Journal of Chromatography A, 1218 (2011) 4856–4862

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Headspace sampling with *in situ* carbodiimide-mediated derivatization for the determination of ibuprofen in water samples

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ARTICLE INFO

Article history:
Available online 27 February 2011

Keywords:
In situ derivatization
Headspace generation
Ibuprofen
Carbodiimide promoted derivatization
2,2,2-Trifluroethylamine

ABSTRACT

A method using headspace generation and *in situ* derivatization with water soluble EDC (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide) and TFEA (2,2,2-trifluoroethylamine) has been optimized for the determination of ibuprofen (2-(p-isobutylphenyl)propionic acid), one of the most common non-steroid anti-inflammatory drug (NSAIDs) residues in surface and wastewater samples. Derivatization was carried out in the vial of the headspace sampler (HS) in only 15 min, after which instrumental measurements were made with gas chromatography–mass spectrometry (GC–MS). As the injection system, a programmed temperature vaporizer (PTV) in the solvent-vent injection mode is proposed in order to increase the sensitivity of the measurements. The effects of the variables affecting HS generation, the derivatization reaction, and the instrumental PTV conditions were studied. A limit of quantification as low as 32 ng/L was achieved, and repeatability values were below 10%. Accuracy of the method was evaluated using spiked ultrapure water at three concentration levels, obtaining apparent recoveries between 96% and 104%. The proposed method was applied to the quantification of ibuprofen in sea water and urban wastewater samples.

1. Introduction

Sample preparation has been considered the most polluting and time-consuming step of analytical procedures, as well as one of the most frequent sources of errors. The use of headspace sampling solves many of these problems by minimizing sample treatment. However, in many cases the limits of detection achieved are insufficient for the detection of the analytes of interest [1]. When this occurs, one possibility involves the use of programmed temperature vaporizers (PTV), which, by injection in the solvent-vent mode, to a large extent allows the solvent and compounds more volatile than the analytes of interest to be removed. Additionally, the formation of more volatile derivatives by derivatization reactions can also be used as a step prior to headspace sampling [2], allowing the use of this technique for compounds for which, owing to their low volatility, it would not be applicable.

Several derivatization reactions in water samples have been proposed using headspace sampling in its different modes, including static headspace (HS), headspace-solid phase microextraction or headspace-single drop microextraction. Acetylation with acetic anhydride in basic medium is commonly used for phenolic compounds [3,4]. Pentafluorobenzaldehyde for the case of amines [5], 2,4-dinitrophenylhydrazine for carbonyl compounds [6], sodium tetraethylborate for organotin compounds [7] and sodium tetraphenylborate for organomercury compounds [8] have also been proposed. For carboxylic acids, reactions with MeOH in acidic medium [9–11], dimethyl sulfate [12] or benzyl bromide [13] have been reported.

To the best of our knowledge, headspace sampling has not yet been used in combination with another common derivatization reaction for carboxylic acids: carbodiimide-mediated amide formation. Carbodiimides have been widely used as reagents to promote condensation, particularly in peptide synthesis [14]. Within the fields of analytical chemistry and bioanalysis, they have been used in the covalent immobilization of proteins on different types of electrodes [15,16] or nanoparticles [17].

As derivatization agents of carboxylic acids, water-insoluble carbodiimides have been used [18–20], pointing to the need to use a phase-transfer step prior to the derivatization reaction. In contrast, the use of water-soluble carbodiimides allows the derivatization reaction to be accomplished without the need for a prior extraction step. In this sense, several works using a water-soluble carbodiimide, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC), have been published, in which later analysis of the derivatized compounds was carried out with liquid chromatography [21,22], high-pressure ionic chromatography (HPIC) [23], or gas chromatog-

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doi:10.1016/j.chroma.2011.02.054

raphy [24]. In the latter case, a subsequent liquid–liquid extraction of the amides with an organic solvent was needed prior to the chromatographic analysis.

In a previous work developed at our laboratory [11], we reported the use of an *in situ* methylation reaction in the vial of a headspace sampler for the determination of ibuprofen (2-(p-isobutylphenyl)propionic acid) in aqueous samples. A programmed temperature vaporizer was used to introduce the sample into the gas chromatographic system.

In the present work we propose a similar approach, using carbodiimide-mediated amide formation with water-soluble EDC and 2,2,2-trifluoroethylamine (TFEA). Ibuprofen was again selected among the NSAIDs due to its frequent detection in environmental waters and the higher concentrations habitually found [25,26], probably as a result of its high prescription extent and wide usage. It has been included in studies aimed to obtain a first overview of contamination with pharmaceuticals [27,28]. With the proposed set-up, it is not necessary to implement previous extraction of the analyte from the aqueous matrix to an organic or hydro-organic medium, this step being mandatory in the types of derivatization most frequently used for NSAIDs prior to their GC analysis, such as silylation [29–32], or alkylation [33–35]. Moreover, headspace sampling makes the liquid–liquid extraction step of the ibuprofen derivative unnecessary to perform the analysis by gas chromatography, thereby simplifying the analytical process.

2. Experimental

2.1. Materials, standard solutions and samples

2-(p-isobutylphenyl)propionic acid (ibuprofen), 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride salt (EDC) and 2,2,2-trifluroethylamine (TFEA) were supplied by Sigma–Aldrich (Steinheim, Germany). Sodium chloride, di-sodium hydrogen phosphate dihydrate and ortho-phosphoric acid were from Scharlau (Barcelona, Spain). Acetonitrile was from Merck (Darmstadt, Germany).

A stock solution (2000 mg/L in acetonitrile) of ibuprofen was prepared and stored at 4 °C in a refrigerator. This solution was used to spike the water samples at the different concentrations analyzed. Optimization of the method was performed with ultra-high quality water (UHQ), obtained with a Wasserlab Ultramatic water purification system (Noain, Spain).

The buffer medium used in the pH studies has been described by McIllvaine and Whiting [36]. The different pH values were obtained by mixing different proportions of two aqueous solutions, one of citric acid (0.25 M) and the other of Na_2HPO_4 (0.50 M). Once the optimum values of pH 6.0 had been selected, a buffer with that pH value was prepared by weighing Na_2HPO_4 and dissolving in water to a concentration of 0.50 M, adjusting to the desired pH with H_3PO_4.

The proposed procedure was used for the determination of ibuprofen in samples of sea water (from A Coruña, N. Spain) and influent and effluent waters from the main wastewater treatment plant (WWTP) of Salamanca, with a population of 260,000 inhabitants.

2.2. Derivatization reaction

NaCl (2.5 g) was added to a 10-mL headspace vial. Then, 0.40 mL of an aqueous solution of EDC 0.40 M was added, followed by 0.40 mL of an aqueous solution of TFEA 0.40 M and 0.40 mL of a buffer solution at a pH 6.0 at a concentration of 0.50 M. Finally, 4.0 mL of an aqueous solution spiked at a given concentration of ibuprofen was added. The vial was sealed hermetically and placed in the headspace sampler.

2.3. Headspace

HS sampling was performed with a PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). This sampler is equipped with a tray for 32 consecutive samples and an oven with positions for six sample vials. Oven temperature was kept at 95 °C and the equilibration time (coincident with the time given to perform the derivatization reaction) was set at 15 min. During this time, agitation of the sample was performed at 750 rpm. A 2.5-mL syringe at 130 °C was used, fixing an injection volume of 2.40 mL. The fill speed and injection speed were fixed to 100 μL/s and 200 μL/s, respectively. After injection, the syringe was cleaned with a flow of He for 2 min. The time between samples was set at 16 min.

2.4. Programmed temperature vaporization

All experiments were carried out with a PTV inlet (CIS-4, Gerstel, Baltimore, MD, USA). A Gerstel CIS-4 liner (71 mm × 2 mm) was used, packed with a chemical sorbent (Tenax-TA). In the optimized method, solvent-vent injection mode was used. Cooling was accomplished with liquid CO_2.

The initial injector temperature was set at 142 °C. Vent flow was adjusted to 30 mL/min, and vent pressure to 34.5 kPa. The purge time was set at 1.20 min. The initial temperature of the liner was maintained for 1.25 min as a safety mechanism so that the heating ramp would start when the split valve was closed. Once venting had finished, the split valve was closed and the liner of the PTV was flash-heated at 12 °C/s up to 300 °C. The analytes were then transferred from the liner to the capillary column (1.5 min). Then, the split valve was opened and the liner temperature was increased at 12 °C/s to 320 °C, and held for 2 min.

2.5. Gas chromatography

To perform the gas chromatography measurements, an Agilent 6890 GC device equipped with a low polarity DB-VRX capillary column (20 m × 0.18 mm × 1 μm, working range −10 °C to 260 °C) from J&W Scientific (Folsom, CA, USA) was used. The carrier gas was helium N50 (99.995% pure; Air Liquide).

The column oven temperature program (starting simultaneously with the PTV injection into the chromatographic column) used an initial temperature of 50 °C for 2.70 min; an increase at 120 °C/min to 70 °C; then an increase at 70 °C/min to 200 °C, and finally an increase at 45 °C/min to 250 °C, then holding for 1.4 min. These temperature ramps are the maximum ones permitted by the instrumental configuration employed. The total chromatographic run time was 7.20 min.

2.6. Mass spectrometry

The detector was a quadrupole mass spectrometer (HP 5973 N). It was operated in the electron-ionization mode using an ionization voltage of 70 eV. The ion source temperature was 250 °C, and the quadrupole was set to 150 °C. A solvent delay of 5.5 min was established, during which the filament was turned off. The m/z range in the scan mode was 50–300 amu; the scan rate was 5.46 cycles/s, and the abundance threshold value was set at 0. This acquisition mode was used for the different optimization studies. Ibuprofen was identified by comparison with the mass spectrum generated by a standard solution of the derivatized compound. In selected ion-monitoring mode (SIM), a single group of ions was used, containing the characteristic ions of the ibuprofen trifluoroethylamide derivative (161, 119, 91). The ions were acquired with a dwell-time

of 30 ms. Data acquisition was performed with Enhanced ChemStation, G1701EA Ver. E.00.00.202 software from Agilent Technologies.

3. Results and discussion

3.1. Variables affecting HS generation and the derivatization reaction

Fig. 1 shows a descriptive mechanism for the carbodiimide-mediated derivatization reaction [37]. The role of the carbodiimide is that of an active coupling agent for the reaction between the carboxylic acid and the amine, with the formation of an intermediate acylisourea and a final ibuprofen amide derivative.

Preliminary experiments were performed in order to study different parameters that might affect the generation of volatiles in the headspace sampler, at an ibuprofen concentration of 500 μg/L. Fig. 2 (upper part) shows the results obtained for the optimization of headspace generation.

First, the variation in the activity coefficient (γ_i) was studied by modifying the sample matrix. In the case of aqueous samples and polar analytes, this effect can be achieved by adding an electrolyte to the medium (*salting-out*). A study was made of the effect of the addition of the electrolyte (NaCl) to the vials at three concentration levels: without NaCl; 1.0 g in 5.2 mL, and 2.5 g in 5.2 mL (supersaturation). The best results were obtained under supersaturation conditions, with a 372-fold increase with respect to the conditions without NaCl, and 11-fold with respect to the intermediate situation.

Regarding the autosampler oven temperature, different values were studied: 40, 60, 80 and 95 °C. The one selected was 95 °C, which is an adequate value for headspace generation in aqueous systems. For lower values, the signal decreased dramatically.

Finally, the time during which the vials were heated in the oven was studied in the 5–90 min range. As well as being the time taken for headspace generation it is also the reaction time. A value of 15 min was chosen, for which the maximum signal observed had been obtained together with good measurement repeatability.

In order to determine the optimum reaction conditions for preparing the amide derivative of ibuprofen, the variables studied were as follows: the addition of reagents, the pH of the medium, and the concentrations of the derivatization reagents (EDC and TFEA). These studies were carried out with an aqueous sample spiked at a concentration of 500 μg/L of ibuprofen. The results are shown in the lower part of Fig. 2.

The mechanism proposed for the reaction involves two consecutive steps, first the activation by the carbodiimide of the carbon bearing the –OH group and then the reaction between the intermediate acylisourea (more reactive than the carboxylic acid) with 2,2,2-trifluoroethylamine. Accordingly, a study was first made of whether there were any differences between the signals generated upon performing the derivatization in one experimental step, mixing the reagents and analyte simultaneously or in two separated steps, adding them separately. In the former case (1 experimental step) (see Section 2.2), the EDC and TFEA reagents, buffer adjusted to pH 5.0, and the aqueous solution of ibuprofen were added and the vial was shaken for 15 min before injection into the chromatographic system. In the second case, only the aqueous solution of ibuprofen, buffer adjusted to pH 5.0, and EDC were added. The vial was hermetically closed and shaken for 5 min with a Vortex device, allowing the first step in the reaction to occur. The vial was then reopened and TFEA was added to the reaction intermediate. We observed that when the process was carried out in two experimental steps, a decrease of about 65% occurred in the signal, such that it was decided to work in the first conditions, which were also simpler to implement.

With respect to the study of pH, this was performed in the pH 2.2–9.0 range. As may be seen in Fig. 2, the reaction was strongly pH-dependent, and the optimum signal was obtained for a pH value of 6.0. Phosphate buffer (see Section 2.1) was chosen to fix this value.

With regard to reagent concentrations, since the reaction stoichiometry is 1:1 it was decided to vary the concentrations of both EDC and TFEA simultaneously, after which we studied values of 0.010, 0.040, 0.10, 0.40 and 1.0 M. The best results were obtained for a concentration of 0.40 M, which was selected as the working concentration. The same experiment was performed for a lower ibuprofen concentration – 10 μg/L – with the same results.

3.2. PTV–GC–MS

3.2.1. Optimization of the programmed temperature vaporizer

The variables optimized in the solvent-vent injection process were venting temperature, venting time, and venting flow. To study the effect of these variables and of their interactions on the analytical signal, a Box–Behnken experimental design was used. This allows the generation of response surfaces by using three levels for each of the factors studied. In all cases, a water sample spiked at the laboratory with a concentration of 50 μg/L of ibuprofen was used. Each of the experiments was performed in triplicate so as to be able to detect any lack of fit of the data in the model generated. The response chosen was the peak area for the *m*/*z* 161 ratio corresponding to the base peak of the spectrum of the ibuprofen derivative. Only the model including the variables, their squares and the interactions among them did not show a lack of fit.

As may be seen in Table 1, all the variables of the experimental design, together with their squares and interactions, are significant, with the exception of the square of the venting time variable (*BB*), indicating that the response was linear with respect to that variable. In the case of the venting temperature, as this increased so did the signal, up to a certain value after which it decreased. This was probably because at high temperatures the analyte is also removed in the venting step. In the case of venting flow, on increasing its value the signal decreased, whereas for venting time, when this value increased an increase in signal was observed. To visualize the effect of the interactions, response surfaces were used. Fig. 3 shows the response surfaces for the *m*/*z* 161 ratio as a function of the different interactions observed. Regarding the response surface of the venting temperature–venting time interaction (*AB*) the best results were obtained at high venting times and temperatures in the 130–150 °C range. In the venting time–venting flow interaction (*BC*), the maximum signal was obtained for high venting times and low venting flows.

Regarding the venting temperature–venting flow interaction (*AC*), this is where the greatest variation in the signal was observed, with optimum values for the venting temperature between 130 and 150 °C and a venting flow of 30 mL/min.

Table 1
Results of the experimental design.

Multiple correlation: 0.955 (cal) R^2: 0.912 (cal)		
	p-Value	Coefficients
Intercept	<0.0001	5.911×10^6
A	<0.0001	-6.849×10^4
B	0.0090	-1.010×10^6
C	<0.0001	-4.532×10^4
AB	0.0144	-3.355×10^5
AC	<0.0001	-7.017×10^5
BC	0.0010	-4.696×10^5
AA	0.0024	-5.669×10^5
BB	0.2968	-1.811×10^5
CC	0.0020	5.795×10^5

A: venting temperature (°C); *B*: venting time (min); *C*: vent flow (mL/min).

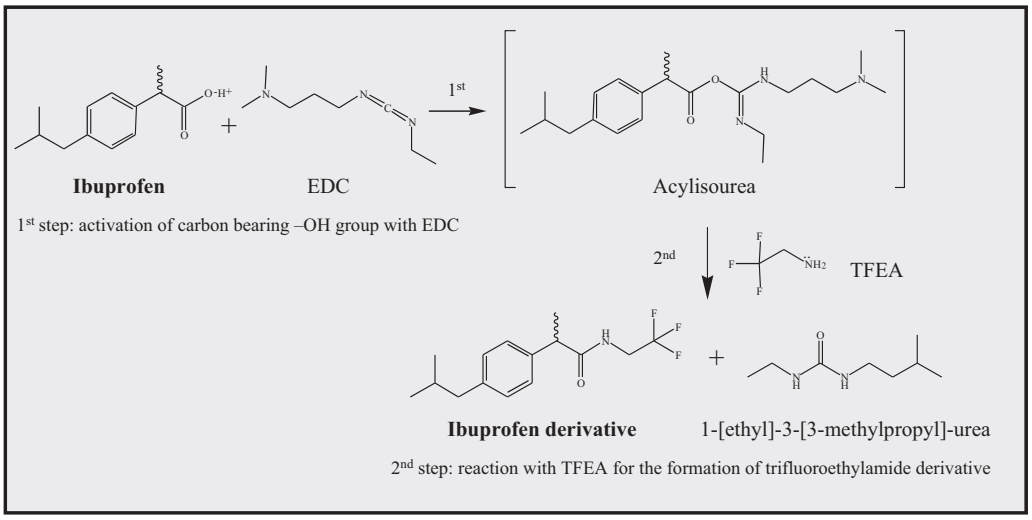

Fig. 1. Scheme of the ibuprofen derivatization reaction with EDC and TFEA.

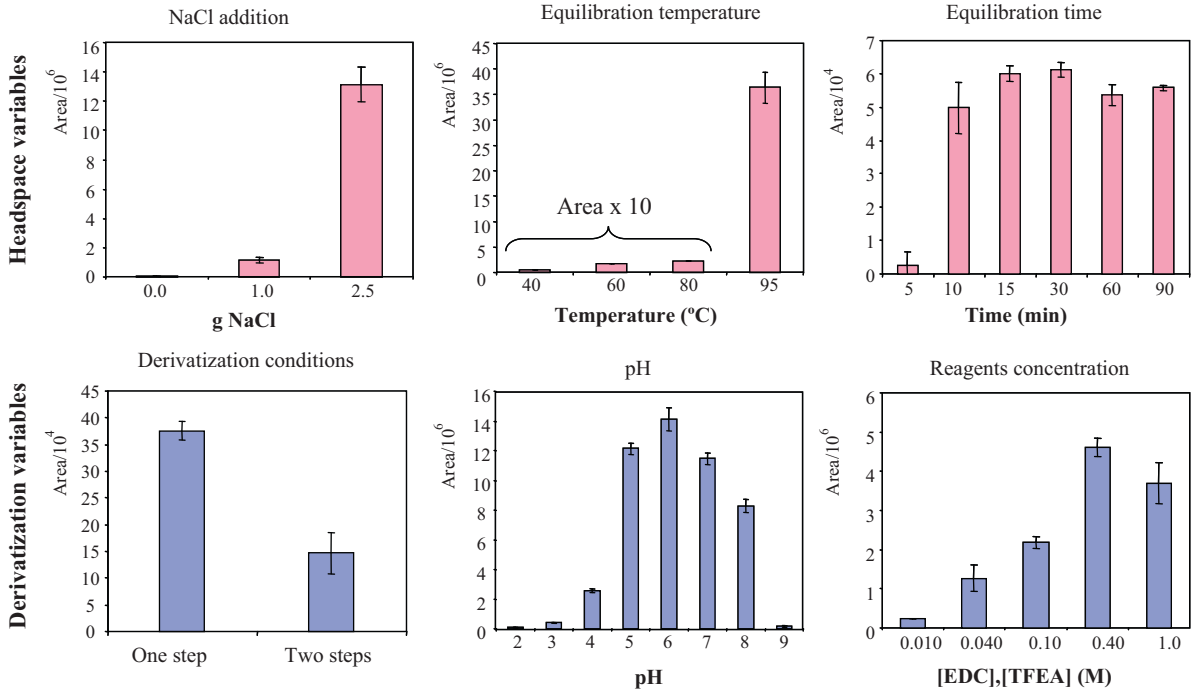

Fig. 2. Study of the headspace variables and those of the derivatization reaction.

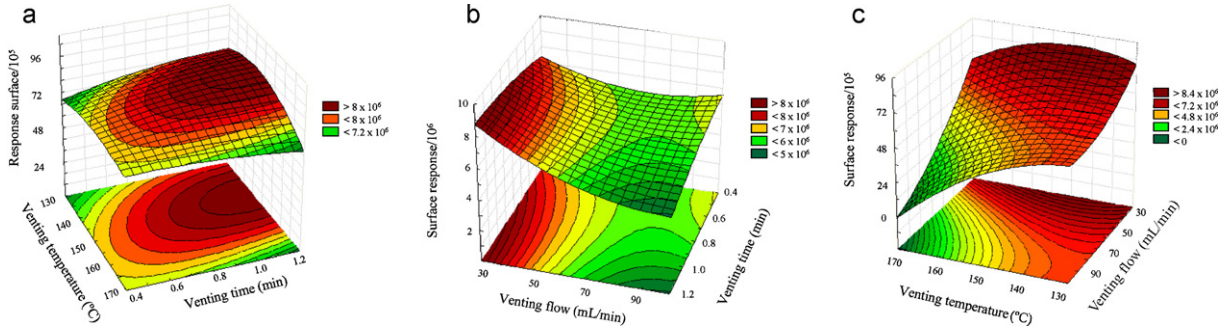

Fig. 3. Experimental design surfaces showing the interactions between venting temperature and venting time (a), venting flow and venting time (b) and venting temperature and venting flow (c) for the ibuprofen derivative at a concentration of 50 μg/L.

Table 2
Analytical characteristics of the proposed method.

Analyte	SIM target ions	Intercept	Slope	LOD (ng/L)	LOQ (ng/L)	R^2	RSD (%)		Prediction		
							0.50 μg/L	5.0 μg/L	0.50 μg/L	2.25 μg/L	5.0 μg/L
Ibuprofen	161, 119, 91	$(7 \pm 6) \times 10^4$	$(65 \pm 2) \times 10^4$	9.6	32	0.9958	6.7	9.2	0.48 ± 0.08	2.3 ± 0.3	5.2 ± 0.3

In light of the results obtained with the experimental design, the values that provided the maximum signal were 1.20 min for the venting time; 142 °C for the venting temperature, and 30 mL/min for the venting flow.

To check that the solvent-vent injection mode was the optimum one, a comparative study was made of the signals obtained with the solvent-vent, split, and splitless injection modes. In the split injection mode, the split ratio was 1:5 and the temperature of the injector was kept at 300 °C. This same temperature was used in the splitless injection mode, with a splitless time of 2.00 min. In the solvent-vent injection mode, the injector conditions were the optimum ones obtained in the experimental design. In both the split injection mode and the solvent-vent mode the injection volume was kept at 2.40 mL. In the case of splitless injection, the injection volume was reduced to 250 μL as a result of the size of the liner used, which does not allow greater injection volumes with appropriate control of the system. It was observed that the maximum signal (about four times higher than the other two) was obtained with the solvent-vent injection mode, such that this was accepted as the optimum one.

3.3. Evaluation of the PTV–GC–MS method

Table 2 shows the analytical characteristics of the proposed method. A calibration curve was obtained with twelve concentration levels, ranging from 0.04 to 10 μg/L. Each level was analyzed in triplicate in SIM mode (161, 119, 91). These fragment ions are characteristic to the ibuprofen trifluoroethylamide derivative, as well as they are to ibuprofen, indicating that molecule fragmentation occurs in a similar way. The molecular peak of the derivative (m/z 287) was also present in its spectrum and it was detected in the scan mode, confirming the identity of the compound, but it was not chosen due to its low abundance. For the ibuprofen trifluoroethylamide derivative the ion 161 corresponds to loss of the fragment O=C–NH–CH_2–CF_3, whereas for ibuprofen the same ion corresponds to the O=C–OH fragment loss. The m/z ratios 119 and 91, found in both spectra, are characteristic of benzyl compounds with alkyl chains. The peak area obtained on extracting the m/z 161 ratio was used as the analytical signal. The calibration model displayed linear behavior. The validity of the model generated was checked using ANOVA, and it was observed that the model generated did not exhibit any lack of fit. The value of the correlation coefficient (R^2) was higher than 0.99. The repeatability of the method was studied at two levels – 0.5 and 5 μg/L – by calculating the relative standard deviation (RSD, %) for 10 replicates. It was found to be 6.7% and 9.2%, respectively.

The limit of detection (LOD) and limit of quantification (LOQ) were estimated using the following formulas:

$$\mathrm{LOD} = \frac{3.3\sigma}{S}; \quad \mathrm{LOQ} = \frac{10\sigma}{S}$$

where σ is the standard deviation obtained upon measuring replicates of a sample with an S/N ratio of approximately 3, and S is the slope of the calibration curve.

The LOD obtained was 9.6 ng/L and the LOQ was 32 ng/L. These values are among the lowest published in the literature and are appropriate for the determination of ibuprofen at the concentrations found in surface waters [33,38]. In comparison with the work of Ford et al. [24], in which the authors used the same derivatization reaction but with liquid–liquid extraction and direct injection, the improvement is very significant, passing from 74.8 μM (15.4 mg/L) to the values obtained with the proposed method. Such an improvement can be attributed to the use of solvent-vent in the PTV and to the SIM detection mode in the mass spectrometer detector. Thus, the limit of detection is improved with respect to that obtained in a work previously published by our research group [11], (LOD 0.23 μg/L), in which derivatization was carried out with methanol in a strongly acid medium, also in the static headspace mode. Additionally, the reaction time and headspace generation were reduced from 60 to 15 min. The values obtained when dimethyl sulfate is used as the derivatization reagent [12] in the headspace mode, with the use of a microextraction fibre in the solid phase, is around 0.3 ng/L. However, this derivatization reaction has the disadvantage that that dimethyl sulfate is known to be highly toxic and carcinogenic [39,40].

When the derivatization reaction is carried out in organic medium, in some cases limits of the order of ng/L are reached [33,35], but the process involves a prior solid-phase extraction step.

To check the accuracy of the model, ultrapure water samples were spiked at three concentration levels: 0.50, 2.25 and 5.0 μg/L. In each case, the model predicted concentration values that were significantly equal to those used to spike the water samples (Table 2). Apparent recoveries, calculated as the ratio of the measured concentration to the spiked concentration (expressed as percentage), were between 96% and 104%.

3.4. Time of analysis

The methodology proposed in the present work required a time of 31 min for the analysis of the first sample 15 min for reaction and the generation of volatiles; 7.2 min for chromatography, and about 8 min to measure the next sample since the column and the PTV had to be cooled down from the final temperature attained (250 °C and 320 °C, respectively, to the initial conditions of 50 °C and 142 °C). However, as from the first injection, it was possible to analyze each sample from the same sequence every 16 min, since the multiposition headspace autosampler oven allows an overlapping of these times.

3.5. Environmental samples

Different types of water samples were analyzed: sea water and wastewater (taken at the influent and effluent streams of the same wastewater treatment plant). No ibuprofen was detected at concentrations above the limit of detection of the method either in the sea water or effluent wastewater, although by contrast it was found in the influent wastewater. Fig. 4 shows the chromatograms obtained for ultrapure water and for the two samples not containing ibuprofen, spiked at a level of 0.50 μg/L, together with the chromatograms corresponding to the influent wastewater without dilution and when diluted at a proportion of 1:1 (wastewater/ultrapure water). This dilution was carried out after observing that for this sample the repeatability in injection decreased considerably and that upon spiking the sample with the analyte no proportional increase in the analytical signal occurred. These effects can be attributed to the complexity of the influent wastewater, which may alter both the conditions under which the derivatiza-

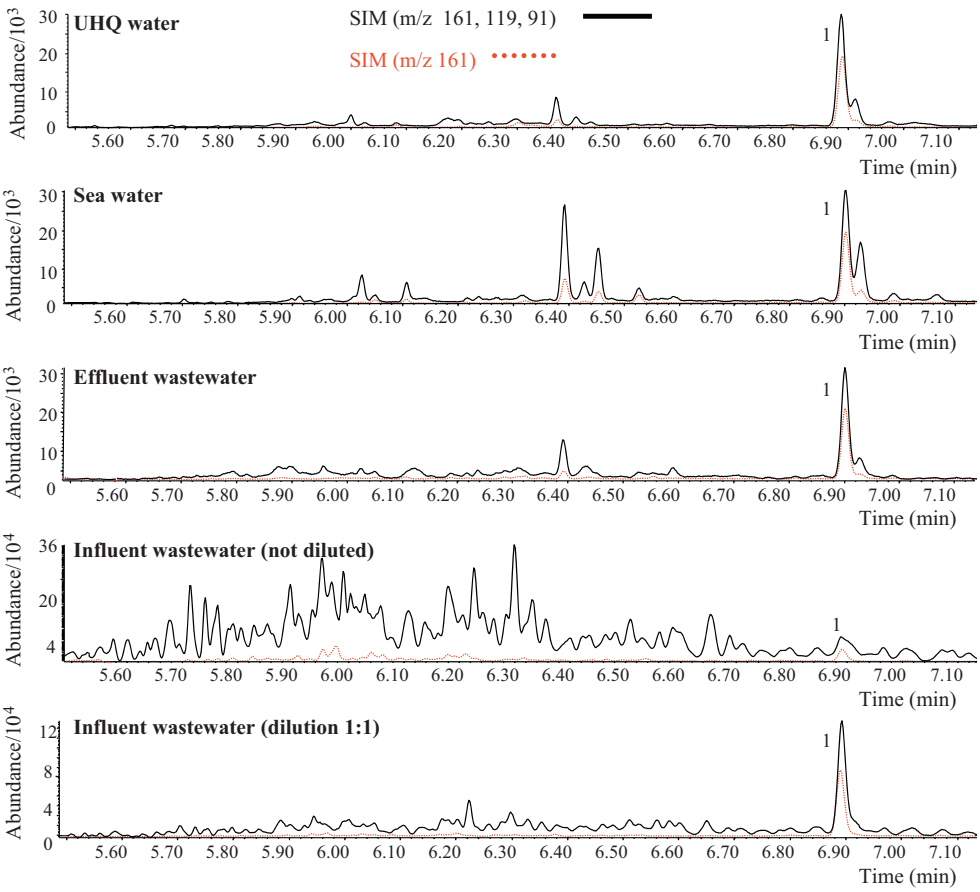

Fig. 4. Chromatograms obtained in the different types of aqueous matrices studied. The samples of ultrapure water, sea water and effluent wastewater were spiked with 0.50 μg/L of ibuprofen. (1) Ibuprofen derivative.

tion reaction occurs and those of the headspace generation. These effects were reduced when the wastewater was diluted with ultrapure water.

Matrix effects were investigated by comparing the slopes of the regression curves obtained in ultrapure water with those provided by the three water samples spiked with the compounds at three concentration levels: 0.50, 2.25 and 5.0 μg/L for sea water and effluent wastewater samples, and 5.0, 10 an 15 μg/L for the diluted influent wastewater sample.

Table 3 shows the slope values obtained for each type of water studied. In each case, the signal corresponding to the blank was subtracted from the signals of the spiked water samples. For sea water and effluent wastewater samples, no matrix effect was observed. However, in diluted influent wastewater samples a significant decrease in the slope of the calibration straight line obtained was noted. Therefore, for quantification of ibuprofen with the proposed method, external calibration was used for the sea water and effluent wastewater samples, and a standard additions protocol was adopted for the diluted influent wastewater sample. Each sample was analyzed in triplicate. The samples of sea water and effluent wastewater were spiked with 0.50 μg/L and the concentration values obtained in their analysis were 0.48 ± 0.08 and 0.47 ± 0.09 μg/L, respectively.

Table 3
Slope values obtained for the different types of water studied.

Water sample	Slope
UHQ water	$(67 \pm 9) \times 10^4$
Sea water	$(60 \pm 7) \times 10^4$
Effluent wastewater	$(64 \pm 7) \times 10^4$
Influent wastewater (dilution 1:1)	$(34 \pm 5) \times 10^4$

For the sample of influent wastewater (diluted at 1:1 (wastewater/ultrapure water)) the quantification of ibuprofen with the standard additions protocol afforded a concentration of 14 ± 4 μg/L. This result is not surprising since ibuprofen is widely used and, in some cases, almost 95% of the dose given to patients may be excreted unmetabolized and eliminated in domestic wastewater [41]. The results obtained suggest that the percentage of elimination of ibuprofen in the wastewater treatment plant is very high, close to 100%, taking into account that ibuprofen was not detected in the effluent sample.

4. Conclusions

Here we optimized an *in situ* derivatization method in aqueous medium for the analysis of ibuprofen in static headspace mode. 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) and 2,2,2-trifluroethylamine (TFEA) were used as derivatization reagents. The instrumental configuration used (HS–PTV–GC–MS) has the advantage that, as from the mixing of the reagents in the HS vial, the whole process takes place on-line, with the consequent reduction in errors associated with sample manipulation. The main limitation to extend this approach to other carboxylic acids present in waters is that their derivatives have to be volatile enough.

The effects of variables affecting HS generation, the derivatization reaction and the PTV instrumental conditions were studied. The proposed method is highly sensitive, with a limit of quantification of 32 ng/L, allowing the determination of this analyte in

both surface and wastewater samples with good repeatability (RSD below 10%) and accuracy values (apparent recoveries between 96% and 104%). In complex aqueous matrices, it may be necessary to perform quantification using the standard additions method.

Acknowledgements

The authors acknowledge the financial support of the DGI (CTQ2007-63157/BQU and CTQ2010-17514/BQU) and the Consejería de Educación y Cultura of the Junta de Castilla y León (Project SA112A08) for this research.

Casas Ferreira A.M. acknowledges an FPU grant from the Spanish Ministry of Education.

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