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Volume 1216, issue 29, 17 July 2009 ISSN 0021-9673

JOURNAL OF CHROMATOGRAPHY A

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Journal of Chromatography A, 1216 (2009) 5489–5496

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Journal of Chromatography A

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Chemometric tools improving the determination of anti-inflammatory and antiepileptic drugs in river and wastewater by solid-phase microextraction and liquid chromatography diode array detection

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ARTICLE INFO

Article history:
Received 31 March 2009
Received in revised form 21 May 2009
Accepted 25 May 2009
Available online 2 June 2009

Keywords:
Pharmaceuticals
Solid-phase microextraction
Liquid chromatography–diode array detection
River water
Wastewater
Second order advantage

ABSTRACT

An analytical method for the simultaneous determination of seven non-steroidal anti-inflammatory drugs (naproxen, ketoprofen, diclofenac, piroxicam, indomethacin, sulindac and diflunisal) and the anticonvulsant carbamazepine in river and wastewater is reported. The method involves pre-concentration and clean-up by solid-phase microextraction using polydimethylsiloxane/divinylbenzene fibers, followed by liquid chromatography with diode array detection analysis. Owing to the fact that river water samples did not contain interferences and no sensitivity changes due to sample matrix were observed, external calibration was implemented. Standardization was also applied in order to carry out the prediction step by preparing only two diluted standards that were subjected to the pre-concentration step and a set of standards prepared in solvent. For the analysis of wastewater samples, in contrast, it was necessary to implement standard addition calibration in combination with the multivariate curve resolution-alternating least squares (MCR-ALS) algorithm, which allowed us to overcome matrix effect and exploit the second order advantage. Recoveries ranging from 72% to 125% for all pharmaceuticals proved the accuracy of the proposed method in river water samples. On the other hand, wastewater sample recoveries ranged from 83% to 140% for all pharmaceuticals, showing an acceptable performance – considering this sample contains no modeled interferences.

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are produced and used in increasingly large volumes every year (as they may be used without prescription) and are among the most frequently detected pharmaceuticals. Their widespread environmental distribution may be justified because most of them are acidic drugs with p*K*as in the range 3–5 [1] and because of their hydrophilicity and stability, they tend to remain in the aqueous phase and are not totally eliminated by sewage treatment plants (STPs). Thus, concentrations up to 1.5 $\mu g\,L^{-1}$ for naproxen and up to 85 $\mu g\,L^{-1}$ for ibuprofen have been found in some STP effluents and low removal efficiencies (between 15% and 69%) for diclofenac have been reported by several researchers [1]. Carbamazepine is used for the treatment of seizure disorders, for relief of neuralgia, and for a wide variety of mental disorders. About 72% of orally administered carbamazepine is absorbed, while 28% remains unchanged and is subsequently discharged through the faeces [2]. Studies conducted to observe the behaviour of pharmaceutical residues in wastewater treatment plants revealed that carbamazepine is persistent, with removal efficiencies below 10%. The low removal efficiency of carbamazepine is due to (i) being found to be resistant to biodegradation at low concentrations [3]; in fact, in the removal status of pharmaceuticals, carbamazepine has been classified as “no removal” [4] and (ii) being hardly attached to the sludge with a distribution coefficient between water and secondary sludge of 1.2 $L\,kg^{-1}$, vs. the 500 $L\,kg^{-1}$ required for significant sorption [5].

Environmental sample matrices such as ground, superficial and wastewaters are complex samples, often containing compounds which can interfere with the compounds of interest and, moreover, pharmaceuticals are generally found in these matrices at trace concentration levels. Therefore, analytical procedures for determining drugs in environmental water samples include an

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doi:10.1016/j.chroma.2009.05.073

initial sample preparation step, which allows purification and concentration of the analytes. Sample preparation for NSAIDS and carbamazepine may be carried out by solid-phase extraction (SPE) using lipophilic sorbents at acidic pH [6] or polymeric sorbents such as Oasis HLB [7,8] or Lichrolut EN cartridges [9] at neutral pH, or by solid-phase microextraction (SPME) [10]. The advantages of both pre-concentration techniques have been discussed in the literature [6].

In a previous paper [10], seven NSAIDs (naproxen, ketoprofen, diclofenac, piroxicam, indomethacin, sulindac and diflunisal) and the anticonvulsant carbamazepine were determined by us in river water samples using SPME and liquid chromatography coupled to diode array detection (LC–DAD), as a simple, efficient, selective and low-cost methodology. In this case, matrix effect was not present and quantitation was carried out using standards prepared in Milli-Q water. However, the SPME approach demands both the samples and the standards be processed in exactly the same way, which involves a time-consuming method as the calibration standards must be subjected to the same pre-concentration step as real samples. This drawback may be overcome by the application of multivariate standardization, which allows finding a mathematical transformation that makes the measured response obtained in a particular set of conditions as similar as possible to the response that would be obtained in a different set of conditions [11]. This strategy has been successfully applied when some sample treatment is necessary, such as extraction or clean-up steps, which are not indispensable during standard calibration. Thus, piecewise direct standardization (PDS) was applied to two-way data to transfer fluorescence spectra obtained after SPE to spectra registered in pure solvent, thus solving the drawback caused by differences between the spectra obtained with and without the pre-concentration step in the determination of multicomponent mixtures by partial least squares (PLS) [11–13]. PDS was also applied to three-way data sets obtained from liquid chromatography with diode array detection (LC–DAD), which allowed the correction of the breakthrough effect observed for the most polar analytes after the SPE step in the determination of eight tetracyclines in wastewater by second order chemometric methods [14].

In spite of the clean-up step, when working with extremely complex matrices such as influent or effluent wastewaters, extracts often contain compounds which can interfere with the analytes of interest leading to lack of selectivity or to matrix effect. According to Massart et al. [15], lack of selectivity occurs when the blank has a response which adds to that of the analyte and would lead to a constant systematic error. Lack of selectivity would be related to additive interferences present in the matrix background, which contribute to increasing baseline or to peak overlapping in chromatographic methods. However, matrix effect, which is also due to the presence of organic and inorganic components in the sample coeluting with the target analytes [16], does not lead to a response as such, but affect the slope of the calibration graph and produces relative systematic errors.

Some drawbacks were found when different authors deal with matrix effect. Quintana and Reemtsma studied matrix effects in the determination of NSAIDs and triclosan in raw and treated municipal wastewater samples after direct injection and after enrichment by SPE and ion-pair reverse-phase liquid chromatography/tandem mass spectrometry [7]. From the response for each of the compounds added to an SPE extract, they found a clear tendency of decreasing signal suppression with increasing retention time, which indicates that matrix effect is related to non-specific interactions of moderately polar matrix components, whose concentrations decrease with increasing retention time [17]. Due to this behaviour it was found not to be possible to reliably compensate for matrix effect by the use of internal standards and so samples were quantified by the method of standard addition applied to the extracts.

In addition, since matrix components in wastewater can be rather variable between samples, in nature and amount, their effects are difficult to predict and to correct [18]. One way this situation may be handled is to use second (and higher) order data from hyphenated techniques coupled to multivariate calibration algorithms involving the second order advantage in combination with some calibration approaches such as standard addition or internal standard. The second order advantage allows accurate quantitation of multiple analytes using a calibration sample containing multiple chemical components without knowledge of the interfering chemical components that may be present in the sample being analyzed [19], thus handling additive unexpected interferences, whereas standard addition or internal standard cope with matrix effect. In this way, Tauler and co-workers [20] evaluated different calibration approaches for quantification of six biocide compounds in mussel samples, undergone to co-elution and to matrix effect in LC–MS, using multivariate curve resolution (MCR-ALS). This chemometric approach is a powerful curve resolution method which allows the efficient resolution of coeluting peaks and simultaneous multicomponent determination without the need for developing specific analytical procedures for each analyte. Among the three calibration strategies used (external calibration, standard addition and internal standard), multivariate extension of the standard addition method using MCR-ALS provided an improvement in the results which was increased when internal standard was additionally used on the same mussel matrix sample.

In this work we propose the use of PDS to transfer data obtained without SPME to data obtained after this pre-concentration approach, thus allowing to carry out the calibration step by injection of standards prepared in solvent, in the determination of seven NSAIDs and carbamazepine in environmental surface water. In addition, we applied the multivariate curve resolution-alternating least squares (MCR-ALS) algorithm [21] in combination with standard addition to determine the above-mentioned pharmaceuticals in effluent wastewater samples, also after SPME. In both cases, separation and detection were carried out by LC–DAD.

2. Experimental

2.1. Chemicals and solvents

Analytical standards (pestanal quality) of naproxen (NAPRO) and ketoprofen (KETO) were available from Riedel de Haën (Seelze, Germany) and sodium diclofenac (DICLO), piroxicam (PIR), sulindac (SUL), diflunisal (DIFLU) and carbamazepine (CBZ) analytical standards were purchased from Sigma–Aldrich (Steinheim, Germany) and indomethacin (INDO) analytical standard was obtained from Fluka (Buchs, Switzerland).

Acetonitrile (ACN) and methanol (MeOH) LC grade were obtained from J.T. Baker (Deventer, The Netherlands). Ultrapure water was obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA).

Hydrochloric acid (37%) and potassium dihydrogenphosphate (KH_2PO_4) analytical grade were purchased from Merck (Darmstadt, Germany).

Aqueous and organics LC mobile phases were filtered through a 0.45 μm cellulose acetate or polytetrafluorethylene (PTFE), respectively and degassed with helium prior to and during use.

2.2. Instrumentation and software

The SPME fiber assembly and SPME–LC interface were purchased from Supelco (Bellefonte, PA, USA). The SPME–LC interface

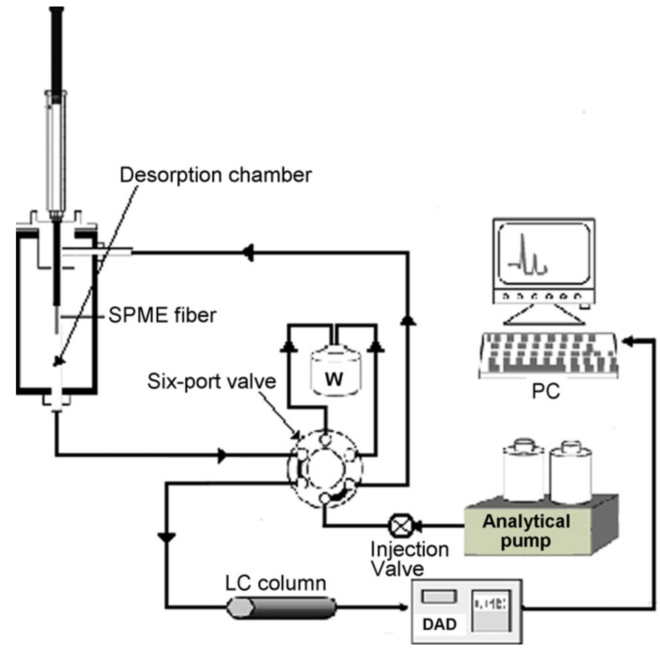

Fig. 1. Schematic diagram of the SPME–LC–DAD instrumentation.

consisted of a six-port injection valve and desorption chamber (chamber volume 60 μL), which replaces the injection loop of a six-port injection system. The SPME polydimethylsiloxane/divinylbenzene (PDMS–DVB, 65 μm) and polyacrylate (PA, 85 μm) fibers were purchased from Supelco.

The LC system consisted of a Waters (Milford, MA, USA) instrument, composed of a gradient Model 600E pump, a Rheodyne 7725i manual injector valve with a 50 μL sample loop and a Mod 2969 DAD. LC separations were performed with a Discovery RP-Amide C16 (150 mm × 4.6 mm, 5 μm particle size) column from Supelco.

A digital venturis FP575 pentium personal computer using a Millenium 32 from Waters was used for data acquisition. Routines for data pre-treatment and processing were written in MATLAB (MATLAB 6.0, The MathWorks, Natick, MA, USA, 2000). Routines for MCR-ALS were available on the Internet (http://www.ub.es/gesq/mcr/mcr.htm). Baseline routines are an adaptation of those described in reference [22] for second order data and were kindly provided by J. Braga (Unicamp, Brazil). PDS was implemented with PLS Toolbox routines.

Fig. 1 shows a schematic diagram of the SPME–LC–DAD instrumentation used in this work.

2.3. *Preparation of standards and spiked samples*

Stock individual standard solutions of pharmaceuticals were prepared in MeOH at concentration levels of 400 μg mL^{-1} and maintained under refrigeration at 4 °C in the dark. Under these conditions they were stable for at least 3 months. Working solutions were prepared daily by dilution of appropriate aliquots in ACN–KH_2PO_4 buffer 0.025 mol L^{-1} at pH 3.00 (50:50, v/v) as solvent and were filtered through Millipore membrane PTFE filters (0.45 μm particle size) before injection into the chromatographic system.

After collection, the river water samples were vacuum-filtered through a 0.45 μm acetate cellulose membrane from Millipore to remove suspended matter and stored at 4 °C in the dark.

For the analysis of river water samples, standard mixtures of the analytes at six concentration levels were prepared in triplicate in ACN–KH_2PO_4 buffer 0.025 mol L^{-1} at pH 3.00 (50:50, v/v) for direct injection in the LC–DAD system. The first concentration level corresponded to the LOQs for each analyte (5.0 μg L^{-1} for SUL and NAPRO, 10.0 μg L^{-1} for CBZ, KETO and DIFLU and 20.0 μg L^{-1} for PIR, INDO and DICLO) and the other concentration levels were 25.0, 50.0, 100.0, 150.0 and 200.0 μg L^{-1} for all analytes. In addition, three diluted Milli-Q water samples containing 5.0, 20.0 and 30.0 μg L^{-1} of each analyte were prepared and subjected to the preconcentration step. The chromatographic signals corresponding to these samples, along with the signals from standards containing 25, 100 and 150 μg L^{-1} for all analytes prepared as described above, were used for standardization by application of PDS. After PDS application, the standardised signals corresponding to the six triplicate standards were used for calibration.

Wastewater final effluent samples were collected from a wastewater treatment plant in Almería (Spain). Before analysis they were centrifuged at 5000 × g and then filtered through a 0.45 μm filter and stored at 4 °C in a refrigerator. The maximum time of storage was 2 days to minimize microbial degradation.

Wastewater samples were spiked at different concentration levels of NSAIDs and CBZ (see Table 2), thus simulating real samples containing the analytes and then, 0.0, 20.0, 30.0, 40.0 and 50.0 μg L^{-1} of each analyte were added to five aliquots of each previously spiked sample for calibration by using the standard addition method.

2.4. *SPME procedure*

SPME experiments were performed using a fused-silica fiber coated with a thin layer of polymeric stationary phase (PDMS–DVB 60-μm film thickness) supplied by Supelco. The SPME methodology includes four main steps: fiber conditioning, extraction of analytes into the SPME fiber from the aqueous sample, automated desorption and transference of analytes from the fiber to the LC system using the SPME interface and, finally, fiber cleaning before extraction of the next sample.

2.4.1. *Fiber conditioning step*

As a previous stage of fiber conditioning is necessary to ensure good selectivity and sensitivity, it was conditioned before first being used according to the supplier's prescriptions. Thus, the fiber was placed into the desorption chamber and was conditioned with the mobile phase (dynamic mode) for 30 min.

2.4.2. *Extraction step*

The SPME procedure was performed by introducing the fiber into 3.00 mL of the water samples adjusted to pH 3.00 with hydrochloride acid, into a 4 mL glass vial sealed with hole-caps and PTFE septa vials. The samples were stirred with a magnetic stirrer at a controlled stirring rate of 1400 rpm before and during extraction. The extractions were performed at room temperature, by direct immersion for an appropriate period of 44 min, without salt or organic solvent.

2.4.3. *Desorption step*

After extraction, the fiber was directly immersed into the chromatographic system by the LC–SPME interface. The fiber was soaked inside the desorption chamber filled with ACN–KH_2PO_4 buffer 0.025 mol L^{-1} at pH 3.00 (50:50, v/v) as solvent for a soaking period of 5 min. Then, the analytes were desorbed and transferred to the analytical column using the mobile phase ACN–KH_2PO_4 buffer (0.025 mol L^{-1} at pH 3.00) (40:60, v/v) at a flow rate of 1 mL min^{-1} (changing the valve of SPME interface to the inject position) for a desorption period of 2 min. Next, the valve was changed to the load position allowing the separation and analysis of analytes by conventional LC analysis.

2.4.4. Fiber cleaning

Before every extraction step, the fiber was kept inside the chamber and was washed (step 1), first with 1 mL of ACN–water (80:20, v/v) in order to avoid any carry-over effect and then with 1 mL of ACN–KH_2PO_4 (0.025 mol L^{-1} at pH 3.00) and 1 mL of Milli-Q water (pH 3.00) before extracting the next water sample.

2.5. Liquid chromatography analysis

The standard solutions and SPME extracts of waste- and surface water were chromatographed by a programmed gradient with ACN as solvent A and 0.025 mol L^{-1} KH_2PO_4 buffer pH 3.00 as solvent B, for 22 min at a flow rate ranged from 1.0 to 1.5 mL min^{-1}. The solvent program was as follows: initially, 8 min isocratic with A:B 40:60 (v/v) at a flow rate of 1.0 mL min^{-1}, then 4 min linear gradient to A:B 50:50 (v/v) at a flow rate of 1.5 mL min^{-1} and 3 min isocratic with A:B 50:50 (v/v) at a flow rate of 1.5 mL min^{-1}, followed by an additional period of 2 min linear gradient to the initial conditions; finally, 5 min in the initial conditions was sufficient time before subsequent analysis runs.

Under the above-described chromatographic conditions, all the analytes were simultaneously analyzed by DAD using a wavelength range between 200 and 350 nm.

3. Results and discussion

3.1. SPME–LC procedure

In a previous work we developed an analytical method for the simultaneous determination of the analytes under study, which involves the pre-concentration and clean-up by SPME using a PDMS–DVB fiber, the main parameters which affect the efficiency of the SPME step (extraction and desorption) being optimized by a multiple response optimization methodology [10]. The best operating conditions were obtained using a stirring time of 44 min at 1400 rpm, a soaking period of 5 min with ACN–KH_2PO_4 0.025 mol L^{-1}, 50:50 (v/v, pH 3.0) and a transferring time of 2 min with mobile phase. After extraction, NSAIDs and CBZ were separated and detected by LC–DAD. Fig. 2 shows chromatograms of a Milli-Q water sample containing 20.0 μg L^{-1} of the pharmaceuticals recorded between 200 and 350 nm obtained by using the SPME–LC–DAD methodology.

The described method showed good performance with recoveries between 71.6% and 122.8%; matrix effect was not present in river water.

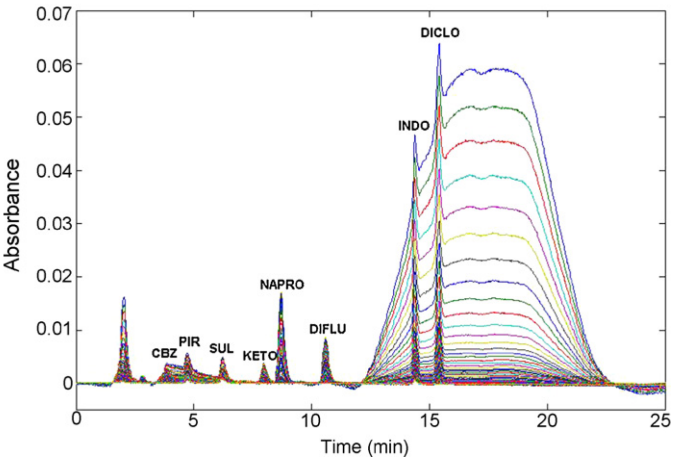

Fig. 2. SPME–LC–DAD chromatograms, each at a single wavelength, of a Milli-Q water sample containing 20.0 μg L^{-1} of each analyte recorded between 200 and 350 nm.

3.2. Calibration transfer for the determination of the analytes in river water

In SPME, standards and samples must be processed in the same way, which implies that every Milli-Q standard sample would spend at least 50 min being processed. An additional drawback when standards undergo SPME would be the increased error in handling, which would lead to the fact that the main source of uncertainty found in the above-mentioned work is that associated with the calibration step [10].

To overcome the above-mentioned problem, in the present work we suggest the application of standardization, a technique that successfully allowed us to prepare only a small number of diluted standards that were subjected to the SPME procedure in such a way that unknown samples, undergoing SPME, were predicted using models built with solvent-based standards.

Several strategies exist for standardization [11]. In this work, we have applied PDS algorithm, which is one of the most widely used transfer methods, showing excellent results when the aim is to make signals, measured by different instruments, look as similar as possible [11].

Concisely, PDS consist of relating the response of a sample measured in "situation A" to its response obtained in another "situation B". If we denote $\mathbf{X}$ as the matrix of a full calibration set built with standards prepared in pure solvents and injected in the LC–DAD system, $\mathbf{X}_A$ the matrix of small subset samples chosen from $\mathbf{X}$ and $\mathbf{X}_B$ the matrix of the same standardization subset built with standards prepared in Milli-Q water and pre-concentrated with SPME, the model transferring $\mathbf{X}_A$ to $\mathbf{X}_B$ is:

$$\mathbf{X}_B = \mathbf{X}_A\mathbf{F} \tag{1}$$

Using the transfer matrix $\mathbf{F}$, the rest of the measurements in situation A (direct measurement of standards prepared in solvent) can be transformed to the situation B (pre-concentration with SPME) by

$$\hat{\mathbf{x}}^T = \mathbf{x}^T\mathbf{F} \tag{2}$$

The signals corresponding to situation A (18 standard calibration samples built in solvent) were corrected according to PDS by using three samples from situation B (three diluted standard samples prepared in Milli-Q water and subjected to the SPME step) and three samples from the situation A (three standard samples prepared in solvent).

Usually, transfer models are established between identical subsets of samples measured under different conditions. However, in this case, to avoid problems related to excessive increases in signals of pre-concentrated samples, which may lie outside linear ranges, and because the concentrations of analytes were increased c.a. five times in the pre-concentration step, we used diluted and standard samples following a 1:5 ratio. Thus, diluted standards of 5.0, 20.0 and 30.0 μg L^{-1} prepared in Milli-Q water and pre-concentrated by SPME (situation B) corresponded to standard samples of 25.0, 100.0 and 150.0 μg L^{-1} prepared in solvent and analyzed by direct injection (situation A). Firstly, for every sample, chromatograms were split into regions corresponding to each analyte elution time. Then, different sizes of window and tolerances were tested. Tolerance values were 0.1, 0.01 and 0.001, while values for window size were odd numbers from 3 to a variable number that corresponded to the width of each chromatogram. The procedure was carried out for every wavelength in each peak and then optimal parameters were used to standardize new samples.

Fig. 3 shows the effect of standardization when analyzing the region corresponding to SUL (peak number 3) in sample 3. The black solid line corresponds to a chromatogram registered at $\lambda = 215$ nm for a concentrated standard solution containing 150.0 μg L^{-1} of SUL.

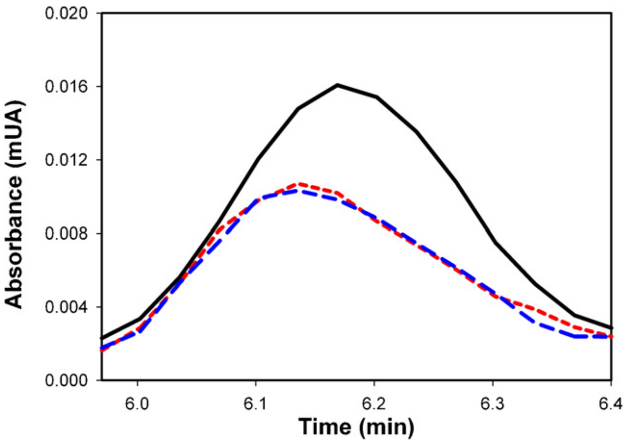

Fig. 3. Chromatograms (λ = 215 nm) restricted to the third peak (SUL) corresponding to a standard solution containing 150.0 μg L^{-1} of SUL (black solid line), a SPME extract from a Milli-Q water sample spiked with 30.0 μg L^{-1} of SUL (red short dashed line) and the former signal after standardization with PDS (blue long dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

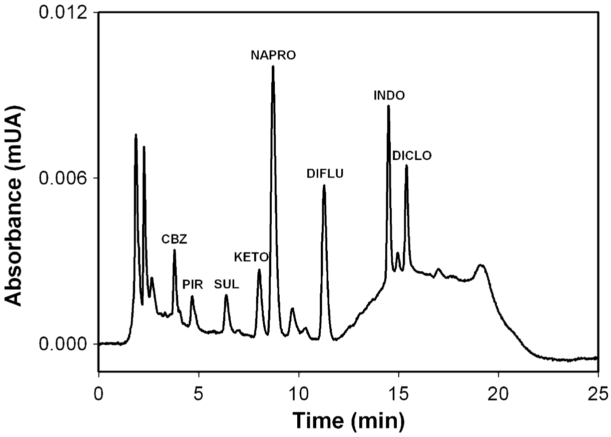

Fig. 4. SPME–LC–DAD chromatogram (registered at λ = 215 nm) of a typical wastewater sample extract obtained from a wastewater sample spiked with 15.0 μg L^{-1} of each analyte.

On the other hand, the red short dashed line corresponds to a SPME extract from a Milli-Q water sample spiked with 30.0 μg L^{-1} of SUL, and the blue long dashed line corresponds to the former signal after standardization with PDS. As can be seen, the strong difference between the concentrated and the pre-concentrated standard signals is evident ($r^2 = 0.867$), including differences in heights and peak retention times. Once the standardization was performed, the corrected signal looks similar to the one corresponding to the pre-concentrated sample ($r^2 = 0.989$), in such a way that chromatographic shifts were even corrected. This procedure was applied to correct the 18 calibration samples and they were used to build a univariate calibration curve as if they were obtained from standards subjected to the pre-concentrations step. Results obtained (shown in Table 1) are comparable to those obtained by using the whole set of standards being subjected to pre-concentration [14], indicating that a considerable amount of time can be saved by using this procedure.

3.3. Second order calibration for the determination of the analytes in wastewaters

3.3.1. Complexity of the matrix sample: application of background correction

Fig. 4 shows a chromatogram (registered at λ = 215 nm) of a SPME wastewater sample extract, obtained after spiking the wastewater sample at 15.0 μg L^{-1} with the seven NSAIDs and CBZ (expected concentration values in these samples) [23]. As can be appreciated in this figure, the high complexity of the analytical problem under study is evident. The names of each compound are indicated in the figure. As can be seen, a large number of compounds are also retained in the SPME step, because their polarity would be similar to the analysts of interest. Owing to the fact that important peak overlapping between analytes and interferents occur, as well as a severe baseline drift, neither identification of the analytes nor application of classical univariate calibration to quantify them is possible as was the case for river water samples [10], although they were conveniently separated by the chromatographic technique.

Elimination of the chromatogram baseline has been shown to be a critical step for reducing the complexity of the matrix sample [14,24]. With this aim, among the different strategies for baseline correction which have been proposed in the literature [25], we chose the methodology proposed by Eilers, i.e. the asymmetric least squares method. The mentioned method is the multidimensional extension of the spline-based approach, a novel algorithm taking advantage of the special structure of both the data as an array and the model matrix as a tensor product [26].

The method consists of obtaining a **B** background correction matrix (dimensions of **B** are the same as those for standard and sample matrices) by using spline basis functions (herein, 10 of them were used), with a single regularization parameter, whose value was 1. Details of the implementation of the algorithm can be found in the literature [26]. Fig. 5A shows the landscape corresponding to a spiked sample (sample number 2 in Table 2) in the time region of 12–25 min. This region was selected to be shown because in it the baseline presents the strongest effect. As can be appreciated in this figure, among the several peaks in the time domain, there are two peaks which correspond to INDO and DICLO. Fig. 5B shows the corresponding background matrix (**B**). A different scale was used in order to show the variation of the baseline at different elution times and wavelengths. Remarkably, the baseline increases with time until a maximum at ca. 20 min is reached, and the same happens with the wavelength (maximum at ca. 260 nm). These facts justify the use of a second-order background correction algorithm. Subtraction of this matrix allowed us to obtain a corrected data matrix. The original chromatogram, its computed baseline and the

Table 1
Predictions for the test samples obtained by spiking different analyte amounts on a real river water sample and using the standardized signals for univariate calibration.

Sample	Added (μg L^{-1})	Found (μg L^{-1})[a]							
		CBZ	PIR	SUL	KETO	NAPRO	DIFLU	INDO	DICLO
1	12	9.1 (75.8)	14.1 (117.5)	10.2 (85.0)	14.8 (123.3)	13.9 (115.8)	13.2 (110.0)	13.8 (115.0)	13.9 (115.8)
2	15	11.6 (77.3)	17.9 (119.3)	11.5 (75.7)	16.2 (108.0)	16.1 (107.3)	11.5 (76.7)	12.9 (85.9)	12.9 (86.0)
3	25	18.0 (72.0)	29.7 (119.0)	21.5 (86.0)	31.2 (124.8)	28.1 (112.4)	24.6 (98.4)	26.3 (105.1)	27.8 (111.2)
4	25	19.6 (78.4)	28.1 (112.3)	24.5 (98.0)	26.9 (107.6)	29.4 (117.6)	25.4 (101.8)	26.3 (105.2)	26.5 (106.0)
5	35	26.1 (74.6)	36.2 (103.4)	31.0 (88.6)	33.8 (96.6)	34.2 (97.6)	26.1 (74.6)	31.0 (88.6)	32.3 (92.3)

[a] Recovery (%) in parentheses.

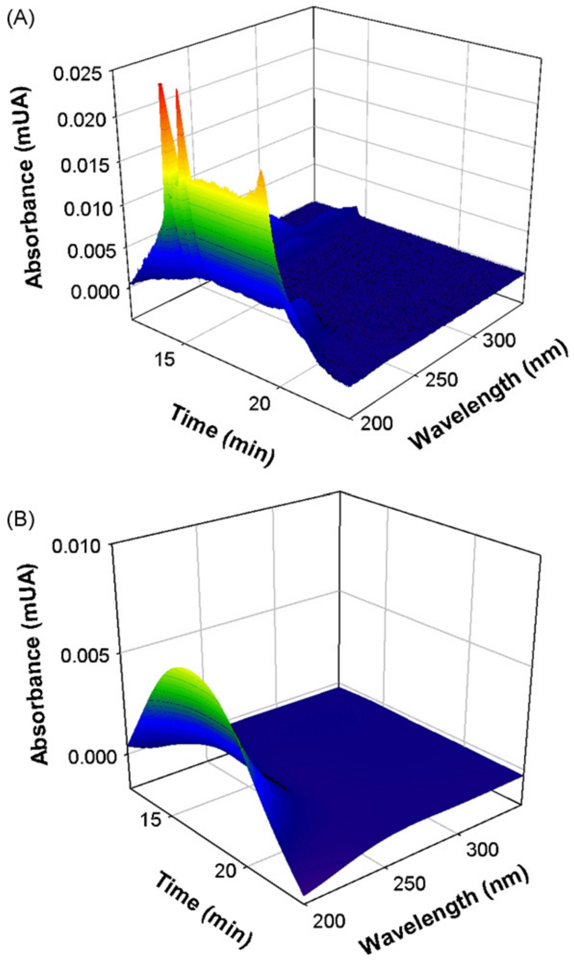

Fig. 5. Landscapes corresponding to: (A) spiked wastewater sample (sample number 2 in Table 2) in the time region of 12–25 min and (B) the corresponding background matrix (**B**).

corrected chromatogram obtained by subtracting the background matrix (**B**), all plotted at 200 nm, are shown in Fig. 6. The simplification of the data complexity is evident.

3.3.2. MCR-ALS modeling: quantitation in spiked samples

The presence of matrix effect was checked by comparing slopes corresponding to univariate calibration curves built for both pure and spiked standard solutions using a hypothesis tests (Statgraphics Plus V 4). Comparison of slopes and intercepts of calibration curves built with Milli-Q and wastewater samples evidenced matrix effect and the presence of systematic constant errors (p-values < 0.1 in both cases) for all the analytes, with the exception of diflunisal.

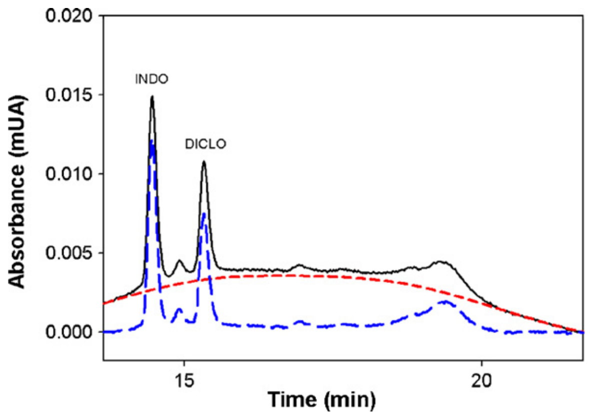

Fig. 6. SPME–LC–DAD original chromatogram (solid black line), its computed baseline (short dashed red line) and the corrected chromatogram obtained by subtracting the background matrix (long dashed blue line), all of them plotted at 200 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Owing to the fact that wastewater samples contained unexpected interferences and sensitivity changes due to sample matrix, a strategy contemplating standard addition calibration using MCR-ALS [20] was implemented considering that this algorithm is able to obtain the second order advantage [27].

As is well known, the MCR-ALS algorithm is capable of dealing with data sets deviating from trilinearity, such as the data analyzed in the present report. Instead of forming a three-dimensional data array, in MCR, the latter is unfolded along the mode which is suspected of breaking the trilinear structure, i.e. if a matrix-to-matrix variation of profiles occurs along the column direction, a column-wise augmented matrix is created. The bilinear decomposition of the augmented matrix **D** is performed according to the expression:

$$\mathbf{D} = \mathbf{C} \times \mathbf{S}^{\mathrm{T}} + \mathbf{E} \qquad (3)$$

where the rows of **D** contain the absorption spectra measured (J wavelengths) as a function of time (K times), the columns of **C** contain the time profiles of the N compounds involved in the process, the columns of **S** their related spectra, and **E** is a matrix of residuals not fitted by the model. Appropriate dimensions of **D**, **C**, $\mathbf{S}^{\mathrm{T}}$ and **E** are thus $(1+I)K \times J$, $(1+\mathrm{I})K \times N$, $N \times J$ and $(1+I)K \times J$, respectively (I = number of training samples). Decomposition of **D** is achieved by iterative least-squares minimization of $||\mathbf{E}||$ under suitable constraining conditions, i.e. nonnegativity in spectral profiles, unimodality and nonnegativity in concentration profiles.

The pure spectra of the compounds should be the same in all experiments, but the profiles in the different **C** sub-matrices need not share a common shape. This is the reason why chromatographic runs can be analyzed together as long as the spectra of the compounds involved in the process remain invariant.

Table 2
Nominal and predicted concentrations[a] (μg L^{-1}) obtained for spiked wastewater samples by using MCR-ALS.

Sample	CBZ	PIR	SUL	KETO	NAPRO	DIFLU	INDO	DICLO	CBZ	PIR
1	10.0/11.7 (117.0)	10.0/11.3 (113.0)	10.0/8.4 (84.0)	10.0/10.8 (108.0)	5.0/5.8 (116.0)	5.0/8.0 (160.0)	5.0/7.5 (150.0)	5.0/4.1 (82.0)	10.0/11.7 (117.0)	10.011.3 (113.0)
2	30.0/36.3 (121.0)	30.0/42.7 (142.3)	30.0/26.9 (89.7)	30.0/30.5 (101.7)	30.0/28.9 (96.3)	30.0/30.5 (101.7)	30.0/28.4 (94.7)	30.0/30.2 (100.7)	30.0/36.3 (121.0)	30.0/42.7 (142.3)
3	20.0/22.7 (113.5)	20.0/25.2 (126.0)	20.0/24.5 (122.5)	50.0/64.7 (129.4)	50.0/65.4 (130.8)	50.0/66.3 (132.6)	50.0/35.7 (71.4)	0.0/0.0 –	20.0/22.7 (113.5)	20.0/25.2 (126.0)
4	50.0/50.4 (100.8)	0.0/0.0 –	50.0/45.8 (91.6)	20.0/28.9 (144.5)	20.0/22.6 (113.0)	20.0/21.7 (108.5)	20.0/23.7 (118.5)	20.0/21.2 (106.0)	50.0/50.4 (100.8)	0.0/0.0 –
5	10.0/14.3 (95.3)	10.0/15.4 (102.7)	20.0/19.2 (128.0)	20.0/19.2 (128.0)	10.0/13.3 (133.0)	10.0/16.7 (111.3)	20.0/21.0 (105.0)	10.0/10.6 (106.0)	15.0/14.3 (95.3)	15.0/15.4 (102.7)

[a] Recovery (%) in parentheses.

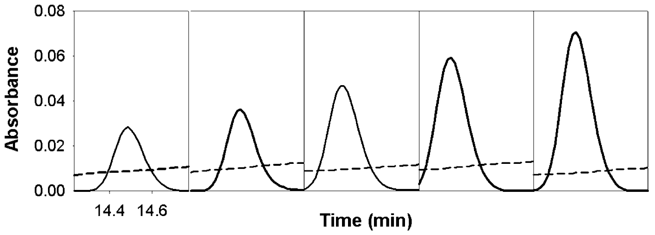

Fig. 7. Time profiles retrieved by MCR-ALS for increasing concentrations of INDO (solid line) and the interference (dashed line) when analysing sample number 2.

The five test samples (sample 1 to sample 5) were spiked with the concentrations corresponding to the eight analytes which are displayed in Table 2 and then, 0.0, 20.0, 30.0, 40.0 and 50.0 μg L^{-1} of each analyte were added to each of the five aliquots of each test sample and used for calibration by the standard addition method. Each of the test samples was analysed in the following way: (i) firstly, in order to simplify the models, the spectral-time matrix for a given test sample was partitioned into eight regions; (ii) then, for each region, the matrix sample was augmented with the four matrices recorded for the spiked calibration samples (all of them containing the eight analytes and partitioned in the same way as the sample matrix).

It is necessary to point out that MCR-ALS requires initialization with system parameters which should not be random numbers. In this case (column-wise augmentation mode), the analyte spectra are required as obtained from either pure analyte standards or from the analysis of the purest spectra based on the so-called SIMPLISMA (simple interactive self-modeling mixture analysis) methodology [28], a multivariate curve resolution algorithm which extracts pure component spectra from a series of spectra of mixtures of varying composition. We have achieved excellent fitting results by using the latter methodology. However, it is important to mention that the generation of suitable initial estimations was only possible when the correct noise level of the data was considered, due to the low signal to noise ratio presents in the analysed samples. In the present case, the noise level was increased from 0.1 up to reaching a consistent selection of spectra (ca. 3%).

Finally, decomposition was performed by imposing the restrictions of nonnegativity in spectral profiles and unimodality and nonnegativity in concentration profiles.

Fig. 7 shows time profiles extracted when analysing analyte number 7 (INDO) in sample number 2. As can be seen, the two extracted profiles present a severe overlapping; however, MCR-ALS is able to correctly decompose the data into the relevant contributions, i.e. signals corresponding to the increasing concentration of analyte related to the standard additions and a constant signal. This allows for isolation of the signal which can be ascribed to every analyte in each studied test sample, and thus used for accurate analyte quantitation. In this case, the use of the relative peak areas for INDO let us build a pseudo-univariate standard addition curve. One interference profile is also observed in Fig. 7. Interestingly, this profile remains constant, showing that it is present in the original sample in a constant way and, therefore, it may be assigned as a constant interfering matrix compound. Another interesting observation that can be made in this figure is the fact that shift peak occurs, indicating lack of trilinearity. On the other hand, Fig. 8 shows the spectral profiles extracted for INDO and the interference. Finally, it should be remarked that the number of estimated components for each peak was: 3, 3, 2, 3, 2, 2, 2 and 2. This fact suggests that interferences were present in all peaks, in a number which depends on the time window selected, and confirms that the choice of a second order strategy was the most convenient option.

Predictions for all the eight analytes in the five samples are displayed in Table 2. A good visualisation of the recoveries computed by using results presented in Table 2 can be made inspecting Fig. 9. In this figure, the plane, corresponding to a 100% of recovery, shows that predictions in most of the samples can be considered acceptable – taking into consideration the complexity of the analytical problem.

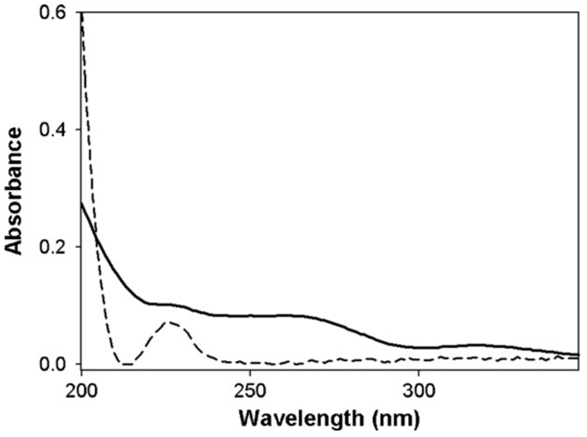

Fig. 8. Spectral profiles retrieved by MCR-ALS for INDO (solid line) and the interference (dashed line) when analysing sample number 2.

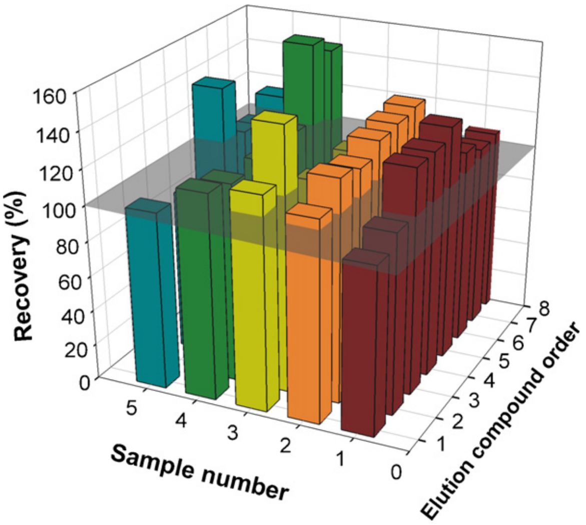

Fig. 9. Bar chart showing the recoveries computed for the eight analytes present in the five validation samples using the results presented in Table 2. The plane corresponds to a 100% recovery.

4. Conclusions

A simple, efficient, selective and low-cost methodology for the determination of seven non-steroidal anti-inflammatories and the anticonvulsant carbamazepine in river water and wastewater samples can be achieved by using solid-phase microextraction and liquid chromatography coupled to diode array detection.

In the case of river water, piecewise direct standardization became a useful tool in reducing the number of standard samples which must undergo SPME for calibration, thus allowing quantifying pre-concentrated river water samples with a considerable reduction in standards handling and time.

For wastewater samples, a baseline correction step can be implemented when signal enhancement is required. Thus, it is possible to reduce both the large baseline drift and additive interferences which are present at the retention times of the analytes. Additionally, second-order data, generated by recording spectra during the chromatographic time evolution, can be successfully handled to assess the content of the analytes, even in the presence of several interferences. This can be done using a strategy which includes data pre-treatment for background correction, and data modeling with the MCR-ALS algorithm in combination with the standard addition calibration mode.

Acknowledgments

The authors are grateful to MINISTERIO DE CIENCIA Y TECNOLOGÍA (Proyecto CTQ2007-65954) for financial support. H.C.G. thanks CONICET (PIP 112-200801-02988), ANPCyT (PICTO 559 05 N° 35124) and Universidad Nacional del Litoral. M.J.C., G.G.S. and L.V-C. thank CONICET for their fellowship.

References

[1] A. Gentili, Anal. Bioanal. Chem. 387 (2007) 1185.
[2] Y. Zhang, S.U. Geiβen, C. Gal, Chemosphere 73 (2008) 1151.
[3] K. Stamatelatou, C. Frouda, M.S. Fountoulakis, P. Drilla, M. Kornaros, G. Lyberatos, Water Sci. Technol.: Water Supply 3 (2003) 131.
[4] A. Joss, S. Zabaczynski, A. Göbel, B. Hoffmann, D. Löffler, C.S. MacArdell, T.A. Ternes, A. Thomsen, H. Siegrist, Water Res. 40 (2006) 1686.
[5] T.A. Ternes, N. Herrmann, M. Bonerz, T. Knacker, H. Siegrist, A. Joss, Water Res. 38 (2004) 4075.
[6] M. Farré, M. Petrovic, D. Barceló, Anal. Bioanal. Chem. 387 (2007) 1203.
[7] J.B. Quintana, T. Reemtsma, Rapid Commun. Mass Spectrom. 18 (2004) 765.
[8] S. Weigel, R. Kallenborn, H. Hühnerfuss, J. Chromatogr. A 1023 (2004) 183.
[9] M. La Farré, I. Ferrer, A. Ginebrada, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova, D. Barceló, J. Chromatogr. A 938 (2001) 187.
[10] L. Vera-Candioti, M.D. Gil García, M. Martínez Galera, H.C. Goicoechea, J. Chromatogr. A 1211 (2008) 22.
[11] R.N. Feudale, N.A. Woody, H. Tan, A.J. Myles, S.D. Brown, J. Ferré, Chemom. Intell. Lab. Syst. 64 (2002) 181.
[12] A. Garrido Frenich, D. Picón Zamora, J.L. Martínez Vida, M. Martínez Galera, Anal. Chim. Acta 477 (2003) 211.
[13] R. Santiago Valverde, M.D. Gil García, M. Martínez Galera, H.C. Goicoechea, Anal. Chim. Acta 562 (2006) 85.
[14] M.D. Gil García, M.J. Culzoni, M.M. De Zan, R. Santiago Valverde, M. Martínez Galera, H.C. Goicoechea, J. Chromatogr. A 1179 (2008) 115.
[15] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Part A, Elsevier, Amsterdam, 1997, pp. 190, 209, 436.
[16] T. Reemtsma, TrAC Trends Anal. Chem. 20 (2001) 533.
[17] B.K. Choi, D.M. Hercules, A.I. Gusev, Fresenius J. Anal. Chem. 368 (2001) 370.
[18] A. Kloepfer, J.B. Quintana, T. Reemtsma, J. Chromatogr. A 1067 (2005) 153.
[19] K.S. Booksh, B.R. Kowalski, Anal. Chem. 66 (1994) 782A.
[20] E. Peré-Trepart, S. Lacorte, R. Tauler, Anal. Chim. Acta 595 (2007) 228.
[21] R. Tauler, Chemom. Intell. Lab. Syst 30 (1995) 133.
[22] P.H.C. Eilers, I.D. Currie, M. Durbán, Comp. Stat. Data Anal. 50 (2006) 61.
[23] M. Klavarioti, D. Mantzavinos, D. Kassinos, Environ. Int. 35 (2009) 402.
[24] L. Vera Candioti, M.J. Culzoni, A.C. Olivieri, H.C. Goicoechea, Electrophoresis 29 (2008) 4527.
[25] M. Daszykowski, B. Walczak, TrAC Trends Anal. Chem. 25 (2006) 1081.
[26] P.H.C. Eilers, I.D. Currie, M. Durban, Comp. Stat. Data Anal. 50 (2006) 61.
[27] G.M. Escandar, N.M. Faber, H.C. Goicoechea, A. Muñoz de la Peña, A.C. Olivieri, R.J. Poppi, TrAC Trends Anal. Chem. 26 (2007) 752.
[28] W. Windig, J. Guilment, Anal. Chem. 63 (1991) 1425.