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MAHMURE ÜSTÜN ÖZGÜR

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A Rapid Spectrophotometric Method to Resolve a Binary Mixture of Food Colorants (Riboflavine and Sunset Yellow)

Mahmure ÜSTÜN ÖZGÜR
Yıldız Technical University, Department of Chemistry,
34210 Davutpaşa, İstanbul-TURKEY
mozgur@yildiz.edu.tr

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A very simple and rapid spectrophotometric method using measurements at zero-crossing wavelength is described for resolving binary mixtures of riboflavine (E-101) and sunset yellow (E-110) in a powder drink. The assay procedure for E-110 and E-101 involves the extraction of the colorants from the powder drink with pH 4.5 acetate buffer, filtration, appropriate dilution, and measurement of the first derivative absorbance values (denoted as ^{1}D). Calibration graphs are linear up to 25.0 μg.mL^{-1} for E-101 and 40.0 μg.mL^{-1}for E-110. The sensitivity and reproducibility of the method were determined by using the synthetic mixtures in different ratios prepared by us, and the proposed method was applied to a commercially available powder drink. The relative standard deviations obtained are 1.39% and 1.15% and the average percentage recoveries are 101.0% and 102.8% for E-101 and E-110, respectively.

Key Words: Derivative spectrophotometry, simultaneous determination, zero-crossing measurements, sunset yellow, riboflavine, colorants, food analysis.

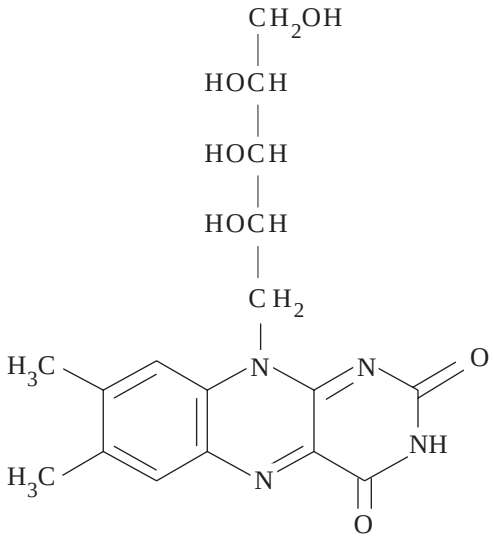
Introduction

Food additives are incorporated in food products to improve their sensory qualities (1,2). Color is the first sensory quality by which food is judged, and food quality and flavor are closely associated with color. Colorants are very important ingredients in many convenience products such as confectionery products, gelatin desserts, snacks and beverages, since without these they would be colorless and appear undesirable.

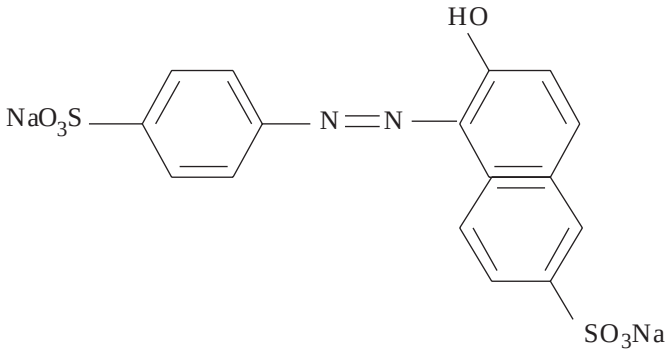
The concentration of these additives must be carefully controlled as they may have various harmful effects on human health. Studies on health-related aspects of the use of synthetic colorants have been reported (3).

E-101 and E-110 are water soluble colorants. E-110 is a synthetic azo dye and the other determined colorant is a natural dye whose toxicological evidence is virtually impossible. At present the USP (U.S. Pharmacopeia, 1990) relies on fluorimetry for E-101 and high-performance liquid chromatography (HPLC) for E-110. The recommended fluorimetric procedure is performed with a product containing only one

colorant. Chromatographic methods are highly suitable when the sample contains very many different colorants. However, in most foods, only 2 or 3 colorants are added. For this reason and taking into account other factors such as economy and rapidity, multi component analysis by a spectrophotometry can be a method of choice for the determination of the presence of colorants in food.



Riboflavine



Sunset Yellow

For the simultaneous determination of E-101 and other vitamins in food products, beverages and pharmaceutical preparations, fluorimetric (4-6), derivative fluorimetric (7), derivative spectrophotometric (8-9), capillary electrophoresis (10-11), HPLC (12-16), voltametric (17) and differential pulse polarographic (18) methods have been reported in the literature. In addition, for the simultaneous determination of E-110 and other food colorants in drinks and foods, derivative spectrophotometric methods (19-24), and partial least-squares (PLS) multivariate calibration (25-26) methods have been used.

The range of application of derivative spectrophotometry is increasing regularly in the field of analysis. Derivative spectroscopy is a relatively modern technique that has proved to be very advantageous in solving particular analytical problems that normal spectroscopy is not able to solve. One of the most extensive fields of application of derivative spectroscopy is the quantitative analysis of a system of 2 or more components (27-33).

In our previous work, we described satisfactory derivative spectrophotometric procedures for the determination of binary (29,33-36) and ternary (37,38) mixtures either as the pure color or in commercial food products.

No method has been published for the simultaneous determination of E-101 and E-110. For this purpose, the first derivative spectrophotometric method has been applied to the direct determination of colorants (E-110 and E-101). The assays were performed without preconcentration, derivatization or chromatographic separation steps.

Experimental

Apparatus

For absorbance and derivative spectrophotometric measurements a Philips model PU 8700 UV-Vis spectrophotometer and 10 mm quartz cells were used. All spectra were recorded from 300 to 550 nm with a 2 nm slit width, 500 nm.min^{-1} scan speed and very high smoothing. The pH values were measured with a Metrohm model pH meter, using a combined glass electrode.

Materials

Pharmaceutical grade samples of E-101 and E-110 were kindly supplied by ATOM KİMYA A.Ş., İstanbul, and the powder drink (LEZZO) was purchased from the market. All chemicals were analytical grade and were purchased from Merck, Darmstad, Germany. Distilled water was used throughout the work.

Stock solution of E-101 and E-110: 200 μg.mL^{-1} of each in pH 4.5 acetic acid/sodium acetate buffer were prepared. E-101 stock solution was prepared daily. An accurately weighed 0.01 g of E-101 was transferred into a 50 mL volumetric flask and 25 mL of pH 4.5 acetate buffer solution was added. The mixture was heated at 70 °C for 10 min, cooled and diluted to volume with pH 4.5 acetate buffer.

Standard solutions for the preparation of the calibration curve were obtained by diluting the stock solutions appropriately. Suitable aliquots of E-110 stock solution (0.4-2.0 mL) were transferred into 10 mL volumetric flasks and 0.5 mL of E-101 stock solution was added to each flask and diluted to an appropriate volume with pH 4.5 acetate buffer solution to make the standard solutions. Similarly, suitable aliquots of E-101 stock solution (0.25-1.25 mL) were transferred into 10 mL volumetric flasks and 0.5 mL of E-110 stock solution was added to each flask and diluted to an appropriate volume with pH 4.5 acetate buffer solution.

Acetic acid/sodium acetate buffer solution (pH 4.5): 5.4 g of sodium acetate was dissolved in 50 mL of water, adjusted to pH 4.5 with glacial acetic acid and diluted with water to 100 mL.

LEZZO powder drink: This product contains sugar, citric acid (E-330), dextrin, anticaking agent (tricalcium phosphate, E-341), nature identical orange flavor, thickener (gum arabic, E-414), antioxidant (ascorbic acid, E-300), oil, colorings [(titanium dioxide, E-171), sunset yellow (E-110), riboflavine (E-101)] and turbidity agent (lecithin, E-322).

Calibration procedure

The first derivative spectra of the standard solutions were recorded between 300 and 550 nm against pH 4.5 acetic acid/sodium acetate buffer solution. The suitable derivative orders with appropriate $\Delta\lambda$ and wavelength, where each colorant could be analyzed in the presence of the other, were determined (suitable settings = slit width 2 nm, scan speed 500 nm.min^{-1}and very high smoothing).

The calibration graphs were prepared by plotting the derivative absorbances of standard solutions against their concentrations (μg.mL^{-1}).

Assay procedure

The sample of powder drink was mixed and powdered. An accurately weighed 25 g of powder was transferred into a 50 mL volumetric flask, treated with 25 mL of pH 4.5 acetate buffer solution and the mixture was

heated at 70 °C for 10 min. The solution was then cooled and completed to volume with pH 4.5 acetate buffer solution, mixed well and filtered through a black filter paper. The absorption spectra of the samples thus prepared were recorded against pH 4.5 acetate buffer solution.

The E-101 content was determined from the first derivative spectrum by measuring the signal at 481.7 nm ($^1D_{481.7}$) (zero-crossing point for E-110) and by using an appropriate calibration graph. The E-110 content was measured from the signal at 445.5 nm ($^1D_{445.5}$), (zero-crossing point for E-101).

Results and Discussion

The absorption (zero-order) spectra of a 20 μg.mL^{-1} solution of E-101 and E-110 in pH 4.5 acetate buffer solution in the 300-550 nm range are given in Figure 1.

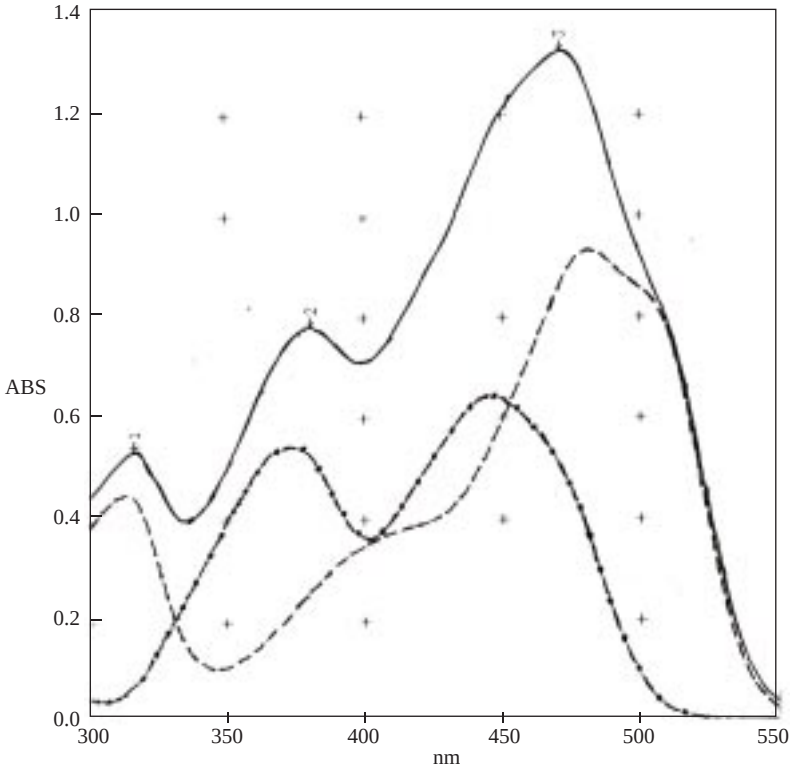


Figure 1. Absorption spectra of 20 μg.mL^{-1} riboflavine (– · – · –·), 20 μg.mL^{-1} of sunset yellow (– – ––) and their mixture (———). Reference: pH 4.5 acetate buffer.

The influence of pH on the absorption spectra for E-101 was studied by Berzas Nevado (22) et al. In this study, E-101 showed 2 maxima at 445.0 and 378.0 nm whose absorbance increased between pH 1.5 and 4.0, whereas between pH 4.0 and 8.0, their spectra remain constant. There was a small decrease in absorbance for higher pH values. In the other study, E-110 showed higher adsorption at pH values between 3.0 and 5.5, decreasing for higher values (41). Therefore we studied pH 4.0, 4.5, 5.0 and 5.5 acetic acid-sodium acetate buffer solution, and the other solution mentioned above for resolving overlapping absorption spectra of E-101 and E-110.

The pH 4.5 (acetic acid/sodium acetate buffer solution) was found to be superior to 0.2 N acetic acid, 0.2 N ammonium acetate, and a methanol:acetate buffer (pH 4.5) mixture in volume ratios of 1:1 and 70:30 for the determination of E-101 and E-110.

Furthermore pH 4.5 (acetic acid-sodium acetate buffer solution) was chosen as optimum because dilute solutions of E-101 and E-110 are stable for long periods (12 h for E-101 and 7 days for E-110) (22,40).

The simultaneous determination of both colorants is not possible by direct absorption measurements because of the spectral overlap of zero-order spectra (Figure 1). The determination of the 2 dyes might be possible by means of multivariant analysis (25,26,29,33,34,39,40). Another possibility that can be suitable to obviate this problem is derivative spectrophotometry (19,21,29,33-36). The last method was chosen to solve this problem. The commonest procedure for the preparation of an analytical calibration graph is zero-crossing.

In practice, the measurement selected as optimum is that exhibiting the best linear response, giving a zero or near zero intercept on the ordinate of the calibration graph, and less affected by the concentration of any other component. The shape of the first derivative spectra is adequate for determining E-110 in the presence of E-101 and vice versa. Figure 2 shows the first derivative absorption spectra respectively of a solution of E-110, a solution of E-101 and their mixture.

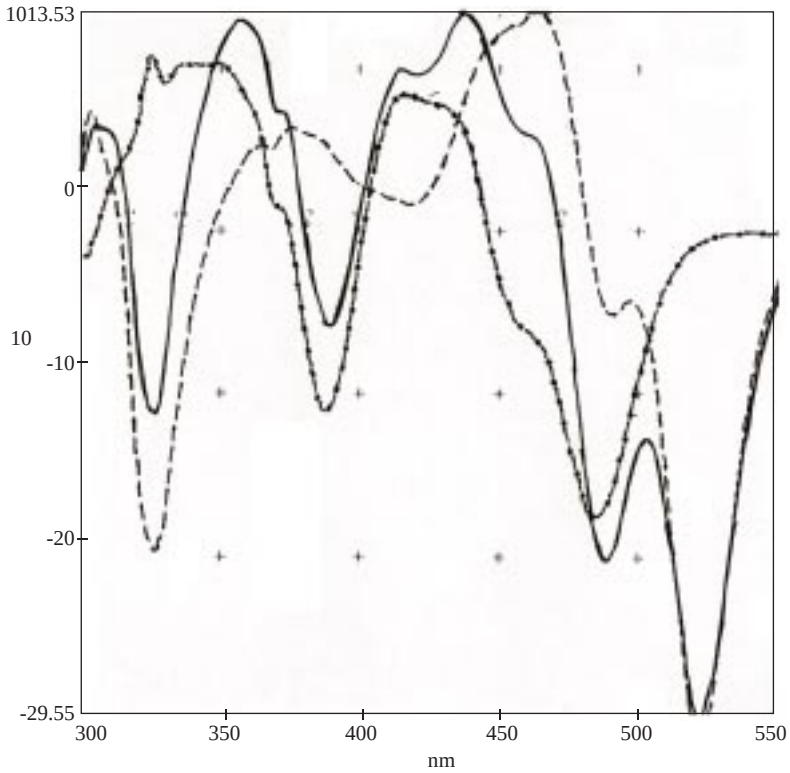


Figure 2. First derivative spectra of 20 μg.mL^{-1} riboflavine (– · – · –·), 20 μg.mL^{-1} of sunset yellow (– – ––) and their mixture (———). Reference: pH 4.5 acetate buffer.

It can be seen that, due to the overlapping spectra of these compounds in a determined region, the zero-crossing method is the most appropriate for resolving mixtures of these compounds and it was used in this work with satisfactory results.

Preliminary experiments showed that the signal of the first derivative at 481.7 nm (working zero-crossing wavelength of E-110) was proportional to the E-101 concentration, whereas the first derivative signal at 445.5 nm (working zero-crossing wavelength of E-101) was proportional to the E-110 concentration.

The main instrumental parameters that affect the shape of the derivative spectra are the wavelength scanning speed, the wavelength increment over which the derivative is obtained ($\Delta\lambda$) and the smoothing. These parameters need to be optimized in order to give a well resolved large peak, i.e. to give good selectivity and greater sensitivity in the determination. In this way, a scan speed of 500 nm.min^{-1}, a $\Delta\lambda = 2$ nm and very high smoothing parameters were selected for obtaining the first derivative spectra.

The calibration graph was tested between 5.0 and 25.0 μg.mL^{-1}of E-101 concentration at 481.7 nm for the first derivative spectra in the presence of E-110. On the other hand, the calibration graph was tested between 8.0 and 40.0 μg.mL^{-1} of the E-110 concentration in the presence of E-101 at 445.5 nm for the first derivative spectra (Figure 3). Good linearities were observed in all cases. Table 1 shows the calibration equations and regression coefficients obtained from the first derivative spectrophotometry for E-101 and E-110.

The proposed method was applied for resolving the determination of E-101 in the presence of E-110 and vice versa in artificial mixtures. The obtained results are given in Table 2. The obtained recoveries were very good in general.

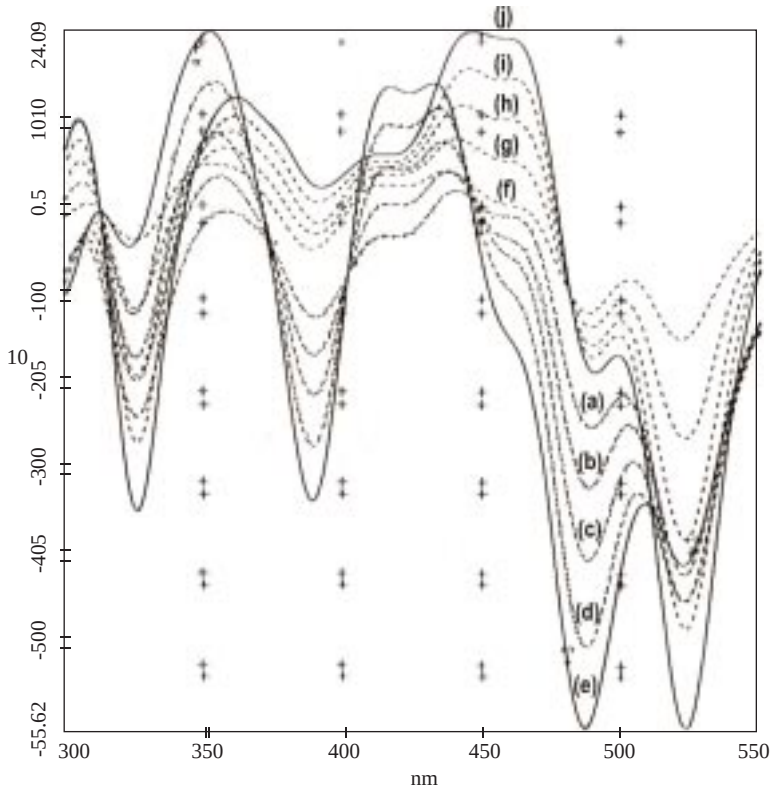


Figure 3. First derivatives for different concentrations of E-101 when E-110 was 10 μg.mL^{-1}. E-101: (a) 5, (b) 10, (c) 15, (d) 20, (e) 25 μg.mL^{-1}, and different concentrations of E-110 when E-101 was 10 μg.mL^{-1}. E-110: (f) 8, (g) 16, (h) 24, (i) 32, (j) 40 μg.mL^{-1}.

Table 1. Calibration equation for the first derivative spectrophotometry.

Equation	Regression coefficient	LOD (μg.mL^{-1})	LOQ (μg.mL^{-1})
$^1D_{481.7}$=-0.827C_{E-101}+0.0539	0.9999*	0.108	0.36
$^1D_{445.5}$=0.514C_{E-110}+0.469	0.9999*	0.267	0.89

C_{E-101}, E-101 concentration (μg.mL^{-1})
C_{E-110}, E-110 concentration (μg.mL^{-1})
*Mean for 5 determinations
LOD: Limit of detection
LOQ: Limit of quantification

Table 2. The results of the analysis of synthetic mixtures containing E-101 and E-110.

Mixtures; Nominal value (μg.mL^{-1})		Concentration Found** (μg.mL^{-1})		Recovery (%)*	
E-101	E-110	E-101 ± RSD	E-110 ± RSD	E-101	E-110
5	40	5.00 ± 2.50	40.52 ± 3.4	100	101.3
10	32	10.24 ± 3.45	32.86 ± 3.18	102.4	102.7
15	24	14.85 ± 4.05	25.11 ± 1.52	99	104.6
20	16	20.50 ± 4.29	16.61 ± 1.87	102.5	103.8
25	8	25.23 ± 1.46	8.13 ± 2.17	100.9	101.6

* Five separate work-ups were performed and the mean calculated

** Mean ± relative standard deviation (μg.mL^{-1}) for 5 determinations

The proposed method was also applied to the commercially available powder drink (Figure 4). The assay results are given in Table 3. The relative standard deviation of the method was 1.39% (n = 10) and 1.15% (n = 10) for E-101 and E-110, respectively.

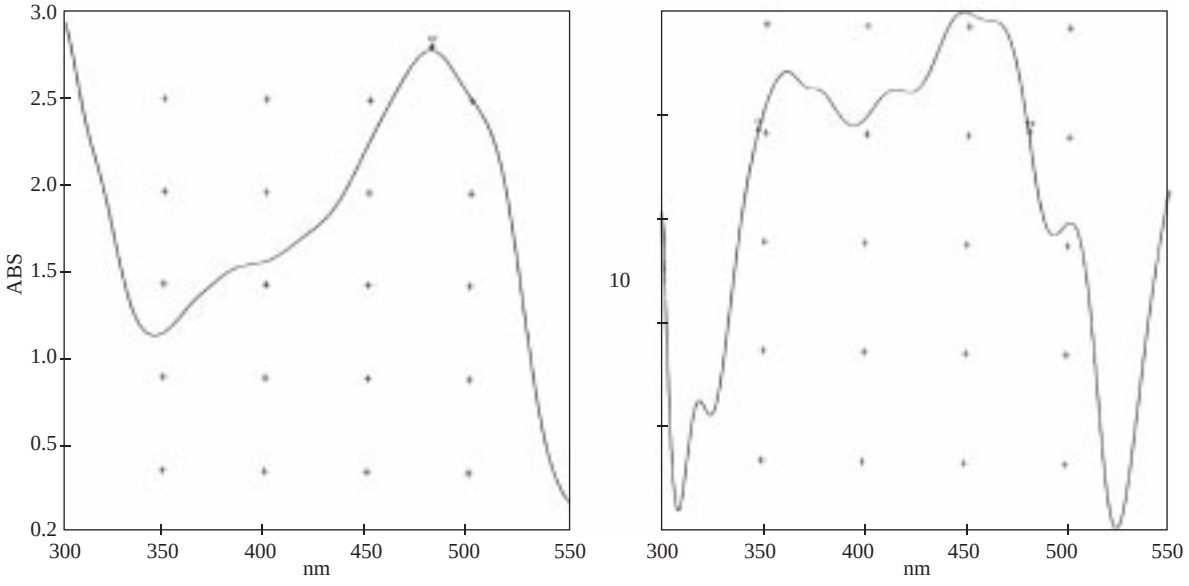


Figure 4. Absorption (a) and first derivative (b) spectra of drink powder.

Table 3. Assay results of the simultaneous determination of E-101 and E-110 in a commercial powder drink (Lezzo).

Sample	E-101 (mg/kg)	E-110 (mg/kg)
	$^1D_{481.7}$	$^1D_{445.5}$
1	12.88	114.61
2	13.22	114.35
3	12.74	113.85
4	12.96	115.95
5	13.08	116.42
6	13.12	116.37
7	12.74	115.28
8	12.68	116.93
9	13.01	117.2
10	13.01	117.95
Mean value ($\overline{X}$)	12.94	115.89
Standard deviation (SD)	0.18	1.34
RSD	1.39	1.15
$\overline{X} \pm$ t.SD/$\sqrt{n}$ P = 0.05, n = 10	12.94 ± 0.13	115.89 ± 0.96

In conclusion, the proposed method is useful for the analysis of E-110 and E-101 mixtures either in pure form or in powder drinks. Assays can be performed routinely by a simple spectral measurement after a direct extraction. Sample preconcentration, derivatization, or chromatographic separation steps, all of which serve to substantially reduce the sample turnaround time, are not part of the experimental procedure.

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