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A simple UV Spectrophotometric method for Quantitative Estimation of Enalapril maleate

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ABSTRACT

A simple UV spectrophotometric method is described here to analyze enalapril maleate in bulk and dosage forms. The method is based on oxidation of enalapril maleate with potassium permanganate and sulphuric acid. The reaction is followed by measuring the absorbance at 267 nm. Beer's law is obeyed in the concentration ranges of 250-500 $\mu g \ ml^{-1}$, respectively. The method has been applied to the determination of enalapril maleate in bulk. Results of analysis are validated statistically and as per ICH guidelines. The method is found to be specific, repeatable, accurate and precise.

Keywords: Enalapril maleate, spectrophotometry, ICH guidelines, oxidation, potassium permanganate

1. INTRODUCTION

Enalapril maleate is a prodrug that is hydrolysed in the body to enalaprilat which is an inhibitor of angiotensin converting enzyme (ACE). Chemically it is ((S)-1-{N-[1-(ethoxy carbonyl)-3-phenylpropyl]-L-alanyl}-L-proline, (Z)-2-butenedioate), a derivative of two amino acids L-alanine and L-proline. The USP 24 describes HPLC method for its quantitative estimation[1].

Quantitative determination of enalapril maleate can be carried out by various reported methods like HPLC[2-8], HPLC-Mass spectrometry[9], Capillary zone eletrophoretic method[10], polarography[11], atomic absorption spectroscopy[12], and membrane selective electrodes[13]. All these methods are costly with regard to instrumentation set up cost and complex with regard to technology.

Some spectrophotometric methods have also been reported for quantitative estimation of enalapril maleate in bulk and in dosage forms. Enalapril maleate after dissolving in distilled water exhibits an absorbance maximum at 207 nm. Most solvents are not transparent in the region of 180-210 nm[14]. So the absorbance in this region is interfered by solvent/ solvent system absorbance. However, this property has been exploited to develop a UV method[15]. Enalapril maleate has also been estimated after derivatization with 2, 4-dinitroflurobenzene at pH 9 to a colored product which absorbs maximally at 356 nm[12]. Spectrophotometric methods, based on the ternary complex formation between enalapril maleate, copper (II), eosin[12]; and enalapril maleate, palladium (II), eosin[16], have been reported. The spectrophotometric methods reported for analysis of enalapril maleate in commercial dosage forms suffered disadvantage of heating at higher temperatures 100 °C and 70 °C[11, 17] and long analysis time[16].

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Nafisur Rahman and Sk Manirul Haque[18] have explained 4 methods based on reaction of carboxylic acid group of enalapril maleate with a mixture of potassium iodate and iodide, and on the charge transfer complexation reaction of enalapril maleate with p-chloranilic acid in 1, 4-dioxan-methanol medium, 3-dichloro 5, 6-dicyano 1, 4-benzoquinone in acetonitrile-1,4 dioxan medium and iodine in acetonitrile-dichloromethane medium. In all these methods harmful and costly organic solvents have been used. Also, a long list of chemicals has been used in all these methods. Difference spectrophotometric method[19] has also been used. But with difference spectrophotometric method, when tried, we did not always obtain the accurate and precise results. Considering these drawbacks, there was a need to develop more advantageous spectrophotometric methods for its quantitative estimation. A simple UV spectrophotometric method is presented here based on oxidation of enalapril maleate with potassium permanganate and sulphuric acid. The method is simple and has been found to be validated as per ICH guidelines Q2 (R1), 2005[20].

2. MATERIALS AND METHODS

2.1 Drug and chemicals used

All chemicals used were of reagent grade. Enalapril maleate was kindly gifted by Sun Pharmaceuticals Pvt. Ltd., Ahmedabad, India and was used as received.

2.2 Preparation of solutions

0.5% $KMnO_4$ was prepared by dissolving 1.0 g potassium permanganate in water in a 200 ml capacity volumetric flask and making the final volume up to the mark with the same solvent.

A 1000 µg/ml stock solution of enalapril maleate was prepared by dissolving accurately weighed 100 mg drug in water in 100 ml capacity volumetric flask and making the volume up to the mark with the same solvent.

2.3 Instrument used

A Shimadzu Pharmspec UV 1800 ultraviolet-visible spectrophotometer was used.

2.4 Procedure

The aliquots of 0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml of the prepared stock solutions were transferred to 10 ml capacity volumetric flasks so as to prepare the dilutions of 0, 250, 300, 350, 400, 450 and 500 µg/ml. To the flasks add 5.0, 2.5, 2.0, 1.5, 1.0, 0.5 and 0 ml water, respectively. Then add 1 ml of 0.5% potassium permanganate solution was added to each flask followed by addition of a volume of concentrated sulphuric acid to each flask so that the solution in each became colourless. Put aside for 10

minutes. The final volume was then made up to the mark with water. The calibration curve was prepared from these dilutions against the prepared blank by taking the absorbances of the prepared standard dilutions at 267 nm and plotting it against concentration.

2.5 Validation

The method was validated as per ICH guidelines Q2 (R1), 2005[20].

2.6 Specificity

The enalapril maleate solutions of known concentrations (300 µg/ml, 375 µg/ml and 450 µg/ml were prepared with and without some common excipients separately in the same manner as the standard dilutions were prepared. All solutions were scanned between 400 to 200 nm and checked for any difference in absorbance of the two types of the solutions at 267 nm. The spectra of the two solutions of were also observed for any change in wavelength of maximum absorbance.

2.7 Linearity

The linearity of the calibration curves prepared was determined using linear regression analysis.

2.8 Precision

Repeatability was assessed by analyzing different levels of drug concentrations from independent stock solutions (n=6). Intraday and interday variations in estimation were determined to assess intermediate precision of the proposed method. Different levels 80%, 100% and 120% of drug concentrations in 6 replicates were analyzed three times in a day for intraday variation. The same method was followed for three different days to study interday variation. The precision was determined as percent relative standard deviation.

2.9 Accuracy

The accuracy studies were performed by standard addition method at three levels i.e. 80%, 100% and 120%. The known amounts of the drug to a known concentration of the standard was added and analyzed.

2.10 Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug by the proposed method were determined using calibration standards. The LOD and LOQ were calculated as per equations 1 and 2 respectively, (ICH Guidelines Q2 (R1), 2005)[20].

$$LOD = 3.3 \left(\frac{SD_{Intercept}}{Slope} \right) \text{----------------------} 1$$

$$LOQ = 10 \frac{SD_{intercept}}{Slope} \text{----------------------} 2$$

where "$SD_{intercept}$" is standard deviation of the intercept of regression line and "Slope" is the slope of the calibration curve.

2.11 Robustness

The robustness was studied by performing the experiments after deliberately varying the reaction time by ±2 minutes, amount of concentrated sulphuric acid by ±0.1 ml and amount of 0.5% potassium permanganate solution by ±0.1 ml.

3. RESULTS AND DISCUSSION

The spectrophotometric determination of enalapril maleate was studied using UV spectrophotometer. This method was based on the oxidation of enalapril maleate by potassium permanganate and sulphuric acid.

Enalapril maleate showed absorbance maximum at 207 nm in aqueous medium. Most solvents being not transparent in the region of 180-210 nm, it was needed to derivatise before evaluation. The reaction of enalapril maleate with potassium permanganate and sulphuric acid was proceeded at room temperature in aqueous medium and it took 10 minutes to complete the reaction. After reaction with potassium permanganate and sulphuric acid, enalapril maleate exhibited a sharp peak at 267 nm (Figure 1) that was considered as λmax for plotting the calibration curve. The calibration curve is presented in Figure 2.
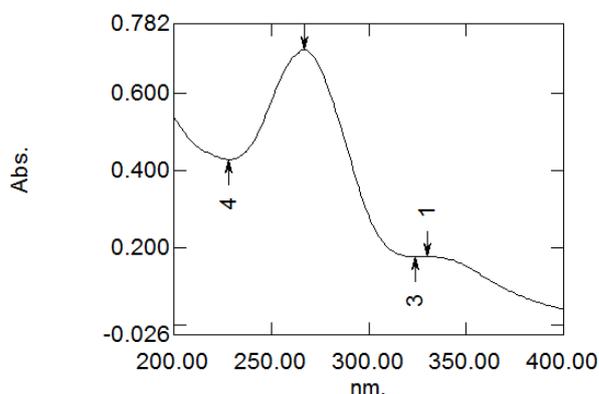
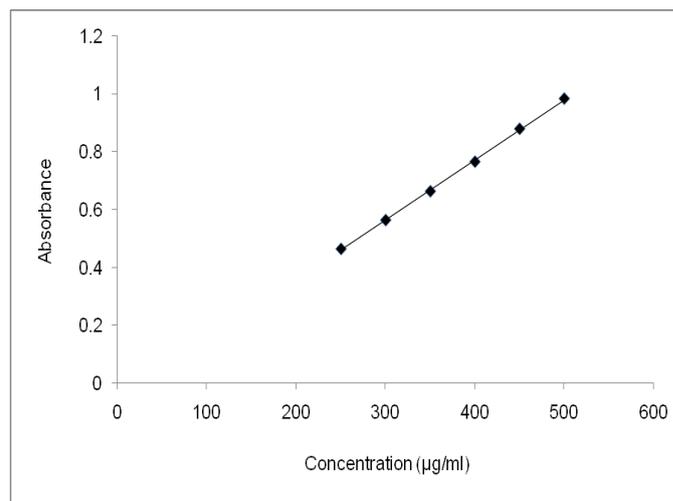

Fig.1: Spectrum of enalapril maleate after derivatization

Fig. 2: Calibration curve of enalapril maleate

The Beer's law was obeyed and validated from 250 µg/ml-500 µg/ml. The linear regression equation was found to be A=0.002C-0.059 (r=0.999, n=6) (Table No. 1), where C is the concentration of enalapril maleate in µg/ml.

The proposed method was validated as per ICH guidelines Q2 (R1), 2005. The results are represented in Table No. 2-6.

The method was found to be specific as indicated by less than 0.43% difference in absorbance of pure and impure (with common excipients) solutions at different concentration levels (300 µg/ml, 375 µg/ml and 450 µg/ml) of the drug (Table No. 2). There was also observed no difference in λmax (267 nm) of the drug in the two types of the solutions.

The low values of RSD (<0.75%, Table No. 3-5) indicated that the developed method was repeatable and precise.

The accuracy was performed by recovery studies (Table No. 6). The percent recovery of 100.01%±0.63%-101.16%±0.68% for the added known amounts of the drug to a known concentration of the sample indicated that the developed method is accurate.

The limit of detection (LOD) and limit of quantification (LOQ) of the drugs by proposed method were found to be 2.19 µg/ml and 6.65 µg/ml respectively.

The developed method was also found to be robust.

Table No.1: Regression parameters for calibration curves

Parameter	Value
Analytical wavelength (nm)	λmax= 267
Linearity range (μg/ml)	250-500
Regression equation[a]	Y= 0.002C-0.059
$SD_{Intercept}$ (n=6)	1.33×10^{-3}
Correlation coefficient (r)	0.999

[a]Y= Absorbance, C= Concentration,

$SD_{Intercept}$= Standard deviation of intercept,

λmax= Wavelength of maximum absorbance

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Table No. 2: Specificity studies for the developed analytical method

Concentration taken (μg/ml)	Absorbance* for		Difference in absorbance (A_c-A_t)	Difference in absorbance (%) (w. r. t. A_c)
	Solution without excipients (A_c)	Solution with excipients (A_t)		
300	0.542±0.0003	0.543±0.0003	-0.001	-0.18
375	0.690±0.0003	0.978±0.0002	0.003	0.43
450	0.841±0.0003	0.844±0.0003	-0.003	-0.36

*Mean±SD

Table No. 3: Repeatability studies for the developed analytical method

Concentration of drug solution (μg/ml)								RSD
Prepared	Found							
	1	2	3	4	5	6	Mean±SD	
10	10.06	09.98	10.00	10.08	10.12	10.04	10.05±0.05	0.50

Table No. 4: Intraday precision of developed analytical method

Concentration of drug solution (µg/ml)					RSD
Taken	Found				
	t1**	t2**	t3**	Mean*	
8	8.04±0.06	8.05±0.04	8.14±0.03	8.07±0.06	0.75
10	10.05±0.05	10.04±0.04	10.07±0.05	10.05±0.05	0.46
12	12.05±0.06	12.07±0.07	12.05±0.05	12.06±0.06	0.48

*Mean±SD of 18 determinations (6 replicate determinations every time for 3 points of time in a day), **Mean±SD of 6 replicate determinations

Table No. 5: Interday precision of developed analytical method

Concentration of drug solution (µg/ml)					RSD
Taken	Found				
	t1**	t2**	t3**	Mean*	
8	8.04±0.06	8.05±0.04	8.14±0.03	8.07±0.06	0.75
10	10.05±0.05	10.04±0.04	10.07±0.05	10.05±0.05	0.46
12	12.05±0.06	12.07±0.07	12.05±0.05	12.06±0.06	0.48

*Mean±SD of 18 determinations (6 replicate determinations every time for 3 points of time in a day), **Mean±SD of 6 replicate determinations

Table No. 6: Accuracy studies for the developed analytical method

C_s (µg/ml)	C_a (µg/ml)	C_t* (µg/ml)	%Recovery*[#]
5	3	8.04±0.02	100.51±0.75
	5	10.03±0.03	100.01±0.63
	7	12.11±0.05	101.16±0.68

*Mean±SD

4. CONCLUSION

The developed method is specific, repeatable, precise and accurate and does not require any laborious procedure. The method shows no interference from the common excipients and additives. Therefore, it is concluded that the proposed methods are simple, sensitive and rapid for the determination of enalapril maleate in commercial dosage forms.

5. ACKNOWLEDGEMENTS

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