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Method Development and Validation of Enalapril Maleate and Hydrochlorthiazide 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 [BDS column. 250mm × 4.6 mm] using mobile phase of, Methanol and water (70:30, pH 3.7) The flow rate was 0.1ml/min and the effluents were monitored at 215nm. 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: *HPLC, reverse phase, hydrochlorothiazide, enalapril maleate, stability, analysis.*

OBJECTIVE

To develop new RpHPLC method for the simultaneous estimation of Enalapril Maleate and Hydrochlorthiazide in pharmaceutical dosage form.

METHODS

Reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Enalapril Maleate(ENL) and Hydrochlorthiazide (HTZ) in combined dosage forms. RP-HPLC separation was achieved by using Hypersil-C_{18} BDS column with 75:25 (v/v) 50mM potassium dihydrogen orthophosphate buffer : methanol as mobile phase at a flow rate of 1.0 ml/min. UV detection at 207nm and 272 nm; HTZ and ENL were eluted with retention times of 2.09 and 5.289min, respectively. The method was continued and validated accordance with ICH guidelines. Validation revealed the method is rapid, specific, accurate, precise, reliable, and reproducible. Isobestic point was 215nm.

INTRODUCTION

Enalapril Maleate (ENL) chemically known as(S)-1- [N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate salt (1:1). It is the maleate salt form of enalapril, a dicarbocyl-containing peptide and angiotensin-converting enzyme (ACE) inhibitor with antihypertensive activity. As a prodrug, enalapril is converted by de-esterification into its active form enalaprilat. Enalaprilat competitively binds to and inhibits ACE, thereby blocking the conversion of angiotensin I to angiotensin II. This prevents the potent vasoconstrictive actions of angiotensin II and results in vasodilation. Enalapril also decreases angiotensin II-induced aldosterone secretion by the adrenal cortex, which leads to an increase in sodium excretion and subsequently increases water outflow.

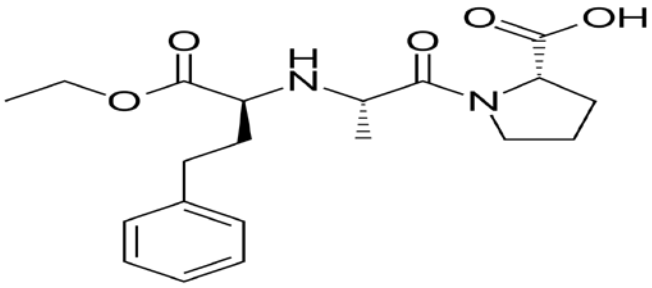


Fig.1: Chemical Structure of Enalapril Malaete

Hydrochlorthiazide (HTZ) is a benzothiadiazine that is 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide substituted by a chloro group at position 6 and a sulfonamide at 7. It is diuretic used for the treatment of hypertension and congestive heart failure. It works by causing a person to make more urine. This helps the body get rid of extra salt and water. This medication also reduces extra fluid in the body (edema) caused by conditions such as heart failure, liver disease, or kidney disease.

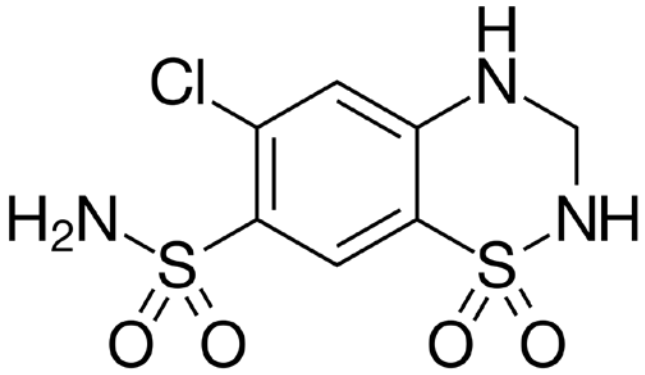


FIG.2: Chemical Structure of Hydrochlorthiazide

There are many UV reported methods to determine either ENL or HTZ alone or in combination with other drugs. NMR spectroscopy, polarographic method, electrokinetic chromatography, HPLC method and UV method are reported for simultaneous estimation of both drugs. But to the best of my knowledge, none has been reported the simultaneous determination of ENL and HTZ by HPLC method in bulk and pharmaceutical dosage forms. Therefore, the present work was aimed to develop and validate a new RP- HPLC method for simultaneous estimation of ENL and HTZ in pharmaceutical dosage forms.

MATERIALS AND METHODS

Enalapril maleate and Hydrochlorthiazide were procured from AHPL, India. A commercial tablet (Enace-D Tablet) used for analysis was procured from pharmacy. HPLC grade Methanol and water were procured from Active Pharma Labs, Hyderabad.

INSTRUMENTS

RP-HPLC was performed using HPLC- Waters Model No. 2690/5 series Compact System consisting of Hypersil-C_{18} BDS column, Electronic balance (SOTORIOUS), Sonicator (FAST CLEAN), Software- Empower 2

CHROMATOGRAPHIC CONDITIONS

A reverse phase column [Hypersil C18 (250x4.6mm, 5µ particle size)], equilibrated with mobile phase. pH adjusted to 3.7 using a OPA. Mobile phase flow rate was maintained at 1ml/min and effluents were monitored at 207nm. The sample was injected using 20 microlitre fixed loop rheodyne injector and run time was 10 minutes.

PREPARATION OF MOBILE PHASE: Methanol:Buffer (70:30)
Phosphate buffer pH2.5 was prepared by Dissolving 100 g of potassium dihydrogen phosphate in 800 mL of water; adjust to pH 2.5 with hydrochloric acid and dilute to 1000.0 mL with water. 600 ml of Buffer was added to 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 Enalapril 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 C18 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.

Table 1: System Suitability parameters

Parameters	Enalapril Malaete	Hydrochlorthiazide
Retention time (mins)	2.8	4.1
Theoritical plates	11456	10366
Tailing factor (T)	1.1	1.3
Resolution (R_s)	2.89	

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.

Table 2: Results for Linearity (n=3)

Parameters	Enalapril Malaete	Hydrochlorthiazide
Slope	32315163	6325416
Y intercept	42801	2325216
Correlation coefficient	0.888	0.888
Regression equation	Y=32315163x+42801	Y=6325416x+2325216

Linearity range	10-30microgram/ml	10-30 microgram/ml
LOD	0.17microgram/ml	0.35 microgram/ml
LOQ	0.5 microgram/ml	1 microgram/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 Malaete	0.72	0.87
Hydrochlorthiazide	0.81	1.14

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				Hydrochlorthoazide			
	Amount Added (ppm)		Amount Found (ppm)	% Recovery	Amount Added (ppm)		Amount Found (ppm)	% Recovery
	Std	Test			Std	Test		
50%	8	2	**10.12**	**101.2**	**5**	**5**	**9.97**	**98.7**
100%	18	2	**19.98**	**99.9**	**15**	**5**	**21.20**	**100.85**
150%	28	2	**29.56**	**98.5**	**25**	**5**	**30**	**99**
Mean recovery	98-101.5%w/w				99-101%w/w			

****n=No. of determinants***

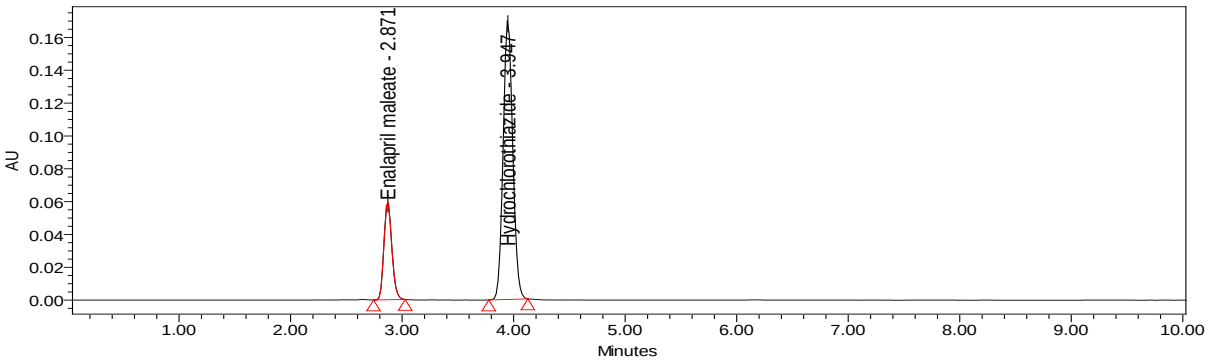
SPECIFICITY

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.

	Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Enalapril Maleate	2.869	81176	15.55	16240		1.154638	7695.204634
2	Hydrochlorothiazide	3.942	440435	84.45	72947	6.168534	1.087624	9701.722314



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

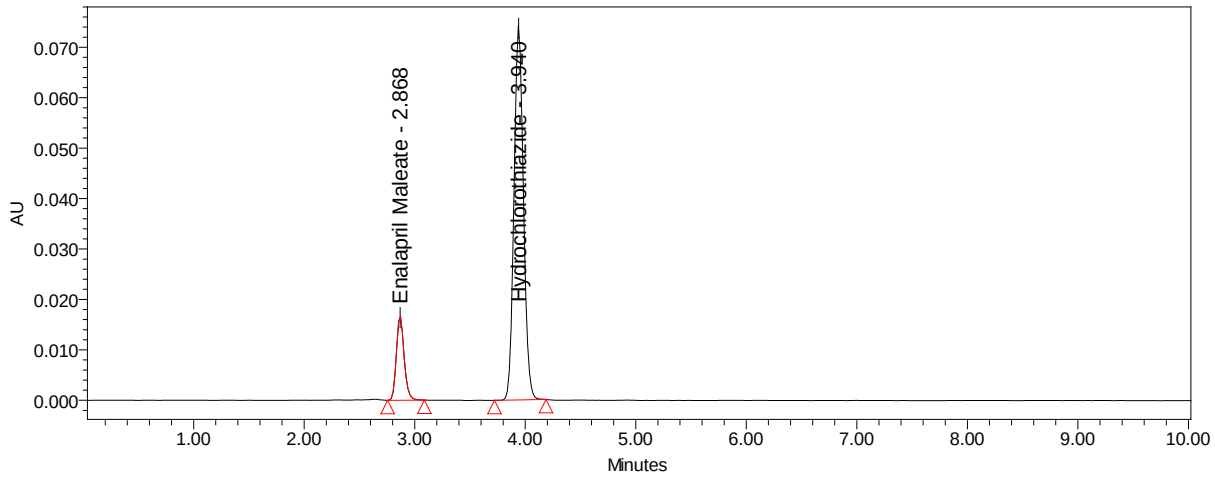
Parameters (n=3)	%RSD	
	Enalapril Maleate	***Hydrochlorthiazide***
Detection wavelength	0.40	0.78
Detection wavelength	0.62	0.40
Flow rate 0.6ml/min	0.38	0.54
Flow rate 1.0ml/min	0.3	0.40

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.

Trial 1

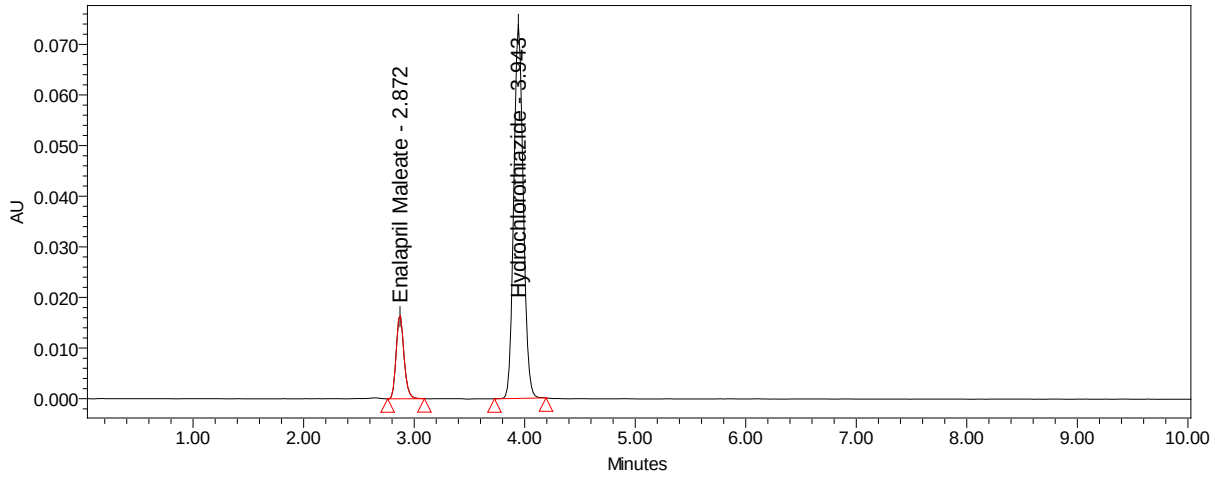
	Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Enalapril Maleate	2.868	81755	15.63	16503		1.144638	7926.034658
2	Hydrochlorothiazide	3.940	441489	84.37	73651	5.286388	1.074698	9905.364456



Chromatogram for Trial 1 using the Marketed sample

Trail 2

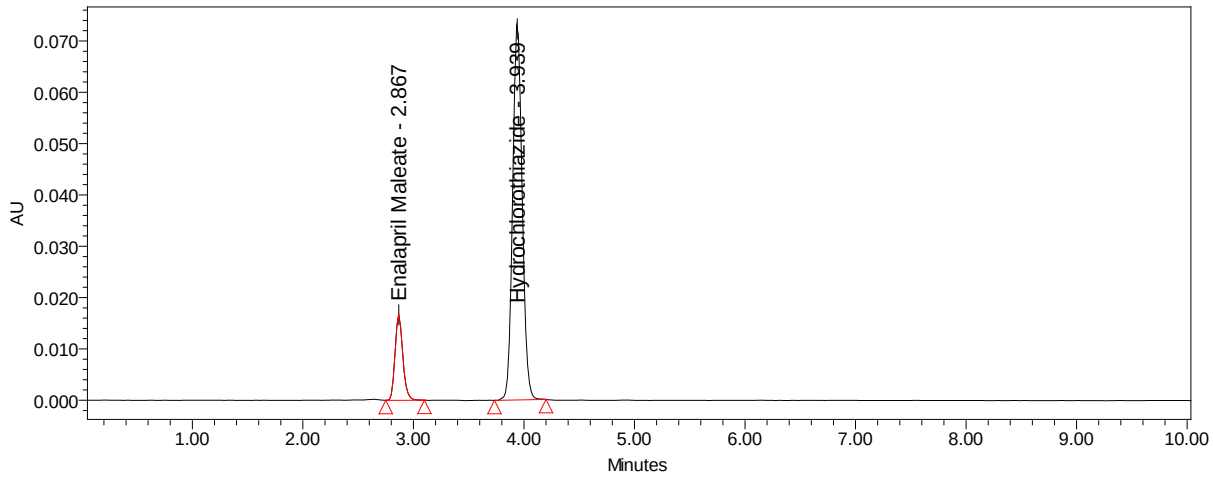
	Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Enalapril Maleate	2.872	81585	15.61	16473		1.157658	7973.334633
2	Hydrochlorothiazide	3.943	440248	84.39	73112	5.345036	1.074358	9872.908630



Chromatogram for Trial 2 using the Marketed sample

Trial 3

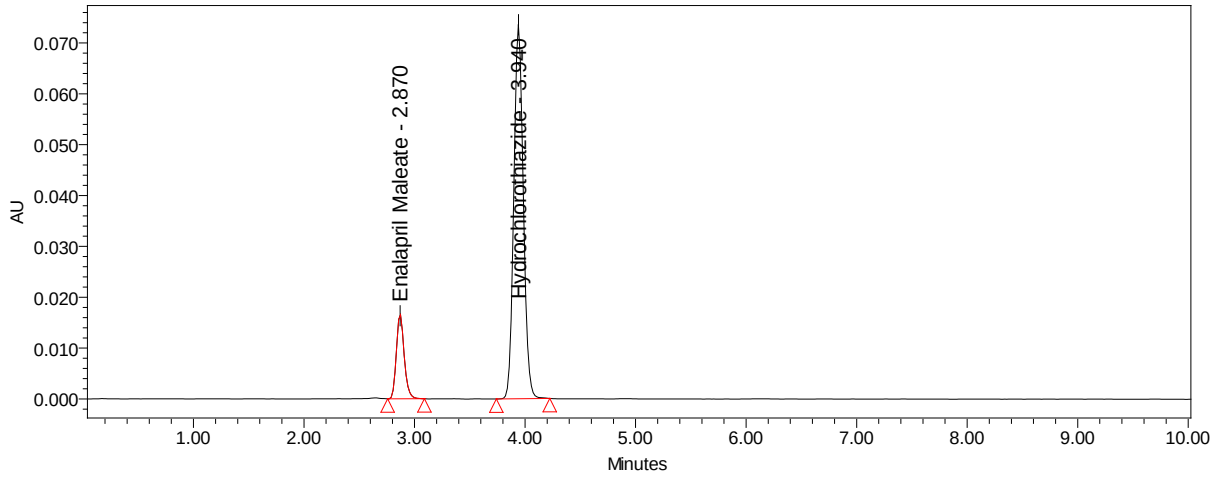
	Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Enalapril Maleate	2.867	81424	15.60	16449		1.167602	7630.144632
2	Hydrochlorothiazide	3.939	441306	84.40	73273	7.184635	1.084635	9728.774328



Chromatogram for Trial 3 using the Marketed sample

Trial 4

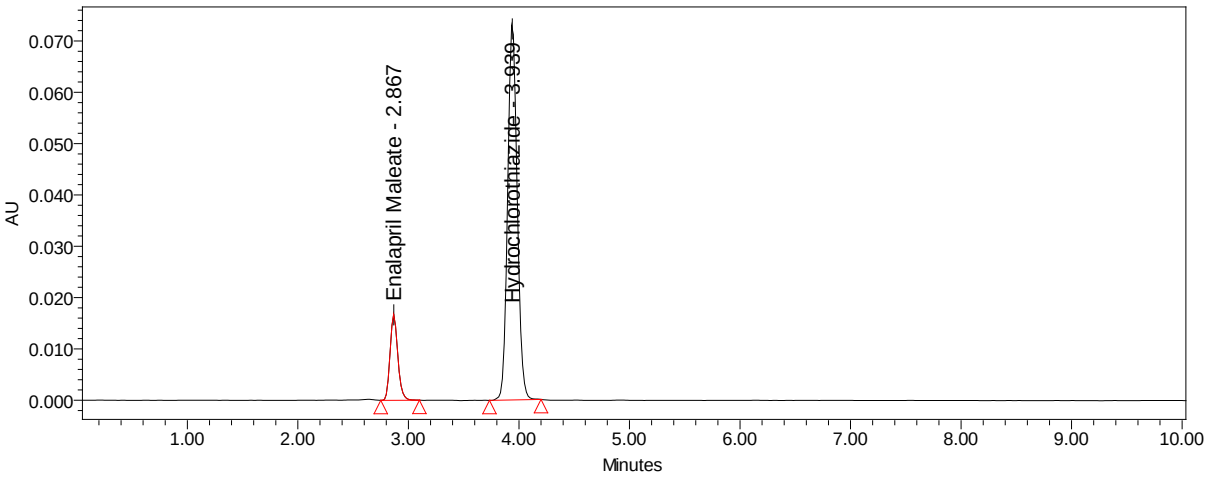
	Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Enalapril Maleate	2.870	81944	15.67	16438		1.165345	7872.774528
2	Hydrochlorothiazide	3.940	440652	84.33	73256	6.272652	1.079463	9827.886529



Chromatogram for Trial 4 using the Marketed sample

Trial 5

	Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Enalapril Maleate	2.867	81444	15.60	16449		1.163546	7630.144563
2	Hydrochlorothiazide	3.939	441632	84.40	73073	6.187625	1.082463	9728.773452



Chromatogram for Trial 5 using the Marketed sample

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, OPA pH 3.7, Methanol : Buffer in the ratio 70:30 was selected as mobile phase because of better resolution and symmetric peaks. Enalapril Maleate and Hydrochlorothiazide were found to show appreciable absorbance at 215 nm when determined spectrophotometrically and hence it was selected as the detection wavelength.

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 10-30ppm for Enalapril Maleate and 10-30ppm for Hydrochlorothiazide were found to be linear with correlation coefficients 0.888 and 0.888 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 to101.5% w/w and 99-101% 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 0.17µg/ml and 0.35µg/ml respectively and the limits of quantitation were 0.5µg/ml and 1µg/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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