ISSN : 0974-7419 Volume 16 Issue 11

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 16(11) 2016 [489-497]

Simultaneous determination of the colorants in foodstuff sample by uv-spectrophotometry and potentiometry using partial least squares method

Güzide Pekcan Ertoku*, Hakan A.Akta
Süleyman Demirel University,Faculty of Science & Art, Department of Chemistry, 32260 Isparta, (TURKEY)
E-Mail: guzideertokus@sdu.edu.tr

ABSTRACT

Two methods are recommended for the simultaneous determination of tthese colorants in foodstuff. The mixtures of food colorants, containing tartrazine, sunset yellow, allura red and caramel were simultaneously analyzed with spectrophotometry without previous chemical separation. The data aquired from experiments were processed by one of chemometric approaches with partial least squares (PLS) using the absorbance and potentiometric data. The linear relationship between the volumes of titrant and the concentrations of analytes was obtained by chemometric methods. The designed model was the used to predict the concentrations of components in unknown sample. In the next step, PLS method, commercial beverage sample caramel, allura red, tartrazine and sunset yellow quantity determinations were performed at the same time. PLS calculated from experimental data, calibration methods, a good fit between the observed and the results were compared statistically.

KEYWORDS

Allura red;
Tartrazine;
Sunset yellow;
Caramel;
Chemometry.

INTRODUCTION

Synthetic sweeteners used in foods for centuries and there are foods that make you more attractive features such as whet your appetite[1]. By reducing the appearance of the party by adding colorants color variations, natural color and natural color enhancers for products that have consumer appeal can be maintained while producing[2]. In recent years, food additives have been investigated to evaluate their toxicity[3]. The amount of these chemicals should be used and sometimes allergy in susceptible individuals, asthma and other health disolders should be checked because it can produce[4]. Various analytical methods have been used for the qualitative and quantitative analyses of food color-, including spectrophotometry[4-6], potentiometry[7,9], chromatography[9,11].

Multivariate calibration methods are utilized in the spectrophotometric and potentiometric data analysis for the mixtures containing two or more compounds.

In this work, Partial Least Squares (PLS) was used for the simultaneous determination of tartrazine, allura red, caramel and sunset yellow in foodstuff without any separation stey. Two methods were used potentiometry and UV-spectrophotometry comparatively.

EXPERIMENTAL

Chemicals and reagents

Analytical reagent grade chemicals were used, unless stated otherwise. Stock solutions 10 mg/ 100 mL of sunset yellow (Fluka), 10 mg/ 100mL of tartrazine (Fluka), 10 mg/ 100mL of allura red (Sigma), 10 mg/ 100mL of caramel (Gurme) (TABLE 1.) were made ready by the exact weighing of the standards and solution with reverse osmosis quality water. Working solutions were prepared from the stock solution diluted with water. The solutions obtained in all the samples is determined for at least a month. To adjust the pH of the standards and samples a 0.1 M HCl aqueous solution was used.

Instruments and software

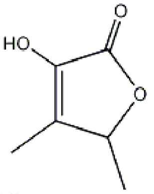
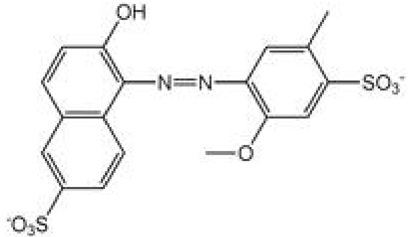
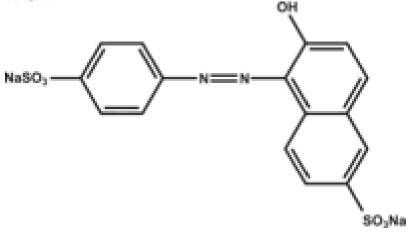
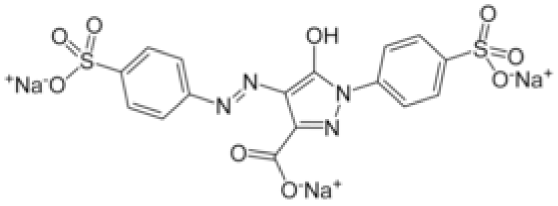
Pharma 1700 Spectrophotometer / SHIMADZU connected to an IBM PS fitted with UV Probe Software was used for all measurements and treatment of data. Absorbance measurements were made two matching 1.0 cm quartz cells.

The potentiometric equipment used was; ORION 5 STAR pH / ion meter equipped with a combined glass-calomel electrode.

Absorbance measurements

Absorbance spectra of the food colorants were recorded between 250-600 nm with a range of 0.1 nm between each two points and were contrasted with the corresponding blanks. The calibration matrix was prepared from 31 solutions containing mixtures of the four components in different ratios._ an aliquat of sample containing between 0,4 and 20 (mg/ mL) (TABLE 2,4.) of the colorants was placed in a 25 mL volumetric flask and 0.1 M HCl was added. The mixture was shaken for 20 min and packed in a

TABLE 1 : Structure of the colorants

The name of compound	The formula of compound
Caramel	
Allura Red	
Sunset Yellow	
Tartrazine	

1 nm cell. The colorant is prepared in the same manner as described for the blind and standards all reagents except for the place.

Procedure for real sample

For this purpose, an amount volume to 20 mL samples in foodstuff. The sample was transferred to 25 mL calibrated flask and dissolved in water mechanically.

Potentiometric measurements

The temperature was adjusted from the outside of the cell at 25ºC ± 0.1 with a cooler system water bath and the test solution was mixed magnetically at the purified nitrogen medium. The e.m.f. measurements to quantify the pH of the solution were applied with a model ORION 5 STAR pH / ion analyser with Ag / AgCl combined pH electrode system (±0.1 mV)

The determination of food colorants in synthetic real samples were nominated by titration, using KOH solution as titrant, in respect of the criteria promoted by IUPAC[12-13].

TABLE 2 : Composition of the calibration set for the food colorants

	Concentration (mg / mL)			
No	**Caramel**	**Allura Red**	**Tartrazine**	**Sunset Yellow**
1	2.0	0.4	0.8	0.4
2	4.0	0.4	0.8	0.4
3	6.0	0.4	0.8	0.4
4	8.0	0.4	0.8	0.4
5	10.0	0.4	0.8	0.4
6	12.0	0.4	0.8	0.4
7	14.0	0.4	0.8	0.4
8	16.0	0.4	0.8	0.4
9	20.0	0.4	0.8	0.4
10	4.0	0.6	0.8	0.4
11	4.0	0.8	0.8	0.4
12	4.0	1.0	0.8	0.4
13	4.0	1.2	0.8	0.4
14	4.0	1.6	0.8	0.4
15	4.0	1.8	0.8	0.4
16	4.0	2.0	0.8	0.4
17	4.0	2.2	0.8	0.4
18	4.0	0.4	1.2	0.4
19	4.0	0.4	1.6	0.4
20	4.0	0.4	2.0	0.4
21	4.0	0.4	2.4	0.4
22	4.0	0.4	2.8	0.4
23	4.0	0.4	3.2	0.4
24	4.0	0.4	3.6	0.4
25	4.0	0.4	0.8	0.8
26	4.0	0.4	0.8	1.0
27	4.0	0.4	0.8	1.2
28	4.0	0.4	0.8	1.4
29	4.0	0.4	0.8	1.6
30	4.0	0.4	0.8	1.8
31	4.0	0.4	0.8	2.0

TABLE 3 : Statistical parameter values for calibration step- simultaneous determination of food colorants using partial least square method

Parameter	Method	Compounds		Compounds	
		Caramel	Allura Red	Tartrazine	Sunset Yellow
RMSEC	PLS	0.1167	0.1158	0.1516	0.1720
PRESS	PLS	0.4223	0.4156	0.7122	0.9178
Regression Coefficient	PLS	0.9995	0.9997	0.9998	0.9989
Intercept	PLS	0.0142	0.0405	0.0205	0.0175
Slope	PLS	0.9962	0.9998	0.9997	0.9995

TABLE 4 : The composition of validation set

	Concentration (mg/ mL)			
No	Caramel	Allura Red	Tartrazine	Sunset Yellow
1	4.0	0.4	0.8	0.4
2	8.0	0.4	0.8	0.4
3	12.0	0.4	0.8	0.4
4	16.0	0.4	0.8	0.4
5	4.0	0.8	0.8	0.4
6	4.0	1.2	0.8	0.4
7	4.0	1.6	0.8	0.4
8	4.0	0.4	0.8	0.8
9	4.0	0.4	0.8	1.2
10	4.0	0.4	0.8	1.6
11	4.0	0.4	1.6	0.4
12	4.0	0.4	2.4	0.4
13	4.0	0.4	3.2	0.4

The standardisation of the electrode system was performed, each time in mixtures studied by Gran’s method[14].

At this step, on conjectural quantity of solution at the same conditions as temprature, ionic strength and solvent composition (hereafter will be called background solution) was placed in a double-walled, thermostated vessel. In first step, the potential was allowed to come to balance after each addition of acid or base and then value was utilized to acquired the standard potential of the cell, E^o. The electrode was dipped in background solution and it was titrated with a strong base in the same experimental conditions of ionic strength and solvent composition. Usually, about 10 or 12 additions is enough for verify E^o to be accurately determined.

In second step, an aliquat of sample containing between 0,4 and 20 (mg/mL) (TABLE 2,4.) of the colorants was titrated by sodium hydroxide.

For real sample; an amount volume of food sample add to the cell and titrated by base.

Partial least Sguares (PLS)

Partial least squares regression (PLS-regression) is one of the most widely used multivariate calibration methods in chemometrics[15].

PLS components obtained using both experimental (or x) and concentration (c) data at the same time. Often PLS is presented in the form of two equations. There are a number of ways of expressing these, a convenient are being;

$$X = T.P. + E \qquad (1)$$

$$c = T.q + f \qquad (2)$$

X points the experimental measurements (e.g. spectra) and c the concentrations. The vector q has some analogy to a loading vector. The matrix T is common to both equations. E is an error matrix for the x block and f an error vector for the c block The scores are orthogonal, but the loadings (P) are not orthogonal, usually they are not normalized[16].

We used Minitab 15 program. Minitab 15 was used for all the process of the absorbance / pH – concentration data and the statistical calculations. Minitab can be used for learning about statistics as well as statistical research[17].

RESULT AND DISCUSSION

The calibration set of 32 standard mixture solutions which contains the concentrations with different ratio of food colorants (TABLE 2).

The prediction residual error sum-of-squres (PRESS) of the calibration step was calculated as;

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

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

C_i^{added} : Prediction Concentration, is the found concentration of colorants.

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

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 3.

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

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

The first step in the simultaneous determination of the colorants by PLS involves constructing the validation set for the quarternary mixtures of them (TABLE 4).

The mean recoveries and the relative standard deviations of partial least squares were calculated and were shown in TABLE 5,6. In this study, the statistical parameters were found saticfactory for the validity PLS method. The reliable accuracy and higher precision in application of PLS method.

In first step; the food colorants were analyzed with UV-spectrophotometry. Tartrazine, sunset yellow, allura red and caramel are all highly absorbing substances in the visible region. Figure 1 shows the absorbance-wavelength (nm) curves of them.

It is seen their highly overlapping peaks in Figure 1. Because of this reason the quantitative analysis of these colorants is not possible by traditional spectrophotometric method. The obtained results of partial least square method were obtained from potentiometry and spectrophotometry.

In second step; the food colorants were exam-

TABLE 5 : The obtained spectrophotometric recovery results by partial least square method

	Concentration (mg / mL)			
No	**Caramel**	**Allura Red**	**Tartrazine**	**Sunset Yellow**
1	101.10	99.85	99.85	100.00
2	100.79	100.12	97.59	100.00
3	100.53	98.03	98.75	99.98
4	99.87	101.78	96.26	100.03
5	100.04	100.55	98.10	100.00
6	99.25	98.14	96.36	100.03
7	97.28	97.73	100.81	99.98
8	98.13	100.73	96.68	100.00
9	100.04	97.78	103.07	99.99
10	104.93	99.75	99.55	100.01
11	102.29	100.93	98.29	100.03
12	99.32	99.23	99.82	100.00
13	97.65	99.43	99.88	100.00
Mean	100.09	99.54	98.85	100.00
SD	2.03	1.31	1.94	0.02
RSD	2.02%	1.32%	1.97%	0.02%

TABLE 6 : The obtained potentiometric recovery results by partial least square method

	Concentration (mg / mL)			
No	**Caramel**	**Allura Red**	**Tartrazine**	**Sunset Yellow**
1	98.70	99.49	98.63	98.78
2	99.49	102.19	96.40	99.28
3	102.76	98.69	100.26	100.00
4	98.31	101.50	100.21	100.34
5	99.76	101.11	100.25	100.08
6	96.36	100.77	97.55	98.90
7	96.69	98.86	96.40	98.06
8	99.79	100.35	100.64	100.55
9	100.79	99.70	100.36	100.11
10	101.59	95.75	97.79	99.31
11	97.57	101.74	100.03	100.60
12	100.79	97.20	99.50	98.69
13	102.40	96.96	99.26	98.11
Mean	99.62	99.56	99.02	99.45
SD	2.05	2.00	1.52	0.89
RSD	2.05%	2.01%	1.54%	0.90%

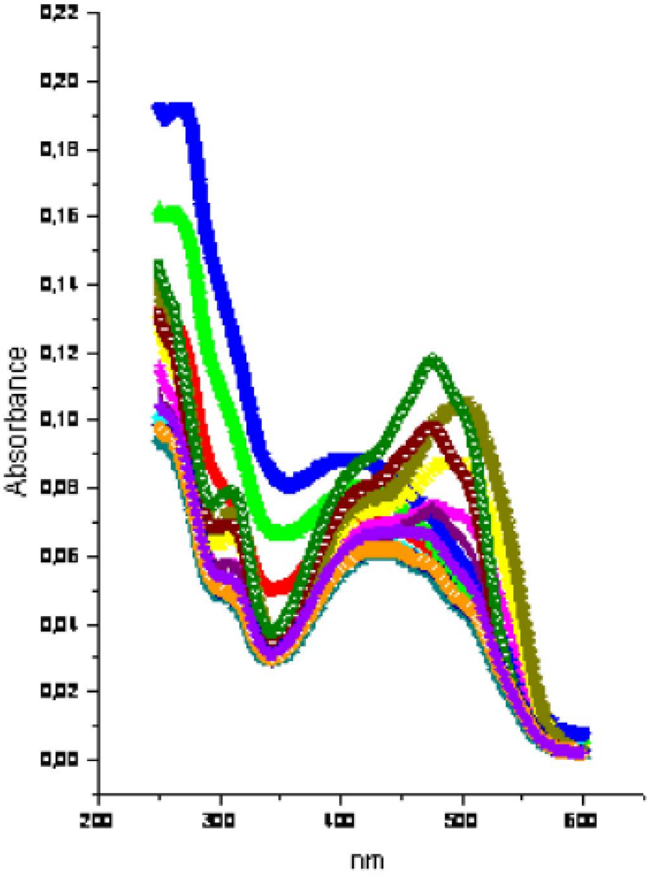


Figure 1 : The spectrum of mixtures

ined with potentiometry. In primary experiments, each colorant and a quarternary mixture of colorants were titrated with 0,1 M potassium hydroxide. The figure shows the titration curves of individual acids and also the titration curve of a mixture of four colorants. For the mixture, the titration curve (Figure 2) shows one inflection point, and, as is obvious, the determination of each acid by usual methods in not possible.

We used partial least square regression of the chemometric methods. Multivariate calibrations are utility on spectrophotometric, potentiometric and chromatographic analysis. Especially, at the same time, including multi-spectral densities, significantly improve the precision and predictive ability.

In this study, the statistical parameters were found saticfactory for the validity PLS method. The reliable accuracy and higher precision in application of PLS method.

Some statistical parameters were defined the ability of a calibration in several ways. The standard error of prediction (SEP) are given by the next 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 con-

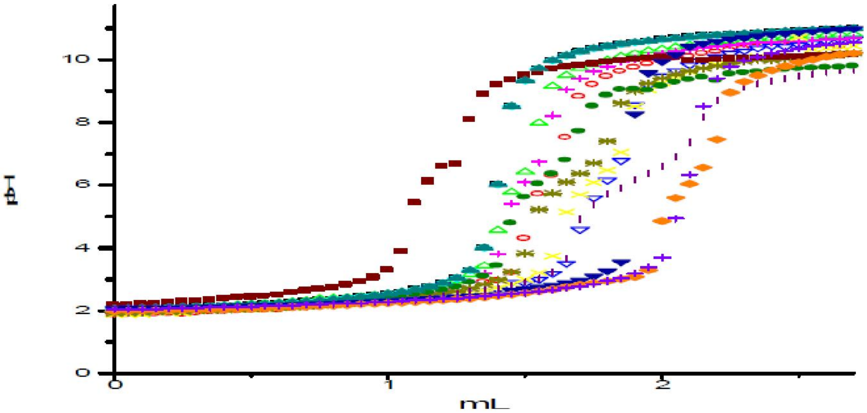


Figure 2 : The titration curve of mixtures

TABLE 7 : Statistical parameters for validation set

Parameter	Method	Spectrophotometric method			
	Caramel	Allura Red	Tartrazine	Sunset Yellow	Caramel
SEP	PLS	1.5775	0.0133	0.0201	0.0001
Regression Coefficient	PLS	0.9996	0.9997	0.9995	1.0000
Intercept	PLS	0.0008	0.0104	0.0123	7.10^{-7}
Slope	PLS	1.0016	0.9735	1.0017	1.0000
Parameter	**Method**	**Potentiometric method**			
		Caramel	**Allura Red**	**Tartrazine**	**Sunset Yellow**
SEP	PLS	0.1297	0.0112	0.0164	0.0054
Regression Coefficient	PLS	0.9989	0.9992	0.9997	0.9999
Intercept	PLS	0.0492	0.0003	0.0051	0.0013
Slope	PLS	1.0073	0.9952	0.9960	0.9977

TABLE 8 : Recovery results obtained standart addition technique using the proposed PLS method

Sample no.	Composition (µg mL^{-1})				% Recovery			
	Caramel	Allura red	Tartrazine	S.Yellow	Spectrophotometric method			
					Caramel	Allura red	Tartrazine	S.Yellow
1	4.0	0.4	0.80	0.4	99.98	99.78	98.90	98.94
2	6.0	0.8	1.00	0.6	99.96	99.89	99.75	98.85
3	8.0	1.20	1.20	0.8	99.82	98.88	98.78	99.42
Mean					99.92	99.52	99.14	99.07
S.D.					0.09	0.55	0.53	0.31
RSD					0.09%	0.56%	0.53%	0.31%
Sample no.	**Composition (µg mL^{-1})**				**% Recovery**			
	Caramel	**Allura red**	**Tartrazine**	**S.Yellow**	**Potentiometric method**			
					Caramel	**Allura red**	**Tartrazine**	**S.Yellow**
1	4.0	0.4	0.80	0.4	99.85	98.72	97.85	99.04
2	6.0	0.8	1.00	0.6	98.86	99.12	98.25	99.15
3	8.0	1.20	1.20	0.8	99.51	98.74	98.98	99.14
Mean					99.41	98.86	98.36	99.11
S.D.					0.50	0.23	0.57	0.06
RSD					0.51%	0.23%	0.58%	0.06%

TABLE 9 : Determination of food colorants in commercial products using PLS method

Spectrophotometric method				
No	**Caramel**	**Allura red**	**Tartrazine**	**Sunset Yellow**
1	0.050	5.230	0.840	0.330
2	0.051	5.440	0.750	0.420
3	0.049	5.350	0.880	0.360
Mean	0.050	5.340	0.820	0.370
SD	0.001	0.105	0.067	0.046
RSD	2.0%	2.0%	8.08%	12.39%
Potentiometric method				
No	**Caramel**	**Allura red**	**Tartrazine**	**Sunset Yellow**
1	0.049	5.390	0.830	0.350
2	0.051	5.470	0.840	0.400
3	0.051	5.500	0.890	0.380
Mean	0.050	5.450	0.840	0.380
SD	0.001	0.057	0.045	0.025
RSD	2.29%	1.04%	5.35%	6.68%

centration of colorants is,

C_i^{added} : Prediction Concentration, is the found concentration of colorants and n is the total number of the synthetic mixtures. The SEP can provide a good measure of how well, on average, the calibration model performs. The SEP values is shown TABLE 7.

The standart of allura red, caramel,tartrazine and sunset yellow was 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 8).

Analysis of real samples

The experimental results of two numerical methods to commercial product –Yedigün®- blood orange (made in Turkey) in this work shown in TABLE 9. We observed that the results sighted in two methods are very close to each other. The commercial product was repeated three times.

CONCLUSIONS

PLS of multivariate calibration methods allow the simultaneous determination of tartrazine, allura red, sunset yellow and caramel in the synthetic mixtures and commercial food sample.

The corresponding calibrations indicated good results both for the mixtures and commercial product. The fit of data to a straight line (with a R^2= 0.9989-1.000) comfirms the excellent predictive ability of the plot used in this investigation.

Mean recoveries of PLS were found between 98.85-100.09 (spectrophotometry) and between 99.02-99.62 (potentiometry). For all points, low prediction errors and high correlations coefficients emhasize the high linear relation ship between the predicted and actual concentrations show excellent predictive ability of this methods.

The proposed chemometric method can be applied for the routine analysis of commercial product without ay a prior chemical separation and without time consuming.This simple and confident chemometric techniques are suitable for the quality control of food colorants in commercial products.

REFERENCES

[1] E.Dinç, E.Baydan, M.Kanbur, F.Onur; Talanta, **58**, 579-594 **(2002)**.

[2] M.Gonzalez, M.Gallego, M.Valcarcel; Analytica Chimica Acta., **464**, 237-247 **(2002)**.

[3] J.J.Berzas, J.R.Flores, M.J.V.Llerena, N.R.Farinas; Anaytica Chimica Acta., **391**, 353-364 **(1999)**.

[4] Berzas J.J.Nevado, Rodriguez J.Flores, Guiberteau C.Cabanillas, Villasenor M.J.Llerena, A.Contento

Salcedo; Talanta, **48**, 933-942 **(1998)**.

[5] Y.Ni, X.Gong; Analytica Chimica Acta, **354(1-3)**, 163-171 **(1997)**.

[6] B.Hemmateenejad, M.Akhond, F.Samari; Spectrochimica Acta Part A. Molecular And Biomolecular Spectroscopy, **67**, 958-965 **(2007)**.

[7] M.Akhound, J.Tashkhourian, B.Hemmateenejad; Journal of Analytical Chemistry, **61(8)**, 804-808 **(2006)**.

[8] A.H.Aktas, S.Yasar; Acta Chim.Slovenica., **51**, 273-282 **(2004)**.

[9] E.Dinç, A.H.Aktas, O.Üstündað; Journal of Aoac International, **88(6)**, 1748-1755 **(2005)**.

[10] E.Dinç, O.Ustündag; Chromatographia, **61**, 237-244 **(2005)**.

[11] E.Dinç, A.Özdemir, H.Aksoy, O.Ustündag, D.Baleanu; Chemical & Pharmaceutical Bulletin, **54(4)**, 415-421 **(2006)**.

[12] S.Rondinini, P.R.Mussini, T.Mussini; Pure Applied Chemistry, **59**, 1549 **(1987)**.

[13] P.R.Mussini, T.Mussini, S.Rondinini; Pure Applied Chemistry, **69**, 1007 **(1997)**.

[14] G.Gran; Part II.Analyst, **77**, 661-671 **(1952)**.

[15] R.B.Kenneth; Chemometrics: A practical guide, John Wiley & Sons.Inc., New York, **(1998)**.

[16] R.C.Brereton; Applied Chemometrics for Scientists, John Wiley & Sons.Inc., New York, **(2007)**.

[17] http://www.causeweb.org/repository/Minitab/Minitab.pdf, accessed on October 20, **(2015)**.