

Simultaneous Determination of Anthocyanin and Ponceau 4R in Drink Powders by Derivative Spectrophotometry and Partial Least-Squares Multivariate Spectrophotometric Calibration

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Summary. Two methods for the simultaneous determination of anthocyanin and ponceau 4R in drink powders by second derivative spectrophotometry and by partial least-squares multivariate spectrophotometric calibration are described. The procedures do not require any separation step. The methods were applied to determine both colorants in commercial gelatin powders; the results obtained are compared.

Keywords. Derivative spectrophotometry; Partial least-squares calibration; Anthocyanin; Ponceau 4R; Food analysis.

Introduction

A number of official methods have been reported for separation, identification, and determination of colorants in food products, among them extractive, chromatographic, gravimetric, titrimetric, and spectrophotometric procedures [1]. The recommended spectrophotometric procedure is based on the absorbance measurements at the wavelength corresponding to maximum of the absorption band of the colorant; accurate results are obtained only for products containing a single colorant. However, in many food products two or more colorants are present [2]. If the absorption spectra of the colorants are well resolved, the direct spectrophotometric determination of colorants is still possible, but if there is spectral overlap, a separation step has to be included in the analytical procedure.

Anthocyanin and ponceau 4R are present together in many drink powders. The absorption spectra of these colorants overlap, and direct measurements of the absorbance without a separation step are not feasible. In this paper, two methods are proposed for the simultaneous spectrophotometric determination of two colorants in

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drink powder: derivative spectrophotometry and partial least-squares (PLS-2) multivariate calibration. The use of derivative spectrophotometry for resolving overlapping spectra produces a considerable loss of accuracy and sensitivity. This problem is due to the fact that the measurements are performed at a very critical wavelength, the localization of which is sometimes difficult and whose extinction may be small. To overcome these problems, the PLS algorithm can be used. This algorithm is very flexible and gives good results for low-precision data, as compared with different multivariate calibration models [3–6].

In recent years, derivative spectrophotometry and PLS have been widely used for the determination of colorants in food products, and satisfactory results have been reported [7–10]. However, the potential of these methods for the simultaneous quantitation of anthocyanin and ponceau 4R in drink powder have not been studied in detail in the past.

Results and Discussion

In Fig. 1, the individual spectra of anthocyanin and ponceau 4R and of a mixture thereof are presented. As can be seen, the absorption spectra of these colorants

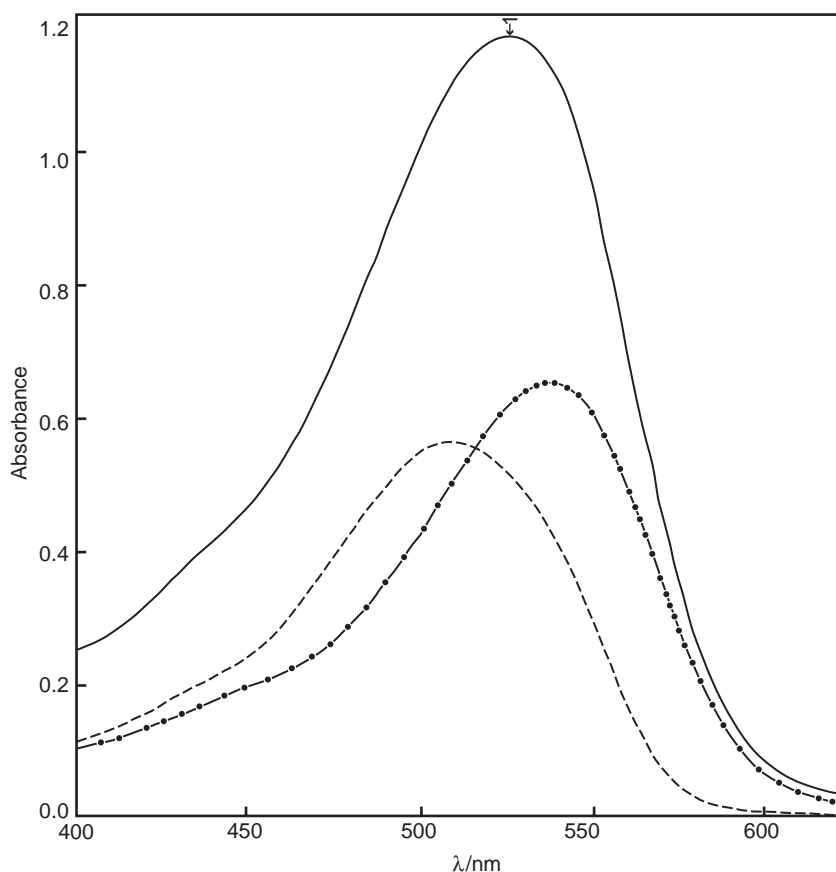


Fig. 1. Absorption spectrum of $200 \mu\text{g} \cdot \text{cm}^{-3}$ of anthocyanin (— · — · —), $20 \mu\text{g} \cdot \text{cm}^{-3}$ ponceau 4R (— — —), and a mixture thereof (——)

Table 1. Calibration equations for derivative spectrophotometry; C_A : anthocyanin concentration, C_P : ponceau 4R concentration ($\mu\text{g} \cdot \text{cm}^{-3}$)

Equation	Regression coefficient
${}^2D_{554} = 0.0019 + 0.0031 C_A$	0.9993
${}^2D_{568.8} = 0.0113 + 0.0252 C_P$	0.9998

Table 2. Results of the determination of anthocyanin and ponceau 4R in commercial drink powders

	Second derivative spectrophotometry	PLS-2
Anthocyanin ($\mu\text{g} \cdot \text{cm}^{-3}$)	80.25 ± 8.795	76.45 ± 3.49
Ponceau 4R ($\mu\text{g} \cdot \text{cm}^{-3}$)	49.34 ± 1.28	50.52 ± 2.18

greatly overlap. In such cases, the exact determination of the components is not possible for reliable direct absorbance measurements.

Table 1 shows the calibration equations and regression coefficients obtained from second derivative spectrophotometry for anthocyanin and ponceau 4R. The correlation coefficients between the true and predicted concentrations found by three-factor PLS-2 solution are 0.9990 and 0.9995 for anthocyanin and ponceau 4R, respectively. Results obtained by PLS-2 and derivative spectrophotometry using ten commercial plum drink powders are shown in Table 2. When *t*- and *F*-tests were applied to the results given in Table 2, it was found that there were no significant differences between two means and variances at the 5% level, except for the variances of the values related to anthocyanin. According to this result, PLS is more precise than derivative spectrophotometry for the determination of anthocyanin.

Conclusions

Both methods described are suitable for the simultaneous determination of anthocyanin and ponceau 4R in drink powder. However, PLS gives better precision than derivative spectrophotometry method for the determination of anthocyanin and also offers the advantage of not having the necessity of selecting critical wavelengths for measurements which causes considerable loss of selectivity and accuracy.

Experimental

Apparatus

A Philips Model PU 8740 UV/Vis scanning spectrophotometer was used for obtaining digitized absorbances. Computations were run on a Pentium 100 computer. A computer program for PLS-2 was written according to the algorithm given by *Martens* and *Naes* [6].

Reagents

All chemicals were of analytical reagent grade. The colorants anthocyanin and ponceau 4R as well as plum drink powders were supplied from Kenton A.Ş. (Istanbul, Turkey). Redistilled water was used

throughout the work. A MeOH:HCl = 95:5 mixture was found to be superior to 0.2 N acetic acid, MeOH:NH₃ = 95:5, EtOH:NH₃ = 95:5, or MeOH:acetate buffer (*pH* = 4.5) = 95:5 for the determination of anthocyanin and ponceau 4R. Stock solutions containing 1000 µg · cm⁻³ of anthocyanin and 200 µg · cm⁻³ of ponceau 4R were prepared in MeOH:HCl = 95:5 from pure samples of the colorants.

Calibration procedures

a) Second derivative spectrophotometry

The calibration samples were prepared in 10 cm³ calibrated flasks containing 50–150 µg · cm⁻³ of anthocyanin and 10–50 µg · cm⁻³ of ponceau 4R and were diluted with MeOH:HCl to the mark. After mixing, the absorption spectra of the samples thus prepared were recorded in 1 cm cuvettes. The anthocyanin content was determined from the second derivative spectrum ($\Delta\lambda = 2$ nm) by measuring the signal at 554 nm ($^2D_{554}$) (zero-crossing point for ponceau 4R) and by using an appropriate calibration graph. The ponceau 4R content was determined in the same way by measuring the signal at 568.8 nm ($^2D_{568.8}$) (zero-crossing point for anthocyanin).

b) Partial least-squares

One of the most important points with the PLS method is the design of a comprehensive set of calibration samples that comprise all the interferences possibly influencing the spectra of samples used for prediction. These include both interactions among the components to be determined and the influence of natural sample components on the spectrum of the analyte.

The calibration set was generated by a 3-level full factorial design [11], and 9 samples were used to construct the model. The levels selected for anthocyanin were 60, 75, and 90 µg · cm⁻³, and those for ponceau 4R 40, 50, and 60 µg · cm⁻³ in this design. By this choice, possible interactions could be accounted for. The absorbances were recorded between 400 and 625 nm every 5 nm. In this way, a signal concentration matrix which could be subjected to data analysis was obtained. The calibration samples were measured in random order to compensate experimental errors due to drift.

Procedure for gelatin powders

An accurately weighted amount of 35 g drink powder was transferred to 50 cm³ volumetric flask. The flask was filled up with MeOH:HCl = 95:5 and agitated for 10 min. The solution was filtered and, the filtrate was diluted to 50 cm³ with the above mixture. The absorbance of this solution was recorded between 400 and 625 nm every 5 nm for PLS analysis of anthocyanin and ponceau 4R.

The anthocyanin content was determined from the second derivative spectrum by measuring the signal at 554 nm; the ponceau 4R content was obtained in the same way utilizing the signal at 568.8 nm.

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Received September 7, 2000. Accepted (revised) November 20, 2000