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Development and Validation of a Reversed Phase HPLC Method for Simultaneous Estimation of Enalapril maleate, Hydrochlorothiazide and Paracetamol in Pure and its Pharmaceutical Dosage Form

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ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for estimation of Enalapril Maleate, (EM), Hydrochlorothiazide (HCT) and Paracetamol in its pure and tablet dosage form. The mobile phase consisting of acetonitrile: water (pH 4.7 adjusted with ortho phosphoric acid) in the ratio of (25:75%v/v) was delivered at the flow rate of 1.2 mL/min and detection was carried out at 220 nm. The separation was achieved using C18 reverse phase column (Hi-Q 250x4.6mm ID; particle size 5µm).The validation of method carried out as per ICH guidelines. The method was tested for linearity, accuracy, recovery study and specificity.

Key Words: HPLC reverse phase, Enalapril maleate, Hydrochlorothiazide and Paracetamol,

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INTRODUCTION

Enalapril maleate (EM) is a derivative of 2 amino acid, L-alanine and L-proline and is an antihypertensive and a vasodilator in congestive heart failure. Various analytical methods for the estimation of Enalapril in the given dosage form were reported in literature which includes high performance liquid chromatography with Ultra violet detection[1] , capillary electrophorosis [2] and flow injection analysis based on the formation of ternary complex [3] . Hydrochlorothiazide (HCT), 6 - chloro - 3, 4 - dihydro - 7 - sulfamoyl- 2H - 1, 2, 4 - benzothia - diazine - 1, 1 – dioxide, is a thiazide Diuretic which reduces the resorption of electrolytes and consequently of water. Many analytical methods alone or in combination with other drugs were reported including UV-spectrophotometric methods[4] ,and using RP-HPLC method in plasma[5] and its combined pharmaceutical dosage form[6] .and Paracetamol, N-acetyl-p-amino phenol is a commonly used analgesic and antipyretic drug. Literature survey reveals many analytical methods for determination of Paracetamol such as UV Spectrophotometry[7] , HPLC[8-10] , and Capillary electrophoresis[11] .There were many analytical methods for simultaneous estimation of Enalapril maleate in combination with Hydrochlorothiazide are reported such as RP-HPLC

method in human plasma[12], RP-HPLC method in its tablet dosage form[13] and simultaneous determination by LC-MS-MS in human serum [14].

Most of these methods are for the determination of either HCT or EM or Paracetamol separately. There were no simple and reproducible methods so far reported for simultaneous determination of Enalapril maleate, Paracetamol and Hydrochlorothiazide by RP-HPLC in solid dosage form. It is essential to developed simple, precise, accurate HPLC method for simultaneous determination of both drugs in solid dosage form. Therefore, in this study we developed reproducible method which can be used in laboratory. The validation of this method carried out as per ICH guidelines [15-16].

MATERIALS AND METHODS

Apparatus:
The apparatus employed was a JASCO HPLC-2000 solvent delivery system with universal loop injector (Rheodyne 7725i) of injection capacity of 20 µL, JASCO UV-2075 Plus intelligent UV-Visible detector and JASCO PU-2080 isocratic HPLC pump. Separation was carried out on a HiQsilC18HS (250 X 4.6 mm I.D., particle size 5µm) under reversed phase partition chromatographic conditions. The equipment was controlled by a PC installed properly with the chromatographic software.

Chemicals:
Generally all chemicals used were the purest grade available and were used as received without further purification. Acetonitrile HPLC-grade (99.8%), water and orthophosphoric acid were used. The active ingredients Hydrochlorothiazide, Enalapril maleate and Paracetamol were obtained from USP and BP as reference standards. The HPLC grade water was used. Commercial tablet purchased from local market.

Chromatographic Conditions:
The mobile phase consists of Acetonitrile: water (25:75 V/V) solution and the pH was adjusted to 4.7 by orthophosphoric acid. The mobile phase was always filter using 0.45µm membrane filter and was degassed by using sonicater for about 15min. The sample solutions were also filter using 0.45 µm membrane filters. The flow rate was 1.2mL/min. The wavelength was used in 220 nm. Total run time was of 10 min.

Preparation of the standard solution:
Standard stock solutions of Enalapril Maleate, Hydrochlorothiazide and Paracetamol were prepared by dissolving 10mg of each in 10 ml of mobile phase. From the above solution 1ml of solution was taken and diluted to 10ml with the same to get a solution containing 100 µg/ml of each drug. From the stock solutions further dilutions were prepared by diluting required volume of mobile phase.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines as follows.

Linearity:
The table 2 presents the equation of the regression line ,correlation coefficient(r^2) values of the slope and intercept for each compounds between the peak areas and concentration of 5-60µg/L

with r^2=0.990, 5-60µg/L with r^2=0.997, 50-400µg/L with r^2=0.998 for EM, HCT and Paracetamol respectively.

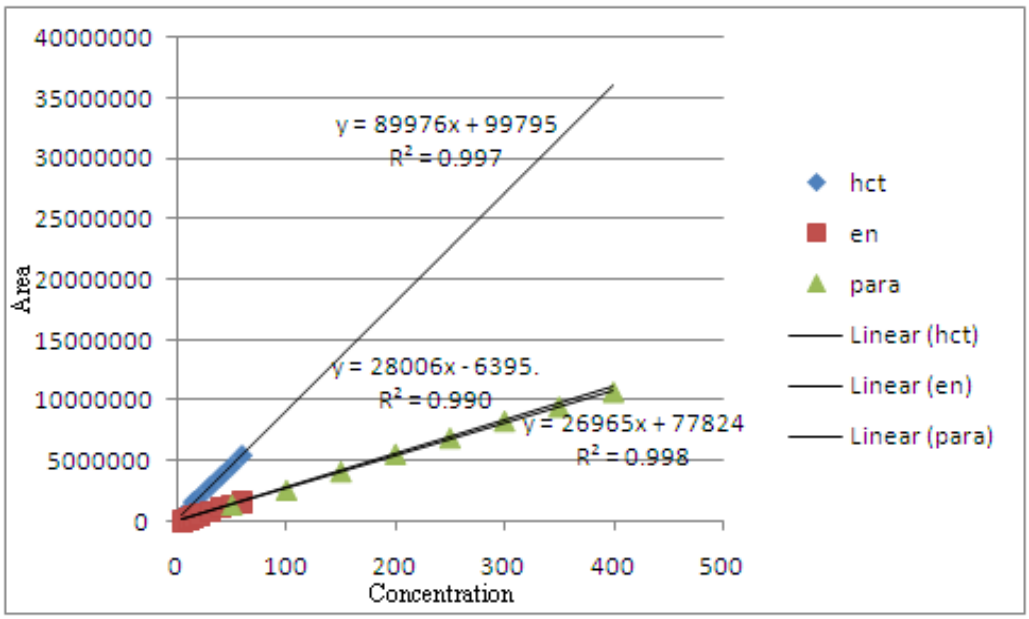

Figure1: Calibration curve of Enalapril maleate, Paracetamol and Hydrochlorthiazide

Table1: Linearity Results, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Compounds	Equations	r^2	LOQ µg/mL	LOD µg/mL
Enalapril maleate	y = 28006x-6395	0.990	0.345	0.567
Paracetamol	y = 26965x+77824	0.998	0.983	0.672
Hydrochlorothiazide	y = 89976x+99795	0.997	0.461	0.124

X= Concentration (µg/ mL); Y= Area

Suitability of the method:

The specificity of this method was determined by complete separation of EM,HCT and Paracetamol as shown in **Fig.2.** Parameters like retention time, resolution and tailing factor are given in **Table 2**. Here tailing factor for peaks of EM, HCT and Paracetamol was less than 2% and resolution was satisfactory. The average retention time ± standard deviation for EM, HCT and Paracetamol were found to be 7.911±0.004 and 1.939±0.005 respectively, for five replicates. The peaks obtained for EM,HCT and Paracetamol were sharp and have clear baseline separation.

Table2: System Performance Parameters of Enalapril Maleate, Paracetamol and Hydrochlorothiazide

Compounds	t_r	Area	R	T
Enalapril maleate	2.458	976275.513	0.00	1.53
Paracetamol	3.683	2101073.422	2.32	1.50
Hydrochlorothiazide	6.000	8072481.780	7.54	1.40

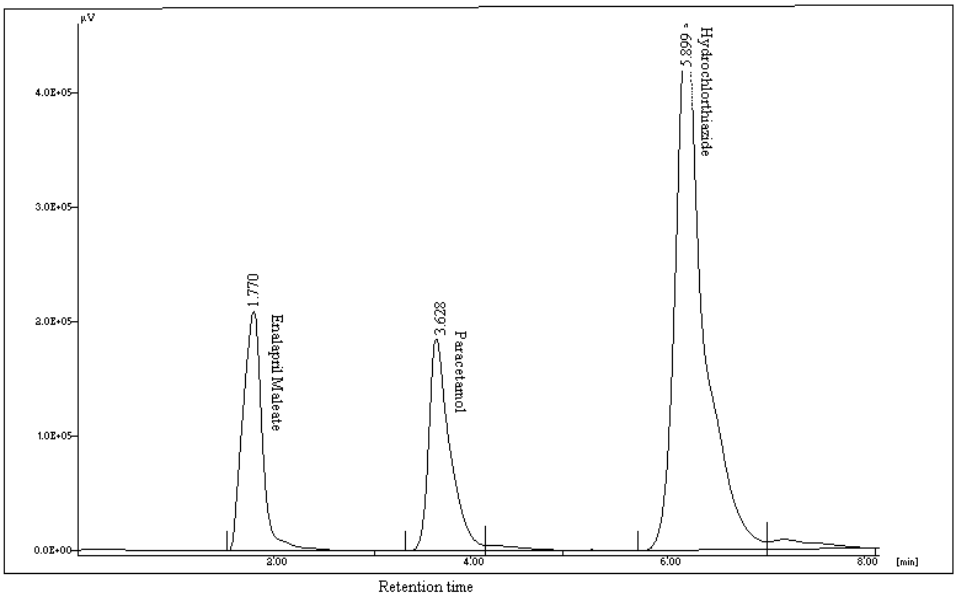

Figure 2: Chromatogram of mixture of Enalapril maleate, Paracetamol and Hydrochlorothiazide

Precision

The precision of the method (within-day variations of replicate determinations) was checked by injecting EM,HCT and Paracetamol 6 times of the stock solution.The precision of the method, expressed as the RSD% of the standard solution was 5.3,0.8,5.8 for EM, Paracetamol and hydrochlorothiazide respectively.

Table3: Precision of the Developed Method

Compounds	Peak area (mean)	RSD%
Enalapril maleate	202512.57	5.3
Paracetamol	9033889.52	0.8
Hydrochlorothiazide	28626227.27	5.8

Accuracy:

A standard working solution containing EM, HCT and Paracetamol yielding final concentrations of each 100 µg/ml was prepared. The prepared mixture of standards was injected 6 times as a test sample. From the respective area counts, the concentrations of the EM, HCT and Paracetamol were calculated using the detector responses. The accuracy, defined in terms of % deviation of the calculated concentrations from the actual concentrations, is listed in Table 4.

Table4: Accuracy of the Developed Method (n= 6)

Compound	Spiked Concentration in g/ml	Measured Concentration in g/ml Mean +*SD*	RSD %	Deviation %
Enalapril maleate	100	100.30	0.950	0.30
Paracetamol	100	97.80	0.778	2.20
Hydrochlorothiazide	100	102.36	0.639	2.36

% Deviation = [(Spiked Concentration - Mean Measured Concentration)/ (Spiked Concentration)] X100

Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis using a standard working solution, the same chromatographic system and the same column on different days. The prepared mixture of standards was injected 6 times as a test sample. Small differences in areas

and good constancy in retention times were observed on different days. The comparable detector responses obtained on different days indicate that the method is capable of producing results with high precision on different days. Similarly, the ruggedness of the method was tested by injecting the standard working solution into a different HPLC unit. The high degrees of reproducibility of detector responses and retention times indicate that the method is fairly rugged.

Table5: Day to Day Variability According to Area

	Day I			Day II		
	Enalapril maleate	**paracetamol**	**Hydrochlorothiazide**	**Enalapril maleate**	**Paracetamol**	**Hydrochlorothiazide**
Area	2701994	2610290	8985659	2710263	2591452	8942563
SD	26740	15843	19611	24563	18562	13512
RSD%	0.989	0.606	0.218	0.9062	0.716	0.151

Table6: Day to Day Variability According to Retention Tim

	Day I			Day II		
	Enalapril maleate	**Paracetamol**	**Hydrochlorothiazide**	**Enalapril maleate**	**Paracetamol**	**Hydrochlorothiazide**
Rt	1.770	3.628	5.889	1.746	3.599	5.876
SD	0.060	0.039	0.278	0.0447	0.0394	0.2735
RSD%	3.389	1.074	4.720	2.560	1.094	4.654

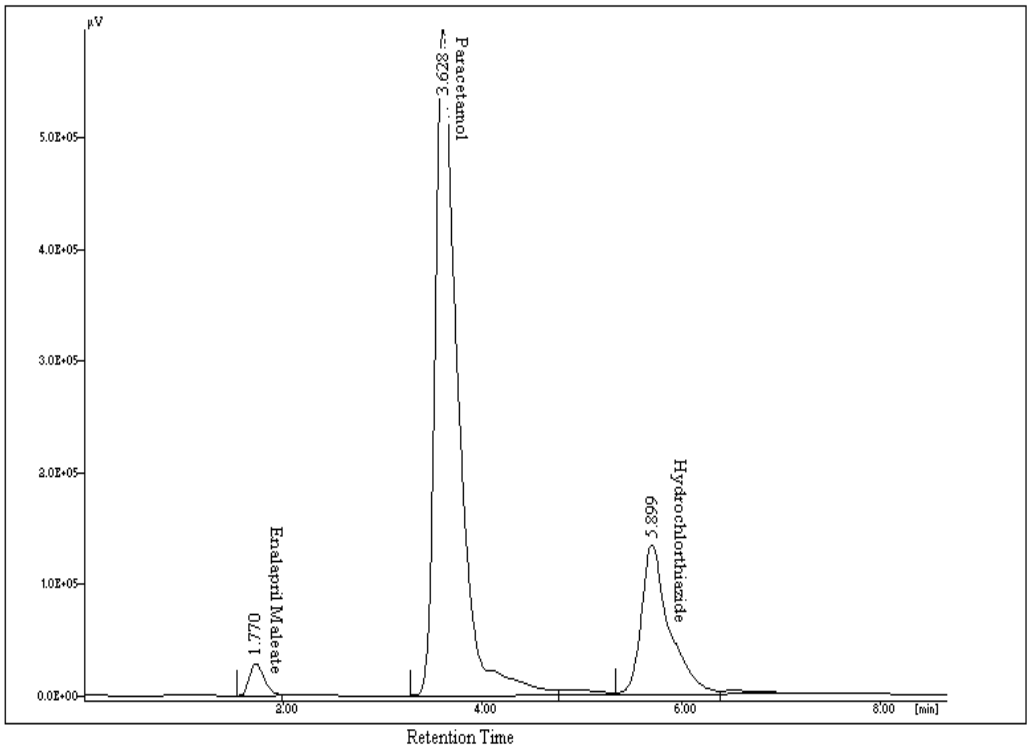

Figure 3: chromatogram of tablet formulation of Enalapril maleate, paracetemol and Hydrochlorothiazide.

Analysis of tablet formulation:

Ten tablets were weighed accurately and powdered. Powder equivalent to 10 mg EM, 25mg HCT and 325mg paracetamol was weighed and transferred to a 100ml volumetric flask. It was dissolved in 60ml mobile phase by shaking the flask for 10min and sonicated for 10 minutes. Then the volume was adjusted up to the mark with the same solvent and mixed well. Then it was first filtered through a 0.45µm whatmman filter paper and then with 0.2µ disk filter. A final concentration of 10µg/ML of EM, 25µg/mL of HCT and 325µg/mL of paracetamol were prepared and concentration of EM, HCT and Paracetamol were calculated from the calibration graph.

Table 7:- Analysis data of tablet formulation

Drug	Lable claim (mg/tab)	Amount found* (mg/tab)	Lable claim (%)	SD*	%RSD
Enalapril maleate	10	10.15	101.50	0.621	0.6126
Paracetamol	325	328.12	100.96	0.673	0.6667
Hydrochlorothiazide	25	25.55	102.20	0.700	0.6849

**Average of six estimation of tablet formulation.*

CONCLUSION

The developed method is suitable for the identification and quantification of the ternary combination of Enalapril maleate, hydrochlorothiazide, and paracetamol.

A high percentage of recovery shows that the method can be successfully used on a routine basis. The proposed method is simple, sensitive, rapid, specific and could be applied for quality and stability monitoring of Enalapril maleate, hydrochlorthazide,paracetamol combinations.

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