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Simultaneous determination of enalapril maleate and hydrochlorothiazide in tablets by derivative UV spectrophotometry and high-performance liquid chromatography

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Summary

A method for the simultaneous determination of enalapril maleate and hydrochlorothiazide in pharmaceutical formulations (tablets) is described. The procedure is based on the use of the high-performance liquid chromatography (HPLC), and of the second-derivative ultraviolet spectra, by utilizing the linear relationship between substances concentration and derivative peak amplitude. The minimum concentration detectable by derivative spectrophotometry was 1 μg ml^{-1} for both drugs, and by HPLC 50 ng ml^{-1} for hydrochlorothiazide and 100 ng ml^{-1} for enalapril maleate. The relative standard deviations observed were approx. 2% for derivative spectrophotometry, and 1.5% for HPLC. The proposed methods, which give thoroughly comparable data, are simple and rapid, and allow precise and accurate results.

Enalapril (**I**) maleate, or (*S*)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline maleate, a synthetic peptidic derivative, is a long-acting oral inhibitor of angiotensin converting enzyme (ACE), which reduces the plasmatic concentrations of angiotensin II and aldosterone, and increases the plasmatic activity of renin (Drummer et al., 1990; Weisser et al., 1991). Hydrochlorothiazide (**II**), or 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, is a diuretic of the class of benzothiadiazines (Beyer, 1958).

The two drugs are used in association in the treatment of hypertension (Sassano et al., 1989; Von Pölnitz et al., 1991). So far no method available for the simultaneous determination of these compounds in pharmaceutical forms or biological fluids has been described.

This paper reports a simple and fast method for the simultaneous quantitation of the two drugs in tablets by derivative UV spectrophotometry and HPLC, providing accurate and precise results.

Enalapril maleate was kindly supplied by Sigma-Tau (Pomezia, Italy); hydrochlorothiazide

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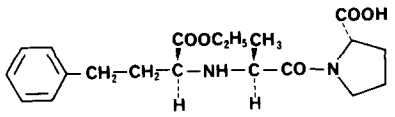

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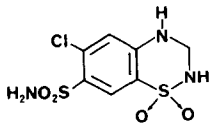

II

Scheme 1

was purchased from Sigma Chimica (Milan, Italy). Methanol and all other chemicals (analytical grade) were obtained from Fluka Chemie (Buchs, Switzerland). HPLC grade acetonitrile was purchased from Farmitalia-Carlo Erba (Milan, Italy). Water was purified and deionized using a Milli-Q ion exchange filtration system (Millipore, Bedford, MA, U.S.A.). Water was filtered through WCN 0.45 μm filters, while acetonitrile was filtered through WTP 0.5 μm filters (Whatman, Maidstone, U.K.).

The chromatographic apparatus (Waters Assoc., Milford, MA, U.S.A) consisted of a model 510 solvent delivery system, and a model 484 spectrophotometric detector connected to a model HP-3396-II integrator (Hewlett-Packard, Rome, Italy). A model 7125 sample injector (Rheodyne, Cotati, CA, U.S.A.) equipped with a 20 μl loop was used.

The separation was performed on a reversed-phase LiChrosorb RP-18 (250 × 4.6 mm, 10 μm particle size) column (Chrompack, Milan, Italy). The mobile phase consisted of a mixture of 0.02 mol dm^{-3} phosphate buffer (pH 3.0)-acetonitrile (50:50 v/v). The mobile phase was prepared daily, filtered, sonicated before use, and delivered at a flow rate of 0.7 ml min^{-1}. The detector wavelength was set at 220 nm.

A Perkin-Elmer model Lambda 5 UV-Vis spectrophotometer was used. Derivative conditions were as follows: scan speed, 60 nm min^{-1}; spectral slit width, 2 nm; $\Delta\lambda$, 6 nm.

Standard solutions for HPLC were prepared with the mobile phase by varying the concentration of a drug in the range 0.5–30 μg ml^{-1}, and maintaining the other one at a constant level of 5 μg ml^{-1}. Standard solutions for derivative spectrophotometry were prepared in methanol by varying the concentration of a drug in the range 1–10 μg ml^{-1}, and maintaining the other one at a constant level of 5 μg ml^{-1}.

Calibration curves for HPLC analysis were constructed by plotting the peak area against the relative concentrations of each drug. Calibration curves for derivative spectrophotometry were obtained by plotting vs the drug concentration the following peak-trough amplitudes in the second-derivative UV spectrum: 198–205 nm (for enalapril maleate), and 225–233 nm (for hydrochlorothiazide).

The equations, obtained through regressional analysis of data for the above standard solutions (each datum average of a minimum number of five determinations), were:

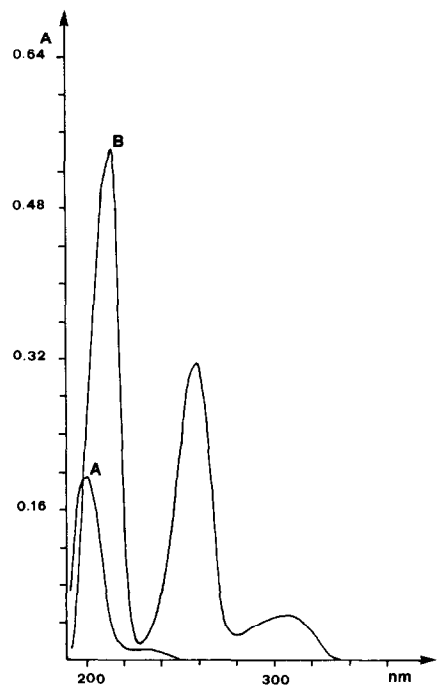

Fig. 1. Zero-order spectrum of a solution in methanol of enalapril maleate (A) (5 μg ml^{-1}), and hydrochlorothiazide (B) (5 μg ml^{-1}).

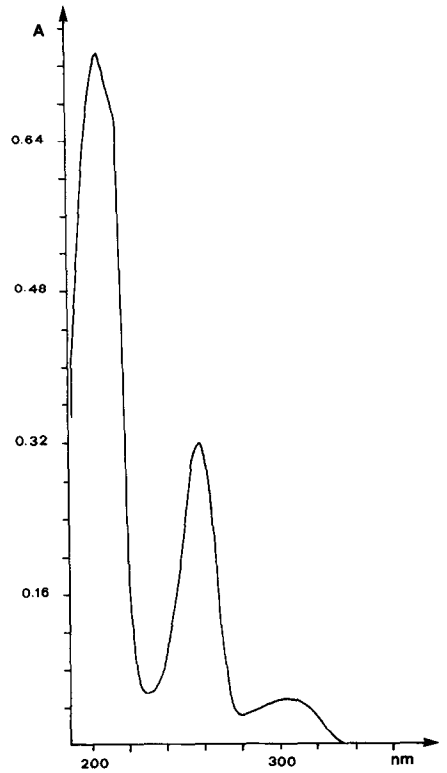

Fig. 2. Zero-order spectrum of a mixture of enalapril maleate (8 μg ml^{-1}) and hydrochlorothiazide (5 μg ml^{-1}) in methanol.

(1) For HPLC

(a) For enalapril maleate

$$y = 2.85 \times 10^4 x - 4.02 \times 10^3 \ (r = 0.9996)$$

(b) For hydrochlorothiazide

$$y = 1.46 \times 10^5 x + 1.02 \times 10^4 \ (r = 0.9995)$$

where y is the peak area in the arbitrary units of the HP-3396-II system used and x denotes the drug concentration (μg ml^{-1})

(2) For derivative spectrophotometry

(a) For enalapril maleate

$$y = 0.508x + 0.178 \ (r = 0.996)$$

(b) For hydrochlorothiazide

$$y = 0.867x + 0.0710 \ (r = 0.9997)$$

where y is the peak-trough amplitude between 198 and 205 nm (enalapril maleate), and between 225 and 233 nm (hydrochlorothiazide) in the second-derivative spectrum, measured on the scale ± 6 and x denotes the drug concentration (μg ml^{-1})

Five tablets were crushed and combined. An amount of material was accurately weighed, added with methanol, transferred in a 100 ml calibrated flask, and completed to volume with methanol. The solution obtained was diluted with methanol so as to obtain a concentration of the two drugs in the range of linearity previously determined, and analysed by derivative spectrophotometry by using the above calibration equations. The same procedure was used for HPLC analysis by utilizing the mobile phase instead of methanol.

Fig. 1 shows the zero-order spectra of enalapril maleate and hydrochlorothiazide alone, and Fig. 2 those of a mixture of the two drugs at the

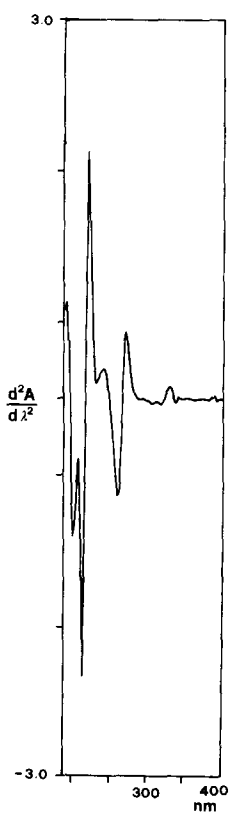

Fig. 3. Second-derivative spectrum of a mixture of enalapril maleate (8 μg ml^{-1}) and hydrochlorothiazide (5 μg ml^{-1}) in methanol.

concentration value of the pharmaceutical forms. The impossibility of proceeding to the simultaneous determination of the two compounds through the ordinary spectrophotometric technique therefore becomes evident because of the overlapping of the relative absorbance spectra.

Fig. 3 demonstrates the possibility of a simultaneous quantitation of the two drugs in pharmaceutical formulations by using their second-derivative spectra, and by utilizing the linear relationship between substance concentration and derivative peak amplitude previously reported.

The minimum concentration detectable by this procedure was 1.0 μg ml^{-1} for both drugs. The relative standard deviation observed was approx. 2%.

Fig. 4 shows a typical HPLC chromatogram; the retention times were 3.7 min for enalapril maleate and 5.0 min for hydrochlorothiazide. The lower detection limit of the method was 50 ng ml^{-1} for hydrochlorothiazide and 100 ng ml^{-1} for enalapril maleate. The relative standard deviation observed was approx. 1.5%.

Table 1 shows the results of the analysis of some commercial tablets. The data obtained by both procedures are thoroughly comparable.

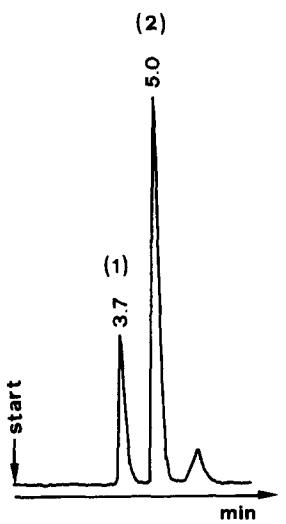

Fig. 4. Chromatogram of a solution of enalapril maleate (1) (4 μg ml^{-1}) and hydrochlorothiazide (2) (2.5 μg ml^{-1}) in the mobile phase.

TABLE 1

Results obtained in the analysis of commercial tablets

Sample	Nominal (mg)		Found (DS) (mg)		Found (HPLC) (mg)	
	I	II	I	II	I	II
A	20	12.5	19.6	12.2	19.7	12.4
B	20	12.5	20.0	12.2	19.8	12.4
C	20	12.5	19.8	12.5	20.0	12.3

DS, derivative spectrophotometry. **I**, enalapril maleate; **II**, hydrochlorothiazide. The data are the average of five determinations for each sample. Relative standard deviations: approx. 2% for derivative spectrophotometry and 1.5% for HPLC.

The proposed methods, simple and rapid, and allowing accurate and precise results, should be of value for the simultaneous determination of enalapril maleate and hydrochlorothiazide in pharmaceutical formulations.

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