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Indirect analysis of urea herbicides from environmental water using solid-phase microextraction

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Abstract

We described here a solid-phase microextraction procedure used to extract six urea pesticides — chlorsulfuron, fluometuron, isoproturon, linuron, metobromuron and monuron — from environmental samples. Two polydimethylsiloxanes and a polyacrylate fiber (PA) are compared. The extraction time, pH control, addition of NaCl to the water and the influence of organic matter such as humic acid on extraction efficiency were examined to achieve a sensitive method. Determination was carried out by gas chromatography with nitrogen–phosphorus detection. The proposed method requires the extraction of 2 ml of sample (pH 4, 14.3%, w/v, NaCl) for 60 min with the PA fiber. The limits of detection range from 0.04 for linuron to 0.1 μg/l for fluometuron and monuron and the relative standard deviations at the 1 μg/l level are between 15% and 9%. The apparent fiber–water distribution constants (K_{fw}) calculated in the proposed conditions were in the order of 10^3. Phenylurea herbicides were indirectly determined in the form of their derived anilines and chlorsulfuron in the form of an aminotriazine as confirmed by gas chromatography–mass spectrometry. Natural waters were utilized to validate the final procedure. However, a unequivocal identification in unknown environmental samples should be done by LC–MS. The presence of dissolved organic matter such as humic acid produces losses during the extraction step. Adding sodium chloride to the sample compensates for this effect.

Keywords: Water analysis; Environmental analysis; Solid-phase microextraction; Pesticides

1. Introduction

Although extraction procedures that use little organic solvent have been developed, techniques that use no solvent have appeared only recently. Solid-phase microextraction (SPME), a solvent-free, easy sample preparation method, has been successfully applied to many contaminants in environmental studies ever since Pawliszyn and Liu [1] perfected it in their work on optical fibers. SPME is based on analyte distribution between the extracting fiber and the water sample volumes. As this process is dominated by the fiber–water (K_{fw}) partition coefficients, identifying the K_{fw} is of great importance in choosing the best extracting fiber. The K_{fw} of some fibers have been studied for several pesticides [2–7] and for other contaminants such as polycyclic aromatic hydrocarbon (PAHs) [8,9], polychlorinated biphenyls (PCBs) [3,9,10] and phenols [11]. The utility of SPME combined with GC using selective detection

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methods has been proven for the three major classes of pesticides, i.e. organochlorine [3,4,12–15], organophosphorus [2–5,13,15–20] and organonitrogen pesticides [2–4,6,13,17–19,21–25].

Polydimethylsiloxane (PDMS), polyacrylate (PA), polydimethylsiloxane–divinylbenzene (PDMS–DVB), Carboxen–polydimethylsiloxane (CAR–PDMS), Carbowax–polydimethylsiloxane (CW–PDMS) and Carbowax–divinylbenzene CW–DVB are commercially available fibers covering a whole range of polarities, thicknesses and affinities to pesticides. PDMS and PA appeared on the market earlier, and consequently are the two most frequently cited. Several papers comparing fibers of different polarities have been reported. A high affinity for N-containing pesticides was found in fibers containing DVB [2]. Therefore, CW–DVB was chosen for an inter-laboratory study to determine triazines [24]. PDMS–DVB was found to have the highest affinity for triazine herbicides [3], whereas PA and PDMS–DVB were the best choice for extraction of several organophosphorus pesticides [3,20]. Regardless of which fiber coating is being worked with, adding sodium chloride to water samples is an extended practice meant to improve the K_{fw} of non-ionized pesticides. This practice has been reported for quite different pesticides such as organophosphorus [5,17,18], triazine [6,17,18,24], other N-containing pesticides [6,17,18], and even for headspace (HS) SPME of low-polarity pesticides such as organochlorine insecticides [12].

Phenyl- and sulfonylureas are extensively used in agriculture as selective herbicides. The residues of these compounds in matrices can be investigated by both high-performance liquid chromatography (HPLC) and gas chromatography (GC) methods [26,27]. Since sulfonylureas are weak acids having pK_a values ranging from 3.3 to 5.2 [28], extraction from waters is affected by the pH. For this reason, solid-phase extraction (SPE) from water is usually done at an acid pH [29]. As phenylureas do not present acid or basic properties, SPE is executed at a neutral pH. Consequently, a change in pH from 7 to 3 in the aqueous mobile phase did not modify their retention times in HPLC determination [30].

SPME of phenylureas has not been extensively studied, and there are few reports in which SPME is coupled with HPLC to test for these compounds [31,32]. It was precisely the lack of studies on SPME of urea herbicides that prompted us to assess the feasibility of SPME coupled with GC using commercial equipment to determine urea herbicides from aqueous matrices.

2. Experimental

2.1. Materials

Two PDMS fibers with thicknesses of 7 and 100 μm and a PA with a thickness of 85 μm (Supelco, Bellefonte, CA, USA) were used with an SPME holder for an 8200 CX autosampler (Varian, Palo Alto, CA, USA).

All the coated fibers were previously conditioned in the injector port according to the manufacturer's instructions until a stable baseline was obtained. The PA fiber was conditioned before initial application by heating it at 300°C for 2 h and the PDMS fibers at 250°C for 1 h.

Salt saturated (35.7%, w/v, NaCl), humic acid sodium salt (10 mg/l), and pH 4 (0.5%, w/v, sodium hydrogentartrate) and pH 9.2 (0.1 *M* sodium tetraborate decahydrate) buffered solutions were used in several experiences. Sodium chloride, sodium hydrogentartrate, sodium tetraborate with purities up to 99.5% and technical-grade humic acid were purchased from Aldrich (Milwaukee, WI, USA).

Methanol (Suprasolv quality) was from Merck (Darmstadt, Germany). Ultra-pure water was obtained from a Milli-Q purification system (Millipore, Milford, MA, USA).

2.2. Standards

The selected pesticides chlorsulfuron, fluometuron, isoproturon, linuron, metobromuron and monuron were purchased from Riedel-de Häen (Seelze, Germany) with purities between 95 and 99%.

1000 μg/l stock solutions of each of the six phenylurea herbicides were prepared separately in methanol. These stock solutions were stored at 4°C and diluted daily with ultra-pure water to prepare 100 μg/l intermediate solutions. Working water solutions were freshly prepared by spiking appropriate amounts of the intermediate solutions. The

methanol contained in the spiked working water samples was always below 0.1%.

2.3. Apparatus

Analysis was carried out in a Varian 3400 CX gas chromatograph equipped with a nitrogen–phosphorus detection (NPD) system working at 3.2 A intensity with hydrogen at 4 ml/min, air at 175 ml/min and nitrogen at 30 ml/min, a septum programmable injector (SPI), a Varian 8200 CX autosampler with an SPME agitation accessory and Varian Star 4.51 software to control the parameters. The SPME agitation accessory provides continuous contact of the fiber with fresh sample by vibrating the fiber protective sheath in the water solution without increasing temperature. A BP10 (30 m×0.25 mm, 0.25 μm 14% cyanopropylphenyl+86% dimethylpolysiloxane) produced by Scientific Glass Engineering (SGE) (Austin, TX, USA) was used with helium as the carrier gas at 2.4 ml/min. SPI and NPD were kept at 300°C. The oven temperature was programmed as follows, the initial temperature (100°C) was increased by 10°C/min to 240°C and held for 15 min. The total run time was 29 min.

To identify of the determined compounds a Fisons 8000 series gas chromatograph coupled with a Trio 1000 quadrupole mass spectrometer (Fisons, Milan, Italy) was used. The transfer line and source temperatures were 250 and 200°C respectively. The mass spectrometer worked in electron impact mode (−70 eV) by scanning from 50 to 450 amu to obtain full spectra of the detected compounds. The spectra were handled with a LAB–BASE data station with NBS, Wiley 6 and NIST spectral libraries. The rest of the chromatographic conditions reproduced those of the Varian 3400 CX.

2.4. Analytical procedure

Two ml autosampler vials were filled with 2 ml of the water samples at pH 4 containing 14.3% w/v sodium chloride and were sealed with hole caps with PTFE faced silicone septa (Supelco). The fiber was immersed in the sample for 60 min under needle agitation at room temperature (22±2°C). After extraction the fiber was directly exposed to the hot injector port for subsequent analysis, and at this time the oven program was started. Thermal desorption of pesticides was held for 5 min. Quadruplicate analyses were performed for all experiments except for the precision study, where six extractions were made. Blank analyses were carried out to ensure the absence of memory effects.

3. Results and discussion

3.1. Fiber coating selection

In contrast with other extraction methods, SPME consists of an equilibrium process in which analytes are not quantitatively extracted from water. The amount of extracted analyte depends strongly on partitioning from the water matrix to the fiber coating, and this process is controlled by the distribution constant (K_{fw}). Since the amount of extracted analyte determines the sensitivity of the method, the choice of an appropriate polymeric coating fiber is of great importance.

We compared three fibers (85 μm PA, 100 μm PDMS and 7 μm PDMS) for extracting the urea herbicides from water. Both PDMS fibers have the same polymer coating and their K_{fw} values are identical. The PDMS fibers differ in film thickness, which results in different coating volumes. The 100 μm fiber presents a larger volume than the 7 μm, and as analytes must diffuse through it, the time needed to reach equilibrium should be longer for the 100 μm fiber. On the other hand, a large fiber volume increases the capacity to retain an analyte, and therefore an improvement in the sensitivity is to be expected using the 100 μm fiber.

For each fiber coating, the maximum sensitivity is reached when the extraction time is almost equal to the equilibrium time. Time absorption profiles for each analyte were investigated by exposing the selected fibers to working water samples (50 μg/l) for times between 5 and 240 min in order to determine the optimum extraction time. Equilibration time was established by plotting the response of the gas chromatograph in area counts for each herbicide against the exposure time. As can be seen in the curves in Fig. 1, the selected herbicides showed similar tendencies with the three assayed fibers. Nevertheless, the PA coating gave the longest

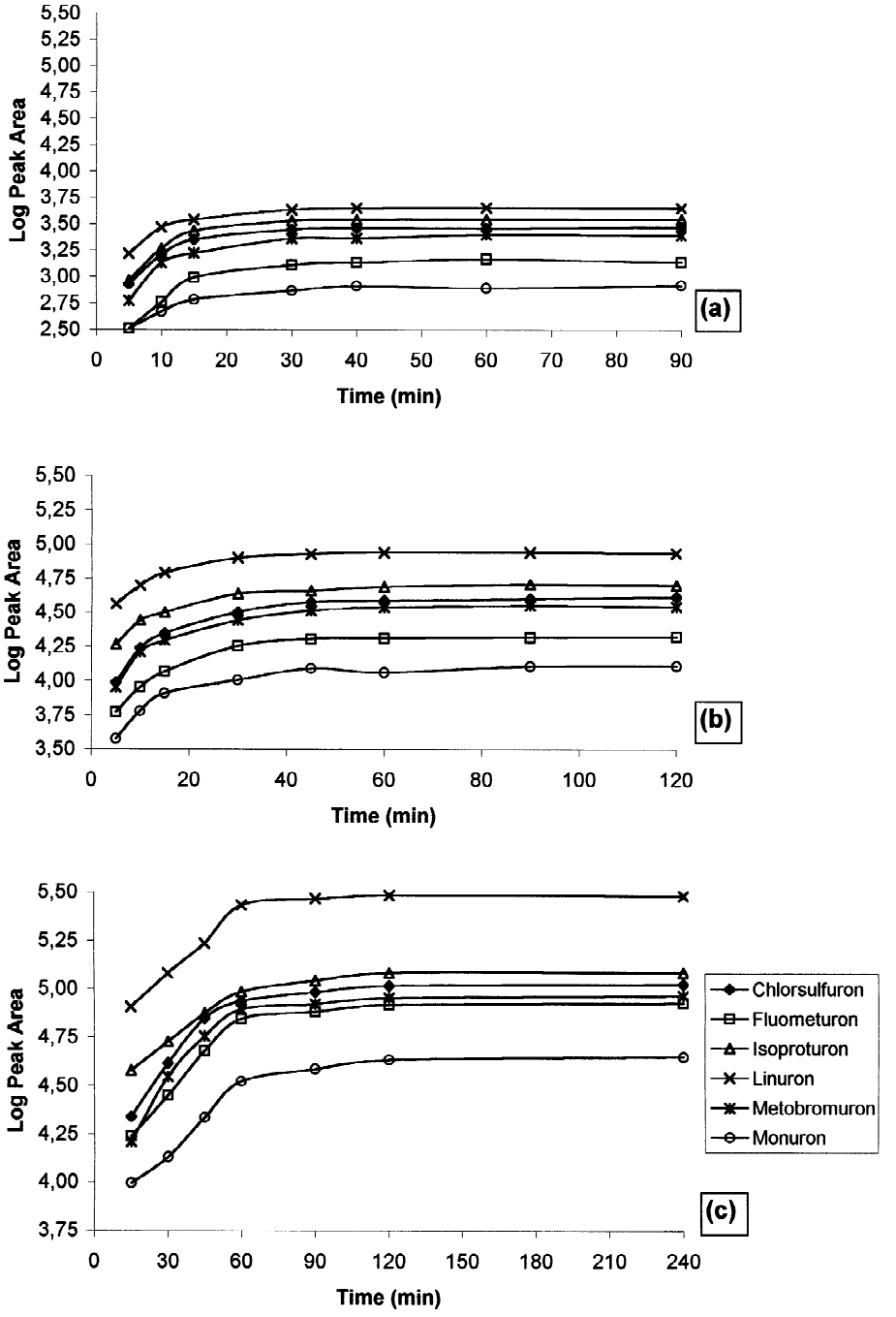

Fig. 1. Absorption time profiles for selected herbicides using 7 μm PDMS (a), 100 μm PDMS (b), and 85 μm PA (c).

equilibrium times (120 min), the 100 μm PDMS reached equilibrium within 50–60 min and the 7 μm PDMS within 30–40 min. The equilibrium times are different for the different fibers, but for a determined fiber they are close for all selected herbicides. The equilibrium times remained unchanged regardless of whether a single pesticide or a mixture of them was extracted.

Because of their water solubility, the selected urea herbicides were considered polar compounds. Therefore, a higher affinity for a polar coating such as PA than for a non-polar one such as PDMS was to be expected. This hypothesis is confirmed in Fig. 1, in which PA extracts the selected herbicides more efficiently than the PDMS fibers. This is true even at 60 min (equilibrium for PA not reached); at this time the amount of extracted herbicide represented 75–90% of the maximum extracted at the equilibrium time (100%).

The volume of the coating was of minor importance. As expected according to the fiber volumes, PDMS 100 μm (0.65 μl) extracts 15 to 19-fold more herbicide than the PDMS 7 μm (0.028 μl).

In order to reconcile good sensitivity with a reasonable extraction time, all further method development and validation were done with the PA fiber at 60 min.

3.2. Optimization of the extraction

The influence of ionic strength (salting out effect) and pH on extraction efficiency were investigated. The ionic strength was modified by diluting NaCl saturated water (35.7%, w/v) with ultra-pure water to obtain more diluted solutions (7.1, 10.8 and 14.3%, w/v). To study the salting out effect, working water was prepared with salted water instead of pure water. The pH effect was studied at pH 4 and pH 9.2, with working water obtained by spiking buffered solutions as opposed to pure water. Extractions at various salt concentrations and pH were compared with control samples without salt or buffer addition.

Fig. 2 shows the salting out effect on SPME of selected herbicides with the PA fiber. The amount of herbicide extracted depended on the salt addition. At 14.3% (w/v) salt addition, the areas increased within 13 and 107%. On the whole, the areas from her-

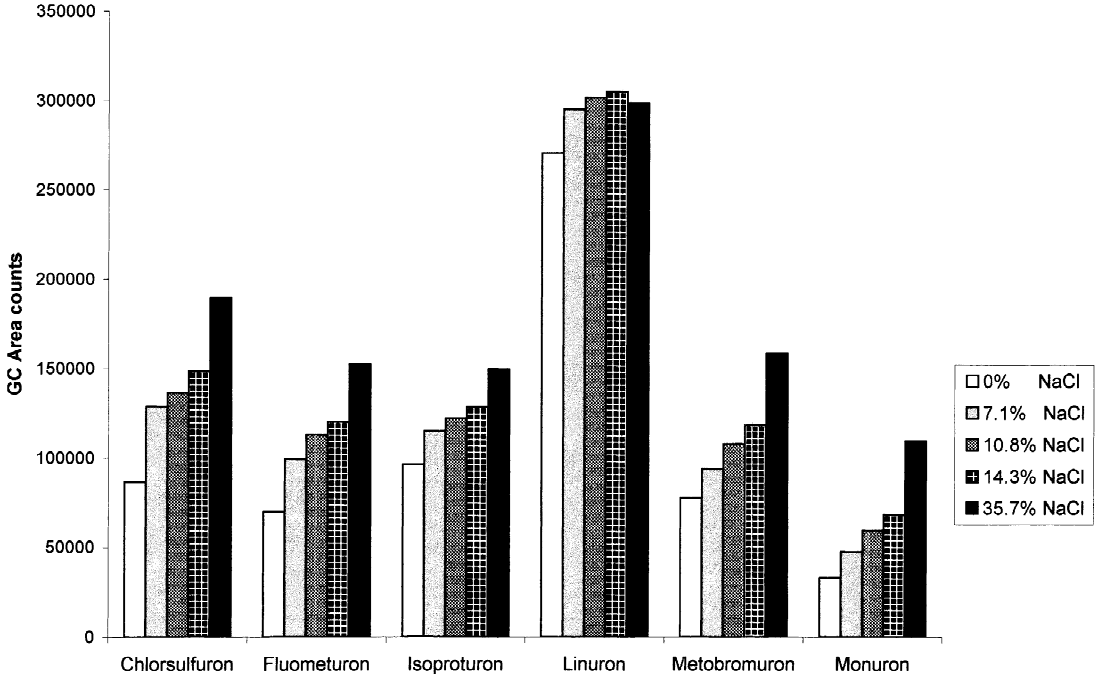

Fig. 2. Effect of NaCl addition on herbicide peak areas with the PA fiber.

Table 1
Physical properties of the selected herbicides. Formula, molecular mass, water solubility, and octanol–water (K_{ow}) and calculated fiber–water (K_{fw}) distribution constants

Herbicide	Formula	Molecular mass	Solubility (mg/l)[a]	K_{ow}	K_{fw} [c]
Chlorsulfuron	$C_{12}H_{12}ClN_5O_4S$	357.78	100–125[d] (25°C)	12.3[e,b]	1028
Fluometuron	$C_{10}H_{11}F_3N_2O$	232.2	105 (20°C)	263[b], 169[a]	624
Isoproturon	$C_{12}H_{18}N_2O$	206.29	72 (20°C)	316[a]	1857
Linuron	$C_9H_{10}Cl_2N_2O_2$	249.1	75–81 (25°C)	575[b], 1000[a]	1613
Metobromuron	$C_9H_{11}BrN_2O_2$	259.11	330 (20°C)	240[b], 257[a]	1343
Monuron	$C_9H_{11}ClN_2O$	198.66	230 (25°C)	96[b]	757

[a] From Ref. [38].
[b] From Ref. [39].
[c] PA fiber, pH 4, 14.3% (w/v) NaCl, 180 min extracting time.
[d] At pH 4.1.
[e] At pH 4.5.

bicides with low K_{ow} such as chlorsulfuron or monuron (see Table 1), which are difficult to extract, were enhanced more. The best extractions were observed at salt saturation, where area growth was between 55 and 230%. Only the most hydrophobic compound (linuron) did not show this improvement. However, working at a high salt concentration facilitates crystal formation thus blocking the fiber protection mechanism and producing a mechanical failure. Other authors [24,31] have already reported this effect. To reduce salt precipitation, further assays were carried out at 14.3% (w/v) sodium chloride and the fiber was submerged in clean water after each run.

On the other hand, buffering at pH 4 increased the chlorsulfuron area by 105% as compared with experiments without pH control, whereas the rest of the herbicides were not affected. Since extraction at pH 9.2 drastically reduced the herbicide areas, all the subsequent SPME extractions were performed at pH 4.0. At acid pH, neutral molecules that have more affinity for the fiber increased in the water sample and extraction improved. In contrast, phenylureas are not acid compounds, and therefore decreasing the pH does not modify the molecular form and extraction remains unaltered.

These observations are consistent with the weak acid properties of sulfonylurea herbicides that have pK_a ranging from 3.3 to 5.2 [28]. In a recent study, Young [29] buffered at pH 3.5 to extract chlorsulfuron and other sulfonylureas from water samples by SPE using a cartridge containing a polymeric sorbent. But Sanchis-Mallols et al. [30] performed SPE of phenylurea herbicides from drinking waters without buffer addition. In that study the authors found no significant changes in the retention factors when the pH of the mobile phase was in the 3 to 7 range, which means that ionization of phenylureas does not occur at these pH.

3.3. Linearity, sensitivity, precision and efficiency of the fiber

Linearity, sensitivity and precision were studied using natural water (Acequia Dreta), which was Whatman No. 1 filtered and then spiked. This water sample was previously analyzed and no herbicide was detected. The results are given in Table 2 The linearity of the method was tested with NPD by extracting spiked samples in quadruplicate at 1, 5, 10, 25, 50, 100 and 250 μg/l levels; correlation coefficients (r) were better than 0.994.

Successive extractions at levels below 1 μg/l provided the information necessary to estimate the limits of detection (LODs) based on the lowest peak with $S/N=3$. The LODs obtained allow the detection of selected herbicides at 0.1 μg/l or less. This is the maximum level of an individual pesticide permitted in the European Union for drinking water. If required, sensitivity can be improved by working at NaCl saturation, by prolonging the extracting time to 120 min, and by using a more sensitive detector system such as mass spectrometry with selected ion monitoring mode (MS–SIM).

As seen in Table 2, the relative standard deviations (RSDs) at three levels calculated from four

Table 2
Gas chromatographic determination of urea herbicides after SPME. Retention times of degradation products identified by mass spectrometry of the following herbicides (t_R)[a]

Herbicide	t_R (min)	Identified compound (molecular mass)	Most significant m/z ions (% relative abundance)	r, (1–250 μg/l)	RSD (%)			LOD (μg/l)	E(%)
					Level 1 μg/l	Level 5 μg/l	Level 50 μg/l		
Chlorsulfuron	8.31	2-Amino-4-methoxy-6-methyl-1,3,5-triazine (140)	140 (73), 110 (72), 69 (100)	0.9980	10.1	8.6	7.2	0.08	26
Fluometuron	5.07	3-Trifluoromethylphenylamine (161)	161 (100), 142 (23), 114 (24) 111 (27)	0.9943	15.4	12.4	10.5	0.10	15
Isoproturon	6.88	4-Isopropylphenylamine (135)	135 (27), 120 (100)	0.9990	13.2	10.8	8.8	0.06	38
Linuron	10.75	3,4-Dichlorophenylamine (161)	165 (10), 163 (64), 161 (100), 126 (11), 99 (15), 90 (13)	0.9997	9.1	7.5	5.9	0.04	32
Metobromuron	8.61	4-Bromophenylamine (171)	173 (98), 171 (100), 92 (80) 65 (89)	0.9959	13.3	12.1	8.2	0.08	26
Monuron	7.25	4-Chlorophenylamine (127)	129 (33), 127 (100), 92 (12) 65 (29)	0.9992	14.5	9.2	7.5	0.10	20

[a] The coefficients of correlation (r), precision (RSD, $n=4$) at three levels and limits of detection (LODs) were calculated from filtered natural water with NPD. The extraction coefficients (E%) were studied from spiked ultra-pure water (pH 4, 14.3%, w/v, sodium chloride) with NPD.

replicates ranged from 5.9 to 15.4%. No carryover effect was observed at blanks within the extractions.

Fiber efficiency was studied with the working water samples in ultra pure water. Calculation of extraction coefficients can not be based on external standard injection because the selected herbicides are degraded at the injector port to anilines and a triazine (as discussed below), whereas injection of ureas prepared in organic solvents generates other compounds such as isocyanates or esters of carbamic acid [33]. For calculation of extraction coefficients (E), the spiked water is successively extracted four times. Assays are performed in quadruplicate.

The extraction coefficient E_1 (per unit) of the first extraction can be calculated from,

$$E_1 = A_1/A_0$$

where A_0 is the area corresponding to the total amount of analyte in the water sample before the extraction (unknown) and A_1 the area corresponding to the amount extracted at the first extraction (known). Similarly, for the second extraction and successive 'i' extractions E can be calculated from,

$$E_2 = A_2/(A_0 - A_1) \text{ and}$$

$$E_i = A_i/[A_0 - (A_1 + A_2 + \cdots + A_{i-1})]$$

Assuming that extraction coefficients remain constant at the assay levels:

$$E_1 = E_2 = \cdots = E_i$$

This allows 'E' to be calculated by re-extracting the same sample twice. Nevertheless, each sample was re-extracted four times to assure that extraction coefficients remained unaltered at the assayed levels. The extraction coefficients (E) calculated in this form are given in Table 2. With the proposed SPME conditions, extraction coefficients ranged from 15 to 38%. These extraction coefficients correspond typically to compounds having a medium affinity for the fiber. This affinity was artificially increased by pH control and adding salt. Affinity and therefore recoveries are lower when the matrix is not modified (ultra-pure water). A similar method allowing the calculation of the amount of analyte extracted by a fiber by running successive SPMEs has recently been published by Urruty and Montury [7].

3.4. Relation between the octanol–water (K_{ow}) and the fiber–water (K_{fw}) distribution constants.

Several authors found the octanol–water (K_{ow}) and fiber–water (K_{fw}) partition coefficients to be directly related in the case of compounds such as

organochlorine compounds [9,34], PAHs [8,9] and triazines [18]. For other contaminants such as PCBs [10] or organochlorine pesticides [3], the K_{ow}/K_{fw} relationship was not evident. These last authors [3,10] therefore suggest that if adsorption processes predominate or coexist with absorption ones, the K_{ow}/K_{fw} interrelation disappears. Other authors disagree and indicate that these adsorption phenomena were due to adsorption onto materials such as stir bars and glass vials but not onto the PDMS fiber [34].

Since K_{fw} is directly related to the amount of extracted analyte, and thus to the signal generated in the detector, we examined the question of whether a similar K_{ow}/K_{fw} association exists in the case of the selected urea herbicides.

Extractions at 50 μg/l level were performed for 180 min (over equilibrium time) and vials were completely filled to avoid headspace. The resulting NPD signal in area counts affected by the number of nitrogen atoms included in the molecule was plotted as a function of the K_{ow} for each herbicide obtained from the literature. Linear relationships with correlation coefficients (r) ranging from 0.98 to 0.96 were found depending on the K_{ow} bibliographic source. This seems to indicate that herbicide extraction using PA fiber is mainly governed by absorption processes, and we therefore expected at this point that the K_{ow} and the apparent K_{fw} would correlate for the selected herbicides. As a consequence, we proceeded to the K_{fw} calculation to confirm this hypothesis.

The following equation,

$$K_{fw} = nV_s/V_f(C_0V_s - n)$$

where 'n' is the amount of the extracted analyte, V_s is the sample volume, V_f is the fiber volume and C_0 is the initial concentration, makes it possible to calculate the K_{fw}. Here, K_{fw} is apparent because the extractions were carried out with modified water (pH 4, 14.3% sodium chloride). According to the extraction coefficients, low K_{fw} were to be expected. The calculated K_{fw} are shown in Table 1 together with the K_{ow} obtained from the literature. The expected linear relationship between K_{fw} and K_{ow} for the selected herbicides presented correlation coefficients ranging from 0.59 to 0.66. This decrease in the linear correlation was attributed to both the different K_{ow} values obtained from the literature and the uncertainty of the indirect K_{fw} calculation.

The selected herbicides showed K_{fw} values ranging from 624 for fluometuron to 1857 for isoproturon, thus indicating an intermediate affinity for the PA fiber. Salting out and pH control had an equalizing effect because affinity for the fiber in the case of the herbicides with low K_{ow} values increased more, whereas herbicides with higher K_{ow} values were influenced less.

3.5. Identification of the determined products

Urea herbicides are decomposed at the injector to products with good gas chromatographic properties. To identify these compounds, the fiber was manually inserted immediately after extraction at the injector port of the Fisons 8000/Trio 1000 gas chromatograph/mass spectrometer. Extractions were performed either with individual herbicides (200 μg/l) or a mixture of them (200 μg/l each).

The homologous anilines from phenylureas and a triazine from chlorsulfuron were identified. The characteristic ions and the abundance of these derived compounds are shown in Table 2. Mass spectra of anilines had intense molecular ions that coincided with the base peak $[M]^+$, except for the aniline formed from isoproturon, in which the base peak corresponded to the loss of a methyl group [120; $M–CH_3]^+$. The typical abundance of some isotopes revealed the presence of halogen atoms on the base peak of the anilines formed from linuron [163 (64%), 2 Cl], metobromuron [173 (98%), 1 Br] and monuron [129 (32%), 1 Cl]. Other characteristic but less intense m/z ions corresponded to the loss of halogen atoms. This was observed in the anilines derived from fluometuron [142; $M–F]^+$, linuron [126; $M–Cl]^+$, metobromuron [92; $M–Br]^+$, and monuron [92; $M–Cl]^+$.

The spectra show low fragmentation with characteristic high masses. These conditions make it possible to reach very good sensitivity using the SIM detection mode. Fluometuron and linuron had the same base peak (m/z 161), but their retention times were clearly different.

3.6. Environmental water analysis

Two and a half litres of water samples were taken at five sites (Gola de Puçol, Racó de l'Olla, Acequia Dreta, Acequia Rodena, and Acequia del Canal)

from the Albufera Lake (Valencia, Spain). The Albufera lake is a protected area of great environmental interest, where an abundant biomass including unique species coexists with intense agricultural activity, mainly rice growing. Sampling was done just before the rice was planted. At that time the organic content of the samples ranged from 3 to 7 mg O_2/l, and the pH from 6.8 to 7.8. The samples were passed through a Whatman No. 1 filter prior to analysis. An appropriate amount of sodium hydrogentartrate and sodium chloride was added to all the samples to reach pH 4 and 14.3% sodium chloride content. None of the selected herbicides were detected in the Albufera water samples. The profiles of the chromatograms were practically as clean as those of pure Milli-Q water, which indicates that other contaminants giving NPD response were not co-extracted. Therefore, the sample from the Acequia Dreta was utilized to study linearity, sensitivity and precision. Fig. 3 shows the chromatogram corresponding to the non spiked Acequia Dreta sample. To prevent sodium chloride precipitation several assays with the spiked sample were performed with lower sodium chloride content (10.8%, w/v) than that proposed in the final method (14.3%, w/v). The corresponding chromatograms are shown in Fig. 3b and c, respectively. In these assays the losses of chlorsulfuron (peak 1) and linuron (peak 6) were greater than those due to the differences in salt content. This negative effect was initially attributed to the dissolved organic matter. To corroborate the hypothesis, an assay with spiked ultra pure water containing 10.8% sodium chloride and humic acid added to 10 mg/l (equivalent to 7.3 mg O_2/l) was run. In this assay extraction of chlorsulfuron and linuron decreased by 53% and 20% respectively, while the use of 10.8% instead of 14.3% sodium chloride without humic acid only produced 9% losses of chlorsulfuron and 2% of linuron. When the assays were performed with 14.3% (w/v) salt saturation, the recoveries from ultra pure water, water containing 10 mg/l humic acid and Acequia Dreta water were indistinguishable. Humic acid was not extracted by the fiber or if it was, it was only to a small extent. Humic acid modifies the fiber–water partition by increasing the water solubility of the herbicides which are bonded to the humic acid macromolecule. The presence of a large amount of ions liberated the herbicides from humic acid union.

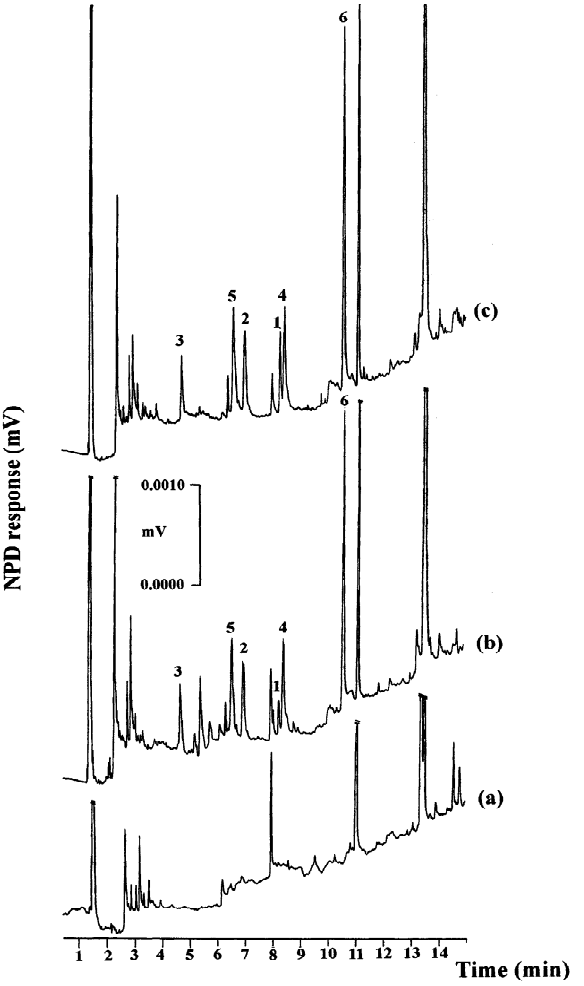

Fig. 3. Chromatograms from the SPME of Acequia Dreta water sample buffered at pH 4; non spiked and containing 14.3% (w/v) NaCl (a), spiked and containing only 10.8% (w/v) of NaCl (b), spiked and containing 14.3% (w/v) NaCl (c). Peak assignment and level of spike; **1**=chlorsulfuron (0.32 μg/l), **2**=monuron (0.5 μg/l), **3**=fluometuron (0.5 μg/l), **4**=metobromuron (0.32 μg/l), **5**=isoproturon (0.30 μg/l), **6**=linuron (0.5 μg/l).

For this reason, when natural water is to be analyzed, the organic matter should be evaluated and sodium chloride may need to be added to correct losses generated by the presence of humic acid. The effect of humic acid was first observed when carbamates were extracted from natural waters by SPE [35], and sodium chloride addition was proposed to improve recoveries. The influence of the presence of organic matter on SPME has also been studied by Pörschmann et al. [36,37] for some organic pollu-

tants. In these studies the bonded fraction of PAHs and phenols to humic organic matter was calculated using PDMS fibers.

In summary, SPME using a PA coating fiber is a sensitive and reproducible technique for the detection of the selected urea pesticides in water. The affinity of the herbicides for the PA fiber is increased by NaCl addition and pH control, and allows detection of almost 0.1 μg/l of the selected urea herbicides. In these conditions the apparent K_{fw} range from 624 to 1857. The presence of humic acid in environmental waters reduces the extraction efficiency, but reinforcing the ionic strength prevents this effect. Since the proposed method is based on an indirect determination, unequivocal identification of herbicides should be done by LC–MS. The proposed method is a simple, rapid and economical way to rule out the presence of selected herbicides in water at trace levels, leaving the more sophisticated equipment for confirming positive samples.

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