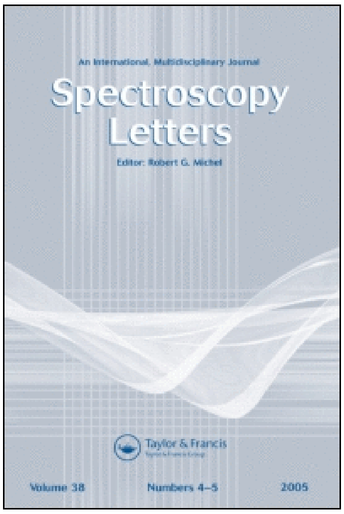

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Spectrophotometric Determination of Ternary Mixtures by the Derivative Ratio Spectrum-Zero Crossing Method

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SPECTROPHOTOMETRIC DETERMINATION OF TERNARY MIXTURES BY THE DERIVATIVE RATIO SPECTRUM-ZERO CROSSING METHOD

Key Words: Ratio spectra; Dipyridamole; Aspirin; Oxazepam; Degradation products; Dosage forms.

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ABSTRACT

A new spectrophotometric method is introduced for the assay of ternary mixtures with overlapping spectra. The method is based on the use of the first derivative of the ratio spectra and measurements of zero-crossing wavelengths. The ratio spectra were obtained by dividing the absorption spectrum of the mixture by that of one of the components. The concentration of the other components are then determined from their respective calibration graphs treated similarly. The method has been applied for the resolution of two ternary mixtures, namely, dipyridamole, aspirin and salicylic acid (I) and dipyridamole, oxazepam and 2-amino-5-chlorobenzophenone (II). Salicylic acid and benzo-

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phenone derivative are the degradation products of aspirin and oxazepam, respectively. The proposed method was applied for the assay of these combinations in synthetic mixtures and in commercial dosage forms. The results obtained were precise and accurate.

INTRODUCTION

The resolution of complex multicomponent systems without separation of the constituent analytes is rather a difficult task. The resolution of binary mixtures of compounds with overlapped spectra by derivative technique is frequently made on the basis of zero-crossing measurements[1-3]. However, in certain cases, the derivative technique cannot cope with the level of interference specially when the spectra are strongly overlapped or in case of ternary mixtures.

A spectrophotometric method for resolving binary mixtures has been reported[4]. The method is based on the use of the first derivative of the ratio spectrum. This method has been extended to determine ternary mixtures by simultaneous use of the zero-crossing method and Salinas method[5].

The approach taken has been applied to analyze ternary mixtures of drugs and their degradation products. The proposed method was applied for the determination of two ternary mixtures, namely, dipyridamole (DIP), aspirin (ASA) and salicylic acid (SA)(I), and dipyridamole (DIP); Oxazepam (OX) and 2-amino-5-chlorobenzophenone (BN) (II).

The DIP-ASA combination is widely used for secondary prevention of myocardial infarction[6], while DIP-OX binary mixture was introduced for the treatment of cardiac diseases and angina pectoris[3]. The literature reveals various methods for the analysis of DIP-ASA, DIP-OX and ASA-SA binary mixtures such as derivative spectrophotometry[3,6-8], fluorimetry[9], HPLC[10,11]. GLC[12] and HPLTC(13).

In literature, there is no method had been described for the analysis of the above mentioned ternary mixtures. Therefore, in this work the aim was to develop a spectrophotometric method for the analysis of ternary mixtures.

EXPERIMENTAL

Instruments

The absorption spectra were recorded on a Perkin-Elmer 3B UV/VIS Spectrophotometer with 1 cm quartz cuvettes. The spectrophotometer is interfaced to an IBM 286 computer loaded with "Perkin-Elmer Computerized Spectroscopy Software" (PECSS) program for spectral acquisition. The computer is connected to a Panasonic impact dot matrix printer KX-P 3626.

The spectrophotometric data were processed on a computer with printer Lazer Jet 4L. The software package used was EXCEL 4.0 under Windows.

Reagents and Chemicals

All the chemicals used were of analytical grade. Pure dipyridamole, oxazepam and acetyl salicylic acid were of pharmaceutical grade. 2-Amino-5-chlorobenzophenone (Sigma, USA) was used. Persantin-Plus capsules (CID Co., Egypt) contain 75 mg dipyridamole and 50 mg aspirin per capsule. Dipyridamole-oxazepam tablets contain 25 mg dipyridamole and 10 mg oxazepam per tablet.

Preparation of standard solutions

About 100 mg of each of DIP and ASA and about 50 mg of each of OX and BN were accurately weighed, transferred into four separate 100-ml volumetric flasks dissolved and completed to volume with methanol.

Calibration graphs

Various aliquots of each standard solution, within the concentration range stated in Table 1&2, were transferred into four sets of 100 ml-volumetric flasks, the solutions were brought to a constant volume with methanol and then were completed to volume with 0.1 N HCl.

TABLE 1

Effect of divisor concentration on the determination of DIP, ASA and SA by the proposed method

Compound	Selected λ nm	Divisor conc. mg%	Regression equation			RSD*
			Intercept (a)	Slope (b)	Corr. Coeff. (r)	
		(ASA)				
DIP 0.2-1.5 mg%	336	0.4	0.5159	79.411	0.9995	1.27
		0.6	0.0382	52.858	0.9995	1.44
		0.8	0.2403	39.844	0.9995	1.89
		(DIP)				
ASA 0.4-1.4 mg%	234	0.2	5.737×10^{-3}	0.481	0.9999	0.36
		0.5	2.428×10^{-3}	0.204	0.9999	0.36
		1.0	1.183×10^{-3}	0.099	0.9999	0.36
		1.5	7.943×10^{-4}	0.066	0.9999	0.37
		(DIP)				
SA 0.2-1.2 mg%	316	0.2	0.0245	0.283	0.9995	1.72
		0.5	0.0117	0.113	0.9994	1.84
		1.0	6.05×10^{-3}	0.056	0.9994	1.84

* Six separate determinations

For SA, different portions of ASA standard solution, within the specified range (Table 1), were transferred into a set of 100-ml volumetric flasks. To each flask, 1 ml of 1.0 N NaOH was added and the solutions were left for 10 min at room temperature. Then 1 ml of 1.0 N HCl was added to each flask and the volume was completed with 0.1 N HCl.

Preparation of Samples

An accurate weight of the mixed contents of ten capsules or powdered tablets, equivalent to the weight of one capsule or two tablets was transferred

TABLE 2

Effect of divisor concentration on the determination of DIP, OX and BN by the proposed method.

Compound	Selected λ nm	Divisor conc. mg%	Regression equation			RSD*
			Intercept (a)	Slope (b)	Corr. Coeff. (r)	
DIP 0.4-1.3 mg%	330	(OX)				
		0.1	0.1931	1.8107	0.9999	0.47
		0.2	0.0982	0.9413	0.9999	0.46
		0.3	0.0462	0.4978	0.9999	0.43
OX 0.1-0.5 mg%	368	(DIP)				
		0.6	$1.099x10^{-3}$	0.1743	0.9995	1.46
		1.0	$6.75x10^{-4}$	0.1201	0.9994	1.60
		1.3	$5.29x10^{-4}$	0.0994	0.9994	1.67
BN 0.1-0.5 mg%	270	(DIP)				
		0.6	$-6.77x10^{-4}$	0.3631	0.9998	1.02
		1.0	$-3.99x10^{-4}$	0.2286	0.9998	1.05
		1.3	$-3.01x10^{-4}$	0.1815	0.9998	1.06

* Five separate determinations

into 100-ml volumetric flask. The powder was extracted with methanol by mechanical stirring for 20 min, the volume was completed with methanol and filtered. Aliquots of the filtrate, within the specified range, were diluted as described under calibration graphs.

Spectrophotometric measurements

The absorbances of the standard and sample solutions were recorded within the wavelength range 200-350 nm and stored.

RESULTS AND DISCUSSION

Mixture (I)

This mixture contains DIP, ASA and SA. The absorption spectra of the three components are strongly overlapped, that the application of the derivative technique failed to resolve it. On the other hand, this spectral overlapping was sufficiently enough to demonstrate the resolving power of the proposed method. For the determination of DIP, the stored absorption spectra of standard solutions of DIP, ASA, SA and a solution of their mixture were divided (amplitude by amplitude at appropriate wave-lengths) by the absorption spectrum of a standard solution of 0.5 mg% ASA, then the first derivative of the obtained ratio spectra were calculated with $\Delta\lambda = 2$ nm (Fig. 1). From this figure, DIP can be determined in this mixture by measuring the amplitude at 336 nm where there is no contribution from ASA or SA.

On the other hand, for the determination of ASA and SA, an analogous procedure was followed. The absorption spectra of DIP, ASA, SA and their mixture were divided by that of a solution of 1.5 mg% DIP, and the first derivative of the developed ratio spectra were calculated with $\Delta\lambda = 2$ nm (Fig. 2). It was shown from this figure that, the first derivative of the ratio spectrum of the mixture consists only of ASA and SA as the corresponding values of DIP are equal to zero. Applying the zero-crossing method, ASA can be assayed by measuring the amplitude at 234 nm (zero-crossing point of SA), while SA can be determined by measuring the amplitude at 316 nm (zero-crossing point of ASA) on using DIP as divisor.

The influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval; $\Delta\lambda = 2$ nm was considered as suitable.

A study was carried out to test for the effect of the divisor concentration on the calibration graphs. Thus the absorption spectra of standard solutions of

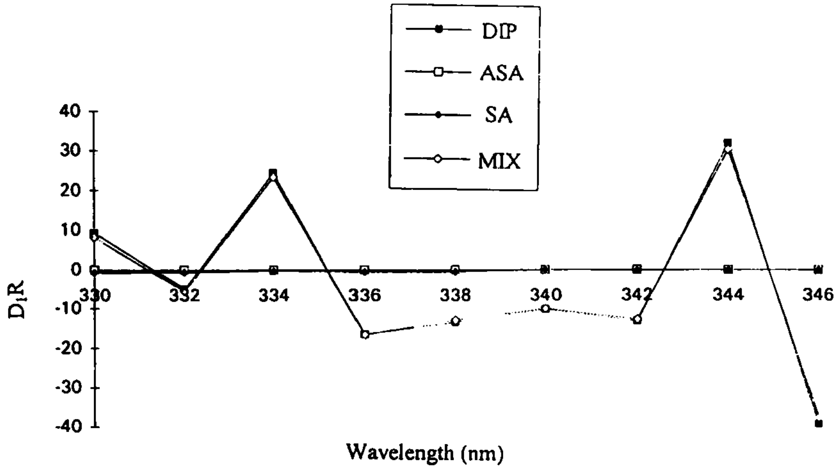

Figure 1: First derivative ratio spectra of (—■—) 1.5 mg% dipyridamole, (—□—) 0.5 mg% aspirin, (—♦—) 0.5 mg% salicylic acid and (—◇—) their mixture; all divided by 0.5 mg% aspirin at Δλ = 2 nm.

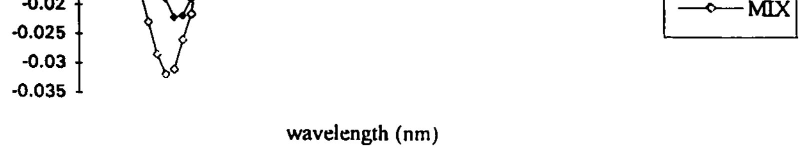

Figure 2: First derivative ratio spectra of (—■—) 1.5 mg% dipyridanole, (—□—) 0.5 mg% aspirin, (—♦—) 0.5 mg% salicylic acid and (—◇—) their mixture; all divided by 1.5 mg% dipyridamole at Δλ = 2 nm.

DIP, ASA and SA of different concentrations (Table 1) were obtained. The amplitudes of these solutions were divided by the corresponding amplitudes of standard solutions of either ASA (0.4, 0.6 and 0.8 mg%) or DIP (0.2, 0.5, 1.0 and 1.5 mg%). The resultant ratio spectra were then differentiated with respect to wavelength using $\Delta\lambda = 2$ nm. The derivative values of each component were measured, at the specified wavelength (Table 1), and plotted against its concentrations. A straight line was obtained in each case. The statistical analysis of these graphs using least square method (Table 1) shows high values of the correlation coefficients and small values of the intercepts which indicates good linearity. The results obtained from this study (Table 1) indicate that the divisor concentration has no effect on the assay. If the concentration of the divisor is increased or decreased, the resulting first derivative values are proportionately decreased or increased, respectively, although the maxima and minima remain at the same wavelength.

Mixture II

Preliminary trials to analyze this mixture by the proposed method showed that DIP can be determined by dividing the amplitudes of the absorption spectra of solutions of DIP, OX, BN and their mixture by the corresponding amplitudes of a solution of OX and calculating the first derivative values for the developed ratio spectra. Now the mixture becomes binary containing DIP and BN where the derivative values of OX are equal to zero. Therefore, DIP can be determined by measuring the derivative value at 330 nm where BN has no contribution (zero-crossing point of BN) (Fig. 3).

On the other hand, OX and BN were determined by dividing the amplitudes of the absorption spectra of the above solutions by those of a solution of DIP and calculating the first derivative values of the developed ratio spectra (Fig. 4a). From this figure, OX can be determined by measuring its

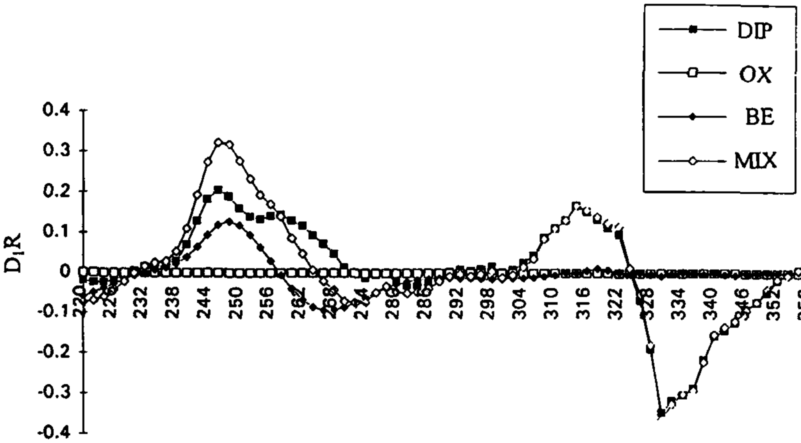

Figure 3: First derivative ratio spectra of (—■—) 1.0 mg% dipyridamole, (—□—) 0.2 mg% oxazepam, (—♦—) 0.2 mg% benzophenone derivative and (—◇—) their mixture; all divided by 0.2 mg% oxazepam at Δλ = 4 nm.

amplitude at 368 nm (zero-crossing point of BN), while BN can be assayed by measuring at 270 nm (zero-crossing point of OX) on using DIP as divisor (Fig. 4b).

To study the assay conditions of this mixture, the influence of Δλ for obtaining the first derivative was tested; Δλ = 4 nm was considered as suitable.

The concentration of the divisor was studied by procedure analogous to that described above. The same conclusion for the effect of the divisor concentration on the analysis was obtained as under mixture I (Table 2).

Separate determinations at different concentration levels were carried out for each drug to test reproducibility. The relative standard deviations (RSD) were found to be less than 2% indicating good reproducibility (Table 1&2).

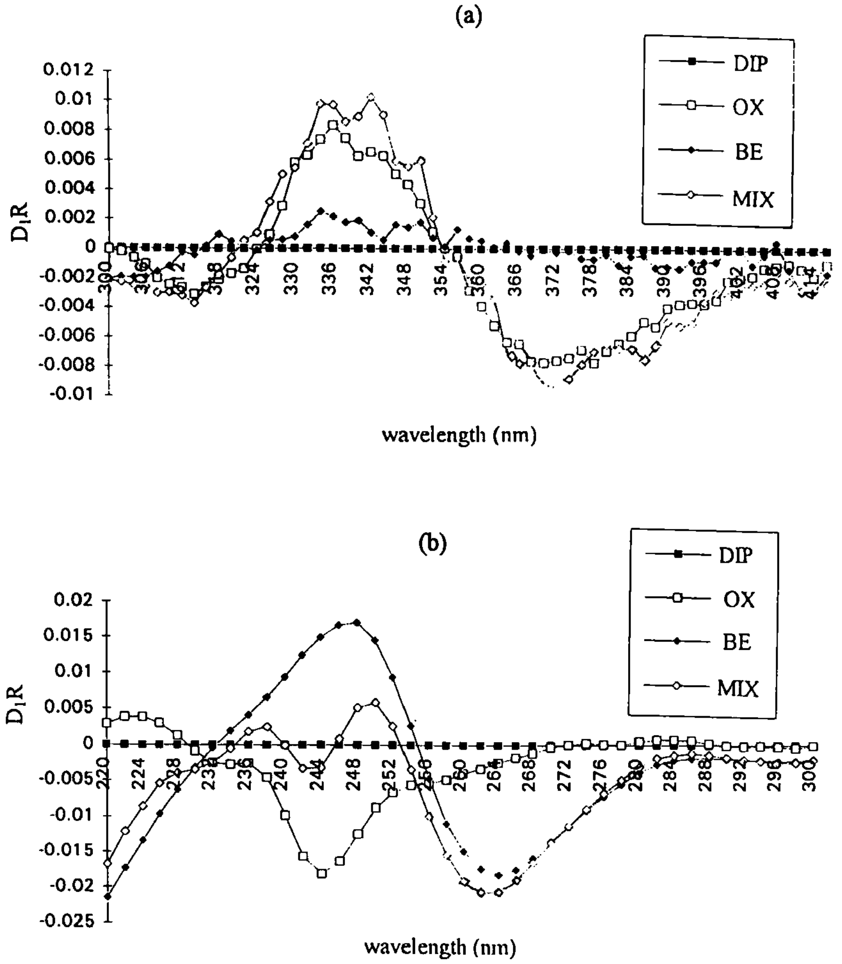

Figure 4: First derivative ratio spectra of (—■—) 1.0 mg% dipyridamole, (—□—) 0.2 mg% oxazepam, (—◆—) 0.2 mg% benzophenone derivative and (—◇—) their mixture; all divided by 1.0 mg% dipyridamole at $\Delta\lambda$ = 4 nm.

TABLE 3

Assay results for the determination of DIP-ASA and DIP-OX in synthetic mixtures and forms by the proposed method

Sample	Content			Mean±S.D.*		
	DIP	**ASA**	**SA**	**DIP**	**ASA**	**SA**
Synthetic mix. (mg%)	1.5	0.5-0.8	0.2-0.5	99.8	100.2	99.9
				0.17	0.88	1.01
Persantin-plus capsules	75	50	-	100.5	99.6	-
(mg/Cap.)				0.78	0.42	
	DIP	**OX**	**BN**	**DIP**	**OX**	**BN**
Synthetic mix. (mg%)	1-1.25	0.2-0.4	0.1-0.2	100.5	99.4	-
				1.14	0.34	
DIP-OX tablets	25	10	-	100.6	100.0	-
(mg/Tab.)				1.34	0.63	

* Mean of 5 separate determinations.

To prove the validity and applicability of the proposed method, synthetic mixtures of DIP; ASA and SA and of DIP, OX and BN, were prepared in different proportions and resolved by the developed procedure. The results were summarized in Table 3. In all cases, good recovery values were obtained.

The present method was applied for the simultaneous determination of the above mentioned mixtures in their available commercial dosage forms. The results obtained (Table 3) were both precise and accurate. The results show the high reliability and reproducibility of the method and conform satisfactory to the lable claim amounts.

The proposed method is simple as there is no need for solvent extraction and direct as it estimates each drug independent of the other. The most striking features of the proposed method is its ability to quantitate degradation products in the presence of intact drugs in ternary mixtures.

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