Simultaneous Determination of Allura Red, Brilliant Blue and Beta-Carotene in Sugar by Statistical Methods

Güzide Pekcan Ertokuş[*]

Department of Chemistry, Faculty of Science & Art, Süleyman Demirel University, Isparta, Turkey
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ABSTRACT

Allura red, brilliant blue and β-carotene are color substances used in foods. In this study, allura red, brilliant blue and β-carotene were simultaneously determined without any chemical pre-separation by the spectrophotometric method. Chemometric methods have evaluated the obtained experimental data. In the first step, the synthetic mixtures containing a three-color material were examined, and the obtained results were applied to the principle component regression-PCR and partial least squares -PLS. In the next step, using PLS and PCR methods, the quantities of allura red, β-carotene and brilliant blue in commercial beverage samples were measured at the same time. A good fit was found between PLS and PCR methods calculated from experimental data and calibration methods when the results were compared statistically. As a result of the determination, high recoveries and low standard deviations were found. For absorbance and concentration values were used in Minitab and other chemometric programs to calculate estimated concentrations with PCR and PLS.

Keywords: Food colorants; Partial least squares regression (PLS); Principal component regression (PCR)
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INTRODUCTION

Today food colorants are added in appropriate amounts to foods and drugs to correct the color of a product or to make it more attractive [1]. The role of food coloring in the food industry is significant [2]. However, when some synthetic colorants are consumed, especially in excessive amounts, they can have pathological effects. Therefore, the use of the dyes must be controlled according to the laws and regulations. Because of this, the analysis is quite important for synthetic colorants [3]. The spectrophotometric method may utilize for the definition of food coloring and processing pre-separation. However, chemical separation is time-consuming and may sometimes fail. To correct this situation, chemometric methods have been developed [4]. Instrumental methods such as spectroscopy, chromatography and quantitative analysis have become full applications of chemometric methods, and are used even in overlapping spectra [5]. Such chemometric methods as PLS and PCR have been implemented in the complex of drug and food mixture successfully [6]. Chemometric methods have been performed in the analysis of many complex mixtures of any pre-separation on the applied numerical methods [7]. A relationship to be established between matrices of chemical data is determinated at chemometric methods [8]. In the first step, chemometric techniques are applied to a known series of concentrations of the calibration sample. At this stage, the traditional mathematical model is used under the same experimental conditions to the unknown samples in the second stage [9]. The purpose of this study was to develop an accurate method for allura red, brilliant blue, and β-carotene in ternary mixtures of any pre-separation before analysis before the advanced method was performed to determine the content of various foods in commercial sugar sample. Mean recoveries (%) and standard deviation of principal component regression (PCR) and partial least squares regression (PLS) methods were calculated for the validation of the methods. The acquired results were statistically compared each other.

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EXPERIMENTAL SECTION

Chemicals and Reagents
Analytical reagent grade chemicals were used unless otherwise indicated. Allura red, brilliant blue and β-carotene are synthetic food colorants. Water, with conductivity lower than 0.05 _ S cm¹, was obtained with a Milli-Q water purification system (Millipore Corp.). Stock solutions of the food colorants, 10 mg/100 mL of each, were prepared in deionized water. Absorbance measurements were taken using Pharma 1700 Spectrophotometer/SHIMADZU.

Absorbance Measurements
Absorption spectra were noted between 420 and 640 nm at intervals of 0.1 nm. The spectra were giving 5500 experimental points for each spectrum. The calibration matrix and validation set were prepared with respectively 24 and 10 solvents containing mixtures of three components in variant rates andoptimized and computed by using PCR and PLS calibrations, both to analyze the spectra obtained and to calculate the concentration of the analytes in the real samples.

Procedure
An aliquot of the sample containing between 0.4 and 20 (mg/mL) of the colorants in the mixture was put into a 25 mL volumetric flask, and deionized water was put in. The blend was mixed for 20 min and placed in a 1 nm cell. Blanks were made ready in the same way as described for the standards and contained all the reagents except the colorants.

Procedure for real sample:
For this purpose, a sugar sample was crushed in a mortar, and this sugar powder was dissolved in distilled water in a beaker. The sample was transposed to a 25 mL calibrated flask and dissolved in water.

Chemometric Methods
In this study; partial least squares (PLS) and principal component regression (PCR) was used as chemometric methods. PLS [10,11] and PCR [12,13] are multivariate calibration methods which have many of the full spectra avantages and have been successfully applied to the spectrophotometric analysis of multicomponent mixtures. PLS method is based on spectral variations without regard for the component concentrations. In PLS method is the spectral decomposition is weighted to the concentration. The significant difference in predictive abilities of these two approaches, in that PLS seems to predict better than PCR methods [14].
The Minitab 17 program (Inova, Ankara, Turkey) was used for the analysis of all the concentration and absorbance data and to do the statistical calculations. Minitab is statistical analysis software. In addition to statistical research, statistics can be used to learn [15].

RESULTS AND DISCUSSIONS

While the definition of food color substances is difficult using conventional spectrophotometric methods, the two chemometric methods were calculated, and these methods were compared with each other. In the chemometric techniques, the concentrated solution was passingly equipped by ternary mixtures. The absorbance data were got by gauging 12 points (from 420 to 640 nm) with spacings of Δλ: 20 nm in the absorbance spectrum from 420 to 640 nm as shown in Figure 1.
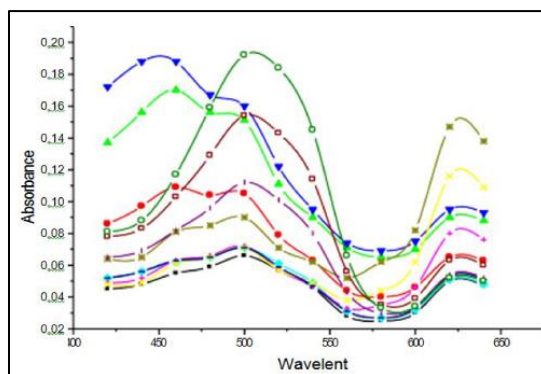

Figure 1: UV-VIS spectra of synthetic mixtures

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The obtained values were computed using Panorama (Lab Cognition) and Minitab 17 software in chemometric methods. For two chemometric techniques (PCR and PLS), the absorbance data matrix for the concentration matrix was acquired by the measurement of absorbance between 420 and 640 nm in the intermissions with Δλ: 0.1 nm at 11 wavelengths in the absorption spectra. In the used chemometric methods, calibration or regression was acquired by using the absorption data matrix for prediction of the unknown concentrations of allura red, brilliant blue, and β-carotene in their ternary mixtures. A concentration set design of allura red (ALL), brilliant blue (BRI), and β-carotene (CAR) combinations as shown Table 1.

Table 1: Concentration set of food compounds for the preparation of the PCR and PLS calibration (mg mL^{-1})

	Concentration (mg mL^{-1})		
No	ALL	BRI	CAR
1	0.4	0.4	4
2	0.4	0.8	8
3	0.4	1.2	12
4	0.4	1.6	16
5	0.4	2	20
6	0.8	0.4	12
7	1.6	0.4	4
8	2.4	0.4	4
9	3.2	0.4	4
10	4	0.4	4
11	0.8	0.4	16
12	0.8	0.8	4
13	0.8	1.2	4
14	0.8	1.6	4
15	0.8	2	4
16	0.8	1.2	4
17	1.6	0.4	8
18	2.4	0.4	12
19	3.2	0.4	16
20	4	0.4	20
21	0.8	0.4	8
22	0.8	0.4	12
23	0.8	0.4	16
24	0.8	0.4	20

The application capability of a calibration model can be clarified in different ways. The predicted residual error sum- of-squares or PRESS is used for explaining the calibration model. The predicted and actual compositions of the samples are compared, and the prediction residual error sum of squares (PRESS) is computed.

$$PRESS = \sum_{i=1}^{n}(C_i^{added} - C_i^{found})^2 \qquad (1)$$

C_i^{added} : Actual Concentration, the joined concentration of colorants

C_i^{found} : Prediction Concentration is the situated concentration of colorants

It is important to emphasize that this is not a correct way to normalize the PRESS values when not all of the data sets contain the same number of samples. PRESS values are close to zero, the degree of accuracy is increasing. The obtained PRESS values are close to zero for PLSR and PCR methods.

The RMSEC can maintain how good a measure of the efficiency of the calibration model. According to the actual and the predicted concentration in samples, RMSEC and PRESS values of allura red and brilliant blue were calculated for the colorants in Table 2.

The root mean square error of cross-validation-RMSEC was calculated for each method as follows; RMSEC =

$$(PRESS/n)^{1/2} \qquad (2)$$

n: the number of predicted samples.

Table 2: Statistical parameter values for calibration step- simultaneous determination of allura red, brilliant blue and β-carotene using chemometric methods

Parameter	Method	Compound		
		ALL	**BRI**	**CAR**
RMSEC	PLS	0.0011	0.0023	0.0012
	PCR	0.0009	0.0018	0.0014
PRESS	PLS	0.00003	0.0001	0.000001
	PCR	0.00015	0.0024	0.000097
Regression Coefficient	PLS	1	1	1
	PCR	0.9998	0.9995	0.9987
Intercept	PLS	0.001	0.0021	0.0012
	PCR	0.0022	0.0049	0.0023
Slope	PLS	1	1.0007	1
	PCR	0.9997	0.9993	0.9989

Regression coefficients for the two techniques, their residuals, and predicted concentration are shown in Table 3. To test the recommended techniques, the sets of synthetic mixtures containing the three colorants were prepared with variable compositions.

Table 3: Composition of prediction set and recovery results obtained in synthetic mixtures for PLS and PCR methods

Sample no.	Concentration (mg mL-1)			% Recovery					
				PLS			PCR		
	ALL	**BRI**	**CAR**	**ALL**	**BRI**	**CAR**	**ALL**	**BRI**	**CAR**
1	0.8	0.4	4	99.96	100.13	100	99.94	100.3	99.98
2	0.8	0.4	8	99.95	100.85	100	99.81	98.99	100.01
3	0.8	0.4	12	100.31	99.83	99.97	100.28	100	100.01
4	0.8	0.4	16	99.98	100.53	100.01	99.85	100.1	99.98
5	0.8	0.8	4	99.89	98.9	100.18	100.11	99.98	100.01
6	0.8	1.2	4	98.86	99.98	100.01	99.83	99.33	100.01
7	0.8	1.6	4	100.06	100.26	100	99.86	100.03	100.01
8	1.6	0.4	4	100.52	101	99.96	99.87	100.23	99.96
9	2.4	0.4	4	99.96	99.95	99.99	100.01	100.15	99.97
10	3.2	0.4	4	99.98	100.4	99.98	99.98	99.99	99.96
Mean				99.95	100.18	100.01	99.95	99.91	99.99
SD				0.407	0.561	0.059	0.141	0.396	0.021

Set of synthetic mixtures of the colorants was analyzed by principal component regression. According to the results, to check against PRESS values for data sets that contain differing numbers of samples, it was converted to the standard error of prediction (SEP).

$$SEP = \sqrt{\frac{\sum_{i=1}^{n}(C_i^{added} - C_i^{found})^2}{n-1}} \qquad (3)$$

C_i^{added} : Actual Concentration
C_i^{found} : Prediction Concentration

The SEP, on average, the performance of the calibration models is a good measure. According to the actual and the predicted concentration in samples; SEP values of allura red, brilliant blue and beta-carotene were calculated for the colorants in Table 4.

Table 4: Statistical parameter values for prediction step- simultaneous determination of food colorants using chemometric methods

Parameter	Method	Allura Red	Brilliant Blue	Beta- carotene
SEP	PLS	0.00484	0.00347	0.00412
	PCR	0.00111	0.0025	0.00398
Regression Coefficient	PLS	1	0.9999	1
	PCR	1	1	1
Intercept	PLS	0.0008	0.0003	0.0033
	PCR	0.0001	0.0014	0.0022
Slope	PLS PCR	1	1.0002	0.9996
		0.9999	0.9971	1.0001

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By using the PCR algorithm, the following equations were obtained;

$C_{allura\ red}$ = - 0,0884 - 40,2 A_1 + 13,3 A_2 + 56,9 A_3 - 99,8 A_4 + 18,6 CA_5 + 67,8 A_6 - 33,3 A_7 - 45,5 A_8 + 117 A_9 - 60,3 A_{10} + 27,4 A_{11}

$C_{brilliant\ blue}$ = 1,12 + 119 A_1 + 400 A_2 - 1181 A_3 + 1132 A_4 + 229 A_5 - 759 A_6 + 198 A_7 + 553 A_8 - 1432 A_9 + 402 A_{10} + 96,9 A_{11}

$C_{beta-karoten}$ = 0,930 - 674 A_1 + 1123 A_2 - 666 A_3 + 1069 A_4 - 665 A_5 + 159 A_6 - 756 A_7 + 1674 A_8 - 2746 A_9- 472 A_{10} + 1558 A_{11}

Where, $C_{allura\ red,}$ $C_{brilliant\ blue}$, and $C_{beta-karoten}$ are the concentrations of food colorants and the measured absorbance values for the solutions introduced into the equation. In conclusion, the composition of each component in the sugar sample was successfully defined.

The standard of allura red, brilliant blue and beta-carotene were added to the product solution for testing the selectivity of the applied methods (standard addition method). When the obtained results were compared with expected results, the recoveries were obtained excellent values (Table 5).

Table 5: Recovery results obtained standard addition technique using the proposed PLS and PCR methods

Sample No.	Composition (mg mL^{-1})			% Recovery					
				PLS			PCR		
	ALL	BRI	CAR	ALL	BRI	CAR	ALL	BRI	CAR
1	0.8	0.4	4	101	100.06	100	99.98	99.89	98.96
2	0.8	1.2	12	100.11	99.85	100.22	99.95	99.93	99.88
3	1.6	0.4	0.4	100.08	99.93	100.2	99.94	99.96	99.87
Mean				100.4	99.95	100.14	99.96	99.93	99.57
SD				0.52	0.106	0.121	0.021	0.035	0.528

Commercial Product Analysis

The experimental results of two chemometric methods to sugar product in this work are shown in Table 6. The results obtained by this method appear to support each other.

Table 6: Determination of allura red and brilliant blue in commercial products using PLS and PCR methods

No	PLS			PCR		
	ALL	BRI	CAR	ALL	BRI	CAR
	(mg mL-1)			(mg mL-1)		
1	0.8056	0.4589	0.179	0.7856	0.458	0.1645
2	0.8254	0.4623	0.1698	0.7944	0.4596	0.1621
3	0.7951	0.4489	0.1767	0.7823	0.4682	0.1596
Mean	0.868	0.457	0.175	0.787	0.461	0.162
SD	0.015	0.007	0.005	0.006	0.005	0.002

The proposed method: PCR and PLS methods successfully processed the spectral data and can be exercised for the simultaneous specification of allura red, brilliant blue, and beta-carotene in synthetic samples and the sugar-containing food colorants. The fit of data to a straight line approves the excellent predictive ability used the correlation coefficients of the plot for individual colorants in this study. The plots accentuate the high linear relationship between the predicted and the actual concentration. For the data, low prediction errors and high correlations coefficients accentuate the high linear relationship between the predicted and actual concentrations. The results obtained with this ternary mixture and some ratios of component concentrations show the excellent predictive ability of these methods.

CONCLUSIONS

The partial least squares method and the main component regression, which were successfully applied, were able to identify the active ingredients in the synthetic solutions separately. For all values, low prediction errors and high correlation coefficients emphasize the high linear relationship between predicted and actual concentrations. The results obtained with this binary mixture and some ratios of component concentrations indicate excellent prediction ability with these methods.

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