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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ASSAY AND RELATED SUSBSTANCES OF ENALAPRIL MALEATE TABLETS

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ABSTRACT

A simple, sensitive, linear, accurate and precise gradient RP-HPLC method was developed and validated for estimation of Enalapril Maleate along with its Impurities (mainly Enalaprilat and Diketopiperazine) in commercial tablet dosage form. The compounds were well separated using Gradient elution mode by using Platinum EPS C8 (250 × 4.6 mm)5µ or equivalent column by using a 2.2 pH buffer : ACN (715:285) as mobile phase A and 2.2 pH buffer : ACN (720:280) as mobile phase B with flow rate of 1.5 ml/min using detection wavelength 215nm. Retention time for Enalapril Maleate, Enalaprilat and Diketopiperazine found to be 6.59, 2.44 & 8.33 respectively. The study showed that the reverse phase liquid chromatography is sensitive and selective for detecting Enalapril Maleate along with its Impurities using Gradient mobile phase.

KEY WORDS

Enalapril Maleate, Enalaprilat, Diketopiperazine, RP-HPLC.

INTRODUCTION:

Reversed-Phase Chromatography is the reverse of Normal-Phase Chromatography in the sense that it involves the use of a non-polar stationary phase and a polar mobile phase[1]. As a result, a decrease in the polarity of the mobile phase results in a decrease in solute retention[2]. Modern Reversed-Phase Chromatography typically refers to the use of chemically bonded stationary phases, where a functional group is bonded to silica, for this reason, Reversed-Phase[3]. Polymeric stationary phases such as polymethacrylate or polystyrene[4], or solid stationary phases such as porous graphitic carbon, are used[5].

Spectrophotometric and chromatographic methods are reported for estimation of Enalapril Maleate in combination with other drugs[6,7,8]. However, there are very few reports yet for determination of Enalapril Maleate alone, with its main impurities by proposed method. The aim of the present work was the development of a Stability indicating RP-HPLC method for estimation of Enalapril Maleate along with its impurities (Enalaprilat and Diketopiperazine) and its validation according to ICH guidelines.

Enalapril Maleate (Fig.1) is an Angiotensin-converting enzyme inhibitor. (ACE)and its Chemical name is :(Z)-but-2-enedioicacid;(2S)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4-phenyl butan-2-yl] amino] propanoyl] pyrrolidine-2-carboxylic acid [9].

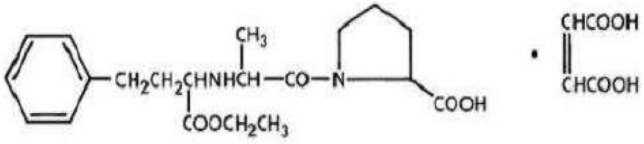


Fig.1: structure of Enalapril Maleate

MATERIALS AND METHODS:

Instrumentation:

An HPLC instrument equipped with a UV-Visible detector (Agilent :1200), weighing balance Sartorius & SE2), P^H meter (TEC: Orion 2 star), Sonicator (PCI Analytical;40LHDTC),

Chemicals and Reagents:

Monobasic Sodium phosphate (AR grade), Methanol (HPLC grade), Acetonitrile (HPLC grade), Orthophosphoric acid (AR grade), Hydrochloric acid (6%) (AR grade), Water (Milli-Q-grade).

Chromatographic Conditions:

Column**:** Platinum EPS C8 (250 × 4.6 mm)5µ, flow rate of 1.5 ml/min at detector wavelength of 215 nm and injection volume of 20 µl at column oven temperature of 65 ^{0}C and at runtime of 35mins of gradient pump mode.

Preparation of Solutions:

Preparation of dilute orthophophosphoric acid (10%v/v): Dilute 10ml of orthophosphoric acid to 100ml with water.

Preparation of Buffer: Dissolved 1.38g of monobasic sodium phosphate in 1000mL water and pH was adjusted to 2.2 with OPA.

Preparation of Mobile phase-A: 715mL buffer mixed with 285mL of Acetonitrile.

Preparation of Mobile phase-B: 720mL buffer mixed with 280mL of Acetonitrile.

Preparation of Diluent: Buffer used as Diluent

Preparation of Enalapril diketopiperazine solution: Weighed accurately 20 mg of Enalapril Maleate standard and transferred into a 100 mL beaker to form a mound on the bottom of the beaker. Place the beaker on a hot plate and set the temperature to 60°C to melt the solid. When melting is observed (after 5-10 min of heating), immediately removed the beaker from the hot plate, and allowed it to cool. Added 50 mL of acetonitrile to the cooled residue in the beaker and sonicated for a few minutes to dissolve the residue.

Note: Avoid overheating beyond the melting initially observed to prevent heat-induced degradation, which would give rise to a brown color.

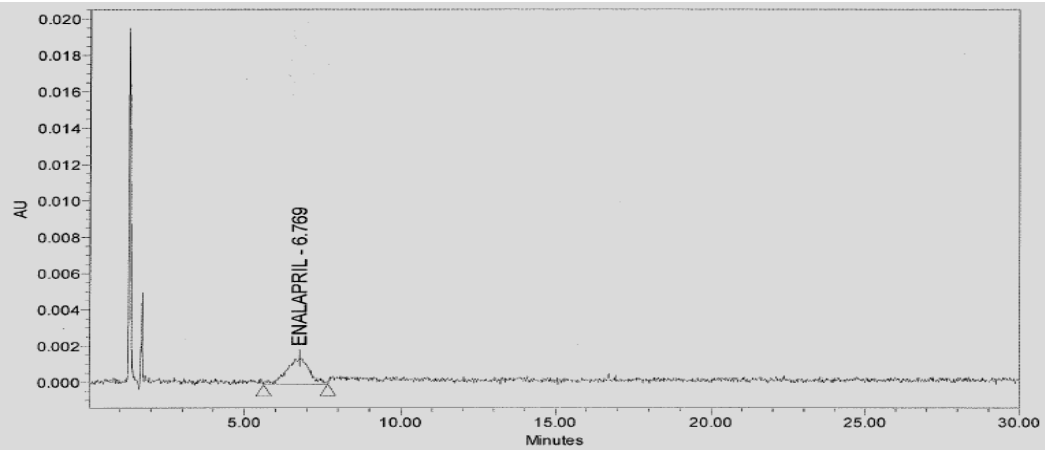
Preparation of Enalaprilat stock solution: Weighed accurately 4mg of Enalaprilat standard into 10mL volumetric flask, dissolve in water and diluted to mark with water, mixed.

Preparation of standard stock solution: Weighed accurately 40 mg of Enalapril Maleate standard into 200 mL volumetric flask, added 100 mL of diluent and sonicated to dissolve. Pipetted 1mL of Enalaprilat stock solution to this solution and diluted to mark with diluent, mix.

Preparation of standard solution: Diluted 1mL of standard stock solution to 100 mL with diluent. (Fig.No.2.1, 2.2 & Table No.1)

Table No 1: Peak results for standard solution

	Name	RT	Area	RRT	USP Resolution	USP Tailing
1	Enalaprilat	2.268	3456	0.42	3.16	1.3
2	Maleic acid	1.368	2876	0.7	1.34	1.1
3	Enalapril	6.599	734	-	-	-



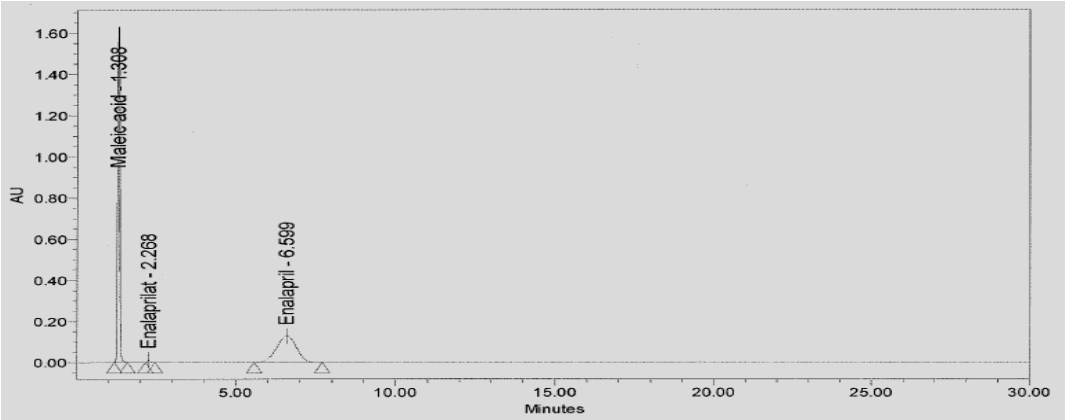


Fig No.2.1 & 2.2: Chromatogram for RS & Assay standard solutions of enalapril Maleate

Preparation of Sample solution: Transferred 10 tablets into a 500 mL volumetric flask. Added 350mL of diluent, sonicated for about 15 minutes, and shake by mechanical means for 30 minutes. Diluted with diluent to volume, shake well, and sonicated for another 15 minutes. Pass the solution through a suitable filter of 0.45µ Nylon filter, and discard the first 2 mL portion of the filtrate. (Concentration of about 0.25mg/mL). (Fig.No.3 and Table No.2)

Procedure: Equilibrate the column for not less than 30 min with initial mobile phase composition at a flow rate of 2.0 ml/min. separately inject 20 micro liter of Blank, standard solution (five injections) and sample solution into the chromatographic system. Record the chromatograms and measure the peak response.

Table no.3: peak results for sample solutions

	Name	Rt	Area	RR_t	USP Resolution	USP Tailing
1	Enalaprilat	2.251	3456	0.42	3.16	1.3
2	Maleic acid	1.392	2876	0.7	1.34	1.1
3	Enalapril	6.497	734	-	-	-

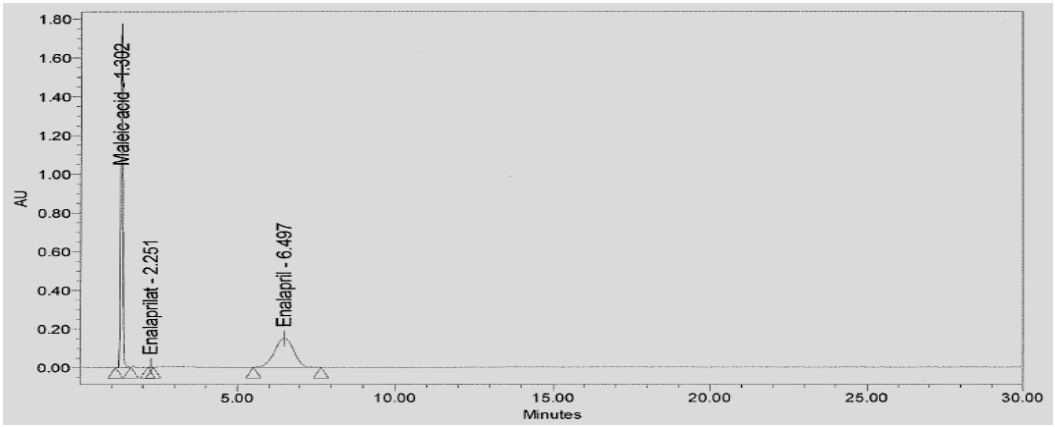


Fig No.3: Chromatogram for sample solutions of Enalapril Maleate

Determination of analytical wavelength:

The detection wavelength of 215.0 nm was selected as drug have good absorbance at that wavelength

Method validation:

According to ICH guidelines the method was validated for parameters such as system suitability, accuracy, precision, linearity, robustness, ruggedness specificity and LOD and LOQ[10].

1. System suitability:

The standard solutions of Enalapril Maleate were prepared and injected 5 replicates as per test method and injected into HPLC system. The system suitability parameters like Retention time, Peak area, USP Plate

count, USP tailing factor were evaluated as per the test method and checked for acceptable limits (Table No.4).

Table No.4: Results of System suitability data of Enalapril Maleate.

S. No	Parameters	Results	Acceptance Criteria
1	USP Plate count	17122	NLT 1000
2	USP Tailing	1.0	NMT 2%

2. Accuracy:

Accuracy of the test method was carried out by spiking known amounts of drug substance of Enalapril Maleate with placebo at 50%, 100% and 150% of target concentration in triplicate for each level. Calculated amount recovery, % recovery, mean % recovery and % RSD at each level and the results were checked for acceptable limits**.**

3. Linearity:

Linearity was performed by preparing Enalapril Maleate standard solution in the range of about 25-225% (0.1816-15.1350 ppm for Enalapril Maleate) of test concentration and injected into the HPLC system. Chromatograms were recorded and measured the peak responses.

Linearity of detector response was established by plotting a graph between concentration and response of Enalapril Maleate. The squared correlation coefficient was calculated.

4. Precision:

To evaluate the precision for assay and RS method, six samples of each Enalapril maleate were prepared and analyzed as per test method. % assay and RSD of six samples are calculated.

5. Specificity:

5.1) Blank interference

Blank was prepared and injected as per test method and it was analyzed for any interference with analytical peaks of Enalapril maleate at their Retention times.

5.2) Placebo interference

Placebo sample was prepared by taking the placebo equivalent to about the weight in portion of test preparation and injected into the HPLC system. No peak should be found at the retention time of Enalapril maleate.

5.3) Impurity interference

All the related compounds are prepared at 1.0% level and injected into the HPLC system and recorded the chromatograms. Impurity solutions of Enalaprilat, Enalapril Diketopiperazine, Cyclo hexyl phenyl alanine, Maleic acid were prepared and injected in chromatographic system and the chromatograms were recorded. No peak should be found at the retention time of Enalapril Maleate with the above prepared samples.

6. Limit of Quantification:

Preparation of LOQ solution:

25 % of Linearity solution

Procedure:

Blank and LOQ solutions were injected into the chromatographic system to determine limit of quantification

7. Limit of Detection:

LOD of each individual impurity was determined by establishing the minimum level at which the analyte can be reliably detected.

Preparation of LOD solution:

3.3ml of LOQ solution was taken into clean dry 10 ml volumetric flask and make up the volume with diluent.

Procedure:

Blank and LOD solutions were injected into the chromatographic system to determine limit of detection.

8. Robustness:

8.1) Effect of variation in flow rate:

A study was conducted to determine the effect of variation in flow rate. The system suitability parameters were evaluated at the flow rate of 1.8 ml/min and 2.2 ml/min. the system suitability results were checked for limits of higher and lower flow rates.

8.2) Effect **of variation in column oven temperature:**

A study was conducted to determine the effect of variation in column oven temperature. The system suitability parameters were evaluated at 60^0C and 70^0C column oven temperatures

8.3) Effect of variation in pH of Buffer in Mobile phase:

A study to establish the Effect of variation in pH of Buffer in mobile phase was conducted. Mobile phases were prepared with Buffer having different pH between 2.2 and 2,4. System suitability parameters were evaluated by using the above mobile phases.

The Robustness of the method with respect to effects of variations in column oven temperature, in flow rates, in pH of buffer in Mobile phase are analyzed for USP plate counting, USP tailing, Retention time, peak area with sample solutions of Enalapril Maleate.

8.4) Effect of variation in Mobile phase composition:

A study to establish the Effect of variation in mobile phase composition was conducted. Mobile phases were prepared with 82.5:17.5%v/v and 67.5:32.5%v/v. System suitability parameters were evaluated by using the above mobile phases.

9. Stress degradation studies:

Stress degradation studies were conducted in acid, base, peroxide, Thermal, photolytic, Water and homogeneity of the peak was assessed in terms of peak purity.

All the related compounds are spiked at 1.0% level with the assay sample preparation, injected into the HPLC system and homogeneity of the peak was assessed in terms of peak purity.

Peak purities of Enalapril Maleate, Enalaprilat, Enalapril Diketopiperazine were analyzed and purity angle and purity threshold are spiked for all the stress conditions-control sample, spiked sample,.1N HCl_60°C for 2 hrs, 1N NaOH_60°C for 2 hrs, 0.1% H2O2_on BT for

5 hrs, 105°C for 1 day, 90% RH for 7 days, 200-watt hours/m^2 and 1.2 million lux hours, 60°C for 2 hrs.

RESULTS AND DISCUSSIONS:

Several trials have made until getting good peak resolution, acceptable plate count and tailing factor. Method was optimized and the retention time was reported as 6.59, 2.44, 8.33 minutes for Enalaprilat, Enalapril Maleate and Diketopioperazine.

1. System suitability:

From the system suitability studies, it was observed that % RSD of peak area of Enalapril was found to be 0.3. Theoretical plates were found to be more than 1000. USP tailing factor of Enalapril was found to be 0.7. All the parameters were within the limits. (Table No.4)

2. Accuracy:

Accuracy for the average of triplicate from each concentration levels Enalapril were within 98.0 to 102.0 %, which shows that the method was accurate.

3. Precision:

The % RSD and Average % Assay of Enalapril were found to be 0.2 and 100.0 respectively, and the % RSD Hence the method is precise for the estimation of Enalapril Maleate with its impurities in enalapril maleate tablets.

4. Linearity:

From the Linearity data it was observed that the method was showing linearity in the concentration range of 25-225 % (Fig no.5-7). Correlation coefficient was found to be 1.000.

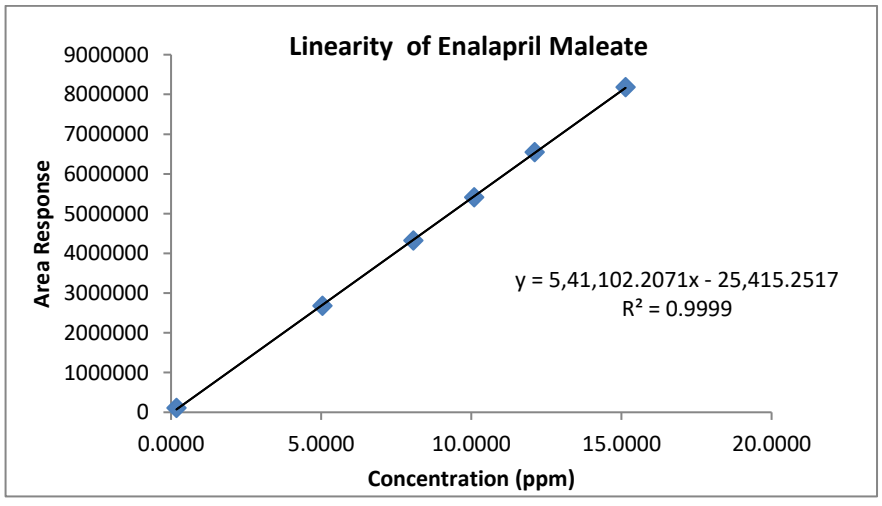


Fig No.5: Calibration graph for Linearity of Enalapril Maleate

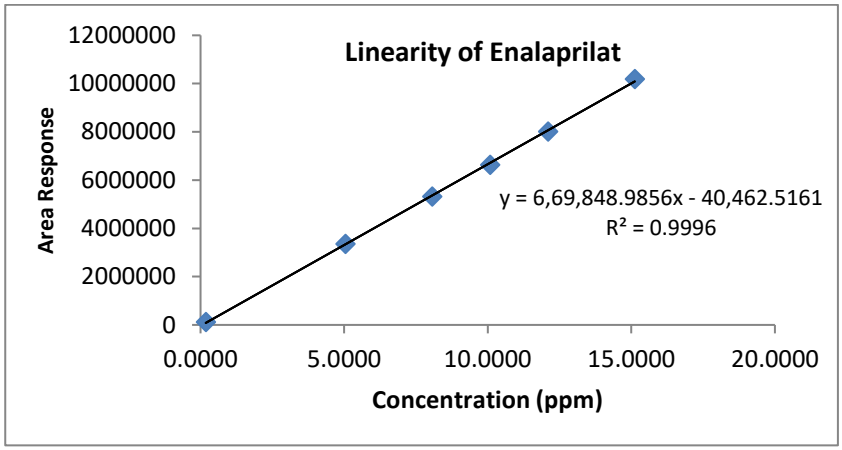


Fig No.6: Calibration curve for Linearity of Enalaprilat

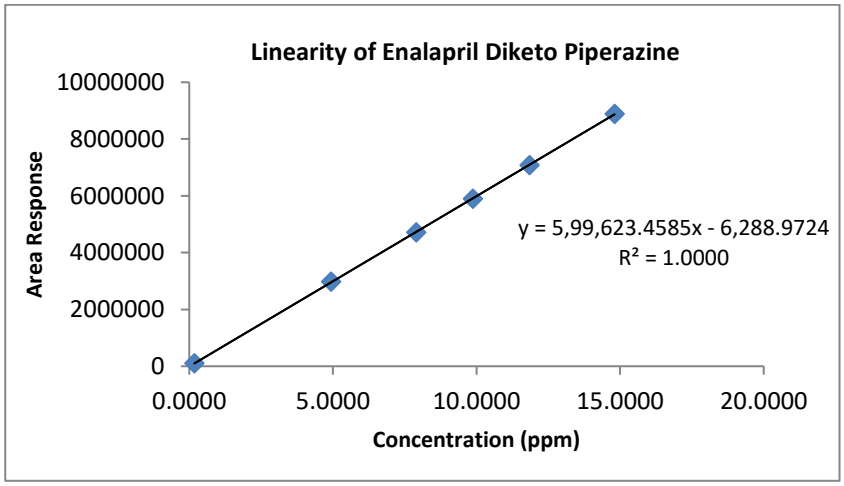


Fig No.7: Calibration curve for Linearity of Enalapril Diketopiperazine

5. Specificity:

The Chromatograms of Standard and Sample are identical with nearly same Retention time. No interference due to Placebo and Samples at the retention time of analyte which shows that the method was specific.

8. Robustness:

As the % RSD of retention time and asymmetry were within limits for variation in flow rate (± 0.2 of the specified flow). Hence the allowable flow rate should be within 1.8 ml to 2.2 ml.

And the influence of variations of mobile phase pH is ± 0.2 of the specified flow. Hence the allowable variation in mobile phase pH is 2.2 ± 0.2. The results for robustness of variations of column temperature were illustrated.

9. Limit of Detection:

The Limit of Detection was calculated from the linearity curve method using slope, and standard deviation of intercepts of calibration curve.

10. Limit of Quantification:

The Limit of Quantification was calculated from the linearity curve method using slope, and standard deviation of intercepts of calibration curve.

11. Stress Degradation Studies:

Purity angle is less than purity threshold for spiked sample and for all the stress conditions.

CONCLUSION:

A simple and precise RP-HPLC method was developed and validated as per ICH guidelines for estimation of enalapril with its impurities in Enalapril Maleate Tablets (5mg).

Good agreement was observed in the assay results of pharmaceutical formulation from developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Enalapril Maleate Tablets.

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