



Science based calibration for the extraction of ‘analyte-specific’ HPLC-DAD chromatograms in environmental analysis

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ABSTRACT

Multivariate science based calibration (SBC) has been applied to the resolution of overlapped peaks in liquid chromatography with diode array detection (LC-DAD). Complex river water samples spiked with 11 pharmaceutical substances resulted in poorly resolved chromatograms containing additional peaks from interfering matrix compounds and a change in the background absorbance due to the mobile phase gradient. Applying the present multivariate approach it was possible to resolve all 11 analytes from overlapping peaks, obtaining linear calibration lines ($R^2 > 0.96$). Recovery percentages on spiked samples ranged between 74.6 and 113.5%, which are quite satisfactory taking into account the low concentration ranges considered to 1–7 $\mu\text{g L}^{-1}$.

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1. Introduction

β -Blockers are drugs extensively used for the treatment of disorders such as hypertension, arrhythmia and heart failure. They are among the most worldwide prescribed medications and therefore most frequently detected in the environment. Its determination in environmental matrices is of importance because of health concerns surrounding widespread exposure to pharmaceuticals [1].

A high performance liquid chromatography-diode array detection (HPLC-DAD) method has been recently reported for the simultaneous determination of nine β -blockers (sotalol, atenolol, nadolol, pindolol, metoprolol, timolol, bisoprolol, propranolol and betaxolol) and two analgesics (paracetamol and phenazone) in river water [2]. The method involved a modified pre-column switching approach replacing the pre-column with a 5 μm C₁₈ chromatographic column (50 mm \times 4.6 mm) for sample pre-concentration discarding early eluting interferences. This method provided univariate method detection and determination limits varying between 0.03 and 0.16 $\mu\text{g L}^{-1}$ and between 0.2 and 0.5 $\mu\text{g L}^{-1}$, respectively. Method precision values found were lower than 9.4% relative standard deviation for spiking levels at the quantitation limits of each analyte and lower than 4.0%, except for bisoprolol (8.3%), for high spiking levels.

As shown by Martínez-Galera et al., by using the aforementioned approach a number of matrix compounds can be retained during the pre-concentration step. The co-elution of these compounds with the analytes leads to strong overlapping and a significant baseline drift. So, due to the complexity of the analytical signal (i.e. HPLC-DAD chromatograms) obtained, chemometric data treatment was found to be mandatory for an accurate identification and quantification of the analytes. The authors proposed a two-steps strategy in which initially the matrix background was reduced in three way data (HPLC-DAD chromatograms) by a baseline correction following the Eilers methodology. Subsequently, multivariate curve resolution-alternating least squares (MCR-ALS) [3] in combination with a standard addition calibration was applied to these data.

The background correction methodology proposed by Eilers consists of obtaining a background correction matrix with the same dimensions as those of samples and spiked samples by using spline basis functions. Details about the implementation of the algorithm can be found in the literature [4]. The aim of this first data pretreatment step was to simplify data complexity through the elimination of the chromatogram baseline.

MCR-ALS is widely used for an iterative optimization chemometric approach for the deconvolution and resolution of hyphenated data. This approach is well suited for resolving overlapping chromatographic signals in the analysis of complex samples, like environmental ones in which it is common that the target analytes co-elute with matrix interferences [5]. Description of MCR-ALS and

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its use in hyphenated systems such as HPLC-DAD can be found elsewhere [3,6–11].

According to this approach each test sample was analyzed in the following way: (i) in order to simplify the models, the spectral-time matrix for a given test sample was divided in six regions; and (ii) for each region, the resulting data matrix was augmented with five matrices recorded for the calibration solutions (i.e. HPLC-DAD chromatograms from the spiked sample). Initial estimates used for the initialization of the MCR-ALS process were obtained either from pure analyte standards or from the analysis of the purest spectra based on the so-called SIMPLISMA (simple interactive self-modeling mixture analysis) methodology [12]. Decomposition was performed by imposing the restrictions of non-negativity in spectral profiles and unimodality and non-negativity in concentration profiles. The number of interferences in each of the peak clusters or elution window analyzed was determined through the number of estimated components calculated by singular value decomposition (SVD) of each data subset.

A new multivariate method [13–16] named science based calibration (SBC) that combines the main features of classical and inverse calibrations has been recently developed. Initially named “Wiener filter”, the methods name was changed to SBC by early users of the pharmaceutical industry [17]. By estimating the spectral signal in a physical way and the spectral noise in a statistical way, the SBC method combines the prediction accuracy of the inverse approach with the low cost and the ease of interpretability of classical models. Results obtained by SBC are based on user-estimates of both, the analyte signal (e.g. spectrum) and spectral ‘noise’, Σ . Although the SBC method has already been deeply explained in previous works [13–16], the basic steps involved for the calculation of the analyte concentration in unknown samples are summarized below.

As described by Marbach [13], each spectrum of k variables ($x^T(1 \times k)$) can be described as:

$$x^T = yg^T + x_n^T \quad (1)$$

where $x_n^T(1 \times k)$ is the absorption in the measured spectrum that is not derived from the analyte, including instrumental noise and interfering spectra, and y and $g^T(1 \times k)$ are the concentration and the spectrum of the analyte, respectively.

For a set of m spectra, $\mathbf{X}(m \times k)$, the signal can be described by a mean $\bar{y}g^T$ and a root mean square value, $\sigma_y g^T$, where σ_y is the standard deviation of y . The spectral noise is also described by a mean value, \bar{x}_n^T , and a covariance matrix, Σ . If the differences between the spectra included in $\mathbf{X}(m \times k)$ are only due to variations in the concentrations of other constituents than the target analyte and to instrumental noise sources, then after mean centering, the measured spectra represent only spectral ‘noise’:

$$\tilde{\mathbf{X}} = \tilde{\mathbf{X}}_n \quad (2)$$

where \mathbf{X} is the mean centered matrix $\mathbf{X}(m \times k)$.

According to this, the covariance of the spectral ‘noise’, $\Sigma(k \times k)$, is calculated as:

$$\Sigma \cong \frac{\tilde{\mathbf{X}}^T \tilde{\mathbf{X}}}{m-1} \quad (3)$$

Subsequently, the optimum b-vector, in the sense of minimum square prediction error is calculated as:

$$b_{opt} = \frac{\sigma_y^2 \Sigma^- g}{1 + \sigma_y^2 g^T \Sigma^- g} \quad (4)$$

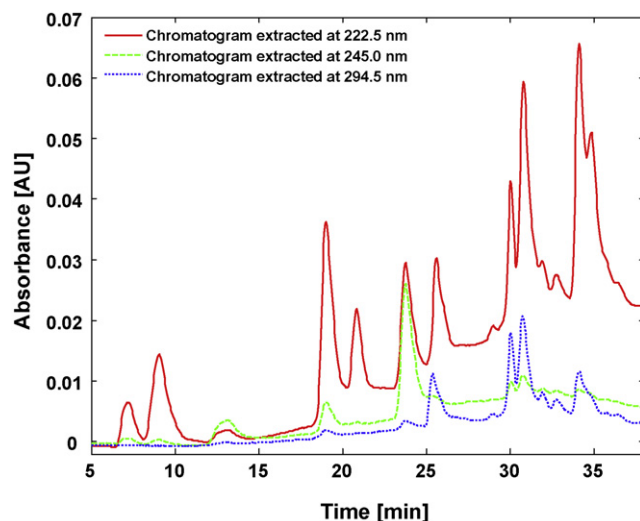


Fig. 1. Chromatograms obtained during the injection of a spiked river water sample extracted at three different wavelengths. Note: (1) SOT, (2) ATE, (3) PARA, (4) PIN, (5) NAD, (6) PHEN, (7) TIM, (8) MET, (9) BIS, (10) PRO, and (11) BEX.

where Σ^- corresponds to the inverse of Σ . If σ_y^2 tends to ∞ , this equation can be simplified to:

$$b_{opt(1)} = \frac{\Sigma^- g}{g^T \Sigma^- g} \quad (5)$$

Finally, the vector $b_{opt(1)}$ is used to predict the concentration of the analyte (y_{pred}) in each unknown sample spectrum (x_{pred}) using the information content of the ‘noise’ matrix and the analyte response as follows:

$$y_{pred} = \bar{y} + (x_{pred} - \bar{x})^T b_{opt(1)} \quad (6)$$

being, \bar{y} the mean value of y and \bar{x} the mean of the ‘noise’ spectra $\mathbf{X}(m \times k)$.

In a recent work, Kuligowski et al. [18] confirmed that the use of SBC presents major practical advantages, over other multivariate methods, for the extraction of analyte-specific chromatograms in LC-IR even in the presence of high spectral and chromatographic overlapping between the analyte of interest, co-eluting sample matrix constituents and the mobile phase components. On the other hand, obviously this approach also presents limitations or conditions of applicability and, accordingly to what is found in different multivariate approaches (e.g. PLS), its benefits are fully exploited when the number of unmodeled interfering compounds is reduced or eliminated. In spite of that it was shown that the method is particularly flexible and well suited for recovering the analyte signal from LC chromatograms.

The objective of this work is to extend the use of SBC to LC-DAD and to evaluate the advantages and drawbacks of its application on real environmental samples. In this sense, the use of the same MCR data set as employed in a previous publication applying MCR-ALS [2] facilitates the comparison of results obtained by both approaches. Moreover the SBC method developed to quantify nine β -blockers and two analgesics could be extended to the detection of other contaminants and pharmaceutical residues that are frequently present in rivers and streams at ppb levels due to discharges by wastewater treatment plants.

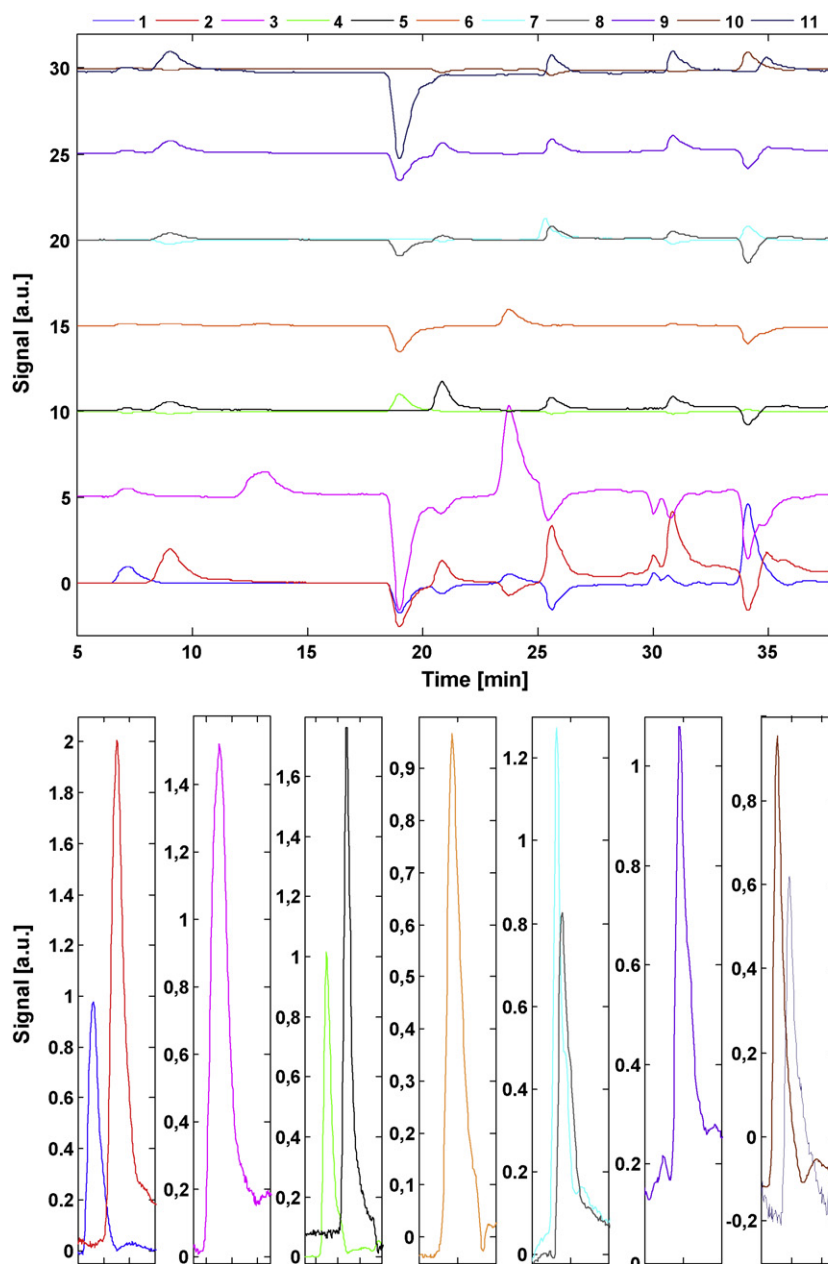


Fig. 2. SBC traces extracted from data obtained during the injection of a spiked river water sample using the conditions described in situation A showing (a) the whole trace and (b) a close-up view of the peaks of interest. Note: If necessary, traces were shifted in the y-axis for a better visualization. (1) SOT, (2) ATE, (3) PARA, (4) PIN, (5) NAD, (6) PHEN, (7) TIM, (8) MET, (9) BIS, (10) PRO and (11) BEX.

2. Materials and methods

2.1. Chemicals and solvents

Analytical standards of sotalol (SOT), atenolol (ATE), paracetamol (PARA), pindolol (PIN), nadolol (NAD), phenazone (PHEN), metoprolol tartrate salt (MET), timolol maleate salt (TIM), bisoprolol (BIS), propranolol hydrochloride (PRO) and betaxolol (BEX) were obtained from Sigma–Aldrich (Germany) in Pestanal quality.

Acetonitrile and methanol of HPLC grade were purchased from J.T. Baker (Holland), ortho phosphoric acid (H_3PO_4 , 85%), potassium dihydrogenphosphate (KH_2PO_4) of analytical grade from Merck (Darmstadt, Germany) and sodium hydroxide (NaOH) from Pan-

reac (Spain). A buffer solution at a concentration of 25 mM was prepared by dissolving an appropriate amount of KH_2PO_4 in water and adjusting the pH to 3.0 with 0.1 M H_3PO_4 . Ultrapure water was obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA). Mobile phase solvents were filtered through a 0.45 μm celluloseacetate or polytetrafluoroethylene (PTFE) membrane and degassed with helium prior and during their use.

2.2. Preparation of standards, spiked samples and standard additions for calibration purposes

A river water sample (R1) from the stream of Nacimiento river (Almería, Spain) was used for recovery studies and calibration. The river water sample did not contain any of the

investigated analytes and was spiked at different concentration levels of β -blockers (1.0, 2.0, 3.0 and 4.0 $\mu\text{g L}^{-1}$ of each analyte) to obtain spiked sample for calibration and recovery studies.

For the on-line pre-concentration of drugs, river water samples or Milli-Q blanks were filtered through a 0.45 μm cellulose acetate membrane. Then 400 μL of methanol were added to 100 mL of each sample or blank, corresponding to an organic modifier concentration of 0.4%, immediately before pre-concentration in the PC-HPLC system.

2.3. Instrumentation and on-line procedure

A detailed description of the set-up can be found elsewhere [2]. On-line sample pre-concentration and separation was performed using a PC-HPLC-DAD system consisting of a high-flow isocratic Model 510 HPLC pump, a quaternary low-flow gradient Model 600E LC pump, both from Waters (Milford, MA, USA), a Type 7000 high pressure column-switching valve from Rheodyne (Berkeley, CA, USA) and a 2996 diode array detector (DAD) from Waters. An Hypersil Gold C_{18} (50 mm \times 4.6 mm, 5 μm particle size, 175 \AA pore size) from ThermoQuest (Waltham, MA, USA) was used as first column for sample pre-concentration. A Gemini C_{18} (150 mm \times 4.6 mm, 5 μm particle size, 110 \AA pore size) from Phenomenex (USA) was used as analytical column to carry out the HPLC separation of drugs.

The on-line procedure for the determination of drugs by PC-HPLC-DAD is based on a column-switching technique using a two position HP valve to connect the two LC columns. In the first position of the HP valve, before processing the first sample, the two columns were coupled in line and conditioned by passing the mobile phase in the initial gradient conditions (KH_2PO_4 buffer: CH_3OH 85:15, v/v) at a flow rate of 1.5 mL min^{-1} using the 600E LC quaternary pump. After 10 min, the HP valve was switched to the second position in such a way that the mobile phase pumped by the 600E LC quaternary pump passed only through the analytical column, while the water sample was loaded into the other column for pre-concentration using the 510 LC pump at a flow rate of 1.5 mL min^{-1} during 20 min. In this step the analytes were retained on the pre-concentration column while most interferences were not retained and eliminated to the waste. When the valve was switched again to the first position, the retained analytes were transferred onto the analytical column where they were separated and subsequently detected in the DAD system. In order to avoid carryover, at the same time the pre-concentration system was cleaned with Milli-Q water for 10 min before loading the next.

Pre-concentration and separation were carried out using a gradient with KH_2PO_4 buffer solution (0.025 mol L^{-1} at pH 3.0) as solvent A, methanol as solvent B and acetonitrile as solvent C at a flow rate of 1.5 mL min^{-1} . The solvent program consisted of a linear gradient from A:B (85:15, v/v) to A:B:C (55:25:20, v/v/v) in 8 min, remaining in isocratic conditions for 4 min, returning to the initial conditions in a linear gradient of 2 min and holding these conditions for 4 min. All signals were simultaneously acquired by a DAD using a wavelength range between 200 and 350 nm. The total analysis time was 38 min.

2.4. Software

EmpowerTM 2 (ChromatographyManager, Waters) software was used for data acquisition. Routines for data processing were written in Matlab 7.7.0 (R2008b) from MathWorks (Natick, MA, USA). Matrix inversion, Σ^{-} , was performed using the *pinv* Matlab function, which returns the Moore–Penrose pseudoinverse of Σ [19].

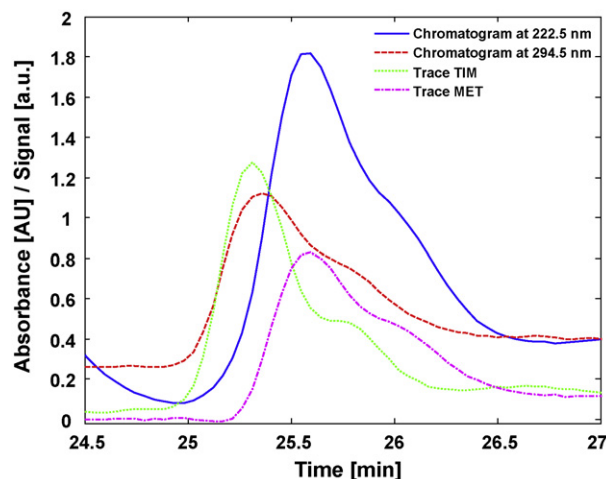


Fig. 3. Comparison of SBC traces obtained for TIM and MET with chromatograms obtained at two different wavelengths. Note: If necessary, chromatograms were amplified and shifted in the y-axis for a better visualization.

3. Results and discussion

3.1. Chromatograms

Fig. 1 shows chromatograms obtained at three different wavelengths recorded during the injection of a river water sample spiked with the 11 considered pharmaceuticals. Due to the high spectral similarities of the eluting substances it was not possible to resolve all the analytes and under the previously described chromatographic conditions, only PARA and BIS showed baseline resolution. From the recorded signal it is evident that the sample matrix is still quite complex after the pre-concentration step. Several matrix components were retained in the pre-concentration step and gave rise to a number of peaks in addition to the analyte signals which also co-eluted or overlapped with analyte peaks. Furthermore, a baseline drift due to gradient elution also hampers the qualitative and quantitative determination of the analytes of interest.

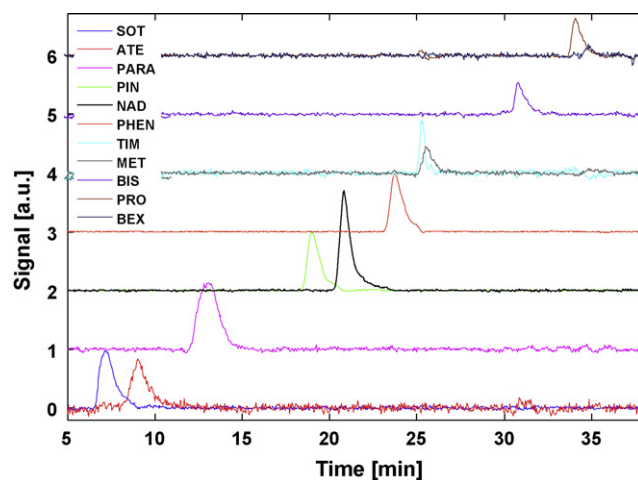


Fig. 4. SBC traces extracted from data obtained during the injection of a spiked river water sample using conditions of Situation B. Notes: (1) If necessary, traces were shifted in the y-direction for a better visualization; (2) See the text for additional details about the previous information available in Situation B.

Table 1
Calibration lines for the HPLC-DAD determination of pharmaceuticals in river water obtained using SBC in situations A¹ and B².

Analyte	Range _{conc} [$\mu\text{g L}^{-1}$]	$t_{\text{Ret}} \pm s$ [min]	Equation: $(a \pm s_a)x + (b \pm s_b)$				R^2	Noise (RMS)	SNR	LOD [$\mu\text{g L}^{-1}$]	LOQ [$\mu\text{g L}^{-1}$]
			$(a \pm s_a)$	$(b \pm s_b)$	s_a 95%	s_b 95%					
Situation A ^a											
SOT	1–5	7.4 \pm 0.4	0.24 \pm 0.01	0.00 \pm 0.02	0.22–0.26	–0.07 to 0.07	0.997	0.004	55	0.1	0.2
ATE	3–7	9.3 \pm 0.4	0.29 \pm 0.01	0.05 \pm 0.03	0.27–0.31	–0.04 to 0.13	0.9990	0.01	149	0.1	0.2
PARA	1–5	13.2 \pm 0.3	0.46 \pm 0.01	–0.04 \pm 0.03	0.43–0.48	–0.12 to 0.05	0.9991	0.02	23	0.1	0.4
PIN	1–5	19.2 \pm 0.3	0.177 \pm 0.003	–0.01 \pm 0.01	0.169–0.185	–0.03 to 0.02	0.9994	0.001	179	0.02	0.1
NAD	3–7	20.8 \pm 0.2	0.183 \pm 0.004	0.04 \pm 0.02	0.172–0.194	–0.02 to 0.10	0.9990	0.01	93	0.1	0.3
PHEN	2–6	23.7 \pm 0.2	0.15 \pm 0.01	–0.02 \pm 0.02	0.13–0.17	–0.10 to 0.05	0.996	0.003	110	0.1	0.2
TIM	1–5	25.3 \pm 0.3	0.126 \pm 0.003	–0.03 \pm 0.01	0.118–0.135	–0.10 to 0.00	0.9990	0.01	10	0.3	1
MET	3–7	25.6 \pm 0.3	0.075 \pm 0.002	0.01 \pm 0.01	0.068–0.081	–0.03 to 0.04	0.998	0.01	31	0.3	1
BIS	2–6	30.9 \pm 0.3	0.103 \pm 0.002	0.00 \pm 0.01	0.095–0.111	–0.03 to 0.03	0.998	0.01	19	0.3	1
PRO	3–7	34.3 \pm 0.4	0.12 \pm 0.01	0.02 \pm 0.03	0.10–0.14	–0.09 to 0.12	0.991	0.002	194	0.05	0.2
BEX	2–6	35.1 \pm 0.4	0.12 \pm 0.01	0.01 \pm 0.04	0.10–0.15	–0.12 to 0.13	0.98	0.01	18	0.3	1
Situation B ^b											
SOT	1–5	7.4 \pm 0.4	0.22 \pm 0.01	0.03 \pm 0.03	0.18–0.26	–0.11 to 0.16	0.997	0.02	14	0.2	1
ATE	3–7	9.2 \pm 0.5	0.14 \pm 0.02	0.0 \pm 0.1	0.08–0.20	–0.2 to 0.3	0.96	0.05	11	1	3
PARA	1–5	13.2 \pm 0.3	0.37 \pm 0.02	0.1 \pm 0.1	0.32–0.43	–0.1 to 0.2	0.993	0.02	18	0.2	1
PIN	1–5	19.1 \pm 0.4	0.177 \pm 0.003	0.00 \pm 0.01	0.168–0.186	–0.03 to 0.03	0.9993	0.002	71	0.04	0.1
NAD	3–7	20.8 \pm 0.2	0.20 \pm 0.01	0.01 \pm 0.04	0.18–0.23	–0.11 to 0.13	0.996	0.01	68	0.1	0.4
PHEN	2–6	23.7 \pm 0.2	0.149 \pm 0.005	0.00 \pm 0.02	0.133–0.164	–0.07 to 0.06	0.997	0.01	45	0.1	0.4
TIM	2–5	25.3 \pm 0.3	0.060 \pm 0.001	–0.006 \pm 0.002	0.058–0.062	–0.013 to 0.000	0.9997	0.03	13	0.5	2
MET	4–7	25.58 \pm 0.25	0.042 \pm 0.002	–0.01 \pm 0.01	0.036–0.049	–0.04 to 0.03	0.994	0.02	10	1	4
BIS	2–6	30.9 \pm 0.3	0.057 \pm 0.004	0.00 \pm 0.02	0.043–0.071	–0.06 to 0.06	0.98	0.02	11	1	2
PRO	3–7	34.3 \pm 0.4	0.08 \pm 0.01	–0.01 \pm 0.05	0.05–0.10	–0.16 to 0.14	0.96	0.01	18	0.5	2
BEX	6	34.84 \pm 0.07	–	–	–	–	–	0.03	3	6	19

Note: Range_{conc} stands for the investigated concentration range. $t_{\text{Ret}} \pm s$ stands for retention time \pm standard deviation. $a \pm s_a$ stands for the slope of the calibration line \pm standard deviation (applying a confidence limit of 95%). $b \pm s_b$ stands for the intercept of the calibration line \pm standard deviation (applying a confidence limit of 95%). R^2 stands for the coefficient of determination. RMS stands for root mean square calculated from the chromatographic noise. SNR stands for signal to noise ratio calculated using the signal obtained from the standard with the lowest concentration. LOD stands for limit of detection and was estimated for a SNR of 3. LOQ stands for limit of quantification and was estimated for a SNR of 10.

^a See text for details.

^b See text for details.

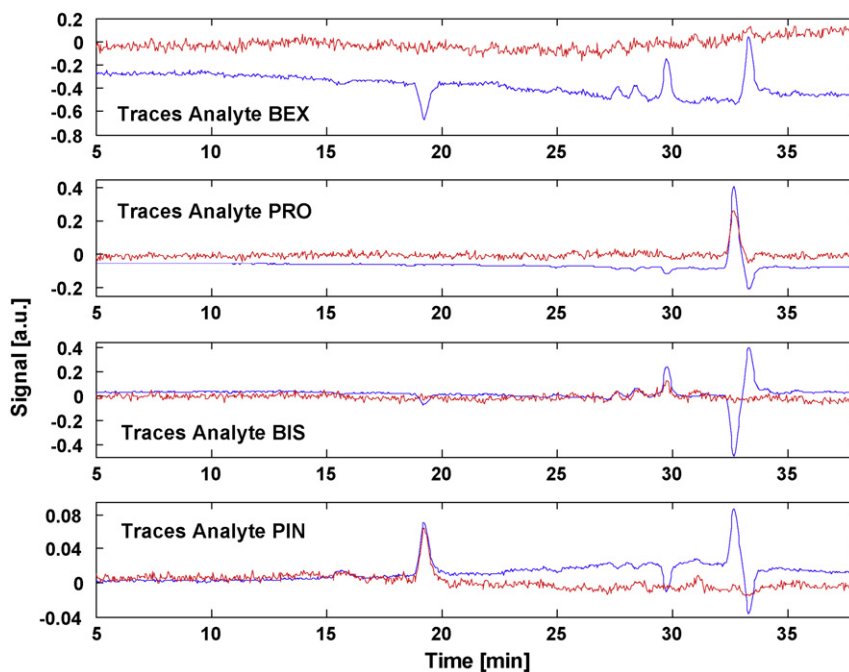


Fig. 5. Traces extracted from data obtained during the injection of a river water sample. *Note:* Traces were extracted as specified before; blue line: using information described by situation A and red line: using the previous information available in situation B (see the text for details). Retention times PIN: 19.2 min, BIS: 29.8 min, PRO: 32.7 min and BEX: 33.3 min. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.2. SBC in on-line LC-DAD

The aim of this study was to demonstrate the suitability of the multivariate SBC approach in HPLC-DAD for the extraction of analyte specific chromatograms with a high spectral and chromatographic overlapping between analytes of interest, matrix compounds and mobile phase absorbance occurs. Therefore, two different situations have been evaluated differing in the previously available information on the investigated system.

3.2.1. Situation A

In situation A the chromatograms were extracted using a set of spectra measured during the injection of Milli-Q water into the chromatographic system employing the same gradient as for the sample analysis as a noise matrix. As aforementioned, accurate SBC results are obtained when the interfering compounds are included in the noise matrix before the calculation of the covariance matrix of the spectral noise for the calculation of the regression vector. Therefore, results based on the use of a solvent injection were found to be highly unspecific. Because of that, the inclusion of interferent spectra in the noise matrix extracted from the sample chromatograms to be analyzed significantly improved the quality of the obtained concentration profiles as it can be seen from results shown in Fig. 2. Fig. 2a shows whole chromatograms of each analyte obtained after the application of SBC in the 5–37 min time range. Positive and negative peaks which did not correspond to any of the target analytes were caused by high spectral similarities between the target analyte and other analytes or matrix compounds and to the use of a noise matrix that was not representative of the data to be analyzed. In spite of that, target analyte peaks could be identified by their retention time and so, additional non-overlapping peaks which do not show chromatographic overlapping did not cause problems in quantitative analysis. Fig. 2b shows close up views of each analyte peak elution window which illustrates that all eleven analytes could be clearly resolved from other overlapping peaks.

Summarizing, for the extraction of the chromatographic traces shown in Fig. 2 a spectrum of each analyte was used as g vec-

tor and, in the case of two overlapping peaks, a spectrum of the overlapping analyte extracted from the sample chromatogram was included in the noise matrix. It has to be highlighted that unknown interferences can be also resolved with this technique. Although in this case, no reference spectra of the interferences were available, appropriate reference spectra (i.e. g vector) could be obtained from sample injection, selecting carefully one spectrum of each one of the unknown interferences that is free from interferences from other eluting analytes.

Fig. 3 shows extracted traces of TIM and MET at two wavelengths and using the SBC approach. The chromatogram extracted at 294.5 nm showed a maximum at 25.36 min with a shoulder at about 25.79 min and the chromatogram extracted at 222.5 nm showed a maximum at 25.6 min with a shoulder at around 26.02 min. As shown in the figure, the maximum SBC signal for TIM shifted from 25.36 to 25.31 min in comparison to the chromatogram extracted at 294.5 nm (for the depicted sample) improving the resolution using SBC. Despite the improvement in resolution, in the SBC trace still a shoulder is observed indicating the overlapping of the TIM peak with an unidentified compound that is not included in the noise matrix. The SBC signal obtained for MET showed the same profile as the chromatogram extracted at 222.5 nm which means that the resolution could not be improved. Results found are in accordance with a previously published paper applying MCR-ALS [2].

3.2.2. Situation B

In situation B different noise matrices were defined for each target analyte: each noise matrix was composed by spectra obtained during the injection of a spiked blank sample, excluding the spectra of the corresponding target analyte. In this situation the noise matrix represents the different sources of noise as it corresponds to the absorption measured during the chromatogram that is not derived from the analyte. It includes the variations of the background absorption and also the spectra of other interfering compounds. Once defined the set of noise matrices, the extraction of the analyte-specific traces was carried out in the set of LC-DAD injections. Fig. 4 shows the analyte-specific SBC traces extracted

Table 2Recovery percentage data obtained for the determination of pharmaceuticals in spiked river water using SBC correction using A and B approaches^a.

Analyte	$\mu\text{g L}^{-1}$ spiked	Situation A		Situation B	
		$\mu\text{g L}^{-1}$ found	Recovery [%]	$\mu\text{g L}^{-1}$ found	Recovery [%]
SOT	1.00	1.01	100.8	1.09	109.1
ATE	3.00	3.15	105.0	3.29	109.5
PARA	1.00	0.92	92.1	1.14	113.5
PIN	1.00	0.96	96.0	0.98	97.7
NAD	3.00	3.21	107.1	3.04	101.3
PHEN	2.00	1.87	93.3	1.95	97.3
TIM	1.00	0.75	74.6	0.88	88.5
MET	3.00	3.11	103.6	2.86	95.2
BIS	2.00	1.99	99.5	1.93	96.5
PRO	3.00	3.13	104.3	2.93	97.8
BEX	2.00	2.05	102.5	<LOD	<LOD

^a See text for details for details.

for each analyte. Due to the specificity of the response, the identification and quantification of the target analyte even in complex mixtures of related compounds was possible.

3.3. Figures of merit

Univariate linear regressions using peak area values obtained from the chromatograms extracted using the multivariate approach were calculated. This approach provided an easy and straightforward calculation of the figures of merit and therefore, facilitated the interpretation of results.

Table 1 shows the figures of merit obtained from calibration lines established for the target analytes in situations A and B. All calibration lines showed high linearity in the investigated concentration ranges ($R^2 > 0.96$). The chromatographic noise was established as the root mean square (RMS) of the trace during 2 min before the elution of the target analyte and the signal-to-noise ratio (SNR) was calculated dividing the signal obtained from the lowest concentration sample by the noise value. To estimate the limits of detection (LODs) and quantification (LOQs) of the method, the concentrations at a SNR of 3 and 10, respectively, were calculated. LODs and LOQs for situation A ranged between 0.02 and 0.3 $\mu\text{g L}^{-1}$ and 0.1 and 1 $\mu\text{g L}^{-1}$, respectively, and were of the same order as those obtained using the MCR based method providing LODs and LOQs ranging between 0.03 and 0.16 $\mu\text{g L}^{-1}$ and 0.2 and 0.5 $\mu\text{g L}^{-1}$, respectively. Using the proposed method, for some analytes the LOD and LOQ could be improved. LODs and LOQs obtained from situation B were slightly higher than those obtained by MCR ranging between 0.04 and 1 $\mu\text{g L}^{-1}$ and 0.1 and 4 $\mu\text{g L}^{-1}$, respectively, except for the analyte BEX that showed higher values due to the strong overlapping with other compounds with similar spectral features in this part of the chromatogram. Although LODs and LOQs in situation B could not be improved in comparison to the MCR based method, the high specificity of the chromatographic signal is remarkable. As summarized in Table 2, for the analysis of spiked samples, recovery values were found to lie between 74.6 and 107.1% for situation A and between 88.5 and 113.5% for situation B. For BEX it was not possible to calculate a calibration line and recovery percentage in situation B, because spiked samples at 1.0, 2.0 and 3.0 $\mu\text{g mL}^{-1}$ were below the detection limit.

3.4. River water analysis

SBC using the noise matrices from situations A and B was applied to data obtained during the injection of a real river water sample. Fig. 5 shows the extracted traces for analytes that could be detected in the sample. It can be seen that situation B is especially useful for the identification of analytes in new samples, because as the sample matrix is complex, slight shifts in retention time can occur which

leads to ambiguous results when several analytes and interferents elute in a reduced interval of time. From Fig. 5 it can be observed that the sloping baseline could be compensated and peak shape and resolution are appropriated for qualitative and quantitative studies.

4. Conclusions

The application of the SBC approach to a complex analytical problem proved that this method is a valuable tool HPLC-DAD chemometrical data treatment. The high versatility of the approach was demonstrated by analyzing two situations with different available spectral information for the calculation of an appropriate regression vector. Specificity of response could be achieved to different extent by using a single blank injection as noise matrix or, to fully exploit the benefits of the method, by employing a spiked sample injection. Both discussed situations were able to compensate the slope caused by the mobile phase gradient resulting in non-sloping chromatograms with a randomly distributed noise around zero. Comparing the results of the present work with those of the previously published paper using the same measurement set-up and samples, the obtained results are in good agreement concerning the obtained linearity, R^2 and recovery values. The applicability of SBC in HPLC could be further extended by combining it with other multivariate techniques: for example, MCR-ALS or SIMPLISMA could be used to obtain the spectrum of an analyte or interfering compound from an HPLC data set and then, this spectrum could be used for an on-the-fly extraction of analyte-specific chromatograms in further measurements as g vector or to improve the calculation of the b vector.

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References

- [1] M. Gros, T.-M. Pizzolato, M. Petrovic, M.J. López de Alda, D. Barceló, J. Chromatogr. A 1189 (2008) 374.
- [2] M. Martínez Galera, M.D. Gil García, M.J. Culzoni, H.C. Goicoechea, J. Chromatogr. A (2010), doi:10.1016/j.chroma.2010.01.082.
- [3] A. de Juan, D. Tauler, J. Chromatogr. A 1158 (2007) 184.
- [4] M. Daszykowski, B. Walczak, Trends Anal. Chem. 25 (2006) 1081.
- [5] E. Peré-Trepat, A. Hildebrandt, D. Barceló, S. Lacorte, R. Tauler, Chem. Intell. Lab. Syst. 74 (2004) 293.
- [6] S. Mas, G. Fonrodona, R. Tauler, J. Barbosa, Talanta 71 (2007) 1445.
- [7] E. Peré-Trepat, R. Tauler, J. Chromatogr. A 1131 (2006) 85.
- [8] E. Peré-Trepat, S. Lacorte, R. Tauler, J. Chromatogr. A 1096 (2005) 111.
- [9] R. Tauler, S. Lacorte, D. Barceló, J. Chromatogr. A 730 (1996) 177.
- [10] S. Lacorte, D. Barceló, R. Tauler, J. Chromatogr. A 697 (1995) 345.
- [11] R. Gargallo, R. Tauler, F. Cuesta-Sánchez, D.L. Massart, Trends Anal. Chem. 15 (7) (1996) 279.

- [12] W. Windig, J. Guilment, *Anal. Chem.* 63 (1991) 1425.
- [13] R. Marbach, *J. Biomed. Opt.* 7 (2002) 130.
- [14] R. Marbach, *J. Near Infrared Spectrosc.* 13 (2005) 241.
- [15] R. Marbach, U.S. Patent No. 6,629,041 B1, 30 September 2003.
- [16] R. Marbach, *Chem. Ing. Tech.* 82 (2010) 453–466.
- [17] Guidance for Industry: PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance, U.S. Department of Health and Human Services, Food and Drug Administration, 2004.
- [18] J. Kuligowski, G. Quintás, S. Garrigues, M. de la Guardia, *J. Sep. Sci.* 32 (2009) 1.
- [19] Matlab R2008b, Version 7.7.0, The Mathworks Inc.