RP-HPLC Method for Simultaneous Estimation of Enalapril Maleate and Chlorthalidone in Synthetic Mixture

VISHWA M. DAVE[1], Dr. DILIP G. MAHESHWARI[*].

*Head of Department, Department of Quality Assurance,
L. J. Institute Of Pharmacy, S.G.Highway, Ahmedabad, Gujarat-382210.
E-Mail:-dgmaheshwari@gmail.com
Phone No:- 9824254740

ABSTRACT

A simple, accurate, precise and rapid RP-HPLC method has been developed for simultaneous estimation of Enalapril Maleate and Chlorthalidone in synthetic mixture. The mobile phase used consisting of Phosphate buffer: Acetonitrile: Methanol (65:25:10 v/v/v) was used at the flow rate of 1.0 mL/min and detection was carried out at 210 nm. The separation was achieved using Hypersil BDS C_{18} (250 x 4.6mm, 5 µm). Linearity was obtained in the range of 5-15 µg/ml and 12.5-37.5 µg/ml for Enalapril Maleate and Chlorthalidone respectively with R^2 value 0f 0.999 for Enalapril Maleate and 0.998 for Chlorthalidone. Retention time for Enalapril Maleate and Chlorthalidone is 7.749 and 4.247 respectively. The recovery studies were found in between 99.83-100.88 for Enalapril Maleate and for Chlorthalidone 99.33-101.11 with relative standard deviation less than 2. The method was validated as per the ICH guidelines Q2(R1).

Key words: Enalapril Maleate, Chlorthalidone, RP-HPLC method

INTRODUCTION

Enalapril belongs to a class of medications called angiotensin converting enzyme inhibitors. Normally angiotensin I is converted to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II constricts blood vessels, increasing blood pressure. By inhibiting ACE, Enalapril decreases levels of angiotensin II leading to less vasoconstriction and decreased blood pressure.

Chlorthalidone is an oral diuretic and inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, Chlorthalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism.

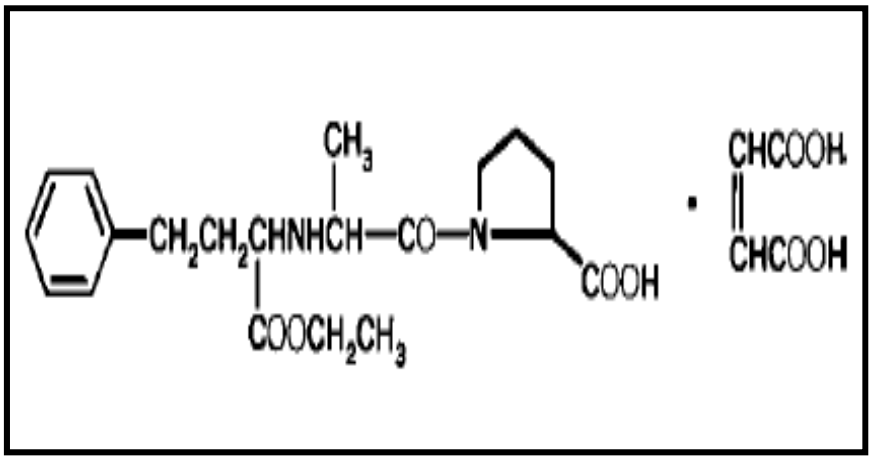

Fig 1: Structure of Enalapril Maleate

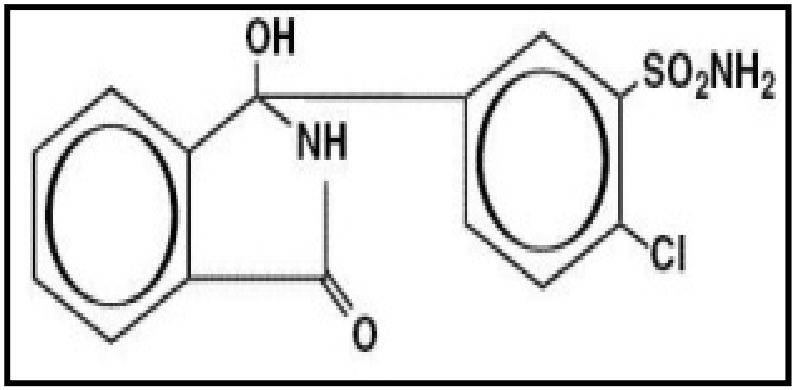

Fig 2: Structure of Chlorthalidone

The combination of Chlorthalidone and Enalapril enhanced antihypertensive effect of Enalapril than using Enalapril individually alone. This combination offers synergistic anti-hypertensive effects and minimizes the sympathetic activity thus decreases Heart rate and contractility over the mono therapy of individual drug. This combination has significantly achieved blood pressure goal in more number of patients.

Literature review reveals that, various methods are reported for the analysis of individual drug and in combination with other drug but no methods were reported for selective estimation of these two drugs in combined dosage form.

Therefore, it was thought worthwhile to develop analytical methods for analysis of Enalapril Maleate and Chlorthalidone in Synthetic mixture.

MATERIALS AND METHODS:

CHROMATOGRAPHIC CONDITIONS:

- **Model:** Waters e2695 Separation (Aliance) Module
- **Stationary phase:** Hypersil BDS C18 (250 x 4.6mm, 5 µm)
- **Detector:** UV detector LC 20AD
- **Software:** Empower 3
- **Mobile phase: Phosphate buffer: Acetonitrile: Methanol (65:25:10)**
- **Flow rate:** 1.0 ml/min
- **Injection volume:** Rhenodyne valve with 20µl fixed loop
- **Column Temperature:** 50°C
- **Sample Temperature**: 25°C
- **Wavelength:** 210 nm

CHEMICALS AND MATERIALS:

- Enalapril maleate (Cadila pharmaceuticals limited)
- Chlorthalidone (Cadila pharmaceuticals limited)
- Water (HPLC grade-Milli-Q)
- Acetonitrile (HPLC grade – RENKEM chemicals)
- Methanol (HPLC grade - Spectrochem chemicals)
- Ortho Phosphoric acid (HPLC grade – RENKEM chemicals)
- Potassium dihydrogen ortho phosphate AR (Cadila pharmaceuticals)
- Malic acid (Cadila pharmaceuticals)

PREPARATION OF MOBILE PHASE:

The mobile phase was prepared by mixing of 65 volumes of Phosphate Buffer adjusted pH 3 with ortho phosphoric acid and 25 volume of Acetonitrile and 10 volume of Methanol and sonicate for 20 minutes

PREPARATION OF CALIBRATION CURVE

An aliquots of standard binary stock solution of Enalapril (500 µg/ml) and Chlorthalidone (1250 µg/ml) take 1ml, 1.5ml, 2ml, 2.5ml, 3ml in a 100 ml volumetric flask which gives 5-15 µg/ml of Enalapril and 12.5-37.5 µg/ml of Chlorthalidone. The chromatogram was recorded under the finalized chromatographic conditions. Peak areas were recorded for all the peaks. Calibration curves of Enalapril and Chlorthalidone were constructed by plotting the peak area versus concentration (µg/ml) respectively

VALIDATION PARAMETERS

- **Linearity and range**

The Linearity was obtained in the range of 5-15 µg/ml and 12.5-37.5 µg/ml for Enalapril Maleate and Chlorthalidone respectively. Each concentration was repeated for the five times. A calibration curve is plotted against the peak area versus concentration and the regression line equation is obtained. The slope and intercept should be determined and %RSD is calculated.

- **Precision**

Repeatability

The concentration of 10 µg/ml of Enalapril Maleate and 25 µg/ml of Chlorthalidone was repeated for six times and the % RSD was determined.

Intraday

Three different concentrations of Enalapril Maleate (5, 10, 15 µg/ml) and of Chlorthalidone (12.5, 25, 37.5 µg/ml) was analyzed for three times at different time interval on the same day and the % RSD was determined.

Interday

Three different concentrations of Enalapril Maleate (5, 10, 15 µg/ml) and of Chlorthalidone (12.5, 25, 37.5 µg/ml) was analyzed at different 3 days and the % RSD was determined.

- **Accuracy**

Accuracy study was carried out by the recovery study. Recovery studies were carried out at three different levels 80, 100 and 120%. Different amount of standard solutions are added to the known amount of solution.

- **Limit of detection and Limit of quantification**

Limit of detection and Limit of quantification were measured by the following equation as follows:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the Intercept and S = slope of the calibration curve.

RESULTS :

A simple, precise, and accurate RP-HPLC method has been developed for simultaneous estimation of Enalapril Maleate and Chlorthalidone in synthetic mixture. Linearity was found to be 5-15 µg/ml for Enalapril Maleate and for Chlorthalidone it was found to be 12.5- 37.5 µg/ml with R2 Value 0.999 and 0.998 for Enalapril Maleate and Chlorthalidone respectively. Precision was found to be less than 2% relative standard deviation (RSD). Accuracy was found to be in the range of 99.83-100.88 and 99.33-101.11 for Enalapril Maleate and Chlorthalidone respectively. LOD was found to be 0.058 and 0.047 for Enalapril Maleate and Chlorthalidone respectively LOQ was found to be 0.178 and 0.143 for Enalapril Maleate and Chlorthalidone respectively.

CONCLUSION:

The simple, precise and accurate RP-HPLC Method has been developed for Simultaneous estimation of Enalapril Maleate and Chlorthalidone in synthetic mixture. This method can be routinely applied for the quality control analysis of Enalapril Maleate and Chlorthalidone.

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TABLES

Linearity data for Enalapril Maleate and Chlorthalidone

Enalapril maleate			**Chlorthalidone**		
Concentration (µg/ml)	**Peak area ± SD**	**%RSD**	**Concentration (µg/ml)**	**Peak area ± SD**	**%RSD**
5	875.888±2.316	0.264	12.5	1104.48±3.299	0.298
7.5	1315.744±1.597	0.121	18.75	1494.234±1.983	0.132
10	1766.028±2.050	0.116	25	2016.81±3.520	0.174
12.5	2205.342±2.631	0.119	31.25	2548.2±4.071	0.159
15	2656.45±2.769	0.104	37.5	3036.744±3.762	0.123

Intraday Precision for Enalapril Maleate and Chlorthalidone

Enalapril maleate			**Chlorthalidone**		
Concentration (µg/ml)	**Peak area ± SD**	**% RSD**	**Concentration (µg/ml)**	**Peak area ± SD**	**% RSD**
5	879.87±1.196	0.136	12.5	1103.09±1.480	0.134
10	1766.03±2.466	0.139	25	2021.853±2.785	0.137
15	2659.77±3.203	0.120	37.5	3039.877±3.296	0.108

Interday Precision for Enalapril Maleate and Chlorthalidone

Enalapril maleate			**Chlorthalidone**		
Concentration (µg/ml)	**Peak area ± SD**	**% RSD**	**Concentration (µg/ml)**	**Peak area ± SD**	**% RSD**
5	874.703±2.630	0.300	12.5	1098.277±2.021	0.184
10	1759.543±3.525	0.200	25	2014.177±3.097	0.153
15	2657.84±4.281	0.161	37.5	3031.44±4.631	0.152

Repeatability for Enalapril Maleate and Chlorthalidone

Enalapril maleate		Chlorthalidone	
Conc. (µg/ml)	**Peak area(mV.s)**	**Conc. (µg/ml)**	**Peak area(mV.s)**
10	1766.03	25	2016.81
10	1766.125	25	2016.325
10	1766.251	25	2016.254
10	1766.547	25	2016.457
10	1766.425	25	2016.145
10	1766.086	25	2016.79
Mean area	1766.244	**Mean area**	2016.464
S.D. (n=6)	0.18731	**S.D. (n=6)**	0.25535
%RSD	0.0106	**%RSD**	0.0126

Accuracy data for Enalapril

Level of concentration	Amount taken (µg/ml)	Amount spiked (µg/ml)	Amount Found (µg/ml)	% Recovery ± S.D	% R.S.D
80	5	4	8.98	99.83±0.251	0.2519
100	5	5	10.03	100.37±0.502	0.5009
120	5	6	11.09	100.88±0.328	0.3254

Accuracy data for Chlorthalidone

Level of concentration	Amount taken (µg/ml)	Amount spiked (µg/ml)	Amount Found (µg/ml)	% Recovery ± S.D	% R.S.D
80	12.5	10	22.34	99.33±0.386	0.389
100	12.5	12.5	24.85	99.43±1.132	1.133
120	12.5	15	27.80	101.11±0.547	0.541

Robustness

By changing mobile phase composition

Change in mobile phase composition	Enalapril			Chlorthalidone		
	Amount taken (µg/ml)	Amount found (µg/ml)	% Assay	Amount taken (µg/ml)	Amount found (µg/ml)	% Assay
75:15:10	10	9.52	95.2	25	24.8	99.2
65:25:10	10	10.2	100.02	25	25.3	101.2

By changing Wavelength

Change in wavelength (nm)	Enalapril			Chlorthalidone		
	Amount taken (µg/ml)	Amount found (µg/ml)	% Assay	Amount taken (µg/ml)	Amount found (µg/ml)	% Assay
208	5	4.93	98.6	25	24.76	98.4
210	5	4.90	98.0.	25	25.02	100.08
212	5	4.94	98.8	25	24.90	99.6

Application to Synthetic Mixture

Enalapril maleate			Chlorthalidone		
Concentration (µg/ml)	**Amount found (µg/ml)**	**% Assay ± SD (n=3)**	**Concentration (µg/ml)**	**Amount found (µg/ml)**	**% Assay ± SD (n=3)**
10	10.04	100.40	25	24.70	98.83

FIGURES

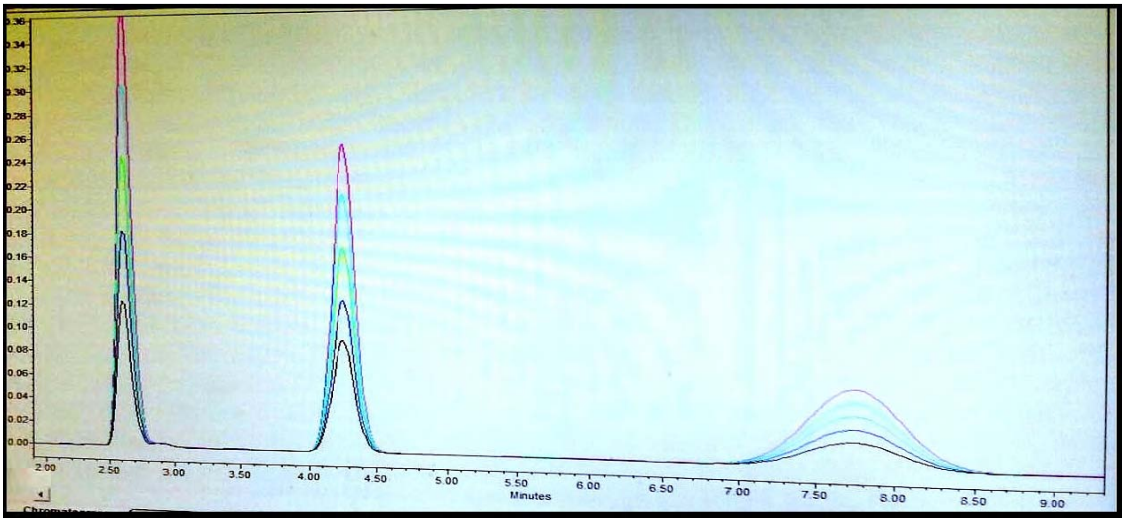

Fig. 1: Chromatogram of calibration curve for Enalapril maleate (5-15 µg/ml) and Chlorthalidone (12.5-37.5 µg/ml)

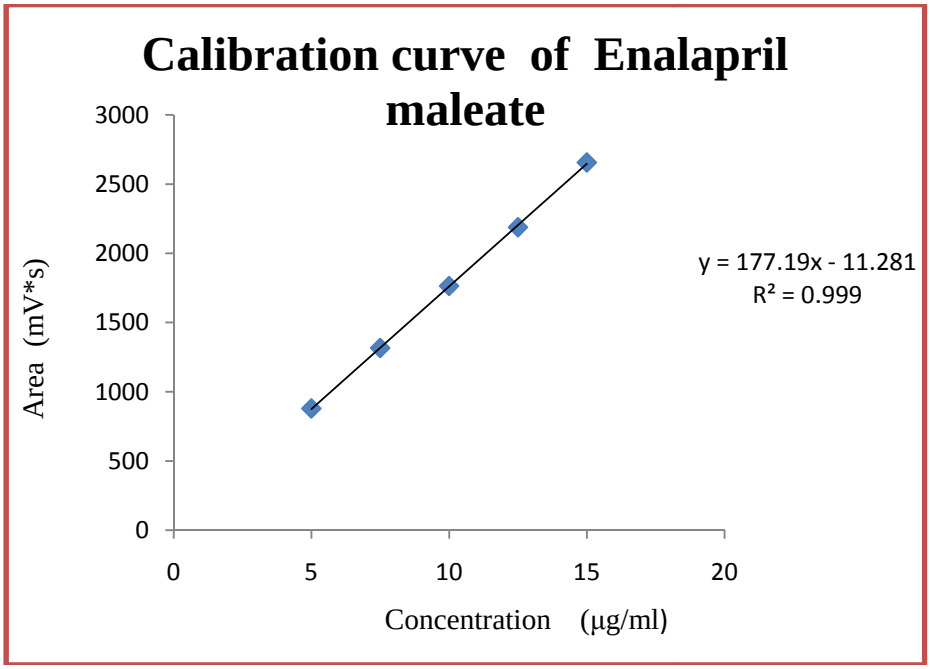

Fig. 2: Calibration curve for Enalapril Maleate (5-15µg/ml)

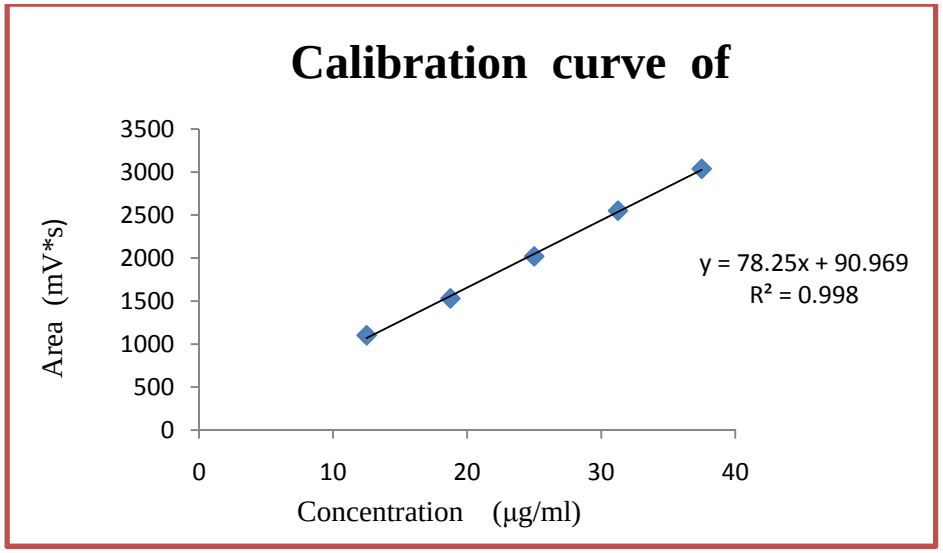

Fig: 3: Calibration curve for Chlorthalidone (12.5-37.5µg/ml)

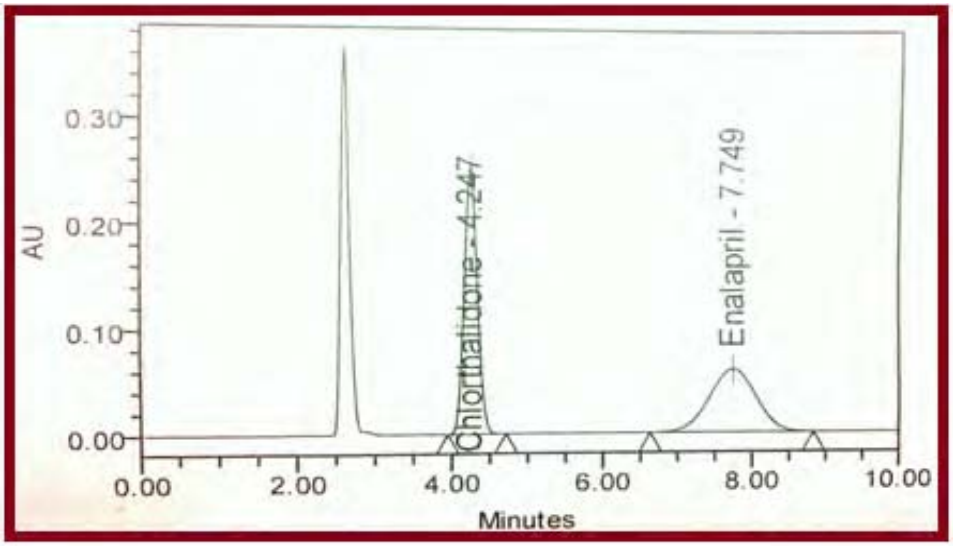

Figure 4 : Chromatogram of Enalapril maleate (10 µg/ml) and Chlorthalidone (25µg/ml)

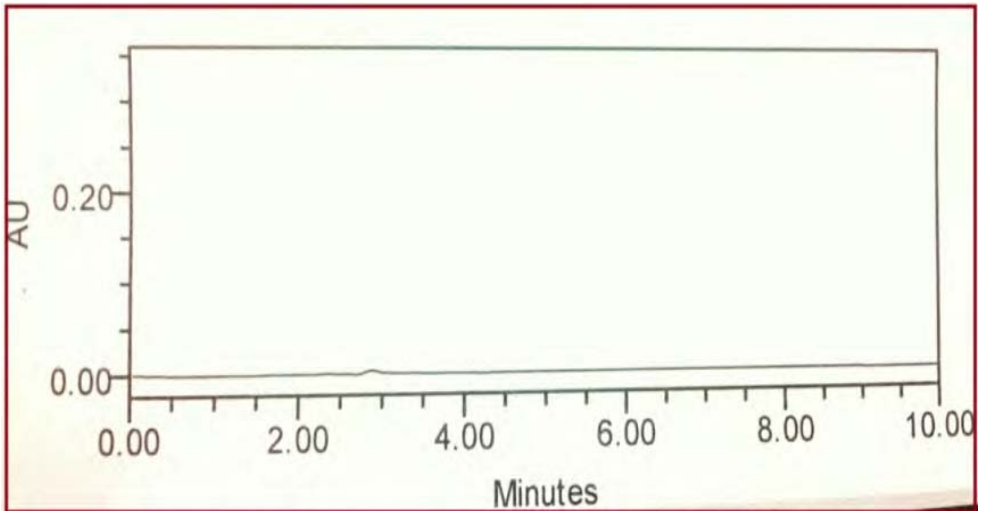

Fig. 5: Specificity Chromatogram of Blank (Mobile phase)

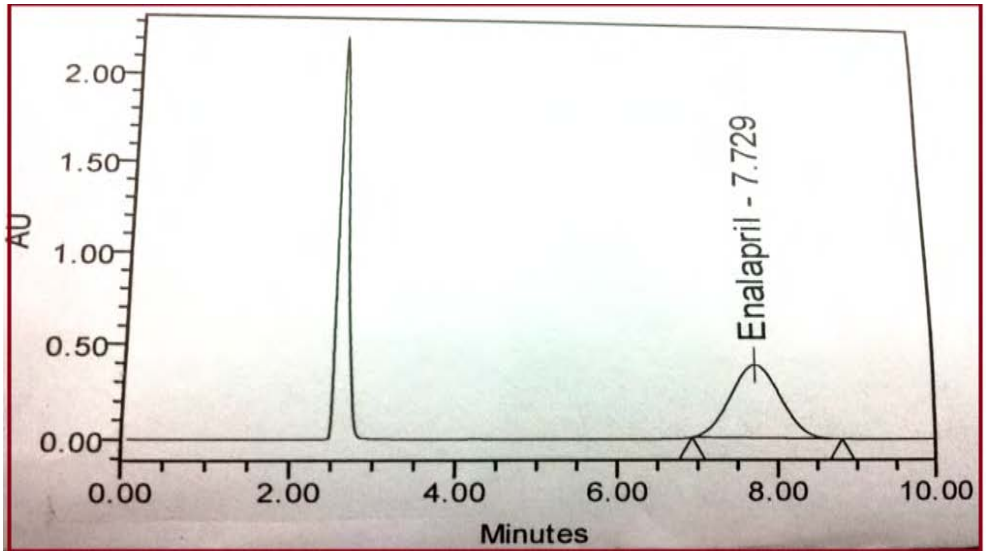

Fig. 6: specificity Chromatogram of standard Enalapril

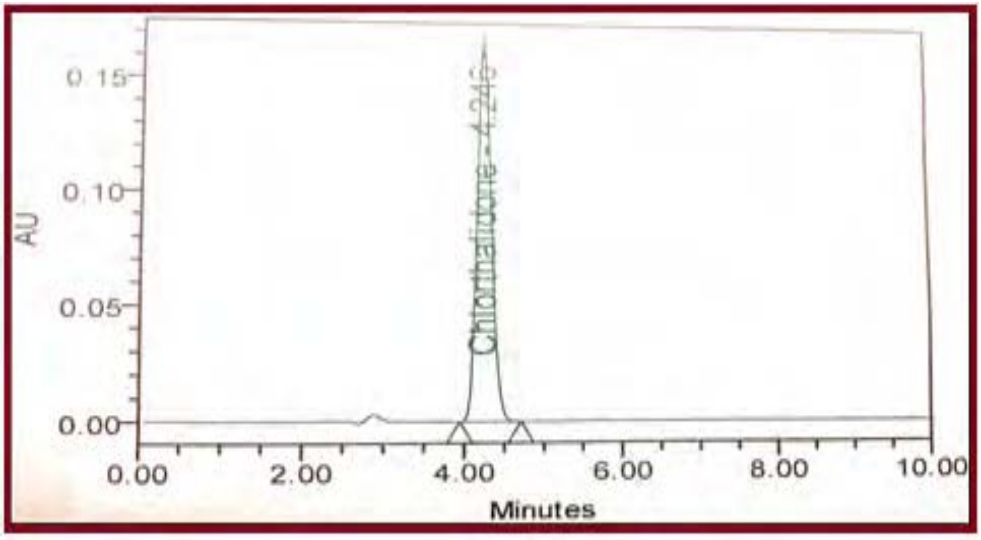

Fig. 7: specificity Chromatogram of standard Chlorthalidone

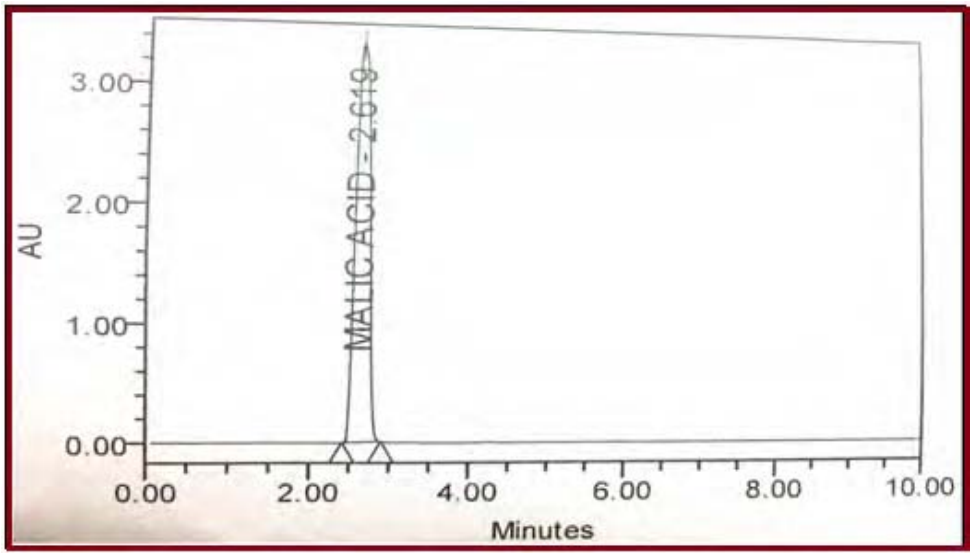

Fig. 8: specificity Chromatogram of standard Maleic acid