

Spectrophotometric Determination of Tartrazine, Riboflavine and Carmoisine in Drinks by Zero-order Spectrophotometric Method using Determinant Calculation and First Derivative Spectrophotometric Method

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Two very simple spectrophotometric methods are described for resolving binary and ternary mixtures of the food colorants tartrazine, carmoisine and riboflavine using the absorption and the first derivative spectra. Calibration graphs are up to 103 mg L^{-1} of tartrazine (E-102), 30.2 mg L^{-1} of carmoisine (E-122) and 30 mg L^{-1} of riboflavine (E-101). Standard deviations of 0.4, 0.7 and 1.4% were obtained for nine standards of 15 mg L^{-1} of tartrazine, 15 mg L^{-1} of riboflavine and 10 mg L^{-1} of carmoisine, respectively. In this study, concentrations of tartrazine (T), riboflavine (R) and carmoisine (C) in binary and ternary mixtures are calculated by Cramer (determinant) method using absorption spectra. T, C and R were also simultaneously determined in their binary mixtures by first derivative spectrophotometry. The method was applied to different drinks. This method was satisfactorily used for determining synthetic mixtures of these colorants in different ratios. It was successfully applied over three commercial products containing the three dyes and did not require any separation step.

Key Words Cramer method, Spectrophotometry, Derivative spectrophotometry, Tartrazine, Carmoisine, Riboflavine.

INTRODUCTION

Food colorants may often be considered simply cosmetic in nature, but their role in the food industry is actually very significant. Colour is the first sensory quality by which foods are judged and food quality and flavour are closely associated with colour. Consumers are conditioned to expect foods of certain colours and to reject any deviation from their expectations. The psychological basis for the need of food colours is well established¹. Colorants also play a significant role in enhancing the aesthetical appeal of food. The term "colour additive" can be applied to any dye, pigment or other substance made (artificial colorant) or obtained from a vegetable, animal, mineral or another natural source that are capable of colouring food, drugs or cosmetics².

Tartrazine (T), carmoisine (C) and riboflavin (R) are the subjects of this work that can be present in common food (drinks, yoghurts, ice cream, sweets, etc.). The structures of these colours are shown in Fig 1. Tartrazine is the food colour that has been most implicated in causing adverse reactions. Tartrazine (FD & C Yellow No. 5) is an approved azo dye present in many drugs and food products.

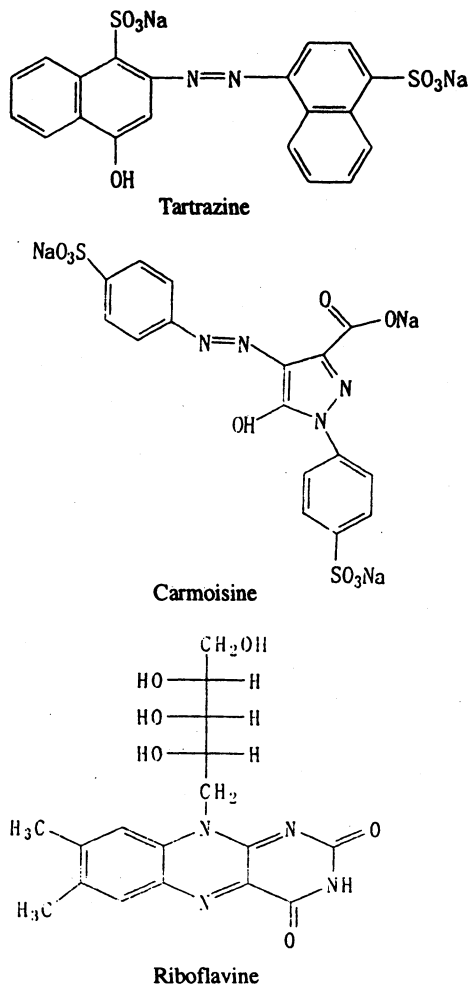


Fig. 1. The structures of food dyes

During the 1970's many cases of tartrazine sensitivity were reported. This led to new regulations that required the listing of azo dyes on package inserts of drugs and on packages of food products. Tartrazine sensitivity is most frequently manifested by urticaria and asthma. Although azo dyes have been implicated in accentuating hyperkinetic syndromes, tartrazine is not considered an offender. Vasculitis, purpura and contact dermatitis infrequently occur as manifestations of tartrazine sensitivity. Cross-sensitivity in aspirin-sensitive and NSAID-sensitive patients may also occur. The mechanism of sensitivity is obscure and has been called pseudoallergic. Management consists mainly of avoidance of drugs and

food products that contain tartrazine³. Carmoisine is a synthetic red azo dye used in foods which must be heat treated after fermentation. Also found in blancmange, marzipan, Swiss roll, jams and preserves, sweets, brown sauce, flavoured yoghurts, packet soups, jellies, breadcrumbs and cheesecake mixes. It appears to cause allergic and/or intolerance reactions, particularly amongst those with an aspirin intolerance. Other reactions can include a rash similar to nettle rash and water retention. Not recommended for consumption by children. The Hyperactive Children Support Group believe that a link exists between this additive and hyperactive behavioural disorders in children. Whilst being a commonly used colour in the UK, its use is banned in Japan, Norway, Sweden and the United States⁴. Riboflavine is a water-soluble vitamin. Its metabolism is controlled by different hormones which regulate its conversion in FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide). These two coenzymes catalyze many oxidation-reduction reactions and are essential for our production of energy. Riboflavin is absorbed through the walls of the small intestine and is carried by the blood to the tissues of our body. Since it is a water-soluble vitamin, it cannot be stored, but small amounts of riboflavin can be found in the liver, heart and kidneys. Otherwise, it is excreted in the urine, feces and sweat. Therefore, this vitamin must be provided daily through our diet⁵.

Dyes are commonly added to industrial foods and laws strictly control their use. The maximum amounts allowed and the acceptable daily intakes (ADI) have been decreased. As these values differ for the various dyes (ranging usually from 30 to 500 ppm), it appears necessary to identify and quantify with accuracy the dyes present in foods. Chromatography, spectrometry and electrochemistry usually perform analysis of dyes and each technique presents some advantages and inconveniences. Chromatographic techniques are appropriated for the analysis of dyes, but raw samples cannot be introduced in the column prior to chemical treatment. Huddleston *et al.*⁶ used chromatography with an ion exchanger for separation and recovery of several common food dyes. Applications in pharmaceutical preparations were developed by Patel *et al.*⁷. Spectrometry was successfully applied to binary and ternary dye mixtures in foods⁸⁻¹⁴ and recently Berzas Nevado *et al.*¹⁵ have developed a mathematical treatment for the spectrometric analysis of a mixture of four red dyes. Application of spectrometry to the analysis of dyes in cosmetic products was developed by Capitán-Vallvey *et al.*^{16,17}. However, it was not possible to distinguish the natural from the synthetic dyes and the similarity of the absorption wavelengths does not allow identification of dyes having the same colour. Since 1960, the electrochemistry of the azo compounds was widely studied¹⁸⁻²⁰ and the electrochemical analysis of the azo dyes present in various commercial products was developed.

In this study, two spectrophotometric methods are reported to accomplish the simultaneous determination of tartrazine (T), carmoisine (C) and riboflavine (R) in drinks without prior separation. One of them is based on derivative techniques, whereas the other one are based on the combination of determinant method (Cramer method) with direct spectral information.

EXPERIMENTAL

A Cary UV-Visible spectrophotometer was used for all absorbance measurements. The derivative spectra were automatically obtained for the spectrophotometer. The optimum $\Delta\lambda$ value was found to be 8 nm for all the first derivatives absorption spectra. Tartrazine (T), carmoisine (C) and riboflavine (R) solutions were prepared from Dr. Marcus GmbH. T, C and R solution stocks had a concentration of 1000 mg L⁻¹ and the working solutions had a concentration of 100 mg L⁻¹.

Standards were prepared in 50 mL volumetric flasks containing 5–20 mg L⁻¹ of T, C and R or binary or ternary mixtures of these dyes and were diluted to volume by distilled water. The absorption spectra were recorded from 350 to 600 nm against a blank in the same way. The absorbance values at the selected wavelengths were obtained from these spectra. First derivative spectra of the same solutions were recorded ($\Delta\lambda = 8$ nm). The concentrations of T, C and R were then found by measurement of the signal and use of an appropriate calibration graph at the selected wavelength. These calibrations were prepared by varying the concentration of one colourant in the absence of the other. Sample solutions were prepared in water by dissolving 10 g powder sample and diluting to 100 mL in a volumetric flask. Soft drinks were used directly in experiments.

RESULTS AND DISCUSSION

The total absorbance of a solution at a given wavelength is equal to the sum of the absorbances of the individual components present. This relationship makes possible the quantitative determination of the individual constituents of a mixture, even if their spectra overlap²⁰. The linear calibration regression function for the spectrophotometric determination of an analyte T at a selected wavelength (λ_i) is given by

$$A_{T_i} = \varepsilon_{T_i} C_T + n_i \quad \dots (1)$$

where ε_{T_i} is the slope of linear regression, C_T is the concentration of analyte T and n_i the intercept value, which reflects the differences between the model and the real system. Zero order spectra of T, C and R are shown in Fig. 2. It can be seen that these spectra are highly overlapped and therefore, the determination of the three dyes is not possible by direct absorbance measurements. Derivative spectrophotometry using zero-crossing method or determinant calculation method (Cramer method) with direct spectral information can be used to solve this problem. Obviously, no wavelength exists at which the absorbance of this mixture is due simply to one of the components; thus, an analysis for either T or C or R is impossible by a single measurement. However, the absorbances of the mixture at the two values of wavelengths λ_1 and λ_2 may be expressed for binary mixtures containing T and C or another combinations as follows:

$$A_{T, C_1} = \varepsilon_{T_1} \cdot C_T + n_{T_1} + \varepsilon_{C_1} \cdot C_C + n_{C_1} \quad \dots (2)$$

$$A_{T, C_2} = \varepsilon_{T_2} \cdot C_T + n_{T_2} + \varepsilon_{C_2} \cdot C_C + n_{C_2} \quad \dots (3)$$

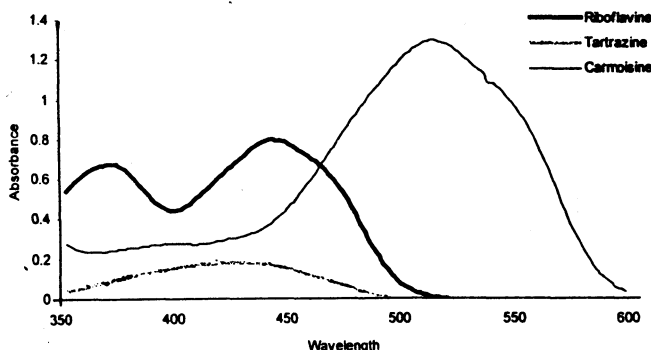


Fig. 2. The zero order spectra of tartrazine, carmoisine and riboflavine

These equations are transformed into the above equations,

$$N_1 = \epsilon_{T_1} \cdot C_T + \epsilon_{C_1} \cdot C_C \quad \dots (4)$$

$$N_2 = \epsilon_{T_2} \cdot C_T + \epsilon_{C_2} \cdot C_C \quad \dots (5)$$

where N_i is equal to $A_i - (n_{T_i} + n_{C_i})$ at a selected wavelength (λ_i). C_T and C_C can be calculated from the following determinant using Cramer method:

$$\begin{vmatrix} \epsilon_{T_1} & \epsilon_{C_1} \\ \epsilon_{T_2} & \epsilon_{C_2} \end{vmatrix} \begin{vmatrix} C_T \\ C_C \end{vmatrix} = \begin{vmatrix} N_1 \\ N_2 \end{vmatrix} \quad \dots (6)$$

Three equations can be derived from ternary mixtures containing T, C and R in the same way (eq. 2 and 3). The concentrations of T, C and R in ternary mixtures can be calculated from these equations using the following determinant:

$$\begin{vmatrix} \epsilon_{T_1} & \epsilon_{C_1} & \epsilon_{R_1} \\ \epsilon_{T_2} & \epsilon_{C_2} & \epsilon_{R_2} \\ \epsilon_{T_3} & \epsilon_{C_3} & \epsilon_{R_3} \end{vmatrix} \begin{vmatrix} C_T \\ C_C \\ C_R \end{vmatrix} = \begin{vmatrix} N_1 \\ N_2 \\ N_3 \end{vmatrix} \quad \dots (7)$$

These equations can be solved using determinant (Cramer) method for ternary mixture by practically an excel program, so concentrations of T, C and R in ternary mixtures can be calculated.

The analytical characteristics for a single component determination were evaluated using wavelengths corresponding to the maximum absorption for each dye and the results obtained are given in Table-1. The two wavelengths corresponding to the maximum absorption were selected for the proposed procedure for tartrazine and riboflavine at binary mixtures or ternary mixtures. Absorption values of carmoisine were measured at 470 nm because the other dyes, tartrazine and riboflavine give no absorption values at the wavelength, 516 nm, corresponding to the maximum absorption for carmoisine. At the selected wavelength, the single component calibration graphs were obtained ($r^2 > 0.9990$) and ϵ_i, n_i values were taken for determinant method (Table-2). Recovery experiments were carried out on 5 solutions containing the two or three

components in different concentration ratios. In Tables 4–7, the results obtained from synthetic mixtures are presented, where each value is the average of triplicate analyses. As can be observed, satisfactory results were obtained. The results from determination of T, C and R in food samples at the selected wavelengths are given in Table-8.

TABLE-1
ANALYTICAL CHARACTERISTICS AND STATISTICAL PARAMETERS
FOR SINGLE-COMPONENT DETERMINATION OF TARTRAZINE (T),
CARMOISINE (C), RIBOFLAVINE (R)

	Tartrazine	Carmoisine	Riboflavine
λ_{\max}	426	470	447
Linearity range (mg L ⁻¹)	0–103	0–30.2	0–30.0
Equation	A = 0.0076 [C _T] + 0.0017	A = 0.03 [C _C] – 0.0052	A = 0.0317 [C _R] – 0.0073
Regression coefficient	0.9989	0.9988	0.9997
RSD (%)	1.27	0.35	0.94
Detection limit (mg L ⁻¹)	0.32	0.14	0.19

*Detection limit = $3s_B/m$; S_B = standard deviation of blank, m = slope of calibration

TABLE-2
LINEAR REGRESSION CALIBRATION FUNCTIONS FOR DETERMINANT METHOD

Component	Wavelength, nm	Equation
Tartrazine	426	A = 0.0076 [C _T] + 0.0017
	470	A = 0.004 [C _T] – 0.002
	447	A = 0.0066 [C _T] + 0.0017
Carmoisine	426	A = 0.0122 [C _C] – 0.0074
	470	A = 0.03 [C _C] – 0.0052
	447	A = 0.0157 [C _C] – 0.0045
Riboflavine	426	A = 0.0319 [C _R] – 0.007
	470	A = 0.0199 [C _R] + 0.0106
	447	A = 0.0317 [C _R] – 0.0073

It must be emphasized that classical derivative spectrophotometry based only on 'zero-crossing' measurements seldom provided the resolution of binary and ternary mixtures of compounds with overlapping spectra. In zero-crossing method, the important point to be considered is, of course, the fact that if one dye gives a signal, the other dye must give no signal at this selected wavelength. The first derivative absorption spectra of solution of T and R are shown in Fig. 3; it can be seen that T can be determined in presence of R at 443 nm and R also can

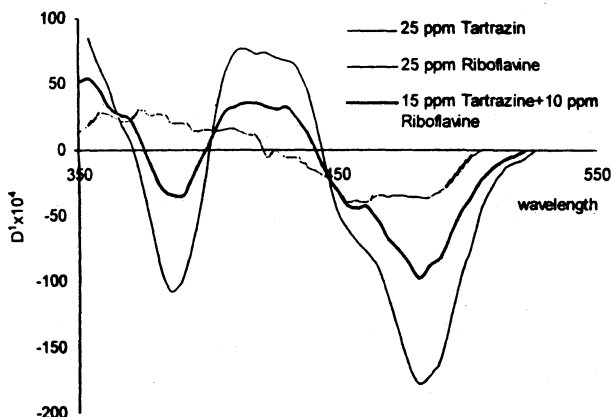


Fig. 3. The first order derivative spectra of tartrazine and riboflavin and their mixture

be determined in the presence of T at 423 nm. In Fig. 4, it can be seen that T can be determined in the presence of C at 403 nm, and C also can be determined in

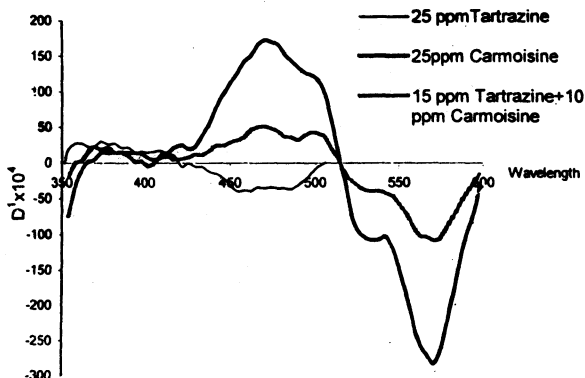


Fig. 4. The first order derivative spectra of tartrazine and carmoisine and their mixture

TABLE-3
LINEAR REGRESSION CALIBRATION FUNCTIONS FOR FIRST DERIVATIVE SPECTROPHOTOMETRIC METHOD USING ZERO-CROSSING METHOD IN BINARY MIXTURE

Binary mixture	Component	Wavelength, nm	Equation
Tartrazine/Carmoisine	Tartrazine	403	$^1D = 6 \times 10^{-5}[C_T] - 4 \times 10^{-5}$
	Carmoisine	569	$^1D = -0.0014[C_C] + 0.0001$
Tartrazine/Riboflavin	Tartrazine	443	$^1D = -8 \times 10^{-5}[C_T]$
	Riboflavin	423	$^1D = 0.0003[C_R] + 0.0004$
Carmoisine/Riboflavin	Carmoisine	569	$^1D = -0.0014[C_C] + 0.0001$
	Riboflavin	406	$^1D = -0.0002[C_R] + 0.0005$

the presence of T at 569 nm. In Fig. 5, it can be seen that C can be determined in the presence of R at 569 nm and R also can be determined in the presence of C at 406 nm. In addition, the selected wavelengths using zero-crossing method and the calibration function equation for each component in the binary mixture are presented in Table-3. In parallel, the same binary mixtures were resolved using first derivative spectra. Using the corresponding equations, recovery results were calculated for the same synthetic mixtures and food samples as in the other method (Tables 4–8). The results obtained using determinant method were in fairly good agreement with those obtained by the zero-crossing technique applied to the first derivative spectrophotometry. First derivative spectrophotometry was not applied for analysis of ternary mixtures because suitable wavelengths for zero-crossing method were not found.

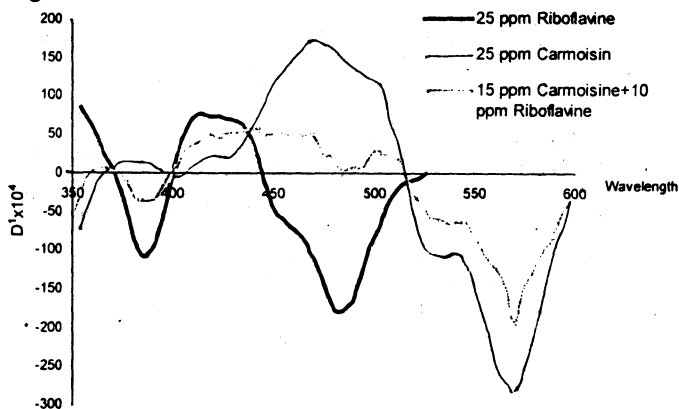


Fig. 5. The first order derivative spectra of carmoisine and riboflavine and their mixture

TABLE-4
COMPOSITION OF BINARY MIXTURES (IN $\mu\text{g/mL}$) CONTAINING
CARMOISINE-RIBOFLAVINE AND RECOVERY RESULTS EXPRESSED AS
A PERCENTAGE USING ZERO-ORDER SPECTROPHOTOMETRIC METHODS
USING DETERMINANT CALCULATION AND FIRST DERIVATIVE
SPECTROPHOTOMETRIC METHODS

Theoretical $\mu\text{g/mL}$		Recovery (R%)			
		Zero order		First derivative	
Carmoisine	Riboflavine	Carmoisine	Riboflavine	Carmoisine	Riboflavine
5	10	106.8	97.7	91.4	95.0
5	5	111.3	97.1	107.1	100.0
15	10	100.7	105.5	91.9	105.0
10	15	98.8	115.2	98.6	106.2
Average		104.4	103.9	97.3	106.6

TABLE-5
COMPOSITION OF BINARY MIXTURES (IN $\mu\text{g/mL}$) CONTAINING
CARMOISINE-TARTRAZINE AND RECOVERY RESULTS EXPRESSED AS
A PERCENTAGE USING ZERO-ORDER SPECTROPHOTOMETRIC METHODS
USING DETERMINANT CALCULATION AND FIRST DERIVATIVE
SPECTROPHOTOMETRIC METHODS

Theoretical $\mu\text{g/mL}$		Recovery (R%)			
		Zero order		First derivative	
Carmoisine	Tartrazine	Carmoisine	Tartrazine	Carmoisine	Tartrazine
5	10	102.4	90.3	102.8	98.3
10	5	95.5	113.6	98.6	96.7
5	5	102.0	97.1	100.0	93.3
10	15	98.5	90.2	101.4	93.3
15	10	100.4	100.2	92.9	98.3
Average		99.8	98.3	99.2	96.0

TABLE-6
COMPOSITION OF BINARY MIXTURES (IN $\mu\text{g/mL}$) CONTAINING
RIBOFLAVINE-TARTRAZINE AND RECOVERY RESULTS EXPRESSED AS
A PERCENTAGE USING ZERO-ORDER SPECTROPHOTOMETRIC METHODS
USING DETERMINANT CALCULATION AND FIRST DERIVATIVE
SPECTROPHOTOMETRIC METHODS

Theoretical $\mu\text{g/mL}$		Recovery (R%)			
		Zero order		First derivative	
Riboflavine	Tartrazine	Riboflavine	Tartrazine	Riboflavine	Tartrazine
5	10	95.6	100.6	93.4	100.0
10	5	100.1	103.2	83.4	100.0
5	5	105.4	102.3	93.4	100.0
10	15	97.9	98.4	93.4	91.7
15	10	98.1	111.1	93.4	100.0
Average		99.4	103.2	91.4	98.4

TABLE-7
COMPOSITION OF TERNARY MIXTURES (IN $\mu\text{g/mL}$) CONTAINING
CARMOISINE-TARTRAZINE-RIBOFLAVINE AND RECOVERY RESULTS
EXPRESSED AS A PERCENTAGE USING ZERO-ORDER SPECTROPHOTOMETRIC
METHODS USING DETERMINANT CALCULATION

Theoretical $\mu\text{g/mL}$			Recovery (R%)		
			Zero order		
Riboflavine	Tartrazine	Carmoisine	Riboflavine	Tartrazine	Carmoisine
5	5	5	108.2	111.0	100.1
10	10	5	103.7	96.9	100.1
5	15	10	102.4	99.2	105.7
10	20	5	101.2	96.9	100.1
10	10	15	106.3	100.7	101.1
Average			106.4	101.0	101.8

TABLE-8
DETERMINATION OF THE RECOVERY FOR FOOD DYES IN PREPARED COMMERCIAL PRODUCT SOLUTIONS

Sample	Compound		Zero order			First derivative				
Sour powder drink (10 g/100 mL)	Tartrazine	Added ($\mu\text{g/mL}$)	—	5.0	10.0	15.0	—	5.0	10.0	15.0
		Found ($\mu\text{g/mL}$)	13.9	18.7	23.4	28.5	12.9	17.3	23.5	29.6
		Recovery (R%)	—	97.1	95.5	97.6	—	96.7	105.7	110.9
	Carmoisine	Added ($\mu\text{g/mL}$)	—	10.0	15.0	20.0	—	10.0	15.0	20.0
		Found ($\mu\text{g/mL}$)	25.4	34.7	40.5	45.6	25.7	35.7	40.7	41.3
		Recovery (R%)	—	93.3	101.3	100.9	—	100.0	100.0	101.5
Lemon powder drink (10 g/100 mL)	Tartrazine	Added ($\mu\text{g/mL}$)	—	10.0	15.0	20.0	—	10.0	15.0	20.0
		Found ($\mu\text{g/mL}$)	10.4	20.6	25.8	30.3	11.3	20.8	26.3	32.1
		Recovery (R%)	—	102.1	102.5	99.5	—	97.7	100.0	102.6
	Riboflavine	Added ($\mu\text{g/mL}$)	—	2.5	5.0	7.5	—	2.5	5.0	7.5
		Found ($\mu\text{g/mL}$)	—	2.4	5.0	7.4	—	2.7	5.1	7.4
		Recovery (R%)	—	93.9	100.1	99.0	—	108.0	102.0	98.7
Strawberry soft drink	Riboflavine	Added ($\mu\text{g/mL}$)	—	10.0	15.0	20.0	—	10.0	15.0	20.0
		Found ($\mu\text{g/mL}$)	—	10.4	16.2	21.8	—	9.5	15.0	19.5
		Recovery (R%)	—	104.4	108.2	109.0	—	95.0	100.0	97.5
	Carmoisine	Added ($\mu\text{g/mL}$)	—	5.0	10.0	15.0	—	5.0	10.0	15.0
		Found ($\mu\text{g/mL}$)	4.2	9.0	13.6	18.1	4.6	9.4	13.7	17.9
		Recovery (R%)	—	95.7	93.9	93.0	—	97.1	91.4	88.6

The proposed methods were applied to the determination of T, C and R in three different drinks. The results obtained using two methods are presented in Table-8. Again, good agreement between the results obtained using the two procedures was found.

TABLE-9
TOTAL TARTRAZINE, CARMOISINE AND RIBOFLAVINE AMOUNTS IN
COMMERCIAL PRODUCTS

Sample	Zero Order			First Derivative		
	Tartrazine	Carmoisine	Riboflavine	Tartrazine	Carmoisine	Riboflavine
Lemon (mg/g)	0.52	—	—	0.56	—	—
Sour (mg/g)	0.70	1.27	—	0.65	1.29	—
Strawberry (mg/mL)	—	0.042	—	—	0.046	—

Conclusions

In this work, a determinant method to calculate concentrations of T, C and R was applied to the simultaneous determination of binary and ternary mixtures of T, C and R in food products by spectrophotometric procedure. The resolution of binary mixtures was also carried out using the first derivative method. The results show that the two methods were in good agreement for both synthetic solutions and real sample solutions. The results show that Cramer method is a good alternative to derivative spectrophotometry with the clear advantage that no mathematical treatment of spectral data is required. This method was also applied for analysis of ternary mixtures while derivative spectrophotometric method was not used because of unsuitable wavelengths for zero-crossing technique.

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