Analytical Methods

Determination of three dyes in commercial soft drinks using HLA/GO and liquid chromatography

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Abstract
Allura Red, Sunset Yellow, and Tartrazine usually present in commercial soft drinks were accurately quantified using HLA/GO method, a net analyte signal-based method, without the need for solutes separation. The obtained percent recoveries (R.S.D.) of dyes were 99.8 (2.8), 102 (3.3), and 100 (4.5) for Allura Red, Sunset Yellow, and Tartrazine, respectively. The calibration method was applied for analysis of food dyes in powdered soft drinks with minimum sample preparation measures. The proposed HLA/GO method was validated against a standard HPLC method. Statistical analyses showed insignificant differences between the results of two methods. The developed HLA/GO method offers a simple and rapid procedure for determination of food dyes in powdered soft drinks in comparison with HPLC methodology.

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1. Introduction

Allura Red (E-129), Sunset Yellow (E-110), and Tartrazine (E-102) are three highly used synthetic dyes which are added to many food products (López-de-Alba, Wróbel-Kaczmarczyk, Wróbel, López-Martínez, & Hernández, 1996; Nevado, Cabanillas, & Salcedo 1995). Allura Red, Sunset Yellow, and Tartrazine are used as additives in pharmaceuticals and cosmetics because they can be easily mixed to give favourable shades and because of their low price compared with natural colourants (López-de-Alba, López-Martínez, Cerdá, & De-León-Rodríguez, 2001). Allura Red, Sunset Yellow, and Tartrazine are probably the most common dyes that applied (individually or together) to colour soft drink powders. According to the legislations of the European Union for food dyes (which is the same legislations adopted by the official food control laboratories in Jordan), the maximum level of Allura Red, Sunset Yellow, and Tartrazine dyes should not be more than 100 μg ml⁻¹ (individually or in combination) in non-alcoholic beverages with added juices and/or flavours (Directive 94/36/EC, 1994). The task of monitoring synthetic dyes in food products should be the responsibility of official food control laboratories and the food manufacturers to ensure safety for the consumers.

In fact, many analytical methods were provided for analysis of synthetic colours in beverages. The direct determination of food dyes is generally impossible. The colours must be separated from foodstuffs and purified, as co-extractives interfere with analysis of the colours. Moreover, the extract must be also concentrated because many food colours are used at low concentrations. The common analytical techniques that frequently used for determination of colours include visible spectrophotometry (Berzas, Rodríguez Flores, Villaseñor Llerena, & Rodríguez Fariñas, 1999), thin-layer chromatography (Oka et al., 1987), and mostly high performance liquid chromatography (Alves, Brum, de Andrade, & Netto, 2008; Chen, Mou, Hou, Riviello, & Ni, 1998). A typical example on using liquid chromatography for food dyes determination is the work of (García-Falcón & Simal-Gándara 2005), the authors provided a simple chromatographic method for analysing five synthetic food dyes in commercial soft drinks within minimal sample clean-up. A recommendation to adopt the earlier method for a serious control of dyes in food products was suggested by the authors.

Recently, multivariate calibration techniques have been employed for simultaneous determination of several analytes in a given sample without the need for analytes separation or even matrix clean-up in some instances (Adams, 2004; Martens & Næs,
were applied to normal-phase chromatographic papers and developed by chromatography. Water-dissolved samples and standard dyes were luted with acetate buffer for chemometric analysis or with methanol–water mixture for HPLC analysis. Qualitative assays were done for 10 min. The obtained mixture was centrifuged for 30 min at water with good mixing. The mixture was heated on a water bath for 10 min. Aliquots of the stock dyes solutions were added to get concentration range between 0–10 for AR and T and 0–7 μg ml⁻¹ for SY. Before final dilution with water, 5.0 ml of acetate buffer solution (pH 5) was added. The spectral measurements for all solutions were carried out over the spectral range 200–800 nm against a blank. The design of the calibration and validation sets is presented as 3D plot (Fig. 1). The design of calibration set was based on four-level fractional factorial design according to Brereton’s procedure (Brereton, 1997). The validity of HLA/GO method was further tested on an independent validation set. Prior analysis of dyes by HLA/GO method, the optimum number of wavelengths was carefully selected as will be shown later. Analysis of dyes in standard solutions and food samples was carried out separately for each dye as following: (a) an average spectrum of all calibration samples (37 spectra) was obtained, (b) $A_n$ matrix was obtained for the dye of interest, (c) NAS projection matrix of this dye was obtained as $P_{NALS} = [I - A_n^T (A_n^T)^{-1}]$, (d) $s_k$ vector, the net sensitivity vector, was then estimated using HLA/GO algorithm, and (e) the prediction of dye concentration in validation or food samples was obtained from the corresponding sample spectrum ($r$) as: $c_{est,k} = \frac{\| r - A_n^T s_k \|}{\| r \|}$, where $r_k$ was estimated as: $[I - A_n^T (A_n^T)^{-1}] r$. In the earlier formulae, $T$, $\| \|$ and $+$ stand for matrix or vector transpose, Euclidean norm, and pseudoinverse, respectively. $I$ is the unity matrix of $n \times n$ order. The adopted algorithm is outlined in the literature (Goicoechea & Olivieri, 1999, 2000).

2.4. Procedures

2.4.1. HLA/GO method

In 25 ml volumetric flasks, aliquots of the stock dyes solutions were added to get concentration range between 0–10 for AR and T and 0–7 μg ml⁻¹ for SY. Before final dilution with water, 5.0 ml of acetate buffer solution (pH 5) was added. The spectral measurements for all solutions were carried out over the spectral range 200–800 nm against a blank. The design of the calibration and validation sets is presented as 3D plot (Fig. 1). The design of calibration set was based on four-level fractional factorial design according to Brereton’s procedure (Brereton, 1997). The validity of HLA/GO method was further tested on an independent validation set. Prior analysis of dyes by HLA/GO method, the optimum number of wavelengths was carefully selected as will be shown later. Analysis of dyes in standard solutions and food samples was carried out separately for each dye as following: (a) an average spectrum of all calibration samples (37 spectra) was obtained, (b) $A_n$ matrix was obtained for the dye of interest, (c) NAS projection matrix of this dye was obtained as $P_{NALS} = [I - A_n^T (A_n^T)^{-1}]$, (d) $s_k$ vector, the net sensitivity vector, was then estimated using HLA/GO algorithm, and (e) the prediction of dye concentration in validation or food samples was obtained from the corresponding sample spectrum ($r$) as: $c_{est,k} = \frac{\| r - A_n^T s_k \|}{\| r \|}$, where $r_k$ was estimated as: $[I - A_n^T (A_n^T)^{-1}] r$. In the earlier formulae, $T$, $\| \|$ and $+$ stand for matrix or vector transpose, Euclidean norm, and pseudoinverse, respectively. $I$ is the unity matrix of $n \times n$ order. The adopted algorithm is outlined in the literature (Goicoechea & Olivieri, 1999, 2000).

2.5. Chromatographic procedure

Analytical separation and quantification of dyes was achieved at 1.3 ml min⁻¹ and 22.6 °C with a 0.1 M phosphate buffer (pH 7) + methanol gradient. Solvent programme was started with 14% methanol, held for 0.5 min, followed by a linear gradient to 25% methanol in 7.0 min, and another linear gradient to 31% methanol in 10 min. Dyes eluting from the column were detected by UV–spectrophotometer set at 475 nm. A 10 μl sample volume was injected in all experiments.
2.4.3. One-component calibration
A series of standard dye solutions in the concentration range from 1 to 40 ppm was prepared for one-component calibration. An adequate volume of the stock dye solution and 5.0 ml of buffer solution were placed in a 25 ml volumetric flask and the flask was filled with pure water. The absorbance was recorded at the wavelength corresponding to maximum of the absorption band of each dye. The calibration equations and the linear dynamic ranges were calculated for each dye.

3. Results and discussion
3.1. Spectral overlap and application of HLA/GO calibration for dyes determination

The absorption spectra of dyes recorded at pH 5.0 are presented in Fig. 2. As indicated in Fig. 2, the three dyes were active in ultraviolet and visible regions and this is attributed to their chemical structure which contains many active functional groups. Generally speaking, the spectral overlap between dyes is high over the entire spectral range. Within the spectral region 350–650 nm, the spectra of AR and SY were highly overlapped, while, a low extent of spectral overlap between T and other dyes in that region was observed. The estimated degrees of spectral overlap between dyes, as obtained from Goicoechea and Olivieri method (Goicoechea & Olivieri, 1998), were 65%, 75%, and 95% between T–SY, T–AR, and SY–AR, respectively. The presence of such high overlapping (particularly between SY and AR) limits the analysis of these dyes in food products by conventional univariate calibration methods. Accordingly, the spectral resolution of food dyes is necessary and this could be established either by: (a) using chromatographic methods like HPLC or TLC or (b) applying multivariate calibration methods.

Recently, many applications of multivariate calibration methods have been reported in chemical (Galeano Diaz, Guiberteau Cabanillas, Alexandre Franco, Salinas, & Viré, 1998), food (López-de-Alba et al., 2001), and pharmaceutical (Goicoechea & Olivieri, 1998; Ni, Wang, & Kokot, 2001) analysis.

López-de-Alba and co-workers were effectively applied PLS (1 and 2 types) and PCR for analysis of R40, Y6, and Y5 in commercial soft drinks and compared their results with a standard HPLC method (López-de-Alba et al., 2001). Nevado, Flores, & Llerena, 1998 have proposed a simple, accurate and rapid PLS calibration method for analysis of Tartrazine, sunset Yellow, and Ponceau in three commercial soft drinks without the need for solutes separation or sample clean-up due to the simple nature of soft drink matrix (Nevado et al., 1998).

Lorber had proposed the common net analyte signal (NAS) concept that permits estimation figures of merit for multivariate data (Lorber, 1986). The estimation of those figures of merit was initially restricted to the CLS model (when pure spectra and concentrations of all components are known (Lorber, 1986)). After that, NAS concept was extended to inverse calibration methods that only need the concentration of the target analyte in the calibration set (Lorber, Faber, & Kowalski, 1997). Recently, new families of multivariate calibration methods have been proposed based on the concept of NAS which are know as NAS-based calibration methods (Berger, Koo, Itzkan, & Feld, 1998; Espinosa-Mansilla, Merás, Rodríguez Gómez, Muñoz de la Peña, & Salinas, 2002; Ferrè & Faber, 2003; Goicoechea & Olivieri, 2000; Mirmohseni, Abdollahi, & Rostamizadeh, 2007; Moore, Cogdill, Short, Hair, & Wildfong, 2008; Xu & Schechter, 1997).

These NAS-based calibration methods used suitable scaling procedures for eliminating the information of the analyte of interest during the calibration step. Removing the spectrum of the pure component from calibration data is the basic step in hybrid linear analysis (HLA) method (Berger et al., 1998). A number of calibration methods have been proposed based on earlier HLA method like HLA/XS (Xu & Schechter, 1997), HLA/GO (Goicoechea & Olivieri, 2000), and HLA/AS (Ferrè & Faber, 2003). In fact, the earlier NAS-based methods have been applied for determination of many analytes in complex matrices. HLA method was used for determination of tetracycline in human serum using fluorescence data (Goicoechea & Olivieri, 1999). A good review on NAS-based calibration methods was provided by Ferré and Faber (2003). Beside their simplicity and ease of implementation, the HLA-based calibration methods can be applied for determination of an analyte in a complex matrix without the need for including the effect of other interferences in the calibration stage (Ferrè & Faber, 2003). Particularly, in HLA/GO calibration method, there is no need to have the pure spectrum of analyte of interest like the case in CLS, HLA, and HLA/XS calibration methods (Ferrè & Faber, 2003; Goicoechea & Olivieri, 2000; Lorber et al., 1997).

In this work, and for the first time, the application HLA/GO method is applied for determination of AR, SY, and T in their mixtures and in food containing these dyes. For sake of validation of HLA/GO method, an independent HPLC method is applied.

3.2. Effect of solution pH on dyes absorption and optimisation of calibration samples

One of most experimental variables that highly affect dyes absorption is solution pH. Therefore, dyes solutions should be maintained at certain pH so that high absorption intensities (for all dyes) should be observed at that pH. The influence of pH on the absorption intensity of food dyes was investigated over a wide pH range: 2–12. Dyes solutions of variable pH were prepared using buffer solutions. Generally speaking, the absorbance values of dyes (at their corresponding λmax) were not changed over the pH range 2–8, after which a considerable reduction in absorbance readings was observed for all dyes. It is highly possible that dyes have been completely ionised at higher pH values and this will affect their light absorption. Based on that, pH 5 was selected as an optimum pH for measuring dyes absorbances. Moreover, dyes solutions were found stable over 30 days when placed in a cold dark place (5.0 °C).

3.3. Designing of calibration mixtures, outlier(s) detection and wavelength selection

The efficiency of multivariate calibration methods is highly dependant on the design of standard mixtures in the calibration
and validation sets and the presence or absence of outliers. Four important issues should be addressed when building calibration and validation sets: (a) the concentration of individual solute should be within its linear range obtained from univariate calibration, (b) calibration set should be orthogonally deigned to reduce the collinearity in calibration matrix and to ensure high prediction power for the method, (c) the absorbances of calibration and validation mixtures should not be higher than the maximum response of the instrument. Usually, in spectrophotometric measurements absorbances higher than 1.0 were not considered and dilution for these mixtures is suggested, and (d) the concentration of solutes in validation set should be within the range used in calibration set. These issues were taken into consideration when building calibration and validation sets for the current dyes system. The obtained dynamic ranges were: 0.3–32, 0.3–35, and 0.4–25 μg ml⁻¹ for AR, SY, and T, respectively. To avoid high absorption intensities for standard mixtures (i.e., absorption values > 1.0), the concentration ranges of dyes were adjusted in away to achieve that aim.

Elimination of outlier(s) from calibration or validation samples is essential prior multivariate calibration. Usually, the detection of outlier(s) can be simply carried out using Mahalanobis distance method (López-de-Alba, López-Martínez, Cerdá, & Amador-Hernández, 2006) or principal component analysis (Adams, 2004; López-de-Alba, López-Martínez, & De-León-Rodríguez, 2002). Cluster plots (derived from principal component analysis PCA) represent a more systematic way to detect outlier(s). Usually, cluster plots obtained by plotting the first principal component (PC1) against the next component (PC2). In the absence of outliers, all samples should form a clear cluster of the data. PCA was carried out for spectral data of calibration and validation sets. For both sets, the variances (94%) in absorption data can be effectively presented using the first two principal factors. PC1–PC2 plot (not shown) clearly indicated that both sets were fall within the same domain and form one homogenous cluster. If an outlier sample is there, then it will be much away from the cluster of samples. Furthermore, cluster analysis indicated the high homogeneity between two sets and this ensures a high prediction power of HLA/GO multivariate calibration method for future samples.

Most of multivariate calibration methods, in fact, are considered as full-spectrum methods because, in some instances, the entire data in a given spectrum would be included in numerical analysis. Including all spectral data points is not necessary to improve the quality of analysis, moreover, it has been shown for many multi-component systems that reduction of data points could improve the accuracy and precision of numerical analysis (Brown, 1992). Hence, various criteria have been developed to allow for effect wavelength selection prior multivariate calibration (Al-Degs, El-Sheikh, Al-Ghouti, Hemmateenejad, & Walker, 2008). In this work, the method proposed by López-de-Alba and co-workers is employed to select the optimum wavelength region(s) for HLA/GO calibration (López-de-Alba, López-Martínez, & De-León-Rodríguez, 2002). For each dye, the correlation coefficients between dye concentration (in calibration set) and the corresponding absorbance at each wavelength were estimated.

The correlation coefficient–wavelength plot of dyes (not presented) indicated that T and AR showed good correlation over the wavelength range 362–455 and 450–550 nm, respectively. On the other hand, SY dye exhibited a poor correlation with the entire spectral range. For HLA/GO calibration, six spectral regions (with 1.0 nm interval) were selected based on the correlation coefficient – wavelength plot: 200–800, 200–600, 200–350, 300–600, 350–550, and 350–800 nm. HLA/GO calibration was carried out separately for each dye for each selected wavelength range. The quality of calibration was assessed by calculating relative error of prediction (REP%) for dyes concentrations (Goicoechea & Olivieri, 1999 and Hemmateenejad et al., 2007).

The prediction power of HLA/GO was satisfactory (total REP < 1%) when the analysis was limited to 350–550 nm spectral range. However, the spectral range 300–600 nm was also useful for HLA/GO calibration where the total REP% value for dyers determination was about 2.0%. It is interesting to notice that including the entire spectral range (200–800 nm, 601 points/spectrum) has decreased the method’s efficiency for prediction as inferred from the high total REP% value (7.32%). The spectral domain 200–350 nm is not recommended for calibration where the maximum values of REP% were reported for that range (13.61%). The modest prediction power of HLA/GO method over 200–350 nm range could be attributed to the high spectral overlap between dyes in that region (Fig. 2).

After checking out outliers and setting the suitable spectral range, HLA/GO calibration method is ready for dyes determination in validation set and food samples and this subject will be investigated next.

3.4. Determination of food dyes in synthetic mixtures by HLA/GO calibration

In this section, HLA/GO method is employed for prediction of dyes contents in calibration and validation sets at the optimum calibration factors (pH 5 and wavelength range 350–550 nm). This goal was achieved by estimating the net sensitivity vectors (s_i) for dyes (separately) and then use these vectors for estimating dyes concentrations in calibration/validation sets and food samples. For each dye in both sets, PRESS (prediction error sum of squares), relative error of prediction (REP%), and square of correlation coefficients (r²) were calculated and the results were summarised in Table 1.

As clearly indicated in Table 1, the HLA/GO method gave satisfactory results for dyes determination. In all cases, r² values were approached to unity which reflects the high closeness between real and predicted values. The average recoveries and R.S.D. values were also satisfactory. In both sets, the average recoveries were within the range: 99.5–102.3% and R.S.D. values were <5.0% in all cases. In summary, the proposed HLA/GO method offers a simple and rapid procedure for dyes determination in water with high precision and accuracy without the need for any previous separation step.

Figures of merit (like accuracy, precision, repeatability, and detection limits) were evaluated for the proposed calibration method as outlined in the literature (Lorber et al., 1997). Analytical intraday precision was determined by analysing a standard mixture of dyes (containing 5, 5, and 6 μg ml⁻¹ of AR, SY, and T, respectively) in six replicates in one day. Interday precision was determined by measuring the same control mixture in duplicate for three days. The concentration of each dye in the control mixture was determined using HLA/GO method on the first day of the study. The standard solution of dyes was prepared and analysed six times within the same day to measure the repeatability of the method. The obtained accuracy and R.S.D. values were

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Assessment of HLA/GO method for dyes quantification in water.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye</td>
<td>Internal Validation</td>
</tr>
<tr>
<td></td>
<td>PRESS</td>
</tr>
<tr>
<td>Allura Red</td>
<td>0.24</td>
</tr>
<tr>
<td>Sunset yellow</td>
<td>0.17</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* PRESS, REP%, and r² values were all estimated for non-zero dye concentrations. The proposed method predicts a very close value to zero when no dye had been added (data not given).
99.2–101.2% and 1.13–2.51 for intraday analysis and 99.1–101.4% and 1.41–1.76 for interday analysis.

The earlier values were satisfactory and reflecting the high accuracy and precision of HLA/GO method for both intraday and interday analysis. The repeatability was also high with a random error of <3%. The obtained detection limits for AR, SY, and T were 0.66, 0.52, and 0.43 μg ml⁻¹, respectively.

3.5. Determination of food dyes in food drinks using HLA/GO method

There are a number of studies that discussed the application of multivariate calibration for food dyes analysis. For example, Neva-do and co-workers have applied PLS-1, PLS-2, and PCR for determination of four food dyes in different commercial beverages (Nevado et al., 1995). López-de-Alba and co-workers have successfully applied PLS-1 and PLS-2 on zero and first order spectral data for sake of determination of three common food dyes in soft drinks (López-de-Alba et al., 2001). Moreover, the pervious authors have validated the proposed chemometric methods against independent chromatographic methods. According to our literature survey, HLA/GO calibration method has not been applied for food dyes analysis in any food type. The HLA/GO method was applied for determination of AR, SY, and T in four commercial soft drinks. The solutions of food samples were prepared as described earlier and scanned using the spectrophotometer. The spectral data were then subjected to HLA/GO calibration to find dyes contents in each sample. The results of analysis were provided in Table 2. The results obtained by HPLC method were given in the same table for sake of comparison.

As indicated in Table 2, food dyes were simultaneously quantified in commercial samples with a reasonable precision using HLA/GO method. For more validation to HLA/GO method, the same food samples were analysed using an independent analytical method. In such cases, one has two options to go through. The first option is to carry out standard addition techniques and the second one is to apply an independent standard method (like HPLC) for dyes determination in food samples. The second option was adopted here because of the high validity of HPLC method in food dyes analysis (Alves et al., 2008).

Under the conditions already outlined earlier for HPLC method, calibration graphs (peak area vs. dye content) were obtained from triplicate determination using five standard solutions of dyes. Table 3 summarised the chromatographic results for dyes separation.

The results obtained for dyes determination by an independent HPLC method was also provided in Table 2. To decide whether the results of HLA/GO and HPLC methods are comparable or not, t and F statistical tests were carried out. For all cases, t-calculated values were lower than t-table values which indicated that no significant difference between HPLC and the proposed method for determination of dyes in food samples. Moreover, F-calculated values were also lower than F-table values which confirmed that both methods are of similar precision. The results in Table 2 indicated that HLA/GO method was able for dyes quantification in four commercial soft drinks without the need for running chromatographic separation. Relatively speaking, the proposed HPLC method has better precision than HLA/GO method as indicated from S.D. values and this was expected because dyes were totally separated before their quantification, while, in HLA/GO method the dyes were quantified directly in the sample matrix. Compare to HPLC method, HLA/GO method was rapid, easy to implement, and of low running cost for dyes quantification. The HLA/GO method could be used for food dyes quantification in cases where the chromatographic ones cannot be implemented owing to cost limitations or lack of analytical instrumentation. It is worth to mention that the levels of AR, SY, and T in soft drinks were below the limit set by European legislations (Directive 94/36//EC, 1994).

The results of HLA/GO and liquid chromatography were, qualitatively, confirmed by simple paper chromatography. The paper chromatographic tests indicated the absence of AR in TANG/Mango, TANG/Orange, and SQUEEZE/Orange and confirmed the existence of all dyes in SQUEEZE/Mango. The separated dyes from food samples were have well-resolved bands and identical Rf values to those obtained from the standards, 0.71, 0.45, and 0.37 for AR, SY, and T, respectively.

It is worth to mention here that the excipients that present in soft drink samples (like citric acid, sucrose, and synthetic flavoring materials) have a low interference with dyes determination. This conclusion was drawn when the spectrum of pure dyes mixture was matched with the spectra of food samples. Fig. 3 showed the spectra of dyes in water and in SQUEEZE/Mango sample. SQUEEZE/Mango sample was selected because of the presence of the three dyes in high amounts (see Table 2).

The similarity between the spectra depicted in Fig. 3 indicated that other food excipients do not interfere in dyes determination by HLA/GO or even HPLC method. It seems that other food excipients are active within the spectral range (250–305 nm) as indi-

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**Table 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Allura Red</th>
<th>Sunset Yellow</th>
<th>Tartrazine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLA/GO</td>
<td>HPLC</td>
<td>HLA/GO</td>
</tr>
<tr>
<td>TANG/Mango</td>
<td>ND</td>
<td>ND</td>
<td>43.0(2.2)</td>
</tr>
<tr>
<td>TANG/Orange</td>
<td>ND</td>
<td>ND</td>
<td>24.2(1.6)</td>
</tr>
<tr>
<td>SQUEEZE/Mango</td>
<td>29.1(1.4)</td>
<td>31.0(1.6)</td>
<td>21.6(2.3)</td>
</tr>
<tr>
<td>SQUEEZE/Orange</td>
<td>ND</td>
<td>ND</td>
<td>17.7(1.6)</td>
</tr>
</tbody>
</table>

*Concentrations are in μg ml⁻¹. Results are average of four experiments, t-table(0.05, 6) = 2.45, F-table(0.05, 3, 3) = 15.44.*

*Not detected in the sample.*

**Table 3**

<table>
<thead>
<tr>
<th>Dye</th>
<th>tₚ/min*</th>
<th>Dynamic range (μg ml⁻¹)</th>
<th>LOD (μg ml⁻¹)*</th>
<th>Calibration equation (Peak Area vs. conc.)</th>
<th>Precision (R.S.D.)</th>
<th>Correlation coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>4.6</td>
<td>0.50–16.0</td>
<td>0.30</td>
<td>PA = 51886 C + 104792</td>
<td>2.2</td>
<td>0.9998</td>
</tr>
<tr>
<td>SY</td>
<td>10.1</td>
<td>0.10–13.0</td>
<td>0.06</td>
<td>PA = 278186 C + 502351</td>
<td>3.2</td>
<td>0.9979</td>
</tr>
<tr>
<td>AR</td>
<td>13.8</td>
<td>0.10–12.0</td>
<td>0.04</td>
<td>PA = 302834 C + 420563</td>
<td>2.8</td>
<td>0.9985</td>
</tr>
</tbody>
</table>

*Retention times of dyes.

*Limits of detection were estimated on the basis of a signal-to-noise ratio of 3 (3S/N method).*
HPLC/GO method, as a multivariate calibration method, was excluded from the chemometric analysis. In case where a high interference is present with target dyes, a suitable extraction and clean-up procedures are necessary to extract dyes prior applying HLA/GO or HPLC methods.

4. Conclusions

HPL/GO method, as a multivariate calibration method, was found useful method for simultaneous determination of AR, SY, and T dyes in pure solutions and in four soft drink samples. The percent recoveries and R.S.D. values for dyes determination in solution were 99.3(4.2), 101.3(3.8), and 102.4(4.6) for AR, SY, and T, respectively. The results obtained by HLA/GO method for determination of dyes were fairly comparable to those obtained by HPLC method which reflects the effectiveness of the former method. Fortunately, the components of soft drinks did not strongly interfere with dyes analysis. In case of presence a strong interference, dyes should be extracted and preconcentrated prior their analysis. HLA/GO calibration method is a reasonable substitute for expensive HPLC method for quantification of dyes in soft drinks.

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