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Multivariate analysis of organic acids in fermented food from reversed-phase high-performance liquid chromatography data

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ABSTRACT

Multivariate calibration coupled to RP-HPLC with diode array detection (HPLC-DAD) was applied to the identification and the quantitative evaluation of the short chain organic acids (malic, oxalic, formic, lactic, acetic, citric, pyruvic, succinic, tartaric, propionic and α-cetoglutaric) in fermented food. The goal of the present study was to get the successful resolution of a system in the combined occurrence of strongly coeluting peaks, of distortions in the time sensors among chromatograms, and of the presence of unexpected compounds not included in the calibration step. Second-order HPLC-DAD data matrices were obtained in a short time (10 min) on a C18 column with a chromatographic system operating in isocratic mode (mobile phase was 20 mmol L^{-1} phosphate buffer at pH 2.20) and a flow-rate of 1.0 mL min^{-1} at room temperature. Parallel factor analysis (PARAFAC) and unfolded partial least-squares combined with residual bilinearization (U-PLS/RBL) were the second-order calibration algorithms select for data processing. The performance of the analytical parameters was good with an outstanding limit of detection (LODs) for acids ranging from 0.15 to 10.0 mmol L^{-1} in the validation samples. The improved method was applied to the analysis of many dairy products (yoghurt, cultured milk and cheese) and wine. The method was shown as an effective means for determining and following acid contents in fermented food and was characterized by reducibility with simple, high resolution and rapid procedure without derivatization of analytes.

1. Introduction

The search for better chromatographic conditions in isocratic mode, particularly as regards the analysis especially the ones that involve analysis time and mobile phase composition, establishes a well-defined limit on the analytical resolution. In general, shorter analysis times for relatively complex mixtures causes little resolution, leading to analytical methods which produces poor selectivity. Moreover, the great number of compounds to be separated as well as the similarities among them also contribute to this poor performance. This in turn is amplified by the complexity of the matrices under study, mainly wines for their polyphenols that are usually important interfering. An alternative to generate good resolutions in short analysis times is the collection of multi-dimensional chromatographic information and the data processing by advanced chemometric algorithms. A quick overview in the latest scientific productions shows that there is abundant evidence on the successful combination of chemometric models with several chromatographic techniques, either for quantitative or qualitative purposes [1–9]. In all cases, giving a chemometric approach to the developed chromatographic methodology makes it possible to resolve analytes whose bands or peaks have similar or even identical retention times [10,11]. This is possible because the chromatographic signal becomes selective, at least from a mathematical point of view, for each of the analytes involved, due to a suitable multivariate analysis [10,11]. Moreover, it is conceivable to quantify the analytes of interest even in the presence of other components not included in the calibration step, provided that the instrumental data has a matrix structure [12]. This property is known as “the second-order advantage”, and its unmistakable impact on the different areas of analytical chemistry accounts for the growing efforts for the development of multi-way analytical data [12]. Examples from recent literature on quantitations aided by the second-order advantage include the determination of sex hormones in environmental waters and sediments [13], agrochemical-residue in vegetables [14], urea herbicides in water and soil samples [15],

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endocrine disruptors in beverages [16] and polycyclic aromatic hydrocarbons in water [17].

As the different chemometric algorithms reach the second order advantage in several ways, it becomes necessary to take into account certain considerations to select the most convenient chemometric strategy [18]. A brief but illustrative classification of the most widespread second-order models can be established in the following list: (1) alternating least-squares (ALS) models, such as multivariate curve resolution–alternating least-squares (MCR–ALS) [19] and parallel factor analysis (PARAFAC) [20] or PARAFAC2 [21], (2) latent structured models, such as unfolded partial least-squares (U-PLS) [22], the term 'unfolded' implies vectorizing the data matrices before mathematical decomposition, and multi-way PLS (N-PLS) [23], both combined with residual bilinearization (RBL) [24,25], and (3) eigenvector–eigenvalue models [26]. The first point to note is that, although all the listed methods can exploit the second-order advantage, only some of them tolerate the presence of chromatographic artifacts. In second order liquid chromatography, the data are considered trilinear when each of the chemical compounds that constitute it presents a unique profile in all samples, in both spectral and temporal dimensions. Nonetheless, successive chromatographic runs can be affected by several experimental conditions, which cause a distortion effect on the data, known as "loss of trilinearity" [18]. MCR-ALS and PARAFAC2 models can, in principle, process non-rilinear data with relative success. However, a recent work shows that for systems with many analytes and interferents, results may not be as satisfactory [27]. On the other hand, it is possible to align the analyte peaks and restore the lost trilinearity in order to obtain a better analytical performance. Numerous methods are available to perform such alignment, which are distinguished in terms of their ability to perform the alignment process in the presence or absence of interferents. The first group include the rank alignment [28], and a suitably initialized and constrained PARAFAC alignment [29], among others. In the second group, on the other hand, the multi-wavelength correlation optimized warping (COW) stands out [30,31]. For all the above reasons, and on the basis that the samples to be analyzed are likely to contain interferents not considered in the calibration step, it was decided to pre-process the data with PARAFAC alignment to restore the trilinearity in order to obtain more satisfactory results in the subsequent chemometric treatment.

In this work, collected data from high performance liquid chromatography with diode array detection (HPLC-DAD) were processed by the PARAFAC and U-PLS/RBL algorithms. The behavior towards the quantitation of the following organic acids in dairy products: oxalic (OXA), citric (CIT), formic (FOR), succinic (SUC), pyruvic (PYR), acetic (ACE), tartaric (TAR), propionic (PRO), lactic (LAC), α-ketoglutaric (CET) and malic (MAL) was thoroughly discussed. Dairy products contain particular organic acids, those naturally present in raw milk (CIT, orotic and uric) and those originated from hydrolysis of fat acid (ACE, butyric), additional acidification (CIT and LAC) or bacterial fermentation metabolisms (LAC, ACE, PYR, PRO and FOR). Table 1 summarizes the main organic acids and the concentration range found in some fermented foods (dairy products and wines) [32–36]. Also, they are the major products of carbohydrate catabolism of lactic acid bacteria (LAB). Their ability to produce acids with resulting pH reduction is the major factor in milk fermentation [37]. Thus, in both dairy products and fermented food in general, acidic conditions avoid the growth and viability of spoilage and pathogenic microorganisms, which constitute one of the main functional advantage, preserving the food safety and quality. However, it should be noted that the decrease in pH and the type of organic acids generated by LAB produce the effective inhibition of undesirable microorganisms [38]. The quantitative determination of organic acids is important to monitor bacterial growth and activity and for nutritional reasons. Organic acids are also important as they contribute to the flavour and aroma of dairy products [39]. In this research work, representative compounds conforming the core organic acids normally present in the fermented foods mentioned above were studied.

Table 1
Organic acids found in fermented foods, their concentrations and main sources.

Organic acid	Concentration in fermented food				
	Wine (mmol L^{-1})			Dairy product (mmol Kg^{-1})	
Tartaric	Grape	10–33		nd[c]	
Malic		1.5–30		nd[c]	
Citric		0–2.6		0.05–100	Milk/
Orotic		nd[c]		0.001–0.008	yogurt
Uric		nd[c]		0.001–0.006	
Hipuric		nd[c]		–[d]	
Lactic		1.1–11[e]	Bacterial growth	9.0–233[f]	
Acetic		3.3–22[g]	and fermentation[a]	8.3–580	FH[b]
Propionic		–[d]		6.7–54	
Succinic		0–17[g]		0.0–26[g]	
Pyruvic		–[d]		0.23–6	
Oxalic		–[d]		0.02–8	
Formic		–[d]		0–35[h]	
Oxaloacetate		–[d]		–[d]	
Fumaric		–[d]		–[d]	
Galacturonic		–[d]		–[d]	
Glucuronic		–[d]		–[d]	
Butiric		ndc		0.0–25	FH[b]

[a] The ranges of organic acid produced by fermentative process.
[b] FH: Milk Fat hydrolysis.
[c] nd: no detectable.
[d] traces.
[e] Žulj et al. reported a LAC concentration of 113 mmol L^{-1} for a Croatian predicate wine [32]. Also, Buglass and Lee informed a LAC concentration until 52 mmol L^{-1} in English red wines [33]. Likewise, Sirén et al. informed a LAC concentration of 60 mmol L^{-1} in commercial wine [34].
[f] Tormo and Izco reported 1.6 103 mmol Kg^{-1} (dry matter) of LAC concentration in yogurt lactic[43].
[g] The succinic acid concentration in Pinot Noir grapes range 0.85–68 mmol L^{-1} [34].
[h] In provolone and blue cheeses the FOR concentrations reported were 13 and 22 mmol Kg^{-1} (dry matter), respectively [35]. In Brick cheese the FOR concentration reported was 35 mmol Kg^{-1} (dry matter) [36].

Most methods developed to analyse organic acids in dairy products are HPLC methods that employ ion-exchange columns [40–42]. In general, these methods have some disadvantages: they use high operating temperatures at about 60 °C and expensive ion-exchange columns [40]. Also, co-elution of some of the organic acids and overlapping peaks have been frequently reported [41]. In a preceding work, the currently studied organic acids were chromatographically resolved with full resolution using ternary solvent gradient elution and reverse-phase columns, requiring approximately 18 min, although such methods present serious difficulties when quantifying the analytes of interest in the presence of interferents [43]. On the other hand, under the currently discussed isocratic conditions, the same compounds in the same type of mixtures eluted in less than 15 min using only phosphoric acid as mobile phase and the second-order advantage. Thus, a reduction in the elution time and consequently in the solvent consumption was achieved when the isocratic mode was used. On the other hand, the overlapping of the analyte retention times was solved with chemometric techniques complementing bidimensional chromatographic-spectral data. A comprehensive summary of the determination of organic acids in dairy products through reverse-phase HPLC (RP-HPLC) methods is presented in Supporting Information (Table 1).

In summary, the aim of this work was to develop a RP-HPLC technique alternative to the ion-exchange and reverse-phase methods for the simultaneous determination of 11 organic acids for being metabolically important components in fermented food and commonly cited in literature. Its application to the analysis of the quality of wine and dairy products has also investigated.

2. Theory

As the PARAFAC and U-PLS theories have been well established and

documented are not described in the present work [20,44]. However, it is necessary to point out that U-PLS reaches the second-order advantage only when coupled to the auxiliary RBL algorithm [24]. RBL is a post-calibration algorithm, which is established from principal component analysis (PCA) to identify the presence of "unexpected" components in a test sample [45]. Therefore, the test matrix data **X**, which can hypothetically have information about both interferents and the analytes of interest, were first vectorized [vec(**X**)] and then expressed as follows:

$$\mathrm{vec(X)} = \mathbf{Lt} + [\mathbf{U}_{\mathrm{int}}\mathbf{S}_{\mathrm{int}}(\mathbf{V}_{\mathrm{int}})^{\mathrm{T}}] + \mathbf{e}_{\mathrm{RBL}} \quad (1)$$

where **L** is the matrix of U-PLS calibration loadings, **t** is the vector of test sample scores, $\mathbf{e}_{\mathrm{RBL}}$ is the residual error RBL term, and $\mathbf{U}_{\mathrm{int}}$, $\mathbf{S}_{\mathrm{int}}$ and $\mathbf{V}_{\mathrm{int}}$ are provided by PCA of a residual matrix obtained after reshaping the residual vector $\mathbf{e}_{\mathrm{RBL}}$ computed, assuming that interferences are absent, by applying the Eq. (2):

$$\mathbf{U}_{\mathrm{int}}\mathbf{S}_{\mathrm{int}}(\mathbf{V}_{\mathrm{int}})^{\mathrm{T}} = \mathrm{PCA}\{\mathrm{reshape}[\mathrm{vec(X)}-\mathbf{Lt}]\} \quad (2)$$

where 'reshape' indicates the reverse operation of the vectorization, i.e., conversion of a $IJ \times 1$ vector into a $I \times J$ matrix (I being the number of time sensors and J the number of wavelengths), and the PCA process is performed using the first N_{int} principal components, where N_{int} indicates the number of interferent test sample components.

The RBL algorithm maintains the matrix of loadings **L** in Eq. (1) constant at the calibration values, and varies **t** in Eq. (2) so that the norm of $\mathbf{e}_{\mathrm{RBL}}$ ($||\mathbf{e}_{\mathrm{RBL}}||$) is minimized. The minimization process is implemented by using the Gauss-Newton method assisted by particle swarm optimization, so as to find good starting values for minimization [46].

The standard deviation (s) of the residuals in Eq. (1) can be considered as a measure of the goodness of fit for the RBL procedure, which is given by:

$$s = ||\mathbf{e}_{\mathrm{RBL}}||/(\mathrm{DOF})^{1/2} \quad (3)$$

where DOF is a suitable number of degrees of freedom. The DOF associated with the matrix reconstruction of Eq. (2) via PCA should be calculated as $[(I-N_{\mathrm{int}})(J-N_{\mathrm{int}})-T]$, where T is an additional loss of degrees of freedom calculated from the number of elements of **t** (equal to the number of calibration latent variables) [47].

The number of interferent components is estimated by examination of the comportment of s as the value of N_{int} increases. It is accepted that s stabilizes at a value which is compatible with the instrumental noise when the correct value of N_{int} is reached [45]. It is true that for more complex cases, that is, where the number of interferents is very large, different criteria should be used [48,49]. However, in this report it was not necessary to use a criterion other than the one commented on.

3. Experimental section

3.1. Reagents and solutions

OXA, CIT, FOR, SUC, PYR, ACE, PRO, LAC, CET, MAL, TAR and phosphoric acids, and sodium phosphate were purchased from Sigma (St. Louis, MO, USA). Milli-Q water (Bedford, MA, USA) was used to prepare buffers, stock solutions of each standard compound and the samples.

All solutions were prepared in standard volumetric flasks. Stock standard solutions of OXA (9.003 mg mL^{-1}), CIT (19.212 mg mL^{-1}), FOR (4.603 mg mL^{-1}), SUC (11,809 mg mL^{-1}), TAR (15.009 mg mL^{-1}), PYR (8.806 mg mL^{-1}), ACE (6.005 mg mL^{-1}), PRO (7.408 mg mL^{-1}), LAC (9.008 mg mL^{-1}), CET (14.611 mg mL^{-1}) and MAL (13.409 mg mL^{-1}) were prepared in analytical grade water (obtained from a MilliQ® water purification system) and stored at 4 °C.

3.2. Equipment and operating conditions

3.2.1. Reversed-phase HPLC method

RP-HPLC was carried out on a liquid chromatograph equipped with a Waters (Milford, MA, USA) 515 HPLC pump and a TCC-240A diode array UV–visible spectrometer (Shimadzu Corporation, Kyoto, Japan) as detector. A 50 µL loop was employed to introduce each sample onto a Zorbax SB C_{18} column (5 µm average particle size, 150 mm × 4.6 mm i.d.). The data matrices were collected using wavelength range from 206 to 288 nm each 2 nm, and times from 0 to 16 min every 1 s. The absorbance-time matrices were of size 42 × 996 and were saved in ASCII format, and transferred to a PC based on AMD Athlon ×2 Dual-Core QL-60 (1.90 GHz) microprocessor for subsequent manipulation.

The mobile phase used for all chromatographic runs was a 20 mmol L^{-1} phosphate buffer adjusted at pH 2.20 with phosphoric acid, delivered at a flow rate of 1.0 mL min^{-1} at room temperature with a chromatographic system operating under isocratic mode. Each chromatogram was accomplished in ca. 16 min.

3.2.2. Ion-exchange HPLC (IE-HPLC) method

Organic acid concentrations were determined in the samples by traditional HPLC method using an Aminex HPX-87H anion-exchange column (Bio-Rad Laboratories, Inc., Richmond, CA). Organic acids were determined by loading an aliquot of 200 µL of standard solution or sample operating at 30 °C in isocratic mode using 0.008 M H_2SO_4 as the mobile phase and a flow rate of 0.6 mL min^{-1}. Quantification of organic acid was carried out using external standard calibration curves. The calibration curves for each organic acid were obtained by triplicate injections of five aqueous solutions of different concentrations. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. Analytical parameters of the calibration curve for each acid (shown in Table 2 in Supporting Information) were used to calculate the acid concentration in real samples.

3.3. Calibration, validation, and test samples

The experimental procedure corresponding to the chemometric analysis was developed preparing a calibration set of 18 samples. Sixteen of these samples corresponded to the concentrations provided by a semi-factorial design at two levels. The remaining two samples corresponded to a blank solution and to a solution containing all the studied organic acids at an average concentration. The concentrations assayed were in the ranges 0–8 mmol L^{-1} for ACE, LAC and MAL, 0–1.5 mmol L^{-1} for PYR and CET, 0–4 mmol L^{-1} for CIT, 0–0.5 mmol L^{-1} for OXA, 0–3 mmol L^{-1} for TAR, 0–12 mmol L^{-1} for FOR and PRO, 0–10 mmol L^{-1} for SUC. These ranges were established based on the analysis of the linear absorbance-concentration range for each analyte. A set of 30 validation samples was prepared employing concentrations different from those used for calibration and following a random design.

Calibration and validation samples were prepared by measuring appropriate aliquots of standard solutions, placing them in 10.00 mL volumetric flasks in order to obtain the desired concentrations, and completing to the mark with mobile phase.

3.4. Sample preparation

Commercial samples of wine, yoghurt, fermented milk and cheese were purchased at local stores. For dairy products, one gram of sample was diluted to 10 mL with water and the preparation was vigorously shaken and blended with a vortex. For wine, one mL of sample was taken. Next, the samples were centrifuged at 3000 × g for 15 min and 1 mL of the supernatant was filtered through 0.45 µm poly(vinylidene difluoride) (PVDF) membranes (Waters) before injecting.

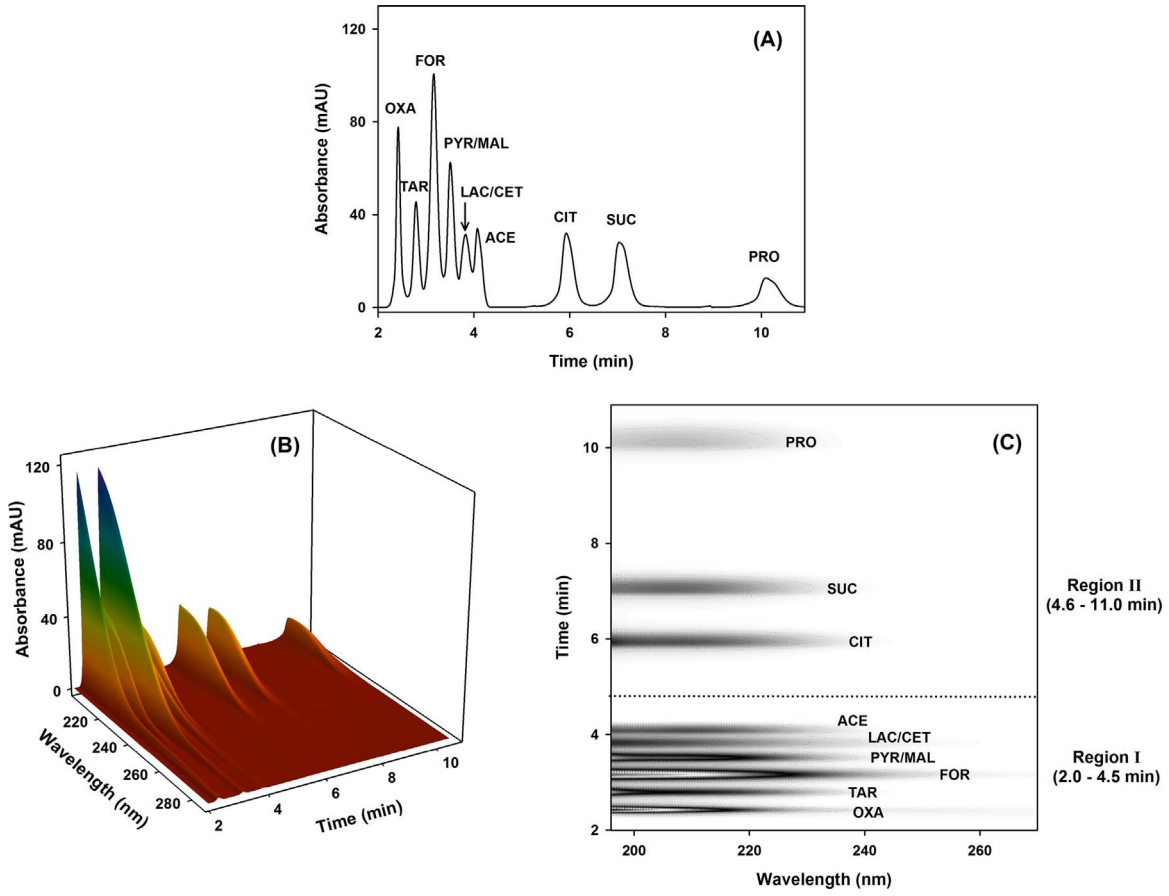

Fig. 1. A) Chromatogram of a sample at 210 nm containing the organic acids studied. B) Three-dimensional plot of a typical chromatogram of a sample containing such organic acids, and C) the corresponding two-dimensional contour plot. Concentrations are as follows (all in mmol L^{-1}): ACE, 5; LAC, 5; PYR, 0.75; CIT, 3; MAL, 5; CET, 0.75; SUC, 0.75.

3.5. Software

The routines employed for PARAFAC (available on the Internet) [50] and U-PLS/RBL are written in MATLAB 7.0 [51]. These algorithms were implemented using the graphical interface of the MVC2 toolbox, which is available on the Internet [52].

4. Results and discussion

4.1. General considerations

Fig. 1A shows a chromatogram of a mixture containing the studied organic acids at 210 nm. On the other hand, Figs. 1B and C show a three-dimensional and a contour plot, respectively, of the complete landscape of absorbance intensity as a function of wavelengths and retention time for a mixture of the eleven studied organic acids. As can be seen in Fig. 1, it is clear that the overlapping of different degrees occurs among the bands and, as discussed below. The situation becomes more serious if additional compounds (such as interferences) which may overlap with any of the peaks, are also present (see Fig. 4). When this occurs, only second-order calibration can be applied for the quantitation of the analytes, since it is essential to exploit the second-order advantage to achieve the desired goal. Therefore, in order to achieve a satisfactory chemometric resolution, the data were split in two different sub sets, according to the time axis: 2.0–4.5 min, and 4.6–11 min. In the first region, the bands of OXA, TAR, FOR, PYR, MAL, LAC, CET and ACE (region I) are detected. The second one includes CIT, SUC and PRO (region II) bands.

Before building the chromatographic time retention–absorbance matrices to be computer processed, some experimental variations must be considered, fundamentally the lack of repeatability in the retention times between successive runs. If the shifts are not corrected, the program will consider these changes take modifications in chemical composition and incorrect results will be obtained because the data not preserve the trilinearity condition (see Fig. 1 in Supporting Information). Due to this fact, preprocessing method was applied to align the chromatographic bands and restore the trilinearity to the system. This alignment process is relatively simple when few bands are involved and both the magnitude and the sense of the shift for all analytes should be the same with respect to a chromatographic run taken as a reference. Bortolato *et al.* developed an algorithm to solve the problem of aligning bidimensional chromatographic matrices in the time dimension. This algorithm is based on the joint processing of the reference and test data matrices with a suitably initialized and restricted PARAFAC model [29]. This procedure enables the alignment of matrices with different number of components in the test samples, due to of the appearance of additional constituents in the calibration matrices. Taking into account all the above mentioned points a suitably initialized and restricted PARAFAC model was selected to pre-process the data.

4.2. Validation samples

After matrix alignment with restricted PARAFAC, second-order multivariate calibration was performed to predict the analyte concentrations in all test mixtures (see Section 3.3). The first second-order multivariate calibration algorithm applied to this analytical problem was PARAFAC with multiple calibration samples (all aligned against one of the calibration data matrices). This algorithm should in principle lead to acceptable results once all data matrices are properly aligned in

Table 2
Component numbers used in PARAFAC and U-PLS/RBL method in Validation and Real samples.

Validation samples	REGION I[a]								REGION II[a]		
	OXA	TAR	FOR	PYR	MAL	LAC	CET	ACE	CIT	SUC	PRO
U-PLS	5	8	3	5	3	5	7	9	4	4	4
PARAFAC[b]	8								3		
Real samples	RBL Components[c]			PARAFAC Uncalibrated components[d]			RBL Components[c]		PARAFAC Uncalibrated components[d]		
Yogurth	3			3			2		2		
Cheese	4			4			2		2		
White wine	4			4			4		4		
Red wine	5			4			5		4		

[a] See the text.
[b] PARAFAC precise a single component number for the complete region data. That number was selected by the so-called core consistency analysis. For further details, see the text.
[c] Unexpected constituents for U-PLS/RBL algorithm, estimated through the so-called generalized cross-validation criterion [45].
[d] Unexpected constituents for PARAFAC algorithm, estimated through the core consistency analysis subtracting previously the calibrated components.

the time dimension. The U-PLS/RBL method was then applied to these data. In order to model the calibration data, the system tested required eight U-PLS latent variables, which were found by leave-one-out cross-validation [48].

Table 2 shows the number of U-PLS latent variables needed to model each analyte in the validation samples, obtained via leave-one-sample-out cross-validation [22], as well as the number of total components per sample required to obtain a satisfactory decomposition according to the PARAFAC model. The number of components when PARAFAC was applied was selected by the so-called core consistency analysis [20], which consists in studying the structural model based on the data and the estimated parameters of gradually augmented models. A PARAFAC model is appropriate if the fit does not improve considerably by the addition of other combination of components [20]. In all cases, PARAFAC was initialized with the best fitting loadings after a small number of trial runs, selected from the comparison of the results provided by generalized rank annihilation and several orthogonal random loadings.

Fig. 2A shows the prediction results corresponding to the application of U-PLS to a set of 30 validation samples different from those used for the calibration step, while Fig. 2B presents the prediction results corresponding to the application of PARAFAC to the same set. As can be observed, the predictions for the eleven organic acids are in good agreement with the corresponding nominal values. If the elliptical joint confidence region (EJCR) is analyzed for the slope and intercept of the above plot (Fig. 2C), it is possible to conclude that ellipse includes the theoretically expected values of (1, 0) in both cases, indicating the accuracy of the used methodology [53]. However, the EJCR for U-PLS is significantly smaller than that corresponding to PARAFAC, suggesting higher precision.

The statistical analysis of the results shown in Table 3, with, root-mean-square error of prediction (RMSEP) and relative error of prediction (REP), which were the worse for PARAFAC, do support this conclusion as well. The LODs obtained by U-PLS, calculated according to the equations proposed by Olivieri [54], they are more than satisfactory considering the complexity of the analyzed samples. The better predictive ability of U-PLS/RBL compared to PARAFAC can be explained by understanding the way that the former algorithm decomposes the data, since it is a more flexible model which tolerates small deviations from trilinearity which may remain in the data set even after the alignment treatment, as already reported [29]. In this sense, it would be reasonable to use the U-PLS/RBL algorithm directly onto the unaligned raw data. However, the experimental limitations of this approach have already been discussed and this strategy was shown to render satisfactory results only in certain ideal occasions (i.e., a large number of calibration samples and a few components) [29].

Fig. 3 shows superimposed standards spectra (Fig. 3A) and chromatograms corresponding to the organic acids analyzed (Fig. 3C), and the experimental profiles retrieved by PARAFAC in the spectral (Fig. 3B) and temporal (Fig. 3D) dimensions for a typical validation sample. As it can be seen, although the system is very convoluted, the spectra are distinguishable, and the chromatographic bands are recognizable and assignable to the analytes of interest.

Table 4 shows the figures of merit of the methods applied to the validation samples, which were calculated according to the work of Olivieri [54]. In general, the analytical performances for both selected algorithms are similar but with a slight advantage for U-PLS. Moreover, an additional figure of merit is defines in PARAFAC, SEL, which has an important diagnostic value. This parameter varies between 0 and 1: zero corresponds to a system totally non-selective for the analyte in question, while the 1 correspond to a completely specific case (pseudo univariated) [20]. It is successfully verified that highly overlapping

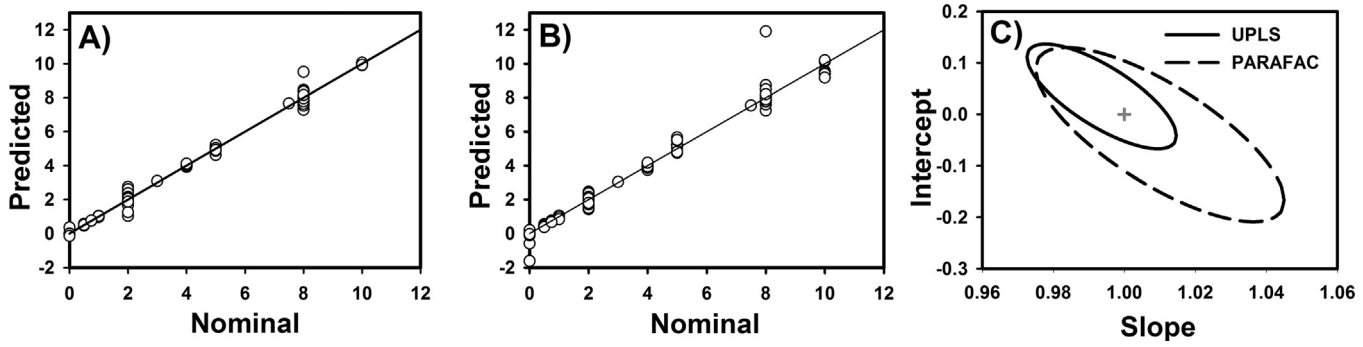

Fig. 2. Plots for predicted concentrations as a function of the nominal values of the eleven studied organic acids in validation samples: A) U-PLS and B) PARAFAC (the solid lines are the perfect fits). C) Elliptical joint regions (at 95% confidence level) for slope and intercept of the regression for validation samples using U-PLS (solid line) and PARAFAC (dashed line). Gray cross marks the theoretical (intercept = 0, slope = 1) point.

Table 3
Statistical results for the studied organic acids in validation samples using PARAFAC and U-PLS/RBL.[a]

	REGION I								REGION II		
	OXA	TAR	FOR	PIR	MAL	LAC	CET	ACE	CIT	SUC	PRO
PARAFAC											
RMSEP[b]	0.02	0.09	0.09	0.05	0.16	0.17	0.05	0.07	0.05	0.11	0.07
REP[c]	9.33	5.81	2.01	10.98	3.59	3.94	10.60	1.72	2.01	2.56	1.56
PARAFAC raw data											
RMSEP[b]	0.07	0.26	0.31	0.16	0.35	0.33	0.16	0.32	0.12	0.24	0.25
REP[c]	**29.2**	**16.3**	6.89	**35.7**	7.56	7.47	**30.8**	7.63	4.81	5.53	5.29
U-PLS											
RMSEP[b]	0.01	0.16	0.12	0.50	0.02	0.26	0.02	0.10	0.15	0.11	0.12
REP[c]	5.33	9.77	2.57	9.86	2.41	4.8	3.26	1.99	5.85	2.56	2.60
U-PLS raw data											
RMSEP[b]	0.05	0.25	0.28	0.08	0.02	0.37	0.02	0.08	0.23	0.25	0.35
REP[c]	**20.6**	**15.5**	6.18	**16.9**	2.58	7.5	3.39	1.70	9.05	5.86	7.40

[a] Number of samples = 30.
[b] RMSEP, root-mean-square error of prediction in mmol L^{-1}.
[c] REP, relative error of prediction in %.

analytes (PYR, MAL, LAC, and CET) present low SEL while OXA, FOR, TAR, ACE, CIT, SUC and PRO show SEL close to one.

4.3. Real samples

According to the results obtained with artificial samples, both U-PLS/RBL and PARAFC were selected for the analysis of real samples.

Four different types of samples (yoghurt, cheese, red and white wine) were selected as examples of real matrices for assaying the proposed methodologies. The concentrations of the eleven studied organic acids in each sample were first determined by a reference method (IE-HPLC). According to the sample sources is possible to distinguish several matrix characteristics (base lines, interferes, spectral and time profiles) and some organic acids as described in Table 1. In dairy samples, where it is expected the absence of MAL and TAR acids and the presence of orotic and uric acids as interferes due they were not

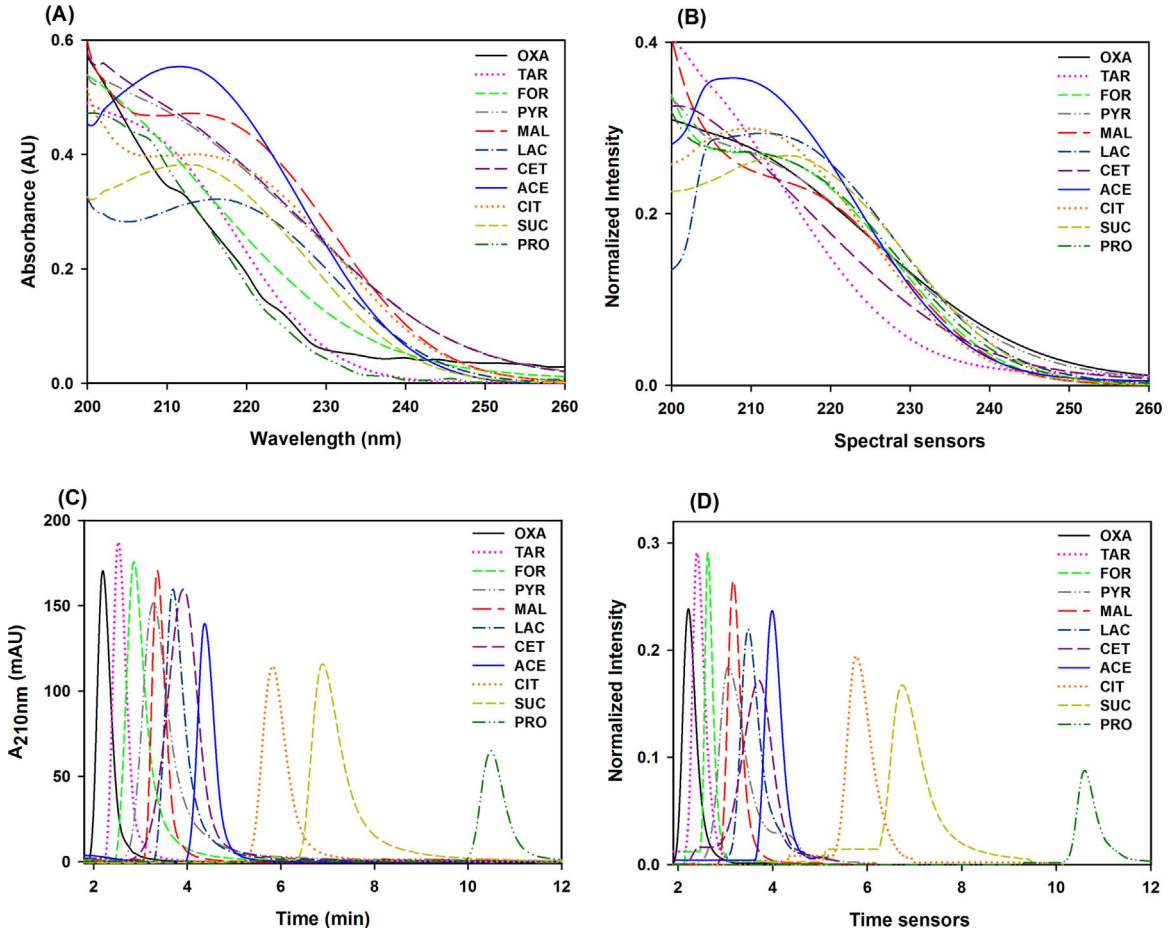

Fig. 3. Profiles retrieved by PARAFAC when processing a typical validation sample. (B) Spectral profiles. (D) Time profiles. In both cases, the profiles are normalized at unity by the algorithm. In contrast, overlapped of standards spectra (A) and chromatograms (C) corresponding to the organic acids analyzed are shown. Concentrations are as follows (all in mmol L^{-1}): OXA, 0.5; TAR, 1.5; FOR, 8; PYR, 0.5; MAL, 2.0; LAC, 5; CET, 0. 5; ACE, 8; CIT, 3.0; SUC, 0.75, PRO, 6.

Table 4
Figures of merit for the studied organic acids in Validation Samples using PARAFAC and U-PLS/RBL.[a]

	REGION I								REGION II		
Validation samples[b]	OXA	TAR	FOR	PYR	MAL	LAC	CET	ACE	CIT	SUC	PRO
PARAFAC											
SEN ($mmol^{-1}$ L)[c]	210	230	230	53	47	68	65	150	140	130	140
γ ($mmol^{-1}$ L)[d]	1600	1800	1700	410	360	520	500	1100	860	800	890
DL (mmol L^{-1})[e]	0.001	0.001	0.001	0.002	0.003	0.002	0.002	0.001	0.001	0.001	0.001
SEL[f]	0.99	0.98	0.99	0.26	0.26	0.38	0.36	0.83	1.00	1.00	1.00
LOD[g]	0.03	0.14	0.23	0.16	0.18	0.15	0.16	0.03	0.13	0.21	0.20
LOQ[i]	0.09	0.42	0.70	0.48	0.55	0.45	0.48	0.10	0.39	0.64	0.61
U-PLS											
SEN ($mmol^{-1}$ L)[c]	960	136	230	562	64	40	295	20	57	28	23
γ ($mmol^{-1}$ L)[d]	310	115	70	149	10	14	152	16	40	31	10
DL (mmol L^{-1})[e]	0.003	0.01	0.01	0.006	0.1	0.07	0.007	0.06	0.025	0.032	0.1
SEL[f]	[h]	[h]	[h]	[h]	[h]	[h]	[h]	[h]	[h]	[h]	[h]
LOD[g]	0.03	0.12	0.35	0.07	0.4	0.4	0.08	0.4	0.14	1.5	0.6
LOQ[i]	0.09	0.36	1.06	0.21	1.21	1.21	0.24	1.21	0.42	4.55	1.82

[a] See the text.
[b] Number of samples = 30.
[c] Sensitivity, is the net signal of the analyte at unit concentration [54].
[d] Analytical sensitivity (calculated as sensitivity/residual).
[e] Minimum increase in analyte concentration which ensures that the analytical signal is significantly different from that corresponding to the original analyte amount, is equal to inverse of analytical sensitivity [54].
[f] Selectivity, is the ratio between SEN and the hypothetical SEN value of the analyte was present in pure form.
[g] LOD, limit of detection calculated according to ref. [54] for PARAFAC, and for U-PLS and U-PLS/RBL it is estimated from samples with very low or zero analyte concentration [54].
[h] Undefined.
[i] LOQ, limit of quantitation defined as (10/3.3) ×LOD.

considering in the calibration set. While in the organic acids composition for wine samples include traces concentration for PRO, PYR, OXA and FOR acids and the absences of the orotic, uric and hipuric acids.

Fig. 4 shows two-dimensional plots of the data matrices corresponding to the study samples. In all samples, besides the analytes of interest, there are species that have not been considered in the calibration step. In the matrices obtained from dairy samples the interferers co-eluted mainly between OXA/FOR - PYR and LAC/ACE - CIT

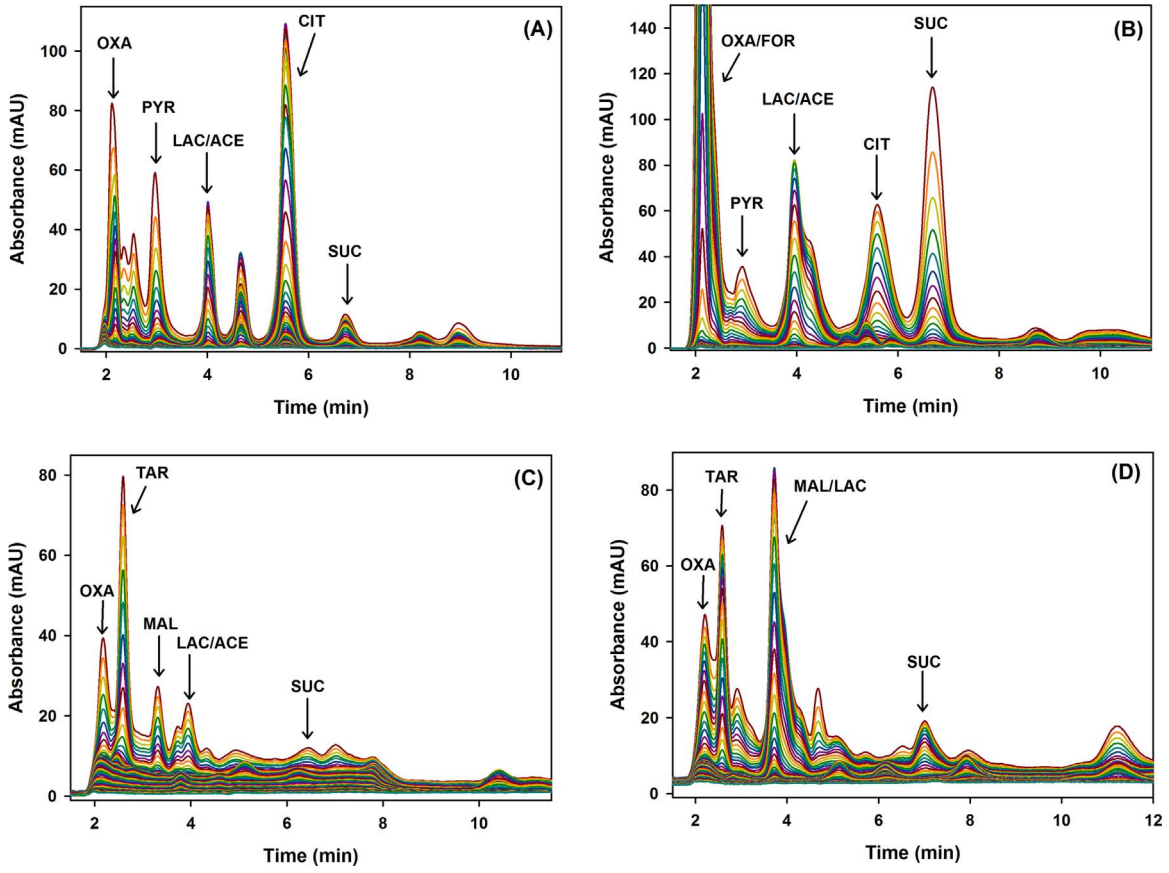

Fig. 4. Two-dimensional plots of the data matrices corresponding to the samples under study: (A) yogurth, (B) cheese, (C) white wine, (D) red wine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Table 5
Determination of organic acid content in fermented samples by the proposed methods and the validation method.

Samples	REGION I								REGION II		
	OXA	TAR	FOR	PIR	MAL	LAC	CET	ACE	CIT	SUC	PRO
Yogurth[a]											
RPLC-PARAFAC[c]	5.20 ± 0.10	ND	ND	2.53 ± 0.71	ND	65.1 ± 5.1	ND	4.00 ± 0.61	115 ± 7	25.5 ± 2.10	ND
RPLC-UPLS/RBL[d]	5.50 ± 0.33	ND	ND	2.52 ± 0.62	ND	67.6 ± 6.2	ND	4.02 ± 0.17	116 ± 6	25.1 ± 2.00	ND
Ion Exch. HPLC[e]	5.03 ± 0.52	ND	ND	2.74 ± 0.83	ND	63.2 ± 4.6	ND	3.96 ± 0.42	109 ± 6	24.7 ± 1.80	ND
Cheese[a]											
RPLC-PARAFAC[c]	3.20 ± 0.44	ND	1.25 ± 0.24	3.23 ± 0.91	ND	100 ± 8	ND	5.50 ± 0.44	14.9 ± 2.40	43.5 ± 6.2	ND
RPLC-UPLS/RBL[d]	3.53 ± 0.42	ND	1.25 ± 0.11	3.64 ± 0.42	ND	102 ± 7	ND	5.70 ± 0.26	14.0 ± 1.40	49.6 ± 2.3	ND
Ion Exch. HPLC[e]	3.64 ± 0.62	ND	1.28 ± 0.31	2.92 ± 0.31	ND	107 ± 9	ND	6.11 ± 0.32	14.4 ± 2.60	41.6 ± 5.6	ND
White Wine[b]											
RPLC-PARAFAC[c]	ND	0.84 ± 0.11	ND	ND	0.95 ± 0.10	7.21 ± 1.40	ND	4.20 ± 0.33	ND	16.5 ± 2.1	ND
RPLC-UPLS/RBL[d]	ND	0.86 ± 0.06	ND	ND	0.95 ± 0.14	7.52 ± 0.91	ND	4.51 ± 0. 2	ND	17.1 ± 0.7	ND
Ion Exch. HPLC[e]	ND	0.79 ± 0.12	ND	ND	0.92 ± 0.12	7.94 ± 1.20	ND	4.16 ± 0. 4	ND	16.1 ± 1.6	ND
Red Wine[b]											
RPLC-PARAFAC[c]	ND	0.87 ± 0.12	ND	ND	0.92 ± 0.24	9.43 ± 1.70	ND	6.20 ± 0. 3	ND	14.8 ± 1.7	ND
RPLC-UPLS/RBL[d]	ND	0.91 ± 0.05	ND	ND	0.94 ± 0.07	9.90 ± 1.00	ND	6.41 ± 0. 2	ND	15.7 ± 0.8	ND
Ion Exch. HPLC[e]	ND	0.80 ± 0.13	ND	ND	0.86 ± 0.19	10.1 ± 1.60	ND	6.16 ± 0. 4	ND	15.2 ± 2.2	ND

ND: not detected.
[a] Number of samples = 4. Concentration in mmol Kg^{-1} of real sample.
[b] Number of samples = 5. Concentration in mmol L^{-1} of real sample.
[c] Mean of replicates, standard deviation between parentheses (the number of total components for each sample are shown in Table 2).
[d] Mean of replicates, standard deviation between parentheses (the number of calibrated components and RBL components for each sample are shown in Table 2).
[e] Mean of replicates, standard deviation between parentheses.

bands, their base lines appearing cleaner in the time profiles, which favors good analytical resolutions (Fig. 4A and B). Instead, in the wine sample matrices a significative background was detected, making it impossible to quantify the acids by single-order analysis (Fig. 4C and D). Even in the presence of these interferences, it is only possible to exploit the second-order advantage.

In Table 2 the additional components required by each model to produce a satisfactory resolution are presented. The selection of additional PARAFAC factors was carried out through the following two tests: (1) analysis of PARAFAC residuals [20], and (2) consideration of the spectral profiles produced by the addition of subsequent components. If the addition of a new component generated repeated profiles, the new component was discarded and the previous number of components was selected. The results obtained by both procedures were consistent and established that the number of total components required by PARAFAC in real samples varied between two and four. In the case of RBL analysis, see Theory section for further details.

Table 5 shows that the results supplied by the presently proposed strategies using U-PLS/RBL and PARAFAC for the samples are in agreement with IE-HPLC. The organic acids levels in these samples correspond with those found in the literature (see Table 1).

The statistical comparison between the results obtained though the two chemometric methods and those provided by the reference method was carried out by the EJCR test for the slope and intercept of the found vs. reference concentration plot. According to Martínez et al. [55], the elliptical region was calculated considering the experimental data corresponding to all analytes, to better estimate the prediction variance. This avoids the oversizing of the joint confidence region due to large experimental random errors and thus the probability of not detecting the presence of bias. The obtained ellipse (Fig. 5) includes the theoretically expected (1, 0) point in both chemometric approaches, supporting that the results obtained with the method here proposed are statistically comparable with those provided by the reference one. In addition, the PARAFAC algorithm yields less accurate results, which is in line with what was discussed in previous sections.

Finally, the advantages of the proposed methodologies in comparison with IE-HPLC were evident in the treatment of real samples: (1) lower required experimental time (4.5 min to resolve 8 organic acids), (2) higher sensitivity, (3) better figures of merits in most cases and (4) RP-columns: wider use, less expensive and robust.

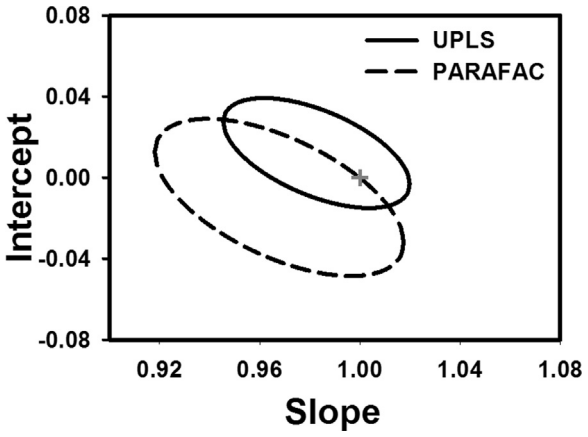

Fig. 5. Elliptical joint region (at 95% confidence level) for the slope and intercept of the regression for U-PLS/RBL and PARAFAC predictions for all analytes studied in the real samples. Gray cross marks the theoretical (slope = 1, intercept = 0) point.

5. Conclusions

Both U-PLS/RBL and PARAFAC combined with reverse phase high-performance liquid chromatography–DAD spectroscopy have demonstrated to be powerful tools to resolve, in a very short time, a complex mixture of analytes of similar absorption spectrum. The determinations are carried out in the presence of unexpected compounds, without the necessity of a complete chromatographic separation. The performance of some analytical parameters of the proposed methodologies on real samples was compared with the routine method (IE-HPLC), yielding a successful result supported by statistical analysis.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2017.09.005.

References

[1] V. Boeris, J.A. Arancibia, A.C. Olivieri, Multivariate curve resolution applied to kinetic-spectroscopic data matrices: dye determination in foods by means of enzymatic oxidation, Talanta 169 (2017) 189–194.
[2] J.M. Padró, J. Osorio Grisales, J.A. Arancibia, A.C. Olivieri, C. Castells, Enantiomeric analysis of overlapped chromatographic profiles in the presence of interferences. Determination of ibuprofen in a pharmaceutical formulation containing homatropine, J. Chromatogr. A 1467 (2016) 255–260.
[3] M.R. Alcaráz, S.A. Bortolato, H.C. Goicoechea, A.C. Olivieri, A new modeling strategy for thirdorder fast high-performance liquid chromatographic data with fluorescence detection. Quantitation of fluoroquinolones in water samples, Anal. Bioanal. Chem. 407 (2015) 1999–2011.
[4] S.A. Bortolato, V.A. Lozano, A. Muñoz de la Peña, A.C. Olivieri, Novel augmented parallel factor model for four-way calibration of high-performance liquid chromatography–fluorescence excitation–emission data, Chemom. Intell. Lab. Syst. 141 (2015) 1–11.
[5] V. Boeris, J.A. Arancibia, A.C. Olivieri, Determination of five pesticides in juice, fruit and vegetable samples by means of liquid chromatography combined with multivariate curve resolution, Anal. Chim. Acta 814 (2014) 23–30.
[6] M. Vosough, M. Rashvand, H.M. Esfahani, K. Kargosha, A. Salemi, Direct analysis of six antibiotics in wastewater samples using rapid high-performance liquid chromatography coupled with diode array detector: a chemometric study towards green analytical chemistry, Talanta 135 (2015) 7–17.
[7] F. Alarcón, M.E. Báez, M. Bravo, P. Richter, G.M. Escandar, A.C. Olivieri, E. Fuentes, Feasibility of the determination of polycyclic aromatic hydrocarbons in edible oils via unfolded partial least-squares/residual bilinearization and parallel factor analysis of fluorescence excitation emission matrices, Talanta 103 (2013) 361–370.
[8] P.L. Pisano, M.F. Silva, A.C. Olivieri, Anthocyanins as markers for the classification of Argentinean wines according to botanical and geographical origin. Chemometric modelling of liquid chromatography-mass spectrometry data, Food Chem. 175 (2015) 174–180.
[9] D.W. Cook, S.C. Rutan, Chemometrics for the analysis of chromatographic data in metabolomics investigations, J. Chemom. 28 (2014) 681–687.
[10] R. Bro, Multivariate calibration: what is in chemometrics for the analytical chemist? Anal. Chim. Acta 500 (2003) 185–194.
[11] J.A. Arancibia, P.C. Damiani, G.M. Escandar, G.A. Ibañez, A.C. Olivieri, A review on second- and third-order multivariate calibration applied to chromatographic data, J. Chromatogr. B 910 (2012) 22–30.
[12] K.S. Booksh, B.R. Kowalski, Theory of analytical chemistry, Anal. Chem 66 (1994) 782–791.
[13] R. Pérez, G.M. Escandar, Multivariate calibration-assisted high-performance liquid chromatography with dual UV and fluorimetric detection for the analysis of natural and synthetic sex hormones in environmental waters and sediments, Environ. Pollut. 209 (2016) 114–122.
[14] M. Carabajal, J.A. Arancibia, G.M. Escandar, A green-analytical chemistry method for agrochemical-residue analysis in vegetables, Microchem. J. 128 (2016) 34–41.
[15] V.A. Lozano, G.M. Escandar, Simultaneous determination of urea herbicides in water and soil samples based on second-order photoinduced fluorescence data, Anal. Met. 8 (2016) 7396–7404.
[16] R. Pellegrino, G. Ibañez, G.M. Escandar, A green method for the quantification of plastics-derived endocrine disruptors in beverages by chemometrics-assisted liquid chromatography with simultaneous diode array and fluorescent detection, Talanta 159 (2016) 336–343.
[17] S.A. Bortolato, J.A. Arancibia, G.M. Escandar, Chemometrics assisted fluorimetry for the rapid and selective determination of heavy polycyclic aromatic hydrocarbons in contaminated river waters and activated sludges, Environ. Sci. Technol. 45 (2011) 1513–1520.
[18] A.C. Olivieri, G.M. Escandar, Practical Three-Way Calibration, Elsevier, MA, USA, 2014.
[19] R. Tauler, Multivariate curve resolution applied to second order data, Chemom. Intell. Lab. Syst. 30 (1995) 133–146.
[20] R. Bro, PARAFAC. tutorial and applications, Chemom. Intell. Lab. Syst. 38 (1997) 149–171.
[21] R. Bro, C.A. Andersson, H.A.L. Kiers, PARAFAC2 – Part II. Modeling chromatographic data with retention time shifts, J. Chemom. 13 (1999) 295–309.
[22] S. Wold, M. Sjostrom, L. Eriksson, PLS-regression: a basic tool of chemometrics, Chemom. Intell. Lab. Syst. 30 (2001) 109–130.
[23] R. Bro, Multiway calibration. Multilinear PLS, J. Chemom. 10 (1996) 47–61.
[24] J. Öhman, P. Geladi, S. Wold, Residual bilinearization. Part 2: application to HPLC – diode array data and comparison with rank annihilation factor analysis, J. Chemom. 4 (1990) 135–146.
[25] G.N. Piccirilli, G.M. Escandar, Partial least-squares with residual bilinearization for the spectrofluorimetric determination of pesticides. A solution of the problems of inner-filter effects and matrix interferents, Analyst 131 (2006) 1012–1020.
[26] E. Sánchez, B. Kowalski, Generalized rank annihilation factor analysis, Anal. Chem. 58 (1986) 496–499.
[27] S.A. Bortolato, A.C. Olivieri, Chemometric processing of second-order liquid chromatographic data with UV–vis and fluorescence detection. A comparison of multivariate curve resolution and parallel factor analysis 2, Anal. Chim. Acta 842 (2014) 11–19.
[28] B.J. Prazen, R.E. Synovec, B.R. Kowalski, Standardization of second-order chromatographic: spectroscopic data for optimum chemical analysis, Anal. Chem. 70 (1998) 218–225.
[29] S.A. Bortolato, J.A. Arancibia, G.M. Escandar, A.C. Olivieri, Time-alignment of bidimensional chromatograms in the presence of uncalibrated interferences using parallel factor analysis Application to multi-component determinations using liquid-chromatography with spectrofluorimetric detection, Chemom. Intell. Lab. Syst. 101 (2010) 30–37.
[30] N.P. Vest Nielsen, J.M. Carstensen, J. Smedsgaard, J. Chromatogr. A 805 (1998) 17–35.
[31] T. Skov, F. van den Berg, G. Tomasi, R. Bro, Automated alignment of chromatographic data, J. Chemom. 20 (2006) 484–497.
[32] M.M. Žulj, I. Puhelek, A.M.J. Korenika, L.M. Bandić, T. Pavlešić, A. Jeromel, Organic acid composition in Croatian predicate wines, Agric. Conspec. Sci. 80 (2) (2015) 113–117.
[33] A.J. Buglass, S.H. Lee, Sequential analysis of malic acid and both enantiomers of lactic acid in wine using a High-Performance Liquid Chromatographic column-switching procedure, J. Chromatogr. Sci. 39 (11) (2001) 453–458.
[34] H. Sirén, K. Sirén, J. Sirén, Evaluation of organic and inorganic compounds levels of red wines processed from Pinot Noir grapes, Anal. Chem. Res. 3 (2015) 26–36.
[35] A.E. Bevilacqua, A.N. Califano, Determination of organic acids in dairy products by High Performance Liquid Chromatography, J. Food Sci. 54 (4) (1989) 1076–1079.
[36] W.J. Mullin, D.B. Emmons, Determination of organic acids and sugars in cheese, milk and whey by high performance liquid chromatography, Food Res. Int. 30 (2) (1997) 147–151.
[37] D. Gonzalez de Llano, A. Rodriguez, P. Cuesta, Effect of lactic starter cultures on the organic acid composition of milk and cheese during ripening-analysis by HPLC, J. Appl. Bacteriol. 80 (1996) 570–576.
[38] J.C. Piard, M. Desmazeaud, Inhibiting Factors Produced by Lactic Acid Bacteria. 1. Oxygen Metabolites and Catabolism End-products, Le Lait, INRA Editions, 71(5), 1991, pp. 525–541.
[39] E.J. Smid, M. Kleerebezem, Production of aroma compounds in lactic fermentations, Annu. Rev. Food Sci. Technol. 5 (2014) 313–326.
[40] A.N. Califano, A.E. Bevilacqua, Multivariate analysis of the organic acids content of Gouda-type cheese during ripening, J. Food Comp. Anal. 13 (2000) 949–960.
[41] S. Kaminarides, P. Stamou, T. Massouras, Comparison of the characteristics of set type yoghurt made from ovine milk of different fat content, Int. J. Food Sci. Technol. 42 (2007) 1019–1928.
[42] A.M.O. Leite, D.C.A. Leite, E.M. Del Aguila, T.S. Alvares, R.S. Peixoto, M.A.L. Miguel, J.T. Silva, V.M.F. Paschoalin, Microbiological and chemical characteristics of Brasilian kefir during fermentation and storage processes, J. Dairy Sci. 96 (2013) 4149–4159.
[43] M. Tormo, J.M. Izco, Alternative reversed-phase high-performance liquid chromatography method to analyse organic acids in dairy products, J. Chromatogr. A 1033 (2004) 305–310.
[44] R.G. Bereton, Applied Chemometrics for Scientists, first ed., Wiley, Chichester, UK, 2007.
[45] A.C. Olivieri, On a versatile second-order multivariate calibration method based on partial least-squares and residual bilinearization: second-order advantage and precision properties, J. Chemom. 19 (2005) 253–265.
[46] S.A. Bortolato, J.A. Arancibia, G.M. Escandar, A.C. Olivieri, Improvement of residual bilinearization by particle swarm optimization for achieving the second-order advantage with unfolded partial least-squares, J. Chemom. 21 (2007) 557–566.
[47] N.M. Faber, Degrees of freedom for the residuals of a principal component analysis – a clarification, Chemom. Intell. Lab. Syst. 93 (2008) 80–86.
[48] P. Craven, G. Wahba, Smoothing noisy data with spline functions, Numer. Math. 31 (1979) 377–403.
[49] R.G. Brereton, Chemometrics Data Analysis for the Laboratory and Chemical Plant, Wiley, Chichester, UK, 2003, p. 199.
[50] ⟨http://www.models.life.ku.dk/source/⟩.
[51] MATLAB 7. 0, The Mathworks, Natick, Massachussets, USA, 2007.
[52] ⟨http://www.chemometry.com/Index/Links%20and%20downloads/Programs.html⟩.
[53] A.G. Gonzalez, M.A. Herrador, A.G. Asuero, Intra-laboratory testing of method accuracy from recovery assays, Talanta 48 (1999) 729–736.
[54] A.C. Olivieri, Analytical figures of merit: from univariate to multiway calibration, Chem. Rev. 114 (2014) 5358–5378.
[55] A. Martínez, J. Riu, O. Busto, J. Guasch, F.X. Rius, Validation of bias in multianalyte determination methods. Application to RP-HPLC derivatizing methodologies, Anal. Chim. Acta 406 (2000) 257–278.