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Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC☆

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Abstract

First-derivative ultraviolet spectrophotometry and high-performance liquid chromatography (HPLC) were used to determine valsartan and hydrochlorothiazide simultaneously in combined pharmaceutical dosage forms. The derivative procedure was based on the linear relationship between the drug concentration and the first derivative amplitudes at 270.6 and 335 nm for valsartan and hydrochlorothiazide, respectively. The calibration graphs were linear in the range of 12.0–36.1 μg ml^{-1} for valsartan and 4.0–12.1 μg ml^{-1} for hydrochlorothiazide. Furthermore, a high-performance liquid chromatographic procedure with ultraviolet detection at 225 nm was developed for a comparison method. For the HPLC procedure, a reversed phase column with a mobile phase of 0.02 M phosphate buffer (pH 3.2)-acetonitrile (55: 45; v/v), was used to separate for valsartan and hydrochlorothiazide. The plot of peak area ratio of each drug to the internal standard versus the respective concentrations of valsartan and hydrochlorothiazide were found to be linear in the range of 0.06–1.8 and 0.07–0.5 μg ml^{-1}, respectively. The proposed methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and commercial tablets.
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1. Introduction

Valsartan (VAL) is a new antihypertensive drug belonging to the family of angiotensin II receptor antagonists. Hydrochlorothiazide (HCT) is one of the oldest thiazide diuretics. More recently, VAL has been marketed in combination with HCT in tablets. The oral administration of VAL with

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HCT has been found to be more effective than either drug alone in the treatment of hypertension in patients whose blood pressure is not adequately controled by monotherapy. This fixed-dose combination should be used as second-line therapy [1,2].

Very few methods appeared in the literature for the determination of VAL individually based on high-performance liquid chromatography (HPLC) [3,4] and gas chromatography–mass spectrometry [5].

There have been several reports on the determination of HCT individually or in its combination with other drugs, including the use of liquid chromatography [6–9], capillary zone electrophoresis [10], spectrophotometry [11–14].

The VAL is not yet offical in any pharmacopoeia either alone or in combinatios with other drugs. To the best of our knowledge, no study has been described for the simultaneous determination of VAL and HCT in pharmaceutical formulations or in biological fluids. Therefore, it was desirable to devolop 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 work, two methods based on UV-derivative spectrophotometry and reversed-phase HPLC are reported for the quantification of both drugs. The utility of the devoloped methods to determine the content of both drugs in commerical tablets is also demonstrated.

2. Experimental

2.1. Apparatus and conditions

Shimadzu UV-160A double beam UV-visible spectrophotometer and its recorder were used. The first derivative spectra of the reference and the sample solution were recorded in 1 cm quartz cells against solvent blank over the range 200–400 nm. The optimal conditions for recording the spectra to achieve good reproducibility included scan speed at 40 nm s^{-1}, slit width at 3 nm. The ordinate maximum and minimum settings were $+0.45$ and -0.30, respectively.

The chromatographic apparatus (Hewlett Packard) consisted of a model 1050 solvent delivery system, and a UV-visible spectrophotometric detector. The separation was performed on a Supelcosil LC 18 column (150×4.6 mm; 5 μm particle size). The mobile phase consisted of a mixture of 0.02 M phosphate buffer (pH 3.2)–acetonitrile (55:45; v/v). The mobile phase was prepared daily, filtered, sonicated before use and delivered at a flow rate of 0.9 ml min^{-1}. The detector wavelength was set at 225 nm. Trimethoprime (IS) was used as an internal standard. The injection volume was 20 μl.

2.2. Chemicals and reagents

VAL and HCT and its dosage forms (Co-Diovan) were kindly supplied by Novartis Pharm. Ind. (Istanbul, Turkey). Internal standard trimethoprime was procured from Sanofi-Doğu Pharm. Ind. (Istanbul, Turkey). Acetonitrile and methanol (Merck) were of chromatographic grade; water was doubly distilled. All the other chemicals were of analytical-reagent grade. All solutions were protected from light and were analysed on the day of preparation.

2.3. Standard solutions and calibration curves

Stock solutions for derivative spectrophotometry were prepared by dissolving VAL and HCT in methanol to obtain a concentration of 1 mg ml^{-1} for each compound. Standard solutions of VAL and HCT containing concentration ranges of 12.0–36.1 and 4.0–12.1 μg ml^{-1} were prepared in methanol, respectively.

For HPLC, stock solutions of 1 mg ml^{-1} of drugs were prepared in methanol. Standard solutions were prepared with mobile phase by varying the concentration of the drugs (0.06–1.8 μg ml^{-1} for VAL; 0.07–0.5 μg ml^{-1} for HCT) and maintaining the concentration of IS at a constant level of 0.29 μg ml^{-1}.

The calibration curves for derivative spectrophotometry were constructed by plotting drug concentration versus the peak trough amplitude in the first derivative ultraviolet spectrum at 270.6 nm for VAL and 335 nm for HCT. Calibration

curves for HPLC analysis were obtained by plotting the peak area ratio of the drug to internal standard against the drug concentration.

2.4. Analysis of tablets

Ten tablets containing VAL and HCT as active ingredients were weighed and finely powdered. Appropriate amount of material was accurately weighed, transferred in a volumetric flask, diluted with methanol, sonicated for 5 min and then completed to volume with the same solvent. After centrifugation, further dilution was made using methanol for derivative procedure or mobile phase with the addition of the internal standard for HPLC procedure.

3. Results and discussion

3.1. Derivative spectrophotometry

In Fig. 1 the zero order spectra of VAL and HCT in the wavelength range 200–400 nm are shown. It can be seen that the absorption spectrum of VAL is overlapped with the HCT spectrum. The determination of HCT directly could be easy at the start, but the small content of this sample and the high content of VAL in commercial tablets (the HCT:VAL ratio is at about 1:6) presumes a large contribution of the spectrum of VAL to the maxima in the spectrum of HCT. When the derivative UV-spectra are recorded, sharp bands of large amplitudes are produced [15–17], which may permit more selective identification and determination of two drugs.

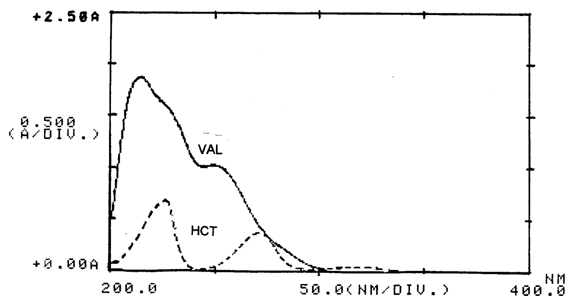

Fig. 1. The zero order spectra of VAL (36 μg ml^{-1}) and HCT (6 μg ml^{-1}) in methanol.

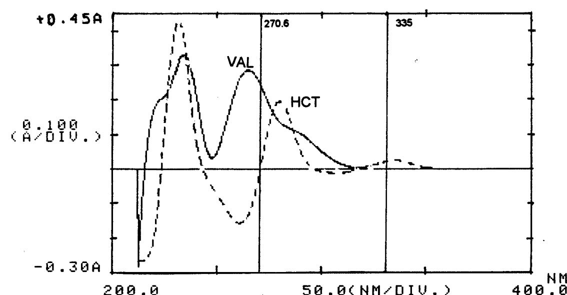

Fig. 2. The first derivative spectra of VAL (36 μg ml^{-1}) and HCT (6 μg ml^{-1}) in methanol.

Fig. 2 shows an example of first derivative UV-spectra of the examined compounds. Simultaneous determination of VAL and HCT was performed simply by reading the dA/dλ values (first derivative absorbances) at 270.6 and 335 nm, respectively. At these wavelengths the analytical signals of the mixture and the compounds to be determined do coincide. Having established the experimental conditions, the calibration graphs were tested between 12.0 and 36.1 μg ml^{-1} for VAL in the absence of HCT at 270.6 nm for the first-derivative spectra. The calibration graphs were also tested between 4.0 and 12.1 μg ml^{-1} of HCT in the absence of VAL at 335 nm for the first-derivative spectra. Good linearity was observed in all the cases (Table 1). The detection and determination limits were also shown in Table 1. The linearity of the calibration graphs and the adherence of the systems to Beer's law are validated by the high value of the correlation coefficient of the regression equations and by the value of the intercepts on the ordinate, which is zero or close to zero.

The repeatability of the method was tested by analysing three replicate samples of 18.06 μg ml^{-1} of VAL and 8.05 μg ml^{-1} of HCT; the relative standard deviations were 0.86 and 0.18%, respectively. The reproducibility of same concentrations of VAL and HCT were evaluated over 3 days by performing three measurements on each day. The relative standard deviations were found to be 1.47 and 1.37% for VAL and HCT, respectively.

In order to assess the validity of the proposed method for assaying each drug in the presence of

each other, synthetic mixtures with different portions of the two drugs were prepared and then assayed using proposed derivative method. Satisfactory results were obtained for the recovery of both drugs (Table 2).

3.2. Chromatography

The reversed-phase HPLC method was developed to provide a specific procedure suitable for the rapid quality control analysis of VAL and HCT as referee method for the developed derivative method. The mobile phase was chosen after several trials with 0.02 M phosphate buffer–acetonitrile and at different pH values with different internal standards. Flow rate was used as 0.9 ml min^{-1}. Using described chromatographic conditions, HCT, IS and VAL were well separated and their retention times 2.20, 2.96 and 6.99 min, respectively. As shown in Fig. 3, the substances were eluted, forming well shaped, symmetrical single peaks, well separated from the solvent front. Capacity factors (k') were obtained as 3.51 for VAL and 0.91 for HCT.

The linearity of the detector response for both drugs was determined by plotting peak area ratios to the internal standard versus concentration. The linearity ranges and analytical data for the calibration graphs are listed in Table 1.

The precision of the method was evaluated by repeating three experiments on the same day (within-day precision) and over 3 days (day-to-day precision). The variability in the peak area ratios on the concentration of 1.5 μg ml^{-1} of VAL and 0.5 μg ml^{-1} of HCT was determined as the precision of the assay. The relative standard deviation values from intra-day and inter-day analysis were found to be 1.21 and 1.76% for VAL, and 0.52 and 1.04% for HCT, respectively.

Results for HPLC analysis of laboratory-prepared mixtures with different proportions of the two drugs are given in Table 2.

3.3. Analysis of tablets

The validity of the proposed methods for pharmaceutical preparations and the effect of possible interferences from common excipients (magnesium stearat, talc, silicon dioxide, polyethylene glycol, crospovidone, hydroxypropylmethylcellulose) were studied by assaying Co-Diovan tablets (labelled to contain 80 mg of VAL and 12.5 mg of HCT per tablet). For the formulation examined the assay results were good agreement with the declared content (Table 2). The results obtained from both proposed methods were statistically compared using Student's t-test. As shown from Table 2, the calculated t-values were less than theoretical value, indicating no significant difference between the mean contents of VAL and HCT obtained by two proposed methods.

Table 1
Statistical analysis of the determination of VAL and HCT in mixture by first derivative spectrophotometry and HPLC methods

	Derivative spectrophotometry		HPLC	
	VAL	HCT	VAL	HCT
Concentration range (μg ml^{-1})	12–36.1	4–12.1	0.06–1.8	0.07–0.5
Slope	6.76×10^{-3}	4.05×10^{-3}	8.58×10^{-4}	3.76×10^{-3}
Intercept	9.0×10^{-3}	0	0.13	2.98×10^{-3}
RSD of slope	0.86	1.49	0.47	0.82
RSD of intercept	0.55	1.24	0.40	0.52
Correlation coefficient	0.9975	0.9995	0.9976	0.9991
Detection limit (μg ml^{-1})	0.51	0.43	0.017	0.02
Quantification limit (μg ml^{-1})	1.7	1.42	0.058	0.067

Table 2
Assay results for the determination of VAL and HCT in laboratory synthetic mixtures and commercial tablet dosage forms

Recovery (mean ± S.D.)[a] (%)				
	Derivative spectrophotometry		HPLC	
VAL	HCT	VAL	HCT	
Synthetic mixtures	99.3 ± 1.3 $t = 1.37$ (2.31)[b]	100.8 ± 2.2 $t = 1.12$ (2.31)[b]	100.6 ± 1.7	99.2 ± 2.3
Commercial tablets	99.2 ± 1.9 $t = 0.96$ (2.31)[b]	102.2 ± 2.6 $t = 0.44$ (2.31)[b]	97.9 ± 2.5	101.5 ± 2.6

[a] Mean of five determinations ± S.D.
[b] Values in parentheses are the theoretical values at $P = 0.05$; each tablet was labeled to contain 80 mg VAL and 12.5 mg HCT.

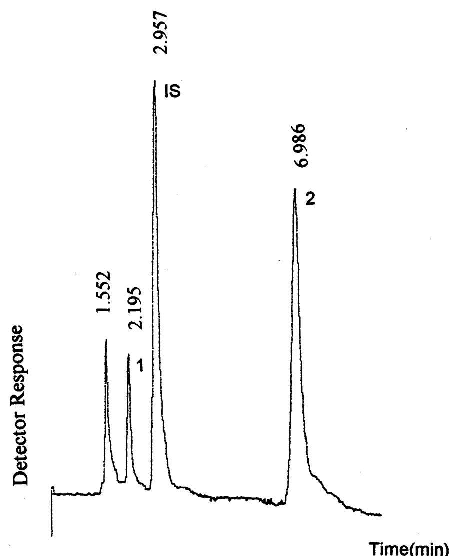

Fig. 3. The chromatogram of a solution of HCT (1) (0.125 μg ml^{-1}) and VAL (2) (0.8 μg ml^{-1}) with IS (0.29 μg ml^{-1}).

4. Conclusion

The described methods give accurate and precise results for determination of VAL-HCT mixtures in tablets without prior separation and are easily applied for routine analysis. The most striking feature of the derivative method is its simplicity and rapidity, no-requiring time-consuming sample preparation such as filtration, degassing that are needed for HPLC procedure.

The HPLC method was shown to be a versatile reference method and may offer advantages over the derivative method for the selective determination of the two intact drugs in a variety of matrices.

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