

1-1-1997

Determination of Ponceau 4R and Tartrazine in Various Food Samples by Derivative Spectrophotometric Methods

Sedat SAYAR

Yüksel ÖZDEMİR

Follow this and additional works at: <https://journals.tubitak.gov.tr/chem>

 Part of the [Chemistry Commons](#)

Recommended Citation

SAYAR, Sedat and ÖZDEMİR, Yüksel (1997) "Determination of Ponceau 4R and Tartrazine in Various Food Samples by Derivative Spectrophotometric Methods," *Turkish Journal of Chemistry*. Vol. 21: No. 3, Article 5. Available at: <https://journals.tubitak.gov.tr/chem/vol21/iss3/5>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Chemistry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Determination of Ponceau 4R and Tartrazine in Various Food Samples by Derivative Spectrophotometric Methods

Sedat SAYAR, Yüksel ÖZDEMİR

*Department of Food Engineering, Faculty of Engineering,
University of Mersin 33160 Çiftlik, Mersin-TURKEY*

Received 11.7.1996

Derivative spectrophotometric methods were originally developed for determining Ponceau 4R and Tartrazine in order to resolve their binary mixtures. The method permits the simultaneous determinations of these food colorants in various food samples. Recovery by derivative methods was nearly 100 %.

Introduction

The criteria for the quality of food are, apart from microbiological aspects, 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, flavourful or well-textured a food, it is unlikely to be eaten unless it is of an appealing colour¹.

Synthetic organic food colours are substances that can be added to food in solution or dispersion. Synthetic organic colours are superior to natural dye extract in tinctorial power, consistency of strength, range and brilliance of shade, stability and ease of application^{2,3}.

In recent years, food additives in general and colours in particular have increasingly come under evaluation of their safety in use. These days in many developed countries only about ten dyes are permitted to be used as food colouring agents, many others having been banned in the last two decades because of toxicity and carcinogenicity^{5,6}. Ponceau 4R and Tartrazine are synthetic organic azo dyes that may be present in common foods (e.g. sweets, drinks, and ice-cream). The analytical control of those compounds is of considerable importance to the food industry.

Many analytical methods have been developed for the qualitative and quantitative analysis of food colour, including Thin Layer Chromatography (TLC)^{3,4,5}, UV/VIS spectrophotometry^{7,8}, mass spectrophotometry⁹, DC-SPE (Dynamic Column-Solid Phase Extraction) system⁵, capillary electrophoresis¹⁰, C₁₈ cartridge¹¹ and various combinations of these techniques. Chromatographic techniques have the disadvantages of the use of expensive equipment and the demand for expert operators. Other methods require long analysis time and are highly complex. Derivative spectrophotometry, on the other hand, is an analytical technique of great utility for resolving mixture with overlapping spectra. The fundamental principles of

derivative spectrophotometry have been described by Talsky et al.¹² and Owen¹³.

In this study, the ease of derivative spectrophotometric methods for circumventing the problem of overlapping spectral bands was shown. The method developed, allowed the simultaneous determination of Ponceau 4R and Tartrazine without prior separation in various food samples. Elimination of the turbid background that come from the samples is another advantage of this method. The method gives accurate and reproducible results.

Experimental

Apparatus and Chemicals

In this study, a Shimadzu 160 A Spectrophotometer was used for absorbance measurements. The derivative spectra were automatically obtained from the spectrophotometer.

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

Material

The samples, sugar candy, sugar-coated pills, strawberry drink, drinking powder and quince desert, were purchased from a local market.

Standards and Sample Preparation

Standard solutions for P and T for the fifth varied concentration ($4.0\text{-}20.0 \mu\text{g.ml}^{-1}$) were prepared from working solutions. Volumes of 2, 4, 6, 8 and 10 ml of working solution were transferred into a 25 ml volumetric flask and diluted to a volume of 25 ml with distilled water. According to dye contents, 5-20 g of the sample was dissolved in distilled water and the solution was transferred into a 100 ml volumetric flask and diluted to a volume of 100 ml with distilled water.

Procedure

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 could 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 was found. These calibrations were prepared by varying the concentration of the colorant, without the presence of the others.

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

Results and Discussion

Figure 1 shows the zeroth order absorption spectra of P and T in the range of 350-700 nm. P can be approximately determined by direct absorbance measurement at 507 nm, but T can not be determined because of the overlapped spectra. The use of derivative spectra can resolve this problem satisfactorily.

First, in order for one of the dyes to be determined in the presence of the other, suitable derivative orders with appropriate $\Delta\lambda$ and appropriate wavelength were decided on. The optimum $\Delta\lambda$ values were found to be 20 and 17.5 nm for the first and second derivative orders, respectively. The first derivative absorption spectra of solution P and T are shown in Figure 2. It can be seen that T can be determined in the presence of P at 507 nm, and P also can be determined in the presence of T at 550 nm.

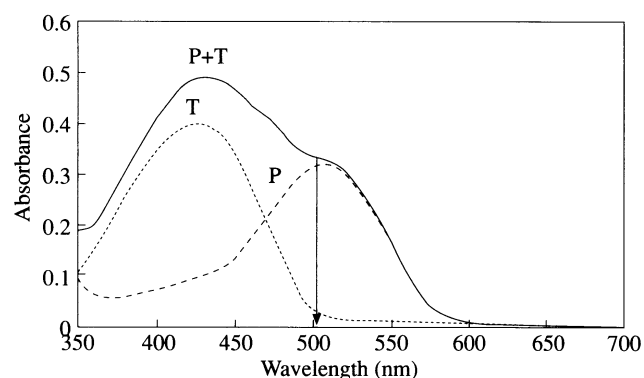


Figure 1. Adsorption spectrum of $10 \mu\text{g.ml}^{-1}$ of Ponceau 4R (P), $10 \mu\text{g.ml}^{-1}$ of Tartrazine (T) and the mixture thereof (P + T)

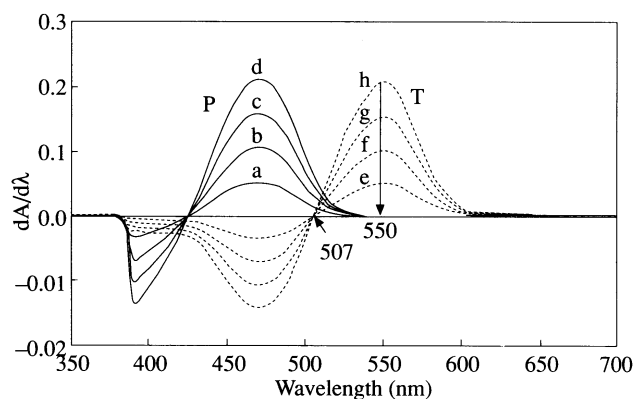


Figure 2. First - derivative spectra of Ponceau 4R (a. $4 \mu\text{g.ml}^{-1}$; b. $8 \mu\text{g.ml}^{-1}$; c. $12 \mu\text{g.ml}^{-1}$; d. $16 \mu\text{g.ml}^{-1}$) and Tartrazine (e. $4 \mu\text{g.ml}^{-1}$; f. $8 \mu\text{g.ml}^{-1}$; g. $12 \mu\text{g.ml}^{-1}$; h. $16 \mu\text{g.ml}^{-1}$)

The second derivative absorption spectra of solutions of P and T are shown in Figure 3. As seen in the figure, T also can be determined in the presence of P at 420 nm by the second derivative method.

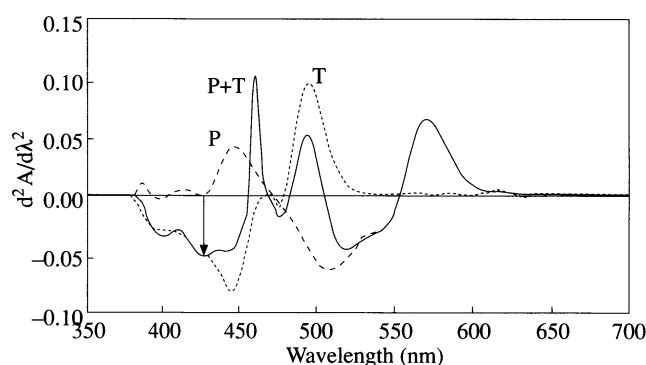


Figure 3. Second - derivative spectra of $10 \mu\text{g.ml}^{-1}$ of Ponceau 4R, $10 \mu\text{g.ml}^{-1}$ of Tartrazine and the mixture thereof

Preliminary experiments showed that the signal of the first derivative at 507 nm and the second derivative signal at 420 nm (working zero-crossing wavelengths of P) is proportional to the T concentration, whereas the first derivative signal at 550 nm (working zero-crossing wavelengths of T) is proportional to the P concentration. P and T are determined simultaneously in synthetic mixtures by measuring the signals at the selected wavelength and in the chosen derivative order. The calibration graphs were obtained by using

the range of concentrations of P and T. The regression equations and the correlation coefficient obtained from calibration graphs are given in Table 1.

The results from the simultaneous determination of P and T in synthetic binary mixtures are given in Table 2. The recoveries for the determination of P and T were nearly 100%. These results indicate that derivative spectrophotometry is suitable for the determination of P and T in different synthetic binary mixtures. However, P can be determined by direct absorbance measurement at 507 nm only at low concentration of T.

Table 1. Statistical data for calibration graphs

Regression equations	Correlation coef. r^2 (n = 5)
${}^0D_{507} = 0.0305 C_P - 0.0027$	0.9999
${}^1D_{550} = 0.0113 C_P + 0.0120$	0.9998
${}^1D_{507} = 0.0035 C_T + 0.0020$	0.9999
${}^2D_{420} = 0.0470 C_T + 0.0001$	0.9999

order derivative $D_{\text{wavelength measure, nm}}$

C_T : micrograms per millilitre of Tartrazine

C_P : micrograms per millilitre of Ponceau 4R

n : number of standards

Table 2. Determination of Ponceau 4R and Tartrazine in synthetic mixture by first and second order derivative spectra

Theoretical, $\mu\text{g. ml}^{-1}$		Rec. %			
Ponceau 4R	Tartrazine	Ponceau 4R	Tartrazine		
		${}^0D_{507}$	${}^1D_{550}$	${}^1D_{507}$	${}^2D_{420}$
20	40	100.3	99.8	100.6	95.8
8	8	109.1	98.4	103.3	100.8
12	12	103.2	97.9	97.2	97.2
8	4	102.2	99.5	93.5	101.1
4	16	116.9	101.9	101.2	102.0
8	12	107.1	100.6	99.6	95.4
4	8	112.1	103.0	103.3	100.8
16	20	104.8	101.0	100.8	102.8
Average		106.9±5.5	100.4±1.9	99.9±3.3	99.5±2.9

The utility of the method was tested on three different types of food samples to which varying amounts of P and T were added. The first and second order derivative spectra for P, T and the sugar candy sample are shown in Figure 4. The results from the determination of P and T in food samples at the selected wavelengths are shown in Table 3. It can be seen that the recoveries for P determination by direct absorbance measurement are higher than 100%. This is because of the turbid background of the samples. The recovery by the first derivative spectra is nearly 100%. This shown that the turbid background was eliminated by first derivative spectrophotometry. The recoveries for T by first and second derivative spectrophotometric methods were in the range of 93.9 to 103.9 % and 95.1 to 100.4 %, respectively.

These results indicate that derivative spectrophotometry is also suitable for the determination of P and T in different food samples.

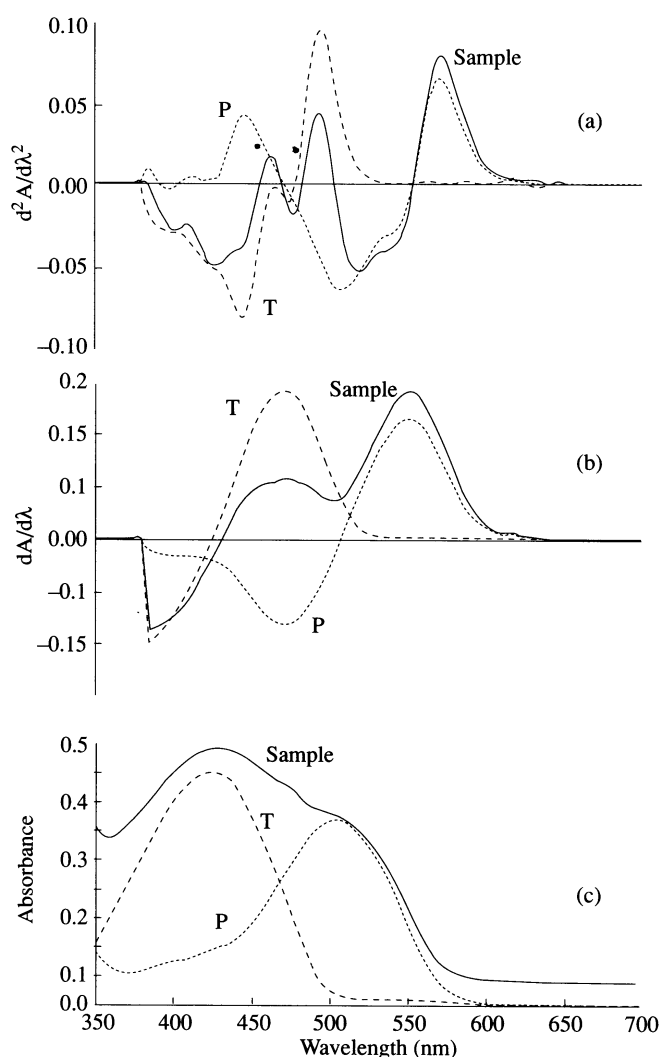


Figure 4. (a) Second, (b) First, (c) Zeroth order derivative spectra of $10 \mu\text{g.ml}^{-1}$ of Ponceau 4R, $10 \mu\text{g.ml}^{-1}$ of Tartrazine and sample (10.07 g of sugar candy dissolved in 100 ml of distilled water)

The first derivative method was also employed for the determination of P and T in six food samples. The results are summarised in Table 4. In all cases, the relative error was less than 5 %. The levels of P and T in all samples (except, P in sugar candy I and II) are higher than the limits ($100 \mu\text{g.ml}^{-1}$) set by the Turkish Food Additive Regulations¹⁴ (GKMY).

In conclusion, a simple derivative spectrophotometric method was developed for the binary determination of P and T in food samples without previous separation. The method provides rapid, accurate and economical analysis of this dyes.

Table 3. Determination of Ponceau 4R and Tartrazine in Sugar Confectionery Products

Sample	Ponceau 4R				Tartrazine			
	Total dye amount, $\mu\text{g.ml}^{-1}$ (in sample+added)		Found, $\mu\text{g.ml}^{-1}$		Total dye amount, $\mu\text{g.ml}^{-1}$ (in sample+added)		Found, $\mu\text{g.ml}^{-1}$	
	⁰ D ₅₀₇	¹ D ₅₅₀	⁰ D ₅₀₇	¹ D ₅₅₀	¹ D ₅₀₇	² D ₄₂₀	¹ D ₅₀₇	² D ₄₂₀
Sugar Candy	-	13.87	-	13.89 (100.1)*	12.54	11.21	12.51 (99.7)	11.03 (98.3)
	-	21.87	-	21.38 (97.8)	20.54	19.21	20.43 (99.5)	12.29 (100.4)
	-	29.87	-	29.98 (100.3)	28.54	27.21	28.36 (99.3)	26.28 (96.6)
Flavoured Sugar	-	10.89	-	10.40 (95.5)	10.73	10.09	10.80 (100.7)	9.60 (95.1)
	-	14.89	-	13.70 (92.0)	14.73	14.09	15.21 (103.3)	14.01 (99.4)
	-	22.89	-	21.10 (92.2)	22.73	22.09	23.10 (101.6)	21.48 (97.2)
Sugar Coated Pill	3.07	3.93	5.06 (165.0)	3.91 (99.3)	8.57	-	8.17 (95.3)	-
	5.07	5.09	7.11 (140.3)	5.10 (100.1)	9.98	-	9.37 (93.9)	-
	6.07	7.73	8.77 (144.6)	7.51 (97.1)	10.95	-	10.57 (96.5)	-

*Percent Recoveries [(Found / Total dye amount) × 100]

Table 4. Total P and T Found in Various Food Products (n = 5)

Sample	Ponceau 4R (mg.kg ⁻¹) ¹ D ₅₅₀	Tartrazine (mg.kg ⁻¹) ¹ D ₅₀₇
Sugar candy I	81.25 ± 1.06	265.8 ± 3.28
Sugar candy II	79.49 ± 1.36	116.11 ± 2.16
Sugar-coated pill	205.40 ± 0.98	208.96 ± 3.57
Strawberry drink	367.21 ± 3.03	–
Drinking powder	151.48 ± 2.27	–
Quince dessert	–	164.02 ± 5.27

Acknowledgement

This study is a part of a research project MF GM(YÖ) 95/1 funded by the Research Foundation of Mersin University.

References

1. F.J. Francis, "Pigments and Other Colorants" in **Food Chemistry**, Ed. O. R. Fennema, pp. 545-546, Marcel Dekker, Inc., New York, 1985.
2. J. Coulson, "Synthetic Organic Colours For Food" in **Development in Food Colours-1**, Ed. J. Walford, pp. 47-48, Elsevier Applied Science Publishers, London, 1980.
3. J. L. Lowe, **New Zealand Journal of Science**, **27**, 113-116 (1984).
4. G. Wadds, "Analysis of Synthetic Food Colours" in **Development in Food Colours-2**, Ed. J. Walford, pp. 24-25, Elsevier Applied Science Publishers, London, 1984.
5. P. Ashkenazi, C. Yarnitzky and M. Cais, **Analytica Chimica Acta**, **248**, 289-299 (1981).
6. FAO Food and Nutrition paper 31/1, "Specification for Identity and Purity of Food Colours", as prepared by the 28th Session of the Joint FAO/WHO Expert Committee on Food Additives, Rome, 1984.
7. J.J.B. Nevada, J.R. Flores and M.J.V. Llerena, **Analytical letters**, **27(5)**, 1009-1029 (1994).
8. J.J.B. Nevada, J.R. Flores and M.J.V. Llerena, **Talanta**, **40(9)**, 1391-1396 (1993).
9. K. Harada, K. Masuda, M. Suzuki and H. Oka, **Biological Mass Spectrophotometry**, **20**, 522-528 (1991).
10. S. Suzuki, M. Shirao, M. Aizawa, H. Nakazawa, K. Sasa and H. Sasagawa, **Journal of Chromatography A**, **680**, 541-547, (1994).
11. H. Horwitz, Editor, **Official Methods of Analysis of the Association of Official Analytical Chemists**, Washington, DC, p. 988. 13 (1980).
12. G. Talsky, L. Mayring and H. Kreuzer, **Angewandte Chemie Int. Ed.**, **17(11)**, 785-874 (1978).
13. T. Owen **International laboratory**, 58-64, October 1987.
14. Turkish Food Additive Regulations (Gıda Katkı Maddeleri Yönetmeliği), T.C. Resmi Gazete. Sayı 18097, 18381 1983/1984.