

Rapid Simultaneous Spectrophotometric Determination of Food Dyes in Soft Drink Using Continuous Wavelet Transform and Multivariate Calibration Methods

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ABSTRACT: In this study, the spectrophotometric technique along with chemometrics methods have been developed for the simultaneous determination of Sunset Yellow (SY) and tartrazine (TZ) in mixtures and soft drinks without any prior separation or purification. The first method is the Continuous Wavelet Transform (CWT), in which various wavelet families were investigated. Eventually, the Gaussian wavelet family of the second order with a scaling factor of 5 for SY and the Daubechies wavelet families of the second order with a scaling factor of 64 for TZ were selected. Both dyes showed good linearity, with the coefficient of determination (R^2) of 0.996 and 0.9981 for SY and TZ, respectively. The Limit of Detection (LOD) and Limit of Quantification (LOQ) values were 0.1794, 0.0003 $\mu\text{g/mL}$ and 0.6066, 0.0983 $\mu\text{g/mL}$ for SY and TZ, respectively. On the other hand, multivariate calibration methods, including Partial Least Squares (PLS) and Principal Component Regression (PCR) were studied. The PLS recovery range was obtained from 100.06% to 103.06% and 100.86 to 103.49 for SY and TZ, respectively. Also, the mean recovery (%) of the PCR method was 101.41 and 102.90 for SY and TZ, respectively. In addition, values of RMSE related to the PLS and PCR were achieved 0.2578, 0.3023, and 0.2635, 0.5602 for SY and TZ, respectively. High-Performance Liquid Chromatography (HPLC) as a reference method was applied for the soft drink, and the results were compared with the proposed methods using a one-way analysis of variance (ANOVA). There was no significant difference between them.

KEYWORDS: Spectrophotometry; Sunset yellow; Tartrazine; Continuous wavelet transform; Multivariate calibration; High-performance liquid chromatography.

INTRODUCTION

One of the important groups of food additives is dyes [1]. The quality and attractiveness of the food depend on the color.

The resistance of natural colors versus the various processes such as heat is low. But synthetic dyes are cheaper and

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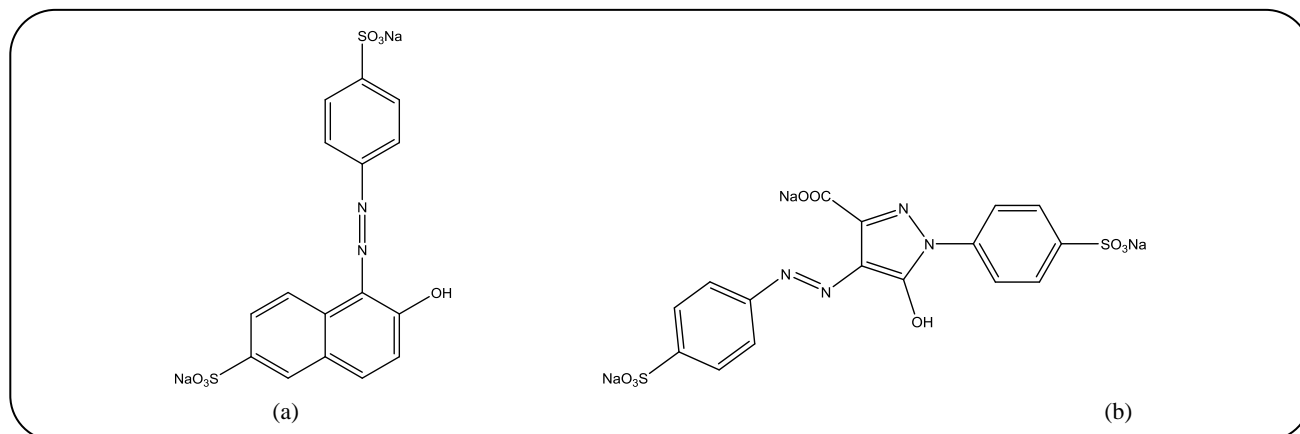


Fig. 1: Chemical structure of (a) SY and (b) TZ.

a more reliable way of providing color for the desired products [2]. Synthetic dyes are used to add to foodstuffs and nonalcoholic beverages to improve their appearance, color, taste, and texture. The advantages of synthetic dyes include high stability against light, oxygen, and pH, as well as uniform color, low microbiological pollution, low cost of production, etc. [3,4]. Sunset Yellow (SY) and Tartrazine (TZ) (Fig. 1) belong to the azo dyes group. Adding these dyes to carbonated beverages, candy, prawn slices, cakes, and so on is usually done simultaneously [5].

Azo dyes contain an azo functional group and aromatic ring that can be harmful to human health. Excessive use of these dyes can cause overactive behavior in children [6,7]. In addition, these dyes can cause allergies and asthma [8,9]. The required amount of SY and TZ when used separately are less than 50 and 100 ppm, respectively. Also, in combination mode no more than 100 ppm [10].

Several methods such as high-performance liquid chromatography (HPLC) [11,12], reversed-phase high-performance liquid chromatography (RP-HPLC) [13,14], Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) [15,16], Thin-Layer Chromatography (TLC) [17], High-Performance Thin-Layer Chromatography (HPTLC) [18], electrochemical [19-21], voltammetry [22], and spectrophotometry [23] have been given in the literature for the simultaneous determination of SY and TZ. The disadvantages of chromatography techniques include time-consuming, sophisticated instruments, and costly. Also, owing to the use of various solvents, they are not environmentally friendly [24,25]. A suitable, simple, inexpensive, and alternative method for routine laboratories is spectrophotometry. Overlapping the spectra

of components is one of the problems of this method, which makes it difficult to measure them simultaneously. Under chemometrics methods along with spectrophotometry procedure, quantitative simultaneous determination of components is possible without the need for separation [26].

In this study, Continuous Wavelength Transform (CWT) and multivariate calibration as robust chemometric methods coupled with spectrophotometry technique was proposed for the simultaneous determination of SY and TZ in a soft drink. The obtained results of the suggested method were compared with the results of the HPLC as the reference method by one-way analysis of variance (ANOVA).

THEORETICAL SECTION

Continuous wavelet transform

These days, signal processing is taken into consideration, and wavelet transform resulting from the Fourier transform is one of the most efficient methods in this field. Cutting up data, functions, or processing into various frequency components is performed by this technique. Afterward, this method can examine each component with a resolution corresponding to its scale. Signal analysis is performed by the foundation of the wavelet theory (set of basic functions). A family of functions, derived from the main function via scaling and translation is the Mother wavelet. When the Mother wavelet was selected by $\psi(\lambda)$, $\psi_{a,b}$ represented a set of functions and it is obtained as Eq (1).

$$\Psi_{a,b}(t) = \frac{1}{\sqrt{a}} \psi\left(\frac{t-b}{a}\right) \begin{cases} a, b \in \mathbf{R} \\ a \neq 0 \end{cases} \quad (1)$$

In this equation, controlling the scaling is indicated by a that shows the variable scale parameter. Also, b and R are the translation parameter controlling the translation and amplitude of actual numbers, respectively.

A certain signal related to the CWT can be defined by Eq (2).

$$\text{CWT}(a, b) = C(a, b) = \int_{-\infty}^{+\infty} f(t) \psi_{a,b}^*(t) dt = \langle f(t), \psi_{a,b} \rangle \quad (2)$$

The superscript $*$ indicates the complex conjugate and $\langle f(t), \psi_{a,b} \rangle$ specifies the inner product of function $f(t)$ on the wavelet function $\psi_{a,b}(t)$. The wavelet ψ will denominate invertible if it fulfills the condition expressed in Eq (3) [27-29].

$$\text{CW} = \int_{-\infty}^{+\infty} \frac{|\Psi(\omega)|^2}{\omega} d\omega < \infty \quad (3)$$

Multivariate calibration method

Partial Least Squares (PLS)

The basis of PLS is the relationship between the multivariable data and specifications of the sample multicomponent analysis. A calibration set of reference samples, containing all expected chemical and physical changes of unknown samples will be collected to create a model. The development of a multiple linear regression between multivariable data and the evolution of kinetic is the aim of this calibration. The fixing of the number of latent variables is enforceable via the cross-validation method. Optimizing the number of hidden variables requires sample prediction independent of the single model. Then, the validity of the model is determined by the prediction set [30,31]. PLS proposes a full-spectrum approach, which can be an efficient outlier detection technique available from spectral residuals. The extraction of chemically interpretable spectral information is possible by this method. This benefit allows for a fast estimation of mixture components without the need for prior separation or sample pre-treatment [32].

Principal Component Regression (PCR)

Two methods containing Principal Component Analysis (PCA) and multiple linear regression (MLR) create the PCR method. Modeling variables of the PCA method have two parts, which include Principal Components (PCs) and

residual components. The weight of modeling variables is shown by PCs that they are not interdependent with each other. The first few independent PCs can develop the MLR model. Hence, using the PCR model, the correlation of the modeling, the variable resolves on the predicted yield [33,34].

EXPERIMENTAL SECTION

Chemicals

SY and TZ were purchased from Merck. The soft drink was obtained from the Khosrovar company. In order to prepare all solutions, deionized water was used.

Apparatus and software

The UV-visible spectrum was recorded by a T90+UV/Vis spectrometer (PG Instruments Ltd). Analysis of HPLC was carried out by the Agilent 1100 series.

Standard dye solutions

Stock solutions of SY and TZ were prepared individually by dissolving 12.5 mg of each dye in deionized water and their volume was made to the mark in 250 mL volumetric flask. Then, standard solutions were obtained by dilution of the stock solutions in the range of 9-20 and 5-20 $\mu\text{g mL}^{-1}$ for SY and TZ, respectively.

Synthetic mixtures preparation

In order to evaluate the validity of the proposed methods, twenty synthetic mixtures with different ratios of SY and TZ stock solutions were made.

Procedure for real sample

The soft drink sample was purchased from a supermarket in Iran and it was provided during the following steps. Three various concentrations of standard solutions related to the SY and TZ were prepared. Then, 1 mL of each standard solution was separately added to the 1 mL of soft drink sample and adjusted to the volume in a 25 mL volumetric flask with deionized water at room temperature. Finally, the absorption of these samples was recorded.

Chromatographic conditions

HPLC analyses were performed using C8 column (250 mm \times 4.6 mm id, 5 μm particle size). The mobile phase system containing acetonitrile, methanol, and ammonium acetate buffer at pH 6.7 (80:10:10 v/v) as mobile phase A, as well as acetonitrile, methanol, and ammonium acetate

buffer (55:20:25 v/v) as mobile phase B, was used. Separation was obtained at a flow rate of 1 mL/min. The UV detector was programmed at 480 and 420 nm for monitoring SY and TZ, respectively.

RESULTS AND DISCUSSION

Spectral characteristics

The colorant spectra of the SY and TZ are overlapped, which is shown in Fig. 2. Therefore, the spectral separation of the dye mixtures is not simple with conventional spectrophotometric techniques. In order to solve this problem, CWT and multivariate calibration procedures were applied along with the UV-Vis method.

Continuous wavelet transform method

In order to find optimum signal processing settings, different wavelet families with diverse scales were evaluated and proper calibration graphs corresponding to the SY and TZ were obtained. Finally, the Gaussian wavelet family of second-order (Gaus2) with scaling factor (a) of 50 at the wavelength equal to 230 nm (related to the zero-crossing point of TZ) and the Daubechies wavelet family of the second-order (db2) using $a=64$ in the wavelength of 373 nm (related to the zero-crossing point of SY) were found as the optimal families for simultaneous estimation of SY and TZ, respectively. The obtained absorption spectra of the standard solutions related to the SY and TZ were transmitted into the wavelet amplitude via gaus2 and db2 wavelets, respectively. The amplitudes versus wavelength of these dyes are illustrated in Fig. 3 (a) and (b).

Also, the calibration curves of the standard solutions for the mentioned components, which were measured at different concentrations and evaluated with the selected wavelet families are exhibited in Fig. 4. As can be seen, the correlation coefficient (R^2) is close to one, which represents the strong intensity of the linear relationship between the two variables.

The statistical results of this method are given in Table 1. According to this table, the calculated values of Limits of Detection (LOD) and Limits of Quantitation (LOQ) of the proposed method are acceptable. Moreover, the plots of predicted concentrations against the actual concentrations are indicated in Fig. 5. As can be observed, R^2 is close to one. In addition, Table 2 represents the ability of this method for predicting because of the admissible recovery percent and low RMSE of SY and TZ.

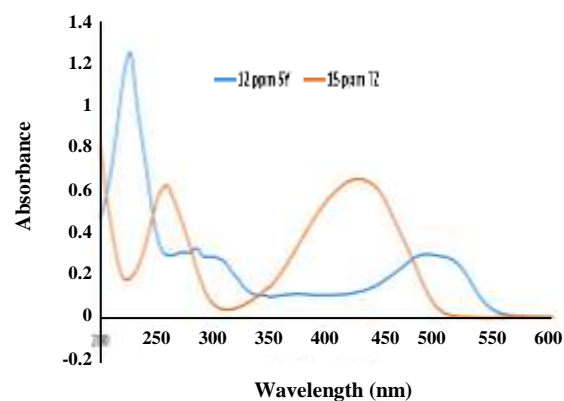


Fig. 2: The absorption spectra of SY and TZ.

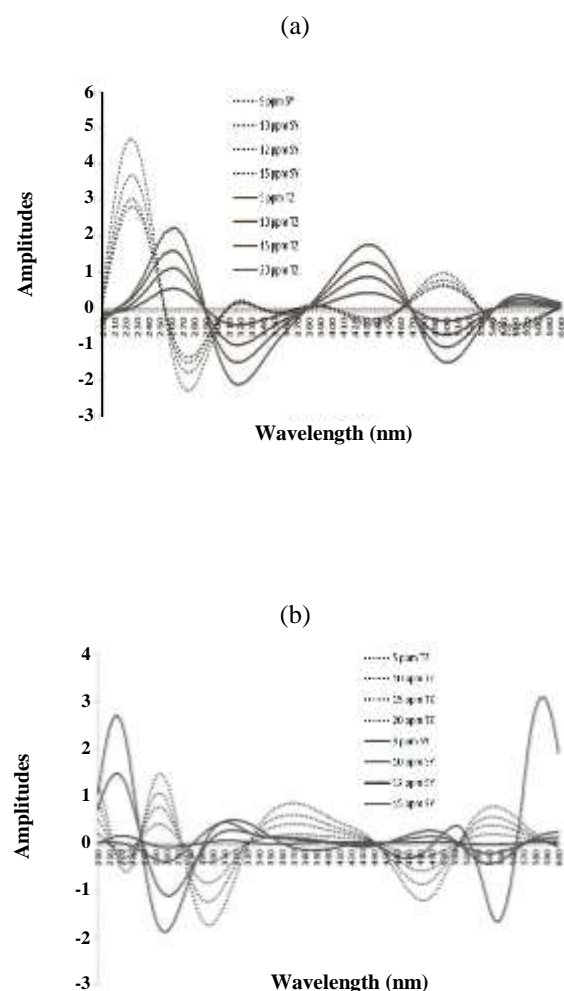


Fig. 3: Spectra of (a) CWT-gaus and (b) CWT-db2 for different concentrations of SY and TZ.

Table 1: The statistical results of calibration standard solutions graphs obtained by the CWT method.

Parameter	CWT	
	SY	TZ
Wavelength (nm)	230	373
Linear range ($\mu\text{g mL}^{-1}$)	9-15	5-20
Slope (a)	0.2977	0.0433
Intercept (b)	-0.0034	-0.0417
Regression coefficient (R^2)	0.996	0.9981
Correlation coefficient (r)	0.99799	0.99904
LOD ($\mu\text{g mL}^{-1}$)	0.1794	0.0003
LOQ ($\mu\text{g mL}^{-1}$)	0.6066	0.0983

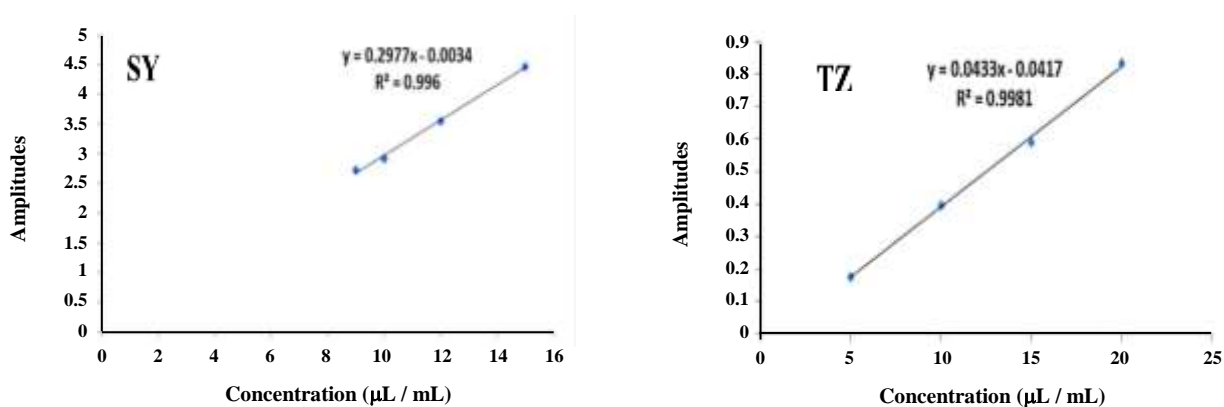
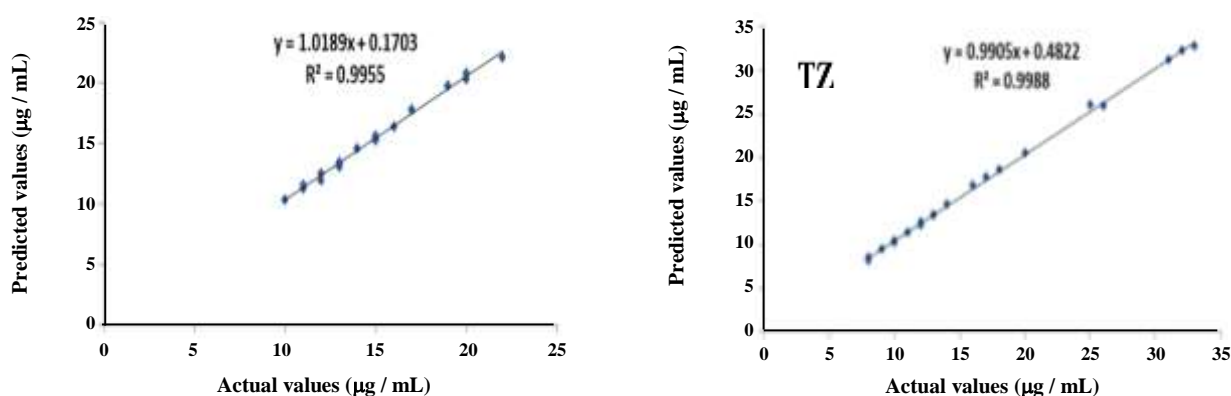
**Fig. 4: Gaus linear calibration graph for determination of SY and db2 linear calibration graph for determination of TZ.****Fig. 5: Actual versus predicted concentrations for SY and TZ.**

Table 2: Recovery data obtained by application of the CWT method in 20 mixtures.

Actual ($\mu\text{g}/\text{mL}^{-1}$)		Found ($\mu\text{g}/\text{mL}^{-1}$)		Recovery (%)	
SY	TZ	SY	TZ	SY	TZ
13	11	13.39	11.34	103.00	103.09
14	12	14.55	12.17	103.92	101.41
20	18	20.8	18.48	104.00	102.66
15	14	15.25	14.6	101.66	104.28
16	12	16.42	12.49	102.62	104.08
17	16	17.75	16.72	104.41	104.50
15	17	15.64	17.62	104.26	103.64
22	13	22.16	13.24	100.72	101.84
17	20	17.8	20.38	104.70	101.90
19	13	19.76	13.4	104.00	103.07
20	26	20.3	25.82	101.50	98.78
15	25	15.62	25.95	104.13	103.80
12	33	12.54	32.7	104.50	99.09
13	32	13.49	32.23	103.76	100.71
15	31	15.35	31.07	102.33	100.22
11	10	11.55	10.07	105.00	100.70
11	9	11.3	9.41	102.72	104.55
12	8	11.97	8.03	99.75	100.37
13	10	13.09	10.32	100.69	103.20
10	8	10.35	8.38	103.50	104.75
Mean Recovery				103.05	102.33
RMSE				0.5100	0.4326

Multivariate calibration methods

In order to perform PLS and PCR methods, cross-validation (k-Fold Cross Validation) was applied to select the optimum number of components based on the minimum mean square error prediction (MSEP). Fig. 6 displays the results of this optimization. It can be concluded that the optimal number of components related to the PLS model are 5 and 2 with MSEP of 0.066 and 0.441 for SY and TZ, respectively. On the other hand, 0.069 and 0.444 are the lowest errors of the PCR method, which are related to the 6 and 5 components for SY and TZ, respectively.

In these proposed methods, 20 mixtures were

divided into the training set (14 mixtures) and a test set (6 mixtures) as calibration and validation sets, respectively. Also, predicted values versus actual values of the validation set were plotted and demonstrated in Fig. 7. It can be seen that R^2 of the PLS and PCR methods are close to one. So, the intensity of the linear relationship between the predicted and actual values is strong. Furthermore, recovery (%), the mean recovery (%), and RMSE of the validation set are given in Table 3. The results showed that the function of the PLS method was a little better than PCR. The results showed that the function of the PLS method was a little better than PCR.

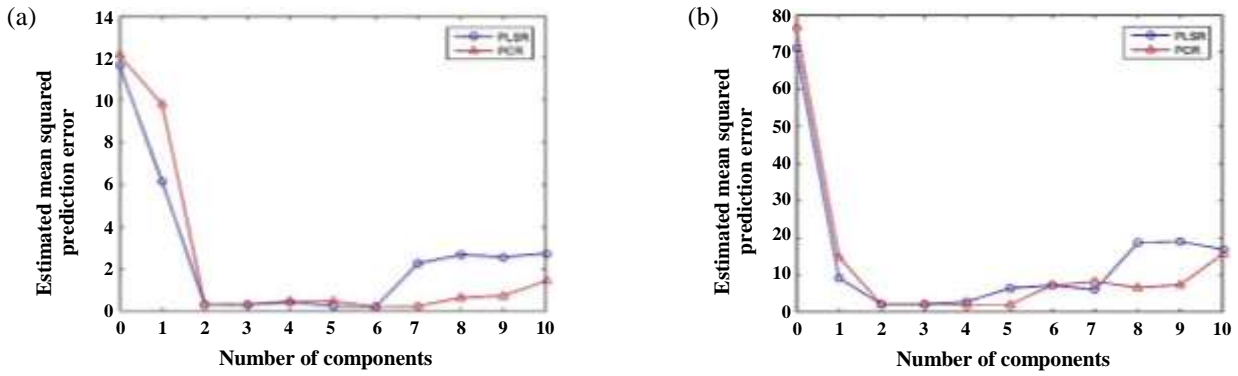


Fig. 6: MSEP versus number of components for (a) SY and (b) TZ in PLS and PCR methods.

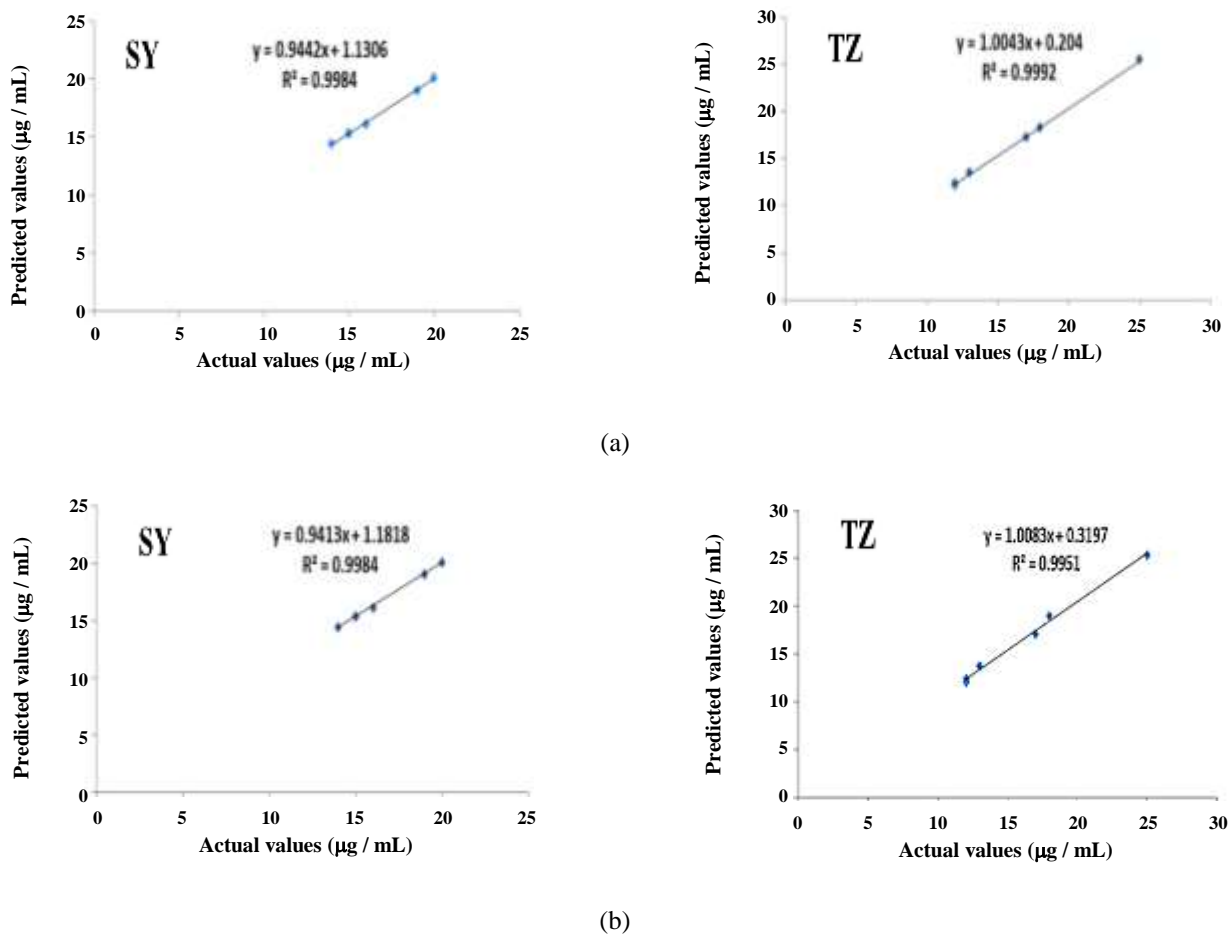


Fig. 7: Actual versus predicted concentrations for SY and TZ by (a) PLS and (b) PCR methods.

High-performance liquid chromatography

Calibration curves of HPLC method were investigated in the range of 10 to 100 ppm for the SY and TZ (Fig. 8). The coefficients of determination (R^2) indicate the linearity. As shown in Fig. 8, R^2 equal to 0.9999 and

0.9996 were obtained for SY and TZ, respectively. After analyzing the soft drink, the retention times (t_R) of the components were achieved at 8.406 and 2.894 min for SY and TZ, respectively. The chromatograms of the soft drink sample are demonstrated in Fig. 9.

Table 3: Recovery results of the mixtures for PLS and PCR.

Mixtures	Add ($\mu\text{g/ml}$)		Found ($\mu\text{g/ml}$)		Recovery (%)	
	SY	TZ	SY	TZ	SY	TZ
1	14	12	14.42875	12.10433	103.06	100.86
2	20	18	20.10763	18.19494	100.53	101.08
3	16	12	16.08835	12.35069	100.55	102.92
4	15	17	15.33182	17.14771	102.21	100.86
5	19	13	19.01174	13.45474	100.06	103.49
6	15	25	15.29206	25.38439	101.94	101.53
Mean Recovery					101.39	101.79
RMSE					0.2578	0.3023
PCR						
1	14	12	14.43992	12.09066	103.14	100.75
2	20	18	20.09969	19.00560	100.49	105.58
3	16	12	16.09584	12.35213	100.59	102.93
4	15	17	15.34042	17.13711	102.26	100.80
5	19	13	19.00924	13.75957	100.04	105.84
6	15	25	15.29645	25.37875	101.97	101.51
Mean Recovery					101.41	102.90
RMSE					0.2635	0.5602

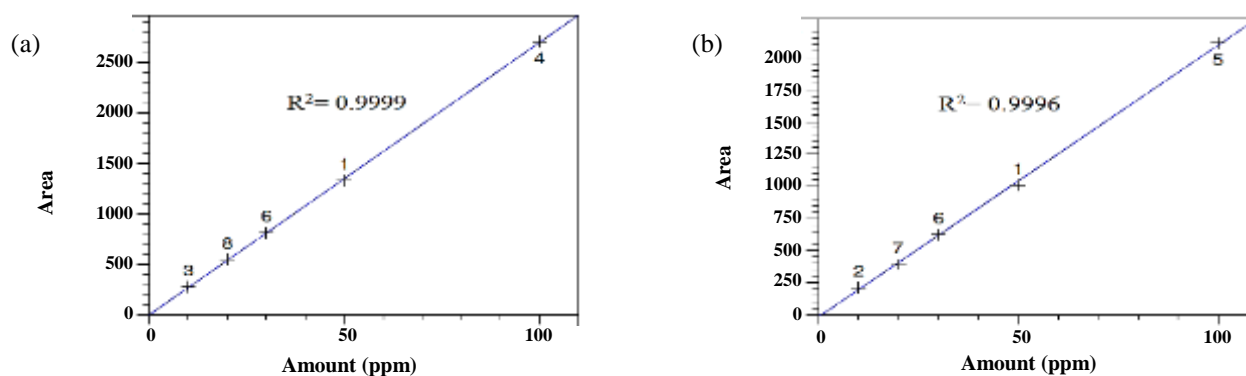


Fig. 8: Calibration curves of (a) SY and (b) TZ for HPLC method.

Analysis of soft drink sample

The proposed methods were used for the simultaneous estimation of SY and TZ in the available commercial beverage sample. The obtained results by the CWT, PLS, and PCR methods were close to the HPLC results. Also, low values of RSD (%) revealed that the developed

methods have high precision (Table 4). According to the ANOVA test, in terms of accuracy and precision, there is no significant difference between the suggested methods and HPLC technique because the values of F calculated (SY=0.000192 and TZ=0.000387) are less than the F critical (4.256494) (Table 5).

Table 4: Results of analyzing soft drink by the proposed and reference methods.

Method	SY				TZ			
	CWT	PLS	PCR	HPLC	CWT	PLS	PCR	HPLC
Recovery ^a (%)	98.25	99.27	98.73	100.44	98.08	99.10	98.11	98.08
RSD (%)	0.105	0.067	0.089	0.051	0.155	0.099	0.114	0.067

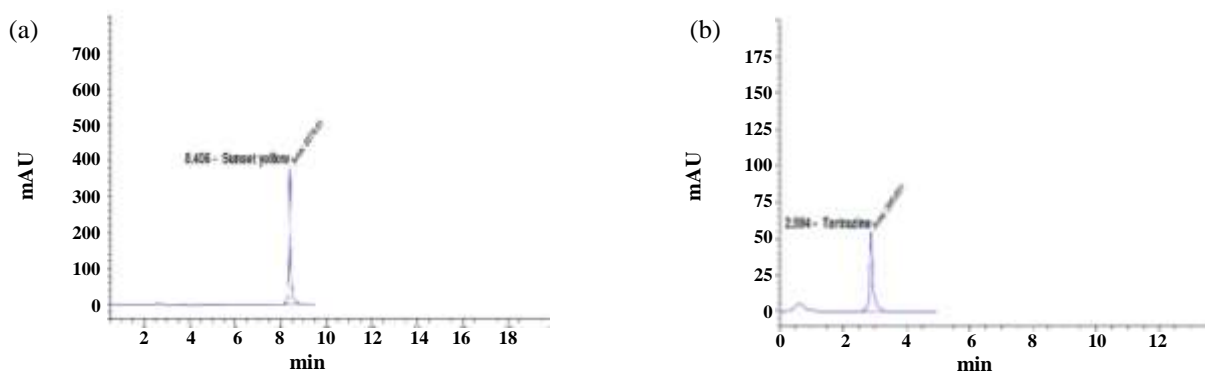
^a Mean value of the three measurements

Table 5: Statistical analysis using One-way Analysis of Variance (ANOVA).

Source of variation	SS	df *	MS	F Calculated	F Critical
Between groups					
SY	0.025108	2	0.012554	0.000192	4.256494
TZ	0.047703	2	0.023851	0.000387	4.256494
Within groups					
SY	586.1404	9	65.12672		
TZ	553.9631	9	61.55145		
Total					
SY	586.1655	11			
TZ	554.0108	11			

SS, sum of squares; df, degree of freedom; MS, mean squares.

* Degree of freedom for between groups: $h-1$; Within Groups: $h(n-1)$; Total: $hn-1$; h , number of methods; n , number of samples of each method.

**Fig. 9: Obtained chromatogram from the soft drink sample.**

CONCLUSIONS

Three analytical spectrophotometric methods were proposed and validated for the quantitative estimation of SY and TZ in mixtures and beverage samples. This paper represents the CWT, PLS, and PCR as accurate, rapid, and simple analytical techniques for the simultaneous determination of colorant compounds in commercial soft drink samples. Also, the HPLC method was applied to determine SY and TZ in the real sample. Compared to the HPLC, the proposed methods have some advantages

such as short analysis time, rapid, low-cost, and environmental protection. Economy and time are necessary for quality control laboratories. So, these methods can be suitable and valid.

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