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Analytical Methods

Determination of tartrazine in beverage samples by stopped-flow analysis and three-way multivariate calibration of non-linear kinetic-spectrophotometric data

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ABSTRACT

The performance of MCR-ALS was studied in the modeling of non-linear kinetic-spectrophotometric data acquired by a stopped-flow system for the quantitation of tartrazine in the presence of brilliant blue and sunset yellow FCF as possible interferents.

In the present work, MCR-ALS and U-PCA/RBL were firstly applied to remove the contribution of unexpected components not included in the calibration set. Secondly, a polynomial function was used to model the non-linear data obtained by the implementation of the algorithms. MCR-ALS was the only strategy that allowed the determination of tartrazine in test samples accurately. Therefore, it was applied for the analysis of tartrazine in beverage samples with minimum sample preparation and short analysis time. The proposed method was validated by comparison with a chromatographic procedure published in the literature. Mean recovery values between 98% and 100% and relative errors of prediction values between 4% and 9% were indicative of the good performance of the method.

1. Introduction

Food dyes are often added to foodstuffs and drinks in order to supply, intensify or restore their colour to create the desired coloured appearance (Código Alimentario Argentino, 2010). Synthetic dyes are widely used as they show several advantages compared with natural dyes such as high stability to light, oxygen and pH, colour uniformity, low microbiological contamination and relatively lower production costs. However, in certain quantities they are harmful to human health (Silva, García, Lima, & Barrado, 2007), hence supervising of synthetic dyes in high consumption products such as beverages becomes an indispensable task.

Tartrazine (E-102) is a highly used synthetic dye often employed as an additive in food, drinks, medicine and cosmetics. Its colour is due to the presence of azo (N=N) functional groups and aromatic ring structures. Argentine Alimentary Code establishes a maximal concentration of 100 and 200 mg L^{-1} of tartrazine in non-alcoholic and alcoholic drinks, respectively, and expresses the obligation of these products to specify its presence in their label (Código Alimentario Argentino, 2010).

The common analytical techniques frequently used for the determination of colourants include visible spectrophotometry (Berzas, Rodríguez Flores, Villaseñor Llerena, & Rodríguez, 1999), thin-layer chromatography (Oka et al., 1987; Soponar, Mot, & Sârbu, 2008), capillary electrophoresis (Dossi et al., 2007a; Dossi, Piccin, Bontempelli, Carrilho, & Wang, 2007b) and mostly high performance liquid chromatography (Dossi, Toniolo, Susmel, Pizzariello, & Bontempelli, 2006; Minioti, Sakellariou, & Thomaidis, 2007; Pereira Alves, Brum, de Andrade, & Netto, 2008; Vachirapatama, Mahajaroensiri, & Visessanguan, 2008; Yang, Yin, & Shao, 2011; Yoshioka & Ichihashi, 2008). However, these methods are known to use toxic solvents, spend long analysis time, and sometimes it is necessary to make sample pretreatments. On the other hand, the combination of simple methodologies, such as spectroscopic methods with chemometric modeling, represents a rapid, simple and cheap strategy for the determination of these colourants (Al Degs, 2009; Dinc, Baydan, Kanbur, & Onur, 2002; Lachenmeier & Kessler, 2008; Llamas, Garrido, Di Nezio, & Fernández Band, 2009).

In the present report, spectral measurements as a function of time were acquired in order to quantitate tartrazine based on its kinetic reaction with potassium bromate. Because of the fact that time period for data collection must be carefully controlled when this kind of determinations are performed, its automation becomes essential (Araújo, Catita, & Lima, 1998). Therefore, a stopped-flow injection system was well suited to accomplish this task as it is based on reproducible timing phenomena. For this purpose, the samples and the oxidant were automatically mixed and injected in the carrier flowing into a mixing coil. The flow was stopped

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and the temporal evolution of spectra was measured. Several interesting applications have been developed in this context (Murillo Pulgarín & García Bermejo, 1996; Muñoz de la Peña, Espinosa-Mansilla, Acedo Valenzuela, Goicoechea, & Olivieri, 2002; Pitonesi, Centurión, Ferenández Band, Damiani, & Olivieri, 2004; Wang & Lu, 2005).

When the temporal evolution of spectra for a reacting system is acquired, the second-order data generated can be successfully handled by second-order multivariate calibration algorithms. As new products are originated in a kinetic process, differences in the spectral or kinetic characteristics from those of the reagents can be used to distinguish components in a mixture (Escandar et al., 2007). Another important item is that second-order data enclose the so-called "second-order advantage", which allows predicting the concentration of the analyte of interest even in the presence of unknown interferents. This also enables several analytes to be determined simultaneously (Booksh & Kowalski, 1994). Recently, an indirect kinetic-spectrophotometric method has been developed for the simultaneous determination of tartrazine and other four synthetic colourants with different chemometric algorithms (Ni, Wang, & Kokot, 2009).

Interestingly, two-way data matrices generated by this type of kinetic methods combined with multivariate calibration make unnecessary the formulation of kinetic models, which is a great advantage over traditional kinetic-spectrophotometric methods, allowing the development of empirical calibration models to predict analyte concentration in unknown samples (Esteves da Silva & Oliveira, 1999). These kind of second-order data have been traditionally processed by the application of a well-known algorithm: multivariate curve resolution-alternating least-squares (MCR-ALS) (De Juan & Tauler, 2001). Recently, attention has been paid to alternative second order multivariate calibration algorithms based on latent-structured methodologies, namely unfolded partial least squares/residual bilinearization (U-PLS/RBL) and multidimensional partial least squares/residual bilinearization (N-PLS/RBL). These algorithms appear to be more flexible and provide better figures of merit than their competitors (Lozano, Ibañez, & Olivieri, 2008).

Unfortunately, non-linearity in the absorbance–concentration relationship occurs in certain kinetic-spectroscopic systems, and this kind of response can be accredited to different causes such as instrumental noise, physical or chemical sources, which can cause curvature in the concentration–response function. This effect is classified as a real non-linear effect (Gemperline, Long, & Gregoriou, 1991). The chemometric algorithms mentioned before in this text are all based on linear models, and hence they are not applicable to the system under study, in which the relationship between the response and the analyte concentration is non-linear.

Recently, unfolded principal component analysis coupled to residual bilinearization (U-PCA/RBL) has been proposed as an algorithm capable of dealing with non-linear second order information (Olivieri, 2005). Furthermore, this method presents the second-order advantage, which allows the elimination of the contribution of the unsuspected components from the total sample data (Culzoni & Goicoechea, 2007; García-Reiriz, Damiani, & Olivieri, 2007; García-Reiriz, Damiani, Culzoni, Goicoechea, & Olivieri, 2008).

The main objective of this work was to study the performance of MCR-ALS applied to the stop-flow kinetic determination of tartrazine as well as to compare the results acquired with this algorithm and U-PCA/RBL, since it appears to be the most appropriate algorithm to process non-linear second-order data.

In order to model the non-linear relationship between the calibration data and the analyte concentration, a non-linear pseudo-univariate calibration graph was obtained with the areas under the kinetic profiles or the scores, if the algorithm applied was MCR-ALS or U-PCA/RBL, respectively, and their associated concentrations. These models were eventually employed to the prediction of tartrazine in validation samples containing also different amounts of brilliant blue (E-133) and sunset yellow FCF (E-110) as possible interferents. Because of the presence of severe overlapped profiles, U-PCA/RBL was not able to obtain the second-order advantage. Therefore, only MCR-ALS was used to the quantitative determination of the dye in real samples. Prediction results obtained for real samples were compared with those acquired using a liquid chromatographic procedure, adapted from the one proposed by Pereira Alves et al. (2008).

2. Theory

2.1. MCR-ALS

The multivariate calibration algorithm MCR-ALS has been extensively described in the literature (De Juan & Tauler, 2001; Saurina et al., 2001; Tauler, 1995) so, only a brief description of it is given here.

The bilinear decomposition of the augmented matrix **D** is performed according to the expression:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{\mathrm{T}} + \mathbf{E} \quad (1)$$

in which the rows of **D** contain the spectra measured for different samples at several decay times, the columns of **C** contain the kinetic profiles of the intervening species, the columns of **S** their related spectra, and **E** is a matrix of residuals not fitted by the model.

Decomposition of **D** is achieved by iterative least-squares minimization of the Frobenius norm of **E**, under suitable constraining conditions during the ALS procedure. MCR-ALS requires initialization with system parameters as close as possible to the final results. In the present work we employed the SIMPLISMA (simple to use interactive self-modeling mixture analysis) methodology (Windig & Guilment, 1991) in all cases.

After MCR-ALS decomposition of **D**, the information contained in **C** can be used for quantitative predictions. If the area under each profile is considered as proportional to each component concentration, then a pseudounivariate model can be built. In this work, a non-linear pseudounivariate graph was obtained and was used to make the predictions on test and real samples.

2.2. Unfolded principal component analysis/residual bilinearization

The essentials of U-PCA/RBL have already been discussed in Refs. Culzoni and Goicoechea (2007); García-Reiriz et al. (2007); García-Reiriz et al. (2008) and Olivieri (2005).

In the present report we proved that calibration scores can be employed to create a pseudounivariate model for further predictions using test scores.

When unexpected components take place in the test samples, its scores will not be suitable for analyte prediction in usual PCA. Therefore, the necessity of an alternative technique arises in order to mark the new sample as an outlier, isolate the contribution of the unexpected constituent from that of the calibrated analytes and then recalculate appropriate scores for the test sample. The RBL procedure is then applied to a given array of test sample data and the outcome scores are free from interferents signal. This step provides the so-called second-order advantage to the methodology.

In the present work, calibration scores were employed to build a non-linear pseudounivariate calibration graph. Afterwards, the scores corrected by the RBL procedure were used for prediction of analyte concentration in the samples.

3. Experimental

3.1. Reagents and solutions

Analytical reagent-grade chemicals and Milli-Q water were used. Solid dye standards were obtained from Ardinet (Buenos Aires, Argentina) with a purity higher than 95% in all cases. Stock solutions of tartrazine (0.002 mol L^{-1}), brilliant blue (0.001 mol L^{-1}) and sunset yellow FCF (0.002 mol L^{-1}) were prepared by dissolving appropriate amounts of each compound in water.

A stock solution of Fe(II) 0.02 mol L^{-1} was prepared by dissolving the appropriate amount of $Fe(NH_4)(SO_4)_2{\cdot}6H_2O$ (Cicarelli, San Lorenzo, Argentina) in water, adding 5.0 mL of H_2SO_4 and diluting to the mark in a 500.00 mL volumetric flask. Solutions of potassium bromate (Cicarelli, San Lorenzo, Argentina) 0.1 mol L^{-1} and phosphoric acid (Cicarelli, San Lorenzo, Argentina) 3.0 mol L^{-1} were also prepared.

Methanol and ammonium acetate were obtained from Sintorgan (Buenos Aires, Argentina) and Cicarelli (San Lorenzo, Argentina), respectively.

3.2. Instrumentation and software

A stopped-flow-injection (FIA) system was developed using five modules (degasser, pump, injection valve, autosampler and DAD detector) of an Agilent 1100 Series instrument (Agilent Technologies, Waldbronn, Germany). The flow-injection manifold was designed to automatically inject 95 μL of the sample solution, previously merged with 5 μL of the reagent (bromate 0.1 mol L^{-1}), into a Milli-Q water carrier flowing at 1.5 mL min^{-1} through a 800 mm length and 0.5 mm i.d. flexible mixing coil. The pump was stopped after 42 s since sample injection, the resultant mixture solution passed through the detecting flow cell and the reaction was monitored during 108 s. Once this time was reached, the flow was restored. Spectra were registered for each FI peak in the range 400–650 nm each 1 nm, at regular steps of 0.4 s for a total time of 150 s. Hence, the size of each temporal-spectral data matrix was 375 × 251, although it was later reduced by appropriate region selection (see below).

The chromatographic method was adapted from the one proposed by Pereira Alves et al. (2008). Chromatograms were recorded using the same Agilent 1100 Series instrument, although the measurements were done on a 3.5 μm ZORBAX Eclipse XDB-C18 column (4.6 × 75 mm) from Agilent Technologies.

The MCR–ALS algorithm was implemented using the graphical interface downloaded from http://www.mcrals.info. A useful interface for data input and parameters setting written by Olivieri, Wu, and Yu (2009) was employed for U-PCA/RBL implementation. The multivariate methods discussed in the present work were run in MATLAB 7.1 (MATLAB 7.1, 2005).

3.3. Analytical procedure

3.3.1. Calibration standards and mixtures of dyes

The experimental procedure was developed preparing a calibration set of ten samples of tartrazine with concentrations equally distributed in the range 2.00–20.00 mg L^{-1}.

Two validation sets were prepared employing different concentrations than those used for calibration and following a central composite design. One of them containing nine samples (set 1) with different concentrations of tartrazine and brilliant blue, and the other one containing twenty samples (set 2) with different levels of tartrazine, brilliant blue and sunset yellow in concentrations ranging from 2.50 to 19.50 mg L^{-1} (see Tables 1 and 2).

Calibration and validation samples were prepared by measuring appropriate aliquots of standard solutions of each dye, placing them into 10.00 mL volumetric flasks to obtain the desired concentrations, adding 200 μL of phosphoric acid 3.0 mol L^{-1} and 100 μL of stock solution of Fe(II) 0.02 mol L^{-1} and completing to the mark with Milli-Q water.

All of the samples were prepared in duplicate. An injection program was developed to automatically merge 95 μL of each sample with 5 μL of bromate 0.1 mol L^{-1}, and to subsequently inject the mixture into the FIA system.

3.3.2. Beverage samples

The analysed samples were three soft drinks ready to consume, one powdered drink and an alcoholic drink, which contain tartrazine among other dyes (see Table 3), purchased from a local supermarket. All of the samples contained citric acid as acidifier. The rest of the declared ingredients were artificial sweeteners such as aspartame, acesulfame potassium, saccharin, sodium cyclamate and sucralose, fruit flavoring, preservatives including sodium benzoate, potassium sorbate, sulfur dioxide and calcium lactate. Other ingredients were sodium citrate, calcium chloride and magnesium sulphate. Water was present in all the samples except in powdered drink. Carbonic gas was added to only two samples.

Samples were homogenised and degassed, if necessary, by ultrasonic bath. In the case of the solid sample, it was previously

Table 1
Composition and prediction results of the validation set 1.

Sample	Tartrazine (mg L^{-1})			Brilliant blue (mg L^{-1})
	Nominal	MCR-ALS[a,b]		Nominal
		Temporal mode	Spectral mode	
1	11.09	11.6 (0.2)	10.6 (1.7)	2.50
2	16.87	15.0 (0.1)	14.8 (0.1)	17.24
3	19.28	17.2 (0.1)	16.8 (0.1)	11.02
4	4.99	5.2 (0.3)	6.2 (0.2)	17.24
5	2.50	2.5 (0.1)	3.6 (0.1)	11.02
6	11.09	9.9 (0.3)	10.3 (0.2)	19.64
7	11.09	11.0 (0.1)	10.90 (0.01)	11.02
8	16.87	16.97 (0.01)	14.58 (0.01)	4.99
9	4.99	5.3 (0.4)	5.3 (0.3)	4.99
Mean recovery[b] (%)		98.1 (7.0)	102.3 (19.1)	
REP[c] (%)		9.0	14.7	

[a] Average of duplicate analysis.
[b] Between parenthesis the standard deviation.
[c] Relative error of prediction, $REP = \frac{100}{\bar{c}}[\frac{1}{I}\sum_1^I (c_{act} - c_{pred})^2]^{1/2}$, where I is the number of samples, c_{act} and c_{pred} are the actual and predicted concentrations, and $\bar{c}$ is the mean concentration.

Table 2
Composition and prediction results of the validation set 2.

Sample	Tartrazine (mg L^{-1})		Brilliant blue (mg L^{-1})	Sunset yellow (mg L^{-1})
	Nominal	MCR-ALS[a,b]	Nominal	Nominal
1	10.99	9.5 (0.8)	11.02	10.94
2	10.99	10.34 (0.01)	11.02	10.94
3	10.99	10.89 (0.01)	11.02	10.94
4	16.00	14.9 (0.1)	16.09 11.02	5.95
5	10.99	11.3 (0.7)	11.02	2.44
6	16.00	15.8 (1.2)	5.94	16.04
7	10.99	10.0 (0.6)	11.02	19.55
8	10.99	11.86 (0.01)	2.49	10.94
9	10.99	10.6 (0.1)	11.02	10.94
10	5.98	4.7 (0.7)	16.09	5.95
11	10.99	11.5 (0.1)	11.02	10.94
12	10.99	10.1 (0.4)	19.54	10.94
13	10.99	11.6 (0.3)	11.02	10.94
14	5.98	6.9 (0.4)	5.94	16.04
15	19.47	19.40 (0.01)	11.02	10.94
16	2.51	2.7 (0.1)	11.02	10.94
17	5.98	6.3 (0.1)	16.09	16.04
18	5.98	7.8 (0.3)	5.94	5.95
19	16.00	17.2 (0.1)	5.94	5.95
20	16.00	13.85 (0.01)	16.09	16.04
Mean recovery[b] (%)		99.9 (11.2)		
REP[c] (%)		6.5		

[a] Average of duplicate analysis.
[b] Between parenthesis the standard deviation.
[c] Relative error of prediction, $REP = \frac{100}{\bar{c}}[\frac{1}{I}\sum_1^I (c_{act} - c_{pred})^2]^{1/2}$ where I is the number of samples, c_{act} and c_{pred} are the actual and predicted concentrations, and $\bar{c}$ is the mean concentration.

Table 3
Results of tartrazine determination in beverage samples.

Sample[a]	Flavour	Declared dyes[b]	Tartrazine (mg L^{-1})	
			HPLC	MCR-ALS
SD1	Orange-peach	E-102, E-110	4.07	4.17
SD2	Green mango	E-102, E-133	6.58	6.69
SD3	Orange-peach	E-102, E-110	0.79	0.73
PD	Apple	E-102, E-110	21.55	21.08
AD	Green evolution	E-102, E-133	7.73	7.75
$t_{calculated}$[c]			0.56	
Mean recovery[d] (%)			–	98.8 (4.2)
REP[e] (%)			–	4.2

[a] SD1-3: soft drinks, PD: powdered drink, AD: alcoholic drink.
[b] E-102 (tartrazine), E-110 (sunset yellow), E-133 (brilliant blue).
[c] Value of t (calculated according to Miller and Miller (2005)) is lower than the critical value 2.78 (95% confidence level and 4 degrees of freedom).
[d] Between parenthesis the standard deviation.
[e] Relative error of prediction, $REP = \frac{100}{\bar{c}}[\frac{1}{I}\sum_1^I (c_{act} - c_{pred})^2]^{1/2}$, where I is the number of samples, c_{act} and c_{pred} are the actual and predicted concentrations, and $\bar{c}$ is the mean concentration.

homogenised in its own package and all of the content was precisely weighted and directly dissolved in 1000.0 mL of ultra-pure water at room temperature.

The sample solutions were placed into 10.00 mL volumetric flasks with 200 μL of phosphoric acid and 100 μL of stock solution of Fe(II). The solutions were then centrifugated at 6000 rpm for 2 min and injected into the FIA system. Generally, the beverages were clear and no sediments or suspension were observed, but the samples were centrifuged to remove any particle which could be present in each of them, in order to prevent any obstruction in the FIA system.

3.3.3. Chromatographic procedure

The concentration of tartrazine in commercial products was verified by the HPLC method adapted from the one proposed by Pereira Alves et al. (2008), at room temperature, using a mixture of (methanol:ammonium acetate 0.08 mol L^{-1}) as mobile phase flowing at 1 mL min^{-1} with ultraviolet detection at 454 nm. In order to achieve a successful resolution, the following gradient program was applied: 15% of methanol for the first 2.5 min, then it was increased to 50% for the next 6.5 min and finally decreased to 15%. In these conditions, the total analysis time for each chromatogram was 10 min.

4. Results and discussion

4.1. Spectral and kinetic characteristics

The reaction between tartrazine and potassium bromate in the presence of Fe(II) has been applied before (Culzoni et al., 2008). During this oxidation reaction, the absorbance of tartrazine (peak at 430 nm) decreases leading to an uncoloured component. Brilliant blue does also react with bromate, suffering a kinetic degradation and yielding a product whose spectrum shares the maximum absorption wavelength of tartrazine. In turn, it can be considered a potential interferent. In the case of sunset yellow,

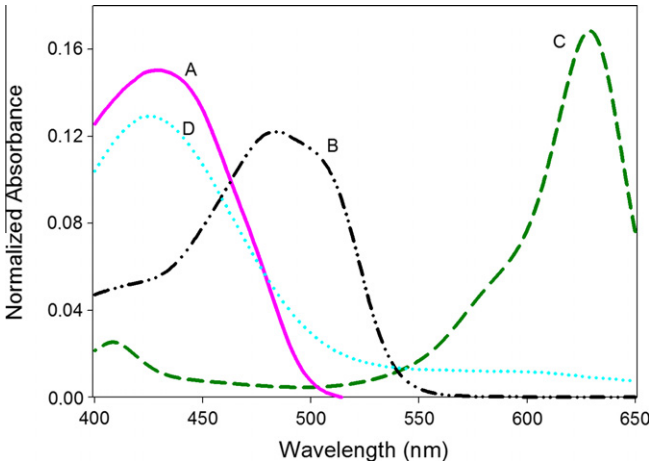

Fig. 1. Normalized absorption spectra of the dyes: (A) tartrazine, (B) sunset yellow, (C) brilliant blue and (D) oxidation product of brilliant blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

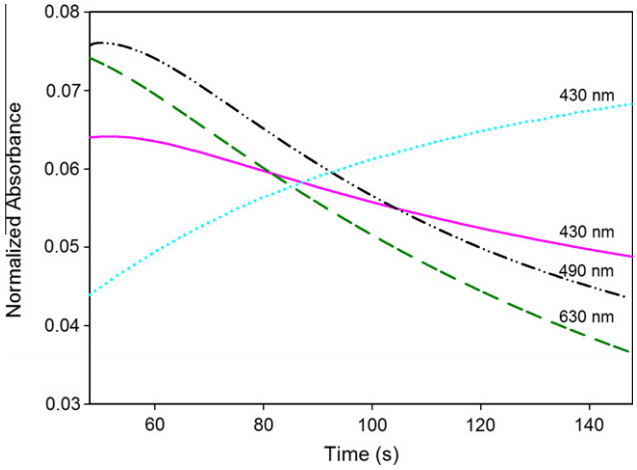

Fig. 2. Normalized kinetic curves obtained for tartrazine, sunset yellow, brilliant blue and its degradation product at their maximum absorption wavelengths. Pink solid line indicates tartrazine, green dashed line brilliant blue, cyan dotted line the product of brilliant blue oxidation and black dashed-dotted-dotted line sunset yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

its spectrum is less similar (although it also presents a minor peak at 420 nm) but its absorbance also decreases in the presence of the oxidant generating an uncolored product. The spectra of the three dyes can be appreciated in Fig. 1, as well as the one from the reaction product of brilliant blue with potassium bromate. Fig. 2 shows the kinetic curves of these compounds at their absorption maxima, i.e.: 430, 630 and 490 nm for tartrazine and brilliant blue product, brilliant blue and sunset yellow, respectively.

4.2. Prediction on validation samples of set 1 and set 2

As mentioned in Section 3, appropriate sensor regions were selected in both dimensions before building the models. Specifically, times were restricted to 46–132 s and wavelengths to 400–500 nm, leading to 216×101 data points per sample. The temporal evolution of the absorbance intensity in the useful range for a sample containing tartrazine during reaction with bromate produces a second order data.

The second-order data for each of the test samples were joined to those for the calibration set and were arranged as column-wise bidimensional data structures to being analysed with MCR-ALS.

To estimate the number of components for each set of samples, preliminary exploratory data analysis using singular value decomposition (SVD) combined with data inspection revealed that appropriate models could be made using three components for set 1 and four components for set 2. The initial estimations for MCR-ALS were obtained by the selection of the purest variables based on SIMPLISMA (Windig and Guilment, 1991).

In the present work, non-negativity in spectral and concentration profiles was applied for all of the analytes. The correspondence among species in the experiments was also used as constraint, indicating the presence or absence of the three or four components in the calibration and test samples of set 1 and 2, respectively.

Firstly, MCR-ALS was applied to set 1 in both the temporal- and spectral-augmentation modes. Unimodality constraint was also used in concentration profiles in the second case.

The areas under the concentration profiles retrieved by MCR-ALS for each sample are proportional to the analyte concentration and those corresponding to the calibration set were employed to build a pseudounivariate graph. As the non-linear behaviour between signal and concentration is transmitted, the relationship was modeled with a second degree polynomial function and was used to predict tartrazine concentration x_T:

$$y_T = a + bx_T + cx_T^2 \tag{2}$$

in which a, b and c are regression parameters and y_T the analyte area under the concentration profile. The least-square fitting gave $a = 4\ (4) \times 10^2$, $b = 148\ (9) \times 10^1$ and $c = -17\ (4)$ (standard deviation in the last significant figure in parentheses) and a correlation coefficient $r^2 = 0.9942$ for the temporal-augmentation mode. In the case of the spectral-augmentation mode, these values were $a = 13\ (3) \times 10^2$, $b = 112\ (7) \times 10^1$ and $c = -17\ (3)$ and a correlation coefficient $r^2 = 0.9924$.

The gathered prediction results are presented in Table 1. As can be seen, better results are obtained when the temporal-augmentation mode is applied, with a REP% of 9.0. On the other hand, a value of REP% of 14.7 is obtained when the spectral-augmentation is implemented. This fact can be ascribed to the degree of spectral and kinetic overlap between the intervening species calculated employing the following expression:

$$S_{12} = \frac{\|\mathbf{s}_1^T \mathbf{s}_2\|}{\|\mathbf{s}_1\| \|\mathbf{s}_2\|} \tag{3}$$

in which $\mathbf{s}_1$ and $\mathbf{s}_2$ are the spectra for components 1 and 2, respectively. The value of S_{12} ranges from zero to one, corresponding to the extreme situations of no overlapping and complete overlapping, respectively. When Eq. (10) is employed to estimate the degree of kinetic overlap between components, the spectra are replaced with the corresponding time profiles.

The degree of spectral overlap between tartrazine and the oxidation product of brilliant blue is 0.9961, while their degree of kinetic overlap is 0.9779. At first sight, these values show a stronger overlap in the spectral dimension in comparison with the time dimension indicating that spectral-augmentation mode would be the best choice, but this is true if there is no lack of synchronization in the temporal profiles. Furthermore, the spectral and kinetic overlap between tartrazine and brilliant blue in the analysed wavelength region, are 0.9017 and 0.9921, respectively, showing the opposite behaviour. Additionally, if sunset yellow is considered, this performance is repeated and the kinetics of tartrazine and this dye are much more overlapped than their spectra (see Figs. 1 and 2). For these reasons, samples of set 2 were only analysed in the temporal-augmentation mode.

Fig. 3 A and B shows both the kinetic and spectral profiles retrieved by MCR-ALS when processing a test sample of set 2 and some of the calibration samples. Notice in Fig. 3A that the profiles

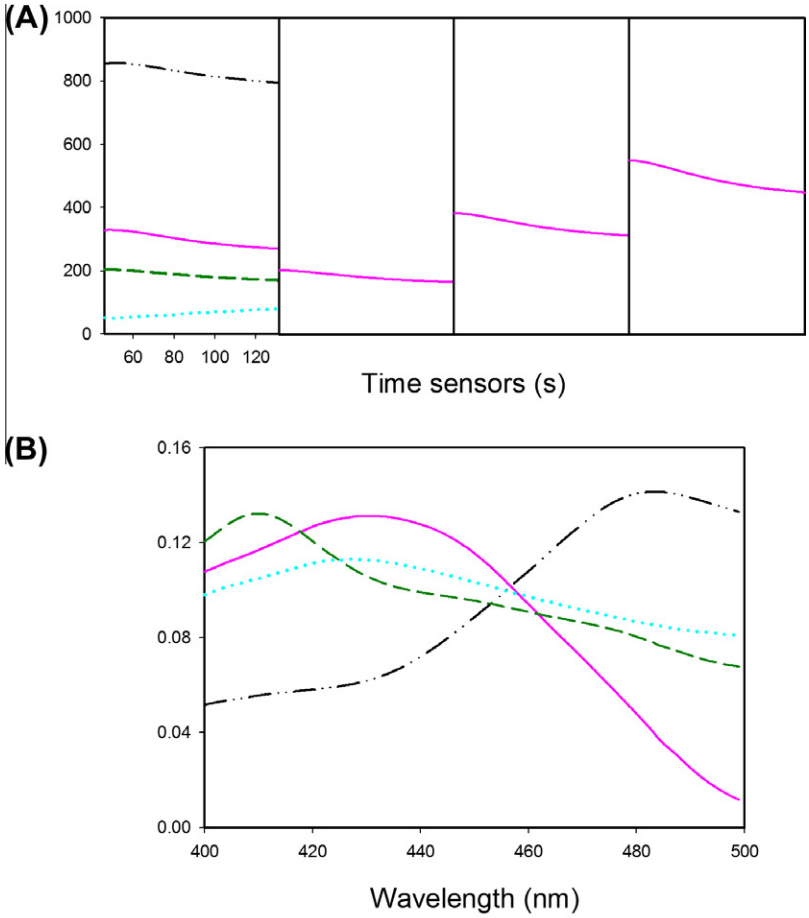

Fig. 3. Profiles retrieved by MCR-ALS when processing a test sample of set 2 together with calibration samples. (A) Kinetic profiles. (B) Spectral profiles. In both cases, pink solid line indicates tartrazine, green dashed line brilliant blue, cyan dotted line the product of brilliant blue oxidation and black dashed-dotted-dotted line sunset yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

show the presence of an increasing signal due to the brilliant blue oxidation product in the test sample. In addition, another two signals with decaying profiles in the working wavelengths can be seen due to the absorption of brilliant blue in this region and to the presence of sunset yellow which produces an uncoloured compound during its degradation. On the other hand, a time-decreasing signal of tartrazine is appreciated in both the test and calibration samples.

The prediction results for both sets (Tables 1 and 2) were reasonably good, leading to a mean recovery of 98 (7)% and 99 (11)% , and a relative error of prediction (REP) of 9.0% and 6.5% based on the average calibration concentration for set 1 and 2, respectively, in the temporal-augmentation mode. These parameters indicate that the proposed method is a feasible methodology for achieving the second-order advantage in cases of sample components with very similar spectra and a non-linear relationship between signal and analyte concentration.

As U-PCA/RBL has been found to be flexible enough to deal with non-linear second-order information (García-Reiriz et al., 2008) and taking into account the non-linear behaviour demonstrated before, we worked under the hypothesis that this algorithm could be useful to model the data obtained for this kinetic system.

The data matrices were unfolded into column vectors of size 21715×1. The scores obtained from the calibration samples were also employed to build a polynomial function [see Eq. (2)], but in this case y_T refers to tartrazine scores. The regression coefficients were $a = 4\ (2) \times 10^2$, $b = 61\ (3) \times 10^1$ and $c = -8\ (2)$, and the correlation coefficient $r^2 = 0.9938$.

The test samples of set 1 were subjected to residual bilinearization in order to eliminate the contribution of the two uncalibrated components and to a PCA analysis in order to obtain the scores. When U-PCA/RBL was applied to the validation set 2 to acquire the scores without the contributions of uncalibrated components only one interferent was found.

Prediction errors were higher than those obtained with MCR-ALS leading to a REP of 14.6% and 16.3% when predicting samples of set 1 and set 2, respectively. This might be due to the observed failure in extracting a convenient interferent profile and can be responsible of the poor ability of the U-PCA/RBL algorithm to model the data under study. As was recently commented, when severe overlapping occurs, RBL may not be a useful procedure for obtaining the second-order advantage (García-Reiriz et al., 2007, 2008). Consequently, the amount of tartrazine in the presence of brilliant blue as unexpected components can not be accurately quantitated following this strategy.

4.3. Prediction of beverage samples

MCR-ALS was employed to analyse drink samples with tartrazine in the presence of other dyes. In order to validate the performance of the new method, the samples were also analysed by the HPLC method and the predicted tartrazine concentrations are displayed in Table 3.

A typical chromatogram of the dyes in one of the studied samples, in which tartrazine and sunset yellow are present, shows the complete separation of the components achieved in six minutes

(figure not shown). Furthermore, samples with tartrazine and brilliant blue needed nearly 10 min to be separated. On the other hand, only 150 s are enough to perform an accurate quantitation of tartrazine when the proposed method is applied.

To decide whether the results of the proposed method and the HPLC method are comparable or not, *t* statistical test was carried out on the basis of paired *t*-statistic technique (Miller & Miller, 2005). As can be appreciated in Table 3, *t*-calculated value (0.56) is lower than *t*-table value (2.78 with 95% confidence level and 4 degrees of freedom), which indicates that there are no significant differences between the reference method and the strategy described in the present report.

The reasonable figures of merit obtained when comparing MCR-ALS results with those obtained by the chromatographic method demonstrate the acceptable performance of the proposed methodology.

5. Conclusions

The combination of stopped-flow analysis and the kinetic reaction with bromate, which generates second-order data presenting non-linear behaviour, can be successfully implemented to determine tartrazine in the presence of other dyes and unexpected sample matrix components.

The most convenient method (U-PCA/RBL), in principle, could not be capable of modeling the existent collinearity between the analyte and interferent spectra, showing a limitation of the method. On the other hand, it is remarkable the ability of MCR-ALS to model non-linear second-order data providing profiles whose areas could be fit with a convenient polynomial function, retrieving satisfactory figures of merit. When this model is applied to the analysis of real samples, the results are satisfactory and statistically comparable with those delivered by HPLC. Furthermore, the levels of tatrazine which were found in the analysed samples were five times lower than its maximum value established by the Argentinian legislation. If future samples with higher concentrations need to be analysed, a simple dilution will make it possible.

The proposed method provides a rapid, accurate, and economical alternative to separation methods.

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