Journal of Chromatography A, 1218 (2011) 6240–6247

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

In situ aqueous derivatization and determination of non-steroidal anti-inflammatory drugs by salting-out-assisted liquid–liquid extraction and gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 13 April 2011
Received in revised form 28 June 2011
Accepted 30 June 2011
Available online 18 July 2011

Keywords:
In situ derivatization
NSAIDs
SALLE

ABSTRACT

A new analytical method for the determination of trace levels of five non-steroidal anti-inflammatory drugs (NSAIDs: clofibric acid, ibuprofen, naproxen, diclofenac and ketoprofen) in water samples is described. The analytical procedure involves *in situ* aqueous derivatization with N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and 2,2,2-trifluoroethylamine hydrochloride (TFEA) and salting-out liquid–liquid extraction (SALLE), followed by gas chromatography–programmed temperature vaporizer–mass spectrometry (GC–PTV–MS). The influence of several parameters on the efficiency of the derivatization (stirring time, reaction time, reagent concentration and pH), and the extraction (solvent, volume, salts and stirring time) and injection steps (liner, injection volume, liner temperature, injection time, venting time and venting flow) was investigated. The detection limits of the method in water varied from 0.042 μg/L for ibuprofen to 1.2 μg/L for ketoprofen. The relative standard deviations (RSD) values were found to be relatively low (<10% for all compounds). The methodology developed was applied to the determination of NSAIDs in several environmental matrices including tap, river, sea and influent and effluent waste water samples. The results obtained show the presence of ibuprofen and naproxen in the influent waste water sample.

1. Introduction

Recent years have seen increased interest in the study of emerging pollutants in different environmental matrices. These substances are considered to be pseudo-persistent compounds, since they continuously enter the environment, although at low concentrations, and they are able to elicit chronic effects in aquatic and terrestrial organisms. Within this group of compounds are pharmaceuticals, since their use is very broad in both animals and humans. Pharmaceutical residues have been detected in different environmental matrices, among them waste waters, river waters, groundwaters and sea waters, sediments and sewage sludge [1–5]. Among pharmaceuticals, one group of particular interest are non-steroid anti-inflammatory compounds (NSAIDs). The importance of this class of substances lies in their physico-chemical properties: high water solubility, low pK_a values, low adsorption coefficients and, often, their persistence.

In recent years, liquid chromatography coupled to mass spectrometry detection (MS) has proved to be the technique of choice for the determination of these compounds [1,2,4–12]. Nevertheless, this technique has certain drawbacks since the signal may be suppressed by the matrix; moreover, the libraries of LC–MS are less complete than those available for GC–MS. Accordingly, gas chromatography continues to be one of the most widely used techniques owing to its separation potential and availability [13–22]. Interlaboratory exercises on NSAIDs in environmental samples have been performed using both techniques [23,24].

Since NSAIDs are polar carboxylic acids with a low vapour pressure, it is necessary to carry out a derivatization prior to their analysis with this technique. There are two routes for derivatization of acids present in aqueous media. First, extraction of the polar acids into an organic solvent immiscible with water, which in most cases involves several later steps of extraction to another organic medium and extract clean-up. Second, to take advantage of an *in situ* approach, using derivatizating agents soluble in water in order to produce non-polar derivatives which can be better extracted into an organic solvent.

One of the derivatization reactions initially used following the first approach transforms the carboxylic acids into alkyl

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doi:10.1016/j.chroma.2011.06.112

Table 1
Acid/base characteristics and octanol/water distribution coefficients of the NSAIDs and their derivatives.

Compound	pK_a[a]	log *Kow*[b]
Clofibric acid	3.61	2.59
Clofibric acid amide		3.37
Ibuprofen	4.53	3.49
Ibuprofen amide		4.25
Naproxen	4.50	3.62
Naproxen amide		3.56
Diclofenac	4.12	4.33
Diclofenac amide		4.98
Ketoprofen	4.35	3.24
Ketoprofen amide		3.95

[a] http://archemcalc.com/sparc/.
[b] http://www.vcclab.org/lab/alogps/start.html.

esters using diazomethane [13]. The yield of this reaction is high, although the problem is that the reagent is highly toxic and polluting, and its stability is low (it is highly explosive), such that alternatives such as methyl chloromethanoate [15,16] and pentafluorobenzyl bromide (PFBBr) [3,25] have been proposed. Currently, the derivatizing agents most widely used when a previous extraction into on organic medium is used are those containing alkylsilyl groups, such as N-methyl-N(tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) [3,7,17,18,26], N-methyl-N(trimethylsilyl)trifluoroacetamide (MSTFA) [19,21] and bis(trimethylsilyl)trifluoroacetamide (BSTFA) [26,27]. The second *in situ* aqueous route has been performed with different derivatizing agents: methanol in acid medium [28]; tetra butyl ammonium (TBA) [29,30] and a water soluble carbodiimide (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, EDC) and 2,2,2-trifluoroethylamine (TFEA) to produce an amide [31,32].

NSAIDs are present in small amounts in the environment such that it is necessary to carry out their preconcentration before determination. The technique most widely used is solid-phase extraction (SPE) [1,3,6–10,13,15–17,19,21]. Lately, the trend has been to reduce the consumption of organic solvents owing to their toxicity. Some of the techniques able to reduce or avoid the use of organic solvents are solid-phase microextraction (SPME) [18,28,30], stir-bar sorptive extraction (SBSE) [2,20] and hollow fibre liquid-phase microextraction (HF-LPME) [30].

Salting-out liquid–liquid extraction (SALLE) is a technique based on liquid–liquid extraction in which an appropriate concentration of salt is added to achieve the separation of the aqueous phase from the partially miscible organic phase. Some of the solvents used in SALLE are acetonitrile, acetone, ethyl acetate and isopropanol. This extraction technique has been successfully used for the extraction of hydrophobic compounds, drugs and metals. Also, it is highly compatible with different analytical techniques, such as: gas chromatography, HPLC and capillary electrophoresis. It has been used above all in biological [33–35] and environmental water samples [36,37]. A variant of the technique, used mainly in the extraction of pesticides from food matrices is the so-called QuEChERS (quick, easy, cheap, effective, rugged and safe) method, which adds a step of dispersive SPE clean-up after the partitioning of the organic phase and aqueous phase in the presence of salts [38–42].

Here we propose the use of salting-out liquid–liquid extraction for the extraction of five NSAIDs: clofibric acid, ibuprofen, naproxen, diclofenac and ketoprofen previously derivatized in aqueous medium. We performed the derivatization reaction with TFEA and EDC. Although these reagents had previously been used for derivatizing ibuprofen, this is the first time that the reaction is applied to the other studied NSAIDs. To increase the sensitivity of the determination, we used a programmed temperature vaporizer (PTV) coupled to a GC–MS for the injection of large volumes of sample in the solvent-vent injection mode.

2. Experimental

2.1. Reagents

The pharmaceuticals studied (clofibric acid, ibuprofen, naproxen, diclofenac and ketoprofen) and the derivatization reagents (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and 2,2,2-trifluoroethylamine hydrochloride (TFEA) and ethylamine hydrochloride (EA)) were from Sigma–Aldrich (Steinheim, Germany). The solvents used were acetonitrile (Merck, Darmstadt, Germany), acetone, and ethyl acetate (Sigma–Aldrich). The salts – magnesium sulphate and sodium chloride – and dihydrated disodium hydrogen phosphate and 84–86% orthophosphoric acid were from Scharlau (Barcelona, Spain). Ultrapure water was obtained using a Wasserlab water purification system (Noain, Spain).

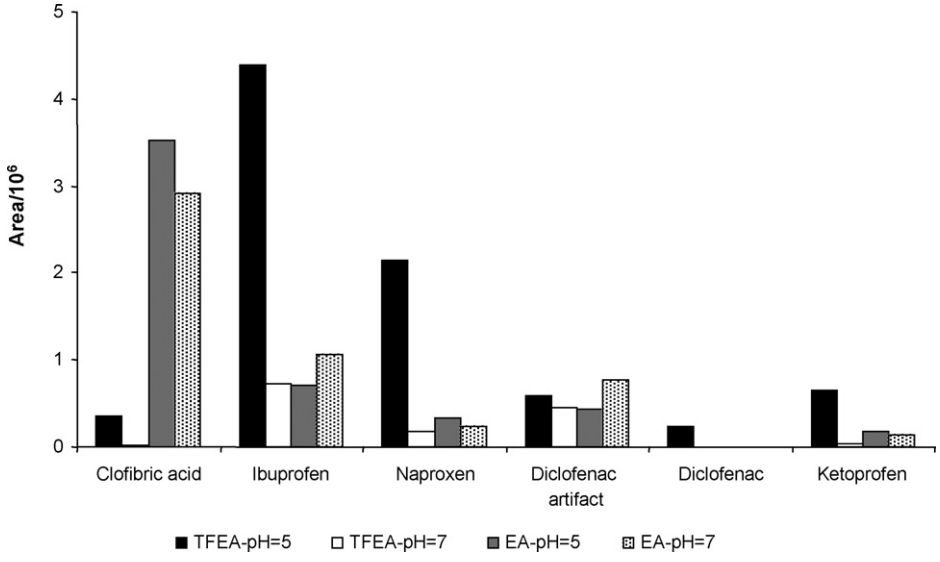

Fig. 1. Comparison of trifluoroethylamine (TFEA) and ethylamine (EA) as derivatizing agents.

2.2. Working solutions

Stock solutions of 1000 mg/L were prepared in acetonitrile, with the exception of diclofenac, which, because it is not soluble in acetonitrile at that concentration, was prepared in ultrapure water. These were kept in a refrigerator at 4 °C and diluted to the desired concentration with ultrapure water. These solutions were used to optimize the method and spike the aqueous samples at different concentrations.

2.3. Samples

The aqueous samples analyzed were collected during August and September 2010. The samples included tap water from the city of Salamanca, water from the river Tormes taken in the city of Salamanca, influent and effluent water taken at the sewage treatment plant in Salamanca and sea water collected from La Manga del Mar Menor, in Murcia (SE Spain). The samples were kept under refrigeration at 4 °C until analysis. All the samples were subjected to analysis without previous filtration.

2.4. Equipment

Chromatographic determination of the derivatized pharmaceuticals was performed on a 7890A Agilent Technologies gas chromatograph (Santa Clara, CA, USA) equipped with an Agilent Technologies 7863 automatic injection system and a Agilent Technologies 6890 PTV injector coupled to an Agilent Technologies 5975C inert XL quadrupole mass spectrometry detector. For separation of the compounds, an HP-5MS (30 m × 0.25 mm, 0.25 μm) (J&W Scientific, Folsom, CA, USA) column was employed. The carrier gas was N50 helium (purity 99.995%, Air Liquide). The oven temperature program was as follows: it started at 50 °C for 3 min, was then raised to 70 °C with a temperature ramp of 120 °C/min, then increasing temperature at 70 °C/min up to 200 °C and then up to 300 °C with a temperature ramp of 45 °C/min, holding this constant for 1 min. Three liners – of 71 mm × 2 mm – (Gerstel CIS-4, Germany) were used in the PTV injector: one baffled empty, another packed with silanized glass wool, and the other with Tenax-TA. The liner selected after optimizing the method was the one packed with glass wool in the solvent-vent injection mode, for which the temperature program consisted in fixing the initial temperature at 70 °C for 0.55 min, thereafter increasing it, once the split valve had been closed, by 12 °C/s up to 300 °C, holding this temperature for 5 min. Cooling was performed with CO_2. The venting flow was adjusted to 50.0 mL/min for 0.5 min. The injection time was set at 2.5 min.

2.5. Mass spectrometry

The mass spectrometer operated in electron ionization mode (EI: 70 eV); the temperature of the transfer line was kept at 280 °C; the temperature of the ionization source at 230 °C, and the quadrupole at 150 °C. Injection was recorded in full-scan mode (in the 50–400 amu range) and SIM, selecting the characteristic ions in each case, with a dwell time of 10 ms. Six groups were used. The first (5.50–5.80 min) contained the most abundant ions of clofibric acid amide (**128**, 168, 295). The second group (5.81–6.94 min) contained the most abundant ions of ibuprofen amide (**161**, 119, 287). In the third group (6.95–7.14 min), the *m*/*z* characteristic of naproxen amide were recorded (**185**, 170, 311). The fourth group (7.15–7.26 min) contained the most abundant ions of diclofenac artifact (**214**, 242, 277). In the fifth group (7.27–7.50 min), the *m*/*z* characteristic of ketoprofen amide was recorded (**105**, 210, 35). Finally, the sixth group (7.51–8.25 min) contained the most abundant ions of diclofenac amide (**214**, 242, 376). Base peaks (in bold) in the spectra of the derivatives were used as quantitation ions; molecular peaks of the derivatives (clofibric acid (295), ibuprofen (287), naproxen (311), ketoprofen (335) and diclofenac (376)) were used as qualifier ions. Data acquisition was performed with an MSD ChemStation, Ver. E.02.00.493 software from Agilent Technologies. The compounds were identified by comparison with the mass spectrum generated by a standard solution of the derivatized analytes and (for the diclofenac artifact) with that of the NIST_98 database (NIST/EPA/NIH Mass Spectral Library, version 2.0).

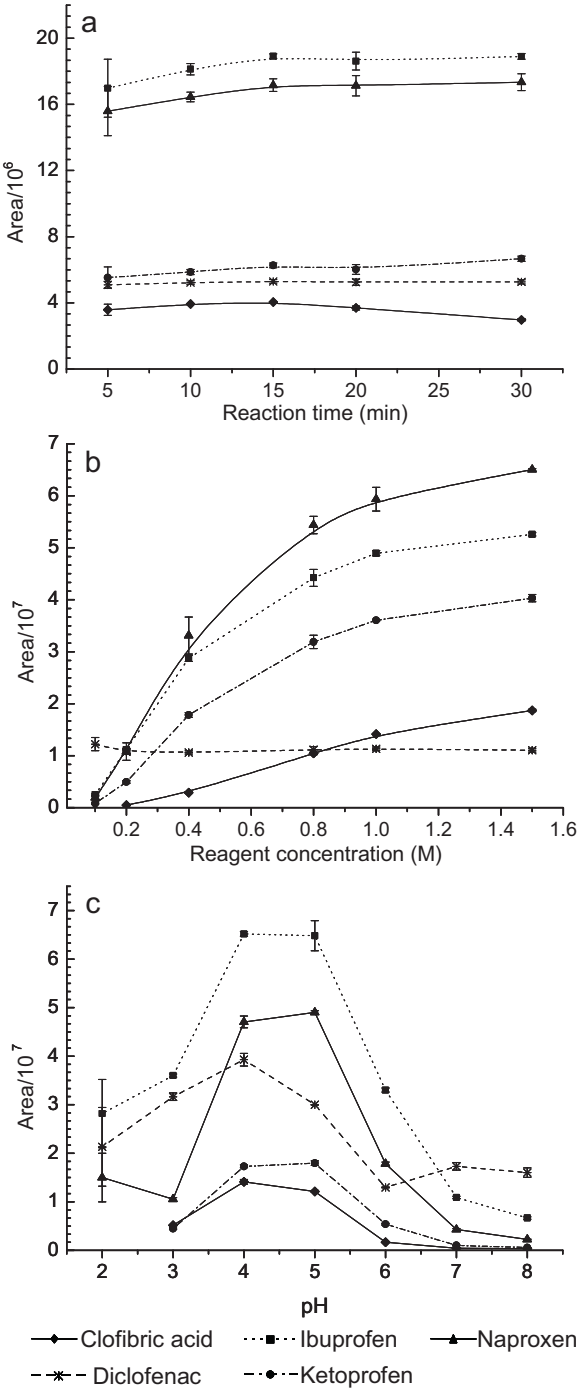

Fig. 2. Influence of variables affecting the derivatization reaction ($n = 4$) (a) reaction time, (b) reagent concentration and (c) pH.

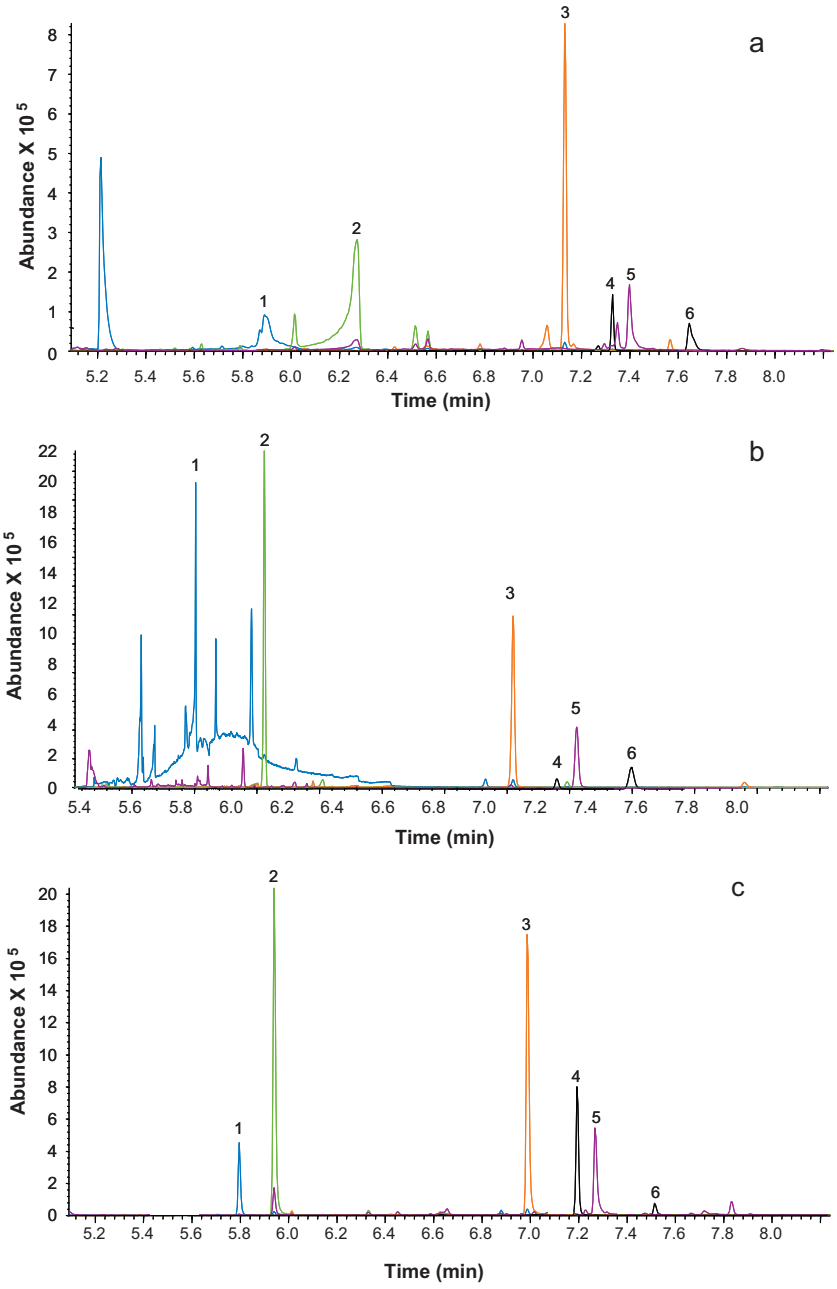

Fig. 3. Chromatograms of the NSAIDs derivatized and extracted with (a) acetone, (b) acetonitrile and (c) ethyl acetate. (1) Clofibric acid derivative, (2) ibuprofen derivative, (3) naproxen derivative, (4) diclofenac artifact, (5) ketoprofen derivative and (6) diclofenac derivative.

2.6. Derivatization and extraction procedure

The following were placed in a pyrex tube in this order: 0.25 mL of EDC, 0.25 mL of TFEA, 0.25 mL of phosphate medium at pH = 5.0 and 2.5 mL of aqueous sample. Once in the tube, the mixture was vortexed for 1 min at 2000 rpm. It was then left to stand during 15 min for the derivatization reaction to be completed; the organic solvent was added (1.5 mL of ethyl acetate) to extract the derivatized compounds, and the mixture was then vortexed again for one more minute at 2000 rpm. Following this, 0.40 g of NaCl was added for a better separation of the organic phase and vortexed again for 1 min at 2000 rpm. Finally, it was centrifuged at 2500 rpm for 5 min to accelerate phase separation and a portion of the organic extract was injected into the gas chromatograph. The analyte concentrations used in the optimization of these conditions were 100 μg/L for clofibric acid; 50 μg/L for ibuprofen and naproxen; 250 μg/L for diclofenac and 200 μg/L for ketoprofen.

2.7. Validation of the method

All the NSAIDs tested showed good linearity in the ranges studied, with good regression coefficients. The limits of detection obtained in SIM mode ranged between 0.042 and 1.22 μg/L. The limits of quantification were within the 0.18–4.1 μg/L range. Reproducibility and repeatability, expressed as coefficients of variation, had satisfactory values (<10%).

3. Results and discussion

3.1. Variables affecting the derivatization reaction

Initially, the variables affecting the derivatization of the analytes were optimized. In all cases, the derivatized compounds were extracted using SALLE with ethyl acetate and the salts sodium

chloride and magnesium sulphate prior to injection into the gas chromatograph.

Table 1 shows the pK_a values of the target analytes, together with the octanol/water distribution coefficients (given as log *K*ow) calculated for them and for their derivatives. *In situ* derivatization results in an enhancement in distribution coefficients as compared to the free acids. Moreover, the acidic nature of NSAIDs makes very difficult to extract them from water in their native form because pH should be quite low. On contrast, *in situ* acylation generally requires milder pH conditions, that are easier to achieve.

As a preliminary test, we compared the signals of the amides corresponding to 2,2,2-trifluoroethylamine (TFEA) and ethylamine (EA), both water-soluble at pH = 5 and pH = 7. The results obtained are shown in Fig. 1. For most of the compounds higher signals were obtained with TFEA. However, in the case of clofibric acid, the opposite was the case, to the extent that no signal from the derivative with TFEA at pH = 7 was obtained at the concentration tested. Diclofenac, which contains an amine group in its molecule as well as the carboxyl, showed a special type of behaviour, described elsewhere [43], upon derivatizing it with pentafluorbenzyl bromide. In that reaction, as well as the ester it was also detected an artifact of diclofenac – 1-(2,6-dichlorophenyl)indolin-2-one – as the result of intramolecular condensation of the amine and carboxyl groups,

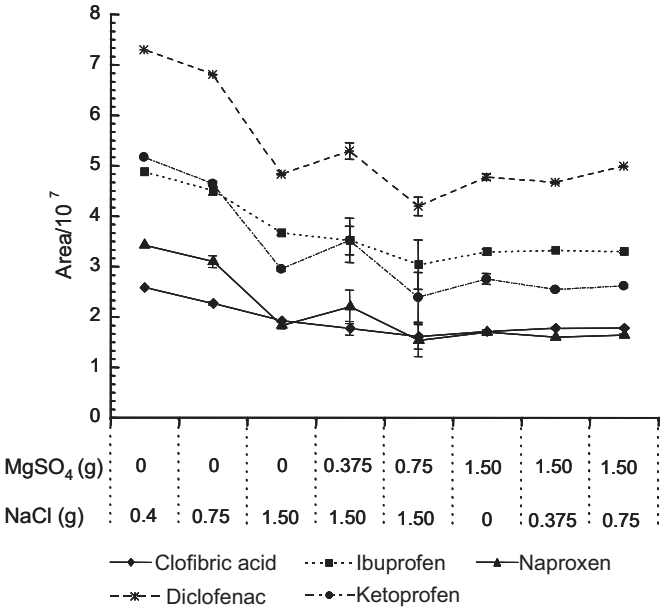

Fig. 4. Influence of the amount of salts used in the extraction ($n = 4$).

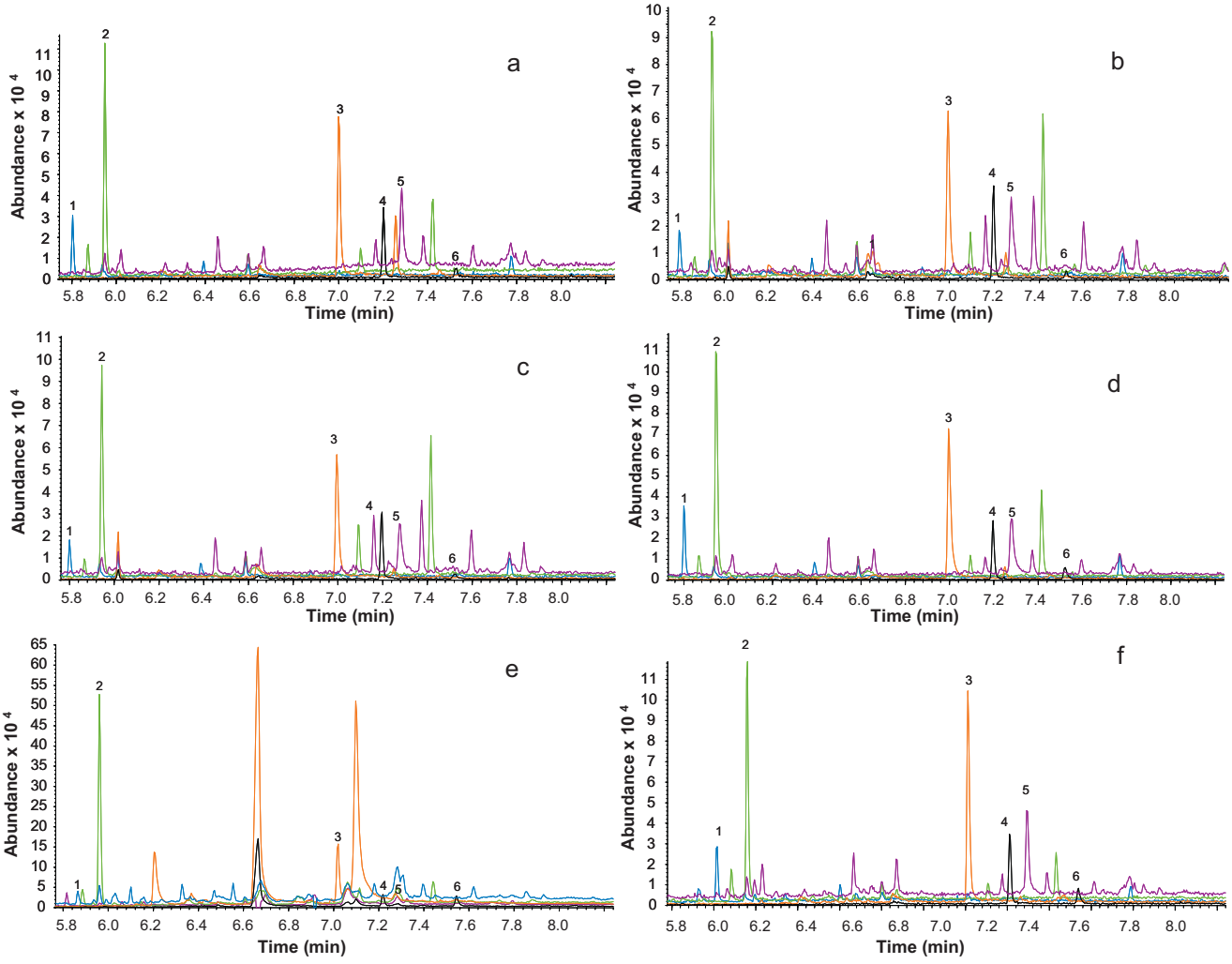

Fig. 5. Chromatograms obtained for the different types of aqueous matrices studied, spiked with 5 μg/L of the analytes, except for ibuprofen and naproxen in the influent sewage water. (a) Ultrapure water, (b) tap water, (c) river water, (d) sea water, (e) influent sewage water and (f) effluent sewage water. (1) Clofibric acid derivative, (2) ibuprofen derivative, (3) naproxen derivative, (4) diclofenac artifact, (5) ketoprofen derivative and (6) diclofenac derivative.

Table 2
Analytical characteristics of the method.

NSAID	Linear range (μg/L)	r^2	LOD (μg/L)	LOQ (μg/L)	5 μg/L		100 μg/L	
					Repeatability (RSD%)	Reproducibility (RSD%)	Repeatability (RSD%)	Reproducibility (RSD%)
Clofibric acid	0.08–1000	0.9988	0.08	0.28	4.5	5.8	3.7	4.0
Ibuprofen	0.04–500	0.9999	0.04	0.14	4.2	7.7	4.0	5.0
Naproxen	0.05–500	0.9997	0.05	0.18	3.3	9.9	4.1	5.0
Diclofenac	0.08–1000	0.9979	0.08	0.27	3.8	9.8	4.1	5.1
Ketoprofen	1.2–1000	0.9994	1.22	4.09	5.4	9.2	4.7	5.6

which prevented its complete derivatization. The mass spectrum of the artifact can be also found in the NIST database.

For the reaction studied here, involving the formation of an amide using a carbodiimide as a condensation agent, we only observed the amide derived from the diclofenac with TFEA at pH = 5, whereas the peak corresponding to the diclofenac artifact was obtained in all the cases compared. Since it was observed that for diclofenac the degree of derivatization could vary, depending on the matrix, it was decided to adopt the criterion proposed by Reddersen and Herberer [43] and obtain its corresponding analytical signal as the sum of those of derivatized diclofenac and the diclofenac artifact.

TFEA was chosen as the most suitable derivatizing agent for the joint determination of the five pharmaceuticals and the following variables affecting the derivatization reaction were studied: stirring time, total reaction time, concentration of the derivatization agents, and the pH at which derivatization took place.

A study was made of the different times of initial stirring from the reagents and analyte mix: 1 min, 2 min, and 5 min, then allowing a reaction time of 15 min. The time of stirring was not seen to affect the reaction, such that it was decided to implement stirring over 1 min. Following this, we assayed different reactions times between 5 and 30 min (Fig. 2a) and an optimum time of 15 min was maintained, since up to that time a slight increase in the signals occurred.

The influence of the concentration of the derivatizing agents (EDC and TFEA) is shown in Fig. 2b. A working concentration of 0.8 M was chosen, since although at higher values the analytical signal continued to increase, it did so much less markedly. Furthermore, an excessive use of reagents was avoided.

Finally, using phosphate medium, we performed a study of the effect of pH in a range between 2 and 8. Fig. 2c shows the results obtained; it may be seen that the maximum signal was obtained at pH = 5 for nearly all the pharmaceuticals, such that this value was chosen for the derivatization. We also studied the possibility of fixing the pH with acetic–acetate buffer or hydrogenoxalate–oxalate buffer at pH = 5, but it was observed that this degraded the shape of some of the analyte peaks, and we decided to use phosphate medium buffer.

3.2. Optimization of salting-out assisted extraction

In this part of the study, the variables studied were the type of solvent, the extraction volume, the type and amount of salts, and the stirring time.

As well as ethyl acetate we also assayed acetonitrile and acetone. It was found that none of them was a suitable alternative for analyte separation, since no good chromatographic signals were obtained (Fig. 3). Different volumes of ethyl acetate (between 500 μL and 2500 μL) were then tested, observing that when the extraction volume decreased a lot the packing of the liner degraded very fast, which can be attributed to the increase in concentration not only of the compounds but also of the salts used in the extraction. Accordingly, an extraction volume of 1500 μL was chosen, with which it

Table 3
Results of the assays to check the accuracy of the proposed method for NSAIDs in spiked environmental water samples.

Analyte	Sample	Spiked (μg/L)	Found[a] (μg/L)	% Recovery[b]
Clofibric acid	Tap water	5	6 ± 1	120
		25	26 ± 1	104
	River water	5	6 ± 1	120
		25	27 ± 1	108
	Sea water	5	6 ± 1	120
		25	31 ± 1	124
	Sewage influent water	5	6 ± 1	120
		25	29 ± 1	116
	Sewage effluent water	5	6 ± 1	120
		25	27 ± 3	108
Ibuprofen	Tap water	5	6 ± 1	120
		25	26 ± 1	104
	River water	5	6 ± 1	120
		25	26 ± 1	104
	Sea water	5	6 ± 1	120
		25	28 ± 1	112
	Sewage influent water	5	c	
		25	c	
	Sewage effluent water	5	6 ± 1	120
		25	26 ± 3	104
Naproxen	Tap water	5	6 ± 1	120
		25	25 ± 1	100
	River water	5	6 ± 1	120
		25	28 ± 1	112
	Sea water	5	6 ± 1	120
		25	28 ± 1	112
	Sewage influent water	5	d	
		25	d	
	Sewage effluent water	5	6 ± 1	120
		25	27 ± 1	108
Diclofenac	Tap water	5	6 ± 1	120
		25	26 ± 2	104
	River water	5	6 ± 1	120
		25	26 ± 1	104
	Sea water	5	4 ± 1	80
		25	20 ± 1	80
	Sewage influent water	5	9 ± 1	180
		25	32 ± 1	128
	Sewage effluent water	5	7 ± 1	140
		25	23 ± 1	92
Ketoprofen	Tap water	5	4 ± 1	80
		25	23 ± 1	92
	River water	5	5 ± 1	100
		25	27 ± 1	108
	Sea water	5	5 ± 1	100
		25	28 ± 1	112
	Sewage influent water	5	5 ± 1	100
		25	35 ± 1	140
	Sewage effluent water	5	4 ± 1	80
		25	24 ± 2	96

c, ibuprofen concentration = 21 ± 1 μg/L.
d, naproxen concentration = 5 ± 1 μg/L.
[a] Average value ± standard deviation of three determinations.
[b] "%Recovery" refers to the NSAIDs concentrations determined rather than the actual percent of analytes extracted by SALLE analysis.

Table 4
Results obtained for the NSAIDs in influent waste water with standard additions calibration.

Sample	Level (μg/L)	Clofibric acid	Ibuprofen	Naproxen	Diclofenac	Ketoprofen
Sewage influent water	5	6 ± 1	a	b	7 ± 2	5 ± 2
	25	25 ± 4	a	b	26 ± 3	28 ± 3

a, ibuprofen concentration 20 ± 1 μg/L.
b, naproxen concentration 4 ± 1 μg/L.

was possible to perform up to 450 injections with no degradation of the liner.

The amounts of salts ($MgSO_4$ and NaCl) and the corresponding signals obtained after extraction are shown in Fig. 4. The largest areas were obtained with 0.4 g of NaCl, such that this value was selected. Additionally, on choosing this amount the degradation of the liner was delayed.

Finally, a stirring time of 1 min was chosen because no significant increase in the chromatographic signal occurred upon increasing the time; even in the case of clofibric acid its signal decreased slightly with the increase in stirring time.

3.3. Optimization of the injection of large volumes of sample in solvent-vent mode

Study of the type of liner and the venting temperature was carried out jointly, testing different temperatures, from 10 °C to 150 °C and three types of liner: an empty baffled liner, a glass wool liner and a Tenax-TA liner. It was observed that in the case of the empty liner the most suitable temperature was 40 °C, since as from this value the signal of the more volatile analytes – clofibric acid and ibuprofen – decreased. The same kind of behaviour was observed for the liner filled with glass wool, but in this case as from a temperature of 70 °C.

In the case of the liner packed with Tenax-TA, the effect of temperature was less marked, with almost stable signals between 30 °C and 150 °C. However, no signal was obtained for the diclofenac artifact, which may have been due to an excessive retention by the Tenax packing. Regarding the injection volume, the optimum value for both packed liners was found to be 25 μL, since as from this volume the peaks split up, especially in the case of ibuprofen and naproxen. In contrast, in the case of the empty liner this occurred at volumes greater than 10 μL.

Upon comparing the signal-to-noise ratios corresponding to each of the liners under their optimum conditions of initial temperature and injection volume, it was concluded that the most suitable one was the glass wool packing, since this provided the best signal-to-noise ratios.

The injection time, venting time and venting flow conditions were also studied, obtaining the values selected in Section 2.

3.4. Validation of the method

Aqueous analyte solutions at different concentrations (0–2000 μg/L) were prepared and subjected to the derivatization and optimized extraction process and injected in triplicate in order to obtain the calibration curves for each of the compounds studied. Table 2 shows the linear ranges, the limits of detection and quantification, and the values of repeatability and reproducibility obtained for the SIM detection mode.

The linear range of the compounds studied was broad. The validity of the models was studied with ANOVA and it was observed that there was no lack of fit. The correlation coefficients were satisfactory for all the drugs studied, with values in the 0.998–0.9999 range.

The instrumental limits of detection were studied as 3 times the standard deviation of the signal of the blank ($n = 8$) divided by the slope of the calibration line and it ranged between 0.042 and 1.2 μg/L. The instrumental limits of quantification were calculated as 10 times the standard deviation of the blank ($n = 8$) divided by the slope of the calibration line. Those limits of quantification were between 0.18 and 4.1 μg/L.

Repeatability was studied by performing extractions at two concentration levels – 5 and 100 μg/L – and injecting 10 aliquots of each of them on the same day. The relative standard deviation of the compounds analyzed was less than 4.5%, indicating good precision. To determine reproducibility, each of the standards were derivatized, extracted and injected on six different days. In all cases, values below 10% were obtained, indicating the good reproducibility of the method.

3.5. Application to environmental water samples

To check the prediction capacity of the model, we analyzed five different water samples: tap water, river water, sea water and influent and effluent waste water of the same sewage treatment plant. Considering the limits of detection of the proposed method it would be mainly adequate for application to waste waters, where high concentrations of the analytes have been found [13,23,24]. However, it could also be applied in cases of contamination of natural waters.

Without performing a prior spiking of the samples, we did not observe significant differences from the signals obtained in the case of the blanks, except for ibuprofen and naproxen in the influent waste water, where their presence was confirmed by their mass spectra. Accordingly, it was decided to spike the samples at two concentration levels – 5 μg/L and 25 μg/L – and carry out prediction (the ibuprofen and naproxen in the influent waste water were predicted without adding them to the sample). Fig. 5 shows the chromatograms of the samples spiked with 5 μg/L, together with the corresponding chromatogram of ultrapure water spiked with the same concentration of analytes.

It was observed that the uncertainty in prediction was very high (up to 50%) if external calibration was applied by comparing the samples with standards prepared in ultrapure water and measured on the same day. A possible solution to this would consist in performing a complete calibration, measured together with the samples daily. However, it was decided to use a multiplicative algorithm of calibration transfer, which allows compensation of the instability in the mass spectrometer signal [44] and use in all cases the same calibrations in ultrapure water obtained on a previous day (Table 2, using the zones of the calibration with concentrations ranging between the blank and 50 μg/L). To accomplish this, on the same day as we analyzed each of the samples we also measured three standards in ultrapure water at concentrations equal to those of the calibration curves.

$$I_{(m/z)_{tr}} = I_{(m/z)_{day_b}} \times \left(\frac{(1/3)\sum_{n=1}^{n=3} I_{(m/z)_{day0}}}{(1/3)\sum_{n=1}^{n=3} I_{(m/z)_{day_b}}} \right)$$

where $I_{(m/z)_{tr}}$ is the intensity resulting from applying the transfer process; $I_{(m/z)_{day_0}}$ corresponds to the intensity at the time of constructing the calibration model; $I_{(m/z)_{day_b}}$ is the intensity measured "b" days after the model has been constructed, and n is the

number of transfer samples, identical to the model, measured on day b; (m/z) corresponds to the quantification ions of each compound.

Table 3 shows the predictions obtained with external calibration, with the multiplicative calibration transfer algorithm. The recoveries obtained ranged between 80 and 120%, except in the case of influent waste water, where the recoveries were in some cases higher than 130%, indicating the existence of a matrix effect. Additionally, it was observed that in this matrix diclofenac was derivatized at higher proportions than in the other aqueous matrices studied, probably due to its greater chemical complexity. Owing to this, it was decided to perform the quantification of this sample spiked with 5 μg/L and 25 μg/L, applying the standard additions method (in the case of ibuprofen, and naproxen, the concentration of the peak corresponding to the unspiked sample was measured). The results are shown in Table 4. With this method it was observed that the predicted concentrations of all compounds were satisfactory.

4. Conclusions

A simple GC–PTV–MS method in combination with *in situ* aqueous derivatization and SALLE for the determination of the NSAIDs drugs clofibric acid, ibuprofen, naproxen, diclofenac and ketoprofen in water samples is reported. The use of a water-soluble carbodiimide and 2,2,2-trifluoroethylamine to form the corresponding amides of the compounds studied represents an easy, comfortable and reliable way for *in situ* derivatization of these priority pollutants. It avoids the need for previous extraction of the compounds into an organic solvent, as well as clean-up of the SALLE extract.

Quantification limits at low μg/L levels were achieved for all the compounds; enabling use of the method for the determination of NSAIDs in waste water or contaminated water samples. Conversely to classic extraction strategies based on liquid–liquid extraction (LLE) or SPE, which require the concentration of large volumes of sample, only 2.5 mL of water is necessary to carry out the extraction. The volume of organic solvent (1.5 mL of ethyl acetate) is equally low.

The proposed method was successfully applied to the analysis of tap water, river and sea water and the influent and effluent water from a sewage treatment plant. The results obtained for real samples reveal the presence of ibuprofen and naproxen in the influent water of the sewage treatment plant. This sample, which was the most complex one, was also the only one in which a matrix effect was seen.

Acknowledgements

The authors acknowledge the financial support of the DGI (CTQ2010-17514/BQU) and the Consejería de Educación y Cultura of the Junta de Castilla y León (GR87) for this research. G. Grueiro Noche acknowledges the financial support of the Xunta de Galicia for the concession of a grant for "Consolidation and structuring of competitive research groups in the University System of Galicia, 2010–12" and Project 09MDS038103PR.

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