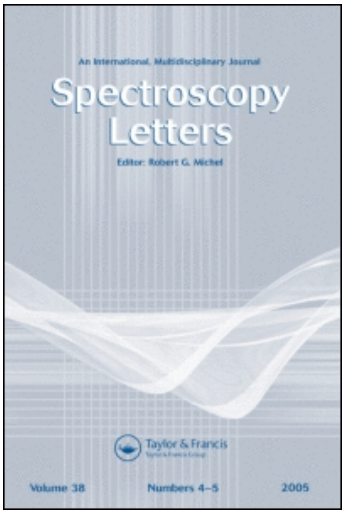

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Comparative Study of the Ratio Spectra Derivative Spectrophotometry, Derivative Spectrophotometry and Vierordt's Method Appued to the Analysis of Lisinopril and Hydrochlorothiazide in Tablets

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To cite this Article Erk, Nevin(1998) 'Comparative Study of the Ratio Spectra Derivative Spectrophotometry, Derivative Spectrophotometry and Vierordt's Method Appued to the Analysis of Lisinopril and Hydrochlorothiazide in Tablets', Spectroscopy Letters, 31: 3, 633 — 645
To link to this Article: DOI: 10.1080/00387019808002756
URL: http://dx.doi.org/10.1080/00387019808002756

SPECTROSCOPY LETTERS, 31(3), 633-645 (1998)

COMPARATIVE STUDY OF THE RATIO SPECTRA DERIVATIVE SPECTROPHOTOMETRY, DERIVATIVE SPECTROPHOTOMETRY AND VIERORDT'S METHOD APPLIED TO THE ANALYSIS OF LISINOPRIL AND HYDROCHLOROTHIAZIDE IN TABLETS

Key Words : Lisinopril / hydrochlorothiazide /pharmaceutical preparations/ratio spectra derivative spectrophotometry / derivative spectrophotometry / Vierordt's method.

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ABSTRACT

Three new spectrophotometric methods are described for the determination of lisinopril and hydrochlorothiazide in their binary mixture:First derivative spectrophotometry ,ratio spectra derivative and Vierordt's method. The procedures do not require any prior separation. In the derivative spectrophotometry, the $dA/d\lambda$ values in the first derivative spectra of the mixture were measured at 269.6 nm for lisinopril and at 279.8 nm for hydrochlorothiazide . The calibration graphs were linear in the range 25.56 - 129.50 $\mu g.ml^{-1}$ for lisinopril and 10.60 - 139.80 $\mu g.ml^{-1}$ for hydrochlorothiazide . In ratio spectra derivative spectrophotometry, the calibration graphs for 15.68-129.50 $\mu g.ml^{-1}$ lisinopril and for 5.98-139.80 $\mu g.ml^{-1}$ hydrochlorothiazide were obtained by measuring the signals at 253.7 nm and 243.6 nm for lisinopril and at 280.1 nm and 270.8 nm for hydrochlorothiazide.In Vierordt's method, A^1_1 (1 %,1 cm) values of lisinopril and hydrochlorothiazide were determined at 259.8 nm and 272.7 nm in the zero - order spectra . The quantity of both compounds were calculated by using the A^1_1 (1 %,1 cm) values . The methods were successfully applied to a pharmaceutical formulation for determination of both active compounds.

INTRODUCTION

Lisinopril and hydrochlorothiazide are present together in commercial diuretic and antihypertensive preparations.

Various methods have been used for the quantitative determination of lisinopril and hydrochlorothiazide in their combinations with other drugs including HPLC (1 - 7) and, spectrophotometry (8 - 12) for the determination of lisinopril and hydrochlorothiazide in pharmaceutical preparations either separately or in combination with other drugs so far.

Salinas et al. (13) developed a new method for the analysis mixtures of compounds with overlapping spectra. This method is based on the use the first derivative of the ratio spectra. In the first derivative of the ratio spectra,the concentrations of active compounds were determined by measuring the amplitudes of the minimum or maximum at points corresponding to the selected wavelengths. Berzas Nevado et al.(14-16) , applied same method to determine the active compounds in different mixtures.

We couldn't find any work on the simultaneous analysis of lisinopril and hydrochlorothiazide mixture in the literature.

In this paper,derivative spectrophotometry, ratio spectra derivative spectrophotometry and Vierordt 's method are proposed for the simultaneous determination of lisinopril and hydrochlorothiazide in a pharmaceutical formulation , tablets , and the results obtained by these three approaches were compared.

EXPERIMENTAL

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 and equipped with an Lexmark printer was used for all the absorbance measurements and treatment of data.

The first derivative curves of the zero-order spectra of references and test solution were recorded in 1 - cm quartz cells over the range 250 to 290 nm ($\Delta\lambda$ = 4 nm) in first derivative spectrophotometry . The ordinate minimum and maximum settings were + 0.070 and - 0.100 . In the first derivative of ratio spectra, ranges were selected as 260.0-300 nm and 250.0-299.9 nm ($\Delta\lambda$ = 4nm) for determining lisinopril and hydrochlorothiazide,respectively.

Pharmaceutical preparation :

A commercial pharmaceutical preparation (SİNORETİK ® tablet , Eczacıbaşı Pharm.Ind.-Turkey , batch no :8) was assayed. Its declared content was as follows:

lisinopril	..... 20,0 mg
hydrochlorothiazide	..... 12,5 mg / tablet

Chemicals used :

Lisinopril and hydrochlorothiazide were kindly supplied by Eczacıbaşı Pharm.Ind.

Methanol was analytical reagent grade (Merck Chem.Ind.).

Reagents :

Standard solutions of 100.0 mg. 100 ml^{-1} of lisinopril and 100.0 mg.100 $ml^{-1}$of hydrochlorothiazide were prepared , respectively in methanol.These solutions were used in the preparation of calibration graphs and for spectra.

Procedures :

20 tablets were accurately weighed and powdered in a mortar.An amount of the tablet mass equivalent to one tablet was dissolved in 50 ml methanol. After 30 min of mechanically shaking, the solution was filtered through whatman No. 42 filter paper to a 100,0 ml volumetric flask. The residue was washed two times with 20 ml methanol, then the volume was completed to 500,0 ml with the same solvent . All the methods explained below were applied directly to this solution.

METHODS

a)Vierordt's method

This method (17-19) is based on the selection of wavelengths at which two compounds in the mixture inversely have an absorpion minimum and maximum , and the determination of these compounds can be made by using A^1_1 (absorbance value of the 1 % solution in a 1 cm cell) values determined at these wavelengths using standards. Absorbances were measured at 259.8 nm (λ_1) and 272.7 nm (λ_2) (maximum absorption wavelengths) in the zero - order spectra of lisinopril and hydrochlorothiazide in methanol . The A^1_1 (1 %,1 cm) values were calculated for each component at these wavelengths (the mean of ten values).By using A^1_1 (1 %,1 cm) values,a system of equations with two unknowns can be written for compounds in binary mixtures.The amount of each ingredient in the pharmaceutical formulation was determined by using the following equations:

$A = \alpha_1 .C$ (path length (1) is equal to 1)

$A_1 = \alpha_1 .C_1 + \beta_1 .C_2$, $A_2 = \alpha_2 .C_1 + \beta_2 .C_2$

where A_1 and A_2 denote the absorbances of the mixture solution measured and α and β represent the values of A^1_1 (1 %,1 cm) calculated for lisinopril and hydrochlorothiazide ,respectively at λ_1 and λ_2.The values C_1 and C_2 are the concentrations of lisinopril and hydrochlorothiazide, respectively,as g/100 ml.The subscripts 1 and 2 refer to λ_1 (259.8 nm) and λ_2 (272.7nm),respectively.

b)First derivative spectrophotometry

In this method , the first derivative spectra were calculated with $\Delta\lambda$ =4 nm interval from the stored zero - order absorption spectra of the prepared samples dissolved in methanol . For the determination of lisinopril and hydrochlorothiazide in their mixtures, the calibration graphs were used , which were obtained by measuring the $dA/d\lambda$ values at 269.6 nm (zero - crossing point for hydrochlorothiazide) and at 279.8 nm (zero-crossing point for lisinopril),respectively.

c) Ratio spectra first derivative spectrophotometry

In this method ,lisinopril was determined as follows , the recorded zero - order spectra of samples were divided by a standard spectrum of 98.70 $\mu g.ml^{-1}$ hydrochlorothiazide solution in methanol. From the ratio spectra thus obtained , first derivative were calculated with $\Delta\lambda$ =4 nm intervals.

The concentration of lisinopril is proportional to the amplitude at 253.7 nm and 243.6 nm . Also, hydrochlorothiazide was determined similarly, the stored zero - order spectra of the samples were divided by a standard spectrum of 109.78 $\mu g.ml^{-1}$ lisinopril solution in methanol.The first derivatives of the ratio spectra of hydrochlorothiazide were obtained by an analogous procedure, when 109.78 $\mu g.ml^{-1}$ lisinopril used as divisor in methanol,with $\Delta\lambda$ = 4 nm intervals.The concentration of hydrochlorothiazide is proportional to the amplitude at 280.1 nm and 270.8 nm.

RESULTS AND DISCUSSION

Vierordt's method :

The zero - order absorption spectra in Vierordt's method were obtained with an interval of $\Delta\lambda$=0.1 nm and a medium level of scanning speed in the spectrophotometer. Under this conditions, the original spectra were recorded in computer. Figure 1 shows that the absorption zero - order spectra of the solutions of lisinopril and hydrochlorothiazide in methanol are overlapping at the region 240 - 300 nm. By using the Vierordt's method,determination of the two compounds is possible using direct absorbance measurements in their zero - order spectra. For this procedure, the absorbances values were measured at 259.8 nm and at 272.8 nm,selecting the maximum and the minumum wavelenghts of the two compounds in a way such that the maximum wavelenght of one compound would correspond to the minumum wavelenght of the second compound. The parameters , shown in Table 1. and the equations used in Vierordt's method are described in *experimental section.*

In this method , Beer's law was valid in the concentration range 15.68 - 129.50 $\mu g.ml^{-1}$ for lisinopril and 5.98 - 139.80 $\mu g.ml^{-1}$ for hydrochlorothiazide .Mean recovery and relative standard deviation of the method were obtained as 98.88 and 0.79 % for lisinopril and 99.29 , and 0.38 % for hydrochlorothiazide , respectively , in the synthetic mixtures prepared by adding known amounts of lisinopril and hydrochlorothiazide (Table 2).

First derivative spectrophotometry:

In the first derivative spectrophotometry experimental conditions were optimized, in order to obtain a sharp peak and zero-crossing point. The influence of the $\Delta\lambda$ for obtaining the best derivative spectra was tested and a value of $\Delta\lambda$ =4 nm was considered as suitable for the determination of both compounds. Figure 2 indicates the first derivative spectra obtained with $\Delta\lambda$ =4 nm interval from the stored absorption curves shown in Figure 1.For the determination of these two compounds, $dA/d\lambda$ values at 269.6 nm and 279.8 nm (zero-crossing points) were measured and calibration graphs over the range 25.56 - 129.50 $\mu g.ml^{-1}$ for lisinopril and 10.60 - 139.80 $\mu g.ml^{-1}$ for hydrochlorothiazide were obtained . Thus, the quantity of lisinopril and hydrochlorothiazide in the mixture can be determined without prior chemical separation and without any interference from one another , in contrast to the zero - order spectra . In the method , mean r ecovery and the relative standard deviation were found to be 98.60 and 0.42 % and, 99.57 and 0.28 % for lisinopril and hydrochlorothiazide , respectively , in the synthetic mixtures prepared by adding known amounts of lisinopril and hydrochlorothiazide (Table 3). Table 4 summarizes the regression

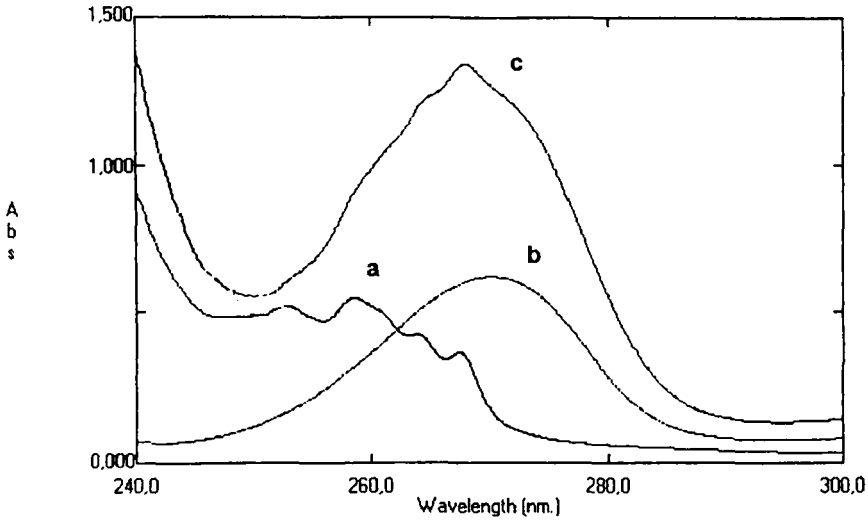

Figure 1. Zero-order spectra of a) 40.0 μg.ml^{-1} lisinopril ,b) 25.0 μg.ml^{-1} hydrochlorothiazide ,and c) their mixture in methanol

TABLE 1. Experimental parameters for Vierordt's method used for the simultaneous determination of lisinopril and hydrochlorothiazide

	Lisinopril		Hydrochlorothiazide	
λ (nm)	α_1	α_2	β_1	β_2
λ_1 = 259.8 nm	978		234.8	
λ_2 = 272.7 nm		678.5		345.7
Linearity range (μg.ml^{-1})	15.68 - 129.50		5.98 - 139.80	

TABLE 2. Recovery data obtained for different mixtures by using the Vierordt's method

	Lisinopril			Hydrochlorothiazide		
Mixture no	Added µg	Found µg	Recovery %	Added µg	Found µg	Recovery %
1	20.00	19.74	98.70	3.00	2.98	99.50
2	20.00	19.67	98.35	6.00	5.96	99.33
3	20.00	19.80	99.00	9.00	8.98	99.78
4	20.00	19.80	99.00	12.50	12.39	99.12
5	20.00	20.10	100.50	15.30	15.30	99.35
6	20.00	19.60	98.00	18.70	18.56	99.25
7	20.00	19.60	98.00	21.30	21.10	99.06
8	20.00	19.89	99.45	24.80	24.76	99.39
			x = 98.88			
			RSD = 0.79 %			
9	5.00	4.97	99.40	12.50	12.37	98.96
10	10.00	9.85	98.50	12.50	12.37	98.96
11	15.00	14.68	97.87	12.50	12.36	98.89
12	20.00	19.83	99.15	12.50	12.45	99.60
13	25.00	24.55	98.20	12.50	12.48	99.84
14	30.00	29.14	97.13	12.50	12.48	99.84
15	35.00	34.89	99.69	12.50	12.39	99.12
16	40.00	39.84	99.60	12.50	12.39	99.12
n = 16						x = 99.29
						RSD = 0.38 %

coefficients and the linearity ranges of the calibration graphs for both active compounds in the selected wavelengths.

Ratio spectra first derivative spectrophotometry :

Figure 3a indicates the ratio spectra of lisinopril at the increasing concentration values , in the solution of methanol , which were obtained by dividing each one with the spectrum of the standard solution of hydrochlorothiazide in methanol. Figure 3b indicates the first derivative of the ratio spectra , which was plotted with intervals of $\Delta\lambda$ = 4nm from the ratio spectra shown in Figure 3a. In Figure 3b , two calibration graphs of lisinopril were established by measuring the signal at 253.7 nm and 243.6 nm corresponding to two minimum .(Table 4.), and were tested between 5.98-139.80 $\mu g.ml^{-1}$ for hydrochlorothiazide in their binary mixtures, as shown in Table 5 .

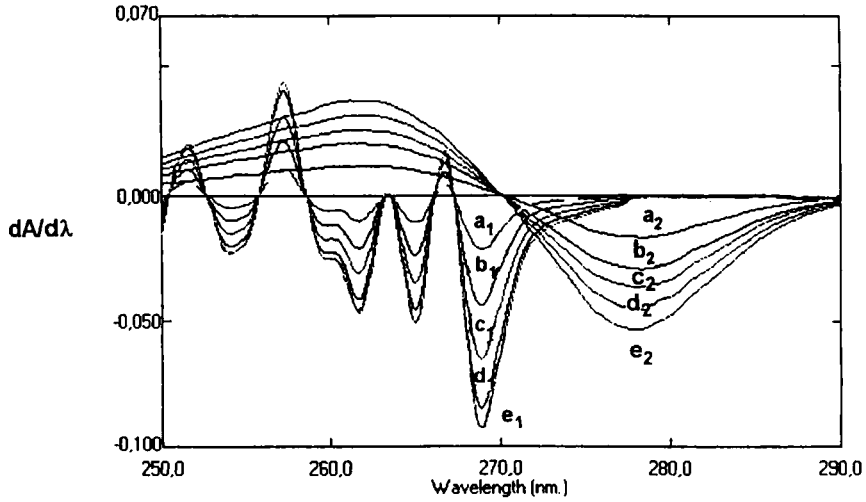

Figure 2. First derivative spectra of lisinopril of a_1) 25.0 μg.ml^{-1} , b_1) 50.0 μg.ml^{-1} , c_1) 75.0 μg.ml^{-1} , d_1) 100.0 μg.ml^{-1} , e_1) 125.0 μg.ml^{-1} and , of hydrochlorothiazide , a_2) 10.0 μg.ml^{-1} , b_2) 40.0 μg.ml^{-1} , c_2) 70.0 μg.ml^{-1} , d_2) 100.0 μg.ml^{-1} , e_2) 130.0 μg.ml^{-1} in methanol (Δλ = 4 nm)

Also, Figure 4a indicates the ratio spectra of hydrochlorothiazide obtained by dividing the amplitudes of the absorption spectrum of the mixture by the standard spectrum of lisinopril in methanol . Figure 4b indicates first derivative of the ratio spectra of lisinopril calculated with Δλ =4 nm intervals from the ratio spectra shown in Figure 4a . Also , in Figure 4b , two calibration graphs of hydrochlorothiazide were established at 270.8 nm and 280.1 nm corresponding to a maximum and a minimum,respectively (Table 4.) and were tested between 15.68-129.50 μg.ml^{-1} for lisinopril in their binary mixtures (Table 5). By using the same method for two compounds, the mean recovery and the relative standard deviation of the method were obtained as 99.91 and 0.91 % for lisinopril and 99.54 and 0.44 % for hydrochlorothiazide in synthetic mixtures prepared by adding known amounts of lisinopril and hydrochlorothiazide (Table 5) . Table 4 summarizes the regression coefficients and linearity ranges of the calibration graphs, obtained by measuring the signals corresponding to the maximum and minimum wavelengths in the first derivative of the ratio spectra for both active compounds . For the determination of lisinopril and hydrochlorothiazide in their synthetic mixtures and in the pharmaceutical

TABLE 3 . Recovery data obtained for different mixtures by using the first derivative spectrophotometry

	Lisinopril			Hydrochlorothiazide		
Mixture no	Added µg	Found µg	Recovery %	Added µg	Found µg	Recovery %
1	20.00	19.80	98.70	3.00	2.98	99.50
2	20.00	19.80	98.35	6.00	5.97	99.67
3	20.00	19.60	99.00	9.00	8.97	99.68
4	20.00	19.70	99.00	12.50	12.46	99.35
5	20.00	19.70	100.50	15.30	15.20	99.63
6	20.00	19.60	98.00	18.70	18.63	99.30
7	20.00	19.80	98.00	21.30	21.15	99.31
8	20.00	19.80	99.45	24.80	24.63	99.84
			x = 98.60			
			RSD = 0.42 %			
9	5.00	4.97	99.40	12.50	12.48	99.84
10	10.00	9.81	98.10	12.50	12.48	99.84
11	15.00	14.90	99.33	12.50	12.40	99.20
12	20.00	19.70	98.50	12.50	12.40	99.20
13	25.00	24.68	98.72	12.50	12.40	99.20
14	30.00	29.80	99.33	12.50	12.47	99.76
15	35.00	34.70	99.14	12.50	12.42	99.36
16	40.00	39.70	99.25	12.50	12.47	99.76
n = 16						x = 99.57
						RSD = 0.28 %

TABLE 4. Calibration data in the determination of lisinopril and hydrochlorothiazide

Methods	λ (nm)	Linearity range (µg.ml^{-1})	Equation	Regression coefficient (r)
First derivative	269.6	25.56 -129.50	$y=6.72.10^{-3}C_I+9.1.10^{-4}$	0.9998
spectrophotometry	279.8	10.60 - 139.80	$y=1.20.10^{-3}C_H+1.00.10^{-4}$	0.9991
Ratio spectra	253.7	15.68 - 129.50	$y=4.30.10^{-3}C_I+6.43.10^{-4}$	0.9997
first derivative	243.6	15.68 - 129.50	$y=8.73.10^{-3}C_I+7.74.10^{-4}$	0.9995
spectrophotometry	280.1	5.98 - 139.80	$y=4.28.10^{-3}C_H+1.31.10^{-4}$	0.9988
	270.8	5.98 - 139.80	$y=8.56.10^{-3}C_H+9.23.10^{-4}$	0.9986

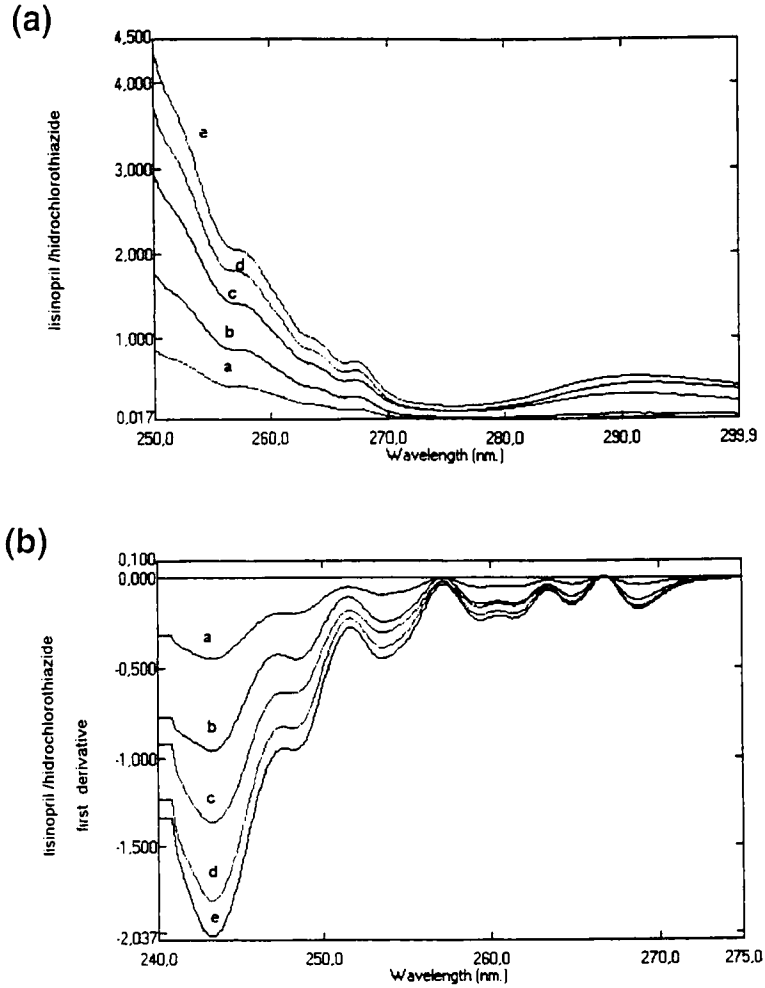

Figure 3. Ratio spectra (a) and first derivative of the ratio spectra (b) of lisinopril of a) 15.0 $\mu g.ml^{-1}$,b) 45.0 $\mu g.ml^{-1}$,c) 75.0 $\mu g.ml^{-1}$,d) 105.0 $\mu g.ml^{-1}$,e) 125.0 $\mu g.ml^{-1}$ when 70.0 $\mu g.ml^{-1}$ hydrochlorothiazide used as divisor in methanol ($\Delta\lambda$ = 4 nm)

TABLE 5. Recovery data obtained for different mixtures by using the first derivative of the ratio spectra

	Lisinopril			Hydrochlorothiazide		
Mixture no	Added µg	Found µg	Recovery %	Added µg	Found µg	Recovery %
1	20.00	19.99	99.95	3.00	2.95	98.33
2	20.00	19.94	99.70	6.00	5.98	99.67
3	20.00	20.30	101.5	9.00	8.97	99.68
4	20.00	20.15	100.75	12.50	12.51	100.00
5	20.00	19.70	100.50	15.30	15.23	99.54
6	20.00	19.74	98.70	18.70	18.68	99.89
7	20.00	19.78	98.90	21.30	21.25	99.76
8	20.00	19.85	99.25	24.80	24.66	99.43
			x = 99.91			
			RSD = 0.91 %			
9	5.00	4.99	99.80	12.50	12.49	99.92
10	10.00	9.96	99.60	12.50	12.48	99.84
11	15.00	14.93	99.53	12.50	12.43	99.44
12	20.00	20.05	100.25	12.50	12.52	100.16
13	25.00	24.68	98.72	12.50	12.40	99.20
14	30.00	29.86	99.53	12.50	12.48	99.84
15	35.00	34.76	99.31	12.50	12.36	98.88
16	40.00	39.87	99.68	12.50	12.38	99.04
n = 16						x = 99.54
						RSD = 0.44 %

formulation only the calibration graphs that were obtained by measuring the signals at 253.7 nm for lisinopril and 270.8 nm for hydrochlorothiazide in the first derivative of the ratio spectra were used The main instrumental conditions were optimized to obtain the most distinct curve of first derivative of the ratio spectra. By selecting a divisor of appropriate concentration. some divisor concentrations were tested in the determination . The standard solutions of 98.70 $\mu g.ml^{-1}$ of hydrochlorothiazide and 109.78 $\mu g.ml^{-1}$ of lisinopril for determining hydrochlorothiazide and formulation only the calibration graphs that were obtained by measuring the signals at 253.7 nm for lisinopril and 270.8 nm for hydrochlorothiazide in the first derivative of the ratio spectra were used The main instrumental conditions were optimized to obtain the most distinct curve of first derivative of the ratio spectra. By selecting a divisor of appropriate concentration, some divisor

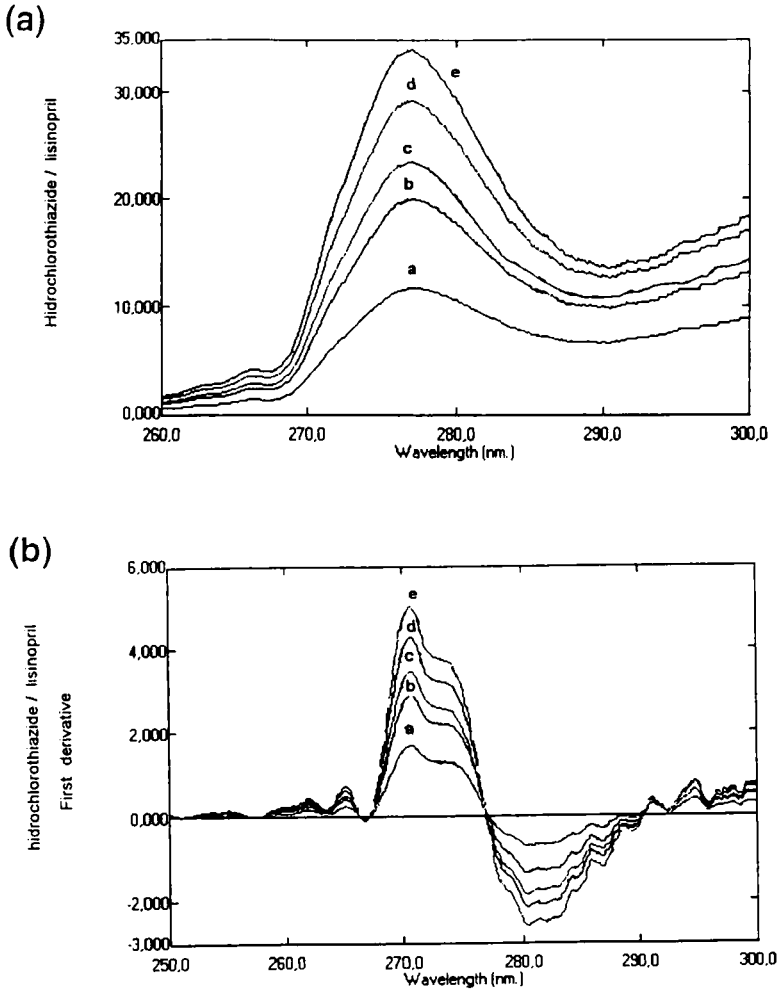

Figure 4. Ratio spectra (a) and first derivative of the ratio spectra (b) of hydrochlorothiazide of a) 5.0 $\mu g.ml^{-1}$,b) 40.0 $\mu g.ml^{-1}$, c)70.0 $\mu g.ml^{-1}$, d)100.0 $\mu g.ml^{-1}$,e) 130.0 $\mu g.ml^{-1}$ when 75.0 $\mu g.ml^{-1}$ lisinopril used as divisor in methanol ($\Delta\lambda$ = 4 nm)

TABLE 6. Assay results in commercial product (mg)

	Lisinopril mean ± SD	Hydrochlorothiazide mean ± SD
Vierordt's method	19.6 ± 0.7	12.3 ± 0.8
First derivative spectrophotometry	20.1± 0.3	12.4 ± 0.5
Ratio spectra derivative spectrophotometry	19.8 ± 0.8	12.3 ± 0.3

- Results obtained are the average of ten experiments for each, -SD = Standard deviation

C_L = $\mu g.ml^{-1}$ of lisinopril ; C_H = $\mu g.ml^{-1}$ of hydrochlorothiazide

concentrations were tested in the determination. The standard solution of 98.70 $\mu g.ml^{-1}$ of hydrochlorothiazide and 109.78 $\mu g.ml^{-1}$ of lisinopril for determining hydrochlorothiazide and lisinopril in their binary mixtures were found to be suitable.The influence of $\Delta\lambda$ on obtaining the first derivative was tested and a value of $\Delta\lambda$ = 4 nm was considered as suitable for both determinations. A good coincidence was observed for the assay results of the pharmaceutical formulation by application of the three methods in this paper (Table 6).

CONCLUSION

The concentration range of both drugs by ratio spectra derivative spectrophotometry,a new and powerful technique for the analysis of mixtures , has been determined. In ratio spectra derivative spectrophotometry separate peaks and higher values of measurements can be obtained owing to the advantages of the selectivity of divisor concentration. The determination limits in Vierordt's method , a classical spectrophotometric method, were found to be the same with ratio spectra derivative spectrophotometry . But in contrast to ratio spectra derivative spectrophotometry,this method requires solving time consuming equations with two unknowns,in contrast to the simplicity of the ratio spectra derivative spectrophotometric method.It was observed that the methods proposed in this paper were more simple and precise than the methods described in the literature . In addition , these spectrophotometric methods have the advantage in comparison with the conventional multicomponent analysis methods, such as HPLC or GC, in not requiring any separation procedures.

These three methods proposed in this paper were found to be suitable for the determination of lisinopril and hydrochlorothiazide in their binary mixtures .Recovery data on added drugs were excellent and the procedures were shown applicable to commercial formulation of these compounds.

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Date Received: November 5,1997
Date Accepted: January 8, 1997