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First-derivative spectrophotometric determination of Ponceau 4R, Sunset Yellow and Tartrazine in confectionery products

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A first-derivative spectrophotometric method was developed for quantitative determination of synthetic organic dyes, Ponceau 4R (E124), Sunset Yellow (E110) Tartrazine (E102), which are used in food products under governmental regulations all over the world because of their toxicity and carcinogenity. In this study, Ponceau 4R with Sunset Yellow, and Tartrazine with Sunset Yellow, were simultaneously determined in their binary mixtures by first-derivative spectrophotometry. The method was applied to different sugar confectionery products. Recoveries were 93.8–101.2%, 92.1–107.9% and 94.9–99.2% for Ponceau 4R, Sunset Yellow and Tartrazine, respectively. The results were also compared by an independent method, thin layer chromatography (TLC). A *t*-test indicates that the differences obtained via the present methods and TLC were insignificant at the 95% confidence level. In addition, the present method is very rapid, economical and accurate. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The quality of food, other than microbiological aspects, is generally based on colour, flavour, texture and nutritive value. However, one of the most important sensory quality attributes of a food is colour. This is because no matter how nutritious, flavoursome or well-textured a food, it is unlikely to be eaten unless it has the right colour (Francis, 1985).

The use of synthetic organic colours has been recognised for many years as the most reliable and economical method of restoring something of the food's original shade to the processed product, such as in canned fruit and vegetables. Equally as important, is the use of colour in those products that have little or no natural colour present, such as mineral waters, dessert powders, table jellies, ice-lollies, sugar confectionery and pickles (Coulson, 1980).

Ponceau 4R, Sunset Yellow (FD and C Yellow No. 6) and Tartrazine (FD and C Yellow No. 5) are synthetic organic azo group food colours that can be found in common food products such as beverages, dry mix products, candies, dairy products, sugar confectioneries and bakery products (Noonan and Meggos, 1980). The structures of these colours are shown in Fig. 1.

In fact, there is no obligation to use food colours, but elimination of these additives would be the end of many food products like carbonated beverages, gelatin desserts, hard candy and many others. Consumers would not be able to decide what they could and could not eat. Therefore, this upheaval in evaluation of the need for and safety of colours and other food additives must be attributed to the science of toxicology and safety testing (Noonan and Meggos, 1980).

In order to prevent indiscriminate use, regulations have been developed by many countries limiting the types, purity, uses and amounts of food colours permitted in foods.

Further analytical methods have been developed for the qualitative and quantitative analysis of food colours. These methods include thin layer chromatography (TLC) with UV/VIS spectrophotometry (Gilhooley *et al.*, 1972; Love, 1984), column chromatography (Bell, 1990), mass spectrophotometry (Harada *et al.*, 1991), DC–SPE (Dynamic Column–Solid Phase Extraction) system (Ashkenazi *et al.*, 1991), capillary electrophoresis (Suzuki *et al.*, 1994), C_{18} cartridge (Bell, 1990) or various combinations of these techniques. Chromatographic techniques have the disadvantages of

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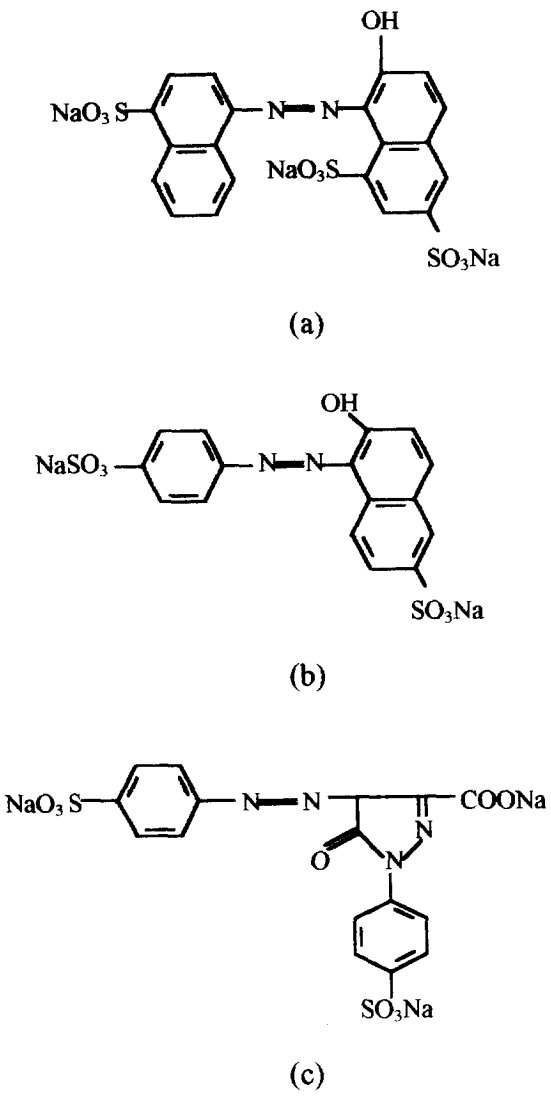

Fig. 1. Structure of (a) Ponceau 4R; (b) Sunset Yellow; and (c) Tartrazine.

requiring expensive equipment and demanding expert operators. The other methods require long analysis times and are complex techniques. Derivative spectrophotometry is an analytical technique of great utility for resolving mixtures with overlapping spectra. The fundamental principles of derivative spectrophotometry have been described by Talsky *et al.* (1978) and Owen (1987).

The aim of this study was to show the ease of the derivative spectrophotometric methods to circumvent the problem of overlapping spectral bands, allowing the simultaneous determination of the binary mixtures of Ponceau 4R–Sunset Yellow and Tartrazine–Sunset Yellow without prior separation. The method provides accurate and reproducible results in different confectionery products.

EXPERIMENTAL

Apparatus and chemicals

In this study, a Shimadzu 160 A spectrophotometer was used for all absorbance measurements. The derivative spectra were automatically obtained from the spectrophotometer. The optimum $\Delta\lambda$ value was found to be 20 nm for all the first-derivative absorption spectrums.

Ponceau 4R (P), Tartrazine (T) and Sunset Yellow (S) aqueous solutions were prepared from Sigma Chemical Product. P, T and S solution stocks had a concentration of 1000 $\mu g\,ml^{-1}$ and the working solutions had a concentration of 50 $\mu g\,ml^{-1}$.

Procedure

Procedure for the derivative methods

Standards were prepared in 25 ml volumetric flasks containing 4–20 $\mu g\,ml^{-1}$ of P, S or T or binary mixtures

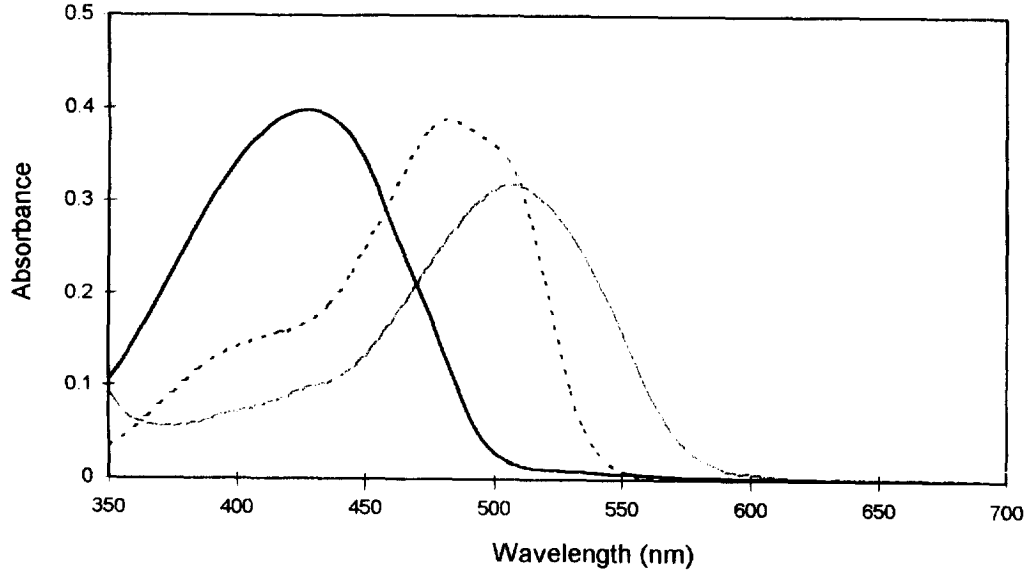

Fig. 2. Absorption spectrum of 10 $\mu g\,m^{-1}$ of Ponceau 4R (—), 10 $\mu g\,ml^{-1}$ of Sunset Yellow (- - - -) and 10 $\mu g\,ml^{-1}$ of Tartrazine (—).

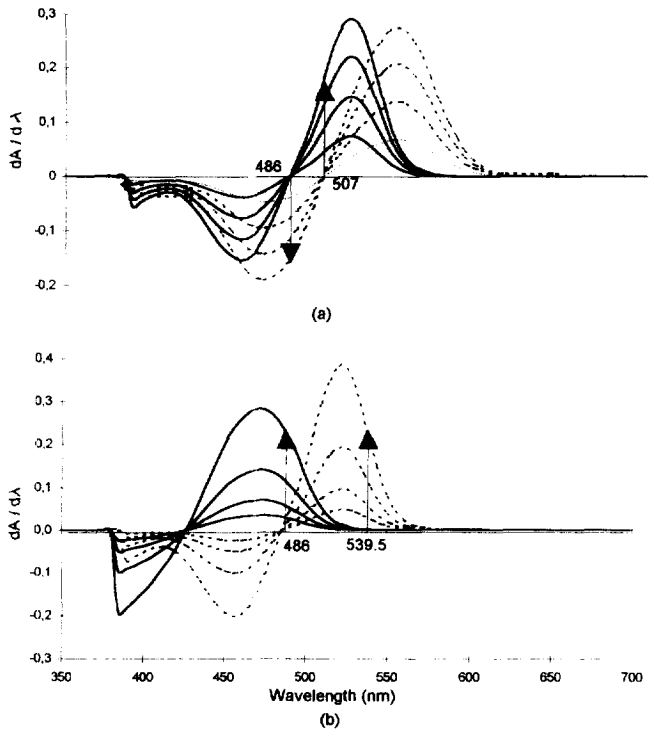

Fig. 3. (a) First-derivative spectra of 6–24 μg ml^{-1} of Ponceau 4R (----) and 2.5–10 μg ml^{-1} of Sunset Yellow (—). (b) First derivative spectra of 5–20 μg ml^{-1} of Tartrazine (—) and 3–12 μg ml^{-1} of Sunset Yellow (– – – –).

of these dyes and were diluted to volume by distilled water. Solid confectionery samples were dissolved in distilled water and prepared as 5–20 g. 100 ml^{-1}, according to dye contents.

The absorption spectra of sample solutions were recorded between 350 and 700 nm. First, the suitable derivative orders with appropriate $\Delta\lambda$ and appropriate wavelength, where one of the dyes can be analysed in the presence of the other, were determined. Then, by measuring the signal and using an appropriate calibration graph at the selected derivative order and wavelength, their concentration could be determined. These calibrations were prepared by varying the concentration of the colorant, without the presence of the other.

To quantify the recovery in confectionery samples, 10 ml of sample solution were transferred into 25 ml calibrated flasks, spiked with different amounts of P, T or S and then diluted to a volume of 25 ml. The P, T and S contents were determined from the first-derivative spectra by measuring the peak at their wavelengths, and total amounts of colorant were calculated from the calibration graph. The recoveries were calculated by dividing the total dye amount found by the present amount (dye in sample + added).

Procedure for TLC

The TLC method was applied in accordence with the method offered by Gilhooley *et al.* (1972). The method is given below.

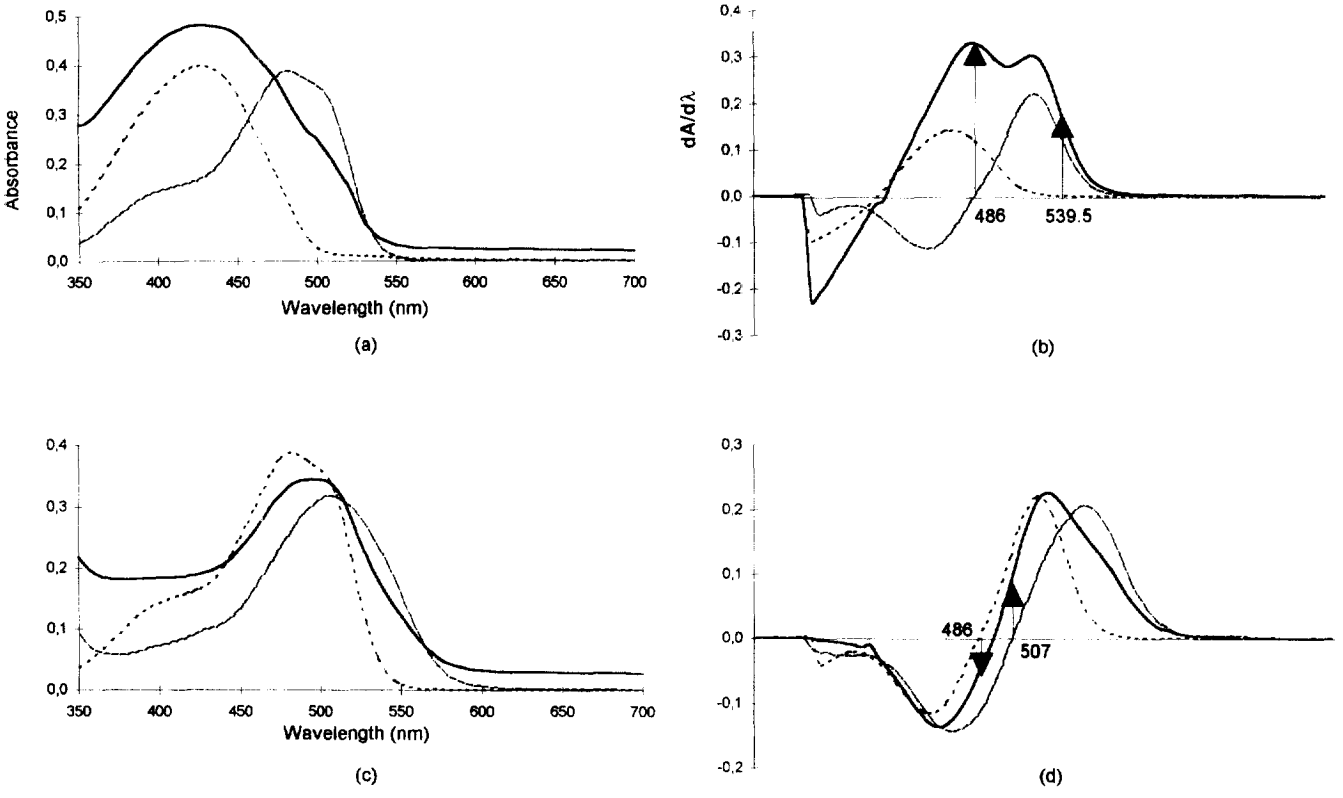

Fig. 4. (a) Absorption (b) first-derivative spectrum of 10 μg ml^{-1} of Tartrazine (----), 10 μg ml^{-1} of Sunset Yellow (—) and 6.8 g per 100 ml of sample (—). (c) Absorption, (d) first-derivative spectrum of 10 μg ml^{-1} of Sunset Yellow (– – – –), 10 μg ml^{-1} of Ponceau 4R (—) and 15.6 g per 100 ml of sample (—).

Table 1. Statistical data for calibration graphs

Regression equation	Correlation coefficient r^2 ($n=5$)
$^1D_{539.5}=0.01245\ C_S-0.00002$	0.9991
$^1D_{486}=0.0123\ C_T-0.00084$	0.9999
$^1D_{507}=0.0154\ C_S+0.00027$	0.9997
$^1D_{486}=0.0071\ C_P+0.00027$	0.9999

$^{\text{orders derivative}}D_{\text{wavelength measured}}$.
C_S: micrograms per millilitre of Sunset Yellow.
C_T: micrograms per millilitre of Tartrazine.
C_P: micrograms per millilitre of Ponceau 4R.

Table 2. Determination of the recovery for Ponceau 4R and Sunset Yellow in synthetic mixture by first-order derivative spectra

Theoretical ($\mu g\,ml^{-1}$)		Recovery (%) Ponceau 4R	Sunset Yellow
Ponceau 4R	Sunset Yellow	$^1D_{486}$	$^1D_{507}$
4	4	101.7	100.2
4	16	99.9	101.7
8	8	99.7	99.6
8	12	101.2	99.6
12	12	100.8	98.9
12	16	100.7	98.9
16	4	101.7	97.3
16	8	102.2	98.7
Average		100.9 ± 0.9	99.4 ± 1.3

Table 3. Determination of the recovery for Sunset Yellow and Tartrazine in synthetic mixture by first-order derivative spectra

Theoretical ($\mu g\,ml^{-1}$)		Recovery (%) Sunset Yellow 4R	Tartrazine
Sunset Yellow	Tartrazine	$^1D_{539.5}$	$^1D_{486}$
4	4	100.4	96.9
4	8	99.4	98.9
4	12	96.4	98.9
4	20	96.8	107.1
8	8	97.4	97.2
12	4	98.5	97.2
16	16	97.9	99.9
20	4	106.4	94.5
Average		99.2 ± 3.2	98.8 ± 3.7

Sample solutions were prepared in 70% ethanol by dissolving 10 g sample and diluting to 100 ml in a volumetric flask. Four hundred microlitres of sample solution were pipetted and spotted on the 20×20 cm Silica gel 60 plate (Merck) by repeated application of adjacent drops on line 16 cm long and dried with gentle air. The plate was placed in a tank containing isopropanol + ammonia(s.g: 0.88) + water (10:1:1) solution and developed until the solvent front reached the top of the plate. The plate was taken off the tank and dried. Adsorbents were scraped carefully from the plate and transferred to glass tubes, then 5 ml of distilled water were added and the adsorbent was removed by centrifugation at 4000 rpm followed by filtration. The concentration was found by measuring the absorbance of the solution and using an appropriate calibration graph.

Results and discussion

Determination of P, S and T in the synthetic binary mixtures

As observed in Fig. 2, the individual spectra of P, S and T overlapped, so their concentrations could not be determined by direct absorbance measurement. Therefore, derivative spectrophotometric methods were studied to assist in resolving this problem.

We have developed simultaneously determination of P and T in binary mixture by first-derivative spectrophotometry at 550 and 507 nm, respectively. The range of recovery for the P and T in three different samples of sugar confectionery products was 92.0–100.3% and 93.9–103.3%, respectively (Sayar and Özdemir, unpublished data).

The first-derivative absorption spectra of solution P and S are shown in Fig. 3(a). It can be seen that P can be determined in the presence of S at 486 nm, and S also can be determined in the presence of P at 507 nm. In Fig. 3(b), it can be seen that T can be determined in the presence of S at 486 nm, and S also can be determined in the presence of T at 539.5 nm.

The calibration graphs are obtained by using a range of concentrations of P, S and T. The statistical data obtained from calibration graphs are summarised in Table 1 and the results obtained from resolution of the synthetic binary mixtures are summarised in Tables 2 and 3. The

Table 4. Determination of the recovery for Ponceau 4R and Sunset Yellow in confectionery sample solutions

Sample	Ponceau 4R				Sunset Yellow			
	Present ($\mu g\,ml^{-1}$)	Added ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	Recovery (%)	Present ($\mu g\,ml^{-1}$)	Added ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	Recovery (%)
Flavoured sugar	3.85	2.00	5.92	101.2	3.49	2.00	5.31	96.7
	3.85	4.00	7.90	100.7	3.49	4.00	7.32	97.7
	3.85	12.00	14.87	95.8	3.49	12.00	14.85	95.8
Jelly	1.42	1.00	2.30	95.0	0.78	1.00	1.74	98.9
	1.42	2.00	3.28	95.6	0.78	2.00	2.61	93.9
	1.42	4.00	5.39	99.4	0.78	4.00	4.40	92.1

Table 5. Determination of recovery for Sunset Yellow and Tartrazine in confectionery sample solutions

Sample	Sunset Yellow				Tartrazine			
	Present ($\mu g\,ml^{-1}$)	Added ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	Recovery (%)	Present ($\mu g\,ml^{-1}$)	Added ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	Recovery (%)
Sugar candy I	0.91	1.00	1.94	101.6	4.81	4.00	8.71	98.8
	0.91	2.00	3.96	101.3	4.81	8.00	12.71	99.2
	0.91	5.00	6.38	107.9	4.81	12.00	16.44	97.8
Sugar candy II	5.68	2.00	8.02	104.4	10.67	4.00	14.56	99.2
	5.68	4.00	9.63	99.5	10.67	6.00	18.37	98.4
	5.68	12.00	18.08	102.3	10.67	16.00	25.31	94.9

Table 6. The comparison of first-derivative spectrophotometric and TLC method for determination of Ponceau 4R, Tartrazine and Sunset Yellow in sample solutions (the mean of the three replicates, $\mu g\,ml^{-1}$)

Sample	First-derivative spectrophotometric			TLC		
	Ponceau 4R	Tartrazine	Sunset Yellow	Ponceau 4R	Tartrazine	Sunset Yellow
Jelly	12.35 ± 0.16	—	7.36 ± 0.05	13.06 ± 0.47	—	7.23 ± 2.01
Sugar candy	—	16.29 ± 0.08	7.89 ± 0.11	—	17.38 ± 0.86	9.37 ± 1.4

Table 7. Total P, S and T in confectionery products (the mean of five replicates, $\mu g\,g^{-1}$)

Sample	Ponceau 4R	Sunset Yellow		Tartrazine
	$^1D_{486}$	$^1D_{507}$	$^1D_{539.5}$	$^1D_{486}$
Jelly I	56.6 ± 5.1	117.2 ± 4.1	—	–
Flavoured sugar	17.0 ± 0.8	9.3 ± 0.6	—	–
Instant fruit tea II	278.2 ± 6.5	—	—	–
Sugar candy III	—	122.0 ± 1.8	—	–
Instant fruit tea I	—	—	125.8 ± 2.0	156.2 ± 4.0
Sugar candy I	—	—	91.2 ± 6.8	163.5 ± 9.1
Sugar candy II	—	—	72.2 ± 1.9	23.4 ± 0.7
Jelly II	—	—	3.2 ± 0.2	–
Jelly III	—	—	—	33.0 ± 1.4

recoveries for determination of the binary mixtures via the first-derivative spectrophotometric method were nearly 100%.

Application

The utility of the first-derivative method was tested in four different types of confectionery samples spiked with varying amounts of P and T or S and T. The results from determination of P, S and T in food samples at the selected wavelengths (Fig. 4) are shown in Tables 4 and 5. In the cases, the recoveries obtained were generally very good. The recoveries via the first-derivative spectrophotomety were of the order 93.8–101.2% for P, 92.1–107.9% for S and 94.9–99.2% for T. These results indicate that the derivative procedure is adequate for simultaneous determination of P and S or S and T in confectionery samples.

Nevado *et al.* (1993) developed a new derivative and ratio derivative spectrophotometric method for the determination of S and T. The method was applied to four different food products and recoveries were found to be higher than 90%. Similar results were obtained in our study for S and T.

When the present method (first-derivative method) was compared with an independent method, thin layer chromatography for determination of P, S and T in confectionery samples (Table 6), a *t*-test indicates that the differences are insignificant ($P < 0.05$), but the standard deviation of the TLC method is higher than the present method.

The proposed method was also applied for the determination of P, S and T in seven confectionery samples. The results are summarised in Table 7. In all cases, the relative error was less than 10%. In conclusion, a simple first-derivative spectrophotometric method was developed for the binary determination of P, S and T in confectionery sample without previous separation. The method also provides rapid, accurate and economic analysis of these dyes.

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