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SIMULTANEOUS DETERMINATION OF CARMINIC ACID, RIBOFLAVINE, CURCUMIN AND ERYTHROSINE BY DERIVATIVE SPECTROPHOTOMETRY AND RATIO SPECTRA DERIVATIVE

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Summary—A quaternary mixture of carminic acid, riboflavine, curcumin and erythrosine can be resolved with a previous extraction step into metyl-isobutyl ketone and, resolving the binary mixtures obtained in the aqueous phase and organic phase, using derivative spectrophotometry on the basis of the zero-crossing measurements in the first derivative spectra as well as the first derivative of ratio spectra. The conditions of extraction established and the proposed methods have been tested to determine these colorants in several synthetic mixtures of four dyes, obtaining good recoveries. The methods have been applied in yoghurt samples spiked with the dyes.

Color is the first sensory quality by which food are judged, and food quality and flavour are closely associated with color. Colorants are very important ingredients in many convenience food such as confectionery products, gelatin desserts, snacks and beverages, since many of these would otherwise be colorless and would thus appear undesirable without the inclusion of colorants. There are four major bodies currently active in the formulation of food additives regulation and specification. In the European Economic Community (EEC), a Scientific Committee has reviewed the safety in use of all compounds proposed for inclusion in a Community List of colorants authorized to be used in foodstuffs where the colorants have been given 'E' numbers. Special attention was given to the sulfonated azodyes. Some of these synthetic dyes were found to be toxicologically acceptable and an Acceptable Daily Intake (ADI) established.

Derivative spectrophotometry is widely used for resolving binary mixtures of compounds with overlapped spectra on the basis of zero-crossing measurements.[1–3] Digital differentiation can be used to obtain the derivative spectra.[4] The use of computers in the treatment of the spectral data permits other suitable alternatives for resolving the mixtures, multicomponent analysis programs[5,6] are used for this purpose. Blanco *et al.*[7,8] resolved the overlapped spectra using multiwavelength linear regression analysis (MLRA). We have developed a new spectrophotometric method for resolving binary mixtures by using the first derivative of the ratio spectra.[9] The absorption spectrum of the mixture is obtained and the amplitudes at appropriate wavelengths are divided by the corresponding amplitudes in the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. Thus, the contribution of the component used as divisor is eliminated in the first derivative and it permits the use of the greatest sensitivity wavelength as a signal of measurements, a maximum or a minimum. The concentration of the other component is then determined from a calibration graph.

Spectrophotometric methods using multiwavelengths measurements,[10–13] or derivative spectrophotometry[14,15] for determining colorants have been proposed by several authors. The use of the zero-crossing in derivative spectrophotometry is not suitable for resolving mixtures of three or more components with overlapped spectra, other multicomponents analysis is required. However, the simultaneous use of liquid–liquid extraction and derivative spectrophotometry can be an alternative procedure.

Carminic acid (E-120), riboflavine (E-101) and curcumin (E-100) are natural colorants, and erythrosine (E-127) a synthetic colorant permitted in the EEC. The ADI are 2 mg/kg (temporary), 0.5 mg/kg, 0.15 mg/kg and 2.5 mg/kg respectively. Because these colorants are together in some food commercial products, a simple and rapid method is desired in order to determine the four colorants in their quaternary mixture. These components have very overlapped absorption spectra. In this paper we propose an extraction procedure and two methods for the simultaneous determination of the dyes in binary mixtures that remain in the aqueous phase (carminic acid and riboflavine) and the binary mixture of dyes extracted in the organic phase (erythrosine and curcumin). These methods are derivative spectrophotometry on the basis of zero-crossing measurements in the first derivative spectra and the derivative ratio spectra method.

EXPERIMENTAL

Apparatus

A Beckman Instruments DU-70 spectrophotometer connected to an IBM PS/2 fitted with Beckman Data Leader Software (16) and an Epson FX-850 printer were used for all the measurement and treatment of data. The pH values were measured with a Crison model 2002.

Reagents

All reagent and solvents used were of analytical reagent grade. Stock reference solutions (200 mg/l.) were prepared in water from pure samples of carminic acid and riboflavine and in ethanol from pure sample of erythrosine and curcumin, from Sigma Chemical Company Products. Buffers of constant ionic strength ($0.1M$) were prepared from sodium hydrogen phosphates except at pH 4–5 where acetate buffer was used.

Procedure

Equal volumes (20 ml) of aqueous and organic phases were shaken 2 min in a 100-ml separating funnel as follows: several amounts of dyes containing up to 100 mg/l. carminic acid, and/or up to 40 mg/l. riboflavine, and/or up to 9 mg/l. erythrosine, and/or up to 9 mg/l. curcumin, buffered at pH 4.8 with acetic acid/sodium acetate solution (ionic strength = 0.1), 25% ethanol and water up to 20 ml, were shaken with 20 ml of methyl-isobutyl ketone (MIBK). The phases were separated. Carminic acid and riboflavine remained in the aqueous phase and erythrosine and curcumin were quantitatively extracted in MIBK. The organic phase was centrifuged in order to eliminate the water. All extractions were carried out in triplicate. The determination of each colorant in the binary mixtures was done by two different methods: first derivative spectrophotometry and first derivative of the ratio spectra.

First derivative spectrophotometry

Carminic acid (CA)–riboflavine (R) binary mixture in the aqueous phase. The first derivative spectra were obtained with a $\Delta\lambda = 4$ nm and smoothed through the use of 13 experimental points. To determine carminic acid, calibration graphs were obtained by measuring the amplitude at 445.5 nm ($^1D_{445.5}$) and 543.5 nm ($^1D_{543.5}$) (zero-crossing points for riboflavine), and gave a straight line up to 100 mg/l. carminic acid. The riboflavine content was determinated by measuring the signal at 492.5 nm ($^1D_{492.5}$) and 391.5 nm ($^1D_{391.5}$) (zero-crossing points for carminic acid) where the concentration of riboflavine was proportional to the amplitude up to 40 mg/l. We use the term $^1D_\lambda$ to indicate the signal of measurement at appropriate wavelength (λ) in the first derivative spectrum (^1D).

Erythrosine (E)–curcumin (C) binary mixture in MIBK. The first derivative spectra were obtained with a $\Delta\lambda = 4$ nm. To determine erythrosine calibration graphs were obtained by measuring the amplitude at 537 nm ($^1D_{537}$), and at 555 nm ($^1D_{555}$) (zero-crossing point for curcumin), and gave a straight line up to 9 mg/l. erythrosine. The curcumin content was determinated by measuring the signal at 432 nm ($^1D_{432}$), at 411 nm ($^1D_{411}$) and at 393.5 nm ($^1D_{393.5}$) (zero-crossing point for erythrosine) where the concentration of curcumin was proportional to the amplitude up to 9 mg/l.

First derivative of the ratio spectra

Carminic acid–riboflavine binary mixtures in aqueous phase. By this method, for determining carminic acid the stored spectra of the mixtures were divided by a standard spectrum of riboflavine of 25 mg/l. The ratio spectra thus obtained were smoothed through the use of 15 experimental points and the first derivatives were calculated with $\Delta\lambda = 4$ nm. The concentration of carminic acid was proportional to the amplitude of the minimum at 518 nm ($^1DD_{518}$) (signal in the first derivative spectrum at 518 nm).

In order to determine riboflavine, stored spectra of the mixture were divided by a standard spectrum of carminic acid of 24 mg/l. The ratio spectra obtained were smoothed through the use of 15 experimental points and the first derivative was calculated with $\Delta\lambda = 4$ nm. The concentration of the riboflavine was proportional to the amplitude of the maximum at 486 nm ($^1DD_{486}$) and to the maximum at 456 nm ($^1DD_{456}$). The term $^1DD_\lambda$ indicates the wavelength (λ) we use as a signal of measurement in the first derivative spectrum ^1DD.

Erythrosine–curcumin binary mixture in phase organic. To determine erythrosine the stored spectra of the mixture were divided by a standard spectrum of curcumin of 6 mg/l. The ratio spectra thus obtained were smoothed through the use of 15 experimental points and the first derivatives were calculated with $\Delta\lambda = 15$ nm. The concentration of erythrosine was proportional to the amplitude of the minimum at 536 nm ($^1DD_{536}$) and to the maximum at 558 nm ($^1DD_{558}$) (signal in the first derivative divided spectrum at 536 and 558 nm).

For determining curcumin, stored spectra of the mixtures were divided by a standard spectrum of erythrosine of 8 mg/l. The ratio spectra obtained were smoothed through the use of 15 experimental points and the first derivative was calculated with $\Delta\lambda = 15$ nm. The concentration of curcumin was proportional to the amplitude of the maximum at 456 nm ($^1DD_{456}$) and to the minimum at 411 nm ($^1DD_{411}$) and at 388 nm ($^1DD_{388}$).

RESULTS AND DISCUSSION

Method development

The influence of pH on the absorption spectra of carminic acid, riboflavine, erythrosine and curcumin was studied. A range of pH values between 1 and 13 were examined. Only negligible changes were experienced between 1 and 5 for carminic acid, 4 and 8 for riboflavine, 1 and 7.5 for curcumin, and 3 and 10 for erythrosine.

Figure 1 shows the absorption spectra of carminic acid, riboflavine, curcumin and erythrosine in the 300–650 nm wavelength range. As can be seen, the spectra are overlapped and the simultaneous determination of them in quaternary mixture is difficult. We resolve that problem with a previous extraction with MIBK step and resolving the binary mixtures of the aqueous phase and organic phase by the proposed methods.

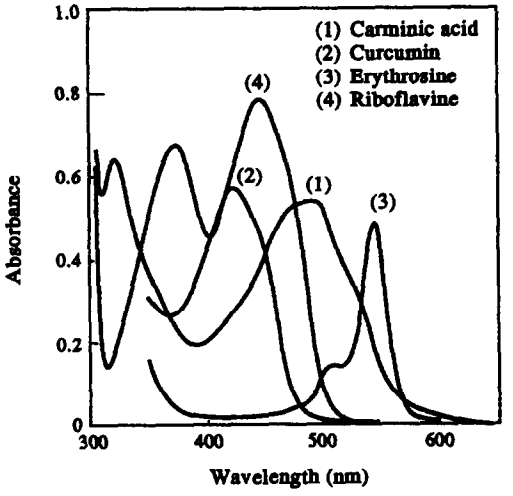

Fig. 1. Absorption spectra of (1) carminic acid (48 mg/l.), (2) curcumin (6 mg/l.), (3) erythrosine (6 mg/l.), (4) riboflavine (24 mg/l.).

Initially, the extractions were tested with different organic dissolvents for carminic acid, riboflavine, erythrosine and curcumin solutions at different pH values. The results obtained are summarized in Table 1. By using MIBK as organic phase and acetic acid/sodium acetate buffer solution as aqueous phase, it is possible to quantitatively separate erythrosine and curcumin (organic phase) from carminic acid and riboflavine (aqueous phase). The extraction step was optimized by testing the influence of the habitual variables on the process, and the following conditions were selected: pH 4.8 (by addition of 5 ml 0.1*M* of HAc/Ac^- buffer solution). $V_0 = V_a = 20$ ml. Shaking times = 2 min. Ethanol in aqueous phase = 25%.

First derivative spectrophotometry

Carminic acid–riboflavine binary mixture in aqueous phase. In Fig. 1, the zero order spectra of carminic acid and riboflavine in the 300–650 nm wavelength range are shown. As can be seen, the absorption spectra of the colorants are very overlapped and because of this the determination of the two colorants is not possible using direct absorbance measurements. The classical derivative spectrophotometry is commonly used to resolve mixtures of compounds with overlapped spectra using the 'zero-crossing' method.

Figure 2 shows the first derivative spectra of carminic acid and riboflavine. Carminic acid can be determined by measuring at 445.5 ($^1D_{445.5}$) and 543.5 nm ($^1D_{543.5}$) (zero-crossing point for riboflavine) and riboflavine at 492.5 ($^1D_{492.5}$) and 391.5 nm ($^1D_{391.5}$) (zero-crossing

Table 1. *Extractions using different organic solvents*

Colorant		Tetrachloromethane	Isoamyl alcohol	Chloroform	Ethyl ether	Methyl Iso-butylketone	Cyclohexanone	n-Butylic alcohol	Toluene	Benzene
Carminic acid	NH_4^+/NH_3	No	No	No	No	No	No	No	No	No
	HAc/Ac^-	No	Distribution	No	No	No	Distribution	Distribution	No	No
	OH^-	No	No	No	No	No	No	No	No	No
	H^+	No	Yes	No	No	Distribution	No	Yes	No	No
Riboflavine	NH_4^+/NH_3	No	Distribution	No	No	No	Distribution	Distribution	No	No
	HAc/Ac^-	No	Distribution	No	No	No	Distribution	Distribution	No	No
	OH^-	No	No	No	No	No	No	No	No	No
	H^+	No	Distribution	No	No	No	No	Distribution	Distribution	No
Curcumin	NH_4^+/NH_3	Distribution	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Hac/ac^-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	OH^-	No	No	No	No	No	No	No	No	No
	H^+	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Erythrosine	NH_4^+/NH_3	No	Distribution	No	No	No	Distribution	Distribution	No	No
	HAc/Ac^-	No	Yes	No	No	Yes	Yes	Yes	No	No
	OH^-	No	Distribution	No	No	No	Distribution	Distribution	No	No
	H^+	No	Yes	No	No	No	Yes	Yes	No	No

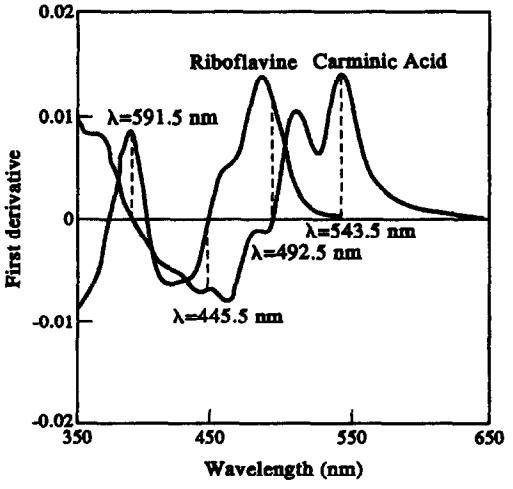

Fig. 2. First derivative spectra of carminic acid (48 mg/l.) and riboflavine (24 mg/l.).

point for carminic acid). At these wavelengths the signals of the mixture and the determined compound are coincident.

The main instrumental parameters that affect the shape of the derivative spectra are scan speed, the smoothing and the wavelength increment over which the derivative spectrum is obtained ($\Delta\lambda$). These parameters need to be optimized to give a well resolved large peak, *i.e.* to give good selectivity and larger sensitivity in the determination. The scan speed used to recorder the absorption spectra was 600 nm/min. Due to the extent of the noise levels a smoothing function was used based on the Savitzky and Golay method[4,17] and 13 experimental points were considered as optimum.

Generally, the noise level of the first derivative spectra decreases with an increase in $\Delta\lambda$ thus decreasing the fluctuations in the derivative spectrum. However, if the value of $\Delta\lambda$ is too large, the spectra resolution is very poor. Therefore, the optimum value of $\Delta\lambda$ should be determined by taking into account the noise level, the resolution of the spectrum and the sample concentration. Various values of $\Delta\lambda$ were tested and 4 nm was selected as the optimum.

The calibration graphs were established by measuring at appropriate wavelengths in the first derivative spectra. It gave straight lines up to 100 mg/l. carminic acid concentration by measuring at 445.5 and 543.5 nm and up to 40 mg/l. riboflavine concentration by measuring the signal at 492.5 and 391.5 nm. Satisfactory regression coefficients were obtained in all cases.

Erythrosine–curcumin binary mixture in MIBK. Figure 3 shows the first derivative

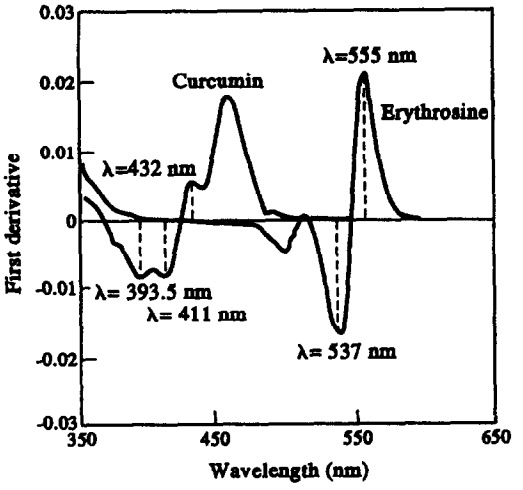

Fig. 3. First derivative spectra of erythrosine (6 mg/l.) and curcumin (6 mg/l.).

spectra of erythrosine and curcumin. Curcumin can be determined by measuring at 432 ($^1D_{432}$), 411 ($^1D_{411}$) and 393.5 nm ($^1D_{393.5}$) (zero-crossing point for erythrosine) and erythrosine at 537 ($^1D_{537}$), 555 nm ($^1D_{555}$) (zero-crossing point for curcumin). At these wavelengths the signals of the mixture and the determined compound are coincident. The scan speed used to recorder the absorption spectra was 600 nm/min. Some values of $\Delta\lambda$ were tested and 4 nm was selected as the optimum. A smooth function was not necessary in this mixture. The calibration graphs were established by measuring at appropriate wavelength in the first derivative spectra. It gave straight lines up to 9 mg/l. curcumin concentration by measuring at 432, 411, and 393.5 nm and up to 9 mg/l. erythrosine concentration by measuring the signal at 537 and 555 nm. Satisfactory regression coefficients were obtained in all cases.

First derivative of the ratio spectra

Carminic acid–riboflavine binary mixture in aqueous phase. In this method the concentration of the divisor must be optimized. In this way if the concentration of divisor increases, the slope of the calibration graph decreases. One of the limitations of this method is that in the wavelength range where the absorbance of the standard solution used as divisor is zero (or below the baseline) the noise is greatly enhanced. In consequence, the useful wavelength range must be selected and if the noise is slightly increased, a smoothing function can be used as in classical derivative spectrophotometry. Logically, the wavelength increment over which the first derivative is obtained ($\Delta\lambda$) has to be optimized.

To determine riboflavine, we selected a carminic acid concentration of 24 mg/l. Due to the noise levels of the ratio spectra a smoothing of 15 experimental points was considered optimum. The influence of the $\Delta\lambda$ in order to obtain the first derivative was tested and a $\Delta\lambda$ of 4 nm was considered as suitable for the carminic acid determination.

Figure 4a shows the first derivative of ratio spectra of different riboflavine standards. The calibration graphs were obtained by measuring at 486 nm ($^1DD_{486}$) and at 456 nm ($^1DD_{456}$) corresponding to two maxima and it was found to be linear up to 40 mg/l. riboflavine.

To determine the other component, carminic acid, an analogous procedure was followed. The

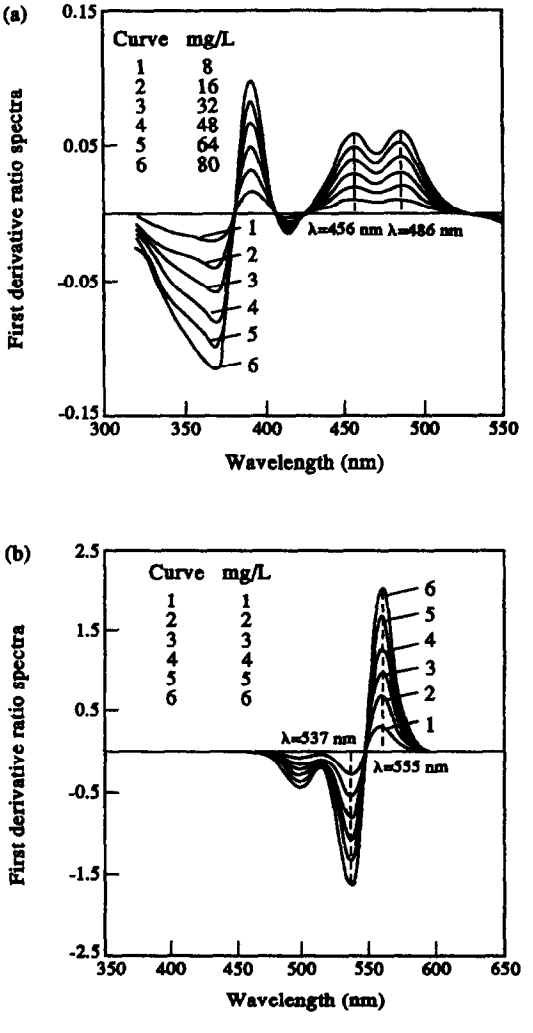

Fig. 4. (a) First derivative of ratio spectra for different concentrations of riboflavine when carminic acid (divisor) was 24 mg/l. (b) First derivative of ratio spectra for different concentrations of erythrosine when curcumin (divisor) was 6 mg/l.

Table 2. Calibration graphs

Equation	Regression coefficient
Carminic acid–riboflavine mixture binary in aqueous phase	
$^1D_{445\,5} = 0.00021 + 0.00010\ C_{CA}$	0.9994
$^1D_{543\,5} = 0.00036 + 0.00020\ C_{CA}$	0.9995
$^1DD_{518} = 0.05460 + 0.0210\ C_{CA}$	0.9997
$^1DD_{555} = 0.05270 + 0.0207\ C_{CA}$	0.9998
$^1D_{391\,5} = 0.00007 + 0.00051\ C_R$	0.9998
$^1D_{492\,5} = 0.000023 + 0.00074\ C_R$	0.9997
$^1DD_{486} = 0.00047 + 0.0130\ C_R$	0.9999
$^1DD_{456} = 0.00081 + 0.0012\ C_R$	0.9998
Erythrosine–curcumin mixture binary in MIBK	
$^1D_{432} = -0.000046 + 0.00092\ C_C$	0.9999
$^1D_{411} = -0.000028 + 0.00114\ C_C$	0.9997
$^1D_{393.5} = -0.00028 + 0.0016\ C_C$	0.9999
$^1DD_{456} = -0.00481 + 0.1725\ C_C$	0.9999
$^1DD_{411} = -0.00520 + 0.08982\ C_C$	0.9999
$^1DD_{388} = -0.01796 + 0.0906\ C_C$	0.9999
$^1D_{537} = -0.00021 + 0.0043\ C_E$	0.9998
$^1D_{555} = -0.00025 + 0.0037\ C_E$	0.9999
$^1DD_{536} = 0.01570 + 0.2700\ C_E$	0.9999
$^1DD_{558} = -0.0210 + 0.3452\ C_E$	0.9999

C_{CA}: mg/l. of carminic acid.
C_R: mg/l. of riboflavine.
C_E: mg/l. of erythrosine.
C_C: mg/l. of curcumin.

concentration of divisor selected was 25 mg/l. of riboflavine, the ratio spectra were smoothed with 15 experimental points and the first derivative was calculated with a $\Delta\lambda = 4$ nm. The calibration graphs were tested by measuring at 518 ($^1DD_{518}$) and 555 nm ($^1DD_{555}$) corresponding to a minimum and a maximum, respectively, and straight lines up to 100 mg/l. of carminic acid were obtained.

Erythrosine–curcumin binary mixture in MIBK. In order to determine erythrosine, we selected a curcumin concentration of 6 mg/l. as divisor, a smoothing of 15 experimental points and a $\Delta\lambda$ of 15 nm to obtain the first derivative of the ratio spectra.

Figure 4b shows the first derivatives of ratio spectra for different erythrosine standards. The calibration graphs were obtained by measuring at 536 ($^1DD_{536}$) and at 558 nm ($^1DD_{558}$) corresponding to a minimum and a maximum, respectively, and it was found to be linear up to 9 mg/l. erythrosine.

To determine the other component, curcumin, an analogous procedure was followed. The concentration of divisor selected was 8 mg/l. erythrosine, the ratio spectra were smoothed with 15 experimental points and the first derivative were calculated with an $\Delta\lambda = 15$ nm. Calibration graphs were tested by measuring at 456 ($^1DD_{456}$), 411 ($^1DD_{411}$), and 388 nm ($^1DD_{388}$) and straight lines up to 9 mg/l. of curcumin were obtained.

Statistical comparative study

The statistical data of calibration graphs obtained for each dye in both binary mixtures by using the two methods are summarized in Table 2. In all cases good regression coefficients were obtained. In order to test the precision of the

Table 3. Statistical data

	Signal Measured	Standard deviation (*mg/l.*)	Relative standard deviation	Determination limit (*mg/l.*)
Carminic acid–riboflavine binary mixture in aqueous phase				
Carminic acid	$^1D_{445\,5}$	0.1359	±0.690	0.930
	$^1D_{543\,5}$	0.2828	±1.069	3.400
	$^1DD_{518}$	0.1845	±0.890	2.100
	$^1DD_{555}$	0.3050	±1.340	1.037
Riboflavine	$^1D_{492\,5}$	0.0315	±0.300	0.010
	$^1D_{391\,5}$	0.0437	±0.420	0.800
	$^1DD_{456}$	0.0589	±0.690	1.280
	$^1DD_{486}$	0.0497	±0.480	0.640
Erythrosine–curcumin binary mixture in MIBK				
Curcumin	$^1D_{411}$	0.0654	±0.027	0.510
	$^1D_{432}$	0.0360	±0.016	0.451
	$^1D_{393.5}$	0.0614	±0.027	0.291
	$^1DD_{456}$	0.0475	±0.021	0.029
	$^1DD_{411}$	0.0650	±0.027	0.030
	$^1DD_{388}$	0.0541	±0.024	0.126
Erythrosine	$^1D_{555}$	0.0221	±1.102	0.110
	$^1D_{537}$	0.0213	±1.100	0.110
	$^1DD_{558}$	0.0245	±1.214	0.040
	$^1DD_{536}$	0.0214	±1.124	0.030

Table 4. Resolution of carminic acid and riboflavine mixtures in aqueous phase by first derivative and ratio spectra derivative methods

Composition of mixture (mg/l.)		Carminic acid				Riboflavine			
		$^1D_{445.5}$	$^1D_{543\,5}$	$^1DD_{518}$	$^1DD_{555}$	$^1D_{391\,5}$	$^1D_{492.5}$	$^1DD_{486}$	$^1DD_{456}$
CA*	R†	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)
24	24	94.25	90.99	95.00	101.80	99.12	101.50	104.87	99.61
48	24	94.56	92.58	99.25	105.47	97.50	99.75	103.59	97.52
72	24	94.66	93.84	103.36	103.30	95.87	98.66	104.55	95.87
96	24	94.72	93.92	101.50	101.30	95.08	98.12	104.46	95.08
100	24	96.40	88.80	101.30	102.50	96.66	98.15	108.40	96.66
24	48	98.37	87.22	95.50	108.30	98.81	99.60	100.52	98.81
16	48	113.30	110.43	113.30	110.90	98.82	99.81	100.72	99.60

*Carminic acid.
†Riboflavine.

methods, 10 individual replicates were measured containing carminic acid (24 mg/l.), riboflavine (20 mg/l.), erythrosine (6 mg/l.) and curcumin (6 mg/l.), respectively (Table 3). The determination limits were calculated for each dye and are shown in the same table.[18,19]

APPLICATIONS

Analysis of synthetic mixtures

In order to test the conditions of extraction established and the proposed methods, we studied separately the resolution of binary mixture of dyes that remain in aqueous phase (carminic acid–riboflavine) and the binary mixture of dyes extracted in organic phase (erythrosine–curcumin).

In Table 4 the results obtained for the carminic acid–riboflavine binary mixture in aqueous phase by two methods are summarized. In Table 5 the results obtained for the erythrosine–curcumin binary mixture in organic phase are shown. The recoveries (%) were very good in general, except when the concentration in mg/l. of dye determined is five times less than the dye interferent, in these cases the error is about 10%.

Once the conditions of extraction and the methods proposed for the binary mixtures in both phases were separately tested, several synthetic mixtures of four colorants were prepared. The extraction procedure was done and the methods proposed were applied for the resolution of the quaternary mixture.

In Table 6 the results obtained for quaternary mixture by first derivative spectra method are summarized and in Table 7 the results obtained by first derivative of ratio spectra are shown. As can be seen the recoveries (%) obtained by first derivative of the ratio spectra method are better than first derivative spectra method.

The methods were applied to yoghurt samples spiked with the four colorants. For each sample about 2 g of yoghurt was weighed, spiked with different amounts of the four colorants and carefully homogenized. Samples were shaken and sonicated with 10 ml of acetone for 1 hr, centrifuged at 5000 rpm for 5 min and the supernatant acetonic solution transferred to a distillation flask. The treatment with acetone was repeated three times and the supernatants collected into a distillation flask. Acetone solution was evaporated to dryness at 50°C in a

Table 5. Resolution of erythrosine and curcumin mixtures in MIBK by first derivative and ratio spectra derivative methods

Composition of mixture (mg/l.)		Curcumin						Erythrosine			
		$^1D_{411}$	$^1D_{432}$	$^1D_{393.5}$	$^1DD_{456}$	$^1DD_{411}$	$^1DD_{388}$	$^1D_{537}$	$^1D_{555}$	$^1DD_{536}$	$^1DD_{558}$
E*	C†	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)
1	9	98.56	101.00	98.92	99.24	97.24	97.33	111.62	108.56	93.52	109.30
3	7	100.82	103.89	101.14	102.10	99.69	102.00	101.50	106.75	93.12	95.23
5	5	98.65	105.47	101.60	102.28	99.64	103.50	101.30	105.67	98.84	101.46
7	3	95.59	106.14	100.70	100.30	97.06	105.59	95.01	98.64	97.29	102.53
9	1	55.79	99.10	72.00	94.50	81.00	115.00	97.66	94.83	98.34	95.30

*Erythrosine.
†Curcumin.

Table 6. Results obtained for the determination of carminic acid, riboflavine, erythrosine and curcumin by first derivative method

Composition of mixture (*mg/l.*)				Carminic acid		Riboflavine		Erythrosine		Curcumin		
				$^1D_{445}$	$^1D_{543}$	$^1D_{391}$	$^1D_{492.5}$	$^1D_{537}$	$^1D_{555}$	$^1D_{393.5}$	$^1D_{432}$	$^1D_{411}$
R*	C†	E‡	CA§	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)
20	2	10	20	94.60	138.62	99.92	101.97	93.72	91.61	63.31	97.52	82.97
20	4	8	40	90.25	104.71	97.27	99.34	86.51	86.02	80.09	88.48	87.89
20	6	6	60	97.49	100.78	97.27	99.34	95.52	95.09	110.32	97.92	95.52
20	8	4	10	97.00	117.68	97.34	99.34	97.05	95.95	88.48	101.48	90.35
30	10	2	10	97.00	109.96	103.65	97.00	102.94	99.26	93.28	102.68	95.95

*Riboflavine.
†Curcumin.
‡Erythrosine.
§Carminic acid.

Table 7. Results obtained for the determination of carminic acid, riboflavine, erythrosine and curcumin by first derivative of ratio spectra method

Composition of mixture (*mg/l.*)				Carminic acid		Riboflavine		Erythrosine		Curcumin		
				$^1DD_{518}$	$^1DD_{555}$	$^1DD_{456}$	$^1DD_{486}$	$^1DD_{558}$	$^1DD_{536}$	$^1DD_{388}$	$^1DD_{456}$	$^1DD_{411}$
R*	C†	E‡	CA§	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)	Recov. (%)
20	2	10	20	95.00	105.62	95.73	97.67	94.45	92.86	97.40	91.46	98.87
20	4	8	40	99.25	103.71	95.36	96.67	95.62	87.55	88.72	101.78	94.60
20	6	6	60	103.36	101.78	97.90	98.00	105.47	97.45	101.27	105.48	104.14
20	8	4	10	108.00	107.68	96.09	96.00	103.74	100.01	90.58	97.36	99.88
30	10	2	10	95.00	96.01	96.91	99.11	109.09	96.34	90.44	99.50	100.67

*Riboflavine.
†Curcumin.
‡Erythrosine.
§Carminic acid.

Table 8. Results obtained for the determination of carminic acid, riboflavine, curcumin and erythrosine by first derivative method in yoghurt

mg/g added				% Recov. CA		% Recov. R		% Recov. C		% Recov. E	
CA*	R†	C‡	E§	$^1D_{543}$	$^1D_{445.5}$	$^1D_{492}$	$^1D_{391}$	$^1D_{432}$	$^1D_{393}$	$^1D_{537}$	$^1D_{555}$
8.0	4.0	1.6	1.6	90.00	86.25	87.50	85.00	87.50	85.12	85.30	87.50
10.0	6.0	3.2	3.2	85.00	89.00	86.66	87.50	86.48	83.20	85.50	85.75
16.0	8.0	3.2	2.4	87.20	90.62	90.62	91.25	87.50	85.41	87.50	96.70
24.0	12.0	2.0	2.0	87.50	83.33	87.50	85.36	84.00	90.00	80.50	84.45

*Carminic acid.
†Riboflavine.
‡Curcumin.
§Erythrosine.

Table 9. Results obtained for the determination of carminic acid, riboflavine, curcumin and erythrosine by first derivative to ratio spectra method in yoghurt

mg/g added				% Recov. C.A.		% Recov. R		% Recov. C			% Recov. E	
CA*	R†	C‡	E§	$^1DD_{518}$	$^1DD_{553}$	$^1DD_{456}$	$^1DD_{486}$	$^1DD_{388}$	$^1DD_{456}$	$^1DD_{411}$	$^1DD_{558}$	$^1DD_{536}$
8.0	4.0	1.6	1.6	92.00	89.35	87.25	88.65	84.37	85.46	82.00	80.01	87.34
10.0	6.0	3.2	3.2	87.58	90.30	88.45	89.32	90.54	87.34	81.34	81.38	88.54
16.0	8.0	3.2	2.4	88.75	91.81	91.84	90.54	88.67	88.98	85.65	85.34	85.32
24.0	12.0	2.0	2.0	87.95	85.45	90.20	91.03	89.58	89.00	85.95	81.03	88.00

*Carminic acid.
†Riboflavine.
‡Curcumin.
§Erythrosine.

rotary still and the residue was dissolved with 6 ml ethanol, transferred to a 25-ml volumetric flask and diluted with 5 ml of acetic acid/sodium acetate, pH 4.8, and water to the mark. The methods described in this paper were applied to this solution.

In Table 8 the results obtained for quaternary mixtures in yoghurt by the first derivative spectra method and in Table 9 by first derivative of ratio spectra are summarized. The recoveries obtained by first derivative of ratio spectra are rather better than first derivative spectra method, as for the synthetic mixtures.

CONCLUSIONS

The experimental results obtained in this paper demonstrate that colorants can be determined in their quaternary mixtures using a previous extractions step with MIBK and by the two methods proposed. The accuracy, precision and rapidity of the proposed extractions by the described methods, as well as the good recoveries obtained, indicate that they are suitable for the analysis of carminic acid, riboflavine, erythrosine and curcumin mixtures.

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