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Enhanced MCR-ALS modeling of HPLC with fast scan fluorimetric detection second-order data for quantitation of metabolic disorder marker pteridines in urine

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ABSTRACT

This work presents the development of a liquid chromatographic method based on modeling entire fast scan fluorimetric detection second-order data with the multivariate curve resolution alternating least squares algorithm, for the simultaneous determination of five marker pteridines in urine samples.

The modeling strategy involves the building of a single MCR-ALS model composed of matrices augmented in the spectral mode, i.e. time profiles remain invariant while spectra may change from sample to sample. This approach allowed us to separate and determine the whole analytes at once.

The developed approach enabled us to determine five of the most important metabolic disorder marker pteridines: biopterin, neopterin, isoxanthopterin, pterin and xanthopterin, three of them presenting emission spectra with the same emission wavelength maxima. In addition, some of these analytes present overlapped time profiles. As a consequence of using the entire data sets, a considerable reduction of the data processing experimental time can be achieved. Results are compared with a previous strategy in which data were split in five different regions, and information about the figures of merit of the new strategy compared with the previously reported strategy is reported.

1. Introduction

When developing a chromatographic method, the use of chemometrics may provide a useful resource for accurate analyte quantitation when the complete separation is not accomplished, or unexpected compounds are present in the sample being analyzed. Some recent reports deal with the combination of multivariate calibration and chromatography [1–3]. Chemometrics has been shown to be useful when second-order data are recorded, for example, using a diode array detector (DAD), a fast scanning fluorescence detector (FSFD) or a mass spectrometry based (MS) detector during the chromatographic time evolution [4–6].

As has been shown in a large number of publications in the last years, an interesting intrinsic property that second-order data possess (if they are modeled with convenient second-order algorithms) is the so called "second-order advantage" [7], which in principle permits analytes quantitation in samples containing unexpected components, i.e. compounds not included in the calibration set [6,8]. This fact allows one to build a predictive model with a limited number of standards, yet quantitating the analyte in the presence of potential interferences [6]. Specifically, several interesting reports have been presented focusing on the resolution of really complex samples by using liquid chromatography and exploiting the mentioned second-order advantage [9–11]. In this context, extremely important issues such as resolution of overlapping peaks or reduction in the time of analysis and consequently costs and amount of contaminant solvents should be considered [4].

Surprisingly, very few literature works concern HPLC with fluorimetric detection in combination with second-order data. Early references on the subject are the pioneering work of Appellof

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and Davidson using a videofluorimeter as liquid chromatographic detector [12], the use of N-dimensional partial least squares (N-PLS) and a comparative analysis of N-PLS and PARAFAC for the determination of polycyclic aromatic hydrocarbons using fast-scanning fluorimetric detection [13,14] and a method of determination of a mixture of 12 naphthalenes and naphthalene-disulfonates by N-PLS, by Beltran et al. [15].

Very recently, a procedure for the determination of ofloxacin, norfloxacin, danofloxacin, enrofloxacin, pipemidic acid, marbofloxacin, ciprofloxacin and lomefloxacin, in the presence of salicylic, salicyluric and gentisic acids, as interferents [16], and a method for the simultaneous determination of 10 polycyclic aromatic hydrocarbons in the presence of interferences [17] have been reported. In addition, benzo[b]fluoranthene and benzo[k]fluoranthene were determined in presence of benzo[j]fluoranthene as interference, using PARAFAC and a new methodology for the alignment of matrix chromatographic data [18], and selected marker pteridins were determined in urine samples, by HPLC-fluorimetric data in combination with second-order MCR-ALS calibration [19].

An important number of algorithms have been used in order to reach the second-order advantage: generalized rank annihilation (GRAM) [20], direct trilinear decomposition (DTLD) [21,22], self-weighted alternating trilinear decomposition (SWATLD) [23], alternating penalty trilinear decomposition (APTLD) [24], parallel factor analysis (PARAFAC) [25], multivariate curve resolution alternating least squares (MCR-ALS) [26], and the most recently implemented bilinear least squares (BLLS) [27], unfolded partial least squares/residual bilinearization (U-PLS/RBL) [28] and artificial neural networks followed by residual bilinearization (ANN/RBL) [29].

Second-order data are trilinear when each compound in all experiments treated together can be described by a triad of invariant pure profiles [30]. In chromatography with fluorescence detection, each analyte should have the same time and spectral profile in all the samples and only differs in the amount in which it intervenes. It is important to note that GRAM, PARAFAC or RBL based algorithms require that the data show the property of trilinearity, which can be lost if chromatographic retention times are not exactly reproducible. In this situation, algorithms such as MCR-ALS (which can solve this type of problems by resorting to the mathematical resource of matrix augmentation) and PARAFAC2, a variant of PARAFAC allowing for distinct time profiles in each experimental sample [31], have been proved to be useful alternatives for treating these data since they are more flexible with regard to trilinearity [32,33]. However, when data are conveniently pretreated in order to alleviate the above-mentioned problems, good results can be obtained by using GRAM, PARAFAC or RBL based algorithms. It is interesting to note that, in order to restore the trilinearity which is lost by lack of reproducibility in retention times, several procedures are available to align the latter, causing all chromatograms for a set of samples to have identical retention time alignment [16,34,35].

Very recently, Olivieri et al. [8] remarked on the problem presented by complete overlapping of profiles in one of the data dimensions, which can be regarded as a special and serious case of linear dependency. In chromatography with fluorescence detection, this problem can occur when two or more analytes being separated in the time domain have the same emission spectra. The latter fact is frequent when similar molecules are being separated, i.e. separating pharmaceutical drugs plus their metabolites, or endogenous metabolites belonging to the same family, such as the pteridines analyzed in this paper.

Biopterin (BIO), neopterin (NEO), pterin (PT), xanthopterin (XAN) and isoxanthopterin (ISO), between others, all are including in the pteridinic family derivatives. These compounds are extensively present in the human and animal organisms. Pteridines are very important cofactors in several cell metabolisms and, in consequence, the amounts of these pteridines in biological fluids have significant importance in clinical diagnostic. The pteridines are excreted in urine and its levels are related with activation of the cellular immune system due to certain diseases such as cancer, viral infections and renal disease [36].

A significant increase in the urinary excretion of XAN, NEO and PT, as well as a significant decrease in ISO and an NEO/BIO increment, was found in cancer patients. In mononucleosis infected children an increase of the NEO amount was also observed [37].

Furthermore, tetrahydrobiopterin (BH4) is the major unconjugated pteridine present in human biological fluids. It is known that alterations on the metabolic mechanism of BH4 give rise to different diseases, named hyperphenylalaninaemia (HPAs). The differential diagnostic of these HPAs is important to establish the adequate therapeutic protocol and is based in the measurement of pteridines and other metabolites excretion. Hence, the classical phenylketonuria produces increments in the urinary levels of NEO and BIO, while the BH4 deficit produces a decrease in the NEO and BIO levels.

In consequence, there is a high interest for the development of methods applied to the determination of the five most important metabolic disorder marker pteridines. Therefore, in a previous work, we developed an approach to determine NEO, BIO, ISO, PT and XAN, by means of a chromatographic method linked to a second order multivariate procedure [19].

The developed method enabled us to determine the listed pteridines, some of them with overlapping profiles, in a short analysis time and with the consequent decreasing of reagent consumption. To solve the problem of strong colinearity between spectra, independent MCR-ALS models were built for each of the five analytes being studied.

In the present report, the focus was put in enhancing the modeling with the object of reducing the time of data processing and the complexity of the problem. The new strategy involves the building of a unique MCR-ALS model composed of matrices augmented in the spectral mode, i.e. time profiles remain invariant while spectra may change from sample to sample. Interestingly, this approach allows us to separate the whole analytes at once, three of them having the same emission spectra, based on differences in their retention times. It is important to note that only one example can be found in the literature in which a similar situation is presented, but in that case, the analytes being separated by capillary electrophoresis have the same migration time [38].

2. Experimental

2.1. Reagents and solutions

NEO, XAN, BIO, ISO and PT were purchased from Sigma (Sigma–Aldrich Química, S.A., Madrid, Spain). Citric acid was supplied by Anedra (San Fernando, Argentina). Acetonitrile HPLC-grade was obtained from Merck (Darmstadt, Germany). HPLC-grade water was obtained from a Milli-Q Biocel System (Millipore SAS, Molsheim, France).

Stock standard solutions of pteridines (15 $\mu g\,mL^{-1}$) prepared by exact weighing and dissolution with ultrapure water (alcalinized to basic pH with NaOH and then adjusted to approximately pH = 7 with diluted HCl) were stable for at least two weeks.

A buffer solution (10 $mmol\,L^{-1}$, pH 5.44) was prepared from citric acid and sodium hydroxide in ultrapure water. Working analyte solutions of different concentrations were prepared by dilution of stock standard solutions with buffer solution.

Fresh urine samples were immediately frozen after collection at −18 °C until analysis.

2.2. Chromatographic system and procedure

The chromatographic studies were performed on an Agilent Model 1100 LC instrument (Agilent Technologies, Waldbronn, Germany), equipped with degasser, quaternary pump, autosampler, oven column compartment, UV–visible diode array detector (DAD), fast scanning fluorimetric detector (FSFD) and the CHEMSTATION software package to control the instrument, data acquisition and data analysis. The analytical column used was a Zorbax Eclipse XDB-C18, 250 mm × 4.6 mm, 5 μm particle size (Agilent Technology).

The column temperature was controlled by an oven at 25 °C. The mobile phase consisted of a 10 mmol L^{-1} citric acid buffer (pH = 5.44)–acetonitrile mixture (94.5/5.5, v/v). The stock standard solutions of pteridines were eluted in isocratic mode. With respect to the urine samples, it was necessary to perform a chromatographic analysis in two steps: in the first one, from 0 to 5.5 min, the pteridines were eluted in isocratic mode, with a 5.5% acetonitrile (ACN), and in the second, a gradient was implemented to elute every compounds of the matrix from the column as follows: from 5.5 to 9.0 min, an increase to 25.0% of ACN; from 9.0 to 11.0 min, 25.0% of ACN; from 11.0 to 14.0 min, a decrease to 5.5% of ACN; and from 14.0 to 16.0 min, 5.5% ACN. In consequence, the complete analysis was carried out in 16 min. The flow rate was maintained at 1.00 mL min^{-1}. Samples were filtered through 0.45 μm nylon membrane filters before injection.

2.3. Calibration and validation samples

Calibration data were built analyzing 15 mixtures of the previously mentioned analytes, under the above selected conditions. Solutions for calibration curves were randomly prepared, by convenient dilutions of the standard solutions, in order to obtain the necessary concentrations. The ranges of concentration were between 0.5 and 10.5 ng mL^{-1} for NEO, BIO and PT; between 4.0 and 8.0 ng mL^{-1} for XAN and between 0.5 and 4.5 ng mL^{-1} for ISO. These ranges were established on the basis of the analysis of the linear fluorescence–concentration ranges for each analyte, according to a recent publication [37]. The specific calibration concentrations are provided in Table 1 of Ref. [19].

A set of 3 unfortified and fortified (with the analytes NEO, XAN, BIO, ISO and PT) urine samples from healthy adults was analyzed, with the object of validating the proposed method. Solutions of urines were prepared by spiking with aliquots of 25.0, 50.0 and 75.0 μL of a mixture of the marker pteridines. After that, they were injected into the chromatographic system, in order to measure the previous analytes in the urine samples. The added concentrations of the analytes, for each fortification, are reported in the Table 1.

2.4. Data generation and software

The data matrices were collected with the excitation wavelength fixed at 272 nm, using emission wavelengths from 380 to 500 nm each 1 nm, and times from 0 to 5 min each 1 s. In this way, the time-emission matrices (TEM) were of size 121 × 300. These matrices were then saved in ASCII format, and transferred to a PC based on an AMD Athlon dual core microprocessor for subsequent manipulation. Additionally, photometric detection at 256 nm was recorded.

Routines employed for the second-order MCR-ALS algorithm are available on the Internet at http://www.ub.es/gesq/mcr/mcr.htm.

Table 1
MCR-ALS predictions, obtained for strategy 1 and strategy 2 on urine samples, spiked with different amounts of the analytes (see Table 2 of Ref. [19]).

Sample	Analyte concentrations (ng mL^{-1})														
	NEO			XAN			BIO			ISO			PT		
	Spiked	S1	S2	Spiked	S1	S2	Spiked	S1	S2	Spiked	S1	S2	Spiked	S1	S2
1–1	1.12	1.05 (93.8)	1.10 (98.2)	1.84	1.75 (95.1)	1.54 (83.7)	1.80	1.98 (110.0)	2.12 (117.8)	0.50	0.51 (102.0)	0.49 (98.0)	1.92	1.83 (95.3)	1.97 (102.6)
1–2	2.24	1.98 (88.4)	2.27 (101.3)	3.68	3.73 (101.4)	3.91 (106.3)	3.60	3.89 (108.1)	4.12 (114.4)	1.00	1.04 (104.0)	0.92 (92.0)	3.84	4.06 (105.7)	3.86 (100.5)
1–3	3.36	3.02 (89.9)	3.58 (106.5)	5.52	5.81 (105.3)	5.75 (104.2)	5.40	5.91 (109.4)	6.13 (113.5)	1.50	1.60 (106.7)	1.56 (104.0)	5.76	5.82 (101.0)	5.73 (99.5)
2–1	1.12	0.99 (88.4)	1.02 (91.1)	1.84	2.39 (113.6)	2.05 (111.4)	1.80	1.97 (109.4)	1.91 (106.1)	0.50	0.48 (96.0)	0.42 (84.0)	1.92	1.88 (97.9)	1.88 (97.9)
2–2	2.24	1.95 (87.1)	2.05 (91.5)	3.68	4.02 (109.3)	3.52 (95.7)	3.60	3.89 (108.1)	3.83 (106.4)	1.00	1.07 (107.0)	1.31 (131.0)	3.84	3.93 (102.3)	3.75 (97.7)
2–3	3.36	3.12 (92.9)	3.12 (92.9)	5.52	5.97 (108.2)	5.51 (99.8)	5.40	5.94 (110.0)	6.01 (111.3)	1.50	1.45 (96.7)	1.32 (88.0)	5.76	4.88 (84.7)	5.83 (101.2)
3–1	1.12	0.90 (80.4)	1.11 (99.1)	1.84	1.83 (99.5)	1.59 (86.4)	1.80	1.97 (109.4)	1.95 (108.3)	0.50	0.46 (92.0)	0.43 (86.0)	1.92	2.05 (106.8)	1.92 (100.0)
3–2	2.24	1.91 (85.3)	2.29 (102.2)	3.68	3.83 (109.0)	3.65 (99.2)	3.60	4.09 (113.3)	4.00 (111.1)	1.00	1.54 (154.0)	0.99 (99.0)	3.84	3.60 (93.8)	3.83 (99.7)
3–3	3.36	2.86 (85.1)	3.34 (99.4)	5.52	5.92 (107.2)	6.06 (109.8)	5.40	5.86 (108.5)	6.06 (112.2)	1.50	1.56 (104.0)	1.52 (101.3)	5.76	5.72 (99.3)	5.77 (100.2)
REP (%)		**5.0**	**2.3**		**5.2**	**4.3**		**8.8**	**11.0**		**18.7**	**12.9**		**8.3**	**1.2**
p-value		**<0.05**			**0.50**			**0.12**			**0.90**			**<0.05**	

3. Results and discussion

3.1. General considerations

Fig. 1A shows a contour plot of the complete landscape of fluorescence intensity as a function of emission wavelength and retention time (registered at λ_{ex} = 272 nm/λ_{em} between 380 and 500 nm and retention time between 2.0 and 5.0 min) for a mixture of the five studied pteridines. The elution order for all the compounds was NEO, XAN, BIO, ISO, and PT, and was indicated by an arrow in Fig. 1A. As can be appreciated in this figure, the time profiles of XAN, BIO and ISO are highly overlapped indicating they possess very close retention times.

The high complexity of the analytical problem under study can be easily appreciated in Fig. 1B, which corresponds to a urine sample after spiking with the five analytes (see Section 2). As can be seen, an unknown peak appears between the NEO and XAN peaks. In addition, several unexpected substances appear in the urine matrix, making necessary to model the data with a second order algorithm capable of exploiting the second order advantage.

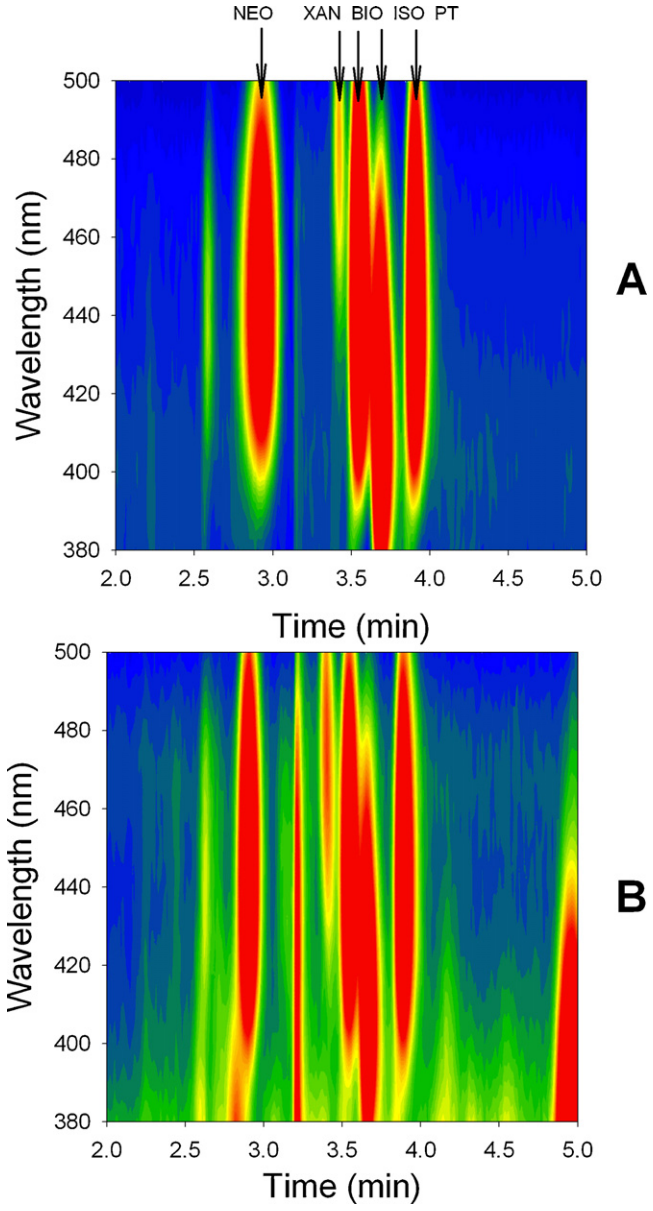

Fig. 1. Two-dimensional contour plots of the time-emission matrices for (A) a mixture of NEO, XAN, BIO, ISO and PT, and (B) a urine sample after spiking with the five analytes.

3.2. Data processing

In Fig. 2, a comparative scheme of the data processing involved in strategy 1 (S1) and strategy 2 (S2) is shown. As can be appreciated in Fig. 2, independently of the methodology followed to process the data, a baseline correction was applied to subtract the background present in the chromatograms [19]. After that, the processing bifurcates in two opposite ways. The more laborious strategy 1 followed in our previous work [19] involves a first step of selection of appropriate regions to quantify one analyte at a time. Specifically, the total chromatographic data were split in five regions (region 1, between 2.75 and 3.12 min, for NEO quantitation; region 2, between 3.35 and 3.58 min, for XAN quantitation; region 3, between 3.45 and

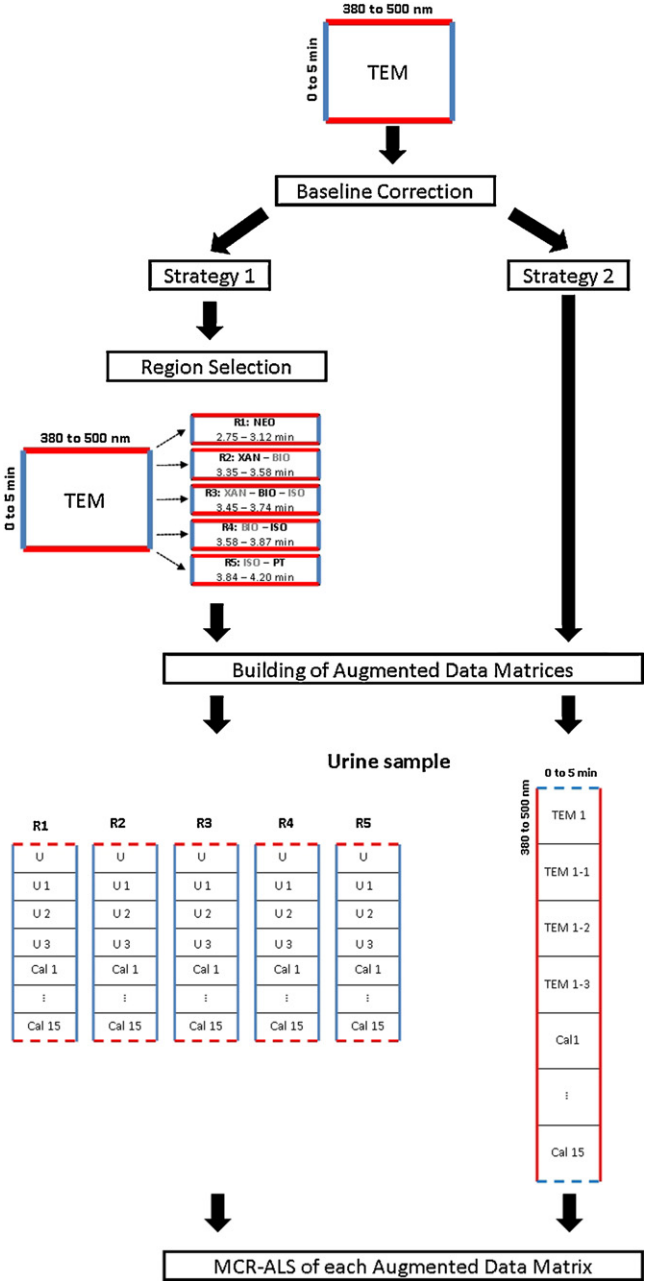

Fig. 2. Comparative scheme of data processing involved in strategy 1 and strategy 2.

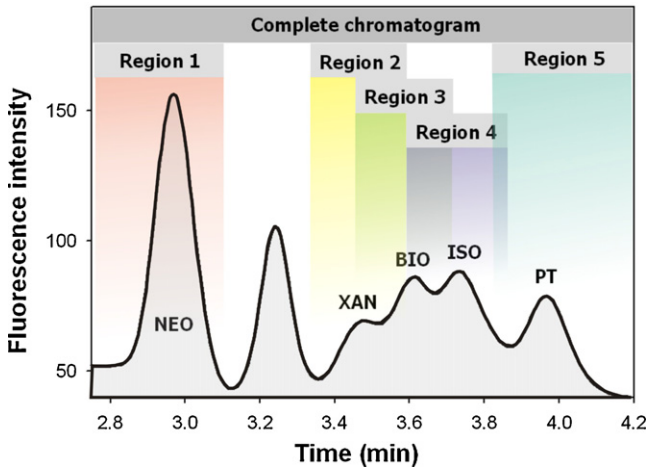

Fig. 3. Five regions generated in strategy 1, in contrast with the entire chromatogram used in strategy 2 to perform the resolution.

3.74 min, for BIO quantitation; region 4, between 3.58 and 3.87 min, for ISO quantitation; and region 5, between 3.84 and 4.20 min, for PT quantitation) and different MCR models were built to carry out the analysis. On the contrary, this procedure was avoided in strategy 2, in which the complete data were used to build the augmented data matrix that allows determining the whole analytes together. Fig. 3 illustrates the five generated regions in strategy 1, which in most of the cases shared part of the information [19], in contrast with the entire chromatogram used in strategy 2.

As can be seen in Fig. 2, the complexity of the processing is extremely reduced if the complete data is conveniently modeled following the strategy 2. In other words, the resolution of each analyte by the strategy 1 requires the building of one particular MCR-ALS model with data belonging to the region in which it is included, i.e. five MCR-ALS models are needed to quantify the five analytes present in one sample.

It is relevant to discuss the validity of the implementation of this new approach, considering that the usual way to resolve this kind of data involves the construction of augmented data matrices from which the MCR-ALS analysis retrieve two matrices: one having the spectra of all the species present in the data and the other including the information useful to perform the quantitation of the analytes, i.e. the area under each chromatographic peak. The satisfactory implementation of this strategy to determine the analytes building only one MCR-ALS model requires that the spectra of all of them and those from the interferences must be different, since the resolution is based on this information (red dashed line in Fig. 2). The latter is not able to perform in our system due to the fact that NEO, BIO and PT present the same emission spectra, losing their selectivity in the spectral mode. On the other hand, as can be appreciated in Fig. 3, although the time profiles of XAN, BIO and ISO are highly overlapped, there exist small differences in their retention times enough to allow the resolution performing only one MCR-ALS model (blue dashed line in Fig. 2). (For interpretation of the references to color in this text, the reader is referred to the web version of this article.) Therefore, the augmentation mode is affected by the time profile overlapping and the quantitation is carried out with the areas under the spectra. It is important to note that this kind of augmentation calls for trilinearity in the data, a fact that in this case is fulfilled because the chromatographic time is not so long, and consequently no shift and warping were present.

Considering the problem presented by complete overlapping of profiles in the spectral dimension, PARAFAC and other trilinear models cannot be applied, even after imposing suitable initialization and restriction conditions. On the other hand, this task could be solved by using U-PLS/RBL (exploiting the selectivity in one of the data dimensions), MCR-ALS and PARAFAC2. However, as will be shown below, owing to the fact that one of the interferences also shows a similar spectrum than those for XAN, BIO and ISO, U-PLS/RBL is not applicable to solve the problem considered here [8].

3.3. MCR-ALS analysis

As was commented before, there exist two algorithms capable in principle of solving this task (MCR-ALS and PARAFAC2). Based on our experience, we chose the first one to accomplish the modeling of several D augmented data matrices built by placing matrices on top of each other corresponding to each sample together with the 15 calibration samples. It is important to state that it is not necessary to build separate D augmented matrices to resolve the analytes presenting matrix effect, i.e. ISO and PT (see Ref. [19]). In these cases, the resolution is performed using the areas under the spectra obtained for ISO and PT in each sample and its corresponding standard additions, in order to apply the standard addition method. The well-known MCR-ALS algorithm has already been described in Ref. [19].

Before starting resolution, the number of contributions to each D data matrix was determined by singular value decomposition (SVD). Taking into account that MCR-ALS needs information as real as possible to start the resolution, the C^T–type initial estimated were built combining the pure analyte chromatograms with those obtained for the interferences by means of the selection of purest time profiles based on SIMPLISMA [39].

Given D and C^T, appropriate constraints (i.e. non–negativity, unimodality, trilinearity) were implemented to drive the iterative optimization to the right solution. Fig. 4 shows the spectra retrieved

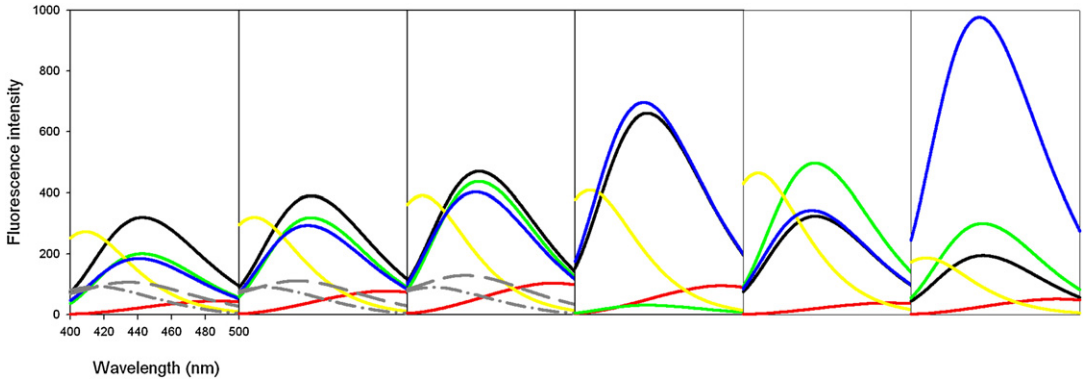

Fig. 4. Emission spectra retrieved by MCR-ALS processing of urines 1–1, 1–2 and 1–3, together with calibration samples 3, 7 and 13. The profiles correspond to NEO (black solid line), XAN (red solid line), BIO (green solid line), ISO (yellow solid line), PT (blue solid line) and two sample interferences (dashed and dashed-dotted gray lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
Figures of merit for MCR-ALS modeling in both strategies.

Analyte	Figures of merit (strategy 1/strategy 2)				
	SEN	Analytical sensitivity	γ^{-1} (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
NEO	438/446	298/303	0.03/0.03	0.02/0.02	0.1/0.1
XAN	97/97	67/66	0.02/0.02	0.1/0.1	0.2/0.2
BIO	420/427	300/291	0.03/0.03	0.02/0.02	0.1/0.1
ISO	561/577	387/392	0.03/0.03	0.01/0.01	0.04/0.04
PT	415/417	277/284	0.04/0.04	0.02/0.02	0.1/0.1

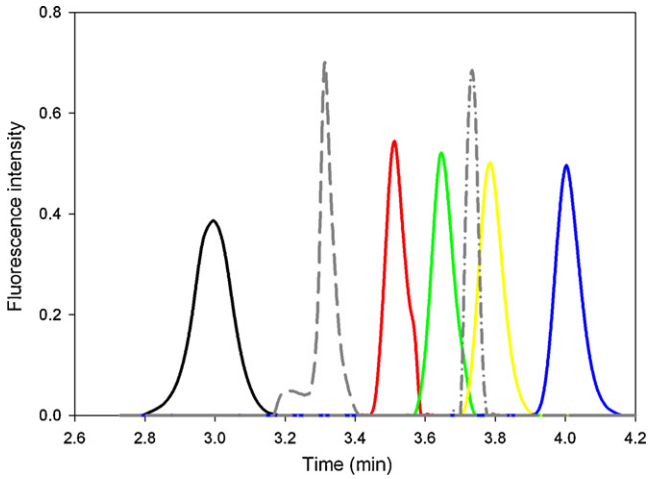

Fig. 5. MCR-ALS retention time profiles corresponding to the analysis of urines 1–1, 1–2 and 1–3, together with calibration samples 3, 7 and 13. The peaks correspond to NEO (black solid line), XAN (red solid line), BIO (green solid line), ISO (yellow solid line), PT (blue solid line) and two sample interferences (dashed and dashed-dotted gray lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

by MCR-ALS processing of urines U1–1, U1–2 and U1–3, together with calibration samples 3, 7 and 13 (see Table 1 in Ref. [19]). As can be appreciated, three profiles (those corresponding to NEO, BIO and PT) present the same shape. In addition, two sample interferences are also retrieved by the algorithm, one of them with a similar spectrum to those from NEO, BIO and PT. Interestingly, this fact makes impracticable the application of U-PLS/RBL as was commented above.

An indicative measure of the quality of the MCR-ALS modeling can be attained by visual inspection of spectral profiles retrieved for the standard solutions in Fig. 4. For example, the three spectra retrieved for analyte BIO, which were drown in green solid lines, corresponding to calibration samples 3, 7 and 13 (0.5, 8.0 and 5.5 ng mL^{-1}, respectively), present differences that can be clearly appreciated and related with their concentrations. Similar considerations can be made for the other four analytes.

Fig. 5 shows the time profiles retrieved by MCR-ALS corresponding to the analysis of urines U1–1, U1–2 and U1–3, together with calibration samples 3, 7 and 13. It can be clearly seen how the two interferences coelute with the analytes, as was noted when analyzing Fig. 1.

Results obtained by the application of the new strategy are displayed in Table 1, as well as those belonging to strategy 1, in order to compare their performances. For every spiked sample, the recovery was computed, and finally, a relative standard error [$\mathrm{REP}(\%) = \frac{1}{\bar{x}_s}\sqrt{\frac{\sum (x_s - x_r)^2}{n}}$, being x_s the amount of analyte spiked to every validation sample, x_r the amount of analyte recovered by both strategies, $\bar{x}_s$ the average and n the number of validation samples] was obtained. The significance of the comparison of REP values was tested using the randomization approach described in Ref. [40]. Specifically, there are no statistical differences between the calculated REPs concerning XAN, BIO and ISO. On the other hand, the estimated significance level associated with the test indicates that the REPs for S1 were larger than the REPs for S2 for NEO and PT, and are lower than 0.05 and therefore significant (calculated using 1999 iterations, for details see Ref. [39]). These results are indicative of a better predictive ability of strategy 2 over strategy 1 for NEO and PT.

A straightforward approach was used for determining the figures of merit in MCR-ALS based on the recovery of the pure response profiles of the analytes after a curve resolution procedure [41]. Consequently, the sensitivity (SEN) is defined as the slope of the calibration curve obtained by plotting the relative responses against the analyte concentration for the standards. The analytical sensitivity was computed as the ratio between the SEN and the standard deviation of differences between predicted and experimental responses for all standards. On the other hand, the standard deviation for a zero concentration sample was computed taking into account the errors of the slope and intercept of the calibration equation, and used for computing the limits of detection (LOD) and quantitation (LOQ) [42]. Following the last reference, analytical sensitivity, its inverse (γ^{-1}), LOD and LOQ were computed for both strategies and presented in Table 2. As can be appreciated, with both strategies similar figures of merit are obtained.

4. Conclusions

A liquid chromatographic method, based on modeling entire fast scan fluorimetric detection second-order data with the multivariate curve resolution alternating least squares algorithm, was presented for the simultaneous determination of five metabolic disorder marker pteridines in urine samples. This new approach was compared with a previous strategy in which the data were split in five regions. It was shown that MCR-ALS is one of the most versatile algorithms available for the management of data presenting similar spectra for analytes and interferences, when time shifts do not occur during the chromatographic runs. The proposed strategy considerably reduces the complexity of the resolution and in consequence the processing time, and similar figures of merit were obtained with both strategies.

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