



# The spectrophotometric multicomponent analysis of a ternary mixture of ascorbic acid, acetylsalicylic acid and paracetamol by the double divisor-ratio spectra derivative and ratio spectra-zero crossing methods

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## Abstract

The double divisor–ratio spectra derivative and ratio spectra–zero crossing methods were applied to the analysis of an effervescent tablet containing the title compounds without using a chemical separation procedure. In the use of both methods, the calibration graphs were linear in the range of 8–28  $\mu\text{g ml}^{-1}$  for three compounds. Comparison of the results obtained by the two methods indicates that both methods gives the best results. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Double divisor-ratio spectra derivative and ratio spectra-zero crossing methods; Ascorbic acid; Acetylsalicylic acid; Paracetamol

## 1. Introduction

For the simultaneous determination of two or more active compounds in the same mixtures without a separation step, several spectrophotometric methods, such as classical derivative spectrophotometry [1–4], Vierordt's method [5] and its modified version [6], orthogonal function method [7], dual wavelength spectrophotometry [8–10], pH-induced differential spectrophotometry [11], and least square method [12], the multi-

component analysis program [13,14] and a method; multi-wavelength linear regression analysis (MLRA) which was referred to by Blanco and co-workers [15] have been utilized.

Salinas et al. [16] proposed a new spectrophotometric method for the simultaneous determination of two compounds in binary mixtures. Two new methods were developed from this theory for resolving ternary mixtures, as explained below.

Berzas Nevado et al. [17], developed a new method for the resolution of ternary mixtures of compounds by the derivative ratio spectra-zero crossing method. In the method, the simultaneous determination of three compounds in ternary mix-

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tures are realized by the measurements of the amplitude at the zero-crossing points in the derivative spectrum of the ratio spectra.

However, recently for the simultaneous determination of the three compounds in ternary mixtures, another new spectrophotometric method has been developed by us, which is very easy to apply, very sensitive and very useful and yet very cheap [18]. This method was called 'the double divisor-ratio spectra derivative method'. The method is based on the use of the coincident spectra of the derivative of the ratio spectra obtained by using a 'double divisor' (sum of two spectra) and the measurements at either the maximum or minimum wavelengths.

The quantitative determination of ingredients in pharmaceutical formulations containing acetylsalicylic acid (ASA), ascorbic acid (ASCA) and paracetamol (PAR), and their mixtures with different active compounds using various methods including spectrophotometry (ASA [19,20], ASCA [23–28] and PAR [30–34]), HPLC (ASA [21,22], ASCA [29]) and TLC (PAR [35]), have been demonstrated for several mixtures and pharmaceutical preparations.

In this paper, two new spectrophotometric methods have been applied successfully to the analysis of the synthetic ternary mixtures and an effervescent tablet containing ASA, ASCA and PAR, which have closely overlapped the spectra. The results obtained by the double divisor-ratio spectra derivative method were compared with those obtained by ratio spectra derivative-zero crossing method.

## 2. Experimental

### 2.1. Apparatus

A Shimadzu 1601PC double beam spectrophotometer with a fixed slit width (2 nm) connected to an IBM computer loaded with Shimadzu UVPC software which was equipped with an HP 1150C printer was used for all the absorbance measurements and treatment of data.

### 2.2. Pharmaceutical formulation

A commercial product AFEBRYL<sup>®</sup> effervescent tablet (produced by Laboratoires SBM Farmaceutica N.V., Belgium, Batch no.B01, containing 300 mg ASA, 300 mg ASCA and 200 mg PAR per tablet) were studied.

### 2.3. Standard solutions

Stock solutions of 100 mg/100 ml of ASCA, ASA and PAR (Bayer and Nobel, Turkey) were prepared in methanol and 0.2 M HCl (1:3). All the solutions were prepared freshly and protected from light. Working standard solutions were prepared in 25-ml volumetric flasks containing 8–28  $\mu\text{g ml}^{-1}$  ASCA, ASA and PAR and their different synthetic mixtures by using the stocks solutions. They were diluted with ethanol–0.2 M HCl (3:1) to the mark.

### 2.4. Procedures

Twenty effervescent tablets, were weighed and powdered in a mortar. An amount equivalent to one tablet was transferred to a 100-ml calibrated flask and dissolved in 50 ml of methanol and 0.2 M HCl (1:3), swirled until effervescence ceases, and diluted with same solvent to the volume. After, these solutions were filtered through a filter paper into a 100-ml calibrated flask and the residue was washed three times with 10 ml of solvent, then the volume was completed to the mark with the same solvent as was used above. The resulting solution was diluted to 1:167 in a 25-ml calibrated flask with methanol and 0.2 M HCl (1:3). The methods were applied to the prepared solutions.

## 3. Application of methods

### 3.1. Double divisor-ratio spectra derivative method

In this method, to determine ASA, the stored spectra of the mixture of ASA, ASCA and PAR were divided by the sum of the spectra of ASCA

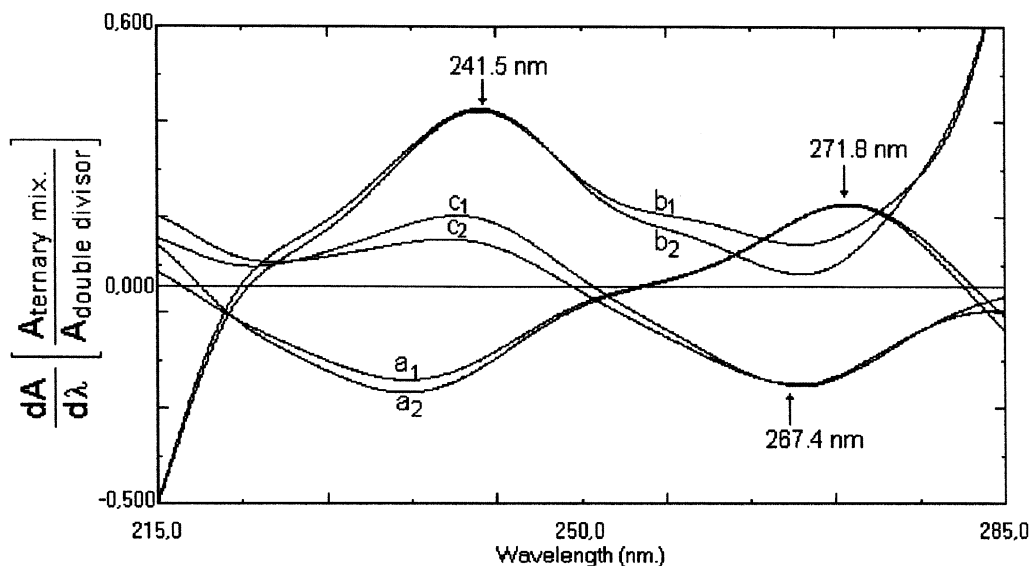


Fig. 1. The coincident spectra of the first derivative of the ratio spectra of: (a<sub>1</sub>) 16 μg ml<sup>-1</sup> pure ASA and (a<sub>2</sub>) ternary mixture (16 μg ml<sup>-1</sup> ASA, 16 μg ml<sup>-1</sup> ascorbic acid (ASCA) and 12 μg ml<sup>-1</sup> paracetamol (PAR)), 28 μg ml<sup>-1</sup> PAR + 28 μg ml<sup>-1</sup> ASCA as a double divisor; (b<sub>1</sub>) 16 μg ml<sup>-1</sup> pure PAR and (b<sub>2</sub>) ternary mixture (16 μg ml<sup>-1</sup> PAR, 16 μg ml<sup>-1</sup> ASA and 16 μg ml<sup>-1</sup> ASCA), 12 μg ml<sup>-1</sup> ASCA + 12 μg ml<sup>-1</sup> ASA as a double divisor; and (c<sub>1</sub>) 12 μg ml<sup>-1</sup> pure ASCA and (c<sub>2</sub>) ternary mixture (12 μg ml<sup>-1</sup> pure ASCA, 16 μg ml<sup>-1</sup> ASCA and 12 μg ml<sup>-1</sup> PAR), 20 μg ml<sup>-1</sup> ASCA + 20 μg ml<sup>-1</sup> PAR as a double divisor in methanol and 0.2 M HCl (1:3) (to save space three curves are shown in the same figure).

and PAR as ‘double divisor’ and their ratio spectra were obtained. First derivatives of the ratio spectra are plotted. At the results of the above mentioned procedure, the amplitudes measured of the maximum at 271.8 nm are dependent only to the concentrations values  $C_{ASA}$  and  $C_{PAR}^0$  ( $C^0$  is standard concentration), but are independent of the concentration values  $C_{ASCA}$  and  $C_{PAR}$  in the ternary mixture. The mathematical expression of this procedure is shown in the following equation:

$$\lambda_i = 271.8 \text{ nm}$$

$$\begin{aligned} \frac{d}{d\lambda} \left[ \frac{A_{\text{ternary mix.}, \lambda_i}}{[\alpha_{ASCA, \lambda_i} + \beta_{PAR, \lambda_i}] C_{PAR}^0} \right] \\ = \frac{d}{d\lambda} \left[ \frac{\gamma_{ASA, \lambda_i}}{[\alpha_{ASCA, \lambda_i} + \beta_{PAR, \lambda_i}]} \right] \frac{C_{ASA}}{C_{PAR}^0} \end{aligned}$$

were the amplitudes measured,  $d/d\lambda (A_{\text{ternary mix.}} / [\alpha_{ASCA, \lambda_i} + \beta_{PAR, \lambda_i}] C_{PAR}^0)$ , were drawn as a graph, versus concentrations of ASA and a straight line was obtained. By using the calibration graph, ASA was determined in the mixture of ASA, ASCA and PAR.

On the other hand, to determine ASCA, the absorption spectra of the mixture containing ASCA, ASA and PAR were divided by the sum of the spectra of ASA and PAR as ‘double divisor’ and the ratio spectra were obtained. First derivatives of the ratio spectra were calculated. The amplitude of the minimum at 267.4 nm are dependent only to the concentrations values  $C_{ASCA}$  and  $C_{ASA}^0$ , but are independent of the concentration values  $C_{ASA}$  and  $C_{PAR}$  in the ternary mixture, as shown below:

$$(\lambda_i = 267.4 \text{ nm})$$

$$\begin{aligned} \frac{d}{d\lambda} = \left[ \frac{A_{\text{ternary mix.}, \lambda_i}}{[\alpha_{ASA, \lambda_i} + \beta_{PAR, \lambda_i}] C_{ASA}^0} \right] \\ = \frac{d}{d\lambda} \left[ \frac{\gamma_{ASCA, \lambda_i}}{[\alpha_{ASA, \lambda_i} + \beta_{PAR, \lambda_i}]} \right] \frac{C_{ASCA}}{C_{ASA}^0} \end{aligned}$$

In this case, also a straight line was obtained by using the amplitudes measured for ASCA. By means of the calibration graph, the content of ASCA was determined in the sample.

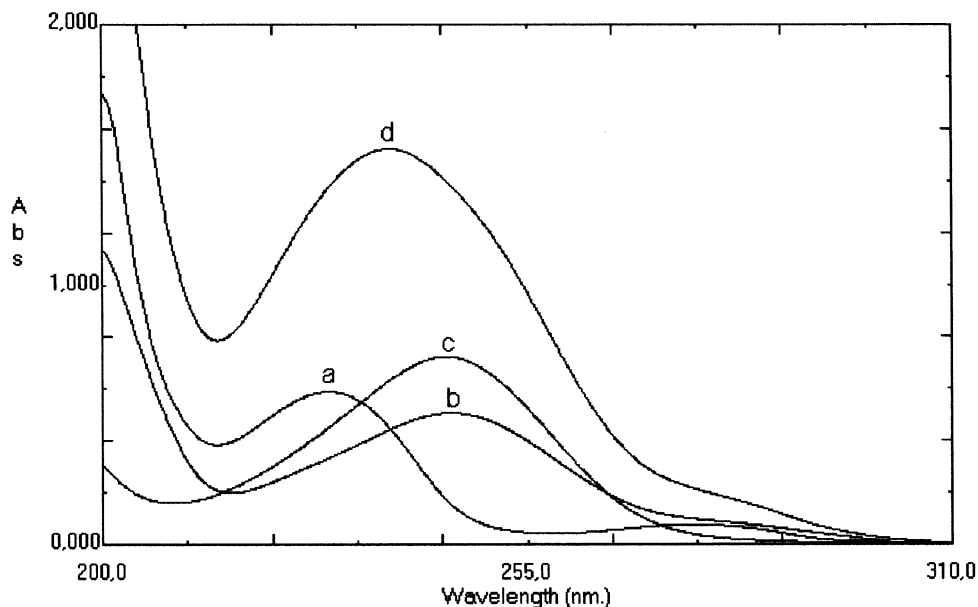


Fig. 2. Zero-order spectra of: (a)  $12 \mu\text{g ml}^{-1}$  acetylsalicylic acid (ACA); (b)  $8 \mu\text{g ml}^{-1}$  paracetamol (PAR); (c)  $12 \mu\text{g ml}^{-1}$  ascorbic acid (ASCA); (d) their ternary mixture in methanol and 0.2 M HCl (1:3).

In the same way, to determine PAR, the stored spectra of the mixture containing PAR, ASCA and ASA were divided by the sum of the spectra of ASA and ASCA as 'double divisor'. From the resulting ratio spectra, the first derivative of the ratio spectra were traced. The concentration of PAR in the ternary mixture was proportional to the amplitude of the maximum at 241.5 nm with respect to the following equation:

$$(\lambda_i = 241.5 \text{ nm})$$

$$\frac{d}{d\lambda} = \left[ \frac{A_{\text{ternary mix.}, \lambda_i}}{[\alpha_{\text{ASA}, \lambda_i} + \beta_{\text{ASCA}, \lambda_i}] C_{\text{ASCA}}^0} \right]$$

$$= \frac{d}{d\lambda} \left[ \frac{\gamma_{\text{PAR}, \lambda_i}}{[\alpha_{\text{ASA}, \lambda_i} + \beta_{\text{ASCA}, \lambda_i}]} \right] \frac{C_{\text{PAR}}}{C_{\text{ASCA}}^0}$$

As explained above, a straight line was obtained and the amount of PAR is determined in the sample containing the above mentioned ternary mixture.

### 3.1.1. Selection of the working wavelength

In the application of this method, the first derivative of the ratio spectra of pure compound and its ternary mixture would be coincided in the

spectral region corresponding to a maximum point or a minimum point of the wavelength as shown in Fig. 1. These coinciding points of the derivative of the ratio spectra can be selected as working wavelengths for the determinations of the subject compounds in the ternary mixture.

### 3.1.2. Establishment of double divisor

The double divisor was obtained either by the sum of the absorption spectra of the same concentration of the two compounds in the same ternary mixture, as is carried out in this paper or it was obtained by preparing the mixed solution of two compounds of the same concentration in the ternary mixture [18]. If the double divisor will be used as the sum of the spectra of the two compounds, this can be performed with the help of Shimadzu UVPC software.

### 3.2. Derivative ratio spectrum-zero crossing method

The absorption spectra of ASA, ASCA and their ternary mixture with PAR, were divided by a standard spectrum of PAR and the first derivative

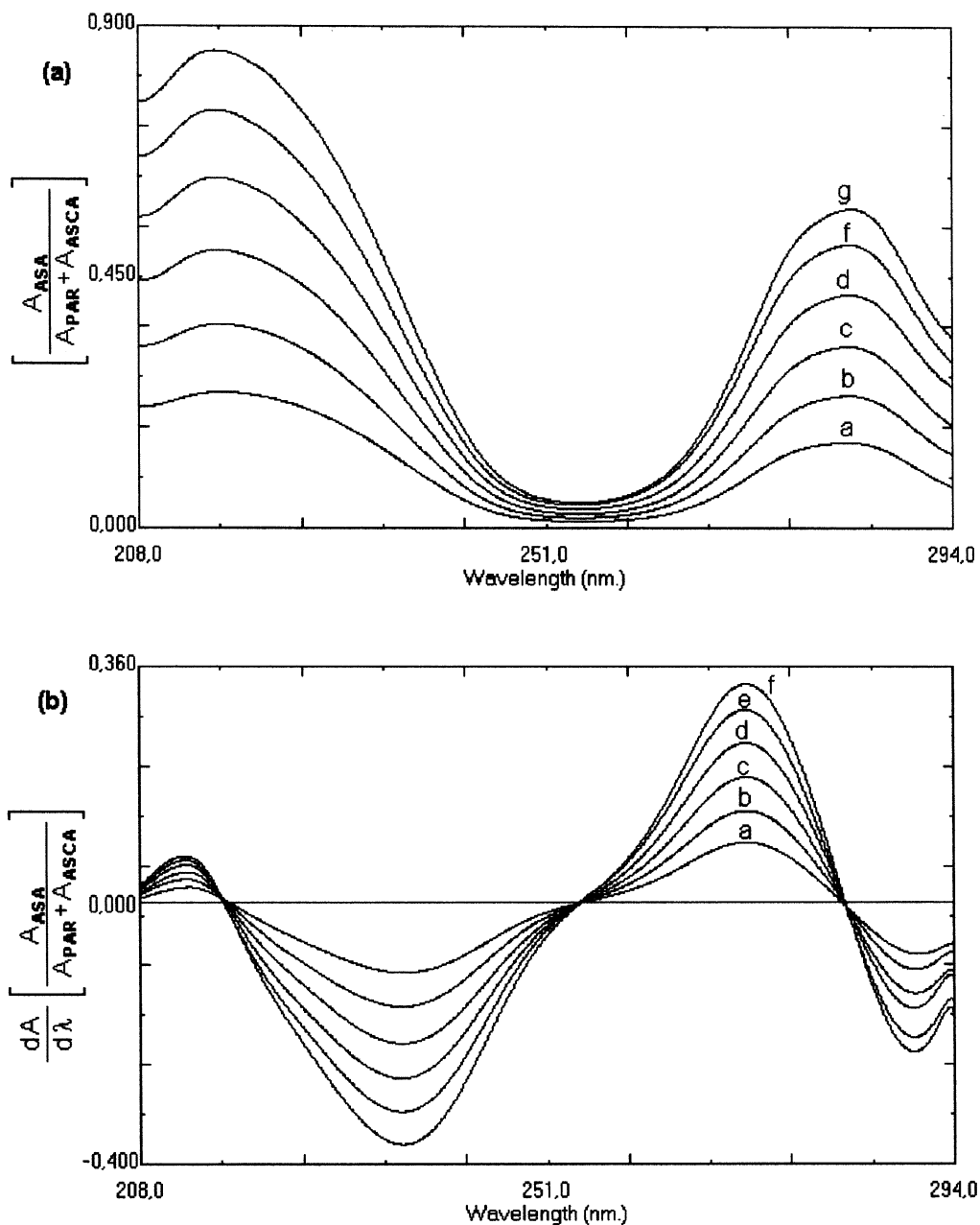


Fig. 3. Ratio spectra (a) and first derivative of the ratio spectra (b) of acetylsalicylic acid (ACA): (a)  $8 \mu\text{g ml}^{-1}$ ; (b)  $12 \mu\text{g ml}^{-1}$ ; (c)  $16 \mu\text{g ml}^{-1}$ ; (d)  $20 \mu\text{g ml}^{-1}$ ; (e)  $24 \mu\text{g ml}^{-1}$ ; (f)  $28 \mu\text{g ml}^{-1}$  ( $28 \mu\text{g ml}^{-1}$  ascorbic acid (ASCA) +  $28 \mu\text{g ml}^{-1}$  paracetamol (PAR) as a double divisor) in methanol and 0.2 M HCl (1:3) ( $\Delta\lambda = 8 \text{ nm}$ ).

of the ratio spectra was calculated. In the ternary mixture, the concentrations of ASCA and ASA were proportional to the first derivative signals at

255.1 and 281.1 nm (zero-crossing point for ASA) and 241.2 nm (zero-crossing point for ASCA), respectively, in the first derivative of the ratio

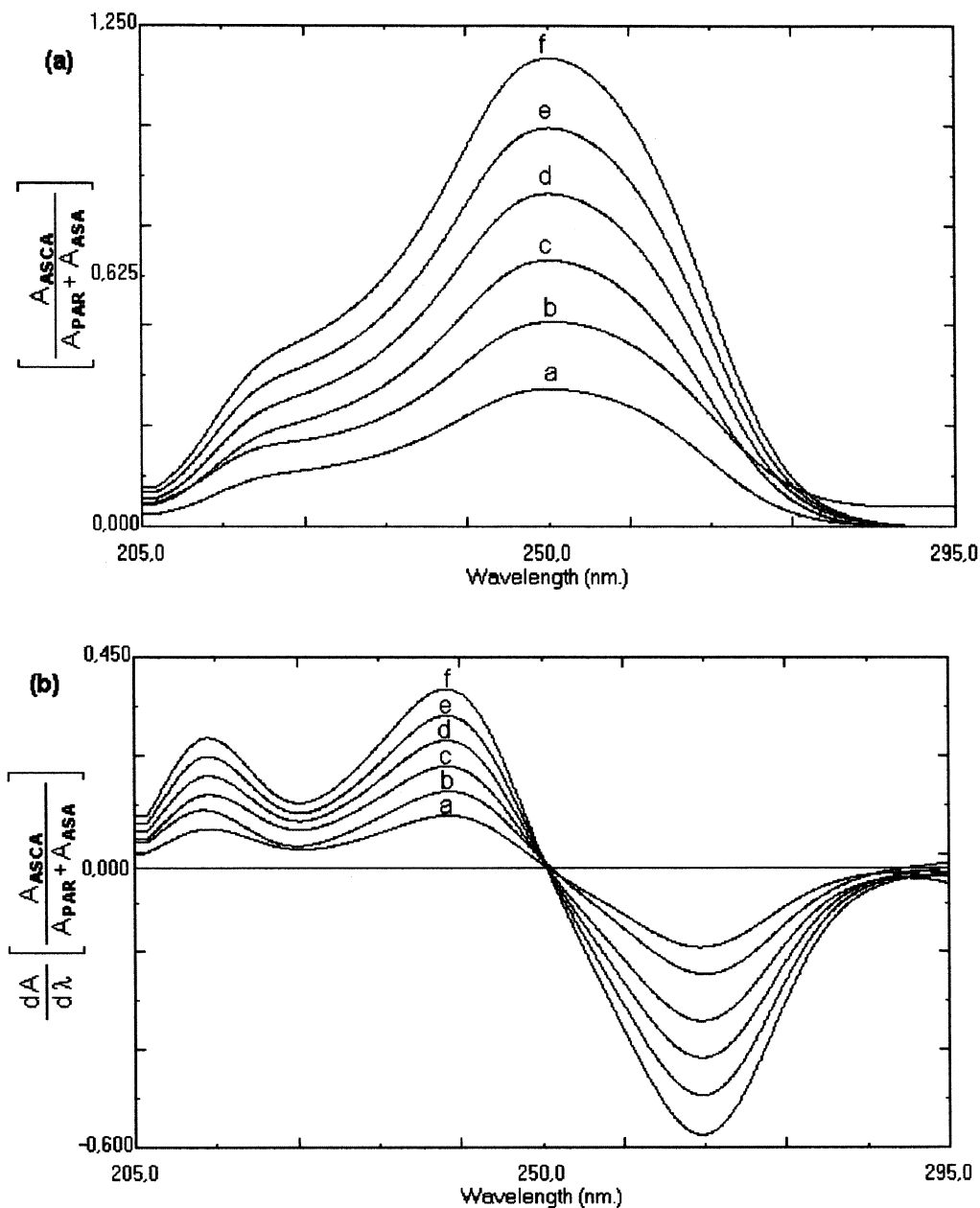


Fig. 4. Ratio spectra (a) and first derivative of the ratio spectra (b) of ascorbic acid (ASCA): (a)  $8 \mu\text{g ml}^{-1}$ ; (b)  $12 \mu\text{g ml}^{-1}$ ; (c)  $16 \mu\text{g ml}^{-1}$ ; (d)  $20 \mu\text{g ml}^{-1}$ ; (e)  $24 \mu\text{g ml}^{-1}$ ; (f)  $28 \mu\text{g ml}^{-1}$  ( $20 \mu\text{g ml}^{-1}$  ASCA +  $20 \mu\text{g ml}^{-1}$  paracetamol (PAR) as a double divisor) in methanol and 0.2 M HCl (1:3) ( $\Delta\lambda = 8 \text{ nm}$ ).

spectra. Two calibration graphs were obtained by measuring the derivative amplitudes against the increasing concentrations of pure ASA and pure ASCA and by using pure PAR as a divisor. The

contents of ASA and ASCA can be determined by use of the above mentioned calibration graphs.

By using the same procedure, the stored spectra of ASA, PAR and their ternary mixture with

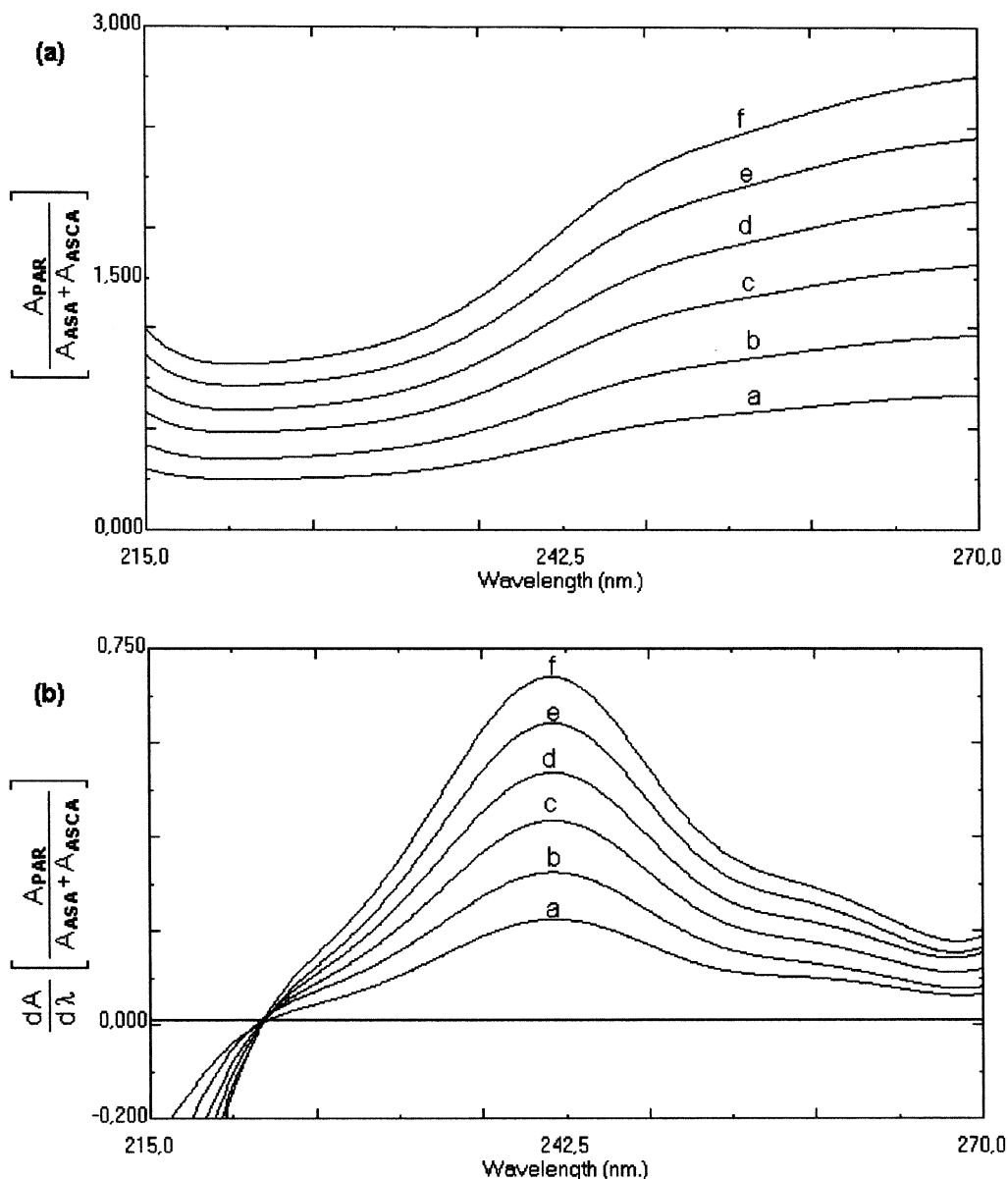


Fig. 5. Ratio spectra (a) and first derivative of the ratio spectra (b) of paracetamol (PAR): (a)  $8 \mu\text{g ml}^{-1}$ ; (b)  $12 \mu\text{g ml}^{-1}$ ; (c)  $16 \mu\text{g ml}^{-1}$ ; (d)  $20 \mu\text{g ml}^{-1}$ ; (e)  $24 \mu\text{g ml}^{-1}$ ; (f)  $28 \mu\text{g ml}^{-1}$  ( $28 \mu\text{g ml}^{-1}$  ascorbic acid (ASCA) +  $28 \mu\text{g ml}^{-1}$  paracetamol (PAR) as a double divisor) in methanol and 0.2 M HCl (1:3) ( $\Delta\lambda = 8 \text{ nm}$ ).

ASCA, were divided by a standard spectrum of ASCA and the first derivative of the result was plotted. ASA and PAR were proportional to derivative signals at 239.3 and 292.4 nm (zero-crossing point for PAR) and 286.1 nm (zero-crossing point for ASA), respectively, in the first derivative

of the ratio spectra. For the determination of ASA and PAR, the calibration graphs were obtained by measuring the first derivative values, versus the increasing concentrations of pure ASA and pure PAR, and by using pure ASCA as a divisor. With this procedure, ASA and PAR can be determined.

Table 1

Recovery data obtained for the synthetic ternary mixtures by using the double divisor-ratio spectra derivative method<sup>a</sup>

Composition mixture:		ASCA and PAR (double divisor)		
ASCA	PAR	ASA	ASA	
Added ( $\mu\text{g ml}^{-1}$ )		Found ( $\mu\text{g ml}^{-1}$ )		Recovery (%)
18.00	12.00	8.00	7.90	98.8
18.00	12.00	12.00	11.80	98.3
18.00	12.00	16.00	15.05	100.3
18.00	12.00	20.00	19.56	97.8
18.00	12.00	24.00	24.05	100.2
18.00	12.00	28.00	27.60	98.6
				$\bar{X} = 99.0$
				RSD = 1.04%
Composition mixture:		ASA and PAR (double divisor)		
ASA	PAR	ASCA	ASCA	
Added ( $\mu\text{g ml}^{-1}$ )		Found ( $\mu\text{g ml}^{-1}$ )		Recovery (%)
18.00	12.00	8.00	8.04	100.5
18.00	12.00	12.00	12.09	100.8
18.00	12.00	16.00	15.90	99.4
18.00	12.00	20.00	20.06	100.3
18.00	12.00	24.00	23.88	99.5
18.00	12.00	28.00	28.59	102.1
				$\bar{X} = 100.4$
				RSD = 0.98%
Composition mixture:		ASA and ASCA (double divisor)		
ASA	ASCA	PAR	PAR	
Added ( $\mu\text{g ml}^{-1}$ )		Found ( $\mu\text{g ml}^{-1}$ )		Recovery (%)
18.00	18.00	8.00	8.00	100.0
18.00	18.00	12.00	11.82	98.5
18.00	18.00	16.00	16.02	100.1
18.00	18.00	20.00	19.75	98.8
18.00	18.00	24.00	24.00	100.0
18.00	18.00	28.00	27.70	99.9
				$\bar{X} = 99.6$
				RSD = 0.71%

<sup>a</sup> RSD, relative standard deviation.

In this case, the amount of ASA in ternary mixture has been determined by both procedures.

#### 4. Results and discussion

The absorption spectra of the three compounds, ASA, ASCA and PAR overlapped closely in the region 200–310 nm in Fig. 2. For this reason, the determination of the above compounds was not

possible from direct measurements of absorbances in the zero-order spectra. On the other hand, also the classical derivative spectrophotometric method was tested (from first to fourth) for simultaneous determination of compounds (ASA, ASCA and PAR) in the same mixture. By these methods, in the same order of derivative spectra and method of direct absorbance measurement could not be realized for the ASA, ASCA and PAR determinations within same mixture.



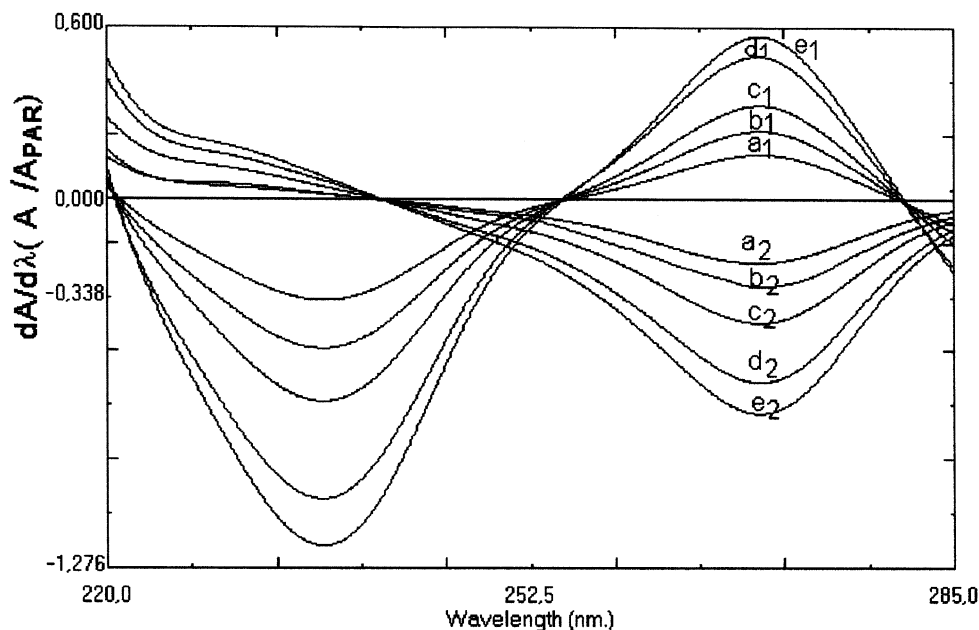


Fig. 6. First derivative of the ratio spectra of acetylsalicylic acid (ACA): (a<sub>1</sub>) 8  $\mu\text{g ml}^{-1}$ ; (b<sub>1</sub>) 12  $\mu\text{g ml}^{-1}$ ; (c<sub>1</sub>) 16  $\mu\text{g ml}^{-1}$ ; (d<sub>1</sub>) 20  $\mu\text{g ml}^{-1}$ ; (e<sub>1</sub>) 24  $\mu\text{g ml}^{-1}$ ; (f<sub>1</sub>) 28  $\mu\text{g ml}^{-1}$  and of ascorbic acid (ASCA) (a<sub>2</sub>) 8  $\mu\text{g ml}^{-1}$ ; (b<sub>2</sub>) 12  $\mu\text{g ml}^{-1}$ ; (c<sub>2</sub>) 16  $\mu\text{g ml}^{-1}$ ; (d<sub>2</sub>) 20  $\mu\text{g ml}^{-1}$ ; (e<sub>2</sub>) 24  $\mu\text{g ml}^{-1}$ ; (f<sub>2</sub>) 28  $\mu\text{g ml}^{-1}$  (16  $\mu\text{g ml}^{-1}$  paracetamol (PAR) as a divisor) in methanol and 0.2 M HCl (1:3) ( $\Delta\lambda = 8$  nm).

#### 4.1. Double divisor-ratio spectra derivative method

The absorption spectra of the solutions of ASA in methanol and 0.2 M HCl (1:3) were recorded in the range 208–294 nm and divided by the double divisor (28  $\mu\text{g ml}^{-1}$  ASCA and 28  $\mu\text{g ml}^{-1}$  PAR) and their ratio spectra was obtained. They were smoothed at  $\Delta\lambda = 8$  nm (Fig. 3(a)). Fig. 3(b) indicates the first derivatives which were calculated with interval of  $\Delta\lambda = 8$  nm and scaling factor of 10 from the ratio spectra. The concentration of ASA was determined by measuring the amplitude at 271.8 nm corresponding to a maximum point.

Then, the absorption spectra of the solutions of ASCA in methanol and 0.2 M HCl (1:3) were recorded in between 205 and 295 nm and divided by the 'double divisor' (20  $\mu\text{g ml}^{-1}$  ASA and 20  $\mu\text{g ml}^{-1}$  PAR). The ratio spectra of the result were smoothed at  $\Delta\lambda = 8$  nm (Fig. 4(a)) and their first derivatives were traced with intervals of  $\Delta\lambda = 8$  nm and scaling factor of 10 (Fig. 4(b)).

The amount of ASCA was determined by measuring the signals at 267.4 nm corresponding to a minimum point of wavelength.

In the same way, the absorption spectra of the solutions of PAR were stored in the spectral range 215–270 nm and divided the 'double divisor' (12  $\mu\text{g ml}^{-1}$  ASA and 12  $\mu\text{g ml}^{-1}$  ASCA). The resulting ratio spectra were smoothed with intervals of  $\Delta\lambda = 8$  nm (Fig. 5(a)). And their first derivatives were traced with  $\Delta\lambda = 8$  nm intervals and scaling factor of 10 (Fig. 5(b)). The content of PAR was determined by measuring the amplitudes at 241.5 nm corresponding to a maximum wavelength.

In this method, various mixtures of PAR, ASA and ASCA were prepared and tested between 8 and 28  $\mu\text{g ml}^{-1}$  for ASA, ASCA and PAR in their ternary mixtures. Mean recoveries and the relative standard deviations of the method were found as 99 and 1.04% for ASA, 100.4 and 0.98% for ASCA and 99.6 and 0.71% for PAR, in the synthetic mixtures prepared by adding known amounts of ASA, ASCA, and PAR (Table 1).

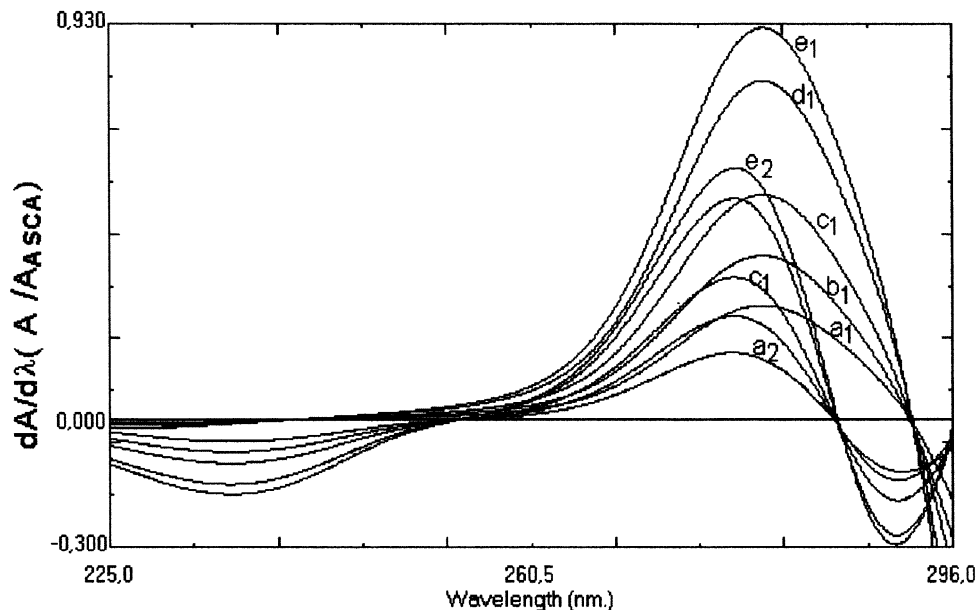


Fig. 7. First derivative of the ratio spectra of paracetamol (PAR): (a<sub>1</sub>) 8  $\mu\text{g ml}^{-1}$ ; (b<sub>1</sub>) 12  $\mu\text{g ml}^{-1}$ ; (c<sub>1</sub>) 16  $\mu\text{g ml}^{-1}$ ; (d<sub>1</sub>) 20  $\mu\text{g ml}^{-1}$ ; (e<sub>1</sub>) 24  $\mu\text{g ml}^{-1}$ ; (f<sub>1</sub>) 28  $\mu\text{g ml}^{-1}$  and of acetylsalicylic acid (ACA) (a<sub>2</sub>) 8  $\mu\text{g ml}^{-1}$ ; (b<sub>2</sub>) 12  $\mu\text{g ml}^{-1}$ ; (c<sub>2</sub>) 16  $\mu\text{g ml}^{-1}$ ; (d<sub>2</sub>) 20  $\mu\text{g ml}^{-1}$ ; (e<sub>2</sub>) 24  $\mu\text{g ml}^{-1}$ ; (f<sub>2</sub>) 28  $\mu\text{g ml}^{-1}$  (12  $\mu\text{g ml}^{-1}$  ascorbic acid (ASCA) as a divisor) in methanol and 0.2 M HCl (1:3) ( $\Delta\lambda = 8$  nm).

The main instrumental parameter conditions were optimized for a reliable determination of the subject matter compounds. For selecting the sum of the spectra as 'double divisor' at an appropriate concentrations, which is a very important factor in practice, some double divisor concentrations were tested in the determinations. The sum of the spectra of 28  $\mu\text{g ml}^{-1}$  ASCA and 28  $\mu\text{g ml}^{-1}$  PAR as a 'double divisor' for determining ASA; of 20  $\mu\text{g ml}^{-1}$  ASA and 20  $\mu\text{g ml}^{-1}$  PAR as a 'double divisor' for determining ASCA and of 12  $\mu\text{g ml}^{-1}$  ASA and 12  $\mu\text{g ml}^{-1}$  ASCA as a 'double divisor' for determining PAR in their ternary mixtures were found suitable. The smoothing function for the ratio spectra and the influence of the  $\Delta\lambda$  for the first derivative of the ratio spectra were tested and found very appropriate to use the values of  $\Delta\lambda = 8$ , for both cases, in the determination of the compounds. Furthermore, the scaling factor of 10 was tested and found suitable as for all the determinations.

#### 4.2. Ratio spectra derivative-zero crossing method

In this method, the stored spectra of the solutions of ASA and ASCA in methanol and 0.2 M HCl (1:3) were divided by the spectrum of the standard solution of 16  $\mu\text{g ml}^{-1}$  PAR and the ratio spectra was obtained in the region 220–285 nm. Fig. 6 indicates the first derivative of the ratio spectra which was plotted with intervals of  $\Delta\lambda = 8$  nm. The concentrations of ASA and ASCA in the ternary mixture were determined by measuring the analytical signals at 241.2 nm for ASA and 255.1 or 281.1 nm for ASCA.

In the same way, the absorption spectra of the solutions of ASA and PAR in methanol and 0.2 M HCl (1:3) were divided by the spectrum of the standard solution of 12  $\mu\text{g ml}^{-1}$  ASCA and their ratio spectra were obtained in the spectral region 225–296. Fig. 7 indicates the first derivative of the ratio spectra which was calculated with intervals of  $\Delta\lambda = 8$  nm. The concentrations of ASA and PAR in the ternary mixture were determined by measuring the signals at

Table 2

Recovery data obtained for the synthetic ternary mixtures by using the ratio spectra derivative-zero crossing method<sup>a</sup>

Composition mixture ASCA	PAR	ASA	ASA	(PAR as divisor)
	Added ( $\mu\text{g ml}^{-1}$ )		Found ( $\mu\text{g ml}^{-1}$ )	Recovery (%)
18.00	12.00	8.00	8.05	100.6
18.00	12.00	12.00	12.30	102.2
18.00	12.00	16.00	15.75	98.4
18.00	12.00	20.00	20.65	103.3
18.00	12.00	24.00	23.80	99.2
18.00	12.00	28.00	28.70	102.5
				$\bar{X} = 101.0$
				RSD = 1.94%
Composition mixture ASA	PAR	ASCA	ASCA	(PAR as divisor)
	Added ( $\mu\text{g ml}^{-1}$ )		Found ( $\mu\text{g ml}^{-1}$ )	Recovery (%)
18.00	12.00	8.00	8.09	101.1
18.00	12.00	12.00	12.20	101.7
18.00	12.00	16.00	16.00	100.0
18.00	12.00	20.00	20.50	102.5
18.00	12.00	24.00	24.10	100.4
18.00	12.00	28.00	28.45	101.6
				$\bar{X} = 101.2$
				RSD = 0.90%
Composition mixture ASA	ASCA	PAR	PAR	(ASA as divisor)
	Added ( $\mu\text{g ml}^{-1}$ )		Found ( $\mu\text{g ml}^{-1}$ )	Recovery (%)
18.00	18.00	8.00	8.05	100.6
18.00	18.00	12.00	12.10	100.8
18.00	18.00	16.00	16.20	101.3
18.00	18.00	20.00	19.80	99.0
18.00	18.00	24.00	24.40	101.6
18.00	18.00	28.00	28.30	101.1
				$\bar{X} = 100.7$
				RSD = 0.91%

<sup>a</sup> RSD, relative standard deviation.

286.1 nm for PAR and 239.3 or 292.4 nm for ASA.

In the method, various mixtures of ASA, ASCA and PAR were prepared and tested between 8 and 28  $\mu\text{g ml}^{-1}$  for ASA, ASCA and PAR in the ternary mixture. Mean recoveries and the relative standard deviations were found to be 101 and 1.94% for ASA, 101.2 and 0.90% for ASCA and 100.7 and 0.91% for PAR, in the synthetic mixtures prepared by adding known amounts of ASA, ASCA, and PAR (Table 2).

The main instrumental parameter conditions were optimized to obtain the most distinct curve of first derivative of the ratio spectra. For selecting a divisor of the appropriate concentration, some divisor concentrations were tested in the determination. The standard solutions of 16  $\mu\text{g ml}^{-1}$  of PAR for determining ASA and ASCA and of 12  $\mu\text{g ml}^{-1}$  of ASCA for the determination of ASA and PAR in their ternary mixtures were found suitable. The influence of the  $\Delta\lambda$  for obtaining the first derivative of the ratio spectra

Table 3  
Calibration data in the determination of ASA, ASCA and PAR by two methods

Methods	$\lambda$ (nm)	Linearity range ( $\mu\text{g ml}^{-1}$ )	Equation	Regression coefficient ( $r$ )
Double divisor-ratio spectra deviation	271.8	8–28	$Y^a = 1.2 \times 10^{-2} C_{\text{ASA}} - 2.5 \times 10^{-3}$	0.9999
	267.4	8–28	$Y^a = 3.0 \times 10^{-2} C_{\text{ASCA}} - 2.7 \times 10^{-3}$	0.9991
	241.5	8–28	$Y^a = 2.5 \times 10^{-2} C_{\text{PAR}} + 5.9 \times 10^{-3}$	0.9998
Ratio spectra derivative-zero crossing	286.1	8–28	$Y^a = 2.4 \times 10^{-2} C_{\text{PAR}} + 7.2 \times 10^{-3}$	0.9995
	239.3	8–28	$Y = 5.6 \times 10^{-3} C_{\text{ASA}} + 9.1 \times 10^{-4}$	0.9998
	292.4	8–28	$Y = 4.7 \times 10^{-3} C_{\text{ASA}} + 8.0 \times 10^{-3}$	0.9980
	241.2	8–28	$Y^a = 3.6 \times 10^{-2} C_{\text{ASA}} + 6.9 \times 10^{-3}$	0.9999
	255.1	8–28	$Y^a = 9.1 \times 10^{-3} C_{\text{ASCA}} + 1.9 \times 10^{-4}$	0.9998
	281.1	8–28	$Y = 1.9 \times 10^{-3} C_{\text{ASCA}} + 2.0 \times 10^{-4}$	0.9994

<sup>a</sup> The calibration graphs were used in the determinations:  $C_{\text{ASA}} = \mu\text{g ml}^{-1}$  of acetylsalicylic acid;  $C_{\text{ASCA}} = \mu\text{g ml}^{-1}$  of ascorbic acid;  $C_{\text{PAR}} = \mu\text{g ml}^{-1}$  of paracetamol.

was tested and a value of  $\Delta\lambda = 8$  nm was considered as suitable for both determinations.

For application of these methods, Table 3 shows the regression coefficients and linearity ranges of the calibration curves for the determinations of ASA, ASCA and PAR in their ternary mixture.

A good coincidence was observed for the assay results of the commercial preparations by application of the two methods in this work (Table 4).

## 5. Conclusion

By applying these methods for the analysis of synthetic ternary mixtures and pharmaceutical effervescent tablets containing the three compounds, successful results were obtained. In spite of the three compounds which produce a perfect overlapping spectrum in the zero-order spectra, without requiring a separation procedure, it was observed

that the methods proposed in this paper were more simple and precise than the methods described in the literature. For example, compared to alternative methods, such as HPLC or GC, these spectrophotometric methods were simple and less expensive, and require neither sophisticated instrumentation nor any prior separation step.

In the first method, for each compound in the ternary mixture, without searching the critical point at the separated peaks, the maximum amplitude of the separated peaks can be measured where this can be considered to be superior to the new method over to alternative spectrophotometric method for the resolution of the ternary mixture. In the case of Berzas Nevado's method, together with the utilization of the derivative of the ratio spectra, at the same time a zero-crossing point is necessary for the determination of compounds in a ternary mixture.

These methods have a very promising field in the

Table 4

Results obtained for the pharmaceutical samples (mg tablet<sup>-1</sup>) by using the two spectrophotometric methods<sup>a</sup>

Methods	ASA Mean $\pm$ S.D.	ASCA Mean $\pm$ S.D.	PAR Mean $\pm$ S.D.
Double divisor ratio spectra derivative	299 $\pm$ 1	301.3 $\pm$ 0.7	199.8 $\pm$ 0.6
Derivative ratio spectra zero-crossing	300 $\pm$ 1	302.3 $\pm$ 0.8	200.8 $\pm$ 0.9

<sup>a</sup> Results obtained are average of ten experiments for each method.

routine analysis of compounds for the multi-mixtures and for the pharmaceutical preparations containing these mixtures.

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