FOOD COMPOSITION AND ADDITIVES

New Liquid Chromatographic-Chemometric Approach for the Determination of Sunset Yellow and Tartrazine in Commercial Preparation

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A new liquid chromatographic (LC)-chemometric approach was developed for the determination of sunset yellow (SUN) and tartrazine (TAR) in commercial preparations. This approach uses LC and chemometric calibration methods, i.e., classical least-squares (CLS), principal component regression (PCR), and partial–least squares (PLS), simultaneously. The combined LC-chemometric approaches, denoted as LC-CLS, LC-PCR, and LC-PLS, are based on photodiode array (PDA) detection at multiple wavelengths. Optimum chromatographic separation of SUN and TAR with allura red as the internal standard (IS) was obtained by using a Waters Symmetry® C18 column, 5 μm, 4.6 × 250 mm, and 0.2 M acetate buffer (pH 5)–acetonitrile–methanol–bidistilled water (55 + 20 + 15 + 10, v/v) as the mobile phase at a flow rate of 1.9 mL/min. The LC data sets consisting of the ratios of analyte peak areas to the IS peak area were obtained by using PDA detection at 5 wavelengths (465, 470, 475, 480, and 485 nm). LC-chemometric calibrations for SUN and TAR were separately constructed by using the relationship between the peak-area ratio and the training sets for each colorant. LC-chemometric approaches were tested for different synthetic mixtures containing SUN and TAR in the presence of the IS. These LC-chemometric calibrations were applied to a commercial preparation of the 2 colorants. The experimental results of the LC-chemometric approaches were compared with those obtained by a developed classical LC method using single-wavelength detection.

Tartrazine (Colour Index No. 19140; TAR) and sunset yellow (Colour Index No. 15985; SUN) are synthetic azo colorants commercially used in coloring food, soft drinks, drugs. and cosmetics (Figure 1). Commercial foods and drinks have contained various colorants for centuries. Some of these synthetic colorants can be harmful to people's health, especially when they are excessively consumed. Consequently, the use of synthetic color additives in commercial foods and beverages has been controlled by governmental laws and regulations. Thus, the quantitative determination of synthetic colorants in commercial preparations is very important for human health, and interest in developing reliable sensitive methods for the determination of these synthetic colorants and their metabolites in food products and human fluids has increased.

SUN and TAR in samples and in mixtures with different colorants or other compounds have been determined by spectrophotometric methods (1–10), liquid chromatography (LC; 11, 12), thin-layer chromatography (13), and polarography with partial least squares (PLS; 14).

The current technological developments of new analytical instrumentation and software offer the use of modern powerful analytical methods for the quality control of products and their routine analysis in analytical chemistry. Usually, LC has been used for quality control or analysis of commercial products in research laboratories or in industrial product laboratories. In some cases, this technique may not be sufficient for the analysis of samples when interferences are present. Therefore, the quantitative analysis of samples needs new analytical applications and approaches based on the simultaneous use of classical analytical methods and powerful mathematical algorithms to provide precise and accurated experimental results.

For these reasons, chemometric calibration methods, in combination with several techniques, have been widely applied in analytical procedures in recent years. Multivariate calibration techniques, i.e., classical least squares (CLS), principal component regression (PCR), and PLS, have been described for the analysis of data obtained from many analytical instruments (15–18). Multivariate calibration models have been applied particularly to the resolution of overlapping spectra for the analysis of multicomponent samples containing 2 or more compounds of interest (19–21).

In this study, TAR and SUN were determined in the synthetic mixtures and commercial product by

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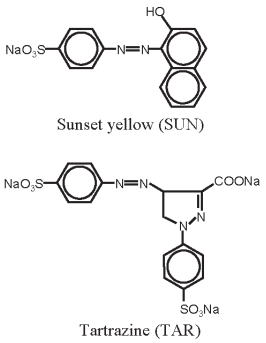


Figure 1. Structures of the synthetic food colorants.

LC-chemometric approaches. In the outline of our proposed approaches, the chemometric calibrations CLS, PCR, and PLS were applied to the LC data set obtained at multiwavelengths by using photodiode assay (PDA) detector responses. The simultaneous use of chemometric calibration and LC were referred to as LC-CLS, LC-PCR, and LC-PLS approaches. The elaborated LC-chemometric approaches are used to eliminate or decrease the inherent disadvantage of using a single regression equation based on a single wavelength. In our case, the LC-chemometric calibrations were applied to the simultaneous determination of both colorants in synthetic and market samples. As an alternative method, classical LC was also used for the analysis of the same samples. The assay results obtained by the LC-chemometric approaches for the determination of SUN and TAR were compared with those obtained by the classical LC method.

Theoretical Outline

In the LC-chemometric study, CLS, PCR, and PLS calibrations were applied to the ratio of the chromatographic peak area of the analyte to that of the IS at 5 wavelengths. The chromatograms of the analytes were plotted and stored in the computer. The detector responses were measured in terms of peak area. The application procedure of the combined multivariate LC calibrations is explained in each of the following sections for multivariate calibration.

LC-CLS Method

This method applies multilinear regression (MLR) to the peak area of individual analytes. If we consider the responses as ratio values of peak-area ratio at the 5-wavelength set (R) and 5-standard series [training set (C)] of analyte, the following equation system can be written:

$$\begin{bmatrix} R_{11} & R_{12} & R_{13} & R_{14} & R_{15} \\ R_{21} & R_{22} & R_{23} & R_{24} & R_{25} \\ R_{31} & R_{32} & R_{33} & R_{34} & R_{35} \\ R_{41} & R_{42} & R_{43} & R_{44} & R_{45} \\ R_{51} & R_{52} & R_{53} & R_{54} & R_{55} \end{bmatrix} \begin{bmatrix} K_{11} \\ K_{21} \\ K_{31} \\ K_{41} \\ K_{51} \end{bmatrix} \times \begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14} & C_{15} \end{bmatrix} \quad (1)$$

where $R_{5 \times 5}$ is the matrix of the ratio of analyte peak areas to IS peak area, $K_{5 \times 1}$ indicates the matrix of the calibration coefficients, and $C_{1 \times 5}$ represents the training set of analyte.

In the compact matrix form, Equation 1 can be rewritten as:

$$R_{5 \times 5} = K_{5 \times 1}\, C_{1 \times 5} \quad (2)$$

By using the matrix calculation, we immediately obtain the values of the matrix $K_{5 \times 1}$ as follows:

$$K_{5 \times 1} = R_{5 \times 1} C_{5 \times 1}^{T} \left[C_{1 \times 5} C_{5 \times 1}^{T} \right]^{-1} \quad (3)$$

where $C_{5 \times 1}^{T}$ is the transpose of $C_{1 \times 5}^{T}$ and $\left[C_{1 \times 5} C_{5 \times 1}^{T} \right]^{-1}$ represents the inverse of $\left[C_{1 \times 5} C_{5 \times 1}^{T} \right]$.

The coefficient $Ka_{1 \times 5}$ for the determination of the amount of analyte in the sample can be calculated by the following equation:

$$Ka_{1 \times 5} = \frac{1}{\left[K_{1 \times 5}^{T} K_{5 \times 1} \right]} \times K_{1 \times 5}^{T} \quad (4)$$

The calculated Ka_{1x5} introduced into the following equation:

$$C_{prediction_{1 \times n}} = Ka_{1 \times 5} \times R_{sample_{5 \times n}} \quad (5)$$

In this way the concentration of analyte in the mixture is predicted by multiplying $Ka_{1 \times 5}$ and $R_{sample_{5 \times n}}$. Matlab 7.0 software can be used for the mathematical treatments.

LC-PCR Method

In the application of the LC-PCR method, the ratio (R) of the peak area of the individual analyte and the analyte concentration set are reprocessed by mean-centering as R_o and C_o, respectively. The covariance dispersion matrix of the centered matrix R_o is computed. The normalized eigenvalues and eigenvectors are calculated starting from a square covariance matrix. The number of the optimal principal components [eigenvectors (P)] is selected by considering only the highest values of the eigenvalues. The other eigenvalues and their corresponding eigenvectors are eliminated. To reach this objective the coefficient b defined as $b = P \times q$ is determined, where P is the matrix of eigenvectors and q is the C–loadings given by $q = D \times T^T \times R_o$. T^T is the transpose of the score matrix T, and D is a diagonal matrix consisting of the inverse of the selected eigenvalues. The content of the relevant compounds in samples is evaluated by using the $C_{prediction} = b \times R_{sample}$. PLS toolbox 3.0 in Matlab 7.0 software is used for the data treatment.

LC-PLS Method

The PLS calibration using the orthogonalized PLS algorithm involves the independent and the dependent variables simultaneously in the data compression and decomposition operations.

In the multivariate LC data analysis, the LC-PLS calibration is performed by decomposing both the concentration and the ratio of the peak-area matrix into latent variables, $R = T \times P^T + E$ and $C = U \times Q^T + F$. The linear regression, $C_{prediction} = b \times R_{sample}$, is used for the estimation of the analytes in the samples. The vector b is given as

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$$\left(b = W \times PT \times W\right)^{-1} \times Q$$

where W is a weight matrix.

Application of this method is performed by using PLS toolbox 3.0 in Matlab 7.0 software.

Experimental

Instrumentation and Chromatography

The chromatograms of standards and samples were plotted by an Agilent 1100 series LC system (Agilent Technologies, Inc., Palo Alto, CA) equipped with a quaternary pump, a thermostatted autosampler, a thermostatted column compartment, and a multiwavelength PDA. The instrument was controlled by using HP ChemStation software for LC (Agilent Technologies, Inc.). The chromatograms were plotted and processed by using the above-mentioned software. The column used was a Waters Symmetry® C18 Column, 5 μm, 4.6 × 250 mm. The flow rate was maintained at 1.9 mL/min, and the injection volume was 30 μL. The mobile phase was prepared daily and filtered through a 0.45 μm membrane filter.

Reagents

(**a**) *Methanol and acetonitrile*.—LC grade from Merck (Interlab, Ankara,Turkey).

(**b**) *Bidistilled water*.—Used throughout.

(**c**) *Colorants SUN (46,522-4) and TAR (20,195-2)*.—Aldrich products (Interlab). All the other chemicals were analytical-grade quality.

Standard Solutions

Stock solutions of 50 mg/100 mL SUN, TAR, and IS were prepared in 0.2 M acetate buffer (pH 5). A series of standard solutions of SUN and TAR over the concentration range of 6–22 μg/mL was prepared from the stock solutions. A validation set of 10 synthetic mixture solutions at the above concentration range of 6–22 μg/mL for SUN and TAR in the presence of the constant concentration of IS at 12 μg/mL was prepared. For the method of standard additions, 5 solutions including the stock solutions and the solutions of the analyzed commercial preparation were used. In all the chromatographic analyses, IS at 12 μg/mL was added to each solution. All the solutions were freshly prepared and protected from light. Under our experimental conditions, all prepared solutions were stable for at least 2 days.

Analysis of Commercial Preparation

An amount equivalent to 2 g soft drink powder (commercial preparation, Tang®, Marsa Kraft Jacobs Suchard Sabanci Food Ind., Adana, Turkey) was transferred to a 100 mL volumetric flask and dissolved in 0.2 M acetate buffer (pH 5). The solution was centrifuged 20 min at 5000 rpm, and the supernatant was used for the analysis after filtration with a 0.45 mm membrane filter. Chromatograms of the resulting solutions were plotted as time vs. detector responses. This procedure was repeated 10 times.

Results and Discussion

Method Development and Optimization

The objective of the present study was to apply the chemometric calibration models (CLS, PCR, and PLS) to the multiwavelength LC data for the determination of TAR and SUN in marketed samples. The simultaneous recording of multiple chromatograms at selected multiple wavelengths was performed by the PDA detector system used in LC. The PDA detector system allows recording of a multiwavelength LC data set of chromatograms having different peak areas. The application of the LC-chemometric approach is a new idea for the simultaneous determination analysis of SUN and TAR in samples.

The selection of a multiwavelength set for optimal chromatographic separation and detection plays an important role in the application of multivariate LC calibrations.

As in a single wavelength LC, the multiwavelength LC data analysis was obtained by calculating the peak-area ratio of each colorant to the IS versus its concentration. The multiwavelength LC data were used to construct the LC-chemometric calibrations as LC-CLS, LC-PCR, and LC-PLS. The elaborated LC-chemometric approaches were validated and compared with a classical LC method based on a single-wavelength detection response.

In our case, the main purpose of LC-chemometric techniques applied to the multivariate LC data was the elimination and reduction of the errors coming from sample injection, instrumental peak fluctuations, and experimental environment. We observed that the classical LC calibration graphs with single-wavelength detection do not produce successful results at every chosen wavelength for the determination of the subject colorants in samples. Although successful results were obtained by using the chromatographic detector response at 465 nm (A), satisfactory results at other wavelength points [470 nm (B), 475 nm (C), 480 nm (D), and 485 nm (E)] were not obtained with the application of the classical LC method. The reason for this experimental contradiction is that each analytical instrument produces noise, which causes error in analyses. Therefore, LC-chemometric calibrations using special mathematical algorithms allow the removal or reduction of the errors and residuals of calibration observed with classical LC based on single-wavelength detection. We observed that the sensitivity, accuracy, and precision of the elaborated LC-chemometric calibrations increased with the simultaneous use of the chemometric technique and multiwavelength LC data in comparison with the results obtained by the classical-LC method.

The application of the elaborated LC-chemometric algorithms given in the theoretical outline is explained below.

LC Data Processing

A training set of 5 mixed solutions containing SUN and TAR in the concentration range of 6–22 μg/mL with a constant amount of IS at 12 μg/mL was prepared in 0.2 M acetate buffer solution (pH 5). The peak areas of the training

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Table 1. Multivariate LC data set corresponding to the concentration set

	Training set, μg/mL			Peak-area ratio, TAR/IS					Peak-area ratio, SUN/IS				
No.	SUN	TAR	IS	465 nm (A)	470 nm (B)	475 nm (C)	480 nm (D)	485 nm (E)	465 nm (A)	470 nm (B)	475 nm (C)	480 nm (D)	485 nm (E)
1	6	6	12	0.4450	0.3443	0.2604	0.1917	0.1354	0.7881	0.7514	0.7111	0.6669	0.6162
2	10	10	12	0.7541	0.5827	0.4403	0.3262	0.2277	1.4110	1.3310	1.2593	1.1893	1.0980
3	14	14	12	1.0169	0.7907	0.5943	0.4411	0.3074	1.9042	1.8281	1.7191	1.6241	1.5031
4	18	18	12	1.2722	0.9690	0.7409	0.5509	0.3808	2.3430	2.2067	2.1015	1.9992	1.8387
5	22	22	12	1.5981	1.2412	0.9354	0.6921	0.4835	2.9932	2.8738	2.7013	2.5518	2.3577

set were recorded at the 5 wavelengths (465, 470, 475, 480, and 485 nm) and at the retention times of 2.012 min for IS, 1.603 min for SUN, and 1.137 min for TAR. The chromatograms of the training set for both colorants with IS are presented in Figure 1. The LC data set and training concentration set are presented in Table 1. The chemometric calibration techniques CLS, PCR, and PLS were applied to the training set and its measured LC area data set. SUN and TAR in synthetic mixtures and marketed samples were determined by the elaborated LC-chemometric approaches and classical LC.

LC-CLS Approach

The LC-CLS algorithm was briefly explained in the theoretical outline. In this approach, the coefficient vector matrix (K) was calculated by using the linear equation system based on the relationship between the peak-area data and the training set (Table 1). These LC data correspond to the chromatograms shown in Figure 2. By placing the coefficient matrix (K) into the linear equation system, LC-CLS calibration was obtained. SUN and TAR in samples were determined by the elaborated LC-CLS calibration. The calibration and data treatment were computed by using the CLS algorithm written in Matlab 7.0 software.

LC-PCR Approach

The LC-PCR approach based on the PCR algorithm as explained above was applied to the square matrix of peak-area data obtained by the decomposition of peak-area values. Linear correlation between the training set and the decomposed peak-area values was used to obtain the LC-PCR calibration. The data set shown in Table 1, which corresponds to Figure 2 was used for the LC-PCR approach. Analogous procedure was repeated for each colorant. The LC-PCR calibration was used for the determination of the 2 colorants in the synthetic mixtures and market samples. The LC-PCR calibration and data analysis were performed by using PLS toolbox 3.0 in Matlab 7.0 and Microsoft Excel, respectively.

LC-PLS Approach

The PLS algorithm described in the theoretical outline was applied to the LC data summarized in Table 1 and corresponding to Figure 2. In this LC-PLS approach, both the peak-area data and the concentration set were simultaneously

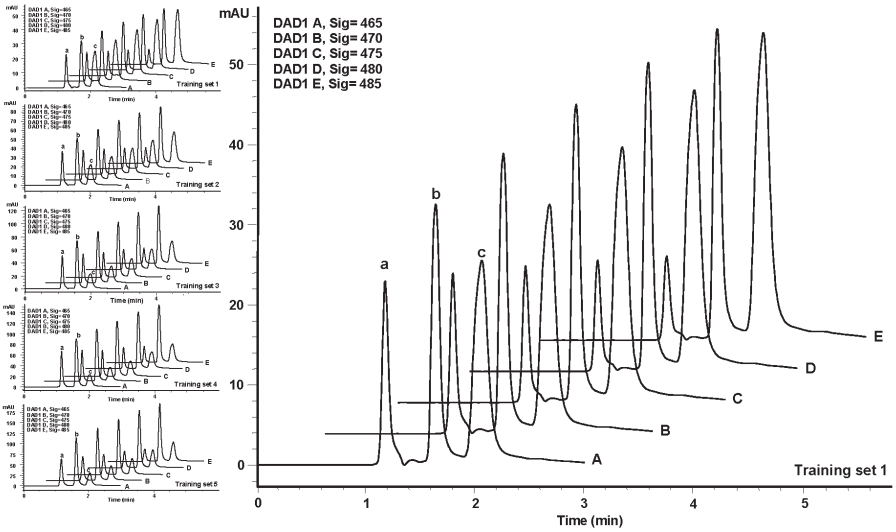


Figure 2. 3D chromatograms corresponding to (a) TAR at 6 μg/mL, (b) SUN at 6 μg/mL, and (c) IS at 12 μg/mL (training set 1). The small chromatograms show training sets 1–5.

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Table 2. Results of linear regression analysis of the classical LC calibration graphs obtained at 465 nm

Analyte	λ, nm	Equation	r[a]	S(a)[b]	S(b)[c]	S(r)[d]	LOD[e]	LOQ[f]
TAR	465	R = 0.0697 C + 0.0454[g]	0.9983	0.0474	0.0029	0.0255	0.4589	1.5298
SUN	465	R = 0.1296 C + 0.0887	0.9964	0.1294	0.0078	0.0696	0.7806	2.6019

[a] Correlation coefficient.
[b] Standard error of the intercept.
[c] Standard error of the slope.
[d] Standard error of the regression constant.
[e] Limit of detection.
[f] Limit of quantitation.
[g] R = Ratio of peak areas; C = concentrations in μg/mL.

decomposed. LC-PLS calibration is based on the use of the relationship between the decomposed peak-area data and the training set. The concentrations of SUN and TAR in the samples were predicted by the elaborated LC-PLS calibration. The mathematical treatments were performed by using the PLS toolbox 3.0 in Matlab 7.0 software and Microsoft Excel.

Classical LC Method

The chromatograms for SUN and TAR the concentration range of 6–22 μg/mL in the presence of IS at 12 μg/mL were plotted from PDA detection data obtained at the 5 wavelengths (Figure 2). The detector responses were measured in terms of peak area. Separation was obtained at ambient temperature on a Waters Symmetry C18 column, 5 μm, 4.6 × 250 mm, with 0.2 M acetate buffer (pH 5)–acetonitrile–methanol–bidistilled water (55 + 20 + 15 + 10, v/v) as the mobile phase. The flow rate was set at 1.9 mL/min with a 30 μL injection volume. Allura red as the IS is suitable for optimum chromatographic separation. In fact, different mobile phases and other chromatographic conditions were attempted, but the above-mentioned chromatographic conditions were found to be suitable for satisfactory separation in the presence of the IS. These same chromatographic conditions were used for both the LC-chemometric approach and classical LC. At a flow rate of 1.9 mL/min, retention times were 1.603 min for SUN, 1.137 min for TAR, and 2.012 min for IS (Figure 2).

The ratios of the peak areas (SUN/IS and TAR/IS) were computed by using the values obtained from the recorded chromatograms obtained for the 5-wavelength set [465 nm (A), 470 nm (B), 475 nm (C), 480 nm (D), and 485 nm (E); Table 1]. In this work, all of these selected wavelengths did not give satisfactory results for the single-wavelength determinations. Successful results were obtained only at 465 nm (A). A linear regression function for each colorant was calculated from the measured LC data obtained at a specific single wavelength (465 nm; A) from the above wavelength set. The 2 equations presented in Table 2 correspond to 465 nm (A) for SUN and for TAR. At the selected wavelength, satisfactory results were obtained for the analysis of the synthetic mixtures and marketed samples.

The linear regression functions and their statistical parameters are presented in Table 2. The slope and correlation coefficient values for the classical LC calibration graphs at 465 nm (A) were found to be more suitable than those at other wavelengths. These calibration equations gave good linearity and successful results.

The classical LC method was validated by applying the standard additions technique for 6 replicates. The results presented in Table 3 indicate that there is no interference from the excipients used in the market samples.

Table 3. Recovery results obtained by the elaborated LC-chemometric approaches[a] and the classical LC method[b]

	LC-CLS		LC-PCR		LC-PLS		Classical LC	
Parameter	SUN	TAR	SUN	TAR	SUN	TAR	SUN	TAR
Mean recovery, %	103.3	102.3	103.1	102.2	102.5	101.7	103.8	102.9
SD[c]	2.85	2.78	2.68	2.64	2.52	2.32	2.99	2.88
RSD, %[d]	2.76	2.72	2.60	2.58	2.46	2.28	2.88	2.79

[a] $n = 6$.
[b] Application of the standard addition technique; $n = 6$.
[c] SD = Standard deviation.
[d] RSD = Relative standard deviation.

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Statistical Parameters

For the LC-chemometric approaches, the predictive applicability of a chemometric calibration model can be defined in various ways. The most general expression of the predictive ability of the calibration is the standard error of calibration (SEC). In the elaborated LC-chemometric approaches, the 5 chromatograms corresponding to the training set with IS were subjected to the calibration steps used for the determination of the colorants. The SEC values of SUN and TAR were computed by using the data obtained from the difference between the added and predicted concentrations in the calibration steps for both colorants. The results of the linear regression analysis including the SEC values are given in Table 4. According to the cross-validation procedure, the first one factor for LC-PCR and LC-PLS gives a good prediction for both colorants.

In the LC-chemometric approaches, another important parameter is the standard error of prediction (SEP). The SEP values and the related statistical values were computed by using a procedure similar to that used to calculate the SEC values. The values obtained for SEP, correlation coefficient (r), slope (m), and intercept (n) are presented in Table 4. The statistical data indicate that the minimum values of SEC and SEP give acceptable results under optimized conditions in the calibration and prediction steps.

Validation of the Developed Methods

The linearity of the LC detector response for the determination of SUN and TAR at the 5 different wavelengths was established out by analyzing a series of different concentrations of each colorant. In accordance with the guidelines of the International Conference on Harmonization (ICH; 12), at least 5 concentrations must be used. In our study, 5 concentrations were planned, ranging from 6 to 22 μg/mL for SUN, and from 6 to 22 μg/mL for TAR. Each concentration was tested 3 times to provide information on the variation in the peak areas samples having the same analyte concentrations. The linearity of the individual calibration functions of the colorants at the 5 different wavelengths was confirmed by the high value of the correlation coefficient (Tables 2 and 4). For the classical LC method, 2 linear regression functions are shown in Table 2.

The precision of the LC-CLS, LC-PCR, LC-PLS, and classical LC methods was tested by 10 replicate determinations at different concentration levels. The relative standard deviations were between 2.26 and 5.29% as shown in Table 5.

The accuracy of the applied methods was tested by analyzing synthetic mixtures containing the analytes at different concentration levels. The average recoveries ranged from 96.9 to 103.2% (Table 5). A good agreement between the results was obtained for the LC-CLS, LC-PCR, LC-PLS, and classical LC methods. During the analytical procedure, interference and systematical error were not evident.

According to the ICH guidelines (12), the limit of detection (LOD) and limit of quantitation (LOQ) are calculated by using the standard deviation of the response and the slope of the calibration equation (Table 2).

Table 4. Statistical data obtained for the LC-chemometric approaches

	LC-PCR		LC-PLS		LC-CLS	
Method parameter	TAR	SUN	TAR	SUN	TAR	SUN
	Calibration step					
SEC[a]	0.1843	0.3517	0.1837	0.2930	0.3866	0.4490
r[b]	0.9994	0.9977	0.9994	0.9984	0.9987	0.9983
m[c]	1.0000	1.0000	1.0000	1.0000	1.0537	1.0283
n[d]	0.0002	0.0002	0.0004	0.0002	–0.7383	–0.3978
SE[e]	0.0225	0.043	0.0225	0.0358	0.0323	0.0370
	Prediction step					
SEP[f]	0.4325	0.3046	0.5648	0.6753	0.6514	0.7382
r	0.9908	0.9967	0.998	0.9995	0.9922	0.9948
m	1.0207	0.9599	1.0144	0.9219	1.0285	0.9494
n	-0.4101	0.5332	0.0488	0.6590	–0.4615	0.2528
SE	0.0519	0.0313	0.0591	0.0553	0.0477	0.0665

[a] Standard error of calibration.
[b] r = Correlation coefficient.
[c] m = Slope.
[d] *n* = Intercept.
[e] SE = Standard error of the correlation coefficient.
[f] Standard error of prediction.

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Table 5. Recovery data obtained by applying the elaborated LC-chemometric approaches and the classical LC method to synthetic mixtures

Added, μg/mL		Recovery, %							
		LC-CLS		LC-PCR		LC-PLS		Classical LC	
SUN	TAR	SUN	TAR	SUN	TAR	SUN	TAR	SUN	TAR
6	14	107.4	93.6	105.3	94.6	105.3	95.9	107.2	99.6
10	14	96.1	96.8	93.9	98.2	93.9	99.0	92.3	97.5
14	14	98.2	92.3	97.3	94.5	97.3	95.1	97.7	92.9
18	14	99.6	96.0	101.5	96.7	101.5	98.1	98.0	95.4
22	14	100.1	96.5	99.8	97.5	98.5	98.5	105.7	104.2
14	6	109.2	102.1	107.5	100.5	106.5	100.5	109.3	108.4
14	10	107.3	94.9	106.2	100.4	106.2	100.4	107.0	108.6
14	14	102.8	98.2	102.6	102.5	102.6	102.5	103.1	103.9
14	18	107.6	100.2	106.8	100.4	106.8	100.4	107.5	101.5
14	22	99.2	98.5	99.8	99.0	99.8	99.5	104.4	103.7
Avg. rec, %		102.8	96.9	102.0	98.4	101.8	99.0	103.2	101.6
SD[a]		4.75	2.98	4.47	2.64	4.43	2.24	5.46	5.22
RSD, %[b]		4.62	3.07	4.38	2.68	4.36	2.26	5.29	5.14

[a] SD = Standard deviation.
[b] RSD = Relative standard deviation.

The calibration range, based on the concentrations of each compound present in market sample product, was designated in the practical range, to give an accurate, precise, and linear response.

The matrix effect of the excipients in the market samples was tested by the elaborated methods. The results and the corresponding standard deviations were calculated (Table 3). The recoveries are averages obtained from the analysis of 5 replicates for each colorant. According to the results of the standard additions assay, we found that the matrix effect was not a source of any error for the determinations.

Analysis of a Commercial Preparation

The elaborated LC-chemometric approaches and the classical LC method were applied to the simultaneous determination of SUN and TAR in samples. The assay results for a commercial preparation are shown in Table 6. Statistical parameters, standard deviation, and percent relative standard deviations are also summarized in Table 6. These values are acceptable for the determination of both colorants in samples. Good agreement was obtained between the elaborated approaches.

The statistical test, *t*-test, and *F*-test were applied to the assay results obtained for market samples to determine the significance of the difference between the applied methods. By using the *t*-test and the *F*-test, LC-CLS, LC-PCR, and LC-PLS were compared with the classical LC method. The critical and calculated results are presented in Table 6. The calculated values from the statistical test did not exceed the critical statistical values, indicating that there was no significant difference among the methods. The numerical values of all the statistical tests indicated that the elaborated approaches are suitable for the determination of SUN and TAR in market samples.

Conclusions

In our study, the LC method based on a single-wavelength detection response was referred to as the classical LC method. In classical LC, it is not possible to find an optimum chromatographic condition for successful chromatographic separation and determination or all analytes in all cases. LC instrumentation, as with other analytical instrumentation, produces several errors derived from different injection conditions, fluctuations in peak shape and area, and noise, as well as from other sources. As demonstrated in this application, chemometric approaches are a promising tool for eliminating the above drawbacks. One of the main advantages of the elaborated LC-chemometric calibrations is the simultaneous data reduction and de-noising of the chromatograms. The LC-chemometric approaches remove or decrease some of the restrictions coming from the single-wavelength calibration used with classical LC methods.

LC-chemometric approaches have an important role in the evaluation of chromatograms obtained by using multiwavelength responses with PDA detection. The elaborated LC-CLS, LC-PCR, and LC-PLS calibration models were successfully applied to the determination of SUN and TAR in market samples.

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Table 6. Experimental results (mg/g) obtained by applying the elaborated LC-chemometric approaches and the classical LC method to a commercial preparation

Parameter	LC-CLS		LC-PCR		LC-PLS		Classical LC	
	SUN	TAR	SUN	TAR	SUN	TAR	SUN	TAR
Average	46.96×10^{-2}	40.85×10^{-2}	46.62×10^{-2}	40.50×10^{-2}	46.50×10^{-2}	40.58×10^{-2}	47.42×10^{-2}	41.12×10^{-2}
SD[a]	0.016	0.013	0.017	0.015	0.014	0.011	0.021	0.018
RSD[b]	3.55	3.252	3.698	3.763	3.093	2.601	4.354	4.257
SE[c]	0.006	0.005	0.006	0.005	0.005	0.004	0.007	0.006
CL[d] ($P = 0.05$)	0.004	0.003	0.004	0.004	0.003	0.003	0.005	0.004
t-test	0.293	0.306	0.070	0.138	0.148	0.168	*t*-critical = 1.895	
F-test	1.534	1.737	1.434	1.319	2.061	2.750	*F*-critical = 3.787	

[a] SD = Standard deviation.
[b] RSD = Relative standard deviation.
[c] SE = Standard error.
[d] CL = Confidence limit.

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