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Simple and Sensitive Titrimetric and Spectrophotometric Determination of Enalapril Maleate in Pharmaceuticals using Permanganate

Kanakapura Basavaiah Vinay, Hosakere Doddarevanna Revanasiddappa, Paraki Ravindra Shantala, Kanakapura Basavaiah*

Department of chemistry, University of Mysore, Manasagangotri, Mysore-57006, India

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Abstract

Two titrimetric and two spectrophotometric methods are described for determination of enalapril maleate (ENP) in pure drug as well as in tablets. In titrimetry, ENP was quantified by either direct oxidation of ENP content with potassium permanganate in H_2SO_4 medium (method A) or oxidation of ENP by a known excess of potassium permanganate in H_2SO_4 medium followed by determination of unreacted permanganate by titration with ferrous ammonium sulphate (method B). In both the methods, the reaction stiochiometry is found to be 1:2 (ENP: $KMnO_4$) and the methods are applicable over the 1.0-10.0 mg. In spectrophotometry, ENP was quantified based on the reduction of potassium permanganate by ENP either in neutral medium (method C, λ_{max} at 340 nm) or in H_2SO_4 medium (method D, λ_{max} at 550 nm) over the concentration ranges, 2.0-12.0 µg mL^{-1} and 7.0-70.0 µg mL^{-1} by method C and method D, respectively. The calculated molar absorptivities are 1.8 x 10^4 and 3.8 x 10^3 L mol^{-1} cm^{-1} for method C and method D, respectively with corresponding Sandell sensitivity values of 0.028 and 0.115 µg cm^{-2}. The limits of detection (LOD) and quantification (LOQ) have also been reported. The interference due to common excipients present in the formulations in method A was successfully overcome by extraction with acetone. The methods were successfully applied to the determination of ENP in tablets and the results were statistically compared with those of a reference method by applying the Student's t-test and F-test. The accuracy and validity of the methods were ascertained by recovery studies via standard addition technique.

Keywords:

Enalapril maleate; titrimetry; spectrophotometry; potassium permanganate; tablets

1. Introduction

Enalapril maleate (ENP), of chemical formula (2S)-1-[(2S)-2-[[(1S)-1- (Ethoxy-carbonyl)-3-phenylpropyl]-amino]propanoyl]pyrrolidine-2-carboxylic acid (Fig 1), is an angiotensin converting enzyme (ACE) inhibitor used in the treatment of hypertension and some types of chronic heart failure [1]. The official methods of analysis of ENP in pharmaceuticals are high-performance liquid chromatography (HPLC) in USP [2] and potentiometric titration using sodium hydroxide in European Pharmacopoeia [3].

Various analytical techniques are available for the determination of ENP in pharmaceuticals when present either alone or in combination with other drugs and they are UV spectrophotometry [4-7], HPLC [5-9], Liquid chromatography/mass spectrophotometry (LC/MS) [10], GC/MS [11], micellar electrokinetic capillary eletrophoresis (MEKCE) [12],

* **Corresponding Author**
E-mail: basavaiahk@yahoo.co.in
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selective membrane electrode potentiometry [13, 14], enzyme-linked immunosorbent assay [15], atomic absorption spectrophotometry [16, 17] and polorography [18]. These methods are sensitive yet the instrumentations are cumbersome and require critical experimental conditions.

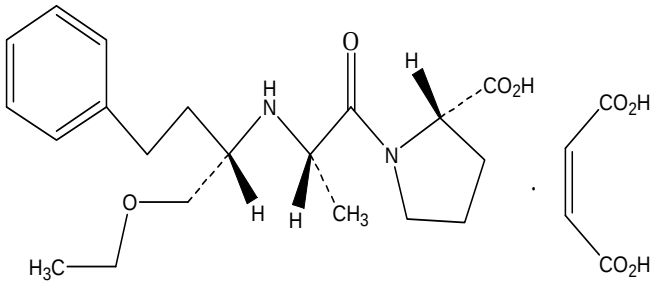

Fig. 1. Structure of Enalapril Maleate.

Titrimetric and visible spectrophotometric procedures are still popular because of their simplicity, fair accuracy and precision, and cost effectiveness. Quite a few researchers have used visible spectrophotometric methods for the determination of ENP in pharmaceuticals when present either alone [19] or in combination with other drugs [16-18, 20, 21], and require pre derivatisation, heating step, use of expensive chemicals and organic solvents as reaction medium. There is only one report, an official method [3], on the use of titrimetric method for the determination of ENP. The method consisted of the titration of the aqueous solution of the tablet with 0.1 mol L^{-1} NaOH potentiometrically and requires fairly large quantities (100 mg of drug for each titration) of ENP. The present manuscript describes two titrimetric and two spectrophotometric procedures for the determination of ENP in both pure form and in tablet form using permanganate as the oxidimetric reagent.

2. Experimetal

2.1. Apparatus

A Systronics model 106 digital spectrophotometric with 1-cm matched quartz cells was used for all absorbance measurements.

2.2. Reagents and Standards

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions.

Potassium permanganate ($1x10^{-2}$ mol L^{-1}): Prepared by dissolving about 0.395 g of the chemical (Merck, Mumbai, India) in water; the solution was boiled for 10 minutes to remove any residual manganese (IV) ions, cooled, filtered and diluted to 250 mL, and standardized using procedure as outlined in literature [22], and used in titrimetric assay. The stock solution was diluted to get 150 and 600 µg mL^{-1} concentrations for use in spectrophotometric method C and method D, respectively.

Ferrous ammonium sulphate (FAS): A 0.05 N FAS for method B was prepared by dissolving 4.9 g of the salt (S.d. Fine Chem, Mumbai, India) in 50 mL of water containing 1 mL of concentrated H_2SO_4, and diluted to 250 mL with water.

Sulphuric acid: Concentrated sulphuric acid (Merck, Mumbai, India, Sp. gr. 1.18) was diluted appropriately with water to get 10 mol L^{-1} for method B, and 5 mol L^{-1} for method A and method D.

Standard ENP solution: Pharmaceutical grade ENP was kindly provided by Micro Laboratory Pvt. Ltd., Bangalore, India, as gift and was used as received. A 1 mg mL^{-1} ENP solution was prepared by dissolving 100 mg of pure ENP in water and diluted to the mark in a 100 mL calibrated flask and used in titrimetry. The stock solution (1000 µg mL^{-1}) was diluted appropriately with water to get working concentrations of 40 and 140 µg mL^{-1} ENP for use in method C and method D, respectively.

2.3. Procedures

2.3.1. Direct Titration (method A)

A 10.0 mL aliquot of standard solution containing 1.0-10.0 mg of ENP was measured accurately and transferred into a 100 mL titration flask, 5 mL of 5 mol L^{-1} H_2SO_4 was added and the flask was kept on hot plate until the solution's temperature reached 80 ^{0}C, and titrated immediately against 0.01 mol L^{-1} $KMnO_4$ to the first appearance of pink color.

The amount of ENP in the aliquot was computed from the formula:

$$\text{Amount (mg)} = \frac{V \times M_w \times S}{N}$$

where V = mL of titrant reacted
M_w = relative molecular mass of drug
S = strength of titrant, M.
N = number of moles of titrant reacting with per mole of ENP.

2.3.2. Indirect Titration (method B)

A 10.0 mL aliquot of pure drug solution containing 1.0-10.0 mg of ENP was measured accurately and transferred into a 100 mL titration flask. The solution was acidified by adding 5 mL of 10 mol L^{-1} H_2SO_4. Then, 10 mL of 0.01 mol L^{-1} $KMnO_4$ was added by means of a pipette and the flask was let stand for 30 seconds at room temperature and the unreacted $KMnO_4$ was titrated immediately with 0.05 mol L^{-1} FAS to a colorless end point. A blank experiment was simultaneously performed.

The amount of ENP was computed from the following formula:

$$\text{Amount (mg)} = \frac{(B-A) \times M_w \times S}{N}$$

where B = mL of titrant in the absence of sample
A = mL of titrant in the presence of the sample.
Mw=relative molecular mass of drug
S = strength of $KMnO_4$, M.
N = number of moles of $KMnO_4$ reacting with per mole of ENP.

2.3.3. Spectrophotometry (method C)

Different aliquots of standard solution (0.5-3.0 mL, 40 µg mL^{-1}) of pure ENP were transferred into a series of 10 mL calibrated flasks by means of micro burette and the total volume was adjusted to 3.0 mL with water. To each flask was added accurately measured 1 mL of 150 µg mL^{-1} $KMnO_4$. The flasks were kept aside for 10 min with occasional shaking before diluting to the mark with water. The absorbance was recorded after 5 min at 340 nm against a reagent blank.

2.3.4. Spectrophotometry (method D)

Into a series of 10 mL calibrated flasks, 0.5-5.0 mL of 140 µg mL^{-1} pure ENP solution were added by means of micro burette and the total volume was brought to 5.0 mL with water. To each flask was added 1 mL of 5 mol L^{-1} H_2SO_4 followed by 1 mL of 600 µg mL^{-1} $KMnO_4$, the latter being measured accurately. The flasks were kept aside for 10 min with occasional shaking and the volume was made up to the mark with water. The absorbance was recorded at 550 nm against the reagent blank.

2.3.5. Assay procedure for tablets

Twenty tablets containing ENP were accurately weighed and ground into a fine powder. An amount of tablet powder equivalent to 250 mg of ENP was weighed into a 250 mL calibrated flask, 100 mL of water added and the mixture shaken for 20 min; then the volume was made up to the mark with water, mixed well and filtered using Whatman No. 42 filter paper. The filtrate equivalent to 1 mg mL^{-1} ENP was subjected to analysis using procedure described under method B. The same stock solution was diluted to get 40 and 140 µg mL^{-1} ENP and analyzed using procedures described under method C and method D, respectively. Another portion of tablet powder equivalent to 50 mg of ENP was weighed into a 50 mL calibrated flask, 30 mL of acetone added and the mixture shaken for 5 minutes. The mixture was filtered using Whatman No. 42 filter paper and the filtrate was evaporated to dryness on a water bath. The residue was dissolved and make upto the mark with water, and was then subjected to analysis using procedure described under method A.

3. Results and Discussion

Permanganate being a very strong oxidizing agent can react with several organic substances [23]. Recently, permanganate has been used to determine pharmaceutical active compounds in formulations both in acid medium [23, 24] and in alkaline medium [25-28]. In the present titrimetric work, ENP was found to react with $KMnO_4$ in 1:2 (ENP:$KMnO_4$) ratio and based on this, a possible reaction scheme is suggested as shown in Fig 2, in which only malealic acid undergoes oxidation reaction to give two moles of oxalic acid.

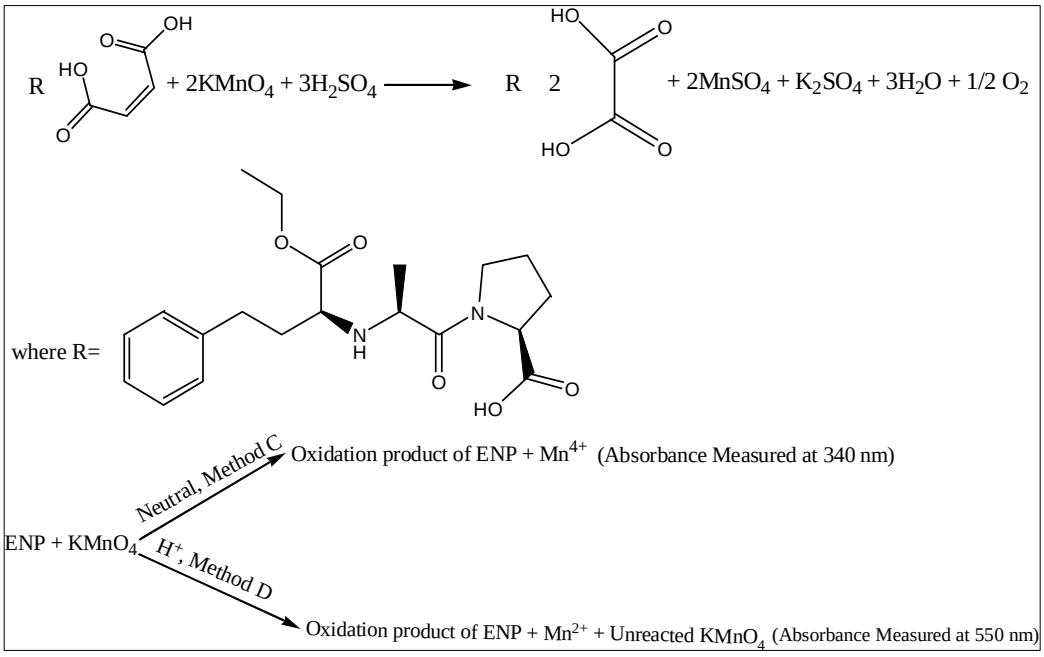

Fig. 2. Probable Reaction Scheme

3.1. Method Development

All the four methods are based on the oxidation of ENP by $KMnO_4$. Oxidation of ENP by $KMnO_4$ in two different medium in case of spectrophotometry quantifies ENP with different detection range.

3.1.1. Titrimetry

The direct titration between ENP and $KMnO_4$ was slow at room temperature. This could be due to a series of slow reduction steps of Mn^{7+} to Mn^{2+}, while forming less stable intermediate products like Mn^{6+} and Mn^{4+}. In order to increase the reaction rate, the titration was performed at 80 ^{0}C. The reaction stiochiometry was found to be 1:2 (ENP: $KMnO_4$) and it did not change when the reaction temperature was maintained between 70 and 90 ^{0}C. In the absence of H_2SO_4 as a reaction medium, the reduction of Mn^{7+} to Mn^{4+} predominates, and the end point is the appearance of brown color which is difficult to detect. Very low concentration of H_2SO_4 also gave indistinct end point. Hence, 5 mL of 5 mol L^{-1} H_2SO_4 acid in a total volume of 20 mL in the beginning was required. At the performance temperature of 80 ^{0}C, slight interference from the tablet excipients was observed and this was successfully overcome by extraction into acetone. In case of method B, measured excess of $KMnO_4$ was allowed to react with ENP in H_2SO_4 medium and the unreacted $KMnO_4$ was subsequently determined by back titrating with FAS. In the presence of excess of $KMnO_4$, the reaction proceeds very fast for the first 30 seconds and then decreases drastically. When measured excess of $KMnO_4$ was allowed to react with ENP for thirty seconds, the reaction stiochiometry was found to be 1:2 (ENP: $KMnO_4$). The reaction was found to proceed rather slowly even after 30 seconds consuming insignificant amount of $KMnO_4$. This could be due to the formation of intermediate product MnO_2, which is a strong catalyst for permanganate decomposition [29]. MnO_2 in acid solution behaves like hydrogen peroxide:

$$MnO_2 + H_2SO_4 \longrightarrow H_2O_2 + MnSO_4$$

$$5H_2O_2 + 2KMnO_4 + 3H_2SO_4 \longrightarrow 2MnSO_4 + 5O_2 + K_2SO_4 + 8H_2O$$

Hence, the reaction time between known excess of $KMnO_4$ and ENP was strictly restricted to 30 seconds in order to overcome erroneous results. The optimum acid concentration for a definite reaction stiochiometry and as well as for a sharp end point detection is 5 mL of 10 mol L^{-1} H_2SO_4 in a total volume of 23 mL.

3.1.2. Spectrophotometry

Oxidation with permanganate under neutral conditions may take place in accordance with the equation [30]:

$$MnO_4^- + 2H_2O + 3e^- \rightleftharpoons MnO_2 + 4OH^-$$

The Mn (IV) ion exhibit brownish yellow color which absorbs maximally at 340 nm (Fig 3). When a fixed concentration of permanganate was reacted with increasing concentrations of ENP in neutral medium, there occurred a concomitant increase in the brownish yellow color product at 340 nm and served as a basis for the quantification of ENP in method C. Effect of $KMnO_4$ concentration was studied by keeping the concentration of ENP fixed. With the increasing concentration of $KMnO_4$, the blank absorbance increases significantly after 3 mL of 50 µg mL^{-1} $KMnO_4$ in a total volume of 10 mL. Therefore, the amount of $KMnO_4$ was restricted to 1 mL of 150 µg mL^{-1} in a total volume of 10 mL. The reaction between ENP and $KMnO_4$ in the neutral medium was complete in 10 min, and soon

after making up to the mark with water, the absorbance of the brownish yellow product was found to be stable between 10-30 min.

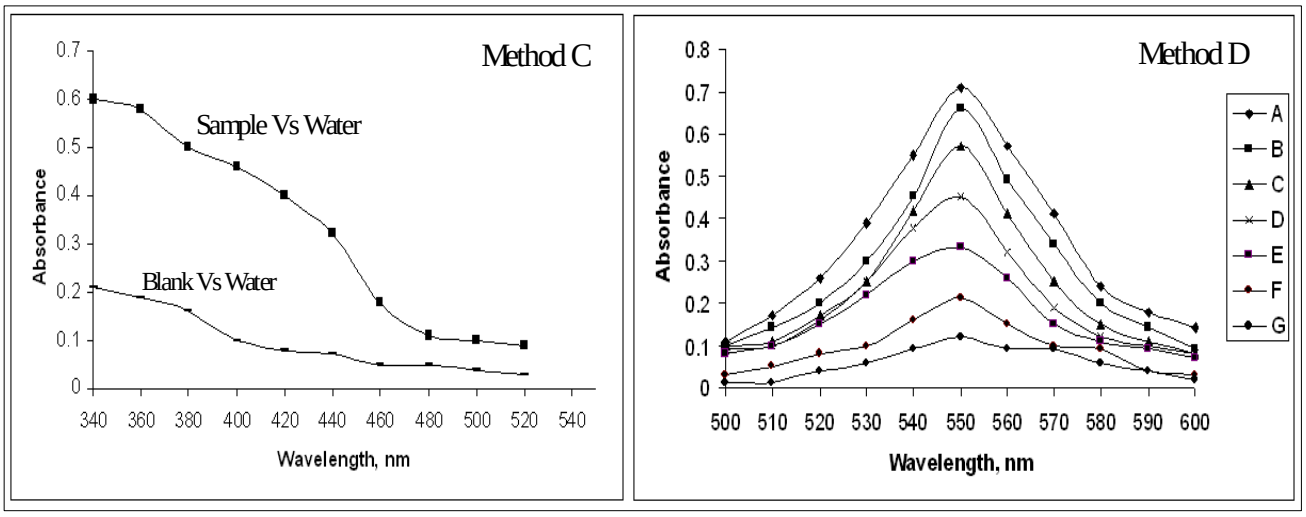

Fig. 3. Absorption spectra for method C and method D
Method C: (Brownish yellow color produced for 10 µg mL^{-1} ENP).
Method D: (A.0.0; B.7.0; C.14.0; D.28.0; E.42.0; F.56.0; G.70.0 µg mL^{-1} ENP).

In method D, when a fixed concentration of permanganate was reacted with increasing concentrations of ENP in H_2SO_4 acid medium, there occurred a concomitant fall in the concentration of permanganate as revealed by the decreasing absorbance at 550 nm (Fig 3), which served as the basis for quantification. A preliminary experiment showed that permanganate can be determined upto 60 µg mL^{-1} (Fig 4) at 550 nm under the optimum acidic conditions of assay. Hence, different concentrations of ENP were reacted with 1 mL of 600 µg mL^{-1} $KMnO_4$ to determine the concentration range over which ENP could be determined. To check the effect of acid concentration on the reaction, 0-5 mL of 5 mol L^{-1} H_2SO_4 was added to the fixed concentration of ENP and $KMnO_4$, and it was observed that there was absolutely no change in the absorbance when 1-5 mL of 5 mol L^{-1} H_2SO_4 were used in a total volume of 10 mL. Effect of hydrochloric acid was not studied since $KMnO_4$ being a strong oxidizing agent would react with HCl to liberate chlorine. The reaction between ENP and $KMnO_4$ in the acid concentration employed was complete in 10 min, and the absorbance of the measured unreacted $KMnO_4$ was found to be stable upto 40 min thereafter. Two blanks were prepared for the study. The reagent blank consisting of acid and permanganate showed maximum absorbance (equal to the intercept). A second blank in the absence of ENP and $KMnO_4$ had negligible absorbance, and hence measurements were made against water blank.

3.2. Method Validation

Method validations were done according to the present ICH guidelines [31].

3.2.1. Analytical parameters of spectrophotometric methods

A linear correlation was found between absorbance at λ_{max} and concentration of ENP in the ranges given in Table 1. Within the Beer's law range for both the methods, the graphs are described by the regression equation:

$$Y = a + bX$$

(Where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in µg mL^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity and sandell sensitivity values of both methods are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [31] are also presented in Table 1. A statistical test was used to see whether the correlation coefficient is indeed significant. This was done by calculating the t-value using the equation [32]:

$$t = \frac{|r|\sqrt{n-2}}{\sqrt{1-r^2}}$$

The calculated t-value was compared with the tabulated value at the 95% significance level, using a two-sided t-test and (n-2) degrees of freedom. The null hypothesis in this case was that there was no correlation between the measured absorbance (Y) and concentration (X). Since the calculated t-values were 80.6 and 19.9 for method C and method D, respectively, which are greater than the tabulated value (2.57), the null hypothesis was rejected and it was concluded that a significant correlation did exist between Y and X. As expected, the closer $|r|$ is to 1, i.e. as the straight-line relationship becomes stronger, the larger the values of t that are obtained.

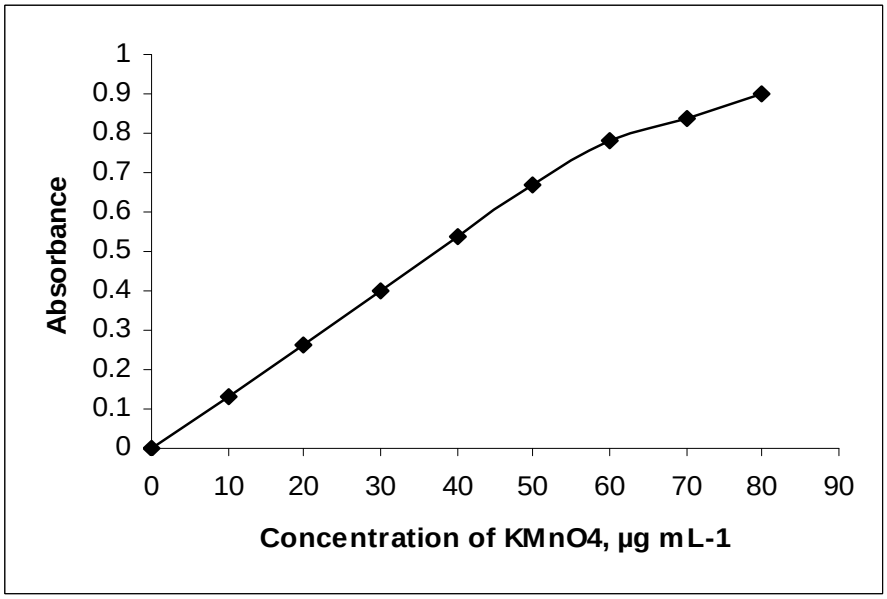

Fig. 4. Linear relation between absorbance at 550 nm and $KMnO_4$ concentration in 0.5 mol L^{-1} H_2SO_4.

Table 1. Regression and Analytical parameters

Parameter	Method C	Method D
λ_{max}, nm	340	550
Beer's law limits, µg mL^{-1}	2.0-12.0	7.0-70.0
Molar absorptivity, L mol^{-1} cm^{-1}	1.8×10^4	3.8×10^3
Sandell sensitivity*, µg cm^{-2}	0.028	0.115
Limit of detection, µg mL^{-1}	0.32	1.58
Limit of quantification, µg mL^{-1}	0.96	4.80
Regression equation, Y**		
Intercept (a)	-0.023	0.690
Slope (b)	0.040	-0.008
Correlation coefficient, (r)	0.9997	-0.9938
Standard deviation of intercept (S_a)	0.0006	0.0642
Variance (S_a^2)	3.6×10^{-7}	4.1×10^{-3}
$\pm tS_a / \sqrt{n}$	6.79×10^{-4}	6.7×10^{-2}
Standard deviation of slope (S_b)	0.0005	0.0015
$\pm tS_b / \sqrt{n}$	5.65×10^{-4}	1.57×10^{-3}

*Is a sensitivity parameter in µg cm^{-2} ENP corresponding to an absorbance of 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and L= 1cm.

**Y = a+bX, where Y is the absorbance and X concentration in µg mL^{-1}.

$\pm tS_a / \sqrt{n}$ =confidence limit for intercept, $\pm tS_b / \sqrt{n}$ =confidence limit for slope.

3.2.2. Assay precision and accuracy

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day) [33]. Three different concentrations of ENP were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day is within 2.51 and inter-day is within 3.50 which showed that the precision was good. The accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for ENP and found to be within the RE (%) values of 2.80 (Intra-day accuracy) and 3.33 (Inter-day accuracy).

3.2.3. Method Selectivity

Placebo analysis was carried out in order to find the interference. A placebo blank consisting of 20 mg sodium alginate, 30 mg magnesium stearate, 20 mg lactose, 20 mg acacia, 50 mg talc and 30 mg starch (manufactured by Loba chemie or Merck, Mumbai, India) but without ENP was prepared and analysed as described under "Assay procedure for tablets". There was absolutely no interference from the placebo in method B, method C and method D, but slight interference was encountered in method A and this was overcome by extraction and evaporation procedure described under assay of tablets. In order to study the selectivity of the methods, a separate experiment was performed with synthetic mixture.

To the placebo blank of similar composition, 100 mg of ENP was added, homogenized and the solution of the synthetic mixture was prepared as done under "Assay procedure for tablets". The percent recoveries of ENP were 99.96, 102.00, 98.90 and 100.70 for method A, method B, method C and method D, respectively. This confirms the selectivity of methods under the optimized conditions.

3.2.4. Robustness and ruggedness

For the evaluation of the method robustness, three important experimental variables such as reaction time, reaction temperature and H_2SO_4 concentration were slightly varied deliberately. The analysis was performed at the deliberately varied experimental conditions by taking three different concentrations of ENP and found to remain unaffected as shown by the RSD values in the range of 0.65 to 3.14 %. Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using three different burettes in case of titrimetric procedures and two different spectrophotometers in case of spectrophotometry. The results are shown in Table 2.

3.2.5. Application to analysis of pharmaceutical formulations

The proposed methods were applied to the determination of ENP in two brands of tablets Envas-10 and Enam-5 purchased from local stores. The results were statistically compared with those obtained by the official European Pharmacopoeia method [3] for accuracy and precision by applying the Student's t-test and variance ratio F-test. The results presented in Table 3 revealed no significant difference between the proposed methods and the literature method at the 95% confidence level with respect to accuracy and precision.

3.2.6. Recovery Study

To confirm the reliability and reproducibility of the proposed methods in the presence of tablet excipients, a standard addition technique was followed. A fixed amount of drug from preparations was taken and pure (standard) drug at three different levels was added and the results are presented in Table 4.

Table. 2. Method robustness and ruggedness

Method	ENP taken*	Robustness (% RSD)		Ruggedness (%RSD)	
		Reaction temperature** /reaction time***	Acid concentration	Inter burette's# /Inter instruments@ $(n=3)$	Inter analysts $(n=4)$
	3.00	0.65	0.78	1.72	1.59
A	6.00	1.12	2.01	2.36	1.90
	9.00	0.78	1.18	3.10	1.20
	2.00	2.10	2.01	2.12	3.14
B	4.00	1.00	2.30	1.28	2.56
	8.00	1.28	1.23	1.85	2.60
	4.00	2.37	--	0.75	3.11
C	6.00	1.56	--	3.01	3.02
	8.00	2.10	--	2.16	2.14
	20.00	1.15	2.13	1.64	1.28
D	30.00	1.18	2.01	2.50	1.96
	40.00	1.46	1.63	2.20	2.30

*mg in method A and method B; µg mL^{-1} in method C and method D.
**In method A, reaction temperature were 75, 80 and 85^{0}C; and H_2SO_4 volume used were 4.5, 5.0 and 5.5 mL.
***In method B, reaction time used were 50, 60 and 70 s, and H_2SO_4 volume were 4.5, 5.0 and 5.5 mL; In method C, reaction time used were 9, 10 and 12 min; In method D, reaction time used were 9, 10 and 12 min, and H_2SO_4 volume were 0.5, 1.0 and 2.0 mL
#In the case of method A and method B.
@In the case of method C and method D.

Table 3. Results of analysis of tablets by the proposed methods.

Tablets analysed**	Label claim, mg/tablet	Found* (Percent label claim ±SD)				
		Reference method	Method A	Method B	Method C	Method D
Enam[a]	5	101.7 ± 0.85	102.5 ± 0.82 **t**= 1.51 **F**= 1.07	101.6 ± 2.00 **t**= 0.08 **F**= 5.54	101.1 ± 1.84 **t**= 0.70 **F**= 4.68	102.4 ± 2.06 **t**= 0.76 **F**= 5.87
Envas[b]	10	100.8 ± 1.20	99.8 ± 0.79 **t**= 1.59 **F**= 2.30	101.8 ± 2.33 **t**= 0.89 **F**= 3.77	102.0 ± 2.01 **t**= 1.18 **F**= 2.80	101.7 ± 1.98 **t**= 0.89 **F**= 2.72
Analysis Time, min		35.0	3.0	4.0	20.0	15.0
Analysis Cost, $		1.50	0.9	0.6	0.2	0.4

*Mean value of five determinations.
**Marketed by: a. Candila Pharmaceuticals, industrial growth center, J & K, (India)
b. Dr. Reddy's laboratories Ltd, Hyderabad, (India)
Tabulated t-value at 95% confidence level for four degree of freedom is 2.77.
Tabulated F-value at 95% confidence level for four degree of freedom is 6.39.

Table 4. Accuracy assessment by recovery experiments.

Tablets studied	**Method A**					**Method B**			
	ENP in tablet, mg	ENP in tablet, mg*	Pure ENP added, mg	Total found, mg	Pure ENP recovered*, Percent± SD	ENP in tablet, mg	Pure ENP added, mg	Total found, mg	Pure ENP recovered*, Percent ± SD
Enam	5.00	4.10	2.00	6.17	103.5 ±0.79	4.06	2.00	6.10	102.0 ± 2.10
		4.10	4.00	8.21	102.8 ±0.85	4.06	4.00	8.18	103.0 ± 1.98
		4.10	6.00	10.26	102.6 ±0.90	4.06	6.00	10.12	101.0 ± 2.00

*Mean value of three determinations.

Table 4. (continued)

Tablets studied	Method C				Method D			
	ENP in tablet, µg mL^{-1}	Pure ENP added, µg mL^{-1}	Total found, µg mL^{-1}	Pure ENP recovered*, Percent ± SD	ENP in tablet, µg mL^{-1}	Pure ENP added, µg mL^{-1}	Total found, µg mL^{-1}	Pure ENP recovered*, Percent ± SD
	4.04	2.00	6.04	100.0 ± 1.80	20.48	10.00	30.73	102.5 ± 2.50
	4.04	4.00	8.10		20.48	20.00	40.44	99.8 ± 2.20
Enam				101.5 ±				
	4.04	6.00	9.99	1.67	20.48	30.00	50.09	98.7 ± 2.43
				99.1 ± 1.83				

*Mean value of three determinations.

4. Conclusions

Very low cost of analysis and short analysis time with minor drawbacks speaks the simplicity, sensitivity and cost-effectiveness of the methods (Table 5). Only the titrimetric method A, requires extraction of ENP from the tablet matrix into acetone. The titrimetric procedures are applicable over 1.0-10.0 mg, against the lone titrimetric method [3], which requires 100 mg of drug for each titration. Of the reported four spectrophotometric methods [19] for the determination of ENP in pharmaceuticals when present alone, except one method (applicable over the range of 2.5-50 µg mL^{-1} ENP), the remaining three methods make use of organic solvents and are comparatively less sensitive (20-560, 5-75 and 10-200 µg mL^{-1} ENP) than the present methods. Statistical analysis of the results confirms high precision and accuracy of the proposed methods; hence the methods can be adopted in industrial quality control laboratories for routine analysis.

Table 5. Important characteristics of the proposed methods

Condition	Method			
	A	**B**	**C**	**D**
Technique	Titrimetry	Titrimetry	Spectrophotometry	Spectrophotometry
Reaction	ENP + H_2SO_4 >< $KMnO_4$	ENP+ H_2SO_4+ $KMnO_4$ >< FAS	ENP + $KMnO_4$, Absorbance measured at 340 nm.	ENP+ H_2SO_4+ $KMnO_4$, Absorbance measured at 550 nm.
Total assay time, min	3.0	4.0	20.0	15.0
Total assay cost/sample, $	0.9	0.6	0.2	0.4
Drawbacks	Reaction temperature, 80 ^{0}C.	Reaction time critical.	Absorbance measured at lower wavelength	Very precise and exact concentration of $KMnO_4$ required.

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References

1. Ferguson R K, Vlasses P H, Swanson B N, Majaverian P, Hichenes M and Irvin J D (1982) Effects of enalapril, a new converting enzyme inhibitor, in hypertension. Clin Pharmacol Ther 32: 48.
2. The United States Pharmacopoeia 24, 2000, The National Formulary, 19 Rockville, USP Convention.
3. The European Pharmacopoeia [1420], 3rd ed., 2001, Council of European, Strasbourg.
4. Prasad C V N, Saha R N and Parimoo P (1999) Simultaneous determination of amlodipine–enalapril maleate and amlodipine–lisinopril in combined tablet preparations by derivative spectrophotometry. Pharm Pharma Comm 5: 383.
5. Carlucci G, Di Giuseppe E and Mazzeo P (1993) Simultaneous determination of enalapril maleate and hydrochlorothiazide in tablets by derivative UV spectrophotometry and high-performance liquid chromatography. Int J Pharm 93: 245.
6. El Walily A F M, Belal S F, Heaba E A and El Kersh A (1995) Simultaneous determination of enalapril maleate and hydrochlorothiazide by first-derivative ultraviolet spectrophotometry and high-performance liquid chromatography. J Pharm Bio Med Anal 13: 851.
7. Bonazzi D, Gotti R, Andrisano V and Cavrini V (1997) Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC) J Pharm Biomed Anal 16: 431.
8. Shetkar P B and Shinde V M (1997) Simultaneuous determination of enalpril maleate and hydrochlorothiazide in tablets by reversed phase HPLC. Anal Lett 30: 1143.
9. Qin X Z, DeMarco J, and Ip D P (1995) Simultaneous determination of enalapril, felodipine and their degradation products in the dosage formulation by reversed-phase high-performance liquid chromatography using a spherisorb C_8 column. J Chromatogr A 707: 245.
10. Pisarev V V, Moskaleva N E, Zverkov Y B, Smirnova L B, Belolipershaya V G and Sukhanov Y V (2005) HPLC/MS determination of enalapril and enalaprilat in the blood plasma. Pharm Chem J 39: 104.
11. Shioya H, Shimojo M, and Kawahara Y (1992) Determination of enalapril and its active metabolite enalaprilat in plasma and urine by gas chromatography/mass spectrometry. Biomed Chromatogr 6: 59.
12. Thomas B R and Ghodbane S (1993) Evaluation of a mixed micellar electrokinetic capillary electrophoresis method for validated pharmaceutical quality control. J Liq Chromatrogr 16: 1983.
13. Aboul-Enein H Y, Bunacin A A, Bala C and Fleschin S (1997). Enalapril and ramipril selective membranes. Anal Lett 30: 1999.

14. Aboul-Enein H Y, Stefan R I and Van-Staden J F (1999). Potentiometric enantioselective membrane electrode for s-enalapril assay. Analusis 27: 53.

15. Matalka K, Arafat T, Hamad M and Jehanli A (2002). Determination of enalapril and enalaprilat by enzyme linked immunosorbent assays: application to pharmacokinetic and pharmacodynamic analysis. Fundam Clin Pharmacol 16: 237.

16. Baraka M M, El-Sadek M, Moussa E M, Abd-Alaty N M (2008) Spectrophotometric and atomic absorption determination of ramipril, enalapril maleate and lisinopril through ternary complex formation with molybdenum (V)-thiocyanate (Mo(V)-SCN). Chem Pharm Bull 56: 1521.

17. Ayad M M, Shalaby A A, Abdellatef H E, Hosny M M (2002) Spectrophotometric and AAS determination of ramipril and enalapril through ternary complex formation. J Pharm Biomed Anal 28: 311.

18. Abdel R O, Belal S F, Bedair M M, Barakat N S and Haggag R S (2003) Spectrophotometric and polarographic determination of enalapril and lisinopril using 2, 4-dinitrofluorobenzene J Pharma Biomed Anal 31: 701.

19. Rahman N and Haque S M (2008) Optimized and validated spectrophotometric methods for the determination of enalapril maleate in commercial dosage forms. Anal Chem Insights 3: 31.

20. Magda M A, Shalaby A, Abdellatef H E and Hosny M M (2003) Spectrophotometric methods for determination of enalapril and timolol in bulk and in drug formulations. Anal Bioanal Chem 375: 556.

21. Salah M B, Heba H A, Fawzy A E and Shaalan R A (2000) Spectrophotometric determination of enalapril maleate and ramipril in dosage forms. Spectroscopy Letters 33: 91.

22. A I Vogel (1961) A Text Book of Quantitative Inorganic Analysis., 3rd edn., The English Language Book Society and Longman, England, p 280.

23. Semaan F S, Nogueira P A and Cavalheiro E T G (2008) Flow injection spectrophotometric determination of furosemide in pharmaceuticals by the bleaching of a permanganate carrier solution. Anal Lett 41: 66.

24. Emara S (2004) Determination of methotrexate in pharmaceutical formulations by flow injection analysis exploiting the reaction with potassium permanganate. Il Farmaco 59: 827.

25. Rahman N, Ahmad Y and Azmi S N H (2004) Kinetic spectrophotometric method for the determination of norfloxacin in pharmaceutical formulations. Eur J Pharm Biopharm 57: 359

26. Abdellatef H E (2002) Kinetic spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulation. J Pharm Biomed Ana 29: 835.

27. Al-Tamrah S A (1999) Spectrophotometric determination of nicotine. Anal Chim Acta 379: 75.

28. Sugathi A, Sivakumar H B, Vijayakumar S C, Ravimathi P and Ravi T K (2006) Visible spectrophotometric determination of valdecoxib in tablet dosage forms Ind. J Pharm. Sc 68: 373.

29. IM Kolthoff and R Belcher (1957) *Volumetric Analysis- Volume III*, Interscience Publishers, Inc., New York. 39.

30. IM Kolthoff and R Belcher (1957) *Volumetric Analysis- Volume III*, Interscience Publishers, Inc., New York. 34.
31. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.
32. J N Miller and Miller J.C., Statistics and chemometrics for analytical chemistry, (2000) Pearson Education Limited: England.
33. SHABIR, G.A. (2003) Validation of high-performance liquid chromatography methods for pharmaceutical analysis: understanding the differences and similarities between validation requirements of the US food and drug administration, the US Pharmacopeia and the international conference on harmonization. J Chromatogr A 987 57.