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Determination of triazines in soil leachates by solid-phase microextraction coupled to gas chromatography–mass spectrometry

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Abstract

A solid-phase microextraction–gas chromatography–mass spectrometry (SPME–GC–MS) method was developed for the evaluation of the leachability order of selected triazines (propazine, terbuthylazine, sebuthylazine, ametryn, prometryn and terbutryn) in soil/sediment samples (organic carbon content ranging from 0.19 to 0.42%), analysing fractions collected from a soil packed microcolumn elution experiments. The procedure is fast, simple, highly sensitive and solvent free. SPME–GC–MS was also employed for the quantitative determination of triazines in the soil leachate, since the method showed good recovery yield. Detection limits were always better than 1 ng ml^{-1}. The method was tested on a contaminated landfill top soil. Prometryn and ametryn were identified through their MS spectra and then quantified. Terbuthylazine was used to assess recovery. Results compared well with those obtained by solvent extraction followed by HPLC–UV detection.

Keywords: Soil; Environmental analysis; Triazines; Pesticides

1. Introduction

Solid-phase microextraction (SPME) is a new technique introduced by Pawliszyn and co-workers [1,2] that allows simultaneous extraction and preconcentration of analytes from sample matrix, eliminating some disadvantages of conventional techniques such as solid-phase (e.g., plugging of cartridges) and liquid–liquid extraction (e.g. use of toxic solvents).

Triazines are among the most widely used herbicides worldwide. Their use has been the cause of great concern because of their mobility and water solubility which allows them to leach into groundwater and surface water. A variable amount of triazines can also remain strongly sorbed onto the soil, depending on the characteristics of the matrix (e.g. soil organic matter content, cation-exchange capacity, pH, surface area, mineralogical composition, clay content).

The majority of the SPME studies published to date for triazines analysis [3–5] reported the determination of these chemicals in water samples.

Recently, SPME coupled to gas chromatography–mass spectrometry (SPME–GC–MS) was successfully used [6] to estimate K_{oc} (soil sorption coefficient) values for some triazines in soil/sediment samples, of different organic matter content.

Leaching of pesticides through soil is of great environmental concern because of the possibility of ground water contamination. The simplest index of leaching is the sorption coefficient itself since chemi-

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cals with lower K_{oc} are typically leached to a higher degree so that a ranking according to the K_{oc} could represent, with a good approximation, a measure of their tendency to leach. Another quantitative index for pesticide leaching is the retention factor value obtained by soil thin-layer chromatography [7,8] or by soil column chromatography [9].

The present paper shows that SPME–GC–MS can be used for the assessment of triazines leachability order, analysing fractions collected from a soil packed microcolumn elution experiments. The procedure is fast, simple, highly sensitive and solvent free.

Since a number of triazines are easily leached and can be recovered with satisfactorily yield, at least from soils/sediments with relatively low organic carbon content, SPME–GC–MS can also be employed for their quantitative determination in soil leachates.

2. Experimental

2.1. Chemicals

A triazines mix containing propazine, terbuthylazine, sebuthylazine, ametryne, prometryne and terbutryn (Dr. Ehrenstorfer, Augsburg, Germany) dissolved in acetonitrile (10 μg/μl) was prepared and stored in the dark at 4°C. More dilute solutions were prepared just before use. Methylene chloride was obtained from Labscan (Dublin, Ireland). Other chemicals were analytical grade reagents.

2.2. Apparatus

GC–MS analysis was performed with an HP 5890 series II gas chromatograph equipped with a HP 5890 GC split/splitless injector and interfaced, by a GC transfer line, to a VG Trio-2000 quadrupole mass spectrometer (VG Biotech, Altrincham, UK). The carrier gas was helium; contaminants were removed by using a drying tube, a Supelpure-HC trap and an OMI-1 indicating purifier (all Supelco) in series.

The GC chromatographic column consisted of a Supelco fused-silica SPB-5 capillary column (30 m×0.20 mm I.D. with 0.25 μm film thickness) connected to the split/splitless injector.

The HPLC system consisted of a Waters (Milford, MA, USA) 600-MS multisolvent delivery system equipped with a Reodyne injector with a 20-μl loop and a 5-μm Supelcosil LC-18-S column (250×4.6 mm I.D.) (Supelco, Bellefonte, PA, USA). A 5-μm Supelguard LC-18-S precolumn (20×4.6 mm I.D.) (Supelco) was used to protect the analytical column. The detector was an HP 1040A photodiode-array spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA) interfaced to an HP 85 computer equipped with an HP dual disk drive and an HP 7470A plotter.

2.3. Chromatographic and detection conditions

Initial experiments to optimise the gas-chromatographic and MS detection conditions were carried out by direct injection of 1 μl of the standard mix in acetonitrile. The oven temperature program was 90°C (4 min) to 220°C at 5°C/min, then 220°C to 280°C at 20°C/min (final temperature held for 10 min). A column head pressure of 100 kPa and an injector temperature of 250°C were used. The GC transfer line was maintained at 250°C. The mass spectrometer was operated in the electron impact positive ion (EI^+) mode with a source temperature of 200°C. The electron energy was 70 eV and the filament current 200 μA.

Mass spectra were acquired in the mass range from m/z 50 to 500, using a scan time of 0.9 s and an inter-scan time of 0.1 s. Detection of analytes was also accomplished in selected ion monitoring (SIM) mode, using the following fragment ions: m/z 229, 172 (propazine); 214, 173 (terbuthylazine); 229, 200 (sebuthylazine); 227, 212 (ametryn); 241, 184 (prometryn) and 226, 185 (terbutryn). The dwell time and the mass span were 0.04 s and 0.3 u respectively, for each fragment.

The mobile phase used for HPLC experiments was acetonitrile–0.1 *M* ammonium acetate (45:55, v/v). The flow-rate was 1 ml min^{-1} and temperature was ambient. The detection wavelength was 220 nm (4 nm bandwidth). Spectra were acquired in the 210–400 nm range at the apex and on the ascending or descending part of each peak. Peak purity could be checked by the technique of spectra overlaying after normalisation.

2.4. Soil samples

Two sediment and one soil specimen having an organic carbon content [6] of 0.42, 0.23 and 0.19%, respectively were used for leachability microcolumn experiments. Samples were air-dried at 35°C and sieved through a 1-mm sieve. A topsoil specimen taken from a landfill and suspected to be contaminated by triazines residues was used to test the developed method.

2.5. Solid-phase microextraction

Silica fibres coated with an 85-μm thick polyacrylate (PA) or a 100-μm thick polydimethylsiloxane (PDMS) film and a manual SPME device (Supelco) were employed as described elsewhere [6]. Standard solutions were prepared by spiking 5 ml of triply distilled water into 7-ml clear vials (Supelco). Then, the vials were sealed with hole caps and PTFE-faced silicone septa (Supelco). The extraction was carried out at room temperature for 30 min under magnetic stirring in order to improve mass transfer from the aqueous sample into the fibre coating. Thermal desorption (5 min desorption time) was performed directly into the GC injection port maintained at 250°C.

2.6. Soil leaching microcolumn experiments

Soil/sediment samples (3.0 g) were premixed with water (5 ml) in order to hydrate the active sites, thus allowing the analytes to evenly distribute over the soil and to interact with active sites. Then, 50 μl of the triazines standard mix in acetonitrile were added and the resulting mixture stirred for 0.5 h and left at room temperature overnight. The samples were then centrifuged (2100 *g*, 5 min); the supernatant was subjected to SPME–GC–MS and an aliquot of the residue soil was packed in a polypropylene filtration tube with polyethylene frits (Supelco). Then, 20 ml of triply distilled water were eluted through the soil packed microcolumn, at a constant flow regulated by an oil free membrane vacuum pump (Millipore), and fractions (5 ml) collected into vials. Pesticides concentration in each fraction was determined, as previously described, by SPME–GC–MS. Finally the soil sample was submitted to a solvent extraction procedure in order to estimate the amount of eventually unleached triazines. Briefly, 6 ml of methylene chloride were added to the soil and vigorously shaken; the organic phase was then reduced to dryness by a gentle stream of nitrogen. The residue was reconstituted with 5 ml of triply distilled water and analysed by SPME.

2.7. Solvent extraction for HPLC–UV analysis

The solvent extraction procedure for HPLC–UV analysis was adapted from Ref. [10]. Briefly, soil specimen (0.5 g) was mixed, in a tapered tube, with 0.5 g of anhydrous sodium sulphate, vigorously shaken with methanol (5 ml) for 15 min and then centrifuged for 5 min at 2100 *g*. The supernatant was then transferred into a different tube and then evaporated at room temperature under a gentle stream of nitrogen. The dried extract was reconstituted with 1 ml of acetonitrile and 20 μl were injected.

3. Results and discussion

The effect of the most important parameters (e.g., extraction time, sample temperature, sample pH) influencing the SPME extraction efficiency has been discussed elsewhere [6]. The role of the fibre coating (PA or PDMS) which can also influence extraction efficiency, has been further investigated. To this purpose the fiber–solution distribution coefficients, K_{f-w}, of each analyte has been calculated as the ratio between the concentration of the analyte in the fiber coating and in the solution. As can be seen from Table 1 the K_{f-w} values obtained for PA coating were

Table 1
Distribution constants (log value) between the fibre coating materials and water and octanol–water partition coefficients [11,12] K_{ow} (log value)

Compound	Log K_{PDMS-W}	Log K_{PA-W}	Log K_{OW}
Terbutryn	4.56	5.32	3.65
Prometryn	4.44	5.00	3.5
Terbuthylazyne	4.25	5.05	3.21
Ametryn	4.33	5.12	3.1
Sebuthylazyne	3.64	4.99	3.31
Propazyne	3.60	4.75	2.94

systematically higher than for PDMS coating indicating that the former was the most suitable (all the results shown in the following refer to PA coated fibres). Furthermore, log K_{PA-W} values seems linearly correlated to the octanol–water partition coefficients [11,12] K_{ow} (i.e. the higher the hydrophobicity the higher the affinity of the investigated triazine towards the polymeric phase of the fiber).

Fig. 1 shows SPME–GC–MS calibration curves obtained for the investigated triazines. For each analyte, the MS response (peak area, SIM mode) was linear over the investigated concentration range (5–250 ng ml^{-1}) with a correlation coefficient better than 0.996 and an intercept not significantly different from zero at 95% confidence level. The different slopes observed in the calibration curves roughly reflect the different values of the distribution constants. The highest and lowest slopes are observed for terbutryn and propazine which have respectively, the highest (5.32) and lowest (4.75) value of log K_{PA-W}; intermediate slope values are observed for the remaining triazines which have log K_{PA-W} values around 5.

Detection limits, estimated at a signal-to-noise ratio of 3, were always better than 1 ng ml^{-1}.

SPME–GC–MS, other than fast and solvent-free, appears sensitive enough to be potentially useful for the assessment of triazines leachability order through the analysis of fractions collected from a soil/sediment packed microcolumn elution experiment (see Section 2).

Cumulative recoveries for the analysed triazines are reported in Fig. 2. After the elution of 15 ml of water, propazine, terbuthylazine, sebuthylazine, ametryn and prometryn were nearly completely recovered from sediments 1 and 2. Similarly, prop-

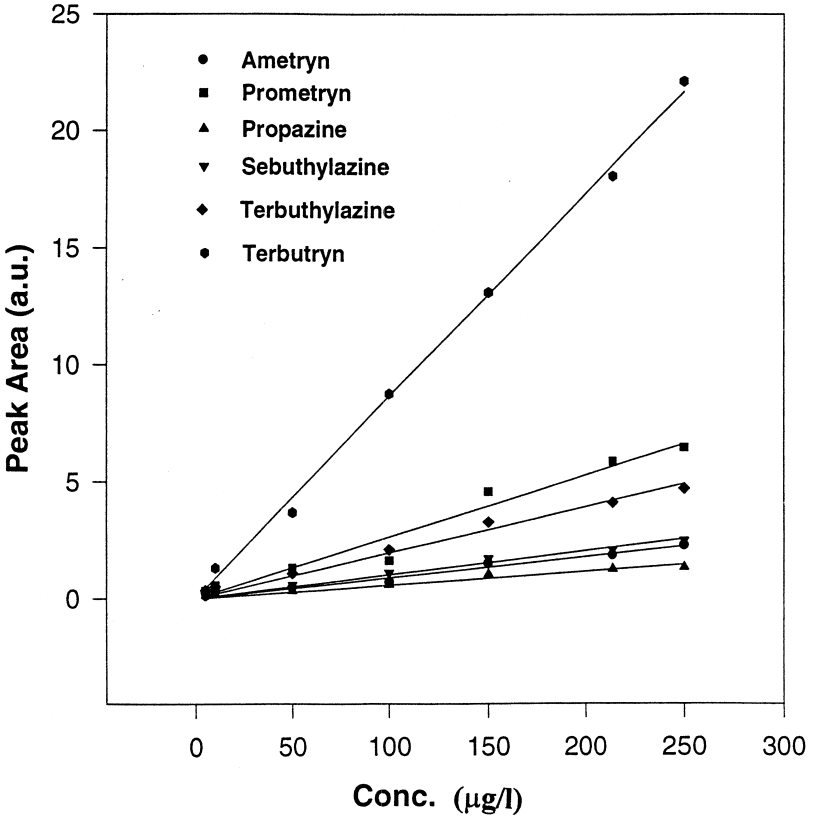

Fig. 1. Calibration curves obtained for the selected triazines by SPME–GC–MS (SIM mode). Fibre coating: 85-μm thick PA; extraction temperature: ambient; extraction time: 30 min under stirring. Fibre desorption conditions in the GC injection port: 5 min at 250°C. For other condititions see text. Each point is the average of three replicates.

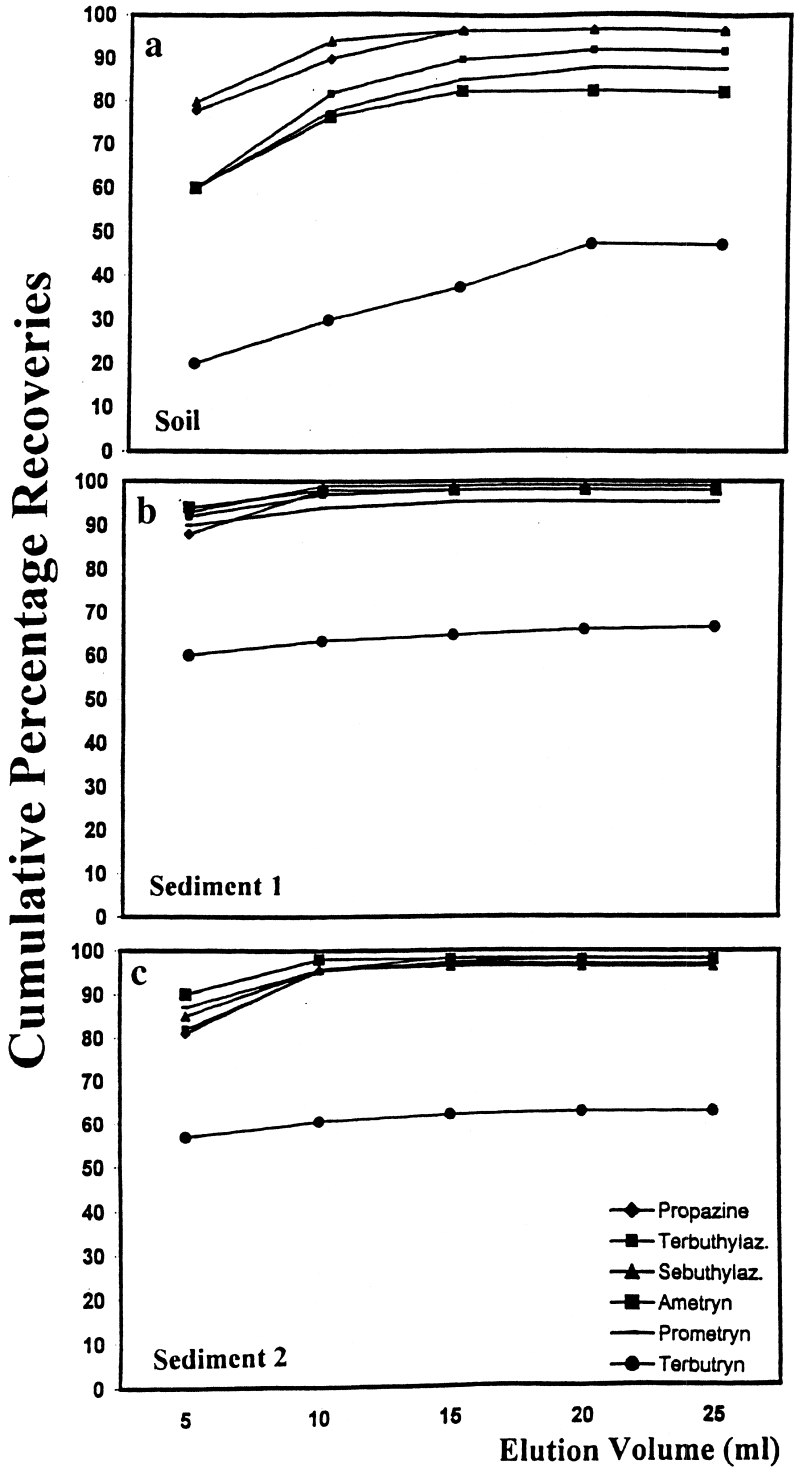

Fig. 2. Cumulative percentage recoveries of the analysed triazines in the water leachate from a microcolumn chromatography experiments. (a) Soil specimen (organic carbon content 0.19%); (b and c) sediments with organic carbon content of 0.42 and 0.23%, respectively.

azine and sebuthylazine were also quantitatively recovered from soil while recovery of terbuthylazine, ametryn and prometryn was not quantitative but still satisfactory (>80%); terbutryn was the only triazine significantly retained on all samples.

In order to have direct evidence of the soundness of results presented in Fig. 2, soil/sediment residues were finally extracted with methylene chloride, the extract reduced to dryness, reconstituted with water and analysed by SPME–GC–MS. As expected, a terbutryn peak was found; however, based on mass balance considerations, terbutryn was not completely recovered even by solvent extraction since ≈30% was still retained on the soil. This means that, expecially for triazines with the highest K_{ow} (see Table 1) or K_{oc} (see [6]), there is a strongly bound fraction which is neither leached nor extracted by organic solvent. It is interesting to observe that triazines (especially the more hydrophobic) tends to be more retained on soil than on sediment even when their organic carbon content (see Fig. 2a and c) is comparable. This seems to indicate that the nature of the organic fraction is an important factor. Indeed the major organic components of agricultural soils and

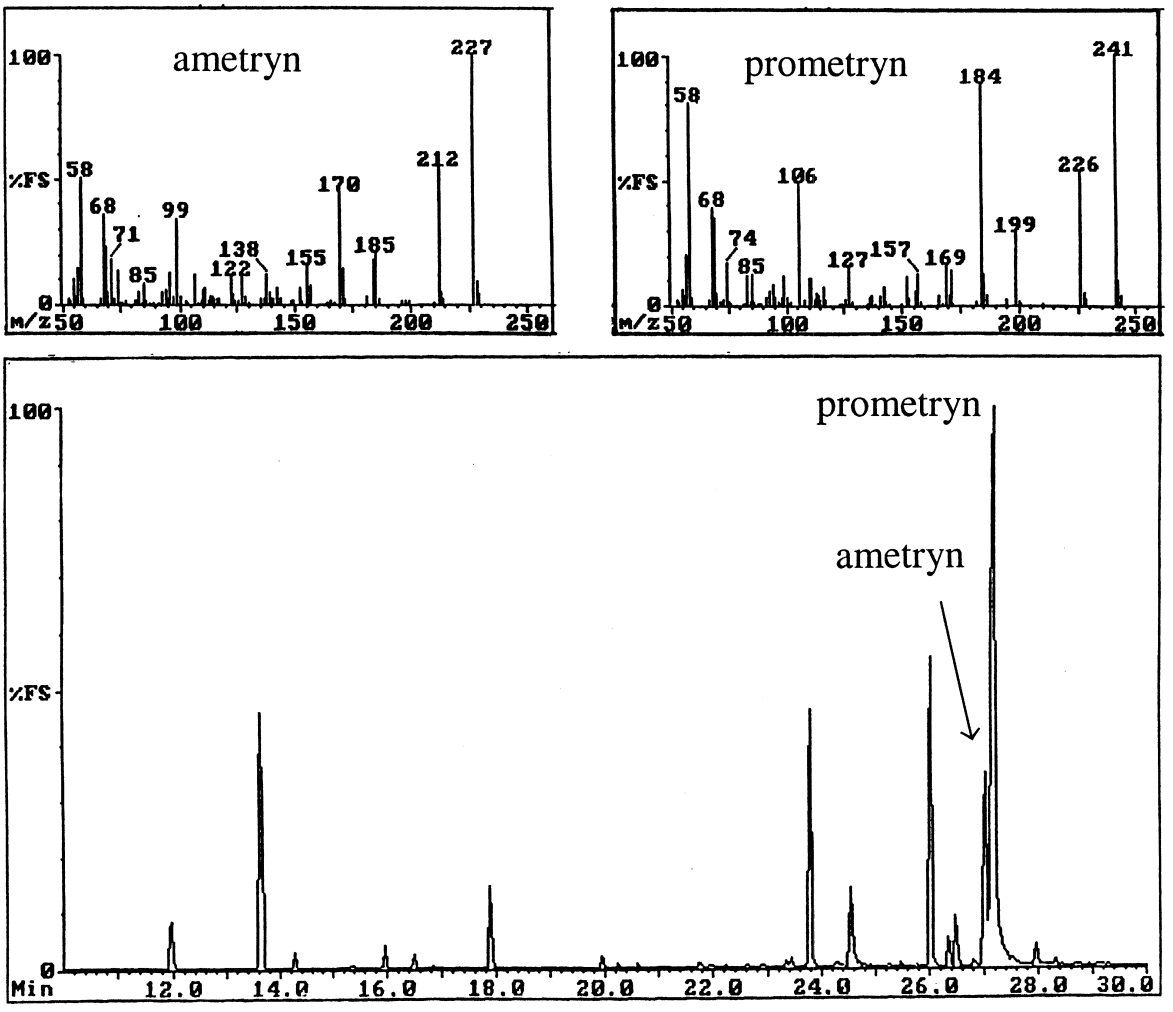

Fig. 3. SPME–GC–MS chromatogram relevant to the leachate of a 'real' soil sample contaminated with triazine residues, acquired in the scan mode. The insets report the EI mass spectra of of ametryn and prometryn.

sediments are represented by humic and fulvic acids, respectively. The difference between the triazines binding observed passing from soils to sediments can likely be ascribed [13] to structure differences between humic and fulvic acids, one being a three-dimensional colloidal gel particle an the other a flexible linear polymer.

Obviously, the described microcolumn experiments cannot quantitatively predict leaching under field conditions since phenomena other than soil sorption (e.g., hydrodynamic dispersion/diffusion and evapotranspiration) are involved. As a consequence, the real usefulness of leachability laboratory test results is to assess the triazines leachability order; thus, a useful picture of the relative mobility of the considered herbicides in a given soil can be obtained, irrespective of the particular eluent (extractant) used. In this respect, $CaCl_2$ is often used [14] in soil column chromatography experiments, but water is also used [15,16]. In our particular case there was a reason for using water since the first step of our experiments was soil equilibration with water containing triazines. Moreover, the present work aimed essentially to demonstrate that SPME–GC–MS can be successfully employed to analyse soil leachates in order to assess a leachability order.

As demonstrated by the described experiments, a number of triazines are easily leached by water and can be, apparently, recovered with satisfactory yield using the above mentioned procedure. This, suggests that SPME–GC–MS could also be used for quantitative determination, at least in the soil leachate.

Fig. 3 shows the SPME–GC–MS chromatogram (acquired in the scan mode) obtained on the leachate of a soil specimen suspected to be accidentally contaminated with triazines residues. A careful inspection of MS spectra (see insets in Fig. 3) revealed that peaks eluted in the time windows 26.5– 28.0 min can be assigned to prometryn and ametryn. An aliquot of the soil sample was then spiked with a known amount (30 μg/g) of terbuthylazine which has a leachability similar to that of prometryn and ametryn and can act as an internal standard; the soil leachate was then analysed (see Fig. 4) by SPME–GC–MS (SIM mode). Terbuthylazine was found to be recovered to an extent of 80% (incidentally the same recovery observed for soil sample in Fig. 2a). The same sample was submitted to a classical

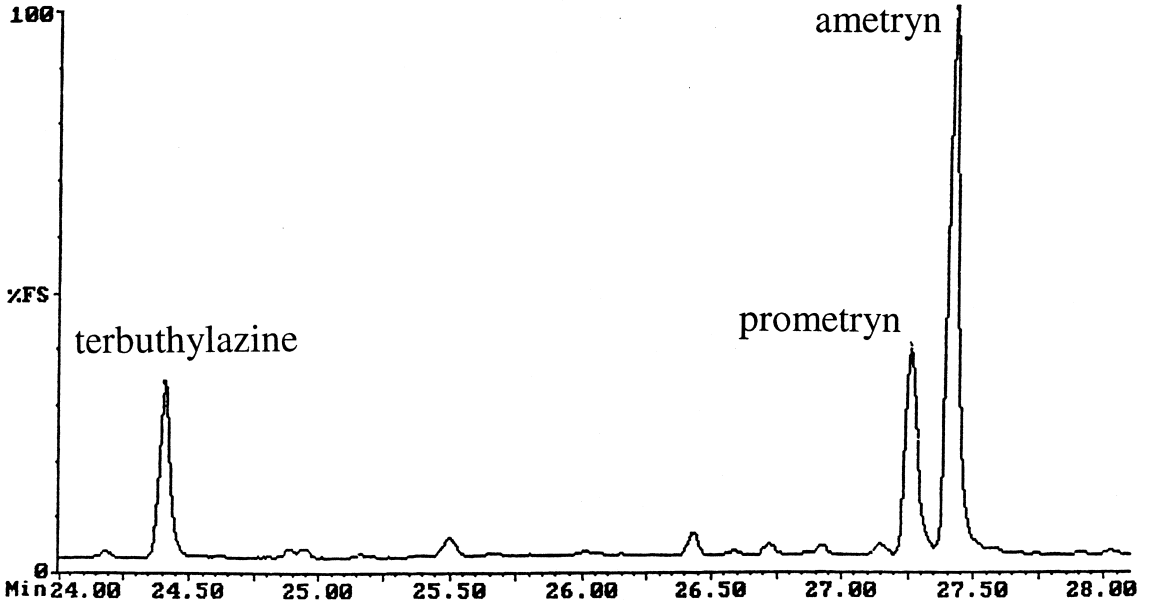

Fig. 4. SPME–GC–MS chromatogram relevant to the leachate of a 'real' soil sample contaminated with triazines residues, spiked with terbuthylazine (30 μg/g), acquired in the SIM mode. Selected ions: m/z 214, 173 (terbuthylazine); m/z 227, 212 (ametryn); m/z 241, 184 (prometryn).

solvent extraction step followed by HPLC–UV detection; Fig. 5 shows the relevant chromatographic trace. Recovery of terbuthylazine from spiked soil, by solvent extraction, was in this case nearly quantitative (97±3%). Estimated concentrations of prometryn and ametryn in soil, (assuming the same recovery as for terbuthylazine) were 47.8 and 47.4 μg/g, respectively.

Interestingly, the prometryn and ametryn content in the analysed soil estimated (after correction for the recovery of terbuthylazine) from the leachate concentrations measured by SPME–GC–MS were 49.7 and 43.6 μg/g (RSD 8%), respectively, which is in good agreement with values given by solvent extraction followed by HPLC–UV. This seems to indicate that, at least in the present case, leachate concentrations measured by SPME–GC–MS seems well correlated to the soil concentrations as measured by a more conventional procedure based on solvent extraction followed by a chromatographic step.

However, it should be stressed that this finding cannot be easily generalised and that the SPME–GC–MS procedure is strictly valid for soil leachates only. This is essentially because, in general, estimation of recovery is not a trivial task especially in the absence of suitable reference materials. In fact

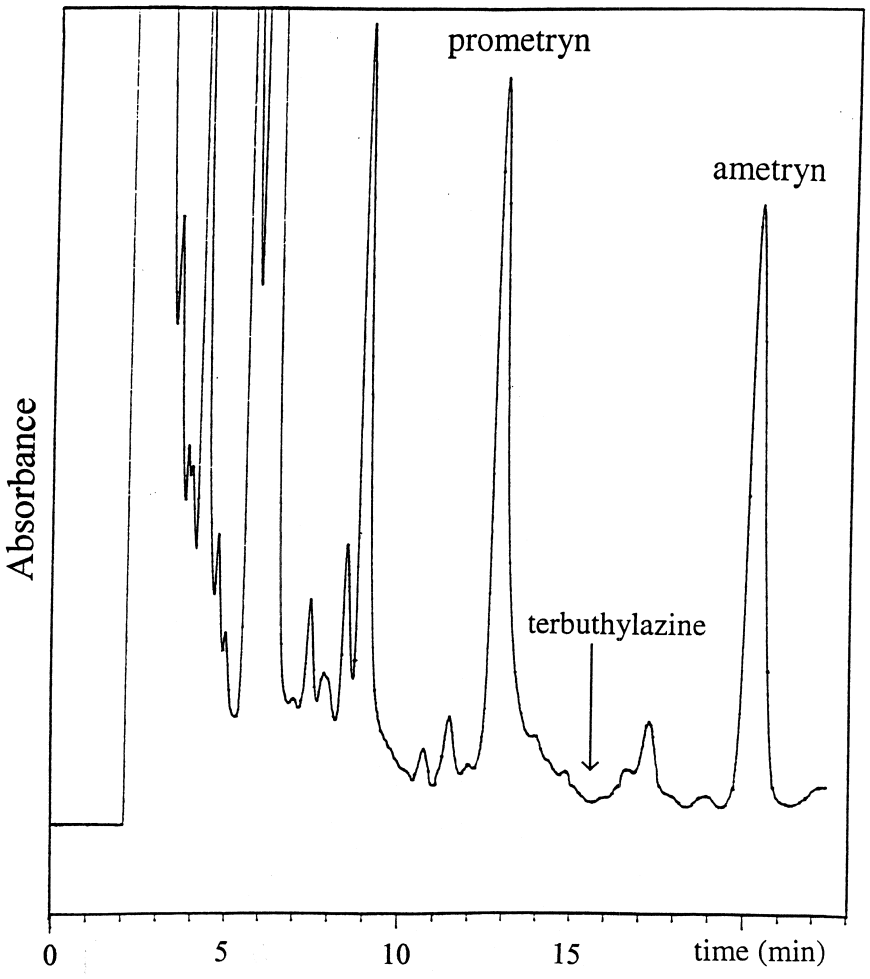

Fig. 5. HPLC–UV chromatogram relevant to the extract of a 'real' soil sample contaminated by triazines residues submitted to the solvent extraction procedure described in Section 2. The arrow indicates the retention time of terbuthylazine. Detection wavelength: 220 nm (4 nm bandwidth). Absorbance axis: 150 mAU full scale.

spiking of samples can results [17] in the analyte being in a 'deposited state' instead of the 'sorbed state' leading to misleading information. On the other hand, simulation of sample weathering by sample spiking well before analysis [18,19] in any case relies on the assumption that any analyte–matrix interactions may have occurred to an extent similar to those in a 'real' contaminated soil.

4. Conclusions

The SPME–GC–MS procedure presented here proved to be a useful tool for the assessment of the leachability order of triazines in selected soil/sediment samples. It possess the advantages of SPME (fast, simple, highly sensitive and solvent free) and could be potentially used to determine the degree of leaching for other classes of pesticides, even in soil specimen having relatively high organic carbon content (work in progress). It was also demonstrated that, for the particular 'real' soil sample analysed, leachate concentration measured by SPME–GC–MS correlated well with the soil concentration obtained by solvent extraction followed by HPLC–UV detection.

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