

SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF MIXTURES OF FOOD COLORANTS

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ABSTRACT

Mixtures of food colorants, containing new cocchine, ponceau 6R and scarlet GN were simultaneously analyzed by spectrophotometry without previous chemical separation. Sixty mixtures of colorants with three-components were evaluated and the spectrograms were smoothed through the use of seven experimental points. The multivariate data consisted of normal, first- and second-derivative absorbance spectra ($\Delta\lambda=5$ nm) registered from 300-650 nm. The data obtained from experiments were processed by PLS method and the proposed method was applied satisfactorily for determination of these colorants in two commercial food products.

Keywords: Colorants, Chemometrics, Derivative technique, Spectrophotometry

INTRODUCTION

Synthetic food colorants such as new cocchine, ponceau 6R, and scarlet GN normally contain azo and aromatic rings and are often used for dyeing foods, drinks, medicine and cosmetics. During the fifties, it was found that these dyes are somewhat harmful to people's health (1). In most countries their uses are strictly limited and, it is important to detect and to analyze them in different products. Generally, spectrophotometry is used for assay of these colorants and due to serious spectral overlapping a prior separation is usually needed (2). Process of chemical separation is most often time consuming and unsuccessful, and the analysis that requires little or no separation is much in demand. In order to avoid the time consuming clean-up procedures, attempts have been made to resolve complex spectra using instrumental approaches and various chemometric methods. Derivatisation techniques (3-5) and multivariate statistical analysis (6-9), such as multiple linear regression (MLR), principal component regression (PCR), partial least squares (PLS), and Kalman filter (10-11), have been successfully applied to the multi-component analysis of mixtures by ultraviolet visible molecular absorption spectrophotometry. In calibration phase of these chemometrics methods by developing a mathematical model that relates the multiple

spectral intensities from many calibration samples, it is possible to predict the analyte contents in the unknown sample. Efficiency of different multivariate calibration models have been compared and have been discussed in many articles (7-8). However it can be resumed that PLS algorithm, although more complicated, has a stronger tendency to overfit in comparison with PCR. Nevertheless, PLS algorithm is very flexible and for low precision data can give better results (8). Derivative ratio spectrum method (12-13), branch and bound algorithm (14) also have been successfully introduced for the determination of mixtures of colorants with three or more component combinations.

In recent years, a combination of derivative techniques and multivariate statistical methods have been proposed and the convenience of such approaches have been evaluated by several spectrophotometric methods (15-17). The intent of the present work was to apply derivative techniques in partial least squares (PLS) method for simultaneous determination of these colorants in a mixture and to compare quantitative prediction abilities of these techniques.

MATERIALS AND METHODS

Apparatus

A UV visible cintra 40 spectrophotometer interfaced to a IBM compatible pentium 100

computer was used. The pH of the solutions were measured with a model 713 pH meter (Metrohm, Switzerland).

For statistical calculations the version of 4.2 of matlab program (Math works Inc) was used.

Reagents

All chemicals were of analytical reagents. New coccine (CI 16255), ponceau 6R (CI 16290) and scarlet GN (CI 14815) were obtained from Institute of Standard of Industrial Research of Iran. Stock solutions of new coccine, ponceau 6R and scarlet GN were prepared at a concentration of 1 mg/ml in a buffer solutions of acetic acid and sodium acetate with pH of 4.5.

Calibration procedure

For calibration procedure suitable amounts of stock solutions were pipeted into a 10 ml volumetric flask and after addition of 2.0ml of acetate buffer solution (pH 4.5) the volume was adjusted to 10 ml with distilled water. The concentration range in standard samples (matrice C) were 1.0-20.0 μgml^{-1} for the new coccine, ponceau 6R and scarlet GN respectively. The concentration ranges were chosen in such a way that the absorbances for all standard samples were 1.5.

The absorbance of these standard mixtures (matrice A) were measured in 10mm cuvettes at wave length range of 300-650 nm with 0.427 nm intervals in comparison with a blank of distilled water containing 2.0ml of buffer solution (pH 4.5). PLS method was applied by using the normal, first- and second-derivative absorbance spectra to predict the spectra of the unknown samples and calculate the concentration of each colorant in the mixture.

Data processing using PLS method

PLS was carried out by simultaneous decomposition of the matrices A and C, and the following equations were obtained.

$$C=UQ', \quad A=TP'$$

Where U and T were the respective score matrices of C and A and Q and P were their loading matrices. When the score matrices U and T were related, the following equation was obtained: $U=TB$, Where B is the diagonal matrix of the regression coefficients for the inner relations. The matrix B was used in prediction step. Estimation of the unknown concentration from the absorbance

spectra(a) of the unknown sample was performed as follow: $c^{\circ} = a^{\circ} (U'A)' BQ$

Since the matrices U', Q and A are known from calibration, c° could be estimated. Data processing were used to obtain the derivative technique:

The obtained spectra were smoothed through the use of seven experimental points and first and second derivative absorption spectra were calculated ($\delta=5\text{nm}$) using the Savitsky Golay procedure (18). The PLS algorithm was used for the resolution of mixtures using the normal spectra and first- and second-derivative. The calibration models obtained were validated using independent set of the test samples. The composition of the test samples were random, but all concentrations were in the range of calibration step.

Determination of colorants in candies

Candies were grounded with a mortar and pestle and were dissolved in distilled water (1gr/50ml) by gentle warming. The pH of this solution was then adjusted to 4.5 by addition of 20% citric acid.

Determination of colorants in beverages

Fifty milliliter of the sample was transferred to a 100 ml flask, boiled and stirred continuously to remove the carbon dioxide. The pH of this sample was then adjusted to 4.5 by addition of 20% citric acid.

RESULTS and DISCUSSION

New coccine, ponceau 6R and Scarlet GN are all highly absorbing substances in the visible region. Absorbance and first- and second- derivative spectra for a solution of three pure colorant are shown in Fig 1 a,b and c, respectively. Because of their highly overlapping peaks conventional spectrophotometry can not be applied satisfactorily for the quantitative analysis. In addition, the overlapping of their first- and second-derivative spectra do not permit the mixture to be resolved by the zero- crossing technique. Multivariate calibrations are useful on spectral analyses since simultaneous inclusion of multiple spectral intensities can greatly improve the precision and predictive ability of Partial least squares (PLS) method. The first step in the simultaneous determination of different colorants by PLS methodology involves construction of the calibration matrix.

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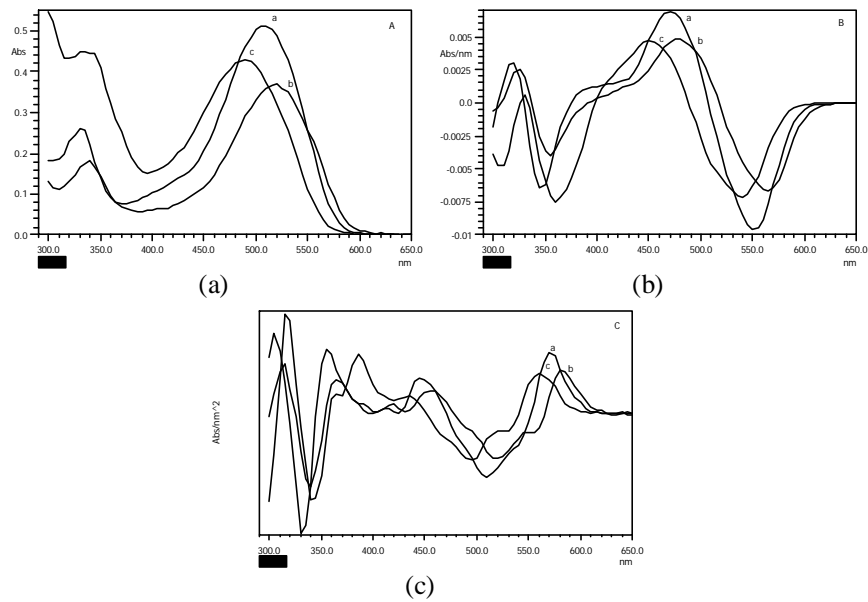


Fig.1. Absorbance spectral profiles for the three food colorant solutions: a, normal absorbance data; b, first-derivative data and c, second derivative data. (a=new cocchine, b=ponceau 6R, c=scarlet GN)

A number of 40 mixtures were selected as the calibration set. Their compositions were randomly designed in order to obtain more information from the calibration procedure. Under these conditions, the calibration models were obtained using the normal, first and second derivative absorbance spectra. The obtained models were validated with a 20 synthetic mixture set containing the considered colorants in different proportions that were randomly selected. To select the number of factors in the PLS algorithm, a cross validation method, leaving out one sample at a time, was employed (7). The prediction error was calculated for each colorant in the prediction samples, which were not participating in the construction of the model. This error was expressed as the sum of square of prediction residual error (PRESS). PRESS was calculated for the first variables, which built the PLS modeling in the calibration step. After that, another latent variable was added for the model building and the PRESS was calculated again. These calculations were repeated for one to 40 latent variables, which were used in the PLS modeling and was performed for each element using calibration models based on the normal, first and second derivative absorbance spectra. For finding the smallest model (fewest number of factors), the F-statistic was used to carry out

the significance of the determination (7). The PLS modeling for each element had a different number of factors. The optimal number of factors for each calibration model (normal absorbance spectra and first- and second-derivative absorbance spectra) was obtained and as it can be observed, the optimum number of factors were generally greater than the number of components in the mixtures for PLS methods. The prediction error of a single component in the mixture was calculated as the relative standard error (R.S.E.) of the prediction concentration (19,20):

$$R.S.E.(%) = 100 * (\hat{O}_{j=1}^N (C_j - j)^2 / \hat{O}_{j=1}^N (C_j)^2)^{0.5}$$

Where C_j was the concentration of the component in the j th matrix and j the estimated concentration. The total prediction error of N samples is calculated as follows:

$$R.S.E.t(%) = 100 * (\hat{O}_{i=1}^M \hat{O}_{j=1}^N (C_{ij} - i_j)^2 / \hat{O}_{i=1}^M \hat{O}_{j=1}^N (C_{ij})^2)^{0.5}$$

Where C_{ij} is the concentration of the i th component in the j th sample and i_j is its estimation. Table 1 shows reasonable single and total relative errors for such a system. As it could be found from Table 1 calibration based on normal absorbance data, improve the precision of the techniques. Recovery of the system was calculated by comparing the

composition of synthetic samples and their prediction by PLS model based on normal absorbance data. The results are shown in

Table 2. In some papers the “derivative of ratio spectrum method” for determination of mixed food dyes has been proposed (12,13).

Table1. Relative error of prediction values for colorants by PLS

Content	PLS		
	Normal	First derivative	Second derivative
New Coccine	2.0714	1.9546	9.1098
Ponceau 6R	3.2381	3.1788	13.2425
Scarlet GN	2.9716	4.1128	9.2408
R.S.E(total)	2.86735	3.2848	10.8958

Table2. Composition of synthetic samples, their prediction by PLS model and statistical parameters for a three component (a=New coccine, b=Ponceau6R, c=Scarlet GN)

Synthetic(μgml^{-1})			Prediction (μgml^{-1})			Recovery(%)		
A	b	c	a	b	c	a	b	c
06	11	14	6.02	11.06	13.54	100.30	100.55	96.73
02	14	12	2.20	12.77	10.56	109.95	91.23	87.99
15	03	19	15.17	2.90	18.65	101.12	96.82	98.14
14	09	01	14.31	8.94	0.65	102.20	99.38	65.32
05	20	12	4.98	19.74	12.15	99.62	98.68	101.28
01	16	05	0.96	16.41	4.88	96.22	102.56	97.58
15	13	16	15.37	13.19	15.96	102.45	101.47	99.78
05	16	08	5.22	15.89	7.77	104.42	99.31	97.08
20	14	08	20.39	13.69	8.23	101.93	97.79	102.84
17	12	08	17.18	11.94	8.13	101.07	99.50	101.62
15	20	18	14.54	20.54	17.94	96.91	102.71	99.69
05	18	11	5.07	18.15	10.54	101.32	100.82	95.80
14	10	17	13.75	10.63	17.09	98.20	106.31	100.51
13	12	18	13.04	12.01	17.89	100.33	100.07	99.41
04	03	13	4.14	3.50	13.47	103.49	116.79	103.63
02	14	16	2.00	14.09	15.54	99.75	100.64	97.10
00	03	05	0.12	2.96	5.88		98.59	117.56
00	04	08	-0.06	4.04	8.56		101.02	107.00
01	03	05	0.99	3.41	5.57	99.31	113.69	111.41
14	10	02	13.91	10.33	2.40	99.36	103.32	120.25
Mean	Recovery					101.00	101.56	100.04

Table 3. Determination of colorants in food samples by absorbtion spectra and PLS method

Food sample	concentration ^{a, b}					
	New coccine		ponceau 6R		scarlet GN	
	result	claimed	result	claimed	result	claimed
Beverage	8.45	8.5±0.18	13.71	14±0.07	ND	0.00
Candy	ND	0.00	16.21	16.5±0.05	1.82	2.00±0.21

^aThe values are given in $\mu\text{g/ml}$ for soft drink and mg/kg for candy, ^bResults are average of the three determination, and claimed values are stated by the manufactures, ^c not detected.

Their method is based on the measurements of signal greatest sensitivity usually a maximum or a minimum in the derivative ratio spectra, and they calibrated each two of dyes in ternary mixtures by the use of the spectrum of the third colorant as divisor. As a result their calibration

procedures were difficult since the system had to be calibrated three times. Another paper (14) also proposed the branch and bound algorithm for determination of six dyes. They claimed that in this method compared with others, it is not required to know the number and spice of

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pigments in samples. However their method is difficult. The PLS algorithm proposed in this paper is rapid and interference free spectrophotometric determination of three food dyes in beverages and candies. Our results (R.S.E=2.87% recovery in the range of 96-109%) clearly confirm that some interferences occurring in spectrophotometric determinations can be eliminated by the PLS algorithm.

Application to food samples

The analytical results of food samples (beverage and candies) obtained by PLS with absorbance spectra are listed in Table 3. As can be seen, results are quite acceptable and are consistent with those of the nominal contents provided by the manufacturers (when determined by chromatography). Compared with synthetic mixtures, food samples are more complex

therefore, serious background due to the matrix effect will generally appear in spectrophotometric measurement.

CONCLUSION

The most important characteristics of this work are: (i) the possibility of simultaneous determination of new coccine, ponceau 6R and scarlet GN in aqueous solution. (ii) application of proposed method for the analyses of real sample; ie. candy and beverage. (iii) No extraction step is required and hence the use of organic solvents is avoided. (iv) the proposed method is very suitable for simultaneous determination of colorants in complex synthetic samples. (v) derivative techniques are compared for the analyses of mixtures with strong signal overlapping.

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