ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 851–856

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Simultaneous determination of enalapril maleate and hydrochlorothiazide by first-derivative ultraviolet spectrophotometry and high-performance liquid chromatography

Abdel Fattah M. El Walily [a,*], Saeid F. Belal [a], Eman A. Heaba [b], Ali El Kersh [b]

[a] *Department of Analytical Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt*

[b] *Quality Control Laboratories, Pharco Pharmaceutical Co., P.O. Box 12 Sidi Gaber, Alexandria, Egypt*

Received for review 23 June 1994; revised manuscript received 29 November 1994

Abstract

Two methods are described for the simultaneous determination of enalapril maleate and hydrochlorothiazide in combined pharmaceutical tablets. The first method depends on first-derivative ultraviolet spectrophotometry, with zero-crossing and peak-to-base measurement methods. The first-derivative amplitudes at 224 and 260 nm were selected for the assay of enalapril maleate and hydrochlorothiazide, respectively. The second method is based on high-performance liquid chromatography on a reversed-phase column using a mobile phase of acetonitrile–water (20:80, v/v) (pH 3.8) with programmable detection at 215 and 275 nm. Both methods showed good linearity, precision and reproducibility. The proposed methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and in commercial tablets.

Keywords: Enalapril maleate determination; First-derivative spectrophotometry; High-performance liquid chromatography; Hydrochlorothiazide determination; Pharmaceutical tablets

1. Introduction

Enalapril maleate is a relatively new oral angiotensin-converting enzyme (ACE) inhibitor. Enalapril maleate, like its first generation relative captopril, has been shown to be effective in the treatment of hypertension and congestive heart failure [1].

Enalapril [(*S*)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline] is a prodrug which is de-esterified in the hepatic system to an active diacid form [2]. The drug is official in the USP XXII [3]. The methods of analysis of the bulk drug and its tablets are high-performance liquid chromatography (HPLC) methods. Recently, enalapril maleate has been marketed in combination with hydrochlorothiazide in tablets. The tablet manufacturer claims that the combined oral administration of enalapril maleate with hydrochlorothiazide has been found to be more effective than either drug alone in the treatment of hypertension.

Other than the published analytical profile [4], a literature survey reveals very few analytical reports for the analysis of enalapril maleate. The analysis of enalapril maleate in pharmaceutical dosage forms has been reported via

* Corresponding author.

SSDI 0731-7085(95)01346-6

flow injection [5], HPLC techniques [6–8] and capillary electrophoresis [9]. Hydrochlorothiazide is one of the oldest and widely used thiazide diuretics. The drug is official in both BP 93 [10] and USP XXII [3] either alone or in binary mixtures with other drugs. The analytical profile of hydrochlorothiazide including several references to analytical methods for the determination of hydrochlorothiazide has been reviewed [11].

The enalapril maleate–hydrochlorothiazide mixture is not yet official in any pharmacopoeia. To our knowledge, no spectrophotometric methods have been described for the simultaneous determination of both drugs in tablets. Therefore, it was desirable to develop a simple and fast procedure that could be applied in quality control laboratories for the determination of both drugs in the presence of each other. In this report, two methods based on UV-derivative spectrophotometry and reversed-phase HPLC are reported for the quantification of both drugs. The utility of the developed methods to determine the content of both drugs in commercial tablets is also demonstrated.

2. Experimental

2.1. Materials

Authentic enalapril maleate and hydrochlorothiazide were used as supplied (Chem Iberica SA, Barcelona, Spain). The purity of both drugs as assessed by the USP XXII methods was 98.5% and 99.1% for enalapril maleate and hydrochlorothiazide, respectively. Caffeine, used as an internal standard in the HPLC method, was an in-house standard and its purity was certified to be 99.2%. Acetonitrile (BDH, Poole, UK) was of HPLC grade; water was doubly distilled from all glass apparatus. All other chemicals were analytical reagent grade.

2.2. Apparatus

Spectrophotometric analysis was performed on a Hewlett-Packard Diode-Array spectrophometer Model HP/8451A using a 1-cm quartz cell and bandpass of 2 nm. The instrument settings were: derivative mode ^{1}D ($dA/d\lambda$) with seven smoothing points and a wavelength range of 300–200 nm. The notation for the amplitude measurements in the derivative mode was made according to Fasanmade and Fell [12].

The high-performance liquid chromatograph was composed of an Isco gradient programmer mixer Model 2360/2361 (Lincoln, Nebraska, USA) and Hewlett-Packard (Avondale, PA, USA) Model HP 1050 pump, an HP Model 1050 variable wavelength detector, an HP Model 1050 autosampler and an HP-3396A integrator.

2.3. Chromatographic conditions

Chromatographic separation was carried out at ambient temperature on a 12-μm Hypersil C-18 column (250 × 4.6 mm i.d.) (Shandon Scientific Ltd, Cheshire, UK). The compounds were separated isocritically with a mobile phase consisting of a mixture of acetonitrile–water (20:80, v/v) with the pH of the water adjusted to 3.8 with acetic acid. The flow rate was 1 ml min^{-1}. The mobile phase was degassed for 15 min in an ultrasonic bath before use. The analysis was usually started after the passage of 20–30 ml of mobile phase to reach equilibrium. The injection volume was 5 μl. The eluted analytes were detected by UV measurements at 215 nm for 3.5 min and then changed to 275 nm to the end of the run.

2.4. Standards solutions and calibration graphs for spectrophotometric measurements

Stock solutions were prepared by dissolving enalapril maleate and hydrochlorothiazide in 0.1 M hydrochloric acid to obtain a concentration of 1 mg ml^{-1} for each compound. The standard solutions were prepared by dilution of the stock solutions in 0.1 M hydrochloric acid to reach concentration ranges of 2–24 and 1.25–15 μg ml^{-1} for enalapril maleate and hydrochlorothiazide, respectively.

2.5. UV measurements

The ^{1}D-curves of the working acidic standard solutions containing varying amounts of each drug were scanned in the range of 300–200 nm against 0.1 M hydrochloric acid as a blank. The values of the amplitudes at 224 (zero crossing of hydrochlorothiazide) and 260 (peak-to-base) nm were measured, and the concentrations vs. their absolute ^{1}D amplitudes were plotted in order to obtain the calibration graphs.

2.6. Standard solutions and calibration graphs for chromatographic procedure

Standard solutions of enalapril maleate and hydrochlorothiazide containing concentration ranges of 50–300 and 30–180 μg ml^{-1}, respectively, and a fixed concentration (500 μg ml^{-1}) of caffeine (internal standard) were prepared in the mobile phase. Triplicate 5 μl injections were made for each solution and the peak area ratios of each drug to the internal standard were plotted against the corresponding concentrations to obtain the calibration graphs.

2.7. Sample preparation

Ten tablets containing enalapril maleate and hydrochlorothiazide as active ingredients were weighed and finely powdered. A portion of the powder equivalent to about 20 mg of enalapril maleate was weighed accurately, transferred to a 100-ml volumetric flask and either suspended in 0.1 M hydrochloric acid or in the mobile phase. The flasks were completed to volume with the same solvent. The HPLC samples were filtered through a 0.45-μm membrane filter, while the acidic aqueous solutions were filtered through wetted filter and then further diluted to suit the calibration graphs for the derivative measurements.

3. Results and discussion

The UV spectrum of an acidic aqueous solution of hydrochlorothiazide showed absorption maxima at about 226 and 274 nm (Fig. 1). The enalapril maleate UV absorption spectrum is typical of an unconjugated phenyl moiety which is not useful for characterization of quantification. Thus, conventional UV spectrophotometry cannot be used for the quantification of both drugs in the presence of each other. Although enalapril maleate does not show any significant absorption in the vicinity of the second maximum of hydrochlorothiazide, it exerts a negative absorption contribution in synthetic mixtures. Because the derivative spectrophotometric technique enhances the detectability of the minor features of the UV absorption spectrum [13], the ^{1}D spectra of both enalapril maleate and hydrochlorothiazide (Fig. 2) display features which may permit the determination of both drugs in the presence of each other. The ^{1}D

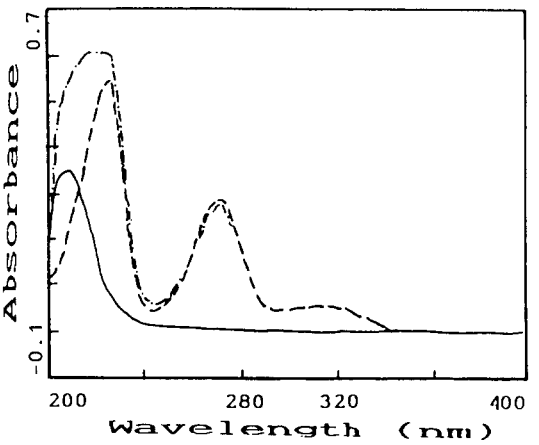

Fig. 1. Zero-order spectra of 8 μg ml^{-1} of enalapril maleate (——); 5 μg ml^{-1} of hydrochlorothiazide (– – –) and a mixture (–·–·–). A 0.1 M solution of hydrochloric acid was used as a blank.

amplitudes at 224 nm (zero-crossing of hydrochlorothiazide) and at 260 nm (peak-to-base line, nil contribution from enalapril maleate) were chosen for the simultaneous determination of enalapril maleate, and hydrochlorothiazide, respectively, in a binary mixture. However owing to the low absorptivity of enalapril maleate and in order to achieve maximum derivative response and accuracy; two different derivative ordinates were used.

Linear relationships between the selected amplitudes from the ^{1}D spectra and drug concentration were observed. Regression analyses were carried out on the slopes, intercepts, correlation coefficient (r) and variances (S_0^2) at $p = 0.05$ level of significance, for $n = 8$ standard specimens (Table 1). Interaction studies by varying enalapril maleate and hydrochlorothiazide concentrations showed that the selected derivative amplitudes were independent of the presence of the other component; in fact, the recovery was, in every instance, very close to 100%. (Table 2).

A critical evaluation of the proposed derivative method was performed by statistical analysis of the experimental data. The linearity of the calibration graphs, the adherence of the system to Beer's law and the negligible scatter of the experimental points were validated by the high values of the correlation coefficients of the regression equations and the values of the intercepts on the ordinate, which were close to zero.

For additional validation, the experimental intercepts, a, of the regression lines were tested for significant deviation from the calculated the

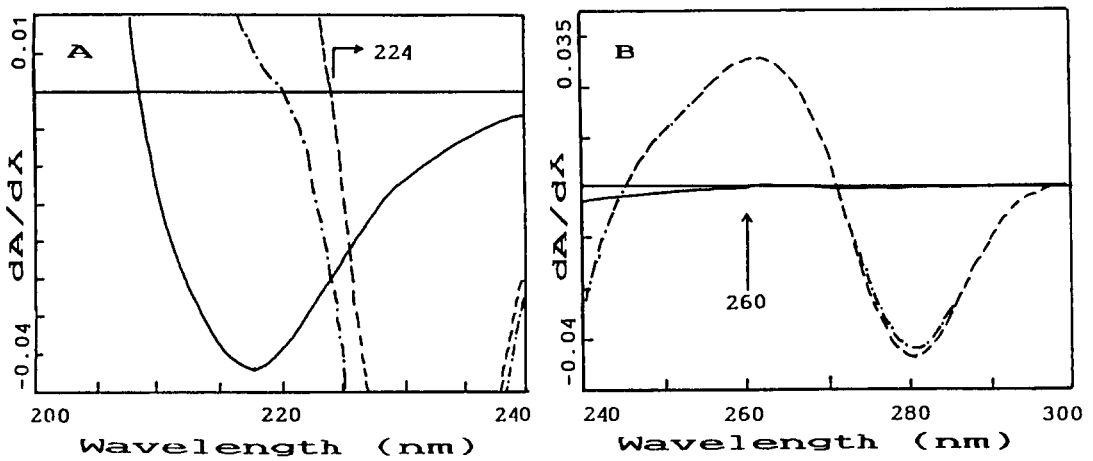

Fig. 2. (A and B) First-order spectra of 16 μg ml^{-1} of enalapril maleate (——); 10 μg ml^{-1} of hydrochlorothiazide (——) and a mixture (– · – · –). 0.1 M of hydrochloric acid was used as a blank.

Table 1
Statistical analysis of the determination of enalapril maleate and hydrochlorothiazide in mixture by derivative spectrophotometry and HPLC methods [a]

Compound	Concentration range (μg ml^{-1})	a	b	r	S_0^2
Derivative method					
EN	2–24	1.40E-4	1.52E-3	0.9999	3.02E-8
HY	1.25–15	2.70E-4	3.02E-3	0.9998	8.43E-8
HPLC method					
EN	50–300	− 1.19E-3	9.04E-3	0.9999	3.37E-6
HY	30–180	− 3.87E-4	9.33E-3	0.9999	1.42E-7

[a] a = intercept, b = slope, r = correlation coefficient and S_0^2 = variance.

Table 2
Assay resuts for the determination of Enalapril maleate and hydochlorothiazide in laboratory synthetic mixture and commercial tablets

Sample	Recovery (mean ± SD)% [a]			
	Enalapril maleate		Hydrochlorothiazide	
	HPLC	Der	HPLC	Der
Synthetic mixtures	100.45 ± 0.41	100.28 ± 0.49	100.20 ± 0.30	100.16 ± 0.24
	t = 0.59		0.23 (2.23) [b]	
	F = 1.43		1.56 (5.19) [b]	
Commercial tablets [c]	99.78 ± 0.34	99.67 ± 0.47	99.87 ± 0.52	100.05 ± 0.74
	t = 0.42		0.44	
	F = 1.91		2.02	

[a] mean and relative standard deviation for six determinations; percentage recovery from the label claim amount.
[b] values in parentheses are the theoretical values at p = 0.95
[c] Co-Renitec tablets are the product of Merck Sharp and Dohme (UK); each tablet was labeled to contain 20 and 12.5 mg of enalapril maleate and hydrochlorothiazide, respectively.

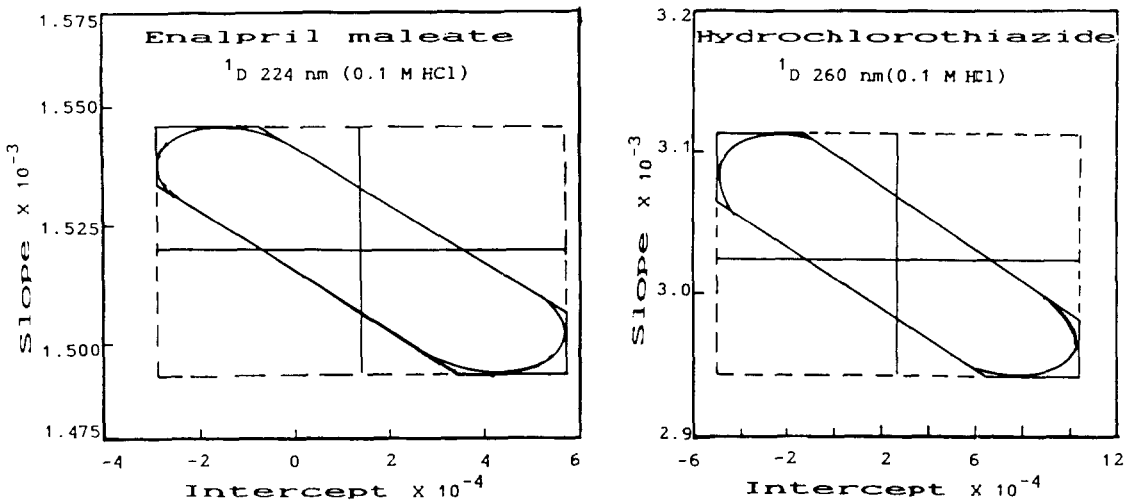

Fig. 3. 95% joint confidence regions for the slopes and intercepts of the regression equations of enalapril maleate and hydrochlorothiazide by the ^{1}D method.

values $t = a/S_a$ [14] and to compare them with the corresponding tabulated data for Student's t-distribution (S_a is an estimate of the accuracy of the determination of a, which is calculated by the expression $S_a = S_0^2\Sigma C^2/(n\Sigma C^2 - (\Sigma C)^2)$, in which S_0^2 is the variance and C is the concentration). The values calculated for t were 1.04 and 1.12 for enalapril maleate and hydrochlorothiazide at 224 and 260 nm, respectively, which do not exceed the 95% criterion $t = 2.31$ ($p = 0.05$).

However, this procedure ignores the strong correlation existing between slopes and intercepts. In a more rigorous approach, the 95% joint confidence regions [15] were drawn for the slopes and intercepts of the regression equations displayed in Table 1. These regions were bounded by an ellipse having the point of best fit as its center (Fig. 3). It can be seen that the points with an intercept of zero fall well within the ellipses, confirming the conclusion that there is no significant deviation from zero.

3.1. HPLC methods

The reversed-phase HPLC method was developed to provide a specific procedure suitable for the rapid quality control analysis of binary mixtures containing enalapril maleate and hydrochlorothiazide, and as referee method for the derivative assay. The method involves use of an RP-C_{18} column and a mobile phase consisting of acetonitrile–water (20:80, v/v) (pH 3.8). The mobile phase was chosen after several trials with acetonitrile–water and methanol–water in various proportions and different pH values. The change in the wavelength of detection during the run was performed to achieve maximum detector response for both drugs. The chromatographic system described allows complete base line separation with a resolution factor better than 2 between adjacent peaks (Fig. 4). The linearity of the detector response for enalapril maleate and hydrochlorothiazide was determined by plotting peak area ratios vs. concentration. The analytical data for the calibration graphs are listed in Table 1.

In order to demonstrate the validity and applicability of the proposed methods (HPLC and UV-derivative), recovery studies were performed by analyzing synthetic mixtures that reproduced the composition of the commercial tablets. The results obtained (Table 2) were statistically compared using Student t- and the F-test. As shown from the Table, the calculated r- and F-values were less than the theoretical values, indicating no significant difference between the two methods. Commercially available tablets containing a mixture of enalapril maleate and hydrochlorothiazide were analyzed using the developed methods. The results are summarized in Table 2.

4. Conclusions

It is concluded that the described methods have the potential for application in quality control laboratories, as they permit rapid, precise and accurate analyses of enalapril maleate–hydrochlorothiazide mixtures in tablets without

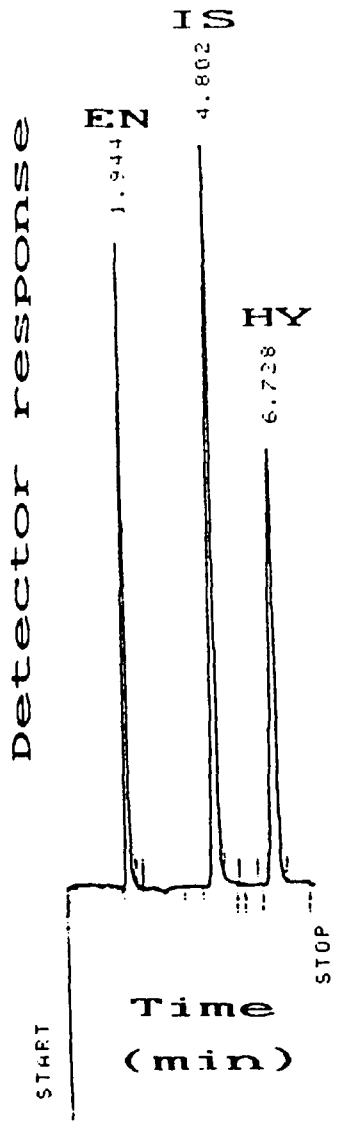

Fig. 4. Typical chromatogram of 5 μl of the two drugs with the internal standard (IS). Enalapril maleate, hydrochlorothiazide and caffeine (IS).

prior separation, and are easily applied for routine use. The most striking fearure of the derivative method is its simplicity, i.e. the fact that sophisticated chromatographic insturmentation is not needed for its performance.

Acknowledgment

The authors wish to thank the authorities of Pharco Pharm. Co., for permission to publish this work, the Shandon scientific Ltd, HPLC division Co. for Supplying the hypersil HPLC column as gift for trials, and Mrs. Ghada Abou Khatwa for helping in the statistical part as well as typing the manuscript.

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