and 98.8-100.75% w/w for Enalapril Maleate and Hydrochlorothiazide respectively. This indicates that the method was accurate. Values obtained were given in table 4.

The limits of detection for Enalapril Maleate and Hydrochlorothiazide were found to be 0.16µg/ml and 0.33µg/ml respectively and the limits of quantitation were 0.49µg/ml and 1.01µg/ml respectively. Values were represented in table 2.

The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination. Values obtained were given in table 6.
The method was found to be robust after changing the conditions like detection wavelength (± 2nm) and flow rate (± 0.2 ml). %RSD was calculated for each variation and reported. Values obtained were given in table 5.

CONCLUSION

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Enalapril Maleate and Hydrochlorothiazide from their formulations. All the validation parameters were found to be within the limits according to ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged and robust and can be involved in the routine analysis of the marketed formulations.

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