Determination of the Colorants in Various Samples by Chemometric Methods Using Statistical Chemistry

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ABSTRACT: *Partial least square and principal component regression methods were applied to various mixtures of Allura Red and Brilliant Blue to determine the concentrations. Colorants, at the same time, were analyzed with UV-spectrophotometry in chemical separation. The obtained experimental data have been evaluated by chemometric methods as Partial Least Squares (PLS) and Principle Component Regression (PCR). In the first step, the synthetic mixtures containing three-color materials were examined, and the obtained results were applied to the PCR and PLS. In the next step, using PLS and PCR methods, the quantities of Allura Red and Brilliant Blue in commercial beverage, samples were measured at the same time. The results were compared statistically.*

KEYWORDS *PLS; PCR; Allura red; Brilliant blue.*

INTRODUCTION

Allura Red (ALL) and Brilliant Blue (BRI) are used as colorants in food products. These colorants obtain attractive colors for the presentation of the product [1]. The different analytical methods used for the qualitative and quantitative analyses of food colorants included spectrophotometry [2-8], potentiometry [9,10], chromatography [11-12] and electrochemical methods [13]. Partial Least Squares (PLS) is a chemometric method which was successfully applied by UV spectrophotometry [14] to the multicomponent mixtures. In this study, the partial least-square method is applied to the analysis of commercial products that contain synthetic blends, Allura Red and Brilliant Blue.

Chemometry is defined as mathematical, computational and statistical methods [15]. The chemometric methods are the most effective way to analyze simultaneously and several analytes [16].

The aim of the study is to develop a simple, precise, accurate and rapid spectrophotometric-chemometric method for the simultaneous determination of food colorants.

Mean recoveries (%) and standard deviation of Principal Component Regression (PCR) and Partial Least Squares Regression (PLSR) methods were calculated for the validation of the methods. The acquired results were statistically compared with each other.

EXPERIMENTAL SECTION
Chemicals and reagents

All materials used were analytical grade. Stock solutions of 30 mg/100 mL of Allura Red (Sigma), 30 mg/100mL of Brilliant Blue (Fluka) were prepared with 0.1 M HCl. A training set and validation set containing the colorants in various proportions, 25 synthetic
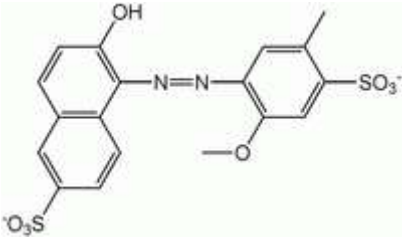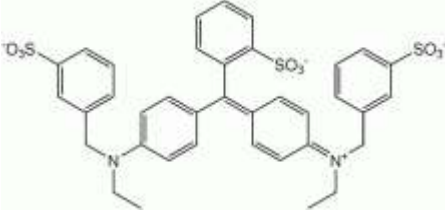
Table 1: Structure of the colorants.

The name of the compound	The formula of the compound
Allura Red	
Brilliant Blue	

mixtures (for calibration), and 10 synthetic mixtures (for validation) were made.

Low conductivity water (0.05 S/cm) was obtained using Millipore's Milli-Q Integral lab water purification system.

Instruments and software

A Shimadzu UV-1700 PharmaSpec Spectrophotometer connected to an IBM PS with UV Probe Software was used for all measurements and data processing. A pair of 1.0 cm quartz cuvettes were used for absorbance measurements.

Absorbance Measurements

Absorbance spectra, food colorant nm both points and the corresponding spaces between contrasting with the 0,1 range 450-700 nm were recorded. The calibration matrix and training and validation sets contain two-component mixtures, at different rates and optimized, and the resulting spectra analysis and analysis of real samples to calculate concentrations have been calculated using PLS.

Samples of 4.0 and 20.00 μg/mL between colorings (alone or in combination) were placed in volumetric flasks, 25 mL and an aliquot containing 0.1 M HCl was added. The mixture was shaken for 20 minutes and filtered. Dry tight standards were prepared in the same manner as described except those with the reagents and colorant. The concentrations prepared from Allura Red and Brilliant Blue for the PLS and PCR calibrations are listed in Fig. 1.

Procedure for real sample:

For this purpose 20 mL samples of foodstuff were transferred into 25mL bottles and mechanically mixed into the water.

Chemometric Method

In this study; Partial Least Squares (PLS) and Principal Component Regression (PCR) was used as chemometric methods. PLS [17,18] and PCR [19,20] are multivariate calibration methods which have many of the full spectra avenges and have been successfully applied to spectrophotometric analyses 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 major difference in the predictive abilities of these two methods in that PLS seems to predict better than PCR methods [21].

Partial least squares regression (PLS-regression) is the most commonly used chemometric multivariate calibration method [22].

PLS is done using both experimental (or x) and concentration (or c) data simultaneously. Usually PLS is presented in the form of equations. There are a few ways to express them, the most suitable for our purpose being:
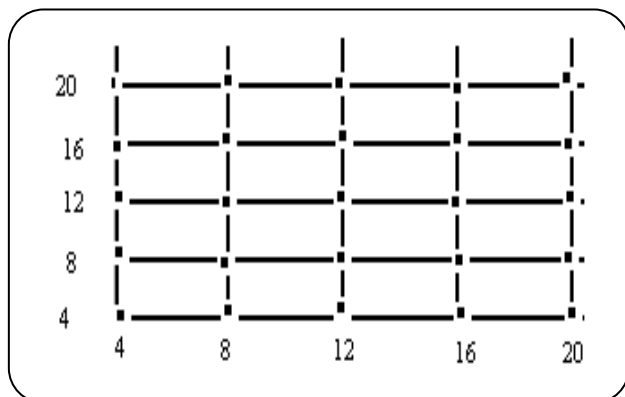
$$X = T.P. + E \tag{1}$$

$$c = T.q + f \tag{2}$$

Fig. 1: Concentration set design for the preparation of the PCR and PCR calibrations.
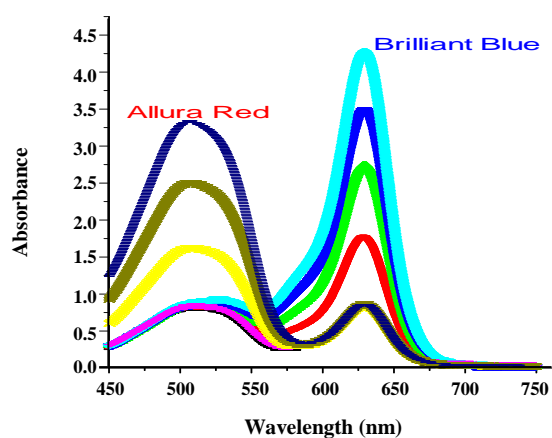

Fig. 2: The spectrum of Allura red and Brilliant blue.

Where X refers to the experimental measurements (e.g. spectra) and c is the concentration. There is a correlation with an installation for vector-vector q. Matrix T is common to both equations. E is an error matrix and error to prevent the x vector c to block scores f orthogonal, but non-orthogonal (P) loads, generally are non-normalized [23].

The Minitab 15 program (İnova, Ankara, Turkey) was used for the analysis of all the concentration and absorbance data and to do the statistical calculations. Minitab is a statistical analysis software. In addition to statistical research, statistics can be used to learn [24].

RESULTS AND DISCUSSION

Allura Red and Brilliant Blue are both in the visible region of high absorbent substances. Fig. 1 shows the absorbance-wavelength (nm) curves. The spectrum of Allura Red and Brilliant Blue are in the range of 450-750 nm.

Fig.2 shows absorption spectra for Allura Red, Brilliant Blue and their binary mixture in 0.1 M HCl.

Our objective in this study is to develop a lower-cost but a more quick and reliable analytical method using chemometry. With this method, active ingredients can be analyzed without pre-separation, and loss of time and work due to the trial and error method will be prevented.

With the spectrophotometric-chemometric method used, it will be easier for the meat industry to identify food colorants that can potentially affect human health.

The PLS method and absorption spectra can be used individually or overlapping for multiple simultaneous detections of very linear components.

Concentrations of ALL-BRI mixtures are listed in Table 2.

The training set of 25 standard mixture solutions contain different concentrations of ALL and BRI.

For calibration, the Prediction Residual Error Sum-of-Squares (PRESS) was calculated as:

$$\text{PRESS} = \sum_{i=1}^{n} (C_i^{\text{added}} - C_i^{\text{found}})^2 \qquad (4)$$

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

C_i^{added}: Predicted Concentration, the calculated concentration of colorant

The RMSEC can provide a good measure of how well, on average, the calibration model performs.

According to the actual and predicted concentrations of the samples, RMSEC and PRESS values of Allura Red and Brilliant Blue were calculated and listed in Table 3.

The root means square error of cross-validation/ RMSEC was calculated for each method as follows:

RMSEC = (PRESS/n)$^{1/2}$ n: the number of predicted samples

Method Validation

Prediction of the calibration methods was done by resolution of ten synthetic mixtures in a working concentration range for food colorants. Predicted concentration data of ALL-BRI mixtures are shown in Table 4. The percentage recovered and relative standard deviations are also listed in Table 4.

In this study, the statistical parameters were found to produce a satisfactory validity for the PLS and PCR

Table 2: Concentration set containing ALL and BRI compounds for the preparation of the PCR and PLS calibration (µg/mL).

	Concentration (µg/mL)			Concentration (µg/mL)	
No	ALL	BRI	No	ALL	BRI
1	4.0	4.0	14	12.0	16.0
2	4.0	8.0	15	12.0	20.0
3	4.0	12.0	16	16.0	4.0
4	4.0	16.0	17	16.0	8.0
5	4.0	20.0	18	16.0	12.0
6	8.0	4.0	19	16.0	16.0
7	8.0	8.0	20	16.0	20.0
8	8.0	12.0	21	20.0	4.0
9	8.0	16.0	22	20.0	8.0
10	8.0	20.0	23	20.0	12.0
11	12.0	4.0	24	20.0	16.0
2	12.0	8.0	25	20.0	20.0
13	12.0	12.0			

Table 3: Statistical parameter values for calibration step- simultaneous determination of Allura red and brilliant blue using partial least square and principal component regression methods.

Parameter	Method	Compound	
		ALL	BRI
RMSEC	PLS	0,0362	0,0303
	PCR	0,0358	0,0378
PRESS	PLS	0,0329	0,0229
	PCR	0,0332	0,0238
Regression Coefficient	PLS	1,0000	1,0000
	PCR	0,9998	0,9989
Intercept	PLS	0,0072	0,0058
	PCR	0,0069	0,0049
Slope	PLS	0,9993	0,9991
	PCR	0,9989	0,9987

Table 4: Composition of prediction set and recovery results obtained in synthetic mixtures for PLS and PCR methods.

Sample no.	Composition (µg/mL)		% Recovery			
	ALL	BRI	PLS		PCR	
			ALL	BRI	ALL	BRI
1	12.0	4.0	99.99	99.95	99.16	99.25
2	12.0	9.0	99.93	99.99	98.33	99.78
3	12.0	12.0	100.00	99.83	99.08	99.50
4	12.0	17.0	99.82	99.93	98.92	99.35
5	12.0	20.0	99.73	100.00	99.00	99.25
6	4.0	12.0	99.48	99.97	95.00	99.67
7	8.0	12.0	99.98	99.87	99.25	99.75
8	12.0	12.0	100.00	100.00	99.83	99.67
9	16.0	12.0	99.93	100.00	99.75	99.50
10	20.0	12.0	99.88	99.99	99.90	100.00
Mean			99.87	99.94	98.82	99.57
SD			0.15	0.062	1.31	0.25

methods. The PLS and PCR methods have reliable accuracy and higher precision.

Some statistical parameters determined the effectiveness of the calibration. The Standard Error of Prediction (SEP) was calculated using the following expression:

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

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

C_i^{added} : Predicted Concentration, the calculated concentration of colorant

n: the total number of synthetic mixtures

The SEP can provide a good measure of how well, on average, the calibration model performs. The SEP values are shown in Table 5.

The standard of ALL and BRI was added to the product solution for testing the selectivity of the applied methods (standard addition method). When the results obtained were compared with the expected results, the values were excellent (Table 6).

Analysis of real samples

Table 7 lists the experimental results of the two numerical methods for commercial products and as you can see the obtained results are very close to each other.

In this study, chemometric methods based on spectral data processing, Allura Red and Brilliant Blue, without interference in each other's mixes and beverages containing a binary mixture of the two can be applied for simultaneous identification.

In order to compare the performances of the investigated chemometric techniques according to UV spectrophotometric method for real samples, we applied Snedecor's *F*-test.

The method used to compare the differences between the one-way ANOVA test was applied to the actual samples for each food colorant. In this study, Snedecor's F-values were calculated and compared with the *F* value (p=0.05). The same computation process was repeated for both colorants. In the standard table, for $n_1=1$ and $n_2=4$ (PLS), the *F*-value is 7.71(PLS) and for $n_1=1$ and $n_2=4$ (PCR), the *F*-value is 7.71(PCR). The results of the ANOVA test for Allura Red were 0.41(PLS) and 0.0045 (PCR) for Allura Red and 0.44 (PLS) and 0.87(PCR) for Brilliant Blue. The experimental (calculated) *F*-values did not exceed the *F*-value in the variance analysis.

Table 5: Statistical parameter values for prediction step- simultaneous determination of allura red and brilliant blue using partial least square and principal component regression methods.

Parameter	Method	Compound	
		ALL	BRI
SEP	PLS	0.0040	0.0023
	PCR	0.0180	0.0330
Regression Coefficient	PLS	1.0000	1.0000
	PCR	0.9998	1.0000
Intercept	PLS	0.0076	0.0047
	PCR	0.2140	0.0473
Slope	PLS	0.9996	0.9998
	PCR	1.0095	0.9917

Table 6: Recovery results obtained standard addition technique using the proposed PLS and PCR methods.

Sample no.	Composition (µg/mL)		% Recovery			
	ALL	BRI	PLS		PCR	
			ALL	BRI	ALL	BRI
1	4.0	6.0	99.76	99.80	98.84	97.84
2	6.0	10.0	99.86	99.91	99.68	96.88
3	8.0	14.0	99.86	98.94	97.53	97.45
Mean			99.83	99.55	98.68	97.39
S.D.			0.06	0.53	1.08	0.48

Table 7: Determination of Allura red and brilliant blue in commercial products using PLS and PCR methods.

No	PLS		PCR	
	ALL	BRI	ALL	BRI
	(µg/mL)		(µg/mL)	
1	23.77	1.73	23.34	1.75
2	24.09	1.77	23.85	1.74
3	23.65	1.71	23.74	1.71
Mean	23.83	1.74	23.64	1.73
SD	0.23	0.03	0.27	0.03

Among all these methods, it was concluded that there was a meaningful difference. All statistical parameters and numeric values are suitable for simultaneous identification in the actual samples.

CONCLUSIONS

The partial least squares method and principal component regression all successfully applied at the same time were able to identify colorants in synthetic solutions and commercial products. For all values, low prediction errors and high correlation coefficients emphasize the high linear relationship between the predicted and actual concentrations. The results obtained with this ternary mixture and some ratios of component concentrations show excellent predictive ability with these methods.

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