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Application of a new simple spectrophotometric method for determination of the binary mixtures of hydrochlorothiazide with either carvedilol or losartan potassium in tablets dosage forms

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ABSTRACT

In this work, a new simple spectrophotometric method is presented for determination of the two antihypertensive binary mixtures of hydrochlorothiazide (HCT) with either carvedilol (CRV) or losartan potassium (LOS) without prior separation. The proposed method is based on generation of ratio spectra of one compound using the other as a divisor followed by measurement of the ratio-difference (peak-to-trough) amplitudes between two selected wavelengths in the ratio spectra. For analysis of HCT/CRV mixture, 20 µg/mL CRV was used as a divisor, and the ratio-difference amplitudes between 287 and 299 nm were plotted against HCT concentration. Similarly, by using 20 µg/mL HCT as a divisor, the peak-to-trough amplitudes between 269.5 and 287.5 nm were found proportional to CRV concentration. Calibration curves were linear in the range 5 - 80 µg/mL for both drugs. For determination of HCT/LOS mixture, a standard solution of LOS 50 µg/mL was used as a divisor, and the ratio-difference amplitudes between 224 and 275 nm were measured and correlated to HCT concentration. Similarly, HCT 50 µg/mL was set as a divisor in LOS determination and the peak-to-trough amplitudes between 242 and 275 nm were recorded. Calibration curves were linear over the concentration ranges 5-50 and 5-60 µg/mL for HCT and LOS, respectively. The developed methods were validated following the ICH guidelines and successfully applied to the determination of the studied drugs in various laboratory prepared mixtures. In addition, satisfactory results were obtained from analysis of the tablets dosage forms with no significant statistical differences from the reference methods.

Keywords: Spectrophotometric analysis; Ratio spectra; Peak-to-trough amplitudes; Hydrochlorothiazide; Carvedilol; Losartan potassium; Tablets dosage forms.

INTRODUCTION

Hydrochlorothiazide (HCT) (Figure 1), chemically known as 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide [1], is a moderately potent thiazide diuretic. It exerts its effect by reducing the re-absorption of electrolytes from the renal tubules, thereby increasing the excretion of sodium and chloride ions, and consequently of water. HCT is used in the treatment of hypertension either alone or with other antihypertensives [1]. Carvedilol (CRV) (Figure 1), chemically known as 1-carbazol-4-yloxy-3-[2-(2-methoxyphenoxy) ethylamino]propan-2-ol [1], is a non-cardioselective beta blocker. It has vasodilating properties, which are attributed mainly to its alpha-1 blocking activity; at higher doses calcium channel blocking activity may contribute. CRV is

used in the management of hypertension, angina pectoris and as an adjunct to standard therapy in symptomatic heart failure [1]. Losartan potassium (LOS) (Figure 1) is chemically known as 2-butyl-4-chloro-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol potassium [1]. LOS is an angiotensin II receptor antagonist with antihypertensive activity due mainly to selective blockade of AT_1 receptors and the consequent reduced pressor effect of angiotensin II. It is used in the management of hypertension, particularly in patients who develop cough with ACE inhibitors and to reduce the risk of stroke in patients with left ventricular hypertrophy, and in the treatment of diabetic nephropathy [1].

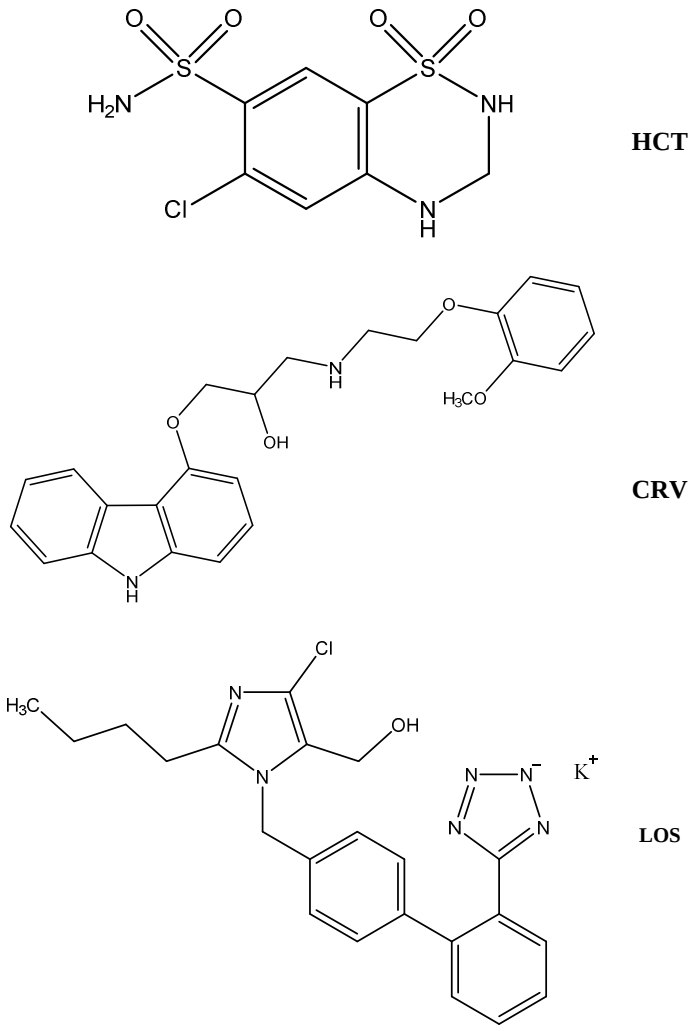

Figure 1: Chemicals structures of hydrochlorothiazide (HCT), carvedilol (CRV) and losartan potassium (LOS)

The simultaneous determination of the binary combination of HCT and CRV was addressed in few analytical reports. These reports described the use of HPLC with UV detection [2-4], HPLC-DAD [5], TLC [4] and capillary electrophoresis [6]. In addition, several spectrophotometric methods were reported including the traditional zero-crossing derivative spectrophotometry [2], derivative ratio spectrophotometry [4], dual wavelength analysis [7] and the Q-analysis (graphical absorbance ratio) method [7]. On the other hand, numerous articles can be found in the literature suggesting various methods for the analysis of HCT/LOS binary mixture. Separation techniques were applied such as HPLC with UV detection [8-10], HPLC-tandem mass spectrometry (LC-MS/MS) [11], HPTLC [12], capillary electrophoresis (CE) [13] and capillary electrochromatography (CEC) [13]. Recently, a differential-pulse voltammetric method was developed for the simultaneous determination of HCT and LOS [14]. Additionally, several spectrophotometric methods were applied for the assay of this mixture such as derivative spectrophotometry

[12,15], derivative ratio spectrophotometry [8], compensation technique [8], simultaneous equations method [16], dual wavelength method [16] and chemometric multivariate analysis [17].

In previous works, we introduced a new *ratio spectra peak-to-trough measurement method* for the simultaneous determination of some selected drugs in their binary mixtures without prior separation [18-20]. The method is based on generation of the ratio spectra of compound X by using a standard spectrum of compound Y as a divisor. The peak-to-trough (ratio-difference) amplitudes between two selected wavelengths are proportional to concentration of X without interference from Y simply because its interference has been converted into a constant which is eliminated by subtraction. Similarly, compound Y can be determined using X as a divisor. In this work, we investigate this simple method for the determination of the binary mixtures HCT/CRV and HCT/LOS. The method was validated and successfully applied to the assay of the investigated drugs in their combined commercial preparations. The simplicity, fast speed and economic affordability of the proposed method support its applicability for quality control purposes.

MATERIALS AND METHODS

Instrumentation

Specord S600 UV-VIS diode array spectrophotometer (scan speed 6000 nm/min and wavelength interval 0.5 nm) associated with WinAspect software version 2.3 (Analytik Jena AG, Germany) was used. Absorbance measurements were recorded using 10 mm quartz cells.

Materials

HCT was kindly donated by Pharco Pharmaceuticals Co., Alexandria, Egypt. CRV was kindly supplied by Chemipharm Pharmaceutical Industries, 6th October City, Egypt and LOS was a gift from El-Amryia Pharmaceuticals Co., Alexandria, Egypt. HPLC-grade methanol (Sigma-aldrich Chemie GmbH, Buchs, Switzerland), analytical grade of hydrochloric acid (El-Nasr Pharmaceutical Chemicals Company, Egypt) and high purity distilled water were used. Pharmaceutical preparations involved in this study are Co-Dilatrend® tablets (Roche Austria GmbH, Wien, Austria, Batch No. M5015M1) labeled to contain 25 mg CRV and 12.5 mg HCT per tablet, Co-Dilatrol® tablets (Chemipharm Pharmaceutical Industries S.A.E., 6th October City, Egypt, Batch No. 110872A) labeled to contain 25 mg CRV and 12.5 mg HCT per tablet and Hyzaar® tablets (Merck Sharp & Dohme BV, Haarlem**,** Netherlands, Batch No. NM10430) labeled to contain 50 mg LOS and 12.5 mg HCT per tablet.

General procedures and construction of calibration graphs

Mixture 1: HCT/CRV

Stock standard solutions of HCT 1000 µg/mL and CRV 1000 µg/mL were prepared in methanol. Portions of both solutions were separately diluted with 0.1M hydrochloric acid solution to attain the concentration ranges specified in Table 1. The absorption spectra of the prepared working solutions were recorded in the range of 200-400 nm against 0.1M hydrochloric acid solution. For the determination of HCT, the recorded absorption spectra were divided, wavelength by wavelength, by the spectrum of a standard solution of CRV 20 µg/mL in 0.1M hydrochloric acid solution. The peak-to-trough (ratio-difference) amplitudes in the obtained HCT ratio spectra between 287 and 299 nm were measured and plotted versus the corresponding concentrations to obtain the calibration graph. Similarly, a standard spectrum of HCT 20 µg/mL was used as a divisor in the determination of CRV and the peak-to-trough measurements between 269.5 and 287.5 nm were recorded.

Mixture 2: HCT/LOS

Stock standard solutions of HCT 1000 µg/mL and LOS 1000 µg/mL were prepared in methanol. Portions of both solutions were separately diluted with 0.1M hydrochloric acid solution to reach the concentration ranges specified in Table 1. The absorption spectra of the prepared working solutions were recorded in the range of 200-400 nm against 0.1M hydrochloric acid solution. For generation of HCT ratio spectra, a standard spectrum of LOS 50 µg/mL was set as the divisor, and the peak-to-trough (ratio-difference) measurements between 224 and 275 nm were used for construction of the calibration graph. As for LOS calibration, the absorption spectra were divided by HCT 50 µg/mL standard spectrum then the peak-to-trough (ratio-difference) amplitudes between 242 and 275 nm were measured and plotted versus the corresponding concentrations.

Assay of tablets dosage forms

Mixture 1: HCT/CRV

Ten Co-dilatrend® or Co-dilatrol® tablets were separately weighed and finely powdered. An accurately weighed amount of the powdered tablets from each brand equivalent to 25 mg CRV and 12.5 mg HCT was separately extracted with 60 mL HPLC-grade methanol with the aid of shaking for 10 min then filtered into a 100-mL volumetric flask. The residue was washed with 2x10 mL methanol and washings were added to the filtrate. Tablet solutions were completed to volume with methanol. Aliquots from the prepared tablet solutions were accurately diluted with 0.1M hydrochloric acid solution to obtain final concentrations within the specified ranges (Table 1) then they were treated as described under general procedures.

Mixture 2: HCT/LOS

Ten Hyzaar® tablets were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 50 mg LOS and 12.5 mg HCT was extracted with HPLC-grade methanol according to the aforementioned extraction procedure. Aliquots from the prepared tablet solution were accurately diluted with 0.1M hydrochloric acid solution to obtain final concentrations within the specified ranges (Table 1) then they were treated as described under general procedures.

RESULTS AND DISCUSSION

Spectral characteristics and development of the methods

Although direct UV spectrophotometry is an appealing simple procedure for the quality control of drugs in their pharmaceutical preparations, it is not applicable when drugs with overlapping spectra coexist in multi-component mixtures. This is the case in the investigated binary mixtures; where both components of mixture 1 (HCT and CRV) and mixture 2 (HCT and LOS) exhibit significant interference in the determination of the other as shown in Figure 2. Hence, the simultaneous determination of the compounds in each binary mixture necessitates a mathematical treatment of the absorption data in order to omit the interference imposed by each drug while determining the other. Accordingly, a simple one-step correction procedure based on generation of ratio spectra was developed.

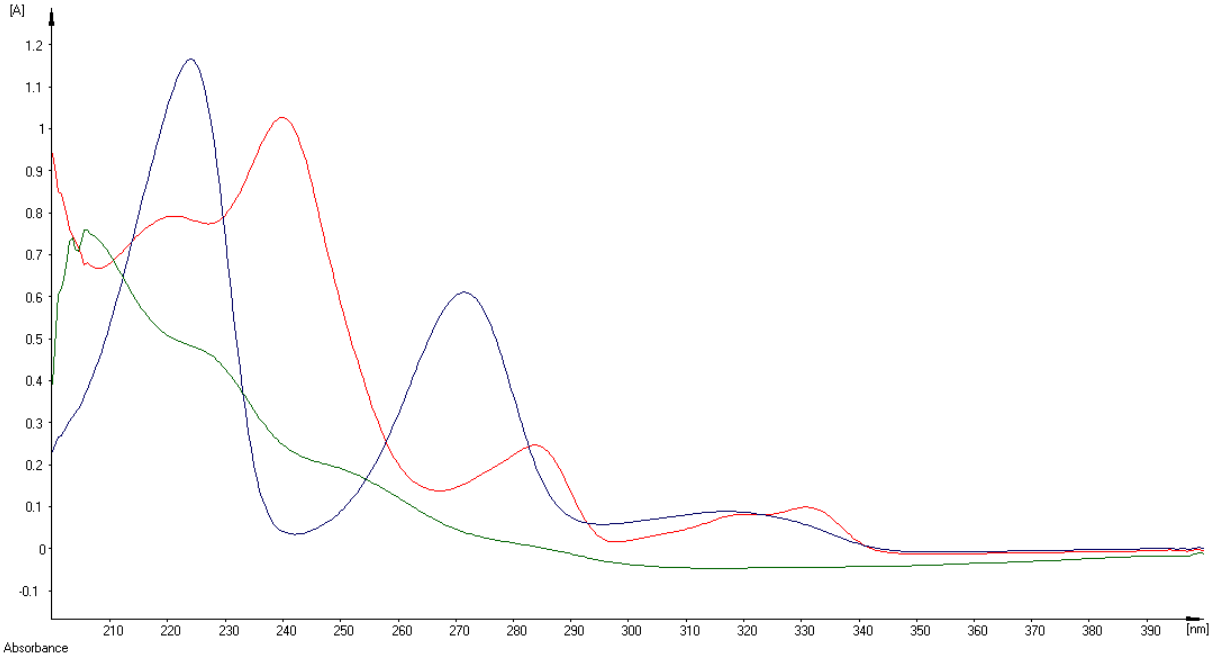

Figure 2: Absorption spectra of 10 µg/mL HCT (——), 10 µg/mL CRV (——) and 10 µg/mL LOS (——) in 0.1 M hydrochloric acid solution

First, a study was carried out to examine the effect of divisor concentration on the generated ratio spectra of the studied drugs. When the concentration of divisor is increased or decreased, the resulting absorbance ratio values

proportionally decrease or increase, respectively, however the positions of the peaks and troughs remain unaffected by changing the divisor concentration. Divisor concentration was tested in the range of 20–30 µg/mL for mixture 1 (HCT and CRV) and in the range 50-80 µg/mL for mixture 2 (HCT and LOS). The best results in terms of sensitivity, accuracy and repeatability of measurements were obtained using divisor concentration 20 µg/mL of either HCT or CRV in case of mixture 1, and 50 µg/mL of either HCT or LOS for mixture 2.

For analysis of HCT/CRV mixture, the ratio spectra of different HCT standards at increasing concentrations in 0.1M hydrochloric acid solution, obtained by dividing each by the spectrum of 20 µg/mL CRV in the same solvent are illustrated in Figure 3. The peak to trough amplitudes between 287 and 299 nm on the generated ratio spectra are proportional to HCT concentration. For the determination of CRV, an analogous procedure was followed. Figure 4 shows the ratio spectra of different standards of CRV using 20 µg/mL HCT as a divisor. The peak to trough amplitudes between 269.5 and 287.5 nm on the produced ratio spectra were measured and found proportional to CRV concentration.

The same principle was applied for the simultaneous determination of HCT and LOS. The ratio spectra obtained from the division of HCT absorption spectra by a standard spectrum of LOS showed a peak at 275 nm and a trough at 242 nm (Figure 5). However, the ratio-difference amplitudes were recorded between the peak at 275 nm and another smaller peak at 224 nm. These selected wavelengths led to better results in terms of accuracy and precision of measurements; therefore they were used for the construction of HCT calibration equation. Similarly by using HCT as the divisor, the peak to trough (ratio-difference) amplitudes between 242 and 275 nm in LOS ratio spectra were selected for its determination (Figure 6).

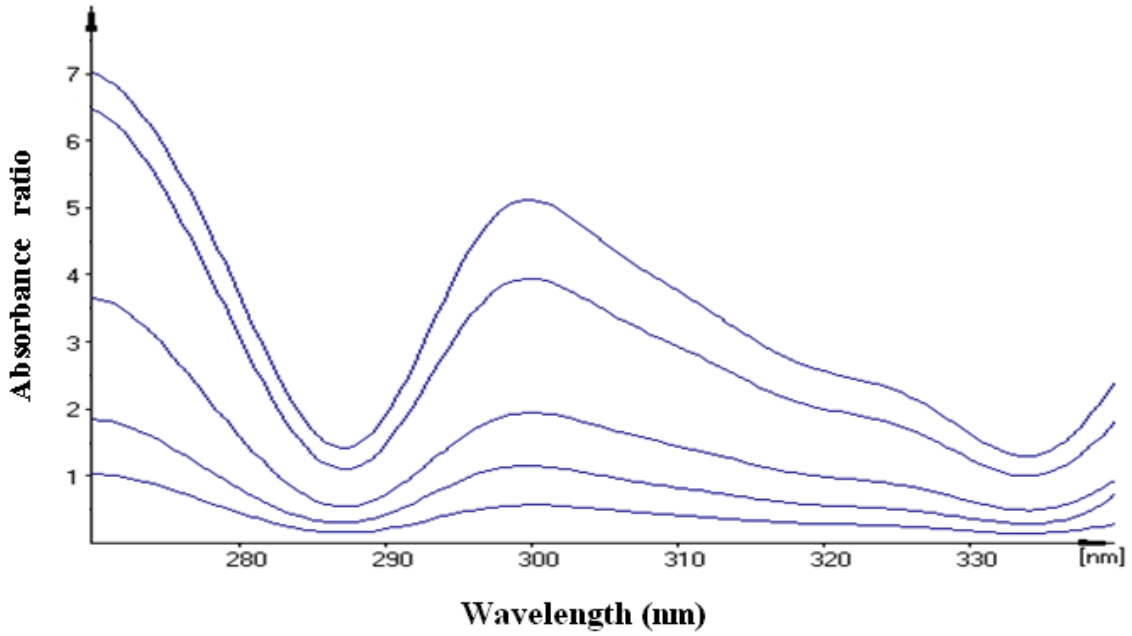

Figure 3: Ratio spectra of 5, 10, 20, 40 and 50 µg/mL HCT using 20 µg/mL CRV as a divisor

Validation of the proposed spectrophotometric methods

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines on validation of analytical procedures [21].

Linearity and concentration ranges

The linearity of the proposed method was evaluated by analyzing serial concentrations of each drug within the concentration range mentioned in Table 1. The measured response was plotted as a function of the corresponding concentration and the regression equation was calculated using the least squares method. Different regression parameters including correlation coefficients (r), slopes, intercepts and their corresponding standard deviations are summarized in Table 1. The high correlation coefficients values (r > 0.9996) associated with the negligible intercepts indicated good linearity of the proposed procedures. In addition,

deviation around the slope can be evaluated by calculation of the RSD% of the slope (S_b%) which were found to be less than 1.6 %. In addition to the previous parameters, linearity can be further guaranteed by the analysis of variance (ANOVA) test [22]. The most important statistic in this test is the F-value which is the ratio of the mean of squares due to regression divided by the mean of squares due to residuals. High F values reveal an increase in the mean of squares due to regression and a decrease in the mean of squares due to residuals. The greater the mean of squares due to regression, the steeper is the regression line. The smaller the mean of squares due to residuals, the less is the scatter of experimental points around the regression line. Consequently, regression lines with high F values (low significance F) are much better than those with lower ones. Good regression lines show high values for both r and F statistical parameters.

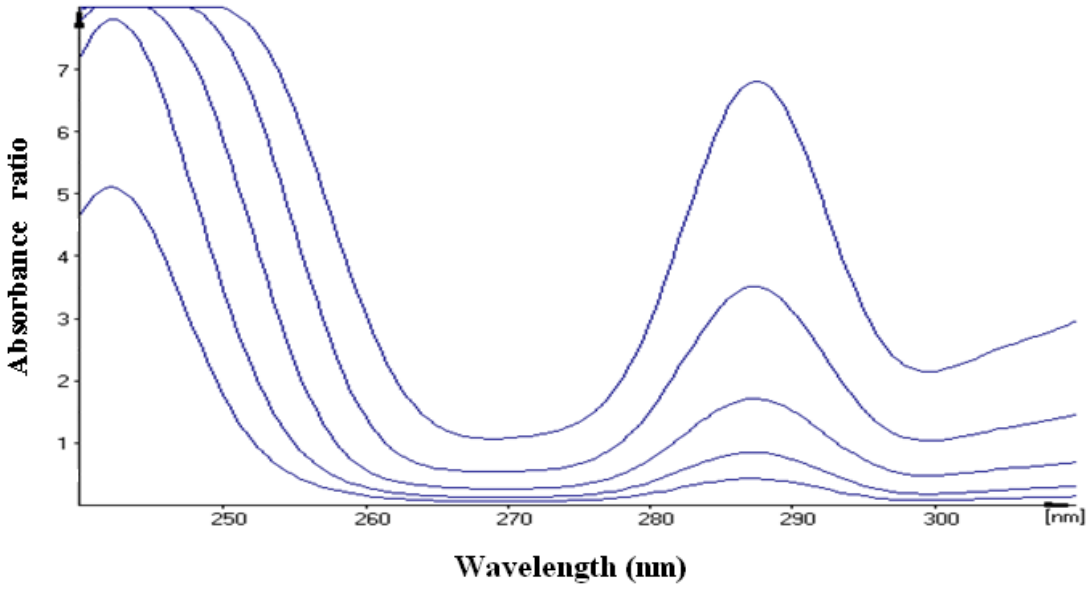

Figure 4: Ratio spectra of 5, 10, 20, 40 and 80 µg/mL CRV using 20 µg/mL HCT as a divisor

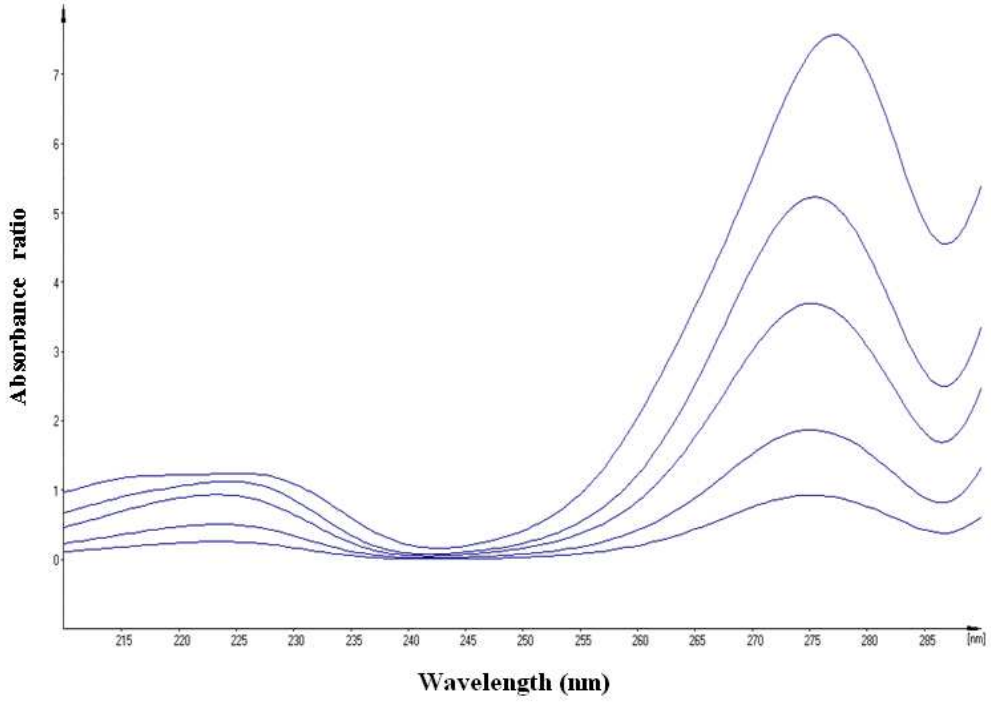

Figure 5: Ratio spectra of 5, 10, 20, 30 and 50 µg/mL HCT using 50 µg/mL LOS as a divisor

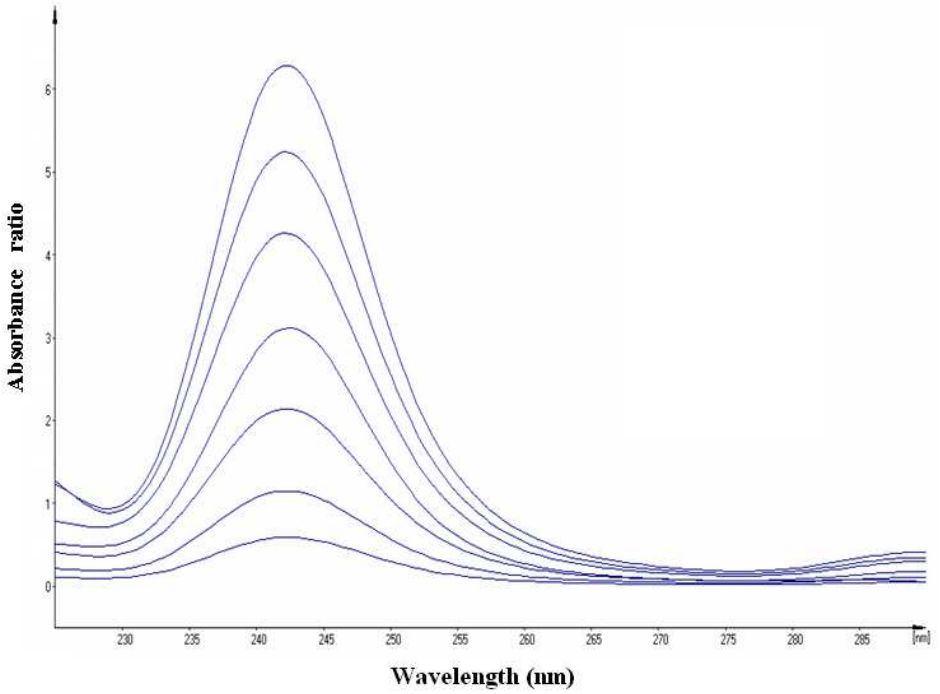

Figure 6: Ratio spectra of 5, 10, 20, 30, 40, 50 and 60 µg/mL LOS using 50 µg/mL HCT as a divisor

Limits of detection and quantification

Limit of detection (LOD) was defined as 3.3 S_a/b and limit of quantification (LOQ) was computed as 10 S_a/b, where S_a is the standard deviation of the intercept and b is the slope of the calibration curve. The calculated values are presented in Table 1.

Table 1. Regression and analytical parameters for the determination of the binary mixtures using the proposed spectrophotometric method

Parameter	Mixture 1		Mixture 2	
	HCT	CRV	HCT	LOS
Wavelengths (nm)	287 and 299	269.5 and 287.5	224 and 275	242 and 275
Concentration range (µg/mL)	5 – 80	5 – 80	5 – 50	5 – 60
Intercept (a)	-0.001	0.008	0.05	0.08
S_a [a]	0.02	0.01	0.06	0.02
Slope (b)	0.07	0.07	0.13	0.10
S_b [b]	0.0007	0.0003	0.002	0.0005
RSD% of the slope (S_b%)	1.00	0.43	1.54	0.50
Correlation coefficient (r)	0.9997	0.9999	0.9996	0.9999
$S_{y/x}$ [c]	0.04	0.01	0.07	0.03
F [d]	10818	70920	5064	30937
Significance F	5.32×10^{-11}	1.89×10^{-13}	2.34×10^{-7}	1.13×10^{-10}
LOD [e] (µg/mL)	0.94	0.47	1.52	0.66
LOQ [f] (µg/mL)	2.86	1.43	4.62	2.00

[a] Standard deviation of the intercept; [b] Standard deviation of the slope; [c] Standard deviation of residuals
[d] Variance ratio, equals the mean of squares due to regression divided by the mean of squares about regression (due to residuals)
[e] Limit of detection; [f] Limit of quantification

Precision and accuracy

The within-day repeatability of the proposed method was assessed through the analysis of three concentration levels for each drug prepared in triplicates. Correspondingly, the between-day precision (also known as the intermediate precision) was studied on the same levels over 3 consecutive days. Table 2 contains the values of percentage relative standard deviation (RSD%) which did not exceed 2% for both mixtures indicating the acceptable level of precision of the proposed method. The adequate recovered concentrations in addition to the low values of percentage relative error (E_r%) gathered in Table 2 also confirm the accuracy of the developed method.

Table 2. Precision and accuracy for the determination of the drugs in bulk forms using the proposed spectrophotometric method

	Analyte	Nominal value (µg/mL)	Within-day			Between-day		
			Found ± SD[a] (µg/mL)	RSD(%)[b]	E_r(%)[c]	Found ± SD[a] (µg/mL)	RSD(%)[b]	E_r(%)[c]
Mixture 1	HCT	10	10.14 ± 0.09	0.89	1.40	10.11 ± 0.08	0.79	1.10
		20	19.97 ± 0.22	1.10	-0.15	20.10 ± 0.38	1.89	0.50
		50	49.57 ± 0.91	1.84	-0.86	49.86 ± 0.79	1.58	-0.28
	CRV	10	9.82 ± 0.10	1.02	-1.80	9.92 ± 0.06	0.60	-0.80
		20	20.29 ± 0.16	0.79	1.45	19.88 ± 0.37	1.86	-0.60
		50	49.93 ± 0.19	0.38	-0.14	49.67 ± 0.31	0.62	-0.66
Mixture 2	HCT	10	10.09 ± 0.06	0.60	0.90	9.88 ± 0.19	1.92	-1.20
		20	20.04 ± 0.16	0. 80	0.20	19.85 ± 0.39	1.97	-0.75
		50	49.19 ± 0.50	1.02	-1.62	49.19 ± 0.64	1.30	-1.62
	LOS	10	10.00 ± 0.07	0. 70	0.00	10.07 ± 0.09	0.89	0. 70
		30	30.57 ± 0.59	1.93	1.90	29.85 ± 0.50	1.68	-0.50
		50	50.22 ± 0.58	1.16	0.44	50.80 ± 0.31	0.61	1.60

[a] Mean ±standard deviation for three determinations.; [b] % Relative standard deviation.; [c] % Relative error.

Stability of solutions

Stability of the working solutions of the studied drugs in 0.1M hydrochloric acid was verified and no significant spectrophotometric changes were noticed for at least 6 hr. Also, the stock solutions in methanol were stable for at least two weeks when stored refrigerated at 4°C.

Applications of the proposed methods

Analysis of laboratory-prepared synthetic mixtures

In order to further assess the applicability of the proposed method for the determination of the selected drugs in their binary solutions, several synthetic mixtures were prepared. These mixtures contained different ratios of each drug pair; both above and below their normal ratios in tablets. The content of each drug was then calculated from the corresponding regression equation. The acceptable recovered concentrations, values of RSD(%) and E_r(%) gathered in Table 3 for both mixtures confirm the accuracy and precision of the method, and demonstrate its analytical power to resolve and quantify the investigated drugs when present in different proportions.

Table 3. Determination of HCT/CRV and HCT/LOS laboratory-prepared mixtures using the proposed spectrophotometric method

Nominal value (µg/mL)		Found ± SD[a] (µg/mL)		RSD(%)[b]		E_r(%)[c]	
HCT	CRV	HCT	CRV	HCT	CRV	HCT	CRV
20	10	19.80 ± 0.17	10.04 ± 0.18	0.86	1.79	-1.00	0.40
10	10	9.98 ± 0.05	10.03 ± 0.14	0.50	1.40	-0.20	0.30
10	20	10.10 ± 0.10	20.05 ± 0.12	0.99	0.60	1.00	0.25
10	30	9.85 ± 0.16	29.87 ± 0.35	1.62	1.17	-1.50	-0.43
10	40	10.19 ± 0.15	39.80 ± 0.12	1.47	0.30	1.90	-0.50
10	50	10.03 ± 0.07	49.63 ± 0.48	0.70	0.97	0.30	-0.74
HCT	**LOS**	**HCT**	**LOS**	**HCT**	**LOS**	**HCT**	**LOS**
20	10	10.02 ± 0.12	19.99 ± 0.09	1.20	0.45	0.20	-0.05
10	10	9.96 ± 0.12	9.95 ± 0.09	1.21	0.91	-0.40	-0.50
10	20	19.66 ± 0.21	10.01 ± 0.06	1.07	0.60	-1.70	0.10
10	30	29.80 ± 0.52	10.04 ± 0.11	1.75	1.10	-0.67	0.40
10	40	40.14 ± 0.17	9.95 ± 0.08	0.42	0.80	0.35	-0.50
10	50	50.19 ± 0.37	10.08 ± 0.03	0.74	0.30	0.38	0.80

[a] Mean ±standard deviation for three determinations.; [b] % Relative standard deviation.; [c] % Relative error.

Analysis of tablets dosage forms

The proposed method was successfully applied to the analysis of both antihypertensive mixtures in their pharmaceutical preparations. The active ingredients were extracted and directly quantified without any interference from the inactive ingredients. Results obtained were precise and in good agreement with the labeled claim as concluded from the satisfactory values of % recovery, SD and RSD(%) gathered in Table 4. A reference HPLC-UV [3] method was applied to the analysis of HCT/CRV binary mixture in its pharmaceutical formulations (Co-Dilatrend® and Co-Dilatrol® tablets). Similarly, a derivative-ratio spectrophotometric method [8] was implemented for the analysis of HCT/LOS binary mixture in its dosage form (Hyzaar ® tablets). Recovery data obtained from the developed spectrophotometric method were statistically compared with those of the reference methods using the

Student's t- and the variance ratio F-tests. In both tests, the calculated values did not exceed the theoretical ones at the 95% confidence level which indicated that there were no significant differences between the recoveries obtained from the proposed method and those of the reference methods (Table 4). It is evident from these results that the proposed method is applicable to the assay of both drug combinations with minimum sample preparation and satisfactory levels of accuracy and precision.

Table 4. Application of the proposed spectrophotometric method for the analysis of HCT/CRV and HCT/LOS binary mixtures in tablets dosage forms

Co-Dilatrend® tablets	**Proposed method**		**Reference method**	
	HCT	**CRV**	**HCT**	**CRV**
%Recovery ± SD[a]	99.85 ± 0.53	99.15 ± 1.01	99.92 ± 0.24	100.10 ± 0.66
RSD(%)[b]	0.53	1.02	0.24	0.66
t	0.28	1.76		
F	4.88	2.34		
Co-Dilatrol® tablets	**Proposed method**		**Reference method**	
	HCT	**CRV**	**HCT**	**CRV**
%Recovery ± SD[a]	100.09 ± 0.76	99.94 ± 0.29	99.10 ± 1.13	99.56 ± 0.53
RSD(%)[b]	0.76	0.29	1.14	0.53
t	1.63	1.38		
F	2.21	3.34		
Hyzaar® tablets	**Proposed method**		**Reference method**	
	HCT	**LOS**	**HCT**	**LOS**
%Recovery ± SD[a]	99.78 ± 0.49	100.01 ± 0.30	100.09 ± 0.26	100.18 ± 0.14
RSD(%)[b]	0.49	0.30	0.26	0.14
t	1.25	1.14		
F	3.55	4.59		

[a] Mean ± standard deviation for five determinations.; [b] % Relative standard deviation.; Theoretical values for t and F at P = 0.05 are 2.31 and 6.39, respectively.

CONCLUSION

A new, simple and rapid method is proposed for the analysis of two antihypertensive binary mixtures with overlapping spectra. The method involves the generation of absorbance ratio spectra followed by measurement of the ratio difference amplitudes between two carefully selected wavelengths. Ideally, these selected wavelengths should correspond to the peak and the trough in the ratio spectrum in order to achieve the highest sensitivity. The proposed method does not require any sophisticated mathematical treatment for the absorption data, and it exhibits several advantages over other spectrophotometric methods for resolution of binary mixtures. Pertaining to the simplicity of the method, it can be considered advantageous over its two lengthier versions; the derivative ratio method; that involves the additional derivative curve generation step, and the ratio subtraction method; which includes a sequence of division, constant subtraction and multiplication steps. Unlike derivative spectrophotometry, this method does not require optimization of the derivative order and searching for zero-crossing points. Finally, compared to the dual wavelength procedure, there is no need for the tedious search for two wavelengths where the interfering compound exhibits the same absorpitivity. Compared to the previously reported spectrophotometric methods for the studied mixtures, the proposed method is advantageous regarding simplicity of operation and sensitivity of measurement. The developed method does not require elaborate treatment, sophisticated experimental setup or consumption of organic solvents usually associated with HPLC and HPTLC methods of analysis. The applicability of the developed method was validated and evaluated through the determination of both drug combinations in several laboratory-prepared mixtures and in pharmaceutical tablets with good accuracy and precision.

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