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Simultaneous Determination of Tartrazine and Allura Red in Commercial Preparation by Chemometric HPLC Method

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ABSTRACT

The concentrations of tartrazine (TAR) and allura red (ALL) in binary mixtures and commercial preparation were determined by the combined use of chemometric (or multivariate) calibrations and HPLC method. In this study, partial least squares (PLS), principal component regression (PCR) and classical least squares (CLS) based on multiwavelength HPLC data were refined as multivariate calibration techniques such as CPLS, CPCR, CCLS. The relation between multiwavelength peak area data (x-block) and concentration set (y-block) were used to obtain the chromatographic multivariate calibrations. Multiwavelength-chromatograms or multiwavelength peak area data were obtained by using photodiode array (PDA) detectors. Waters Symmetry® C18 Column 5 μm 4.6 × 250 mm and a combination of 0.2 M acetate buffer (pH = 5), acetonitrile, methanol and bidistilled water (55:20:10 v/v) at the flow rate of 1.9 mL/min were used to obtain a good chromatographic separation between TAR and ALL in presence of sunset yellow (Internal Standard (IS)). These chromatographic multivariate techniques were validated by analyzing the different synthetic mixtures and by using standard addition technique. These methods were applied to the commercial soft drinking powder samples containing TAR and ALL. The results from these chromatographic multivariate techniques were compared with each other as well as obtained by alternative single HPLC method.

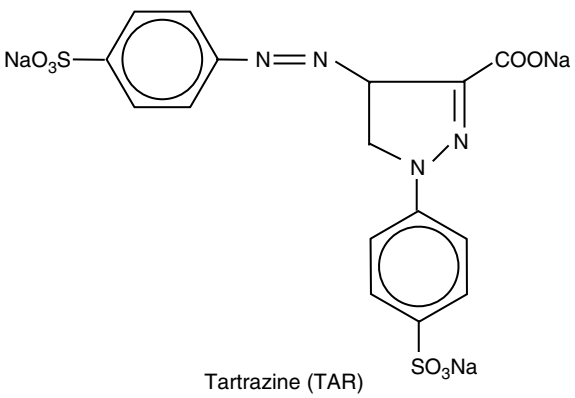
Key words: Chromatographic multivariate techniques, Simultaneous determination, Multiwavelength HPLC data processing, tartrazine, allura red

INTRODUCTION

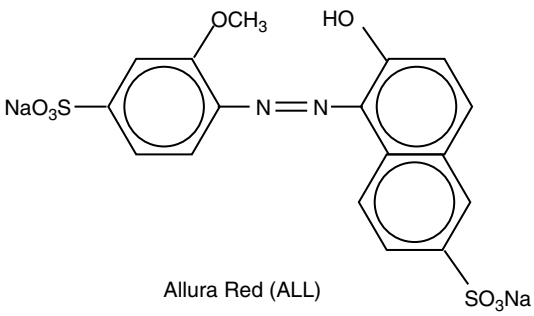
Tartrazine (TAR) and allura red (ALL) shown in scheme 1 are azo colorants used commercially in some coloring foods, such as soft drinks powders, drugs and cosmetics in order to make them attractive. These colorants cause toxic effects an human health if their quantities exceed the limits set by the laws and regulations. Therefore, the quantitative determination of TAR and ALL in synthetic mixtures and commercial soft drink powder is an interesting issue to be investigated.

The determination of TAR and ALL in samples with other colorants or other active compounds has been carried out by spectrophotometric method[(1-6)], chemometric techniques[(6-7)], polarography[(8)], capillary zone electrophoresis[(9)], HPLC[(10)] and bivariate calibration by spectrophotometry[(11)]. The investigations on the analytical applications reveal that the current efforts towards the development of the new methods having high selectivity and sensitivity have been continued for more quantitative resolution of the mixtures containing two or more active compounds in the presence of the sample matrix.

Although traditional analytical methods such as



Tartrazine (TAR)



Allura Red (ALL)

Scheme 1. Structure of the synthetic food colorants

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spectrophotometry, and HPLC method have been used for solving the above mentioned problems, these classical methods cannot provide successful results in some cases. For example, the traditional HPLC method is based on the peak area recorded at one specific wavelength to construct the linear regression equation. Of course, this traditional or classical HPLC method get more sensitive and selective determination than the spectrophotometric methods, but the chromatographic determination based on one specific wavelength brings calibration graph errors in the linear regression analysis due to chromatographic area errors coming from injection and instrumental fluctuations, etc sources. These mentioned reasons affect a deviation from the real analysis results. We believe that the combination of multivariate calibrations and multi-chromatograms recorded at multi-wavelengths PDA detector responses will be used to eliminate or reduce the above mentioned drawbacks.

The aim of this study is to apply simultaneously multiwavelength HPLC and multivariate calibration techniques to the multi-colorant determination for accurate, sensitive and reproducible analysis results. A good coincidence was observed in the application of CCLS, CPCR and CPLS methods to the simultaneous quantitation of TAR and ALL in samples.

MATERIALS AND METHODS

I. *Materials*

Chromatography was performed with an Agilent 1100 series HPLC system (Agilent Technologies, Inc., California, and USA) provided with a quaternary pump, a thermostatted autosampler, a thermostatted column compartment, and a multiwavelength diode array detector (DAD). Data was processed using HP Chem Station for LC (Rev. A0.01 [403]) software from Hawlet-Packard. The column used was a Waters Symmetry ® C18 Column 5 µm 4.6 × 250 mm. Flow rate was maintained at 1.7 mL/min and the injection volume was 30 µL. The mobile phase was prepared daily and filtered through a 0.45 µm membrane filter.

Stock solutions of 25 mg/50 mL TAR, ALL and IS (sunset yellow was used as the internal standard, IS) were prepared in 0.2 M acetate buffer (pH = 5). A series of standard solutions in the concentration range of 6-22 µg/mL for TAR and ALL in the presence of 10 µg/mL IS was prepared. Afterwards, an independent validation set consisting of various synthetic mixtures of two colorants and constant concentration IS was obtained from stock solutions.

II. *Methods*

During chemometric HPLC analysis, CLS, PCR and PLS calibrations[12-16] were applied to the ratio of the peaks area of analyzed colorants to IS at the five wavelengths using a PDA detector. The chromatograms of analyzed colorants are plotted and stored in computer. 10 µg/mL of IS was added to the injection samples for the chromatographic study. The detector responses were measured in terms of peak area. Chemometric calibration followed the procedure of the combined HPLC-multivariate calibrations as described below.

In the commercial preparation analysis, 2000 mg of soft drinking powder (commercial preparation) was transferred to a 100 mL calibration 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 analysis after filtration with 0.45 µm membrane filter. Chromatograms of the resulting solutions were plotted as time versus detector responses. The above procedure was repeated ten times.

The limit of detection (LOD: signal-to-noise ratio of 3:1) and the limit of quantitation (LOQ: signal-to-noise ration of 10:1) were calculated using the data obtained from six replicates for 14 µg/mL of standard solutions for both colorants. These results are shown in Table 2.

III. *Chromatographic Classical Least Squares Technique*

This approach is based on the application of multi linear regression (MLR) to the ratio of the peak area of individual colorants. Considering the responses as ratio values of peak area at five wavelengths (R) and five standard series (concentration set (C)) of analyzed colorants, the following linear system was obtained:

$$\begin{bmatrix} R_{11} & R_{12} & R_{13} & R_{14} & R_{15} \\ R_{21} & R_{22} & R_{23} & R_{24} & R_{25} \\ R_{31} & R_{32} & R_{33} & R_{34} & R_{35} \\ R_{41} & R_{42} & R_{43} & R_{44} & R_{45} \\ R_{51} & R_{52} & R_{53} & R54 & R55 \end{bmatrix} = \begin{bmatrix} K_{11} \\ K_{21} \\ K_{31} \\ K_{41} \\ K_{51} \end{bmatrix} x$$

$$\begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14} & C_{15} \end{bmatrix} \quad (1)$$

Here, R_{5x5} represents the matrix of the peak area responses (ratio of the peak area of analyte to the peak area of the internal standard), K_{5x1} is the matrix of the calibration coefficients and C_{5x1} denotes the concentration set of the analyzed compound.

The compact matrix form the equation (1) becomes

$$R_{5x5} = K_{5x1}\, C_{1x5} \quad (2)$$

By using the matrix calculation, the values of the matrix K_{5x1} is obtained by

$$K_{5x1} = R_{5x5}\, C^{T}_{5X1} \left[C_{1x5}\, C^{T}_{5X1} \right]^{-1} \quad (3)$$

where, C^{T}_{5X1} denotes the transpose of C_{1x5} and

$\left[C_{1x5}\, C^{T}_{5X1} \right]^{-1}$ represents the inverse of $C_{1x5}\, C^{T}_{5X1}$.

The mathematical computation is carried out by the following algorithm:

$$Ka_{1x5} = \frac{1}{[K^{T}_{1x5}\, K_{5x1}]} \times K^{T}_{1x5} \quad (4)$$

Ka_{1x5} is then introduced into the following equation

$$C_{prediction_{1xn}} = Ka_{1x5} \times R_{sample_{5xn}} \quad (5)$$

Finally, the concentration of the content of analyte in the mixture is determined by multiplying Ka_{1x5} by $R_{sample_{5xn}}$.

IV. *Chromatographic Principal Component Regression Technique*

The ratio (R) of the peak area of individual colorant and the colorant concentration set were reprocessed by mean-centering as R_o and C_o, respectively, within HPLC-PCR method and the covariance dispersion matrix of the centered matrix R_o was calculated. The normalized eigenvalues and eigenvectors were obtained starting from square covariance matrix. The number of the optimal principal components (eigenvectors (P)) is selected by taking into account only the highest values of the eigenvalues. The remaining part of eigenvalues and their corresponding eigenvectors are eliminated. To fulfill the above requirements, 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 having the components the inverse of the selected eigenvalues. The colorant content in samples was obtained using $C_{prediction} = b \times R_{sample}$. PLS toolbox 3.0 in Matlab 7.0 software was used for the data processing.

V. *Chromatographic Partial Least Squares Technique*

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

During the HPLC data analysis, the HPLC-PLS calibration is obtained by decomposition of both concentration and the ratio of 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 colorants in the samples. The vector, b is given as $b = W \times (P^T \times W)^{-1} \times Q$, where W represents a weight matrix. By using of PLS toolbox 3.0 in Matlab 7.0 software the above approach was performed.

RESULTS AND DISCUSSION

I. *Method Development and Optimization*

Improvement of multiwavelength PDA detector systems used in the HPLC instrument allows simultaneous detection of samples at multiwavelengths. The obtained multiwavelength detections produce different peak area information. Simultaneous data collection at multiwavelengths provides application of multivariate calibration technique to these multiwavelength HPLC data for quantitative studies. The application of multivariate methods CLS, PCR and PLS to the obtained chromatographic data is a new concept for the simultaneous chemometric analysis of TAR and ALL in samples.

The application of multivariate methods to the multiwavelength HPLC data requires collection of peak area at multiwavelengths of good absorption and good peak separation on the chromatograms as well as classical HPLC method.

Chromatographic multivariate calibrations require the same data process as single wavelength HPLC calculations. The peak area ratio to IS peak area was calculated for each colorant peak area. These peak area ratios as HPLC data set were used to obtain the multivariate calibrations CCLS, CPCR and CPLS.

To compare these HPLC-multivariate calibrations, the classical HPLC method using a single wavelength detection response was also applied to analyze mixtures of the two colorants. The experimental results of chromatographic multivariate calibration methods were compared with that of the classical-HPLC method.

Multivariate calibration techniques reduce or eliminate the errors from sample injection and experimental environment that affect the peak area. Therefore, chromatographic calibration permits to remove the errors and residuals of calibration of the classical HPLC based on a single wavelength. Sensitivity, accuracy and precision of the chromatographic multivariate calibrations increase with the combined use of chemometric algorithms and multiwavelent HPLC data.

The application of the multivariate calibration algorithms explained in theoretical section was given in the following section.

II. *Processing of HPLC Data*

The concentration set containing the mixture solution with the concentration of 6-22 μg/mL for both ALL and TAR and 10 μg/mL for IS was prepared. The peak area of concentration set was recorded at a five-wavelength set (465, 470, 475, 480 and 485 nm) and at the retention time of 1.14 min. for TAR , 2.01 min. for ALL, and 1.60 min. for IS. The multiwavelength chromatograms of concentration set in the working range for both colorants with IS are shown in Figure 1. The HPLC data set corresponding to the concentration set is presented in Table 1. CLS, PCR, and PLS were subject to the prepared concentration set and the measured HPLC data set. The concentration of TAR and ALL in samples were determined by the constructed chromatographic multivariate calibrations.

III. *Chromatographic Classical Least squares Technique*

In this mathematical approach, the coefficient

vector matrix (K) was calculated by using the linear equation system based on the relationship between the peak area data and concentration set (Table 1). This multiwavelength HPLC data set corresponds to the chromatograms shown in Figure 1. Replacing the coefficient matrix (K) into the linear equation system, the CCLS calibration was obtained. The prediction of unknown concentration of TAR and ALL in samples was performed by the CCLS calibration. The calibration and data treatment were calculated by using CLS algorithm written in Matlab 7.0 software.

IV. *Chromatographic Principal Component Regression Technique*

In this technique, the square matrix of peak area data was obtained by decomposition of peak area data. Linear correlation between concentration set and decomposed peak area was used to obtain the CPCR calibration. This procedure was separately repeated for both colorants. The obtained CPCR calibration was used for the determination of the above colorants in the synthetic mixtures and soft drink powder samples. The corresponding data in Table 1 and Figure 1 was used to obtain the CPCR calibration. Calculation of calibration and data processing was accomplished by the PLS toolbox 3.0 in Matlab 7.0.

V. *Chromatographic Partial Least Squares Technique*

PLS calibration algorithm was applied to HPLC data summarized in Table 1, which corresponds to Figure 1. In this calibration model, both peak area data and concentration set were decomposed. CPLS calibration was obtained by using the relationship between the decomposed peak area data and concentration set. The quantitative determination of the two colorants in samples was performed by the CPLS calibration. The mathematical calculations were using the PLS toolbox 3.0 in Matlab 7.0.

VI. *Classical HPLC Technique*

The multiwavelength chromatograms of the concentration range of 6-22 μg/mL for both TAR and ALL with 10 μg/mL IS were recorded by using diode array detector at the five-wavelength set as shown in Figure 1. The detector responses were measured in terms of peak area. Separation was obtained at the ambient temperature on Waters Symmetry ® C18 Column 5 μm 4.6 × 250 mm and mobile phase containing 0.2 M acetate buffer (pH = 5), acetonitrile,methanol and bidistilled water (55:20:10 v/v). The flow rate was set 1.9 mL/min with 30 μL as injection volume. IS was found suitable in our case as seen in Figure 1. In fact several mobile phase and other chromatographic parameters were tested. However, the above chromatographic conditions were found to be suitable for the separation and determination of TAR and ALL in their mixtures. The same conditions were used for the chromatographic multivariate calibrations. At a flow rate of 1.9 mL/min, retention time was 1.14 min. for TAR, 2.01 min. for ALL and 1.60 min. for IS (Figure 1).

For the calibration, the ratio of peak area of analyte to IS was recorded versus the concentration of TAR and ALL. Table 1 indicates the data of the ratio peak area obtained at the five wavelength set 465 (A), 470 (B), 475 (C), 480 (D), and 485 (E). In the above wavelength points, five straight lines for each colorant were obtained from the HPLC data given in Table1.

The calculated straight lines and their statistical parameters are shown in Table 2. The correlation coefficients of regression equations were generally higher than 0.99. At the subject wavelength point, the calibration equations gave good linearity and successful results for TAR and ALL. As shown in Table 2, two equations having the highest regression coefficients at 480 nm from the calculated calibration equations were chosen for analyzed procedure of TAR and ALL.

VII. *Statistical Analysis*

In the chromatographic multivariate calibrations, the predictive ability of a regression model can be defined by various ways. The most general expression for a calibration is the standard error of calibrations (SEC). In our case, five chromatograms corresponding to the concentration set with IS were used in calibration steps for both colorants. The SEC values of TAR and ALL were calculated by the data obtained from difference between added and predicted concentrations in the calibration steps of two colorants. The linear regression analysis and its other statistical results based on the relationship between added and predicted concentrations were obtained (Table 4). According to the cross validation procedure, the first two factors for CPCR and CPLS were found suitable for the prediction of TAR and ALL. The above SEC values and other statistical values, correlation coefficient (r), slope (m) and intercept (n) were calculated by the CPCR and CPLS calibrations obtained from the first two factors.

Standard error of prediction (SEP) is another important parameter of the multivariate calibration techniques. The SEP and their statistical values were calculated based on the difference between added and predicted concentrations in the synthetic mixtures. The obtained results, SEP, correlation coefficient (r), slope (m) and intercept (n) are presented in Table 4. All the statistical data indicated that the minimum values of SEC and SEP give satisfactory results under optimized conditions in the calibration and prediction steps.

VIII. *Validation of Applied Techniques*

The performance of CCLS, CPCR, CPLS and classical-HPLC was validated to control the reliable results of analysis. For this reason, ten different synthetic mixtures in the concentration range of 6-22 μg/mL for both ALL

Table 1. Multiwavelength HPLC data set corresponding to the concentration set

Concentration Set (μg mL-1)				The ratio of peak areas (TAR/IS)					The ratio of peak areas (ALL/IS)				
No	TAR	ALL	IS	465 (A)	470 (B)	475 (C)	480 (D)	485 (E)	465 (A)	470 (B)	475 (C)	480 (D)	485 (E)
1	6	6	10	0.3449	0.2805	0.2449	0.1700	0.1302	0.3219	0.3337	0.3219	0.3714	0.4035
2	10	10	10	0.5472	0.4445	0.3578	0.2811	0.2141	0.5354	0.5589	0.5902	0.6272	0.6806
3	14	14	10	0.7708	0.6257	0.5000	0.3931	0.2955	0.9345	0.9753	1.0231	1.0868	1.1786
4	18	18	10	0.9789	0.7935	0.6382	0.4983	0.3742	1.1680	1.2219	1.2825	1.3641	1.4803
5	22	22	10	1.2050	0.9951	0.7853	0.6168	0.4598	1.4475	1.5163	1.5920	1.6935	1.8373

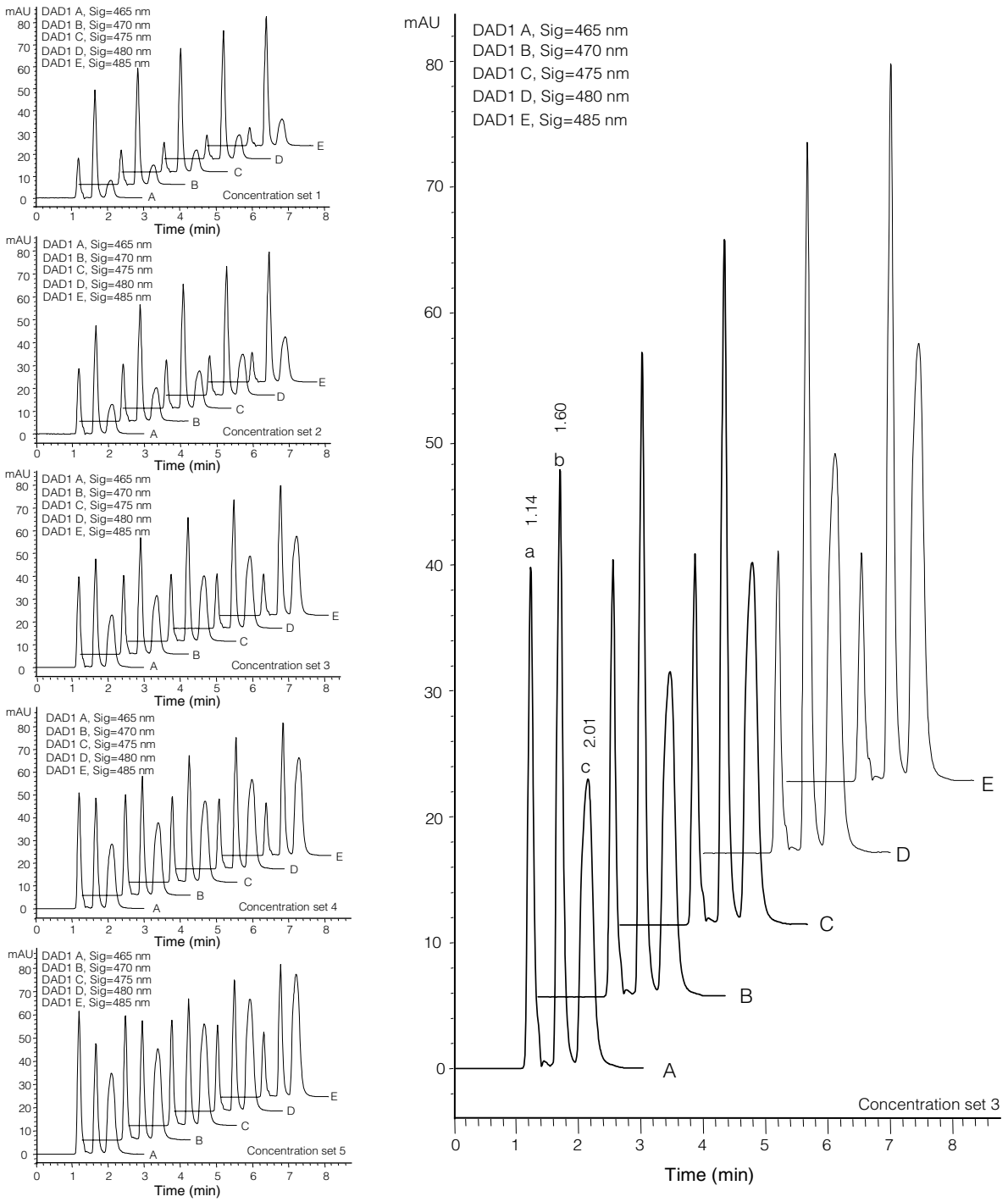

Figure 1. Chromatograms corresponding to the concentration set at five-wavelength sets, a) 14 μg/mL TAR, b) 10 μg/mL IS and (c) 14 μg/mL ALL.

and TAR with 10 μg/mL IS at a constant concentration were analyzed by proposed calibration techniques. The mean recoveries and the relative standard deviations of methods were calculated and presented in Table 3. Their numerical values were found satisfactory for the validity of CCLS, CPCR, CPLS and classical-HPLC. Accuracy and higher precision in application of these methods were observed for the analysis of both colorants., No interference and systematical error was reported during the analysis procedure.

In order to test the effect of excipients in commercial samples on the quantitative analysis, another parameter for validity of developed approaches is the standard addition technique after the analysis of commercial samples. Standard solutions of two pure colorants of equal content of the commercial samples were added to the commercial preparation in the working concentration range. All the proposed techniques were tested by applying the standard addition technique for five replicates. The percent recovery and relative standard deviations for ALL are 100.9% and 2.89 for classical HPLC, 99.5% and 2.78 for CCLS, 100.4% and 2.21 for CPCR and 99.8% and 1.85 for CPLS, respectively. For TAR we found the percent recovery and relative standard deviations to be 100.7% and 2.86 for

Table 2. Calculated straight lines and statistical parameters

	λ	Equation	r	S(n)	S(m)	S(r)	LOD (μg/mL)	LOQ (μg/mL)
	465	A = 0.0161 C + 0.0538	0.9998	0.0083	0.0006	0.0070	0.3370	1.1234
	470	A = 0.0445 C + 0.0055	0.9994	0.0136	0.0009	0.0114	0.6639	2.2129
TAR	475	A = 0.0340 C + 0.0288	0.9990	0.0132	0.0009	0.0111	0.8449	2.8163
	480	A = 0.0278 C + 0.0031	1.0000	0.0038	0.0003	0.0032	0.3007	1.0022
	485	A = 0.0205 C + 0.0080	0.9999	0.0022	0.0001	0.0018	0.2328	0.7760
	465	A = 0.0721 C - 0.1279	0.9961	0.0558	0.0037	0.0467	0.8620	2.8735
	470	A = 0.0757 C - 0.1387	0.9963	0.0573	0.0038	0.0480	1.0373	3.4578
ALL	475	A = 0.0808 C - 0.1694	0.9969	0.0552	0.0037	0.0462	0.9736	3.2454
	480	A = 0.0845 C - 0.1584	1.0000	0.0612	0.0041	0.0513	0.9911	3.3038
	485	A = 0.0917 C - 0.1675	0.9990	0.0662	0.0044	0.0554	0.7255	2.4185

SE(m) : Standard error of slope,
SE(n) : Standard error of intercept,
SE(r) : Standard error of regression constant
C : Concentration (μg/mL)
A : Peak area
r : Regression coefficient

Table 3. Recovery results obtained by applying the proposed methods to the synthetic mixtures

Added		Recovery (%)							
μg/mL		Classical HPLC		CCLS		CPCR		CPLS	
ALL	TAR	ALL	TAR	ALL	TAR	ALL	TAR	ALL	TAR
6	8	106.0	101.2	104.0	100.8	102.3	100.6	101.8	100.3
10	8	100.6	97.0	100.4	98.5	100.3	98.6	100.1	99.4
14	8	104.6	101.4	103.6	101.0	103.2	100.8	101.8	100.3
18	8	97.3	101.6	97.9	101.3	98.6	100.9	99.2	100.6
22	8	105.4	101.4	105.2	101.3	105.2	100.9	104.8	100.6
18	6	102.0	103.1	102.3	103.0	102.3	101.8	101.8	101.3
18	10	97.2	103.1	97.8	102.7	97.8	101.8	98.7	101.3
18	14	99.6	106.4	99.8	105.7	99.8	104.3	99.9	98.7
18	18	101.0	96.2	100.8	97.0	100.8	98.1	100.7	98.3
18	22	100.0	99.7	100.1	99.8	100.1	99.9	100.1	100.0
	Average	101.4	101.1	101.2	101.1	101.1	100.8	100.9	100.1
	SD	3.12	2.97	2.51	2.43	2.23	1.73	1.76	1.01
	RSD	3.08	2.93	2.48	2.40	2.21	1.72	1.74	1.01

SD: Standard deviation
RSD: Relative standard deviation

classical HPLC, 99.7% and 2.70 for CCLS, 100.5% and 2.19 for CPCR and 99.9% and 1.87 for CPLS, respectively.

The above mentioned recovery results were obtained as the average of five replicates for each colorant. A good agreement was reported for the standard addition assay results by application of methods. In addition, the results indicated that there is no effect of matrix or excipients on the analysis of commercial preparation containing the subjected colorants.

IX. *Sample analysis*

CCLS, CPCR, CPLS and classical-HPLC techniques were applied to the quantitative analysis of TAR and ALL in commercial samples. The experimental results of soft drink form are presented in Table 5. The results of all the applied technique were very close to each other, indicating precision and accuracy. Consistency was observed for all the proposed techniques.

CONCLUSIONS

HPLC method is a commonly used reference method for the analysis of samples. This study employed a classical HPLC method. For a good separation and determination, it is not possible to find chromatographic condition and optimization in all cases. For this reason, chromatographic multivariate calibration technique plays an important role for the evaluation of chromatograms at the multiwavelength points in the presence of PDA responses. As an alternative combined calibration technique, CCLS, CPCR and CPLS calibration models were proposed to different calibration approaches for simultaneous predic-

Table 4. Statistical calculations of HPLC-multivariate calibrations

Method		Classical HPLC		CCLS		CPCR		CPLS	
Parameter		TAR	ALL	TAR	ALL	TAR	ALL	TAR	ALL
Calibration step	SEC	0.0776	0.8201	0.2008	0.4456	0.0924	0.4689	0.0796	0.3737
	r	0.9999	0.9937	0.9997	0.9986	0.9999	0.9972	0.9999	0.9984
	m	0.9998	0.9509	1.0195	0.9659	1.0000	1.0000	1.0000	1.0098
	n	0.0021	0.9024	-0.3195	0.6491	0.0000	0.0000	0.0000	-0.2241
	SE	0.0896	0.8192	0.1754	0.3819	0.1067	0.5415	0.0919	0.4107
Prediction step	SEP	0.4084	0.5386	0.3474	0.4113	0.2623	0.3792	0.1317	0.3325
	r	0.9972	0.9943	0.9980	0.9995	0.9990	0.9948	0.9998	0.9999
	m	1.0138	0.9845	1.0114	0.9219	1.0058	0.9494	1.0132	0.7911
	n	-0.2375	0.0784	-0.2142	0.6590	-0.1318	0.2528	-0.1280	1.6814
	SE	0.4159	0.5318	0.3494	0.5538	0.2492	0.6658	0.1178	1.0424

SEP : Standard error of prediction
SEC : Standard error of calibration
n : intercept
m : slope

Table 5. Experimental results of commercial preparation by the proposed methods

	TAR (μg/g)				ALL (μg/g)			
	Classical HPLC	CCLS	CPCR	CPLS	Classical HPLC	CCLS	CPCR	CPLS
	0.5125	0.5135	0.5137	0.5138	0.5983	0.6169	0.6146	0.6136
	0.4871	0.4875	0.4884	0.4885	0.6038	0.6109	0.6161	0.6141
	0.4951	0.4944	0.4954	0.4955	0.6058	0.6251	0.6163	0.6163
	0.4649	0.4871	0.4862	0.4964	0.5957	0.6139	0.6070	0.6070
	0.4907	0.4900	0.4918	0.4921	0.5940	0.6265	0.6052	0.6002
Mean	0.4901	0.4945	0.4951	0.4973	0.5995	0.6186	0.6118	0.6102
SD	0.0153	0.0099	0.0098	0.0087	0.0046	0.0061	0.0048	0.0059
RSD	3.1239	1.9953	1.9828	1.7558	0.7624	0.9895	0.7770	0.9658
SE	0.0054	0.0035	0.0035	0.0031	0.0016	0.0022	0.0017	0.0021
CL (P = 0.05)	0.0037	0.0024	0.0023	0.0021	0.0011	0.0015	0.0011	0.0014
t-test	2.1318	0.1884	0.1428	0.1503	0.0000	0.0045	0.0001	0.0009

tion of colorant amount in samples. In this study, a good chromatographic separation and higher peak area were obtained with satisfactory optimization and condition. In addition, consistency was observed for the results of the chromatographic multivariate approaches. This new application of chemometric calibration technique to the HPLC data set is an alternative model for the minimization of experimental errors in chromatographic analysis.

The chromatographic multivariate calibration techniques can be successfully applied to the routine quality control analysis of colorants in commercial samples.

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