Acta Pharmaceutica Sciencia
48: 195-203 (2006)

Determination of Enalapril Maleate in Pharmaceutical Preparations Using Methyl Orange

Enalapril Maleatın Metiloranj Kullanılarak Farmasötik Preparatlarda Miktar Tayini

Sevgi Tatar Ulu

Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, 34116-Beyazıt, Istanbul-Turkey

Abstract

A simple and sensitive spectrophotometric method has been developed for the determination of enalapril maleate in pharmaceutical preparations. This method is based on the formation of a yellow colored ion-pair complex by the reaction of methyl orange with enalapril maleate in acidic (pH 3.5) medium. Under optimised conditions, they showed absorption maxima at 425 nm. Linear relationship was obtained over the enalapril maleate concentration ranges of 4.0-20 μg/mL. The detection and quantification limits of enalapril maleate were 1.32 and 4.0 μg/mL, respectively. The proposed method is suitable for the routine analysis and quality control of enalapril maleate in pharmaceutical preparations.

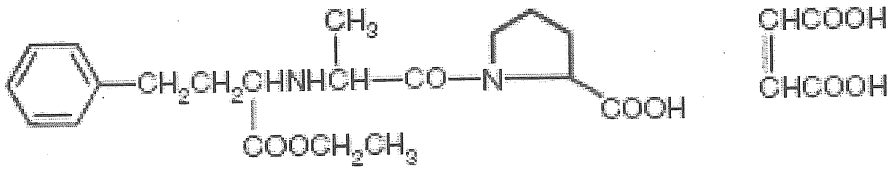
Keywords: Enalapril maleate, Methyl orange, Extraction spectrophotometry, Ion-pair complex, Pharmaceutical preparations

Introduction

Enalapril maleate (ENA), (N-[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl-L-proline hydrogen maleate) (Merck Index, 2001) (Fig.1).

Corresponding author: e-mail: sevgitatar@yahoo.com

(a)



(b)

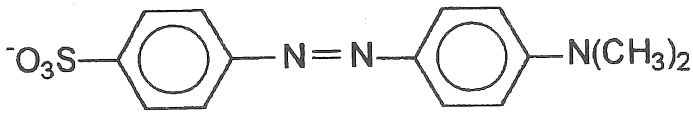


Fig.1. Chemical structures of enalapril maleate (a) and methyl orange (b)

ENA is an angiotensin-converting enzyme inhibitor widely used in the treatment of hypertension and heart failure. ENA is a pro-drug. Following oral administration, it is bioactivated by hydrolysis of the ethyl ester to enalaprilat, which is the active angiotensin-converting enzyme inhibitor (Dominic *et al.*, 1987).

Several methods have been presented in the literature for the determination of ENA in pharmaceutical preparations. Determination and rotamer separation of ENA by capillary electrophoresis have also been described (Qin *et al.*, 1992).

Derivative UV spectroscopic and liquid chromatographic methods have been developed for the determination of ENA in tablets (Carlucci *et al.*, 1993; Walily *et al.*, 1995; Bonazzi *et al.*, 1997).

^{1}H NMR spectroscopic method for quantitative analysis of ENA in tablets was studied (Zoppi *et al.*, 1995). Spectrophotometric and atomic absorption spectrometric methods were studied for determination of ramipril and ENA through ternary complex formation (Ayad *et al.*, 2002).

ENA was determined in tablets by spectrophotometry and polarography (Abdel Razak *et al.*, 2003).

The aim of this study was to develop a simple and sensitive spectrophotometric method for determination of ENA in pharmaceutical preparations by ion-pair

complex formation between the drug and methyl orange. The reaction conditions and the application of the method for the determination of ENA in pharmaceutical preparations have been established. The proposed method is simple, rapid, economic and provides sensitivity.

Materials and Methods

Apparatus: UV-VIS spectrophotometer (Shimadzu UV-160 A) with data processing system was used for solutions in 1 cm quartz cells. A WTW pH meter was used for pH measurements.

Reagents: ENA and its tablets (Enapril ® 5 mg) were kindly supplied from Ilsan-Iltas (Istanbul, Turkey). Methyl orange (MO) was purchased from Merck (Darmstadt, Germany). All chemicals were of analytical reagent grade and were provided by Merck (Darmstadt, Germany).Ultra pure water (aquaMAX™ ultra, Young instrument, Korea) was used for the experimental work.

Solutions: The stock solutions of ENA were prepared in methanol (1.0 mg/mL). Standard solution was prepared with the concentration of ENA 100 µg/mL. This solution was used as a standard for the determination of ENA.

MO solution was prepared in water with a concentration of 0.02 % (w/v). Phthalate buffer was prepared by dissolving 1.280 g of potassium hydrogen phthalate in 50 mL of water. The pH was adjusted to 3.5 with 0.2 M HCI solution and the volume was completed to 250 mL with water.

Hydrochloric acid solution was prepared by appropriate dilution of concentrated hydrochloric acid.

General procedure: Into 5 mL glass tubes, 1.0 mL of buffer solution of pH 3.5 and 1.0 mL of MO were placed. An appropriate volume of 100 µg/mL working solution (0.2–1.0 mL) was added and mixed. After 2 min vortexing, the tubes were allowed to stand. The ENA-MO complex was extracted three times with 1.5 mL of dichloromethane. The combined dichloromethane extracts were dried over anhydrous sodium sulphate and the volume was adjusted to 5 mL with dichloromethane and the

absorbance of the extract was measured at 425 nm, against a blank, which was prepared similarly without ENA.

Procedure for tablets: The contents of twenty tablets of ENA were accurately weighed and powdered. The powder with weight equivalent to 100 mg of ENA was dissolved in methanol by thorough mixing, then filtered and completed to volume in a 100 mL volumetric flask. A 1 mL volume of the filtrate was adjusted to 10 mL with methanol in a calibrated flask (100 µg/mL). This solution was analyzed as in the general procedure.

Results and Discussion

The absorption spectrum of ENA-MO complex under optimum conditions is shown in Figure 2. The maximum absorbance of the complex was observed at 425 nm. The reagent blanks prepared under similar conditions showed no absorption.

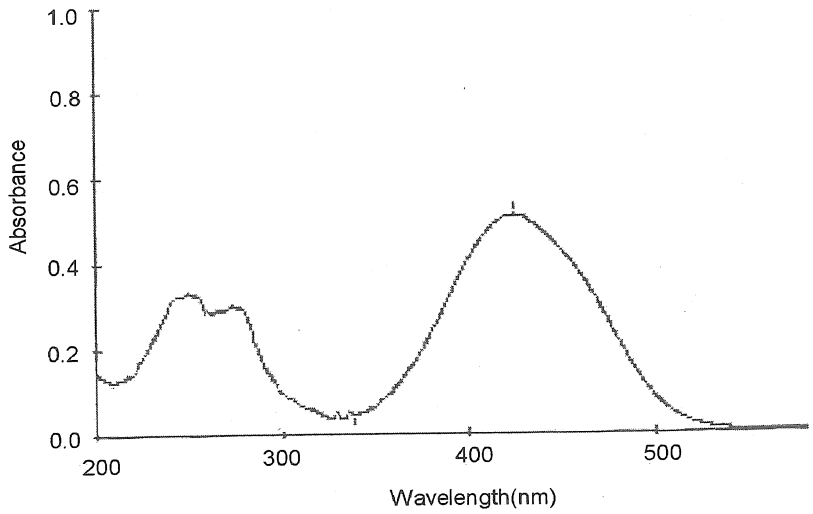


Fig. 2. Absorption spectrum of ENA-MO ion pair complex

The effect of pH on the drug–reagent complex was investigated over the pH range 2.5–4.5 using phthalate buffers, where the maximum absorbance was obtained at pH 3.5 as shown in Figure 3.

When the general procedure was followed with varied amounts of 0.02% MO concentration, maximum and constant absorbance was obtained with 1.0 mL.

The absorption spectrum of ENA-MO complex was studied in different organic solvents (dioxane, acetonitrile, chloroform, dichloromethane). Dichloromethane was preferred to the other solvents for proposed method for its selective and quantitative extraction.

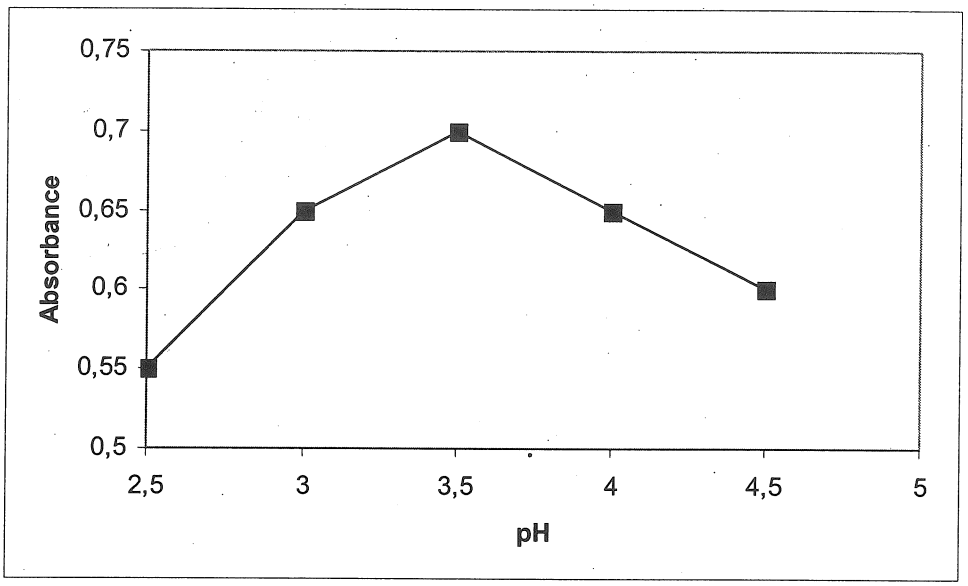


Fig. 3. Effect of pH on the reaction of ENA-MO

The optimum reaction time was investigated by following the color development at ambient temperature (25±2 °C). Complete color intensity was attained after 5 min of mixing for the complex.

The molar ratio of the drug to the reagent in the complex formed was investigated by Job's method of continuous variations which were found to be 1:1 (Figure 4). The absorbance values were then plotted against the molar ratio 3.5.

Linear correlations were observed between absorbances and concentrations over the range of 4.0-20 µg/ mL. The equations of the calibration curves were obtained by the least - squares linear regression analysis and calculated as: $A= 0.026C -8.6x10^{-1}$ with r=0.9999 (Table 1)

The concentration range is quite low when compared to the methods in the literature (Bonazzi *et al.*, 1997; Ayad *et al.*, 2002; Abdel Razak *et al.*, 2003).

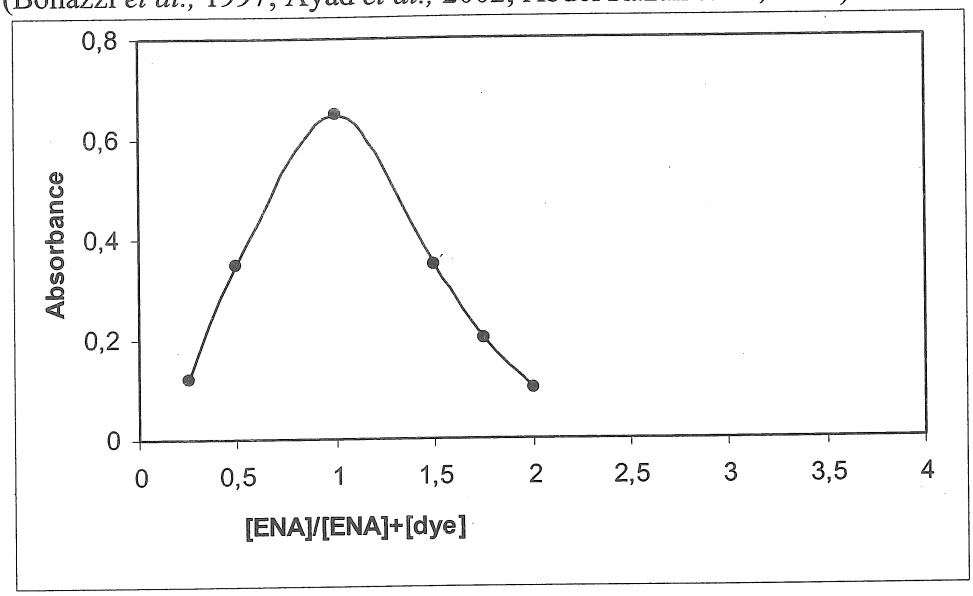


Fig.4. Job's method

Table 1. Results of regression analysis of the linearity data of ENA

	Mean ± SD (n = 6)
Slope	$0.026 \pm 2.86 \times 10^{-4}$
Intercept	$8.6 \times 10^{-1} \pm 0.0104$
Correlation coefficient(r)	0.9999 ± 0.0012

The limit of detection was calculated by $LOD = 3.3\sigma/S$, where σ is the standard deviation of the response of the blank or intercept and S is the slope of the calibration curve. The limit of quantification was calculated by $LOQ = 10\sigma/S$ under the ICH guidelines (Methodology ICH Harmonised Tripartite Guideline, 1996). LOD and LOQ were 1.32 and 4.0 µg/mL, respectively. In order to determine the precision of the method, solutions containing known amounts of drug were prepared and analyzed in six replicates. The intra-day RSD at 4.0, 10.0 and 20.0 µg/mL of ENA were 1.12%, 0.78% and 0.65% (n = 7), respectively, indicating good intra-day

precision. The inter-day RSD at the above concentrations were 1.28%, 0.83% and 0.76 % ($n = 7$), respectively.

A recovery study was performed to establish the accuracy of the procedure. This study was performed by adding known amounts of the studied compounds to a known concentration of the commercial pharmaceutical tablets (standard addition method). The value of the mean recovery obtained by the standard addition method was 99.34% with standard deviation of 0.67% (the analytical measurements were repeated five times).

Ingredients of tablets such as starch, lactose, glucose and stearic acid did not interfere in the proposed method.

Having been done a research into the stability of ENA-MO complex, it was observed that while it remained stable in dichloromethane at 4°C in the dark for 4 days, the ENA-MO complex lacked the absorbance at room temperature in the dark and in daylight for 12 hours.

Analytical applications: The proposed method was successfully applied to determine ENA in its tablets. The results obtained were compared statistically by Student's *t*-test and variance ratio *F*-test, with the reference method (Bonazzi et al., 1997) at 95% confidence level as recorded in Table 2. The results showed that the *t*- and *F*-values were smaller than the critical values indicating that there was no significant difference between the proposed and reference methods.

Table 2. Determination of ENA in tablets (Enapril® 5 mg) using the proposed method compared statistically to a reference method (n=6)

Statistical value	**Proposed method**	**Reference method**
Mean	4.97	4.95
Recovery (%)	99.40	99.00
RSD (%)	0.67	0.72
t-test of significance *	0.34	
F-test of significance *	1.15	

p = 0.05, t = 2.23, F = 5.05

Conclusion

The proposed method is simple, sensitive, rapid, precise and accurate for determination of ENA in pharmaceutical preparations when compared with the other methods, especially with HLPC (Carlucci *et al.*, 1993; El Walily *et al.*, 1995; Bonazzi *et al.*, 1997; Ayad *et al.*, 2002; Zoppi *et al.*, 2005). The proposed method may be applied for routine analysis and in quality control laboratories for the quantitative determination of the ENA in pharmaceutical preparations.

Özet

Enalapril maleatın farmasötik preparatlarda tayini için basit ve duyarlı spektrofotometrik bir yöntem geli□tirildi. Bu metot, enalapril maleatın metiloranj ile asidik ortamda (pH 3.5) sarı renkli iyon çifti kompleksi olu□turması esasına dayanmaktadır. Optimize edilmi□ko□ullarda maksimum absorbsiyon 425 nm'dir. Enalapril maleatın lineer konsantrasyon aralı□ı 4.0 -20 µg/mL olarak saptandı. Enalapril maleatın te□his ve tayin sınırları sırasıyla 1.32 ve 4.0 µg/mL dır. Geli□tirilen yöntem enalapril maleatın farmasötik preparatlardaki rutin analizleri ve kalite kontrolü için uygundur.

References

Abdel Razak, 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. Pharm. Biomed. Anal.* 31: 701-711.

Ayad, M.M., Shalaby, A.A., Abdellatef, H.E. and Hosny, M.M. (2002). Spectrophotometric and AAS determination of ramipril and enalapril through ternary complex formation. *J. Pharm. Biomed. Anal.* 28: 311-321.

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: 431-438.

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: 245-248.

Dominic, P.I., Gerald, S.B. and Florey, K. (1987). *Analytical Profiles of Drug Substances* 16: 207–243.

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. Biomed. Anal.* 13:851-856.

Methodology ICH Harmonised Tripartite Guideline "Validation of analytical procedures" Having reached Step 4 of the ICH Process at the ICH Steering Committee meeting on, 6 November, 1996.

The Merck Index (2001). Merck & Co. Inc., NJ, USA, pp. 630-631.

Qin, X.Z., Ip, D.P. and Tsai, E.W. (1992). Determination and rotamer separation of enalapril maleate by capillary electrophoresis. *J. Chromatogr. A* 626: 251-258.

Zoppi, A., Linares, M. and Longhi, M. (2005). Quantitative analysis of enalapril by ^{1}H NMR spectroscopy in tablets. *J. Pharm. Biomed. Anal.* 37: 627-630.

Received: 07.06.2006
Accepted: 05.09.2006