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## DEVELOPMENT AND VALIDATION OF A RP- HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ENALAPRIL MALEATE AND RAMIPRIL IN BULK AND TABLET DOSAGE FORM

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### ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Enalapril maleate and Ramipril in bulk and tablet dosage form. Chromatographic analysis was performed on an Oyster BDS C18 column (250x 4.6 mm, 5 $\mu$ m) column temperature 65 $^{\circ}$ C with a mixture of buffer A and buffer B in the ratio 50:50 [Buffer A preparation: 2gm of sodium perchloride in 800ml water and add 0.5 ml tri ethyl amine, adjust the pH to 3.6 $\pm$ 0.1 with phosphoric acid and add 200 ml of acetonitrile. Buffer B preparation: 2 gm of sodium perchloride in 300 ml water and add 0.5 ml tri ethyl amine, adjust the pH to 2.6 $\pm$ 0.1 with phosphoric acid and add 700 ml of acetonitrile] as mobile phase, at a flow rate of 1.0 mL min $^{-1}$ . UV detection was performed at 208 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Enalapril maleate and Ramipril were 4.197 and 5.819 min, respectively. Calibration plots were linear over the concentration ranges 5–30  $\mu$ g mL $^{-1}$  and 5–30  $\mu$ g mL $^{-1}$  for Enalapril maleate and Ramipril, respectively. The Limit of detection was 0.571 and 1.090  $\mu$ g mL $^{-1}$  and the quantification limit was 1.733  $\mu$ g mL $^{-1}$  and 3.303  $\mu$ g mL $^{-1}$  for Enalapril maleate and Ramipril, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98.06% to 100.47%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of Enalapril maleate and Ramipril in bulk and tablet dosage form.

**Keywords:** Enalapril maleate, Ramipril, RP-HPLC.

### INTRODUCTION

Enalapril is a prodrug which is hydrolysed in the body to Enalaprilat, which is an inhibitor of angiotensin-converting enzyme (ACE). It is indicated for treatment of hypertension, treatment of symptomatic heart failure and prevention of symptomatic heart failure in patients with asymptomatic left ventricular dysfunction (ejection fraction <35%) [1]. Chemically it is ((S)-1-{N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl}-L-proline, (Z)-2-butenedioate (1:1), a derivative of two amino-acids, L-alanine and L-proline (fig.1). It is a white to off-white crystalline, odourless powder which melts in the range of 143–144  $^{\circ}$ C [2]. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II, which stimulates aldosterone secretion by the adrenal cortex. Blocking the

conversion of the angiotensin I to the angiotensin II, leads to a reduction in vasopressin activity and a decrease in peripheral vascular resistance [3, 4]. Oral enalapril is rapidly absorbed, with peak serum concentrations of enalapril occurring within one hour. The extent of absorption of enalapril from oral enalapril tablet is approximately 60%. The absorption of oral enalapril is not influenced by the presence of food in the gastro-intestinal tract. The determination of enalapril has been carried out by numerous analytical methods such as HPLC [5–12], UV spectroscopic method [13].

Ramipril is chemically (2S, 3aS, 6aS)-1-[(2S)-2-[[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino] propanoyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid [14], (fig.2).

It is white or almost white crystalline powder. Freely soluble in Methanol, Slightly soluble in Water. Which melts in the range of 109 °C . It is an angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure It is a prodrug and is converted to the active metabolite ramiprilat by liver esterase enzymes. It is an official drug in IP [15] and BP [16]. There are very few analytical methods reported for the estimation of Ramipril which includes RP-HPLC [13-24]. But these methods are sophisticated, expensive and time consuming when compared to simple HPLC method. There is need for a interest to develop simple, accurate, specific, sensitive, precise and reproducible simultaneous RP-HPLC method for the estimation of Enalapril maleate and Ramipril in bulk and its formulation.

## MATERIALS AND METHODS

Pure standard of Enalapril maleate and Ramipril (Assigned purity 99.98%) was obtained as a gift sample from Hetero labs Pvt. Ltd, Baddi, India. The gift samples were used as standard without further purification. HPLC grade water, methanol (Qualigens), sodium per chloride, phosphoric acid and tri ethyl amine (S.D. fine chemicals, Mumbai, India), were used throughout the experiment. Commercial pharmaceutical preparation (ENVAS RB (Enalapril, Ramipril), VOLTA (CADILA)) which was claimed to contain 10mg of Enalapril maleate and 10 mg of Ramipril is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies. HPLC grade Acetonitrile from Merck specialties Pvt Ltd, Mumbai. Water HPLC grade was obtained from Rankem laboratories.

### Instrumentation and Chromatographic Conditions

High performance liquid chromatograph, HPLC (WATERS 2695), UV-VIS detector was used. Isocratic elution of mobile phase comprising of Chromatographic analysis was performed on a Oyster BDS C18 column (250x 4.6 mm, 5µm) column temperature 65°C, with a mixture of buffer A and buffer B in the ratio 50:50 [Buffer A preparation: 2 gm of sodium perchloride in 800ml water and add 0.5 ml tri ethyl amine, adjust the pH to 3.6±0.1 with phosphoric acid and add 200 ml of acetonitrile. Buffer B preparation: 2gm of sodium perchloride in 300 ml water and add 0.5 ml tri ethyl amine, adjust the pH to 2.6±0.1 with phosphoric acid and add 700 ml of acetonitrile] as mobile phase, at a flow rate of 1.0 mL min<sup>-1</sup>. UV detection was performed at 208 nm. The retention times of Enalapril maleate and Ramipril were 4.197 and 5.819 min. The column temperature was maintained at ambient and the volume of injection was 20 µL. Prior to injection of analyte, the column was equilibrated for 30 min with mobile phase.

### Preparation of mobile phase

The HPLC grade solvents were used for the preparation of mobile phase, isocratic elution of mobile phase comprising of with a mixture of buffer A and buffer B in the ratio 50:50 [Buffer A preparation: 2 gm of sodium perchloride in 800ml water and add 0.5 ml tri ethyl amine, adjust the pH to 3.6±0.1 with phosphoric acid and add 200 ml of acetonitrile. Buffer B preparation: 2 gm of sodium perchloride in 300 ml water and add 0.5 ml tri ethyl amine, adjust the pH to 2.6±0.1 with phosphoric acid and add 700 ml of acetonitrile] as mobile phase and filtered before use through a 0.45µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 mL min<sup>-1</sup>.

### Standard solution

Standard stock solutions 1 mg mL<sup>-1</sup> of Enalapril maleate and Ramipril were prepared in mobile phase and further diluted in mobile phase. The working standard solutions were prepared in mobile phase to contain mixture of Enalapril maleate and Ramipril in over the linearity range from 5-30 µg mL<sup>-1</sup> and 5-30 µg mL<sup>-1</sup>.

### Assay in formulation

Twenty tablets each containing and their average weight was calculated. The tablet were crushed to furnish a homogeneous powder and a quantity equivalent to one tablet were weighed in to a 100 mL volumetric flask, dissolve in mobile phase, sonicated for about 15 min and then made up to volume with mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 mL volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 mL of mobile phase, and then the volume was completed to 100 mL with the same solvent. Further add mobile phase to obtain an expected concentration of 10 µg mL<sup>-1</sup> Enalapril maleate and 10 µg mL<sup>-1</sup> Ramipril. All determinations were conducted in triplicate.

## RESULTS AND DISCUSSION

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of Enalapril maleate and Ramipril were shown in (Fig.3). There was clear resolution between Enalapril maleate and Ramipril with retention time of 4.197 and 5.819 minutes, respectively.

### Linearity

The response was determined to be linear over the range of 5µg mL<sup>-1</sup> to 30µg mL<sup>-1</sup> (5, 10, 15, 20, 25, 30) for Enalapril maleate and 5- 30 µg mL<sup>-1</sup> (5, 10, 15, 20, 25, 30) for Ramipril. The solutions were injected into HPLC system. Each of the concentration was injected to get reproducible response. The run time was 15 min and the peak areas were measured (Table 1 & 2). The calibration

curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated by statistical study. They were represented by the linear regression equation (Fig 4 and 5 calibration curve).

$Y_{\text{Enalapril}} = 53087x - 98013$  Coefficient of correlation ( $r^2$ ) value = 0.999

$Y_{\text{Ramipril}} = 65905x - 16789$  Coefficient of correlation ( $r^2$ ) value = 0.999

### Accuracy

The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 50%, 100%, 150% wt of sample drug solution were pipetted into each of three volumetric flasks. To this 5ml of Enalapril standard drug solution of 200  $\mu\text{g mL}^{-1}$  was added to each volumetric flask respectively. To this 5mL of Ramipril standard drug solution of 200 $\mu\text{g mL}^{-1}$  was added to each volumetric flask respectively. The volume was made up to 100 mL with mobile phase. 20  $\mu\text{L}$  of each solution was injected and chromatograms were recorded. The range was found between 98.06 % to 100.47 % respectively. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients (Table 3).

### Limit of Detection and Quantification

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

$$\text{L.O.D.} = 3.3(\text{SD}/\text{S})$$

$$\text{L.O.Q.} = 10(\text{SD}/\text{S})$$

Where, SD = Standard deviation of the response, S = Slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

The LOD was found to be 0.571  $\mu\text{g mL}^{-1}$  and 1.090  $\mu\text{g mL}^{-1}$  and LOQ was found to be 1.733  $\mu\text{g mL}^{-1}$  and 3.303  $\mu\text{g mL}^{-1}$  for Enalapril and Ramipril respectively which represents that sensitivity of the method is high.

### Precision

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts, and different equipments. The repeatability study which was conducted on the solution having the concentration of about 10  $\mu\text{g mL}^{-1}$  for Enalapril and 10  $\mu\text{g mL}^{-1}$  for Ramipril showed a RSD of 0.09 % for Enalapril and 0.17 % for Ramipril. It was concluded that the analytical technique showed good repeatability (Table 4).

### Reproducibility and Ruggedness

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst, and different dates (Table 5).

### Robustness

The robustness of the method was determined by deliberate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the wavelength. The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust.

### Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there are no peaks of diluents and placebo at main peak's. Hence, the chromatographic system used for the estimation of Enalapril and Ramipril is very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Fig.6)

**Table 1. For Peak Area of Enalapril maleate**

Conc $\mu\text{g/ml}$	area
0	0
5	2431068
10	5161578
15	7885747
20	10589529
25	13206310
30	15781198

**Table 2. Peak area of Ramipril**

Conc µg/ml	Area
0	0
5	2967718
10	6325745
15	9721440
20	13133747
25	16235347
30	19641463

**Table 3. Result of recovery studies**

	Enalapril	Ramipril
<b>Accuracy std</b>	10588754	13154765
	10587895	13161276
	10578943	13170432
Avg	10585197.33	13162157.67
<b>Accuracy sample</b>	10586587	13157863
50%spike	15720959	19763154
	15821783	19778034
	15830256	19768790
Avg	15790999.33	19769992.67
amt.recovered	<b>49.17</b>	<b>50.24</b>
%Recovery	<b>98.33</b>	<b>100.47 %</b>
100%spike	20825663	26358495
	21031548	26364587
	21041432	26370032
Avg	20966214.33	26364371.33
amt.recovered	<b>98.06</b>	<b>100.34</b>
%Recovery	<b>98.06</b>	<b>100.34 %</b>
150%spike	26760044	32893798
	26762154	32893798
	25770843	32891084
Avg	26431013.67	32892893.33
amt.recovered	<b>149.68</b>	<b>149.94</b>
%recovered	<b>99.79</b>	<b>99.96 %</b>

**Table 4a. Results of repeatability analysis**

Enalapril			Ramipril		
S.No.	RT	Area	S.No.	RT	Area
1	3.602	10591539	1	6.897	13176780
2	3.601	10553534	2	6.893	13138144
3	3.601	10569019	3	6.887	13172805
4	3.602	10592134	4	6.897	13169845
5	3.602	10565347	5	6.895	13141542
6	3.602	10570126	6	6.895	13153265
Avg	3.601667	10573617	avg	6.894	13158730
Stdev	0.000516	15291.34	stdev	0.003742	16712.44
<b>%RSD</b>	<b>0.01</b>	<b>0.14</b>	<b>%RSD</b>	<b>0.05</b>	<b>0.13</b>

**Table 4b. Results of repeatability analysis**

Enalapril			Ramipril		
S.No.	RT	Area	S.No.	RT	Area
1	3.608	10616395	1	6.933	13170043
2	3.603	10597338	2	6.915	13175060
3	3.604	10603294	3	6.905	13204350
4	3.606	10621453	4	6.935	13165763
5	3.602	10605438	5	6.902	13211543
6	3.602	10602543	6	6.902	13212154
avg	3.604167	10607744	avg	6.915333	13189819
stdev	0.002401	9200.346	stdev	0.01524	21769.45
%RSD	<b>0.07</b>	<b>0.09 %</b>	%RSD	<b>0.22</b>	<b>0.17 %</b>

**Table 5. Results of reproducibility**

	Enalapril	Ramipril
Average Percentage Recovery	98.71%	100.25%
SD between set of analysis on same date	15291.34	16712.44
SD between set of analysis on different date	9200.34	21769.45
RSD between set of analysis on same date	0.14%	0.13%
RSD between set of analysis on different date	0.09%	0.17%

**Table 6. Results of system suitability parameters**

S.No.	Parameters	Enalapril values	Ramipril values
1.	Theoretical Plates (N)	4155.00	7641.00
2.	Resolution (Rs)	5.03	12.36
3.	Tailing factor (Tf)	1.11	1.02

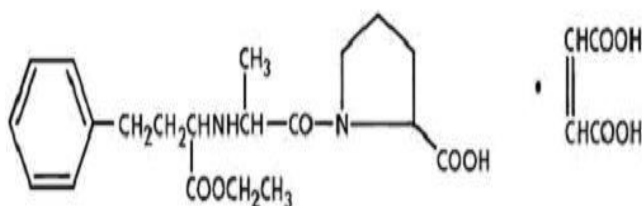
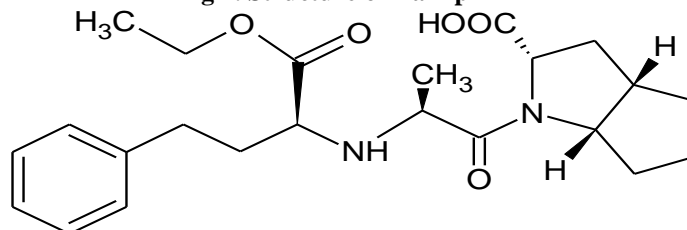
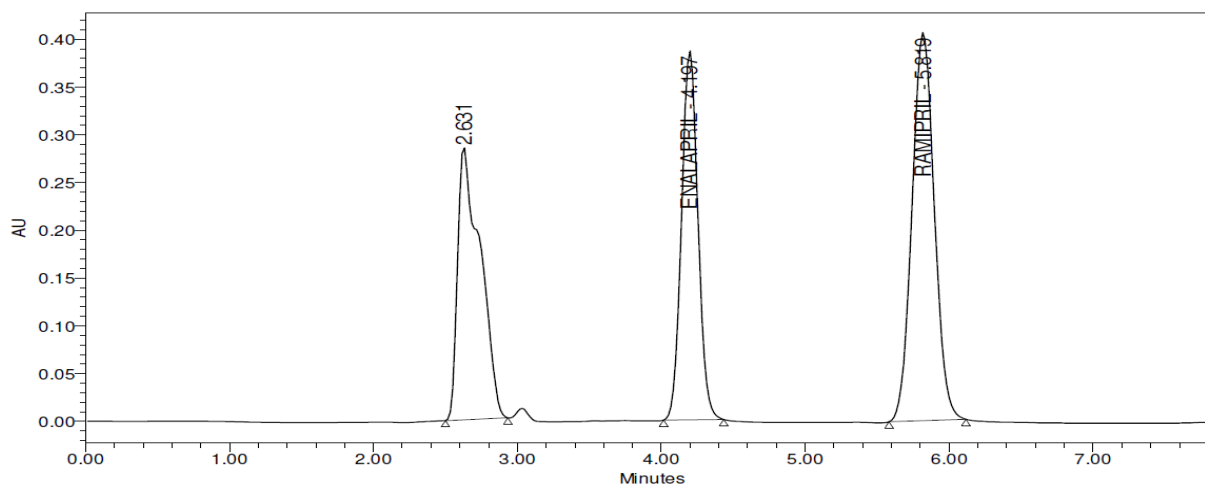
**Fig.1. Structure of Enalapril maleate****Fig 2. Structure of Ramipril****Fig. 3. Typical chromatogram showing Enalapril maleate and Ramipril**

Fig. 4. Calibration curve for Enalapril

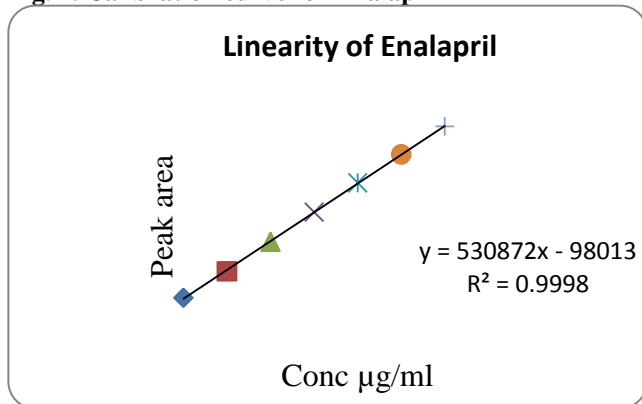


Fig. 5. Calibration curve for Ramipril

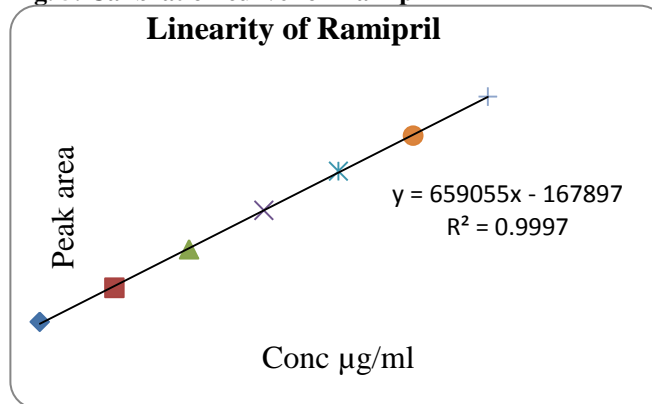
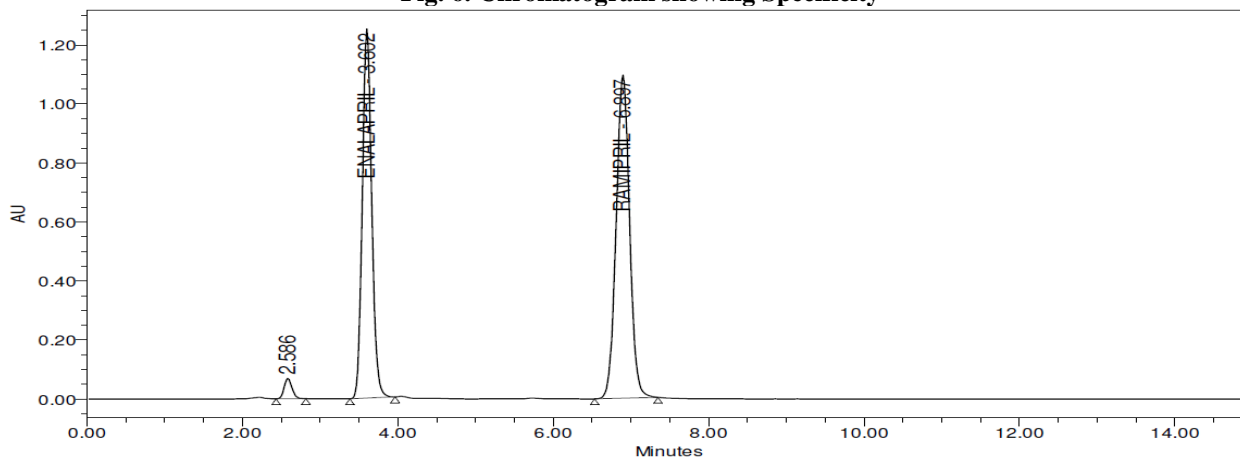


Fig. 6. Chromatogram showing Specificity



### System Suitability

A binary solution of 10  $\mu\text{g mL}^{-1}$  of Enalapril and 10  $\mu\text{g mL}^{-1}$  of Ramipril (in triplicate) was prepared and same was injected, then the system suitability parameters like resolution factor ( $R_s$ ), tailing factor ( $T_f$ ) and theoretical plates ( $N$ ) were calculated and recorded in Table 6. The values for system suitability parameters showed feasibility of this method for routine pharmaceutical application.

### CONCLUSION

The proposed RP-HPLC method is found to be simple, accurate, precise, linear, and specific for quantitative estimation of Enalapril and Ramipril in bulk and its formulation. The proposed RP-HPLC method is cost effective and less time consuming. The values for

system suitability parameters showed feasibility of this method for routine pharmaceutical application. Hence the present HPLC method is suitable for routine assay of Enalapril and Ramipril in raw materials and in pharmaceutical formulations in the quality control laboratories.

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