



Second order advantage in the determination of amaranth, sunset yellow FCF and tartrazine by UV–vis and multivariate curve resolution-alternating least squares

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ABSTRACT

A direct spectrophotometric method for the determination of three artificial colors – amaranth, sunset yellow FCF and tartrazine – in beverages samples is proposed. The spectra were recorded between 359 and 600 nm. The spectra of the samples (just filtrated), pure dyes (concentrations ranged between 0.01 and 1.8 mg L⁻¹ for amaranth, 0.08 and 4.4 mg L⁻¹ for sunset yellow and 0.04 and 1.8 mg L⁻¹ for tartrazine) and synthetic mixtures were disposed in a column-wise augmented data matrix. This kind of data structure, analyzed by multivariate curve resolution-alternating least squares (MCR-ALS) makes it possible to exploit the so called 'second order advantage'. MCR-ALS algorithm was applied to the experimental data under the non-negativity and equality constraints. As a result, the concentration of each dye in the samples and their corresponding pure spectra were obtained.

The results were validated using internal reference materials and no significant differences were found ($\alpha = 5\%$) between the reference values and the ones obtained with the proposed method. The second order advantage made it possible to obtain unbiased results even in the presence of interferences.

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1. Introduction

Synthetic dyes are usually added to foodstuffs and soft drinks not only to improve appearance, colour and texture but also to maintain the natural colour during process or storage. Synthetic dyes 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, many of them may exhibit adverse health effects (allergy, respiratory problems, thyroid tumours, chromosomal damage, urticaria, hyperactivity, abdominal pain, etc.) [1,2].

On the other hand, in some cases the use of food dyes is also indicative of foodstuff adulteration such as in their addition to fruit juices. Thus, the use of synthetic dyes is strictly controlled by laws, regulations and acceptable daily intake (ADI) values [3]. These regulations frame the role of the analytical chemist who has to test for the levels of dyes added to food. Amaranth (E123), sunset yellow FCF (E110) and tartrazine (E102) are among the synthetic dyes mainly used in non-alcoholic beverages and the ADI values are between 0 and 0.5 mg kg⁻¹ for amaranth, 0 and 2.5 mg kg⁻¹ for sunset yellow and 0 and 7.5 mg kg⁻¹ for tartrazine [3]. Also, Argentine Alimentary Code establishes a maximal concentration

for non-alcoholic drinks of 50 mg L⁻¹ of amaranth and 100 mg L⁻¹ for sunset yellow FCF and tartrazine [4].

Some problems found in artificial colors determination are related to the variety of dyes mixtures and the potential interferences present in the commercial samples. Therefore, the analyses have traditionally been focused on separation methods. The analytical methods frequently used for the determination of amaranth, sunset yellow and tartrazine include thin layer chromatography (TLC) [5], capillary electrophoresis (CE) [6–8], and mainly high-performance liquid chromatography (HPLC) [9–12]. However, some disadvantages arise from these methods, such as usage of toxic solvents, spending of time, and the need of sample pre-treatments. The direct UV–vis spectrophotometric determination represents a rapid, simple, and cheap method for the determination of these colorants. In spite of this, the direct spectrometric measurements show lack of specificity because the spectra are strongly overlapped. In such cases the chemometric techniques become an indispensable tool to overcome these problems. In this sense, Ni and coworkers [13] have carried out a kinetic spectrophotometric analysis of some food colorants in drinks and jellies (previously reacted with a suitable chromogenic reagent) with the aid of several chemometric tools: Iterative target factor analysis (ITFA), principal component regression (PCR), partial least squares (PLS) and principal component-radial basis function-artificial neural network (PC-RBF-ANN). Also, Lachenmeier and Kessler [14] have compared PLS and multivariate curve resolution (MCR) in the study of artificial food colors by UV–vis, but only reported qualitative

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results, i.e. they used the multivariate models to parametrically judge the presence or absence of the food colours.

The first order calibration methods (such as PLS) need that both unknown samples and standards have the same chemical and physical characteristics, even the eventual interferences. Second order calibration methods make it possible to carry out the determination using standards that contain only the analyte of interest and so, the interferences do not need to be present in the calibration standards. This is universally recognized as the second order advantage [15].

In the current study, a direct spectrophotometric method for the determination of three artificial colors – amaranth (AM), sunset yellow (SY) and tartrazine (TA) – in beverages samples is proposed. The resolution of the mixtures was carried out by multivariate curve resolution optimized by alternating least squares (MCR-ALS) [16,17]. Sample spectra were disposed in augmented data matrices in order to achieve the second order advantage. In this way, calibration can be carried out with few standards instead of a large set of calibration standards and, in addition, it is possible to quantify the analytes in the presence of potential interferences. To the best of our knowledge, it is the first time that multivariate curve resolution-alternating least squares is applied to the direct spectrophotometric quantitative determination of dyes in real samples of soft drinks and isotonic drinks.

2. Materials and methods

2.1. Reagents and solutions

All solutions were daily prepared. Analytical reagent-grade chemicals and ultra pure deionized water ($18.3 \Omega \text{ cm}^{-1}$, Barnstead, Dubuque, USA) was used.

Amaranth, sunset yellow FCF and tartrazine 0.10 mol L^{-1} stock solutions (all from Aldrich) were prepared in ultra pure water. Standard solutions and mixtures of dyes were freshly prepared by appropriated dilution of stock solutions with HCl 0.10 M .

Hydrochloric acid solution, concentration 0.10 mol L^{-1} , was prepared with a suitable volume of hydrochloric acid (Merck) dissolved in ultra pure water.

2.2. Apparatus and software

A UV–vis Agilent 8453 spectrophotometer with diode array detector was used. All measurements were carried out at room temperature using a Hellma 178-010-QS quartz cell (10 mm light path).

A Ross Sure Flow 8172 ion selective electrode was used for potentiometric measurements of pH.

The UV–vis spectra were recorded between 190 and 800 nm, in steps of 1 nm. All spectra were exported in ASCII format, and the data treatment was carried out using MATLAB® [18] and the multivariate curve resolution-alternating least squares subroutines [19].

2.3. Procedure

2.3.1. Calibration standards and mixtures of dyes

Standard calibration solutions of the analytes (five standards for each dye) were prepared in the concentration ranges between 0.01 and 1.8 mg L^{-1} for amaranth, 0.08 and 4.4 mg L^{-1} for sunset yellow and 0.04 and 1.8 mg L^{-1} for tartrazine.

On the other hand, nine mixtures of the analytes were prepared in order to evaluate the prediction error of the chemometric method.

The overall prediction error was calculated using the following expression [20]:

$$\text{Error}(\%) = \frac{\sqrt{\sum_{i=1}^{\text{sample}} (C_{i\text{true}} - C_{i\text{calc}})^2}}{\sqrt{\sum_{i=1}^{\text{sample}} (C_{i\text{calc}})^2}} \times 100 \quad (4)$$

where $C_{i\text{true}}$ was the true concentration of the analyte in the synthetic mixture i and $C_{i\text{calc}}$ was the concentration calculated by the proposed method.

2.3.2. Beverage samples

The analyzed samples were seven soft drinks and two isotonic drinks that were purchased on a local supermarket. Samples were previously homogenized and filtered through glass fiber filters ($0.45 \mu\text{m}$ in pore size). Then, appropriated dilutions were made with HCl 0.1 M . The expanded uncertainties for the samples were calculated considering the standard deviation obtained from the calibration curves and the standard uncertainties of the volumetric materials used in the sample preparation.

2.3.3. Internal reference materials

Six internal reference materials were prepared in our laboratory by mixing defined aliquots of commercial beverages, in order to ensure the presence of the matrix components. Thus, the reference material IRM1 was prepared by mixing aliquots of soft drinks containing AM and SY (as indicated in the label of the commercial beverages), the reference material IRM2 was prepared by mixing aliquots of soft drink containing SY and TA, and the reference material IRM3 was prepared by mixing isotonic drinks. Also, reference material IRM4 was prepared by adding 5 mg L^{-1} of both AM and SY dyes to the reference material IRM1, reference material IRM5 was prepared by adding 5 mg L^{-1} of both SY and TA to the reference material IRM2 and reference material IRM6 was prepared by adding 5 mg L^{-1} of both SY and TA to the reference material IRM3.

All reference materials were filtered through glass fiber filters ($0.45 \mu\text{m}$ in pore size) and kept at -4°C .

The resulting internal reference materials have similarity with the sample matrix, are homogeneous and their stability was checked, and so they are useful for three months. The traceability was assessed by an external laboratory. The reference procedure used in the external laboratory was adapted for the HPLC method proposed by Pereira Alves et al. [11]. Chromatograms were recorded at room temperature, using a mixture of methanol:ammonium acetate 0.08 mol L^{-1} (23:77) as mobile phase flowing at 1 mL min^{-1} with ultraviolet detection at 454, 484 and 550 nm for tartrazine, sunset yellow and amaranth, respectively.

2.4. Data analysis

2.4.1. Pretreatment and data arrangement

Previously to the data analysis, wavelengths of interest were selected. The spectral region where absorption bands of dyes were not observed (i.e. wavelengths higher than 600 nm), was removed. Also, those wavelengths related to the absorption bands of other species present in the beverages samples (such as sweeteners and preservatives) were eliminated (wavelengths lower than 359 nm) because of the strong overlapping with the dyes absorption bands in this spectral region. In this way, the resulting spectra ranged between 359 and 600 nm (242 variables).

After wavelength selection, spectra were arranged in matrices. Thus, three matrices (5×242 in size) were constructed; one for each set of spectra associated to the standard solutions of each analyte. Each individual spectrum corresponding to the mixtures (nine), samples (nine) or reference materials (six) was considered

as a matrix (1×242 in size). For quantification, the data matrices corresponding to the samples, mixtures and internal reference materials have to be simultaneously analyzed with those of the standards. So, all the matrices (27) were disposed in a column-wise augmented matrix (39×242 in size).

2.4.2. Rank analysis

The number of chemical species present in augmented matrix was first estimated by singular value decomposition (SVD) [21], since it was assumed that the singular values associated to the chemical components are much larger than other possible contributions such as instrumental drift or experimental error. Therefore, the chemical rank was estimated by simply inspecting of the tables of singular values for the augmented matrix. The number of species finally chosen was checked to provide a chemically reliable resolution of the system.

2.4.3. Initial estimates of the spectra

Initial estimates of the pure spectra were obtained by using the SIMPLISMA algorithm, a technique based on selecting of the purest variables [22]. SIMPLISMA was applied to the augmented matrix to search for spectral estimates of the different components.

2.4.4. Alternating least squares (ALS) optimization

The ALS optimization algorithm for curve resolution has been described in detail elsewhere [16,17]. A series of constraints was applied in an attempt to improve the optimization and to restrict the number of possible solutions: (a) the pure spectra of each component must be non-negative; (b) the concentration profiles of each component must be non-negative, (c) the pure spectra of the studied dyes were fixed throughout the iterations of the algorithm. Since the spectra for TA, AM and SY standard solutions were available, the algorithm was forced (as an equality constraint) to keep these spectral shapes for the dyes during the MCR-ALS optimization. Moreover, when multiple matrices are simultaneously analyzed by column-wise matrix augmentation, additional conditions are fulfilled: the pure spectra of common components in the different samples (matrices) are equal and there is correspondence of common components between samples.

3. Results and discussion

3.1. Selection of the experimental conditions

Fig. 1 shows the spectra of AM, SY and TA in acidic medium. There is no variation among the spectra recorded in acidic and neutral media, whereas at pH higher than 7.0 an hypsochromic shift takes place and the spectral bands of the three dyes are more overlapped. The beverages samples studied are acidic, so this medium was preferred for the dyes determination. As can be seen in Fig. 1, the spectra for the three analytes (i.e. AM, SY and TA) are considerably overlapped, except for AM at wavelengths higher than 560 nm.

3.2. Rank analysis

A good resolution of the system strongly depends on the correct selection of the number of components that, in absence of other sources of variability such as instrumental drift or noise, could be assumed as equal to the number of absorbing species present in the system. Table 1 shows the singular values obtained when SVD was applied to the augmented data matrix. A visual inspection of the singular values suggests that there are five significant factors instead of the three expected components (i.e. AM, SY and TA). It could indicate the existence of other absorbing species that are different to

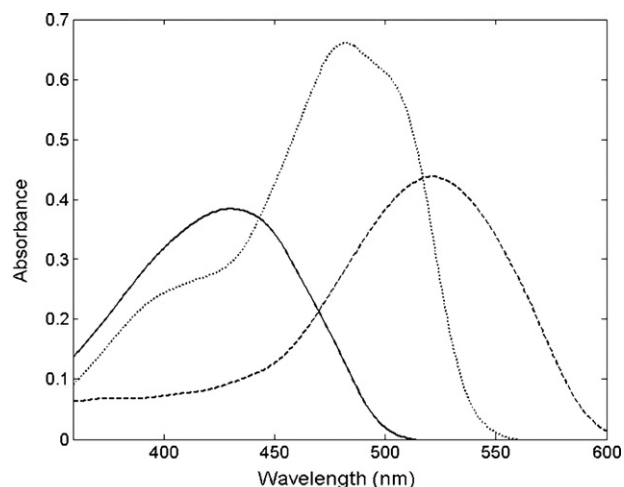


Fig. 1. Pure spectra of the studied dyes: tartrazine 1.8 mg L^{-1} (solid line), sunset yellow 4.4 mg L^{-1} (dotted line) and amaranth 1.8 mg L^{-1} (dashed line).

Table 1

Singular values corresponding to the SVD of the augmented data matrix.

Singular values
44.4
11.4
5.2
3.0
1.2
0.3
0.1
<0.10

the analytes under study, or some spectral artefacts (interactions, spectral distortions, etc.) that contribute to the observed variability.

3.3. ALS optimization

Fig. 2 shows the five purest spectra recovered by SIMPLISMA when applied to the augmented data matrix. The first recovered spectrum corresponds to TA standard solution, the second one to AM and the fourth to SY. The third and fifth spectra are selected among the beverages samples and presumably are related to interferences present in the sample matrix. Thus, the spectra recovered

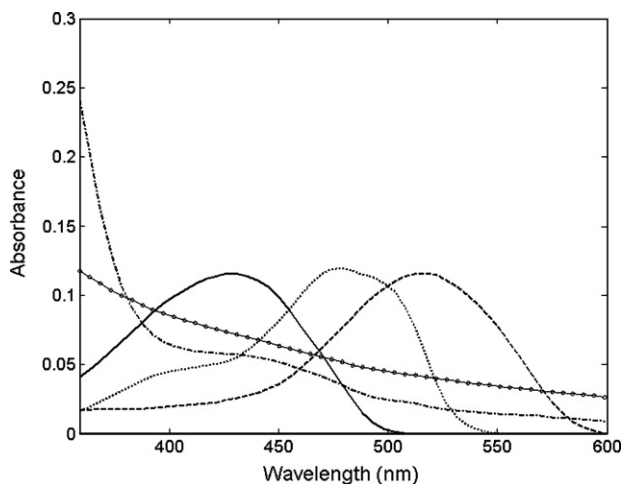


Fig. 2. Spectra recovered when SIMPLISMA was applied to the augmented data matrix. Tartrazine (solid line), sunset yellow (dotted line), amaranth (dashed line), interference 1 (dash-dotted line), interference 2 (line with circles).

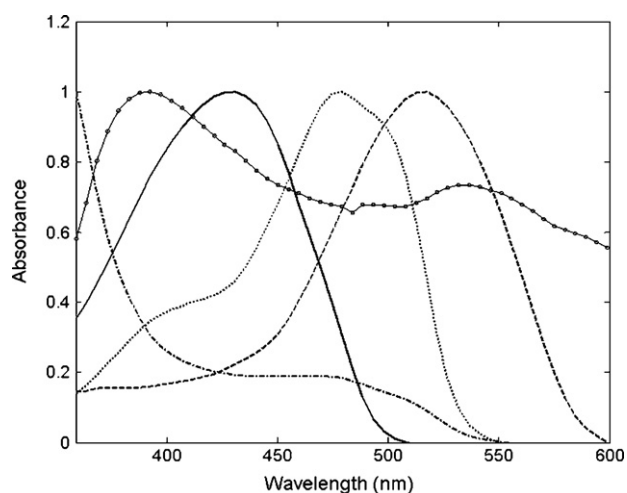


Fig. 3. Spectra recovered by MCR-ALS. Tartrazine (solid line), sunset yellow (dotted line), amaranth (dashed line), interference 1 (dash-dotted line), interference 2 (line with circles).

by SIMPLISMA were used as spectral initial estimates for the ALS optimization.

During the optimization, the constraints listed in Section 2.4.4 were applied. Information about the presence/absence of the analytes in the samples was given and it was based on the labels of the commercial samples. It was assumed that interferences were present in all the beverages samples and internal reference materials.

The applied equality constraint, which consists of fixing the pure spectra of the dyes during the optimization process, contributed to break-up the rotational and intensity ambiguities that are intrinsic to the MCR-ALS methods. In this way, quantitative results could be obtained.

It is important to note that the *second order advantage* is achieved by simultaneously analyzing the calibration data and the samples data. This large data set was decomposed into contributions from the analytes and the potential interferences, and prediction was made in a pseudounivariate manner [23].

After optimization the values of lack of fit was 8.36% and the explained variance was 99.3%. Fig. 3 shows the recovered spectra in the matrix S^T after the ALS optimization. Besides the spectra of AM, SY and TA, the spectra corresponding to the interferences were recovered.

Quantification was performed by regressing the scores values obtained in the matrix C for each standard against the known concentration values. The following straight line equations were obtained for each analyte:

$$\begin{aligned} \text{Amaranth} &: Y = 0.0322 C + 0.0002, \\ \text{Sunset yellow} &: Y = 0.0465 C + 0.0065, \\ \text{Tartrazine} &: Y = 0.0281 C + 0.0017, \end{aligned}$$

Table 4

Comparison between the reference values of the internal reference materials and the concentrations obtained for the three dyes by MCR-ALS. Values of concentration are reported with their respective confidence intervals.

Reference material	Proposed method (mg L ⁻¹)			Reference values (mg L ⁻¹)		
	AM	SY	TA	AM	SY	TA
IRM1	0.14 ± 0.02	15.5 ± 0.4	–	0.14 ± 0.02	15.6 ± 0.3	–
IRM2	–	6.2 ± 0.2	2.2 ± 0.2	–	6.3 ± 0.3	2.3 ± 0.2
IRM3	–	15.2 ± 0.3	1.0 ± 0.1	–	14.4 ± 0.3	0.9 ± 0.2
IRM4	5.79 ± 0.04	19.7 ± 0.3	–	5.42 ± 0.03	20.0 ± 0.2	–
IRM5	–	11.2 ± 0.1	6.7 ± 0.3	–	11.2 ± 0.2	7.1 ± 0.1
IRM6	–	20.2 ± 0.4	5.7 ± 0.2	–	19.2 ± 0.3	6.0 ± 0.1

Table 2

Actual values for studied dyes in the synthetic mixtures, concentration values obtained by MCR-ALS and the corresponding relative errors (%).

	Predicted values (mg L ⁻¹)			True values (mg L ⁻¹)			% Error		
	AM	SY	TA	AM	SY	TA	AM	SY	TA
1	5.5	5.8	–	5.4	5.6	–	1.8	3.6	–
2	–	5.6	–	–	5.6	–	–	0.0	–
3	–	5.7	5.6	–	5.6	5.4	–	1.8	3.7
4	–	–	5.5	–	–	5.4	–	–	1.8
5	5.4	5.3	5.3	5.4	5.6	5.4	0.0	5.4	1.8
6	–	5.6	5.7	–	5.6	5.4	–	0.0	5.6
7	–	–	5.3	–	–	5.4	–	–	1.8
8	–	5.7	5.2	–	5.6	5.4	–	1.8	3.7
9	5.2	5.4	–	5.4	5.6	–	3.7	3.6	–

Table 3

Concentrations and expanded uncertainties of AM, SY and TA obtained by MCR-ALS in the analysis of beverages samples.

Sample	Concentration (mg L ⁻¹)		
	AM	SY	TA
SD1	2.1 ± 0.3	11.7 ± 2.1	–
SD2	–	30.2 ± 3.3	–
SD3	–	29.1 ± 3.2	–
SD4	16.1 ± 0.6	18.5 ± 2.5	–
SD5	–	–	8.2 ± 1.2
SD6	–	12.1 ± 2.1	6.4 ± 1.0
SD7	–	9.2 ± 1.8	8.7 ± 1.2
ID1	–	25.4 ± 3.0	–
ID2	–	–	5.6 ± 0.9

$n=3$.

where Y are the values of the C matrix obtained for each analyte and C are the concentration values in mg L⁻¹.

Table 2 shows the results obtained for the analysis of the synthetic mixtures prepared to evaluate the prediction error. The individual errors are below 5.6% and the calculated overall prediction error was 3.0%.

3.4. Beverages samples

The proposed method was applied to the simultaneous determination of AM, SY and TA in beverages samples. Table 3 shows the values obtained for each dye in the seven samples of soft drink (SD1 to SD7) and the two samples of isotonic drink (ID1 and ID2) and their corresponding expanded uncertainties. None of the samples exceed the maximum established by the Argentine Alimentary Code. A warning should be made in reference to the sample SD4. A person who weighs about 30 kg (typically, a child), which consumes 1 L of soft drink per day, could exceed the ADI established for amaranth.

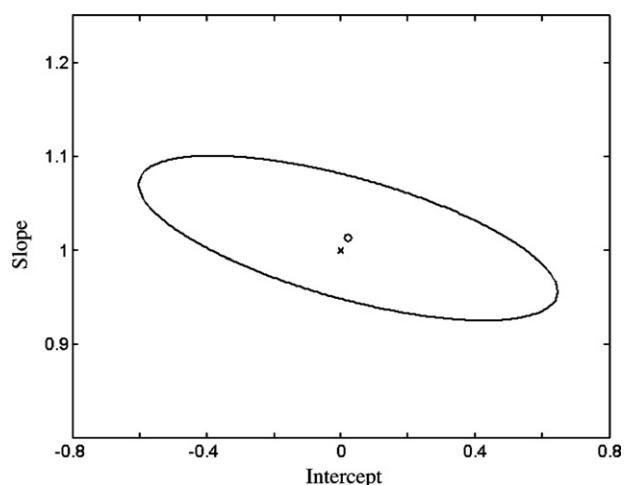


Fig. 4. Elliptical joint confidence region for the BLS regression at an overall level of significance of 5%. (○) Represents the obtained values of slope and intercept and (×) corresponds to the theoretical value of zero intercept and unity slope.

3.5. Method validation

The analytical results obtained by the proposed method were regressed on the results obtained by a reference method. In this case we used HPLC [11]. In this way, a straight line is expected. Bivariate least squares (BLS) could be regarded as being an appropriate technique to perform the regression because it considers errors in both axes [24]. In order to test whether there are significant differences between the regression coefficients and the theoretical values of zero intercept and unity slope, the joint confidence interval test for the slope and the intercept can be used [25]. The joint test verifies the presence of the theoretical point zero intercept and unity slope within the limits of the elliptical-shaped joint confidence region centered on the slope and intercept values of the straight regression line obtained.

Table 4 shows the concentration for AM, SY and TA obtained by the proposed method when internal reference materials were analyzed and the reference values. When the concentrations recovered by MCR-ALS were plotted versus the reference values of the internal reference materials, the bivariate least square technique gave a straight regression line, with a slope near to 1 and an intercept near to 0 ($y = 1.01x + 0.02$). The qualitative similarity between the concentration values obtained by the proposed method and the reference values was evaluated in terms of the correlation coefficient of this straight line. The value obtained was 0.996. Because the validation involved comparing values at different concentration levels, the level of significance had to be corrected by using the Bonferroni adjustment [21]. Thus, to carry out the test at an overall significant level of $\alpha = 0.05$, each individual comparison had to be performed at a significance level of $\alpha' \approx 0.004$, considering 12 comparisons.

The results show (see Fig. 4) that there is no significant statistical difference between the values obtained by the proposed method and the reference values, considering an $\alpha = 0.05$ as the overall significance level.

From these results it can be concluded that, in comparison to the reference concentrations, there is no significant bias in the values obtained by MCR-ALS. The intrinsic intensity ambiguity, that normally affects MCR-ALS, was overcome by applying the equality constraint.

4. Conclusions

The determination of amaranth, sunset yellow and tartrazine in isotonic and soft drinks was successfully performed in binary mixtures of the dyes by combined direct UV–vis spectrophotometric measurements and multivariate curve resolution-alternating least squares (MCR-ALS).

The second order advantage was achieved by simultaneously analyzing the calibration data and the sample data, and the resolution and determination was possible even in presence of some interferences contained in the almost untreated samples.

The trueness of the results was appropriately validated by analyzing internal reference materials manufactured in our laboratory. No bias was detected in the obtained results.

The proposed method could be applied to the food quality control, and represents an interesting, rapid, environmental friendly and cheap alternative to separation methods.

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