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Use of Solid-Phase Microextraction for the Quantitative Determination of Herbicides in Soil and Water Samples

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An in-depth study of SPME optimization and application has been made, considering not only aqueous (surface water and groundwater samples) but also the more complex soil samples. Seven herbicides widely used in the area of study have been selected including five triazine herbicides (atrazine, simazine, terbumeton, terbuthylazine, terbutryn), molinate, and bromacil. Linearity range was between 0.1 and 10 ng/mL and the repeatability below 10% when applying the optimized SPME procedure to water samples. Reproducibility was found to be lower than 20% at the 1 ng/mL level, and the limits of determination in environmental water samples using GC/MS (SIM mode) were well below 0.1 ng/mL (values ranging from 10 to 60 ng/L). Extraction of selected herbicides from soil was carried out by microwave-assisted solvent extraction using methanol in screw-capped vials, leading to recoveries over 80% in spiked soil samples at the 5−200 ng/g level. SPME application over methanolic soil extracts required a 10-fold dilution with distilled water. The recommended procedure was found to be fully applicable for quantitative determination of selected herbicides in soils containing low organic matter content with coefficients of variation below or around 10% and limits of determination ranging from 1 to 10 ng/g. Both procedures were applied to real-world surface water and soil samples where several pesticides were detected including atrazine, simazine, terbuthylazine, and molinate.

The presence of pesticides in the environment is a problem that has caused great social and scientific concern in the last years as can be deduced from the restrictive legislation developed by various governments. As a result, both EPA regulations and EU legislation established a maximum pesticide residue level in water supply samples[1] that is in the range of low parts-per-billion. This has forced the scientific community to develop analytical procedures that can be used to determine not only the presence of pesticides in environmental samples but also their concentrations with a good accuracy. These methods have to be robust, precise, and sensitive to be used in regulatory situations. In most cases, determination of pesticide residues in water, or other environmental samples, relies on the use of liquid−liquid extraction, solid-phase extraction, or supercritical fluid extraction as described in many papers and as is referenced in several EPA methods.[2−7] These procedures are usually expensive and labor- and time-consuming because in most of cases typical environmental samples cannot be directly analyzed by the usual chromatographic methods applied. Sample matrixes range from relatively simple, as in groundwater, to more complex as in surface water, wastewater, or soil samples. Additionally, the concentration levels are too low to allow the determination without a preconcentration step. In this way, some papers have been published dealing with the trends and strategies in sample preparation for organic micropollutant determination in environmental samples.[8−10] It is widely accepted that, at the moment, a great number of research activities are oriented to develop simple (preferably in one step) sample preparation procedures that could be automated and coupled on-line with the final analytical measurement step. Sample treatment simplification accounts for several aspects related to cost saving (by reduction of time, laboratory staff, and solvent consumption) and ecological and toxicological concern (by dramatically decreasing solvent residues and by eliminating the use of highly toxic chlorinated solvents), taking profit from the amazing progress made in analytical instrumentation.

As a result of the effort devoted in this research field of sample treatment reduction, Pawliszyn and co-workers developed in the early 1990s the solid-phase microextraction technique (SPME),[11,12] which provides a simple solvent-free approach for organic pollutant

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(1) EC Drinking Water Guideline, 98/83/CE, Brussels, November 1998.

(2) DFG. *Manual of Pesticide Residue Analysis;* WCH: Weinheim, Germany, 1992; Vol. II.

(3) Clesceri, L. S., Greenberg, A. E., Trussell, R. R., Eds. *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association, American Water Works Association, Water Environment Federation: Washington, DC, 1998.

(4) Hernandez, F.; Morell, I.; Beltran, J.; Lopez, F. J. *Chromatographia* **1993**, *37*, 303−312.

(5) Beltran, J.; Lopez, F.; Forcada, M.; Hernandez, F. *Anal. Chim. Acta* **1997**, *356*, 125−133.

(6) Font, G.; Manes, J.; Molto, J. C.; Pico, Y. *J. Chromatogr.* **1993**, *642*, 135−161.

(7) EPA Method 565.1. Determination of organic compounds in drinking-water by liquid solid extraction and capillary column GC/MS. Revision 2.2, May **1991**, Environmental Monitoring System Laboratory, US EPA, Cincinnati, OH, 45268, 1991; p 323.

(8) Balinova, A. *J. Chromatogr., A* **1996**, *754*, 125−135.

(9) Poole, C. F.; Poole, S. K. *Anal.Commun.* **1996**, *33*, 11H-14H.

(10) Hogendoorn, E. A.; Dijkman; E., Baumann, B.; Hidalgo, C.; Sancho, J. V.; Hernandez, F. *Anal. Chem.* **1999**, *71*, 1111−1118.

(11) Arthur, C. L.; Pawliszyn, J. *Anal. Chem.* **1990**, *62*, 2145−2148.

(12) Arthur, C. L.; Killam, L. M.; Buchholz, K. D.; Berg, J. R. *Anal. Chem.* **1992**, *64*, 1960−1966.

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determination. This technique is based on extraction using a polymeric-coated fused-silica fiber, usually housed in a modified syringe, which allows easy manipulation from sample extraction to final desorption in the inlet port of a gas chromatograph. This technique has revealed itself as a good alternative to traditional extraction procedures, mainly when dealing with water samples, with some already known advantages (no solvent used, low sample volume, use of the whole sample for determination, single-step sample preparation).[13,14] Although the extraction procedure can be applied in an automated mode (using a modified autosampler device), most applications rely on manual extraction of aqueous (mainly water) samples for the determination of a great variety of compounds including BTEX, halocarbons, VOCs, food components, PAH, PCBs, or even organometallic compounds. SPME has an increasing interest in the field of pesticide residue analysis as shown in the literature.[15] It has been applied to the determination of several organophosphorus,[16−18] organochlorine,[19,20] or triazine compounds,[20,21] among others.[22] In most of cases, SPME is performed by direct dipping of the fiber into the aqueous sample, but it can also be carried out by sampling the headspace of the sample contained in a hot vial.[20,23]

While application of SPME to water samples can be easily achieved, determination of pesticides in soil samples poses additional questions about SPME use. In fact, there are only a few references on SPME application to the determination of pesticides in soils. Extraction can be achieved by direct dipping of the fiber in an aqueous suspension of soil[24] or by dilution with water of the organic extract obtained by a conventional solid−liquid extraction. Several disadvantages can be pointed out to both approaches related with fiber stability (physical or chemical) and sensitivity.

In this paper, an in-depth study on the applicability of SPME to herbicide determination in water and soil samples has been done. Optimization of the variables involved in the SPME procedure has been carried out by a well-structured step-by-step approach using spiked water samples. Determination of herbicides in soil samples required the extraction of compounds from the soil prior to SPME. Therefore, this preliminary step was studied in order to obtain soil extracts compatible with the subsequent SPME using efficient extraction systems. Microwave-assisted solvent extraction (MASE) using organic solvents as described in several papers[9,25,26] and water (used in recent papers describing the extraction of low-polarity compounds by using subcritical water)[27,28] has been assayed. Moreover, extraction by ultrasonics has also been performed.

We propose two multiresidual methods for the determination of seven selected herbicides widely used nowadays (five triazines, molinate, and bromacil) in water and soil samples by applying SPME based on the use of a recently developed fiber (Carbowax/divinylbenzene). The characteristics of this fiber, its robustness, and its behavior under different experimental conditions are also discussed. The selected herbicides can easily be analyzed by GC and they are relevant from an environmental point of view as they have been often detected in soils and water samples from agricultural areas.

EXPERIMENTAL SECTION

1. Chemicals. A stock standard mixture containing seven herbicides (Table 1) (Dr. Erhenstorfer) was prepared in acetone in a concentration of 50 μg/mL and stored at 4 °C. Working standard solutions (0.1−5 μg/mL) were prepared by volume dilution in acetone, hexane, or methanol and renewed every two weeks. Spiked water samples were prepared weekly by adding the appropriate volume of the methanol pesticide standard solution to the water sample, keeping the final concentration of organic solvent in the sample at a maximum of 1%.

Organic solvents (hexane, acetone, acetonitrile, methanol) were of ultratrace analysis quality (Scharlau). Sodium chloride (analytical grade, Scharlau) was purified by overnight heating at 300 °C. All glassware was cleaned with laboratory detergent, rinsed with tap and distilled water, rinsed with acetone, and finally baked in an oven at 300 °C overnight. Clean glassware was stored wrapped with aluminum foil. Purified NANOpure HPLC water was used for SPME optimization experiments.

2. Equipment. (a) The SPME device for manual extraction used, consisting in a holder assembly and several replaceable fiber assemblies, was purchased from Supelco. Four different fiber types were compared: from nonpolar poly(dimethylsiloxane) (100 μm) to more polar polyacrylate (85 μm), Carboxen/PDMS (75 μm), and Carbowax/divinylbenzene (65 μm). The fibers were conditioned prior to use as recommended by the manufacturer by heating them at different temperatures (250−300 °C) for periods between 30 min and 2 h in the gas chromatograph injection port.

(b) Clear glass vials (4 mL, Supelco) were used to perform SPME under magnetic stirring using a Teflon bar (10 mm × 6 mm o.d.) and a magnetic stirrer (model A-159, SBS). A microwave oven (OI Analytical 7195), controlled with an external computer, and an ultrasonic bath (Ultrasons Selecta) were used for the extraction of pesticides from soil samples.

(c) Chromatographic analyses were performed using a Hewlett-Packard 5890 Series II gas chromatograph equipped with an splitless injector and mass spectrometric detector (Hewlett-Packard 5971 MSD). The GC was fitted with a 25 m × 0.2 mm i.d., film thickness, 0.33 μm Ultra 2 (5% phenyl methyl silicone) column (Hewlett-Packard). Injector temperature was investigated in the study, while the temperature program used was as follows: 90 °C (5.5 min); then 30 °C/min to 170 °C; then 4 °C/

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(13) Ai, J. *Anal. Chem.* **1997**, *69*, 1230−1236.

(14) Dean, J.; Tomlinson, W.; Maskovskaya, V.; Cumming, R.; Hetheridge, M.; Comber, M. *Anal. Chem.* **1996**, *68*, 130−133.

(15) Beltran, J.; Lopez, F.; Hernandez, F. *J. Chromatogr., A*. In press.

(16) Lopez-Avila, V.; Young, R.; Beckert, W. F. *J. High Resolut. Chromatogr.* **1997**, *20*, 487−492.

(17) Beltran, J.; Lopez, F. J.; Cepria, O.; Hernandez, F. *J. Chromatogr., A* **1998**, *808*, 257−263.

(18) Valor, I.; Molto, J. C.; Apraiz, D.; Font, G. *J. Chromatogr., A* **1997**, *767*, 195−203.

(19) Jackson, G. P.; Andrews, A. R. J. *Analyst* **1998**, *123*, 1085−1090.

(20) Eisert, R.; Levsen, K. *J. Chromatogr., A* **1996**, *737*, 59−65.

(21) Eisert, R.; Levsen, K. *Fresenius J. Anal. Chem.* **1995**, *351*, 555−562.

(22) Lee, M.; Lee, R. J.; Lin, Y.; Chen, C. M. *Anal. Chem.* **1998**, *70*, 1963−1968.

(23) Lee, X. P.; Kumazawa, T.; Sato, K.; Suzuki, O. *Chromatographia* **1996**, *42*, 135−140.

(24) Boyd-Boland, A. A.; Pawliszyn, J. *J. Chromatogr., A* **1995**, *704*, 163−172.

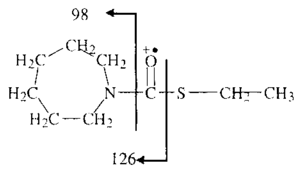
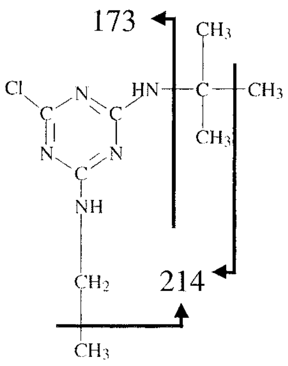
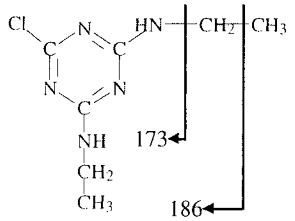
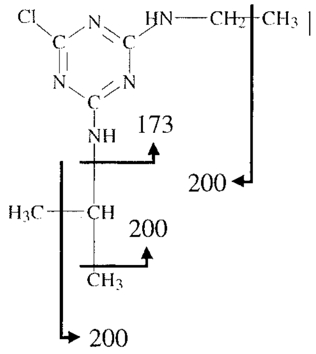
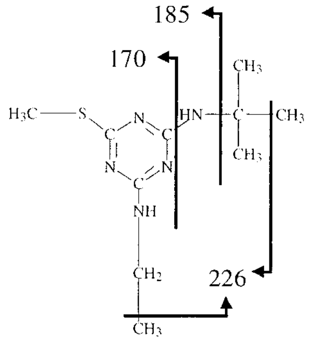
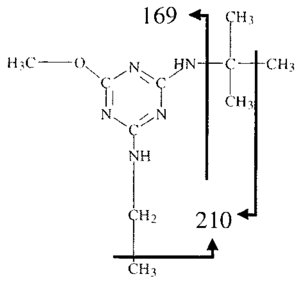
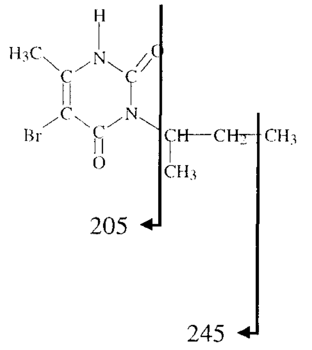
(25) Font, N.; Hernandez, F.; Hogendoorn E. A. Baumann, R. A.; van Zoonen, P. *J. Chromatogr., A* **1998**, *798*, 179−186.

(26) Molins, C.; Hogendoorn, E. A.; Heusinkveld, H. A. G.; van Beuzekom, A. C.; van zoonen, P.; Baumann, R. A.*Chromatographia* **1998**, *48*, 450−456.

(27) Wang, Y. W.; Bonilla, M.; Mcnair, H. M.; Khaled, M.*J. High Resolut. Chromatogr.* **1997**, *20*, 213-216.

(28) Hawthorne, S. B.; Grabanski, C. B.; Hageman, K. J.; Miller, D. J. *J. Chromatogr., A* **1998**, *814*, 151−160.

Table 1. List of Compounds Studied, Molecular Structure, and *m*/*z* Selected for SIM Mass Detection

Herbicide	Molecular structure	m/z	Herbicide	Molecular structure	m/z
Molinate		126[a], **187**[b]	Terbuthylazine		214, 173, **229**
Simazine		**201**, 186, 173			
Atrazine		200, **215**, 173	Terbutryn		226, **241**, 185, 170
Terbumeton		210, **225**, 169	Bromacil		205, 207, (**260**)

[a] Underline indicates spectrum base peak. [b] Boldface indicates molecular mass peak.

min to 270 °C (hold time 5 min). Helium was used as carrier gas with a flow of 0.5 mL/min (linear velocity ~40 cm/s). MSD conditions were as follows: transfer line temperature 280 °C; 70 eV electron impact; electron multiplier voltage 2100 V; mass range for full-scan experiments 80−350 *m*/*z*. Several *m*/*z* values were used for selected ion monitoring (SIM) mass detection as shown in Table 1.

3. SPME Procedure Optimization. The development of the SPME procedure required the optimization of a series of variables involved in both absorption and desorption steps, as referenced by several authors.[18,24,29] The fibers used included, as indicated, the PDMS, PA, CX/PDMS, and CW/DVB, covering a wide range of polarities. Desorption of herbicides previously retained in the fiber was optimized considering both desorption time and temperature. The effect of chemical composition of the samples on the extraction efficiency was investigated by controlling ionic strength (adding NaCl to reach concentrations up to 30%) and organic solvent content (acetone and methanol in concentrations up to 10%). All these experiments were carried out in triplicate. Simultaneously, the adsorption time profiles for the studied herbicides were obtained, covering a range between 5 min and 2 h. Special attention has been given to the stability of the CW/DVB fiber under different sodium chloride concentrations (used for salting-out effect). Once the optimum conditions were determined, the analytical characteristics of the procedure were obtained for precision, linearity range, reproducibility, and limits of determination.

4. Soil Extraction Optimization. The SPME procedure was applied to the determination of the studied herbicides in soil samples. The general extraction procedure consisted in two separate off-line steps: first, extraction of herbicides from the soil and then SPME over 3 mL of soil extract. Extraction of herbicides from soil samples was carried out by using either water or

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(29) Magdic, S.; Pawliszyn, J. *J. Chromatogr., A* **1996**, *723*, 111−122.

Table 2. Physicochemical Characteristics of the Soil Samples

sample	soil 1	soil 2	soil 3
pH	8.43	8.42	8.44
clay, %	22	32	14
silt, %	18	20	22
sand, %	60	48	64
organic matter, %	5.05	0.95	2.53

methanol by heating in a microwave oven (MASE) or by ultrasonic agitation. Extraction of 3−5 g of dried soil was carried out using the solvent in a 1:1 ratio (solvent/soil). Variables studied included extraction time and, in the case of MASE, the microwave power applied over the extraction chamber (closed glass vials). Soil suspensions were separated by centrifugation, and the liquid phase was separated using a Pasteur pipet. The aqueous soil extracts were directly subjected to SPME using the procedure described for water samples. When the extraction was carried out using organic solvents, the extract was centrifuged, an aliquot was diluted with distilled water, and then an aliquot of 3 mL was subjected to SPME.

Soil samples used in this study included three soils taken from agricultural areas at different depths. Table 2 gives some details of their composition according to organic matter content, pH, and texture.

5. Recommended Procedures for Water and Soil Samples. Extraction of water samples was carried out by direct dipping of the fiber into the sample (3 mL, 10% NaCl) contained in a 4-mL clear glass vial under magnetic stirring for 30 min at ambient temperature. As recommended by Eisert and Pawliszyn,[30] the fiber was situated off-center in the vial so the sample flows perpendicular to the fiber axis. Desorption of pesticides was carried out at 240 °C for 5 min in the hot split−splitless injector.

Extraction of pesticides from soil was carried out over 5 g of soil (previously dried and sieved through 2-mm mesh) with 5 mL of methanol in glass vials (10 mL) with Teflon-lined screw caps in a microwave oven (1.5 min at 20% of maximum power). The soil suspension was centrifuged, and then 2 mL of the methanolic extract was taken and diluted with distilled water up to 20 mL. Finally, 3 mL of this aqueous extract was subjected to SPME as indicated before.

6. Application to Real-World Water and Soil Samples. Surface water samples collected from three different wetland areas in the Comunidad Valenciana (Spain) during 1998 and 1999 were analyzed in triplicate using the recommended procedure. First, a chromatographic method based on full-scan detection was applied in order to determine the presence of pollutants. Then quantitation of pesticides detected was carried out by using a SIM program method prepared with some characteristic ions of the pesticides quantified. Quantitation of the samples was achieved by solid-phase microextraction, comparing peak areas to aqueous standard solutions prepared by spiking NANOpure water using external the standard method.

A soil sample in which atrazine residues were expected to be found was also analyzed by MASE (methanol) and then SPME.

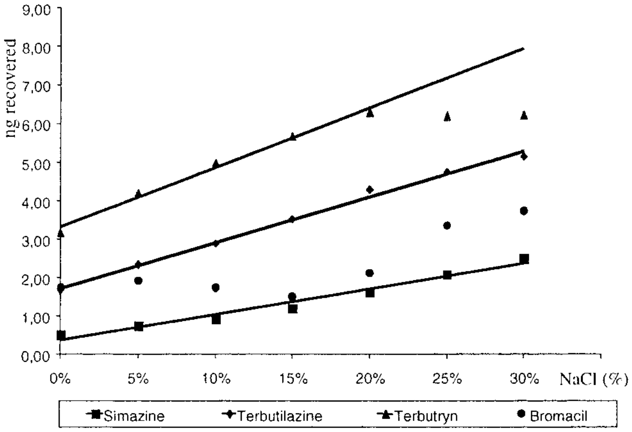

Figure 1. Effect of NaCl content on SPME efficiency for selected herbicides.

The final chromatographic determination was performed by GC/MSD in full-scan mode to both identify and quantify atrazine.

RESULTS AND DISCUSSION

SPME Optimization. As indicated in the introduction, the aim of this study was the development of a multiresidue procedure for the determination of seven herbicides with a variety of chemical characteristics (Table 1). Therefore, the choice of the fiber was of outstanding importance in order to achieve the higher extraction efficiency for all compounds studied.

In a first approach, and following the scheme described in a previous paper,[17] a simultaneous optimization of desorption conditions and type of fiber was carried out after fixing the absorption characteristics (30-min extraction of 3 mL of water sample containing 10% NaCl using magnetic stirring). The best global results were obtained with the recently developed CW/DVB fiber, as it allowed the extraction of all pesticides studied, including simazine, atrazine, and bromacil, which showed low extraction efficiencies with the other fibers tested. This fiber was selected to further proceed with method development, with desorption temperature fixed at 240 °C, during 5 min. The desorption temperature was lower than the recommended working temperature indicated by the manufacturer and it was selected to avoid thermal degradation of the fiber.

The first variable considered during absorption optimization was, as indicated in many papers, the ionic strength in order to use the salting-out effect that has been extensively used in liquid−liquid and solid-phase extraction. The effect of NaCl (or other ionic salts) on SPME efficiency has been widely discussed, but some contradictory results have been reported.[16,29] In our case, all pesticides studied, except the more polar bromacil (water solubility 815 mg/L), showed an increase in extraction efficiency with NaCl concentration up to 30%, following a linear relationship between extracted amount (ng of pesticide) and NaCl percentage (Figure 1). From the results obtained it was evident that a 30% NaCl content allowed a more efficient extraction of the herbicides. However, we proved that a very fast degradation of the fiber occurred under these conditions, it being possible to use the same fiber for only about 10−20 injections. This unexpected fact, not referenced previously, forced us to perform many additional experiments until we found the reasons for the excessively short duration of the fiber. Finally, by using an optical microscope (Uni

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(30) Eisert, R.; Pawliszyn, J. *J. Chromatogr., A* **1997**, *776*, 293−303.

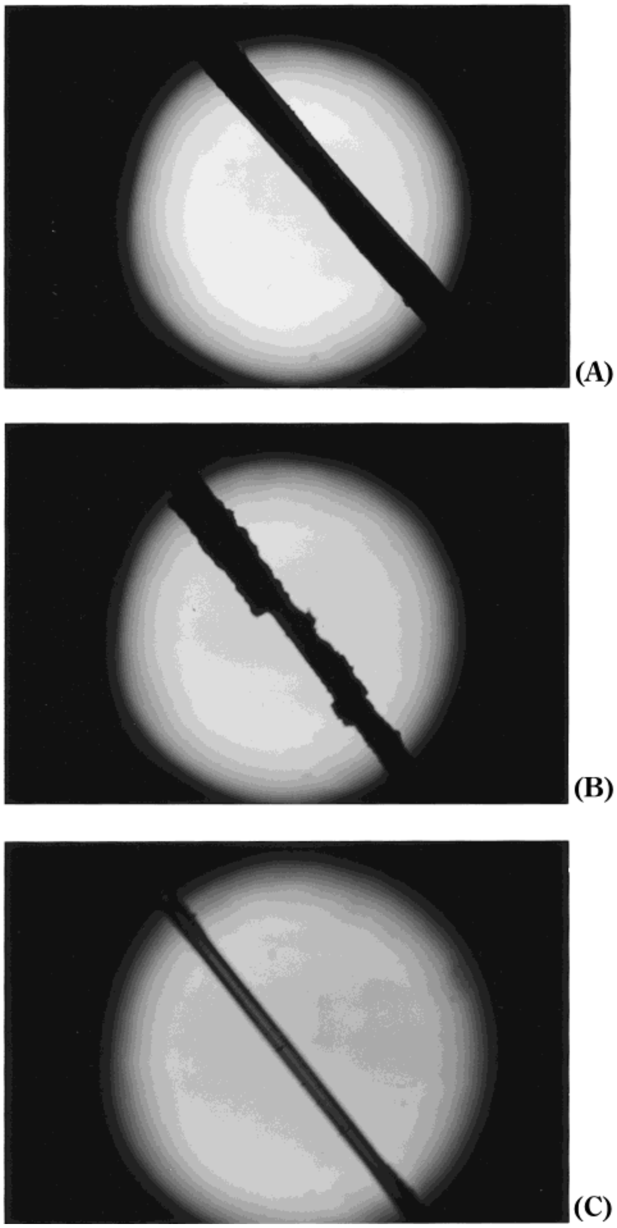

Figure 2. Photographs of the CW/DVB fiber obtained using an optical microscope: (A) new fiber, (B) after 6 extractions using 30% of NaCl, and (C) after 15 extractions using 30% of NaCl.

Iberica SL), it was possible to determine that a completely new fiber (Figure 2A) suffered stripping of the polymeric phase after only 6 injections (Figure 2B), leading to a complete removal of the fiber coating after 15 extractions using 30% of NaCl (Figure 2C). This results forced us to use a maximum concentration of 10% NaCl, and under this condition, it was feasible to use a single fiber for extraction of over 100 samples without significant degradation of the fiber coating. After lowering the salt content in the sample, a good compromise between extraction efficiency and fiber duration was reached, and results obtained in subsequent experiments were satisfactory. While writing this paper we have read the work of Moder et al.,[31] where they obtained high extraction yields for selected pesticides in the CW/DVB fiber, but very low stability of this coating, lasting only 10−15 extraction cycles. Although the authors did not mention the reason for the low stability of this fiber, the high salt content of the water (25%) was possibly the cause of the lower ruggedness of the CW/DVB coated fiber.

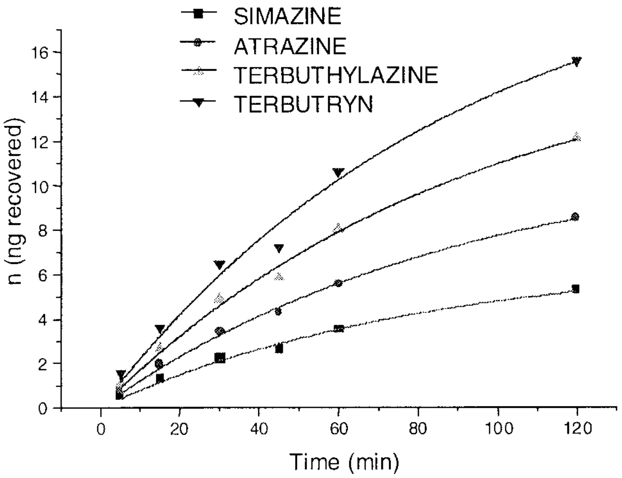

Figure 3. SPME absorption time profiles for four of the herbicides studied from the aqueous solution using the CW/DVB fiber. Plotted lines are regressions using eq 1.

The next step in method development was to obtain absorption time profiles for the herbicides selected. Thus, a number of spiked water sample aliquots were extracted using a CW/DVB fiber (3 mL of 10 ng/mL water sample containing 0.3 g of NaCl, desorption at 240 °C during 5 min) for times ranging from 5 min to 2 h.

Figure 3 shows the curves obtained when plotting the amount extracted for each pesticide versus extraction time. It can be deduced from the curves that most compounds are clearly away from equilibrium conditions even after 120 min. Experimental curves were fitted to an equation (eq 1), formerly proposed by

$$n = n_0[1 - \mathrm{e}^{-at}] \qquad (1)$$

Ai[13] and tested by us in a previous paper,[17] using MicroCal Origin in order to calculate the theoretical equilibrium time and the maximum extractable quantity (n_0).

Results obtained (Table 3) indicate that equilibrium should require extraction times ranging between 2.2 h (molinate) and 4.6 h (terbumeton). These results are consistent with the fact that compounds under investigation have relatively high partition coefficients (K_{SPME}), and thus, longer equilibration times should be expected.[24,29] The K_{SPME} values were calculated by extracting 30 mL of sample during ~15 h and by applying eq 2 as described

$$K_{\mathrm{SPME}} = \frac{n_{\mathrm{S}} V_{\mathrm{aq}}}{V_{\mathrm{S}}(V_{\mathrm{aq}} C_{\mathrm{aq}} - n_{\mathrm{S}})} \qquad (2)$$

in several papers,[14,24,29,32]where n_{S} is the mass of analyte extracted at the equilibrium, V_{aq} is the sample volume, V_{S} is the volume of polymeric stationary phase (0.35 μL for the CW-DVB), and C_{aq} is the initial concentration of analyte in solution.

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[31] Moder, M.; Popp, P.; Eisert, R.; Pawliszyn, J. *Fresenius J. Anal. Chem*. **1999**, *363*, 680−685

[32] Dugay, J.; Miege, C.; Henion, M. C. *J. Chromatogr., A* **1998**, *795*, 27−42.

Table 3. Parameters Obtained by Mathematical Fitting (Eq 1) of Absorbed Amount vs Extraction Time[a]

herbicide	WS (mg/L)[b]	K_{OW}[c]	n_0 (ng)[d]	a	t_{eq} (h)[e]	K_{SPME}
simazine	5	91.2	6.8	0.0122	4.1	2320
terbuthylazine	8.5	1096	16.6	0.0108	4.6	15801
terbutryn	25	3070	21.1	0.0111	4.5	35086
atrazine	30	219	11.5	0.0111	4.5	6324
terbumeton	130	1097	13.3	0.0115	4.3	9483
bromacil	815		9.6	0.0232	2.2	1930
molinate	880	760	12.2	0.0122	4.1	9317

[a] A 3-mL water sample spiked at the 10 ng/mL level. Extraction constant for SPME obtained after extraction of 30 mL of water sample for 15 h using CW/DVB fiber. [b] Water solubility. [c] Octanol−water partition coefficient. [d] Maximum extractable amount in equilibrium conditions. [e] Calculated as the time needed for extraction of 95% of the amount extracted at the equilibrium.

Values obtained for K_{SPME} ranged between 1900 for bromacil and 35 000 for terbutryn. These values are comparable to those reported in the literature.[24,32] Using the statistical software included within the Microsoft Excel, it was possible to obtain the fitted relationship between K_{SPME} and octanol−water partition coefficient (K_{OW}), which is shown in eq 3.

$$K_{SPME} = 10.63K_{OW} + 1833.8 \qquad R^2 = 0.96 \qquad (3)$$

This equation should allow us to obtain some indication of the extraction behavior of the herbicides by comparing their K_{OW} values.[33] Moreover, although a good fit can be obtained between K_{SPME} and K_{OW}, it was not possible to correlate the K_{SPME} values with those of water solubility of the studied pesticides. The correlation between K_{SPME} and K_{OW} has been observed by several authors, such as Dean and co-workers, who studied a large number of organic compounds.[14] However, other authors state that solubilities and/or hydrophobicities are not sufficient in most cases to explain observed affinities of pesticides for fiber coatings.[32]

From a practical point of view, it was not feasible to use a method based on equilibrium conditions, so a compromise extraction time of 30 min was selected to carry out the experiments. Using this extraction time (clearly lower than equilibrium time which ranged from 2 to 4 h; see Table 3), between 30 and 50% of the equilibrium amounts were recovered. In addition, it is highly desirable that the extraction time is shorter than the chromatographic run time (in our case 40 min), to achieve a maximum sample throughput when manual extraction is applied. The application of SPME in nonequilibrium conditions has been largely used for the quantitative determination of several organic compounds in aqueous matrixes with satisfactory results,[17,18,24,29,32] the main disadvantages being the relatively lower extraction precision (compared with the equilibrium situation) and the need for strictly controlling extraction time.

After optimization of all the variables considered, the recommended procedure was that indicated in the Experimental Section: extraction of 3 mL of water sample containing 10% NaCl under magnetic stirring during 30 min using a CW/DVB fiber coating and subsequent desorption at 240 °C during 5 min.

Table 4. Precision, Expressed as Repeatability and Reproducibility, of the Recommended Procedure Applied to Groundwater and Surface Water Samples. Limits of Determination

	repeat. (CV %)				
herbicide	10 ng/mL	1 ng/mL	0.1 ng/mL	reprod (CV %) 1 ng/mL	LOD (ng/mL)
		Groundwater Samples			
molinate	4	6	8	8	0.02
simazine	4	8	10	9	0.06
atrazine	3	6	13	8	0.04
terbumeton	3	8	7	14	0.04
terbuthylazine	3	5	6	14	0.02
terbutryn	4	4	6	19	0.02
bromacil	5	13	21	16	0.01
		Surface Water Samples			
molinate	13	7	8	7	
simazine	4	2	14	6	
atrazine	4	1	13	6	
terbumeton	4	1	7	5	
terbuthylazine	4	20	5	19	
terbutryn	3	7	4	6	
bromacil	14	36	39	31	

Analytical Characteristics. Considering the quantitative requirements of the procedure and the need for validation of analytical methods, some experiments were carried out to obtain the analytical characteristics of the method: linearity range, precision, reproducibility, and limits of determination. The linearity of the method was tested over a range between 0.1 and 10 ng/mL using five concentration levels and analyzing each level in triplicate. After plotting the mean peak areas versus sample concentration to generate the calibration curves, a statistical regression model was applied to obtain the corresponding values for slope and intercept for each compound. Square regression coefficients (R^2) were higher than 0.999 for all compounds except for bromacil (0.96). Quantitation of this compound was found to be quite difficult due to the fact that its electron impact (70 eV) mass spectrum showed only two main peaks at m/z 205 and 207, which were seriously interfered with by the presence of a column bleeding spectrum.

Following the indications for method validation given by Huber,[34] the precision of the method was obtained by analysis of six spiked groundwater and six spiked surface water replicate samples at three concentration levels each (0.1, 1, and 10 ng/mL). The results obtained are shown in Table 4 and led to calculate coefficients of variation below 10% in most cases, except for bromacil, which gave worse precision probably due to quantification problems as indicated before. In any case, the values obtained are comparable or even lower than those reported in the literature for SPME determination of pesticides[16,21,24,29,32] or by application of off-line LLE or SPE extraction procedures combined with GC determination. Reproducibility was also studied by obtaining coefficients of variation for the analysis of three replicates of water samples spiked at 1 ng/mL on three different days ($n = 9$). Values obtained are also shown in Table 4 and were lower than 20% for all compounds studied except bromacil. These values are in the range indicated as acceptable (<30%) for

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(33) Noble, A. *J. Chromatogr.* **1993**, *642*, 3−14.

(34) Huber, L. *LC-GC Int.* **1998**, *11*, 96−105.

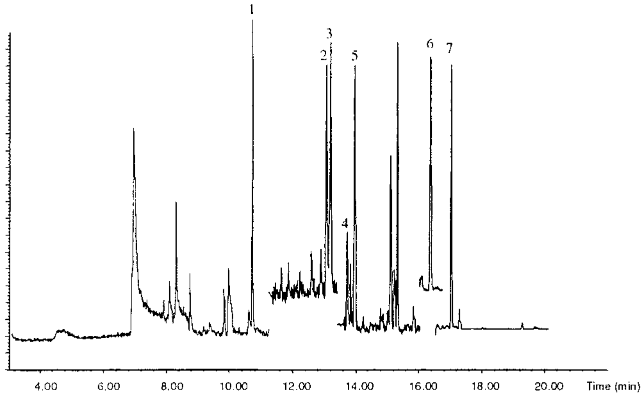

Figure 4. GC/MS chromatogram of a spiked groundwater sample (0.1 μg/L) obtained after SPME. Peaks: (1) molinate, (2) simazine, (3) atrazine, (4) terbumeton, (5) terbuthylazine, (6) terbutryn, and (7) bromacil.

environmental analysis at the 1 ng/mL level by the AOAC manual[35] as referenced by Huber.[34]

Validation of the analytical procedure was completed by calculating the limits of determination (LODs) of the herbicides studied. Table 4 shows the LODs obtained from the chromatograms corresponding to the solid-phase microextraction and GC/MSD (SIM mode) determination of three replicates of spiked groundwater samples at the 0.1 ng/mL level, calculated as the peaks having a signal-to-noise ratio of 3. As can be seen, the experimental LODs are in the range of 10−60 ppt; thus the procedure developed is fully applicable to the determination of these herbicides in groundwater and surface water, meeting the restrictive requirements of EU directives for public supply water.[1] As an example, Figure 4 shows some typical chromatograms used for LOD calculation as indicated above.

Analysis of Real-World Water Samples. To complete the study on the applicability of the recommended SPME procedure to water samples, it was applied to several surface water samples obtained from three different wet areas at the Comunidad Valenciana (Spain) during 1998 and 1999, as part of a more extensive monitoring program. This area is of great agricultural importance, the most relevant crop being citrus, and thus different herbicides are usually applied. Glyphosate, MCPA, bromacil, triazines, and phenylureas are widely used. Moreover, molinate is extensively applied in rice fields of the area. The samples were analyzed using the recommended SPME procedure and subsequent GC/MSD determination. To emphasize the potential of the SPME technique for multiresidue analysis, detection was carried out both in SIM (for quantitative determination of the herbicides included in this paper) and in full-scan mode (in order to detect any other pesticide present in the sample). Results are shown in Table 5 , where they are compared to those obtained when a conventional liquid−liquid extraction with dichloromethane, as previously used in our laboratory, is applied.[4] Quantitation of the samples in the SPME procedure was carried out by a three-point level external standard calibration using spiked NANOpure water samples (levels 0.1, 1, and 10 ng/mL, containing 1% MeOH) and analyzing every sample in duplicate. As it can be seen, most of results obtained when both methods are applied are comparable, giving concentrations in the same order of magnitude for the herbicides selected. In the samples taken from Almenara (Castellon) and Albufera (Valencia), high concentrations of molinate were found with values between 45 and 110 μg/L. All these results evidence that SPME technique can be successfully applied to pesticide residue determination in real-world water samples. Most of results are consistent with those obtained with well-established techniques such as LLE, and the multiresidual character of the developed SPME procedure has been demonstrated by the detection of nontarget compounds in the samples, belonging mainly to the OP and OC insecticides. Quantitation of these insecticides was carried out by single-level calibration in SIM mode. In spite of the fact that SPME was not optimized for these groups of pesticides, the results could be considered as acceptable, and in several cases, the findings by SPME were corroborated by the LLE method. All these data show the applicability of the SPME technique for the quantitative analysis of selected herbicides and also for the quantitative (or semiquantitative) analysis of nontarget compounds.

Application of SPME to Soil Samples. Once the procedure for the determination of herbicides in water was developed, we intended to extend the applications of the SPME to other sample matrixes. The determination of herbicides in soil was of interest, not only because of its environmental relevance but also because the determination of herbicides in soil samples requires an extensive sample handling, including the application of complex extraction procedures usually followed by cleanup based on liquid−liquid partition or solid-phase extraction. SPME appears as a good alternative in order to avoid (or at least greatly reduce) sample manipulation for this type of complex sample.

The first experimental approach selected was the extraction of herbicides from the soil using hot water and then the submission of this aqueous extract to SPME prior to GC determination. Although the use of water for the extraction of nonpolar compounds is not well defined, several references can be found dealing with this subject[9,27,28] that made us to consider this approach. In addition, the application of SPME for aqueous extracts is preferred to the use of organic solvent extracts. The first step was to prepare spiked soil samples, which was accomplished by adding 0.3 mL of a methanolic standard mixture of the compounds studied to 3 g of soil (sieved through 2-mm mesh), shaking for 2 h, and finally leaving the sample for 12 h for drying and aging. After optimization of the experimental conditions (sample size, water volume, extraction time, microwave power), extraction of the samples was accomplished by mixing 3 g of soil with 6 mL of distilled water and then submitting the resultant mixture to heating in a microwave oven at 20% of maximum power for 1.5 min in a glass vial with Teflon-lined cap. Then, the samples were centrifuged, and 3 mL of the aqueous supernatant was subjected to SPME as described for water samples. The results obtained for the overall procedure led to extraction recoveries that ranged between 30 and 68% for all compounds studied (coefficient of variation less than 10% at the 10 ng/g spiking level). These results were discouraging because the low recoveries of the procedure made difficult the quantitative application of the method. However, the described procedure was considered useful as a rapid screening method for selected

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(35) AOAC Peer Verified Methods Programme, Manual of Policies and Procedures, Arlington, VA, November 1993.

Table 5. Analysis of Real-World Surface Water Samples (Results in μg/L) Obtained by Application of SPME (CW/DVB) or LLE with Dichloromethane Followed by GC/MSD Analysis[a]

pesticide	Almenara 1 (29/05/98)	Almenara 2 (29/05/98)	Albufera 1 (29/05/98)	Albufera 2 (29/05/98)	clot 1 (29/05/98)	clot 2 (22/07/98)	clot 3 (22/02/99)	torrelasal (22/03/99)
molinate	70.6	82.6	45.0	110	–	–	–	–
terbuthylazine	–[b]	0.13 (–)[c]	–	–	0.10 (–)	0.05 (–)	0.17 (0.15)	–
symazine	–	–	–	–	–	0.39 (0.80)	–	0.16 (0.19)
terbutryn	–	–	–	–	–	0.56 (1.02)	0.21 (–)	–
bromacil	–	–	–	–	–	0.48 (0.51)	–	–
malathion	–	–	–	–	20.9 (15.1)	–	–	–
thiobencarb	0.29 (2.00)	0.49 (3.40)	–	–	0.20 (–)	–	–	–
fenthion	–	–	0.40 (–)	0.12 (–)	8.18 (8.80)	–	–	–
chlorpyrifos	–	–	0.11 (–)	3.90 (–)	3.01 (1.03)	–	–	–
endosulfan	–	–	–	1.72 (–)	2.41 (1.02)	–	–	–

[a] Samples were collected in 1998 and 1999 in four different wet areas in the Valencian area of Spain. [b] –, not detected. [c] (–), results obtained by LLE-GC/MSD.

herbicides in soil samples with very good detection limits (below 0.5 ng/g).

The next consideration was directed toward the use of organic solvents for herbicide extraction from soil samples, which has been extensively used in the literature. As is well known, it is not feasible (nor recommendable) to directly dip the fiber into the organic solvent, mainly because of stability of the fiber and extraction efficiency, and thus dilution of the organic soil extract with water was necessary. However, it was evident that the higher the dilution factor with water the lower the sensitivity that could be achieved. Therefore, a compromise between stability/efficiency of the fiber and sensitivity of the overall procedure should be reached.

Some authors state that the presence of high concentrations of organic solvents in aqueous samples lead to an important decrease in extraction efficiency.[18,21,36] So this concentration should be maintained at relatively low levels, generally less than 1%. To investigate the effect of acetone, acetonitrile, and methanol concentrations on the extraction efficiency of selected herbicides in water, several aqueous samples were spiked at the 1 ng/mL level and then different volumes of each solvent were added (1, 5, and 10%). After the SPME procedure was applied to 3 mL of each aqueous/organic sample, results obtained (Figure 5) indicated that the best extraction efficiency always corresponded to the lowest percentage of organic solvent assayed, i.e., 1%. This fact was observed for all pesticides and organic solvents studied. In addition to this general behavior, when the effect of the three solvents tested was compared, both acetone and acetonitrile showed an important decrease in extraction efficiency when solvent concentration was increased from 1 to 5 and 10% (besides, acetone had the strongest effect in lowering pesticide recoveries). However, when methanol concentration was increased to 5 and 10%, the decrease in efficiency was only ~20%. This fact allowed us to conclude that methanol was the best choice for herbicide extraction in soil samples considering the subsequent dilution of the soil extracts with water prior to SPME application. Methanol allows the use of soil extracts containing up to 10% of organic solvent without important decrease in extracted masses. In addition, the strong effect of acetone on the SPME extraction efficiency indicates that methanol is preferred to acetone when spiked water samples are prepared.[18,29,30] The fact that the presence of organic solvents in the aqueous sample has such an important influence over extraction efficiency also makes it necessary that, when quantitative determinations (by external standard comparison using spiked aqueous solutions) are performed, real samples and aqueous standards contain the same organic solvent content. In the present work, we have fixed a content of 1% MeOH that is added to the real-world water samples under investigation before SPME.

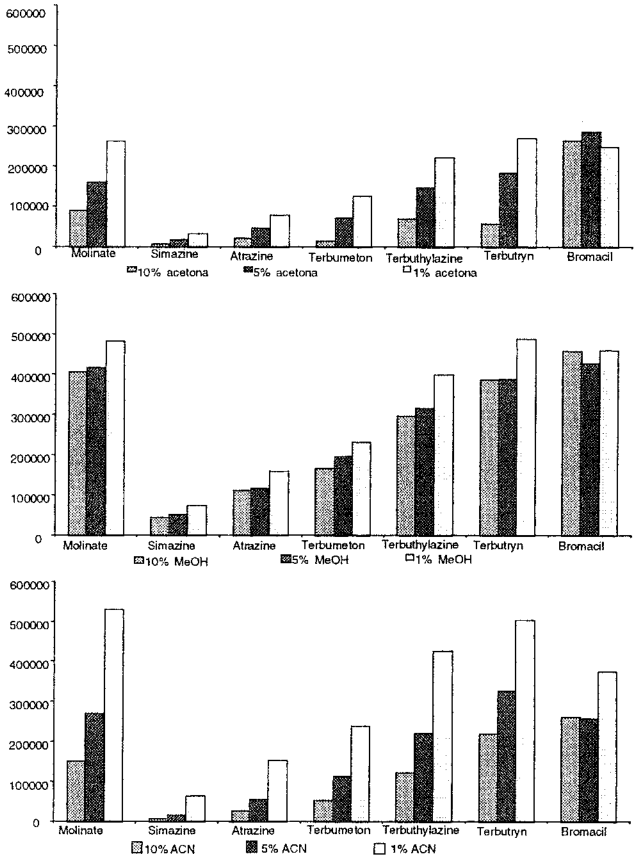

Figure 5. Influence of the organic solvent content in aqueous samples on SPME efficiency.

Once it was established that the maximum operative concentration of methanol that should be present in aqueous extracts was 10%, the next step was to study the efficiency of methanol

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(36) Batlle, R.; Sanchez, C.; Nerin, C. *Anal. Chem.* **1999**, *71*, 2417–2422

Table 6. Recoveries of Selected Herbicides in Spiked Soil Samples (200 ng/g) after Extraction with Methanol under Ultrasonic Shaking and Microwave Heating, and Subsequent Application of SPME in the Water-Diluted Extracts ($n = 6$)

	rec, % (CV %)	
	ultrasonics	MASE
molinate	57 (10)	84 (5)
simazine	62 (4)	86 (5)
atrazine	61 (6)	82 (3)
terbumeton	67 (6)	81 (4)
terbuthylazine	61 (5)	82 (4)
terbutryn	59 (4)	80 (6)
bromacil[a]	81 (7)	77 (14)

[a] Spiked level at 600 ng/g.

Table 7. Recoveries and Coefficients of Variation (in Parentheses) Obtained after Application of a Microwave-Assisted Extraction Procedure Followed by SPME to Spiked Soil Samples. Limits of Determination

	SPME on extrd soil ($n = 6$) 200 ng/g	soil extrn and then SPME ($n = 6$)			LOD (ng/g)
		200 ng/g	20 ng/g	5 ng/g	
molinate	75 (5)	84 (5)	70 (4)	64 (20)	2[a]
simazine	79 (5)	86 (5)	74 (9)		10[b]
atrazine	80 (4)	82 (3)	70 (8)	67 (20)	3[a]
terbumeton	80 (6)	81 (4)	82 (7)	94 (12)	5[a]
terbuthylazine	81 (4)	82 (4)	82 (6)	73 (17)	1[a]
terbutryn	80 (4)	80 (6)	83 (3)	69 (26)	1[a]
bromacil[c]	74 (9)	77 (14)	86 (20)		60[d]

[a] Calculated from the chromatogram of the sample spiked at 5 ng/g level. [b] Calculated from the chromatogram of the sample spiked at 20 ng/g level. [c] Spiked levels 600 and 60 ng/g levels. [d] Calculated from the chromatogram of the sample spiked at 600 ng/g level.

for the extraction of pesticides in soil samples. Two procedures were considered: extraction using an ultrasonic bath and MASE. In both cases, extraction was carried out on 5 g of spiked soil samples with 5 mL of methanol. Table 6 shows the recoveries obtained when ultrasonics (10 min) and microwave heating (20% power, 1.5 min) were applied to soil samples spiked at the 200 ng/g level (soil 2). After centrifuging, 2 mL of methanolic extracts was diluted up to 20 mL with distilled water prior to SPME application. As it can be seen, there is a substantial improvement in extraction efficiency when microwave-assisted extraction was used (recoveries ~80%). Thus, the use of MASE for soil sample extraction was selected to continue the present study.

Precision and recoveries were first obtained for the SPME procedure applied to six aliquots of an homogeneous extract obtained from soil 2 spiked at 200 ng/g. Then, the overall procedure was tested by extracting six replicate soil samples followed by SPME. The overall precision was obtained at three concentration levels (200, 20, and 5 ng/g) (Table 7). Results were similar in both cases with average recoveries of ~80% and coefficients of variations below 10%. This means that deviations come from the application of SPME itself more than from the methanolic extraction of the soil samples. As expected, deviations at the lowest assayed level of 5 ng/g were considerably higher, reaching coefficients of variation up to 25%.

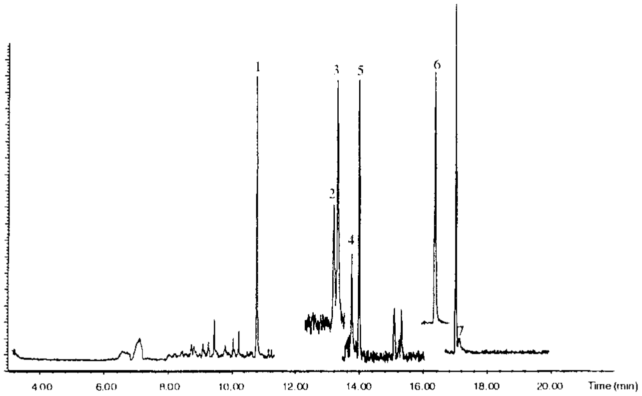

Figure 6. GC/MS chromatogram of a spiked soil sample (20 ng/g) obtained by methanol MASE followed by SPME. Peaks: (1) molinate, (2) simazine, (3) atrazine, (4) terbumeton, (5) terbuthylazine, (6) terbutryn, and (7) bromacil (60 ng/g spiked level).

Table 8. Comparison of Results (Recovery and Coefficient of Variation) Obtained When Applying Three Procedures for Herbicide Determination in Soil Samples ($n = 3$)[a]

	concn found, ng/g (CV %)		
	methanol extrn	acetone extrn	
	SPME	SPE cleanup	LLE
molinate	18 (2)	9 (8)	11 (16)
simazine	53 (3)	42 (4)	43 (8)
atrazine	58 (13)	40 (18)	37 (6)
terbumeton	48 (6)	40 (16)	40 (7)
terbuthylazine	53 (6)	35 (17)	38 (6)
terbutryn	49 (6)	38 (16)	40 (5)
bromacil[b]	94 (46)	145 (6)	188 (15)

[a] Spiking level 50 ng/g. [b] Spiking level 150 ng/g.

Finally, limits of determination for the studied herbicides were obtained using the chromatograms corresponding to spiked soil samples (Figure 6). The values obtained (Table 7) ranged between 1 and 10 ng/g (except for bromacil, 60 ng/g) and are similar to those obtained by applying some of the most common procedures described in the literature despite the previous dilution of the methanolic extract by a factor of 10. Some additional advantages can be pointed out as the simplification in sample handling, reduction in sample size and solvent volume, and the absence of additional cleanup procedures to sample extracts (as accomplished by the SPME procedure).

To fully validate the SPME-based procedure, its performance was compared to that of two well-established conventional procedures based on extraction of 50 g of soil sample with acetone (150 mL) and subsequent cleanup by liquid−liquid partition or C_{18} solid-phase extraction.[37] The procedures were applied to spiked soil samples (soil 2 at 50 ng/g level) and the pesticide concentration obtained as the mean value for three replicates. Using a statistical *t-paired* test, it was deduced that the concentrations calculated with the three procedures were comparable, except for bromacil. The low recovery values for molinate in the three procedures were surely due to losses by volatility during drying of the wet sample in the spiking of soil. In any case, results

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(37) Hernandez, F.; Beltran, J.; Forcada, M.; Lopez, F. J.; Morell, I. *Int. J. Anal. Chem.* **1998**, *71*, 87−103.

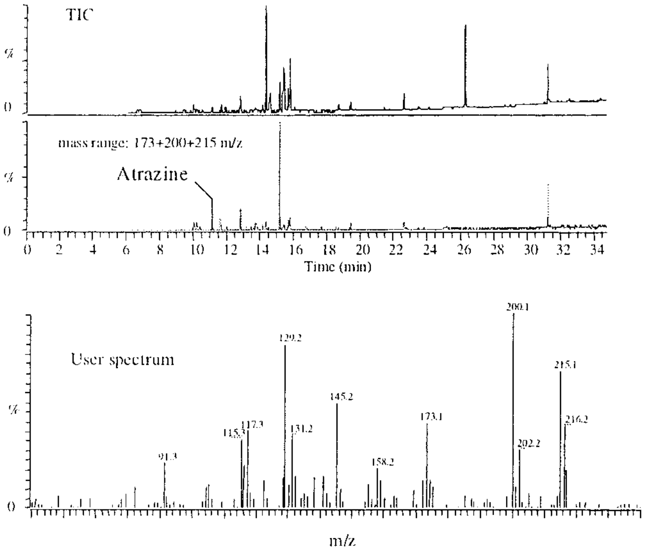

Figure 7. GC/MS chromatogram of a real-world soil sample. Spectrum of atrazine as detected in the sample (21 ng/g).

obtained for measured concentrations of molinate were consistent when all procedures were used (Table 8).

Our developed procedure has been applied to a soil in which atrazine residues were suspected to be found. Analysis performed allowed us to detect and unequivocally identify atrazine at a level of 21 ng/g as shown in Figure 7.

In our latest experiment, we have proved that the application of the developed procedure to soil samples with high organic matter content (such as soil 1) is not satisfactory because of the low recoveries obtained (~60% in most cases). This could be due to the lowest efficiency of method extraction for this type of samples compared to acetone or, more possibly, to the lower efficiency of SPME in extracts with high organic content, as has been recently referenced by other authors.[38]

CONCLUSIONS

The Carbowax/divinylbenzene fiber efficiently extracted all herbicides studied (five triazines, molinate, and bromacil), but it showed low ruggedness when the salt content of the samples was increased. The practical maximum limit was 10% NaCl; under these conditions the use of this fiber coating was feasible for extraction of more than 100 samples.

The developed procedure allowed the multiresidue determination of selected herbicides in water under nonequilibrium conditions, with sample throughputs around 10−12 samples per day. The application of the optimized procedure to real-world surface water samples allowed us to detect some of the selected herbicides and also several nontarget pesticides (OP or OC insecticides), thus confirming the multiresidual character of the SPME technique.

It has been demonstrated that the combination of MASE with methanol and SPME allowed the quantitative determination of selected herbicides in soil samples. The control of organic solvent content prior to SPME was of paramount importance, a dilution with distilled water and the use of external standards with the same organic solvent content as sample extracts being necessary.

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(38) Lee, M.-R.; Yeh, Y.-C.; Hsiang, W.-S.; Hwang, B.-H. *J. Chromatogr., A* **1998**, *806*, 317−324.