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Determination of active ingredients in the pharmaceutical formulations containing hydrochlorothiazide and its binary mixtures with benazepril hydrochloride, triamterene and cilazapril by ratio spectra derivative spectrophotometry and vierordt's method

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Abstract

Procedures were developed for the simultaneous determination of pharmaceuticals in binary mixtures, containing hydrochlorothiazide–benazepril hydrochloride, hydrochlorothiazide–triamterene and hydrochlorothiazide–cilazapril by ratio spectra derivative spectrophotometry and Vierordt's method. Mean recoveries, relative standard deviations and linearity ranges in calibration graphs of the methods were compared. These procedures were successfully applied to three pharmaceutical formulations for the determination of active ingredients. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Hydrochlorothiazide; Benazepril hydrochloride; Triamterene; Cilazapril; Simultaneous determination; Pharmaceutical preparations; Ratio spectra derivative spectrophotometry; Vierordt's method

1. Introduction

The binary mixtures of hydrochlorothiazide–benazepril hydrochloride, hydrochlorothiazide–triamterene and hydrochlorothiazide–cilazapril are widely used in diüretic and antihypertensive pharmaceutical formulations.

Several analytical procedures have been described for the simultaneous determination of hydrochlorothiazide, benazepril hydrochloride, triamterene and cilazapril in their mixture with other drugs including spectrophotometry [1–13] and HPLC [14–18] pharmaceutical preparations either separately or in combination with other drugs so far.

Salinas et al. [19] method based on the use of the first derivative of the ratio spectra is very useful. Berzas Nevado et al. [20], applied same method to determine the active compounds in the their mixtures.

In this study, ratio spectra derivative spectrophotometry and Vierordt's method are proposed for the simultaneous determination of ingredients containing hydrochlorothiazide and its

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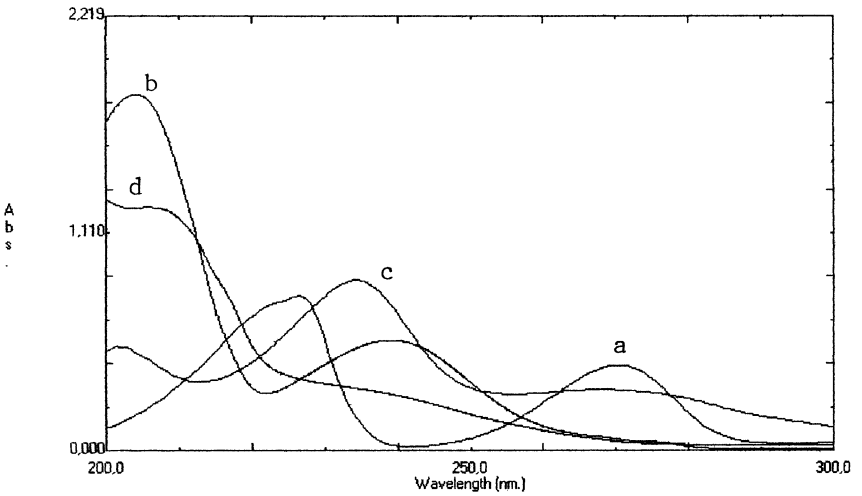

Fig. 1. Zero-order spectra of (a) 12.0 μg ml^{-1} hydrochlorothiazide; (b) 20.0 μg ml^{-1} benazepril hydrochloride; (c) 20.0 μg ml^{-1} triamterene and (d) 20.0 μg ml^{-1} cilazapril in methanol: 0.1 N HCl (1:1).

binary mixture with benazepril hydrochloride, triamterene and cilazapril in pharmaceutical formulations, and the results obtained by these three approaches were compared[1].

2. Material and methods

2.1. Apparatus

A Shimadzu 1601 double beam spectrophotometer with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with Shimadzu UVPC Software equipped with a Lexmark printer was used for all the absorbance measurements and treatment of data.

2.2. Chemicals used

Hydrochlorothiazide and benazepril hydrochloride were kindly supplied by Ciba-Geigy Pharm. Ind. Triamterene was kindly supplied by ibrahim Ethem Pharm. Ind. Cilazapril was kindly supplied by Roche Pharm. Ind. Methanol and HCl were of analytical reagent grade (Merck Chem. Ind.)

2.3. Pharmaceutical preparation

A commercial pharmaceutical preparation (CiBADREX® film tablet Ciba-Geigy Pharm. Ind. Turkey, batch no: 1) was assayed. Its declared content was as follows: benazepril hydrochloride (5.00 mg), hydrochlorothiazide (6.25 mg per film tablet).

TRiAMTERiL® capsule was assayed. Its declared content was as follows: triamterene (50.0 mg), hydrochlorothiazide (25.0 mg per capsule).

INHiBACE® plus tablet was assayed. Its declared content was as follows: cilazapril (5.0 mg), hydrochlorothiazide (12.5 mg per tablet)

2.4. Reagents

Standard solutions of 100 mg 100 ml^{-1} of hydrochlorothiazide (H),benazepril hydrochloride (B),triamterene (T) and cilazapril (C) were prepared, respectively in methanol: 0.1 N HCl (1:1).These solutions were used in the preparation of calibration graphs and for spectra.

[1] These spectrophotometric methods can be regarded as a useful alternative to chromatographic techniques in the routine quality control of pharmaceutical formulations, allowing qualitative and quantitative measurements to be simultaneous and rapid at relatively low costs.

2.5. Procedures

Twenty tablets (from CiBADREX® film tablet) were accurately weighed and powdered in a mortar, an amount equivalent to one tablet, was dissolved in methanol: 0.1 N HCl (1:1) in 100 ml calibrated flasks. After 30 min of mechanically shaking, the solution was filtrated in a 100 ml calibrated flask through Whatman no. 42 filter paper. The residue was washed three times with 10 ml of solvent then the volume was completed to 100 ml with methanol: 0.1 N HCl (1:1) (I). An analogous procedure as explained above was applied to INHIBACE® plus tablet and TRiAMTERiL®, the sample solution (II) and (III), were prepared. Appropriate solutions of (I), (II) and (III) were prepared by taking suitable aliquots of the clear filtrates and diluting both of them (III) with methanol. All the methods were applied to the solutions thus prepared.

3. Application of methods

3.1. Vierordt's method

Using methanol: 0.1 N HCl (1:1) as a blank, the absorbance for the standard working solutions of hydrochlorothiazide (H) and benazepril hydrochloride (B) at 271.7 nm (λ_1) and 238.1 nm (λ_2), hydrochlorothiazide (H) and triamterene (T) at 271.7 nm (λ_1) and 234.1 nm (λ_2) and also hydrochlorothiazide (H) and cilazapril (C),at 271.7 nm (λ_1) and 210.7 nm (λ_2) was measured. The absorbance A (1%, 1 cm) for each drug at the two anaytical wavelengths was calculated and the mean values determined. Similarly the absorbance of the mixed sample solutions was measured; and the concentration of each compound calculated from the following simultaneous equations:

$$A_1 = \alpha_1 \cdot C_1 + \beta_1 \cdot C_2,$$

$$A_2 = \alpha_2 \cdot C_1 + \beta_2 \cdot C_2$$

where A_1 and A_2 denote the absorbances of a mixture solutions of H and B and α and β represent the values of A_1^1 (1%, 1 cm) values calculated for H and B, respectively, at λ_1 and λ_2. C_1 and C_2 are the concentrations of H and B, respectively, in g 100 ml^{-1}. The subscripts 1 and 2 refer to λ_1 (271.7 nm) and λ_2 (238.1 nm), respectively. In a

Table 1
Experimental parameters for Vierordt's method used for the simultaneous determination of ingredients in binary mixtures containing hydrochlorothiazide–benazepril hydrochloride, hydrochlorothiazide–triamterene and hydrochlorothiazide–cilazapril

Mixture of hydrochlorothiazide and benazepril hydrochloride	Hydrochlorothiazide		Benazepril hydrochloride	
(λ nm)	α_1	α_2	β_1	β_2
$\lambda_1 = 271.7$ nm	41.7	–	28.7	–
$\lambda_2 = 238.1$ nm	–	34.6	–	36.4
Linearity range (μg ml^{-1})	2.0–28.0		8.0–36.0	
Mixture of hydrochlorothiazide and triamterene	Hydrochlorothiazide		Triamterene	
$\lambda_1 = 271.7$ nm	37.9	–	42.9	–
$\lambda_2 = 234.1$ nm	–	24.7	–	57.9
Linearity range (μg ml^{-1})	2.0–28.0		4.0–36.0	
Mixture of hydrochlorothiazide and cilazapril	Hydrochlorothiazide		Cilazapril	
$\lambda_1 = 271.7$ nm	26.4	–	30.5	–
$\lambda_2 = 210.7$ nm	–	35.9	–	38.8
Linearity range (μg ml^{-1})	2.0–28.0		4.0–36.0	

Table 2
Recovery data obtained for synthetic different mixtures by using Vierordt's method

Mixture of hydrochlorothiazide and benazepril hydrochloride	Hydrochlorothiazide	Benazepril hydrochloride
Mean*	99.7	99.4
RSD (%)**	0.33	1.12
Mixture of hydrochlorothiazide and triamterene	Hydrochlorothiazide	Triamterene
Mean*	99.7	99.1
RSD (%)**	0.98	0.50
Mixture of hydrochlorothiazide and cilazapril	Hydrochlorothiazide	Cilazapril
Mean*	99.5	99.2
RSD (%)**	1.20	1.80

* Mean for five experiments.
** RSD Relative standard deviation.

similar manner, the mathematical explanation of procedure can be written for the binary mixture containing H and T where λ_1 (271.7 nm) and λ_2 (234.1 nm), and for the binary mixture containing H and C where λ_1 (271.7 nm) and λ_2 (210.7 nm), respectively.

3.2. Ratio spectra derivative spectrophotometry

3.2.1. Mixture containing hydrochlorothiazide and benazepril hydrochloride

The absorption spectra of the solutions prepared at different concentrations of H in its binary mixture with B were recorded with 1 nm resolution against methanol: 0.1 N HCl (1:1) and stored in the IBM.The stored spectra of the binary mixtures, H and B of the binary mixtures were divided by a standard spectrum of B of 10.0 μg ml^{-1}. The ratio spectra were obtained and these were smoothed $\Delta\lambda = 4$ nm intervals. In the binary mixtures, H can be determined by measuring at 269.5 nm (derivative divided spectrum at 269.5 nm). On the other hand, stored spectra of binary mixtures were divided by a standard spectrum of H of 8.0 μg ml^{-1}. Similarly, the content of B was determined by selecting the first derivative of the ratio spectra measuring the signals at 238.8 nm corresponding to a maximum wavelength.

3.2.2. Mixture containing hydrochlorothiazide and triamterene

The absorption spectra of the solutions prepared at different concentrations of H in its binary mixture with T were recorded with 1 nm resolution against methanol: 0.1 N HCl (1:1) and stored in the IBM.

The stored spectra of the binary mixtures, H and T of the binary mixtures were divided by a standard spectrum of T of 10.0 μg ml^{-1}. The ratio spectra were obtained and these were smoothed at $\Delta\lambda = 4$ nm intervals. In the binary mixtures H can be determined by measuring at 260.7 nm (derivative divided spectrum at 260.7 nm). On the other hand, stored spectra of binary mixtures were divided by a standard spectrum of H of 8.0 μg ml^{-1}. Similarly the first derivatives were obtained from smoothed ratio spectra. The content of T was determined by selecting the first derivative of the ratio spectra measuring the signals at 284.4 nm corresponding to a maximum wavelength.

3.2.3. Mixture containing hydrochlorothiazide and cilazapril

The absorption spectra of the solutions prepared at different concentrations of H in its binary mixture with C were recorded with 1 nm resolution against methanol: 0.1 N HCl (1:1) and stored in the IBM.

The stored spectra of the binary mixtures, H and C of the binary mixtures were divided by a standard spectrum of C of 10.0 μg ml^{-1} The ratio spectra were obtained and these were smoothed at $\Delta\lambda = 4$ nm intervals. In the binary mixtures H can be determined by measuring at 273.7 nm (derivative divided spectrum at 273.7 nm). On the other hand, stored spectra of binary mixtures were divided by a standard spectrum of H of 8.0 μg ml^{-1}. Similarly, the first derivatives were obtained from smoothed ratio spectra. The content of C was determined by selecting the first derivative of the ratio spectra measuring the signals at 236.8 nm corresponding to a maximum wavelength.

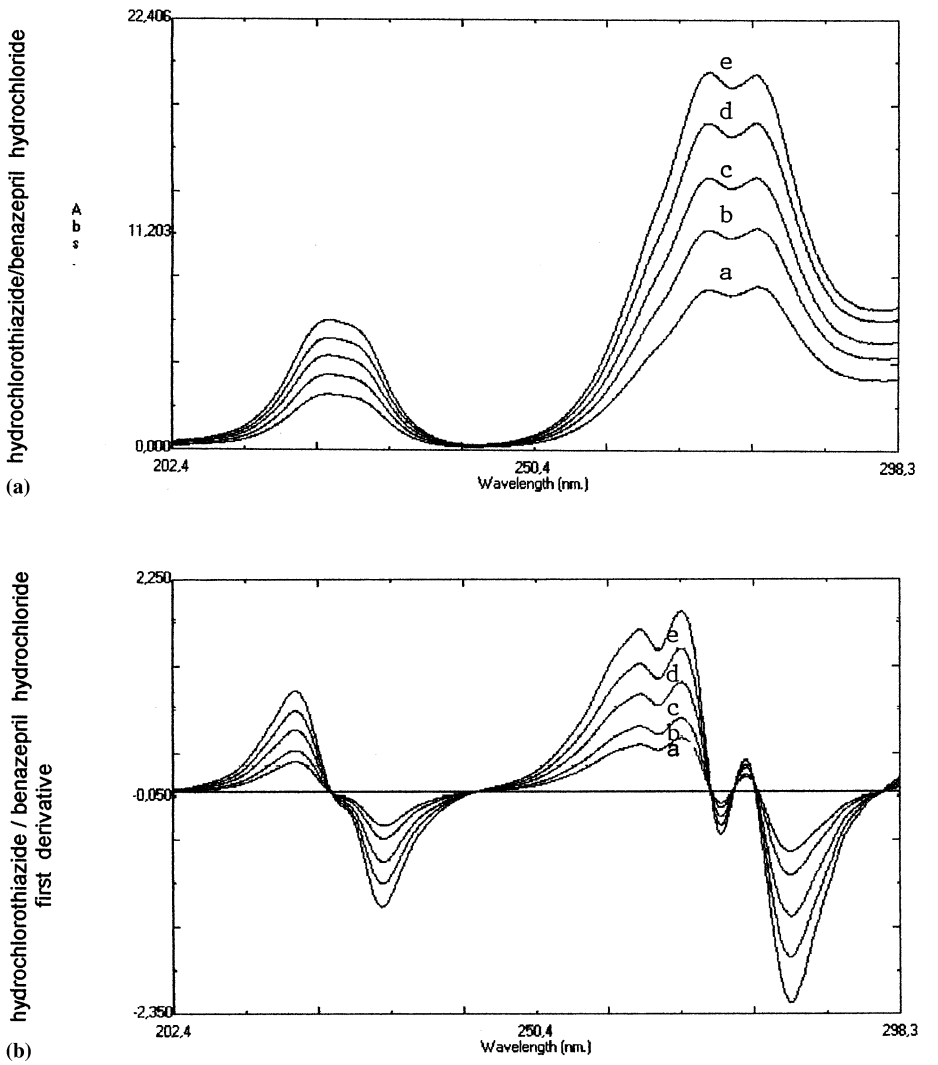

Fig. 2. Ratio spectra (a) and first derivative of the ratio spectra (b) of hydrochlorothiazide of (a) 2.0; (b) 8.0; (c) 14.0; (d) 20.0; (e) 28.0 μg ml^{-1}, with 10.0 μg ml^{-1} benazepril hydrochloride used as divisor in methanol: 0.1 N HCl (1:1) ($\Delta\lambda = 4$ nm).

Table 3
Calibration data in the determination of ingredients in pharmaceutical preparations containing hydrochlorothiazide and its binary mixture with benazepril hydrochloride triamterene and cilazapril[a]

Methods	λ (nm)	Linearity range (μg ml^{-1})	Equation	Regression coefficient (r)
Mixture of hydrochlorothiazide and benazepril hydrochloride				
Ratio spectra first derivative spectrophotometry	269.5	2.0–28.0	$y = 1.2 \cdot 10^{-3}\ C_{hid} + 4.7 \cdot 10^{-4}$	0.9991
	238.8	8.0–36.0	$y = 4.2 \cdot 10^{-3}\ C_{ben} + 1.8 \cdot 10^{-4}$	0.9998
Mixture of hydrochlorothiazide and triamterene				
Ratio spectra first derivative spectrophotometry	260.7	2.0–28.0	$y = 2.9 \cdot 10^{-3}\ C_{hid} + 4.3 \cdot 10^{-4}$	0.9986
	284.4	4.0–36.0	$y = 5.6 \cdot 10^{-3}\ C_{tri} + 1.2 \cdot 10^{-4}$	0.9998
Mixture of hydrochlorothiazide and cilazapril				
Ratio spectra first derivative spectrophotometry	273.7	2.0–28.0	$y = 5.2 \cdot 10^{-3}\ C_{hid} + 1.3 \cdot 10^{-4}$	0.9996
	236.8	4.0–36.0	$y = 4.2 \cdot 10^{-3}\ C_{cil} + 3.6 \cdot 10^{-4}$	0.9993

[a] C_{hid} = μg ml^{-1} of hydrochlorothiazide, C_{ben} = μg ml^{-1} of benazepril hydrochloride, C_{tri} = μg ml^{-1} of triamterene, C_{cil} = μg ml^{-1} of cilazapril.

4. Results and discussion

4.1. Vierordt's method

4.1.1. Mixture containing hydrochlorothiazide and benazepril hydrochloride

As seen in Fig. 1, the spectra of the two compounds, H and B are overlapped. In the application of Vierordt's method, H and B have absorption peaks that are well separated in terms of wavelength. The choice is then very simple; that is, λ_{max} of H are chosen as λ_1; whilst λ_{max} of B is chosen as λ_2. By using Vierordt's method, the determination of the two ingredients is possible for direct measurements of absorbances measured at 271.7 nm and 238.1 nm in the zero-order spectra. In the method, the parameters used, shown in Table 1 and Vierordt's method used have been explained in the methods section. Beer's law was obeyed in the concentration range 2.0–28.0 μg ml^{-1} for H and 8.0–36.0 μg ml^{-1} for B in the synthetic mixture containing H and B. Mean recoveries and relative standard deviations of the method were obtained as 99.7 and 0.33% for H, 99.4 and 1.12% for B, respectively, in the synthetic mixtures prepared by adding known amounts of H and B (Table 2).

4.1.2. Mixture containing hydrochlorothiazide and triamterene

Fig. 1 shows the absorption zero-order spectra of the solutions of H and T in methanol:0.1 N HCl (1:1) are overlapped. In above mentioned application of Vierordt's method, the determination of the two compounds is possible for direct absorbance measurements in their zero-order spectra. For this procedure, the absorbance values were measured at 271.7 and 234.1 nm, selecting the maximum and the minimum wavelengths of the two compounds in a way that the maximum wavelength of one compound would be corresponding to the minimum wavelength of the second compound. In Vierordt's method, the parameters used, shown in Table 1 and the equations used have been explained in the methods section. In the method, Beer's law was valid in the concentration range 2.0–28.0 μg ml^{-1} for H and 4.0–36.0 μg ml^{-1} for T in the synthetic mixture containing H and T. Mean recoveries and relative standard deviations of the method were obtained as 99.7 and 0.98% for H, 99.1 and 0.5% for T, respectively, in the synthetic mixtures prepared by adding known amounts of H and T (Table 2).

4.1.3. Mixture containing hydrochlorothiazide and cilazapril

Fig. 2 shows the absorption zero-order spectra of the solutions of H and C in methanol:0.1 N HCl (1:1) are overlapped at the region 200.0–300.0 nm. In above mentioned application of Vierordt's method, the determination of the two compounds is possible for direct absorbance measurements in their zero-order spectra. For this procedure, the absorbance values were measured at 271.7 and 210.7 nm, selecting the maximum and the minimum wavelengths of the two compounds in a way that the maximum wavelength of one compound would be corresponding to the minimum wavelength of the second compound. In Vierordt's method, the parameters used, shown in Table 1 and the equations used have been explained in the methods section. In the method, Beer's law was valid in the concentration range 2.0–28.0 μg ml^{-1} for H and 4.0–36.0 μg ml^{-1} for C in the synthetic mixture containing H and C. Mean recoveries and relative standard devia-

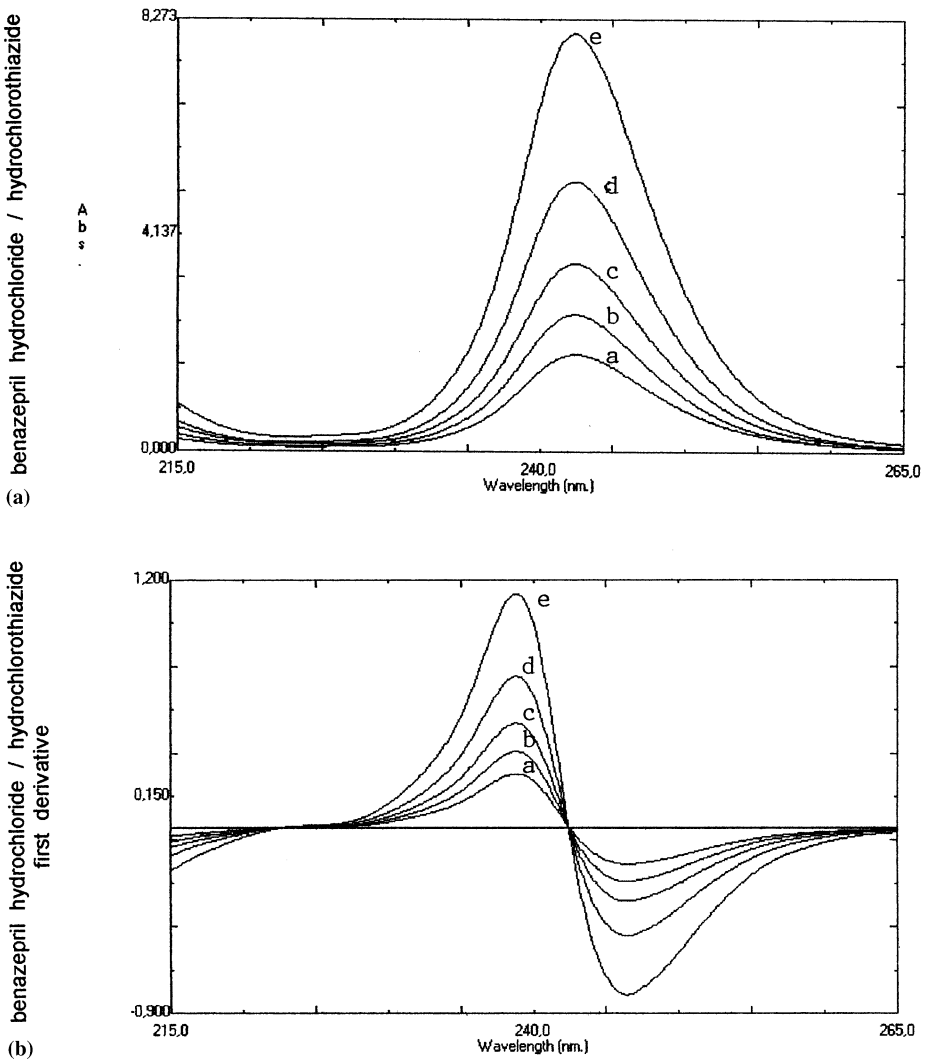

Fig. 3. Ratio spectra (a) and first derivative of the ratio spectra (b) of benazepril hydrochloride of (a) 8.0; (b) 10.0; (c) 20.0; (d) 30.0; (e) 36.0 μg ml^{-1}, with 8.0 μg ml^{-1} hydrochlorothiazide used as divisor in methanol: 0.1 N HCl (1:1) ($\Delta\lambda = 4$ nm).

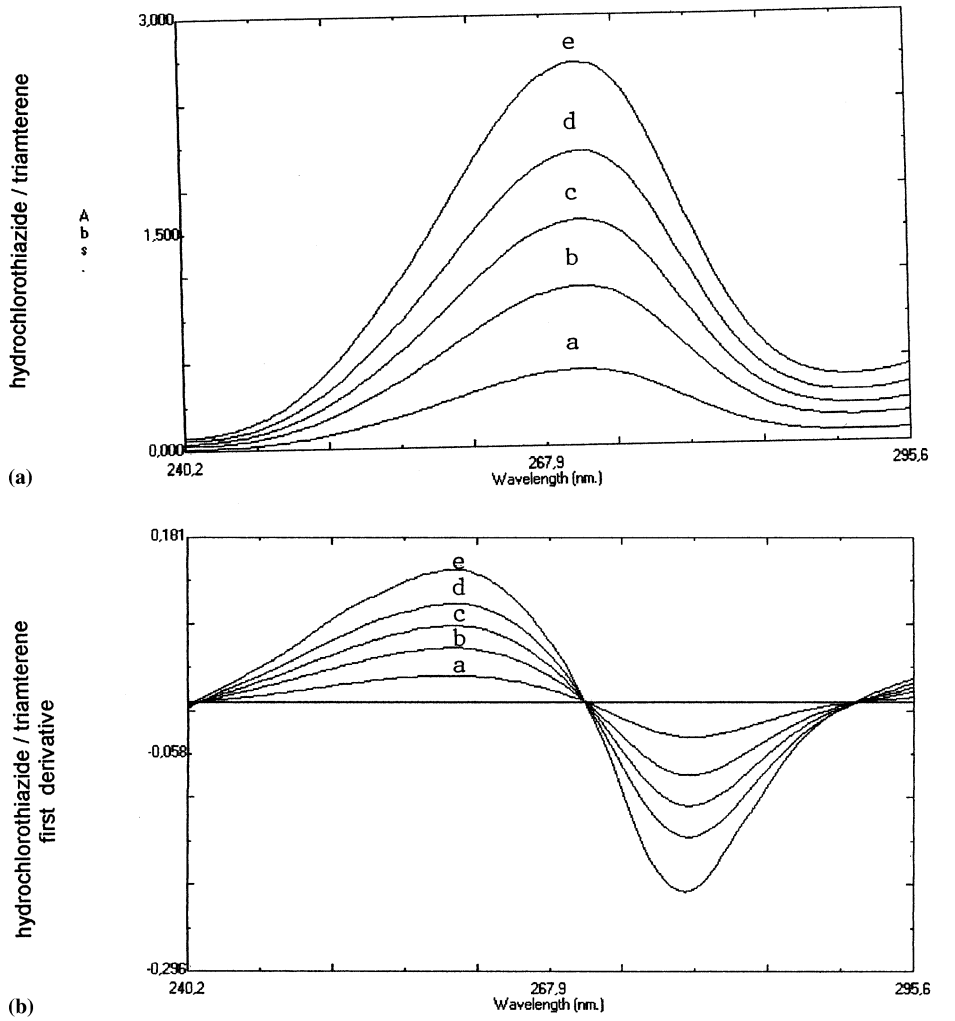

Fig. 4. Ratio spectra (a) and first derivative of the ratio spectra (b) of hydrochlorothiazide of (a) 2.0; (b) 8.0; (c)14.0; (d) 20.0; (e) 28.0 μg ml^{-1}, with 10.0 μg ml^{-1} triamterene used as divisor in methanol: 0.1 N HCl (1:1) ($\Delta\lambda = 4$ nm).

tions of the method were obtained as 99.5 and 1.2% for H, 99.2 and 1.8% for C, respectively, in the synthetic mixtures prepared by adding known amounts of H and C (Table 2).

4.2. Ratio spectra derivative spectrophotometry

4.2.1. Mixture containing hydrochlorothiazide and benazepril hydrochloride

Fig. 2(a) shows the ratio spectra of different H standards (spectra divided by the spectrum of a 10.0 μg ml^{-1} B solution) and their first derivatives were calculated. The first derivative amplitudes at a given wavelength are proportional to the H concentration. The influence of $\Delta\lambda$ for obtaining the first derivative was tested; $\Delta\lambda = 4$ nm was considered as suitable. The concentration of divisor (H in this case) can be modified, and different calibration graphs are then obtained. A B concentration of 10.0 μg ml^{-1} was selected. The calibration graph was established by measuring at the amplitude at 269.5 nm corresponding to a maximum wavelength in Fig. 2(b). Several mixture compositions of H and B were prepared and

tested between 2.0–28.0 μg ml^{-1} for H in their binary mixtures shown in Table 4. For determining the other component B an analogous procedure was followed. The ratio spectra of different B standards (spectra divided by the spectrum of a 8.0 μg ml^{-1} H solution) was obtained and their first derivatives were calculated. The calibration graph was obtained by measuring the amplitude at 238.8 nm corresponding to a maximum wavelength (Fig. 3b).

4.2.2. Mixture containing hydrochlorothiazide and triamterene

Fig. 4(a) shows the ratio spectra of different H standards (spectra divided by the spectrum of a 10.0 μg ml^{-1} T solution) and their first derivatives were calculated. The first derivative amplitudes at a given wavelength are proportional to the H concentration. The influence of $\Delta\lambda$ on obtaining the first derivative was tested; $\Delta\lambda = 4$ nm was considered as suitable. The concentration

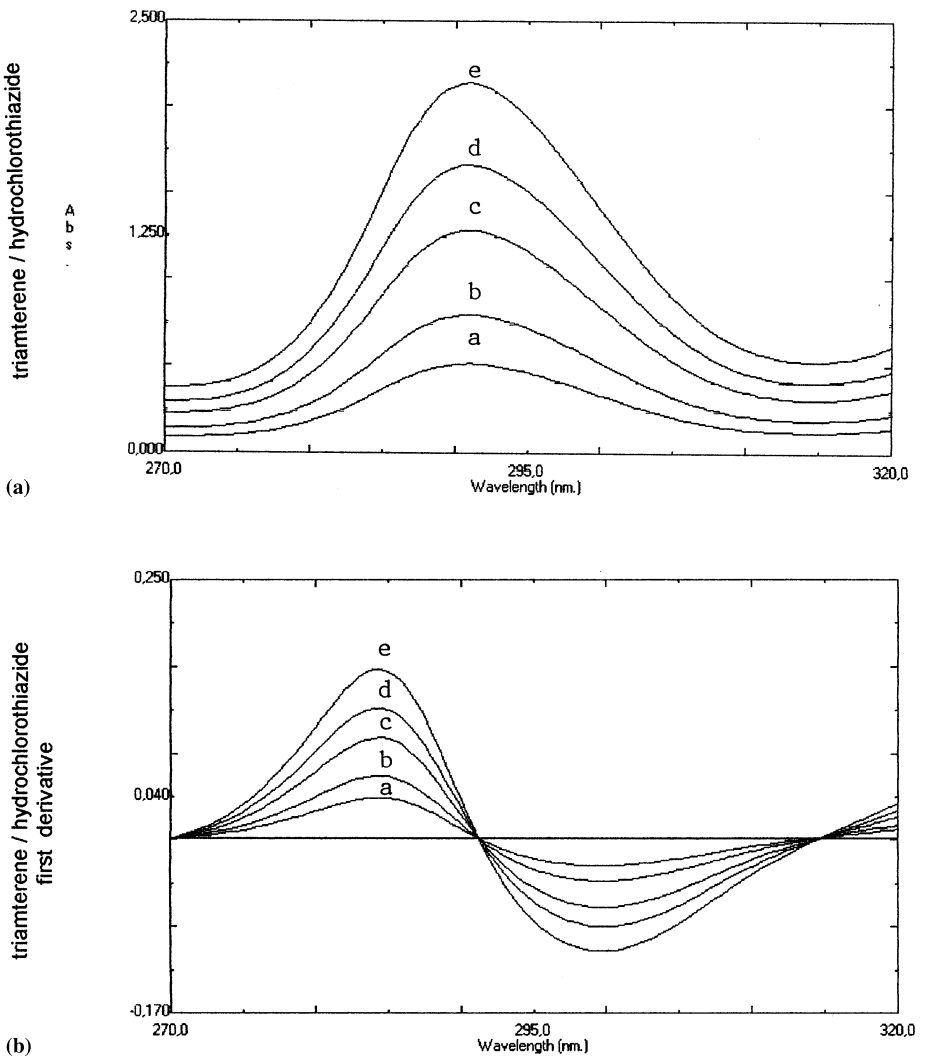

Fig. 5. Ratio spectra (a) and first derivative of the ratio spectra (b) of triamterene of (a) 4.0; (b) 10.0; (c) 20.0; (d) 30.0; (e) 36.0 μg ml^{-1}, with 8.0 μg ml^{-1} hydrochlorothiazide used as divisor in methanol: 0.1NHCl (1:1) ($\Delta\lambda = 4$ nm).

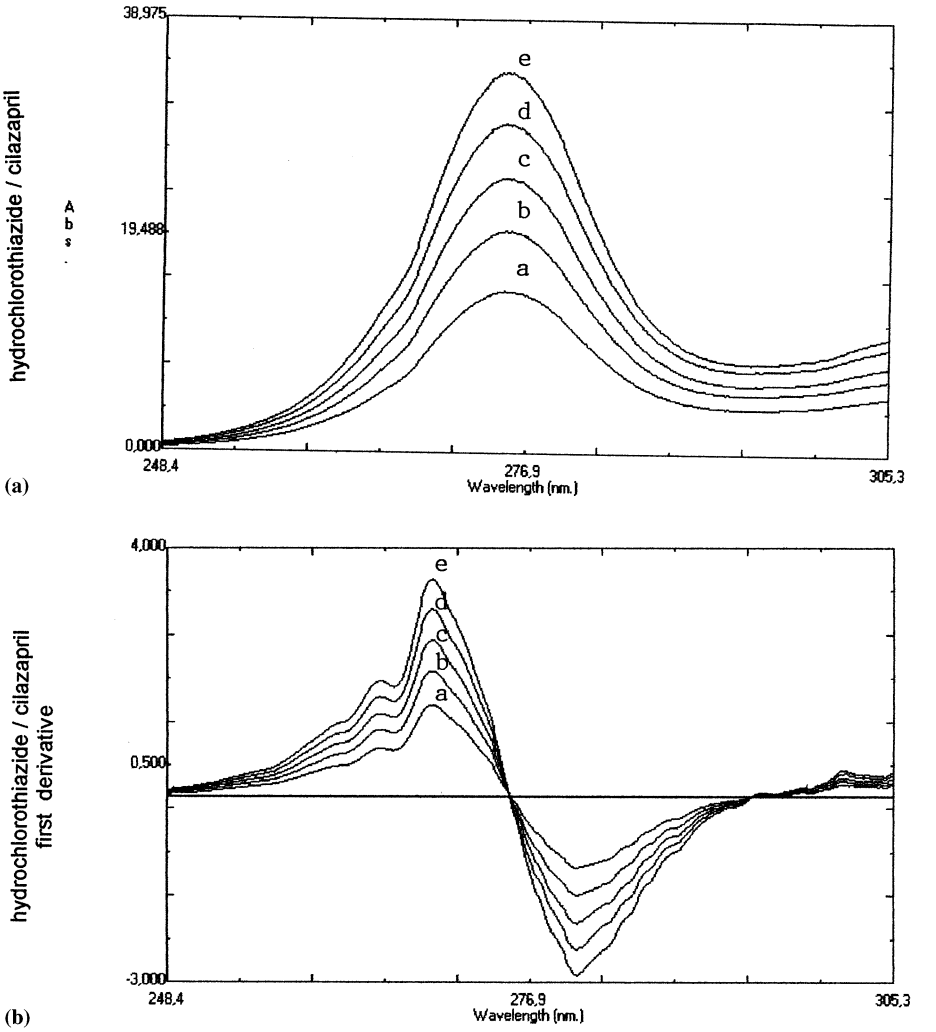

Fig. 6. Ratio spectra (a) and first derivative of the ratio spectra (b) of hydrochlorothiazide of (a) 2.0; (b) 8.0; (c) 14.0; (d) 20.0; (e) 28.0 μg ml^{-1},with 10.0 μg ml^{-1} cilazapril used as divisor in methanol: 0.1 N HCl (1:1) ($\Delta\lambda = 4$ nm).

of divisor (H in this case) can be modified, and different calibration graphs are then obtained. A T concentration of 10.0 μg ml^{-1} was selected. The calibration graph was established by measuring at the amplitude at 260.7 nm corresponding to a maximum wavelength in Fig. 4(b). Several mixture compositions of H and T were prepared and tested between 2.0–28.0 μg ml^{-1} for H in their binary mixtures shown in Table 4.

For determining the other component T an analogous procedure was followed. The ratio spectra of different T standards (spectra divided by the spectrum of a 8.0 μg ml^{-1} H solution) was obtained and their first derivatives were calculated. The calibration graph was obtained by measuring the amplitude at 284.4 nm corresponding to a maximum wavelength (Fig. 5b).

4.2.3. Mixture containing hydrochlorothiazide and cilazapril

Fig. 6(a) shows the ratio spectra of different H standards (spectra divided by the spectrum of a 10.0 μg ml^{-1} C solution) and their first derivatives were calculated. The first derivative ampli-

tudes at a given wavelength are proportional to the H concentration. The influence of $\Delta\lambda$ on obtaining the first derivative was tested; $\Delta\lambda = 4$ nm was considered as suitable. The concentration of divisor (H in this case) can be modified, and different calibration graphs are then obtained. A C concentration of 10.0 μg ml^{-1} was selected. The calibration graph was established by measuring at the amplitude at 273.7 nm corresponding to a maximum wavelength in Fig. 6(b). Several mixture compositions of H and C were prepared and tested between 2.0–28.0 μg ml^{-1} for H in their binary mixtures shown in Table 4.

For determining the other component C an analogous procedure was followed. The ratio spectra of different C standards (spectra divided by the spectrum of a 8.0 μg ml^{-1} H solution) was obtained and their first derivatives were calculated. The calibration graph was obtained by measuring the amplitude at 236.8 nm corresponding to a maximum wavelength (Fig. 7b).

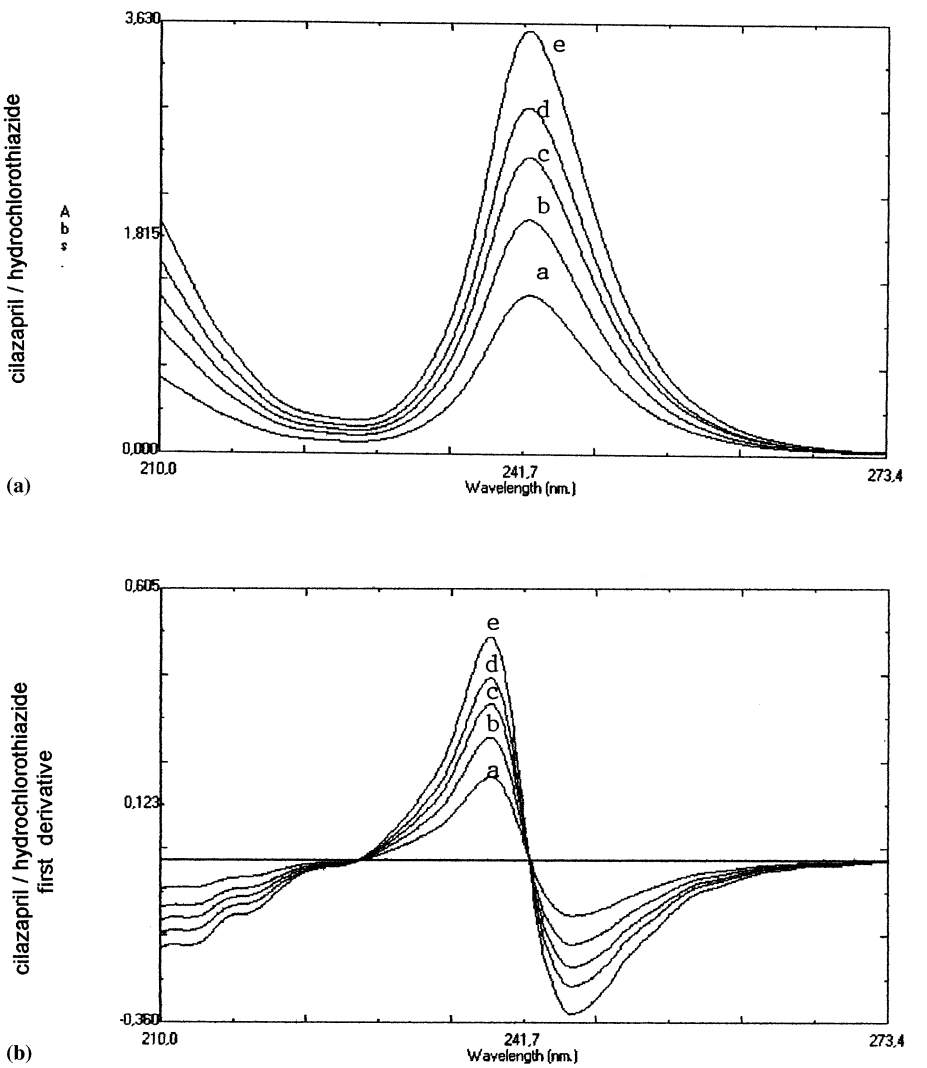

Fig. 7. Ratio spectra (a) and first derivative of the ratio spectra (b) of cilazapril of (a) 4.0; (b) 10.0; (c) 20.0; (d) 30.0; (e) 36.0 μg ml^{-1}, with 8.0 μg ml^{-1} hydrochlorothiazide used as divisor in methanol: 0.1 N HCl (1:1) ($\Delta\lambda = 4$ nm).

Table 4
Recovery data obtained for synthetic different mixtures by using the first derivative of the ratio spectra

Mixture of hydrochlorothiazide and benazepril hydrochloride	Hydrochlorothiazide	Benazepril hydrochloride
Mean*	99.3	99.9
RSD (%)**	0.36	1.30
Mixture of hydrochlorothiazide and triamterene	Hydrochlorothiazide	Triamterene
Mean*	99.7	98.6
RSD (%)**	0.80	1.50
Mixture of hydrochlorothiazide and cilazapril	Hydrochlorothiazide	Cilazapril
Mean*	99.5	99.6
RSD (%)**	0.90	1.70

* Mean for five experiments.
** RSD Relative standard deviation.

Table 5
Assay results in commercial product (mg)[a,b]

	Benazepril hydrochloride (mean ± SD)	Hydrochlorothiazide (mean ± SD)
Vierordt's method	4.7 ± 0.8	6.23 ± 0.8
Ratio spectra derivative spectrophotometry	4.9 ± 0.3	6.19 ± 0.5
	Triamterene (mean ± SD)	Hydrochlorothiazide (mean ± SD)
Vierordt's method	49.1 ± 1.9	24.8 ± 1.5
Ratio spectra derivative spectrophotometry	49.8 ± 0.8	24.8 ± 1.2
	Cilazapril (mean ± SD)	Hydrochlorothiazide (mean ± SD)
Vierordt's method	4.8 ± 1.1	12.3 ± 0.7
Ratio spectra derivative spectrophotometry	4.9 ± 0.9	12.5 ± 0.5

[a] Results obtained are the average of ten experiments for each method.
[b] SD = Standard deviation.

Table 3 shows the regression coefficients and the linearity ranges of the calibration graphs for active ingredients at the suitable wavelengths for the determinations of H, B, T and C in their binary mixtures.

A good coincides was observed for the assay results of the commercial preparations by using the two methods in this paper (Table 5).

5. Conclusion

The ratio spectra derivative spectrophotometry and Vierordt's method permits simple, rapid, and direct determination of these binary mixtures without previous separations. Moreover, it has some advantages over other separation techniques such as high-performance liquid chromatography or gas chromatography. These two methods proposed in this paper were found to be suitable for the determination of ingredients in the three different pharmaceutical preparation containing hydrochlorothiazide and its binary mixture with benazepril hydrochloride, triamterene and cilazapril.

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