Analysis of some pesticides in water samples using solid-phase microextraction–gas chromatography with different mass spectrometric techniques

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Abstract

A solid-phase microextraction (SPME)–GC procedure has been developed for the analysis of four selected pesticides (propanil, acetochlor, myclobutanil and fenoxycarb) in water samples. Mass spectrometry (MS) was used and two different instruments, a quadrupole MS system and an ion trap operating in the MS–MS mode, were compared. A Carbowax–divinylbenzene SPME fiber was used. The performances of the two GC–MS instruments were comparable in terms of linearity (in the range of 0.1–10 μg/l in water samples) and sensitivity (limits of detection were in the low ng/l range); the quadrupole MS instrument gave better precision than the ion trap MS–MS system, but generally the relative standard deviations for replicates were acceptable for both instruments (<15%). Specificity with these two instruments was comparable in the analysis of ground water samples. Recovery tests were made to assess the applicability of the SPME procedure in the quantitative analysis of contaminated groundwaters. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Solid phase microextraction (SPME) is a recent sample preparation technique that is proving increasing useful in organic analytical chemistry [1]. The device is simple and easy to use, with expanding possibilities for the analysis of liquid samples. The option of extracting the sample and injecting the adsorbed analytes into the analytical instrument using the same device is particularly useful when monitoring pesticide pollution in water. There is a great demand for high-sensitivity methods for this topic, on account of the 0.1 μg/l admissible level for pesticides in water in the European Union (EU) [2]. Gas chromatography–mass spectrometry (GC–MS) has been widely applied [3,4], because of its high specificity and sensitivity and for the potential of multiresidue and multiclass analyses.

SPME has been introduced for pesticide analysis in water samples [5–9], and GC–MS has been recently used together with this technique to detect pesticides in waters [10–14]; method validations in inter-laboratory tests have been also done [15,16], demonstrating the wide applicability of the SPME technology in this field.

Another attractive technique for pesticide detec-

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tion is gas chromatography–tandem mass spectrometry (GC–MS–MS). The tandem MS technique allows highly specific MS analyses, with the possibility of directly analyzing complex environmental samples without extensive clean-up steps. The last generation of low-cost benchtop ion trap instruments can operate in the MS–MS mode: a specific ion, formed by electron ionization, is isolated in the ion trap and subsequently dissociated, increasing its collisions with the GC carrier gas molecules. Product ions are detected after this step, ejecting these ions from the trap by applying a radiofrequency (RF) voltage ramp to the trap electrodes.

Few applications of GC–MS–MS in pesticide analysis are reported [17,18] and its use is limited to residue confirmation [19]. The recent application of the MS–MS function in ion trap instruments could in the future increase the number of applications, considering its ease of use and the relatively low cost of the instruments.

Within the framework of a project funded by the EU, dealing with the validation of immunoenzymatic methods using "traditional" instrumental proce-

dures, our laboratory has developed an analytical method for testing water samples for four target pesticides, representative of four different chemical classes: propanil (anilide postemergent herbicide), acetochlor (chloroacetanilide preemergent herbicide), myclobutanil (azole fungicide) and fenoxycarb (carbamate insecticide) (Fig. 1). The analytes were chosen on the basis of their wide use in the EU, in the Central European countries and in the new independent states of the former Soviet Union.

GC–MS with solid-phase extraction (SPE) of the water sample was considered for propanil [20]. Recent analytical methods for myclobutanil quantitation in water samples are an immunoenzymatic method [21] and thin-layer chromatography (TLC) with SPE preconcentration [22]. Screening water samples for the herbicide acetochlor has recently become important in the USA [23,24], since this has been introduced as a replacement for the widely used alachlor, atrazine and metolachlor pesticides. Thus, the development of new acetochlor analytical procedures is an important task; current methods are principally based on SPE and GC–MS [23–25].
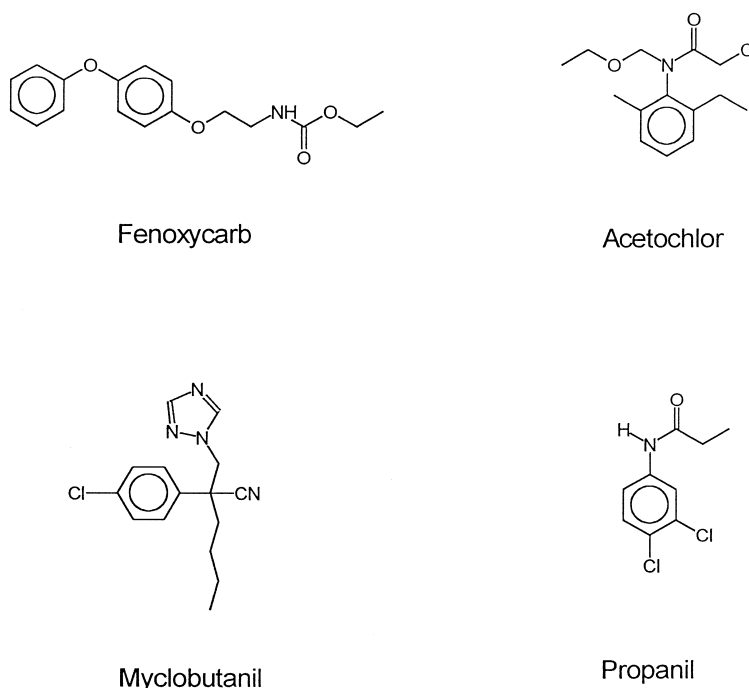

Fig. 1. Structures of the pesticides investigated in this study.

Reports of the analysis of fenoxycarb in water samples have not been found. In general, no SPME procedures are reported for these pesticides in water.

We developed an SPME-based method for these compounds, choosing GC–MS as the instrumental technique; we used two different MS instruments, to explore the capabilities of the MS–MS function in the ion trap, compared to a quadrupole detector operating in the selected ion monitoring (SIM) single-MS mode. The choice of the tandem MS detector is interesting, since no GC–MS–MS methods have been reported for these pesticides.

2. Experimental

2.1. Chemicals

All solvents were analytical grade from Carlo Erba (Milan, Italy). Pure standards of myclobutanil and fenoxycarb were kindly provided by A. Széckàs (Plant Protection Institute, Budapest, Hungary). Certified analytical grade acetochlor was from Dr. Ehrenstorfer (Augsburg, Germany). A propanil analytical standard was provided by Farmoplant (Milan, Italy). Standard solutions were prepared in methanol and stored in a freezer.

2.2. SPME material and procedure

Different SPME fibers (Supelco, Bellefonte, PA, USA) were tested for an initial fiber selection: Carbowax–divinylbenzene (CW–DVB), thickness 65 μm, polyacrylate 85 μm, polydimethylsiloxane 100 μm and polydimethylsiloxane–divinylbenzene 65 μm. All the extractions were done at room temperature in 12-ml dark glass vials; 10 ml of distilled water per vial was used in all the experiments. The sample was stirred vigorously magnetically and the fibers were immersed directly in the liquid phase. Injection of the extracted components into the GC–MS system was by thermal desorption, exposing the fiber at 250°C for 15 min into the GC split/splitless injector. The injection was splitless for 1 min and then the split valve was open for the rest of the analytical run. The SPME fiber was kept in the hot injector for 14 min with the split valve open, to purge any residue of the extracted compounds not completely desorbed from the fiber during the split-less phase. After this desorption, fibers were washed for 5 min in distilled water, to remove salt particles on the adsorption surfaces, then dried at 250°C in a GC injector for 5 min. This last step was important when using the CW–DVB fiber, since this phase swelled considerably after immersion in water.

2.3. GC–MS conditions

All the SPME optimization experiments were done only on a HP5871 MSD quadrupole mass spectrometer interfaced to a HP5890 gas chromatograph, MS operated in the SIM mode. Experiments on linearity, precision and sensitivity of the method were done with this quadrupole and also in a Varian Saturn 2000 ion trap mass spectrometer, coupled with a Varian 3800 GC; this ion trap was operated in the MS–MS mode. To develop the MS–MS method, we selected a specific parent ion for each analyte (the ion with greatest abundance in the single-MS spectrum of each pesticide). The optimum wave amplitudes for collision-induced dissociation (CID) of these parent ions were found using the MS–MS toolkit software, operated in the automatic method development (AMD) mode. Daughter ions considered for the quantitative analyses were the most intense in the resulting CID daughter ions spectra. GC–MS and GC–MS–MS conditions are shown in Table 1.

2.4. Quantitative method

Calibration curves were plotted analyzing distilled water spiked with different levels of the pesticides: 0, 0.1, 1 and 10 μg/l were considered for the quadrupole MS analysis and the ion trap MS–MS procedure. These linearity tests were repeated on three different days to obtain mean values. The precision of the method was calculated by analyzing three different water samples spiked with 1 μg/l on a single day; limits of detection (LODs) for the procedures were calculated on a signal-to-noise basis of 3:1, with extrapolation from the signal obtained for a 10 or 70 ng/l spiked sample.

Table 1
Experimental conditions for the GC–MS and GC–MS–MS analyses

	GC–quadrupole MS		GC–ion trap MS–MS
Instrument	HP 5890 Plus (GC)		Varian 3800 (GC)
	HP 5971 MSD (MS)		Varian Saturn 2000 (MS)
Capillary column	Supelco PTA5		Hewlett-Packard HP 5-MS
	30 m×0.25 mm I.D., 0.50 mm d_f		30 m×0.25 mm I.D., 0.25 mm d_f
GC oven	120°C for 1 min		90°C for 3 min
temperature	15°C/min up to 300°C		8°C/min up to 240°C
programme	300°C for 1 min		15°C/min up to 300°C
			300°C for 0.25 min
Injector temperature	250°C		250°C
Injection mode	Splitless for 1 min		Splitless for 1 min
GC–MS interface	280°C		280°C
temperature			
MS source temperature	180°C	Trap temperature	240°C
GC head pressure	0.09 MPa (helium)		0.069 MPa (helium)
Electron energy	70 eV		70 eV
MS mode	Selected ion monitoring (SIM)	Excitation method	Resonant
Ions monitored (m/z)	Propanil (161, 163)	CID amplitude	0.4 V
	Acetochlor (146, 162)	Reactions monitored	Propanil (m/z 161→126, 134)
	Myclobutanil (150, 179)		Acetochlor (m/z 223→146)
	Fenoxycarb (88, 116)		Myclobutanil (m/z 179→125, 152)
			Fenoxycarb (m/z 116→88)

3. Results and discussion

Fig. 2 presents the ion trap mass spectra for the selected pesticides and the corresponding daughter ions mass spectra obtained from the isolation of a specific parent ion from every analyte spectrum. These daughter ions are produced at a specific CID amplitude. This parameter was optimized to obtain a daughter ion mass spectrum with a minimal abundance of the parent ion signal, indicating the maximum yield for the dissociation of this ion.

Table 2 shows the proposed structures for the parent ions and for the corresponding daughter ions selected for the MS–MS quantitative method. In a preliminary fiber selection experiment, we compared different SPME fibers for the extraction of a spiked water sample. The polar CW–DVB fiber was the phase that performed best in terms of sensitivity for the majority of the pesticides, considering the mid-polarity of these molecules.

The carry-over of the analytes in this fiber phase was evaluated with blank fiber analyses after extraction of a 10 µg/l sample and desorption in the injector at a specific temperature and time. A desorption time of 15 min with a high temperature (250°C)

was adopted to minimize this effect to less than 2% for each pesticide. The SPME water extraction time profile revealed that equilibrium was not reached, using this fiber, even after 2 h of extraction (data not shown). These long extraction times for pesticides were reported by other authors [26], using another SPME phase made with a viscous polymer (poly-acrylate).

In order to avoid excessive process times for each sample, we adopted a 70-min extraction period in subsequent experiments.

The influence of addition of salt and the pH of extraction were also studied (data not shown): salt was added up to 30% (w/w) (close to the saturation concentration), at a neutral and a basic pH. A salt concentration of 30% (w/w) and neutral pH were the best conditions for analysis. The use of these high salt concentrations led to some problems in the SPME analyses: presence of salt particles on the fiber surface was observed after the sample desorption in the GC injector. Consequently, the fiber was cleaned after every sample injection, exposing it in distilled water for 5 min, to prevent salt accumulation and to increase fiber lifetime.

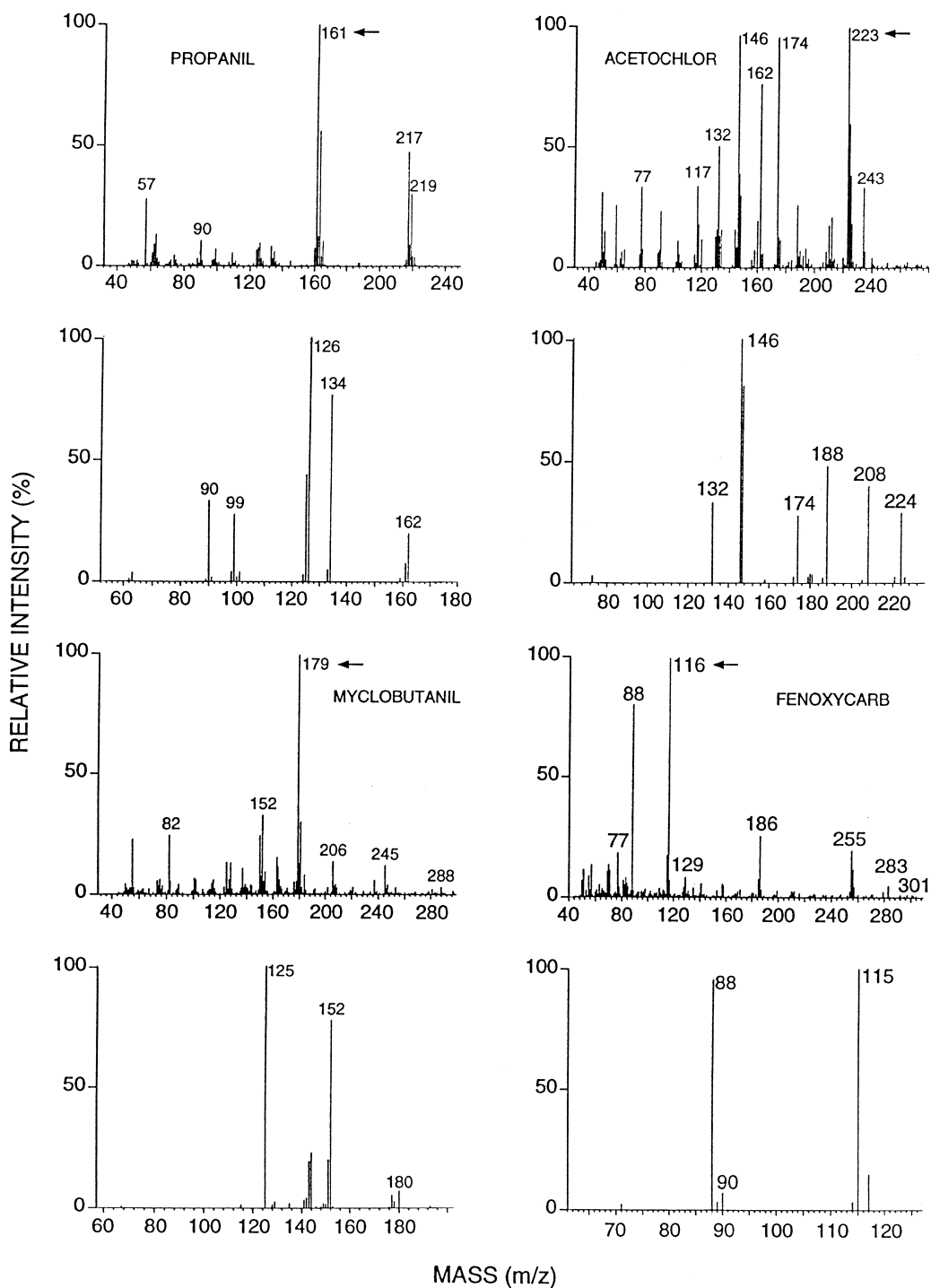The linearity of the method was studied in the 0.1

Fig. 2. Ion trap single-MS mass spectra and corresponding MS–MS spectra (below) for the four pesticides. Each selected parent ion is shown by the arrow. CID voltage: 0.4 V.

Table 2
Proposed structures of the MS and MS–MS spectral signals on the basis of specific fragment losses from the molecular ion (M) or a parent ion (m/z values for these ions are in parentheses)

Compound	Parent ion	Daughter ions	
Propanil	M-COCHCH$_3$ (161)	161-HCN (134)	161-Cl (126)
Acetochlor	M-CH$_3$CH$_2$OH (223)	223-COCH$_2$Cl (146)	
Myclobutanil	M-CH$_2$(C$_2$H$_2$N$_3$)-HCN (179)	179-CHCH$_2$ (152)	179-(CHCH$_2$)$_2$ (125)
Fenoxycarb	M-C$_6$H$_5$OC$_6$H$_4$O (116)	116-CH$_2$CH$_2$ (88)	

to 10 µg/l range. Results are shown in Table 3: both instruments gave good linearities, as demonstrated by the correlation coefficients. Analysis of a 100 µg/l calibration point was considered for the GC–MS–MS curves, but at this concentration the calibration was found non-linear. Consequently, the quantitative range of this method was limited to 10 µg/l.

The precision of the method was calculated on a single day basis (Table 3); relative standard deviations (RSDs) for the GC–MS–MS procedure were higher than with the quadrupole MS; this might be explained by the different performances of the two MS detectors, since the SPME fiber and the preparation procedure for the water samples were the same in all these experiments. The precision was still acceptable and consistent with that reported by other authors [13] for the SPME–GC–MS of pesticides from water samples.

The LODs are shown in Table 3; sensitivities at the low ng/l levels were comparable for propanil and acetochlor. MS–MS gave the best performance for myclobutanil, but not for fenoxycarb. In this case it must be considered that MS–MS analyses were done on a special low-bleed apolar GC column, as recommended by the ion-trap manufacturer; this could not be the best phase for analysis of the carbamate pesticide. Consequently, the sensitivities of the two techniques can be considered comparable.

Specificity was tested by analyzing a real sample: a groundwater contaminated with some aromatic solvents (total benzene, toluene and xylene levels of 14 mg/l) was taken from an industrial site, and preliminary SPME–GC–MS analysis revealed the absence of the four selected pesticides. The chromatograms for extraction of this sample, spiked at the 1 µg/l level, are shown in Fig. 3 for GC–MS and in Fig. 4 for GC–MS–MS. No significant amounts of coextracted compounds were observed in the chromatogram traces near the analyte retention times with both the detectors.

Accuracy of the SPME method was determined calculating the recovery in the extraction of a series of groundwater samples, spiked at different levels

Table 3
Linearity, precision and limit of detection of the SPME method using the two different GC–MS techniques

Technique	Propanil	Acetochlor	Myclobutanil	Fenoxycarb
Linearity[a] (mean regression coefficient±SD for the curve)				
GC–MS	0.9947±0.004	0.9961±0.002	0.9967±0.003	0.9960±0.004
GC–MS–MS	0.9997±0.0004	0.9997±0.0003	0.9998±0.0004	0.9990±0.0009
Precision (RSD of the replicates, %, $n=3$)				
GC–MS	5%	3%	3%	3%
GC–MS–MS	10%	12%	4%	8%
Limit of detection[b] (ng/l)				
GC–MS	2	18	30	7
GC–MS–MS	2	15	10	15

[a] Concentration range in the water sample: 0.1–10 µg/l.
[b] Based on a signal-to-noise ratio of 3:1.
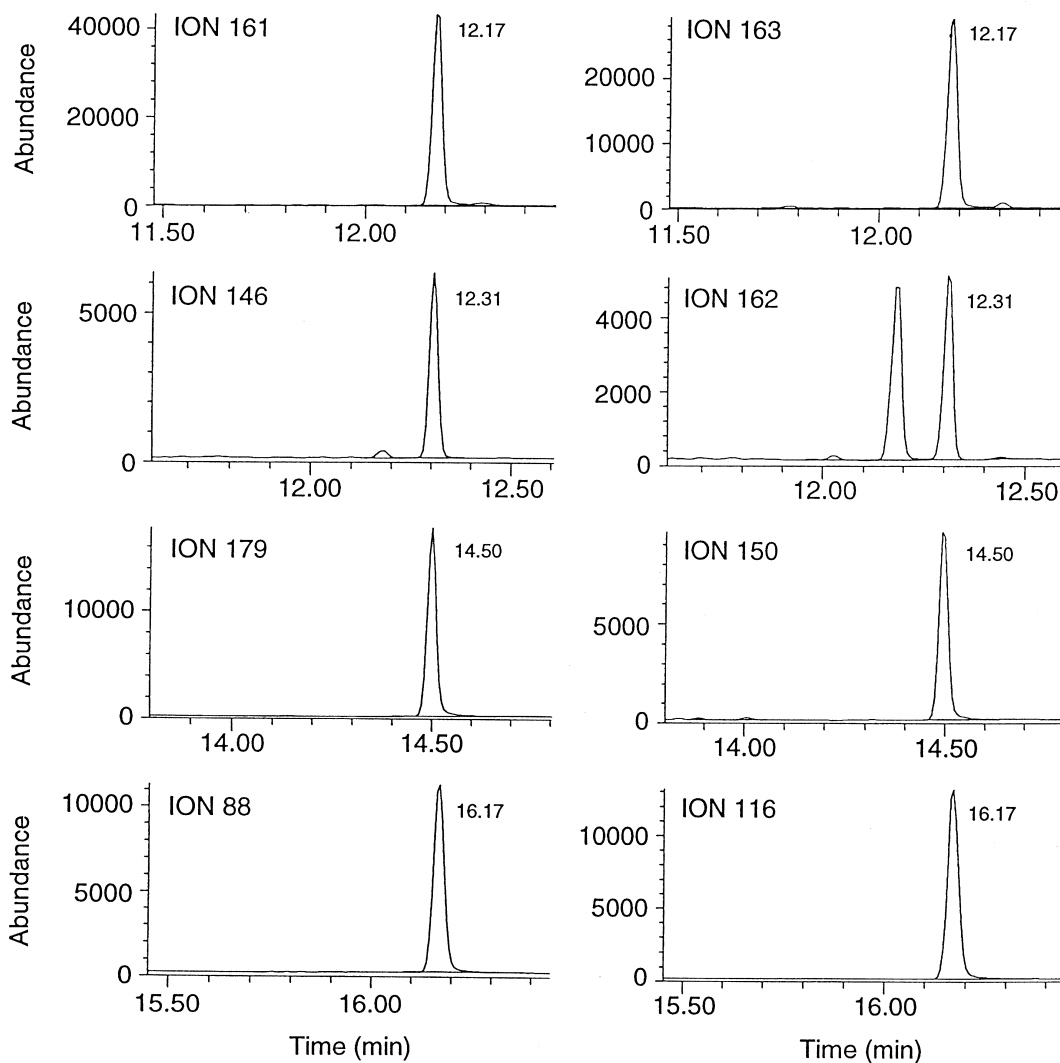
Fig. 3. GC–quadrupole MS selected ion chromatograms for SPME extraction of a ground water sample spiked with 1 μg/l of each pesticide. None of these pesticides were detected in the original unspiked sample.

(0.1, 1 and 10 μg/l), and analyzed using the GC–MS instrument. Peak areas for each pesticide were compared to those obtained extracting distilled water samples, spiked at the same levels.

Mean recoveries were 81% for propanil (RSD=20%), 99% for acetochlor (RSD=22%), 97% for myclobutanil (RSD=12%) and 110% for fenoxycarb (RSD=14%): extraction efficiencies in the real matrix and in distilled water are generally comparable for acetochlor, myclobutanil and fenoxycarb.

Consequently, quantitative SPME analysis of groundwaters in the 0.1 to 10 μg/l range is possible using an external standardization made with spiked distilled water.

4. Conclusions

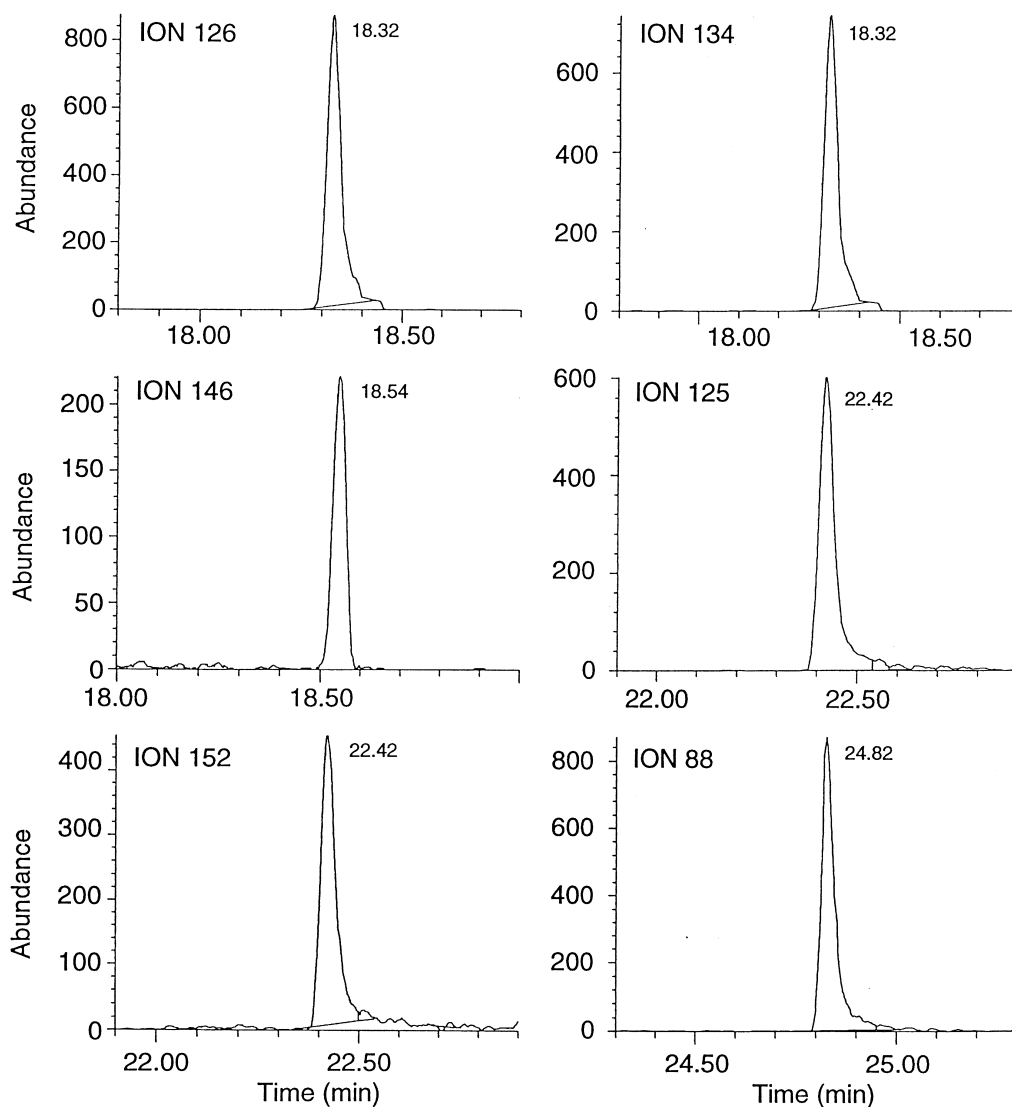The applicability of the SPME method has been shown for the analysis of some mid-polar pesticides

Fig. 4. GC–ion trap MS–MS daughter ion chromatograms for SPME extraction of the same sample shown in Fig. 3.

in water. The LODs fully satisfy the requirements of the EU (0.1 μg/l). CW–DVB was the best SPME phase for these purposes. This fiber can be successfully used in the development of a large multiclass method, since the pesticides considered in our study were representative of four chemical classes. The two GC–MS systems used in this study provided comparable results in terms of sensitivity, but the quadrupole detector yielded higher precision. Both instruments were satisfactory in the analysis of standard water samples in a concentration range of 0.1 to 10 μg/l, giving acceptable linearities for the quantitative method. Screening the spiked ground water indicated that both instruments and the SPME procedure are sufficiently specific for analysis of these environmental samples. Recoveries founded in

the extraction of this real matrix demonstrate that our SPME procedure can be applied to the quantitative analysis of contaminated groundwaters.

Acknowledgements

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