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Solid-phase microextraction method for the determination of atrazine and four organophosphorus pesticides in soil samples by gas chromatography

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Abstract

A simple and rapid solid-phase microextraction (SPME) based method is presented for the simultaneous determination of atrazine and four organophosphorus (i.e. parathion-methyl, chlorpyriphos, methidathion and carbophenothion) in soils. After optimisation of the different experimental variables affecting the SPME of the target compounds from aqueous solutions by using an experimental design, a consensus strategy was adopted which allowed the use of the SPME method developed for the simultaneous screening of all the analytes selected. The complete analytical procedure finally proposed consisted of a 15-min ultrasonic extraction of the target compounds from a 0.5-g soil sample with 5 ml of methanol and the dilution of this extract to up 10% methanol followed by the addition of NaCl to a final concentration of 10% (w/v). The analytes in this aqueous extract were preconcentrated for 30 min in the SPME fiber and subsequently desorbed by heating of the fiber at 260°C for 5 min in the gas chromatograph injection port. Final determination was carried out with an electron-capture detector. The recoveries of the pesticides studied in soils ranged from 72 to 123%, except for atrazine, and the SDs were below 16%. The feasibility of the procedure finally proposed for the screening of the endogenous pesticides irrespective of the properties of the soil selected has been shown. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Solid-phase microextraction; Atrazine; Pesticides; Organophosphorus compounds

1. Introduction

The social concern about the levels of pesticides in the environment and the constant trend observed in current legislations to reduce their maximum residue levels allowed in a variety of matrices is increasing the number of samples to be analysed as well as the need for their accurate determination at very low levels. Solid-phase microextraction (SPME) has been regarded as a valuable (solvent-free) alternative analytical technique to more traditional procedures which reduces the laboratory-generated waste and time for sample preparation while providing adequate accuracy [1].

The SPME technique was initially used for the determination of volatile compounds [2,3] and for preconcentration of a variety of organic compounds from aqueous samples [4–7]. However, it was only recently that its feasibility for the determination of pollutants in soil samples started to be investigated.

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Those studies have shown the direct dipping of the fiber in an aqueous suspension of soil [8] and, especially, in a diluted organic extract previously obtained by a conventional solid–liquid extraction method [4] as the best strategies for SPME of pesticides from soils. Surfactant mediated SPME is a novel approach which attempts to overcome the usually reported lack of sensitivity of the quoted above extraction procedures while preventing the use of an organic extraction solvent. In spite of the promising results reported when this method was applied for the analysis of pesticides in fruit slurries [9], to our knowledge, its feasibility for soil analysis has not been tested yet.

The objective of this work was to develop an SPME-based procedure for fast simultaneous screening of atrazine and four organophosphorus pesticides in soils. A factorial experimental design was used for the optimisation of the experimental variables affecting the SPME step as this approach provides a maximum of information with a low number of experiments and allows us to study the (possible) cross effect among the parameters investigated. The optimised method was applied to the determination of the endogenous pesticide levels in different soils.

2. Materials and methods

2.1. Chemicals

Atrazine, methyl parathion, chlorpyriphos, fenamiphos and methidathion were obtained from Riedel-de-Haen (Hannover, Germany). Standard stock solutions (1000 mg/l) were prepared in acetonitrile (Scharlau, Barcelona, Spain) and stored at −20°C in the dark. Working standard solutions were prepared daily. Dodecylsulphate sodium salt (SDS, 99% purity) and sodium chloride (99.5–100%) were purchased from Merck (Barcelona, Spain), acetone, *n*-hexane, methanol from SDS (Peypin, France). All reagents used were analytical grade.

Four soil samples from the Madrid region, Spain, with sand and clay contents ranging from 55 to 92% and from 0.3 to 26%, respectively, were used in this study. Soils were air dried and sieved to 35 mesh before using.

2.2. Apparatus and experimental conditions

The SPME fiber holder for manual extraction and the fibers of polydimethylsiloxane (PDMS, 100 μm film thickness) were from Supelco (Madrid, Spain). SPME fibers were conditioned by heating at 250°C for 2 h in the gas chromatograph (GC) injection port according to the manufacturer recommendations and reconditioned between two consecutive extractions at 260°C for 4 min.

An ultrasonic bath (Ultrasons Selecta, J.P. Selecta, Barcelona, Spain) and a Hermle Z-320 centrifuge (Hermle, Germany) were used for the extraction of the pesticides from the soils and to separate the soil particles from the supernatant, respectively.

GC separation was performed using an HP 5890 series II GC (Hewlett-Packard, Alto Palo, CA, USA) equipped with an ECD, on a DB-5 60-m fused-silica capillary column (0.25 mm I.D., 0.25 μm film thickness) supplied by J&W Scientific (Folson, CA, USA). The GC oven program started at 60°C (6 min) and increased at 15°C/min to 280°C (11 min). The detector temperature was set at 300°C. Nitrogen was used as a carrier gas at a column head pressure of 22.4 p.s.i. and as make-up gas (60 ml/min).

Final confirmation of the pesticides in the soil extracts was performed by GC (HP 5890 Series) with mass spectrometer (HP 5890 Series) detection (GC–MS) in the selected ion monitoring (SIM) mode. In this part of the study, 4 ml of the soil extract were concentrated to 100 μl and a 1-μl sub-sample was injected in the splitless mode (splitless time, 1.0 min) in a capillary OV-1 column (25 m×0.25 mm I.D., 0.25 μm film thickness) from J&W Scientific. The oven was programmed from 70°C (1.3 min) to 280°C (5 min) at a rate of 15°C/min. Helium was used as the carrier gas at a column head pressure of 8 p.s.i. For each compound, the two most abundant ions produced by electron ionisation (EI) at 70 eV were monitored, i.e. ions 215/200 for atrazine, 109/125 for methyl parathion, 197/97 for chlorpyriphos, 145/125 for methidathion and 342/157 for carbophenothion.

2.3. Procedures

SPME procedure for water. Preliminary experiments were carried out to optimise the main parame-

ters affecting the SPME of the pesticides investigated from aqueous solutions (i.e. extraction time, ionic strength and magnetic stirring rate of the solution) and their subsequent quantitative thermal desorption in the GC injection port (i.e. temperature and time of desorption). In these studies Milli-Q water samples spiked with the appropriate amount of the standard solution to give a final concentration of 3 ng/l and equilibrated for 15 min were used. Optimisation was carried out by factorial experimental design [10].

After optimisation, a typical experiment consisted of the direct immersion of the conditioned fiber into the spiked water sample (3 ml with 10% NaCl, w/v) contained in a 4-ml amber glass vial. SPME was carried out under magnetic stirring for 30 min at ambient temperature. Desorption of pesticides was carried out at 260°C for 5 min in a splitless injector. Efficiency of the SPME of pesticides from water samples under the experimental conditions finally proposed was evaluated by direct comparison of the SPME peak areas with those of the calibration lines obtained by 1-μl splitless injection of the pesticides selected in *n*-hexane (calibration range 0.25–7.5 ng/ml with coefficient of correlation better than 0.994). Then, new calibration lines were constructed by SPME of the target compounds from water samples spiked at concentration levels ranging from 0.1 to 10 ng/ml (0.03–3.3 ng/ml for chlorpyrifos) under these experimental conditions. These calibration lines were used for quantification in subsequent experiments.

SPME procedure for soil. The feasibility of the method developed for SPME of pesticides from aqueous solutions was investigated for the analysis of the analytes selected in soil aqueous extracts. In this part of the study, sub-samples of a sandy soil (92% sand and 0.3% clay) were spiked at the 100 (or 75) ng/g level with the target compounds by pre-soaking the sample in methanol containing the pesticides at the appropriate level for 5 min. Then, methanol was allowed to evaporate in a fume hood overnight. Feasibility of two soil extraction methods for obtaining the aqueous solutions subjected to SPME was evaluated: ultrasonic extraction with methanol and surfactant mediated extraction with SDS. In the former method, a 0.5-g soil sample spiked at the 100 ng/g level was extracted for 15 min by ultrasonic agitation with 5 ml of methanol. Methanol was chosen because of its high efficiency for the extraction of the target compounds from soil allowed to minimise the volume of the organic solvent used in this step. After the extraction, the solid particles were separated from the supernatant by centrifugation (15 min at 2000 g). A 2-ml volume of this methanolic extract was diluted to 20 ml with Milli-Q water as it has been proved that percentages of methanol up to 10% did not affect the SPME of polar pesticides from aqueous solutions [4]. A 3-ml aliquot of this aqueous solution was then used for SPME and subsequent analysis. In the surfactant mediated extraction approach, a 0.5-g soil sample spiked at the 75 ng/g level with the target compounds was extracted by shaking for 10 min with 5 ml of a 10% NaCl (w/v) extraction solution containing 3 mg/ml of SDS [9]. Then, the supernatant was removed and the extraction repeated. Both extracts were mixed and diluted to 12 ml to ensure that the micelles were broken [11] and pesticides available for SPME. A 3-ml aliquot of this aqueous solution was used for SPME and subsequent analysis. Efficiency of the two soil extraction methods tested was evaluated by comparison of the SPME peak areas obtained in each case with those of the calibration lines constructed by SPME of spiked water samples. Finally, the optimised extraction method plus SPME procedure was applied to the analysis of pesticides in non-spiked soils.

Blank samples, i.e. non-spiked water and soil samples, were analysed to check any contamination throughout the analytical procedure. No background interference was found to be introduced by the proposed methodology. Otherwise specified, experiments were carried out in duplicate.

3. Results and discussion

3.1. Optimisation of SPME of pesticides from water by factorial experimental design

On the basis of the result previously published for the target compounds, a PDMS fiber was chosen as this material has been reported to have a satisfactory extraction efficiency for a variety of compounds, including atrazine [4] and some of the organophosphorus pesticides selected in this study [5].

Some preliminary experiments were carried out to

determine the boundary levels for the parameters affecting the desorption of the analytes from the SPME fiber. In these experiments, 3 ml of Milli-Q water containing 10% NaCl (w/v) and spiked at the 3-ng/ml level were extracted under magnetic stirring for 10 min at ambient temperature. According to these preliminary assays (results not shown), desorption temperatures in the range 200–260°C and desorption times ranging from 2 to 5 min were selected for the central composite design used to further proceed with method optimisation. As regards the variables affecting the SPME sorption, the extraction time and the ionic strength were considered to be the most relevant according to previously reported data [5] and, therefore, included in the experimental design used in this study. Extraction times ranging from 5 to 40 min were investigated. Due to the contradictory results published for the salting-out effect, i.e. ionic strength — as percentage of NaCl, w/v — in SPME methods [4,12,13], percentages of NaCl ranging from 0 to 30% were considered in the experimental design. The rest of the parameters were kept identical to those used in previous experiments. As an example of the results found in this part of the study, Figs. 1, 2 and 3 summarise, respectively, those obtained for pesticides with relatively high, intermediate and low polarity (i.e. atrazine, chlorpyriphos and carbophenothion, respectively).

The pareto chart shows the influence that each factor investigated has on the response obtained for every particular compound studied as well as the possible cross effect among these factors. As expected, and in agreement with previous observations [4], the pareto chart obtained for atrazine (Fig. 1A) shows that the ionic strength has a major effect on the SPME efficiency of the most polar pesticides investigated. The extraction time, the desorption temperature as well as the interaction between these factors and the ionic strength were also found to be statistically significant. However, the desorption time had not significant effect since this bar did not cross the vertical line in Fig. 1A that represents a 95% test for significance. Fig. 1B shows that the higher the percentage of NaCl and the longer the extraction time, the higher the response obtained for atrazine when using a desorption temperature of 230°C and a desorption time of 3.5 min. Rather similar results

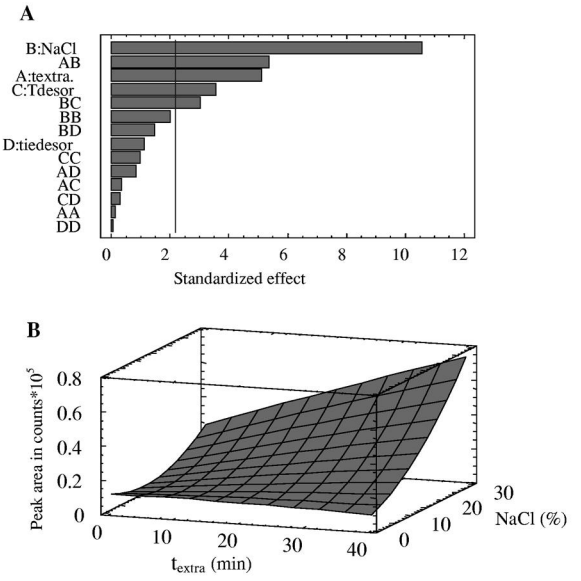

Fig. 1. Atrazine (A) pareto chart for the standardised main effects in the central composite design, and (B) response surface estimated from the factorial design by plotting extraction time versus NaCl% with a desorption *T* of 230°C and a desorption time of 3.5 min.

were obtained for pesticides with intermediate polarity as chlorpyriphos (Fig. 2A). However, as it can be read out from both the pareto chart (Fig. 2A) and the response surface obtained when using a desorption temperature of 230°C and a desorption time of 3.5 min (Fig. 2B), the ionic strength did not have as dramatic an effect as in the case of more polar pesticides. Finally, in the case of the most apolar pesticide investigated (carbophenothion) again the time of desorption did not show any significant effect (Fig. 3A) and the SPME efficiency was found to increase with the desorption temperature. Nevertheless, a negative effect was observed for the ionic strength: the SPME efficiency increased as the percentage of NaCl added to the aqueous solution decreased. Competition between this apolar compound and the more polar ones, for which sorption on SPME fibers clearly improved with the ionic strength, could be proposed as a possible explanation for this result. This finding could also explain the contradictory results published for authors as regards the salting-out effect on SPME methods [4,12,13].

As somewhat divergent optimum conditions were

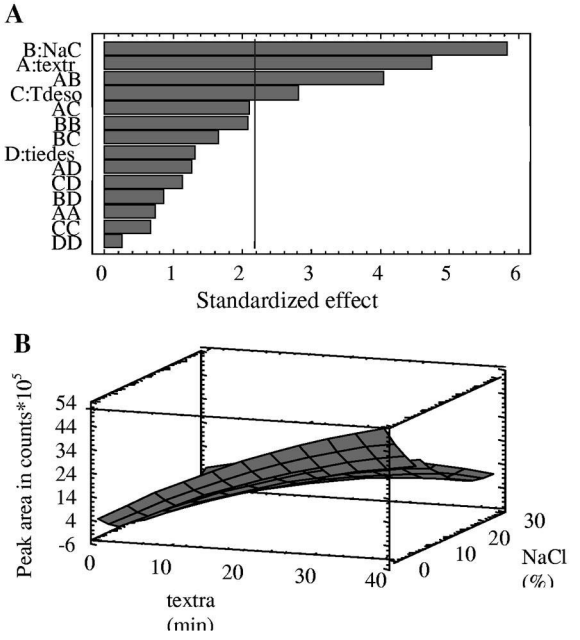

Fig. 2. Chlorpyriphos (A) pareto chart for the standardised main effects in the central composite design, and (B) response surface estimated from the factorial design by plotting extraction time versus NaCl% with a desorption *T* of 230°C and a desorption time of 3 min.

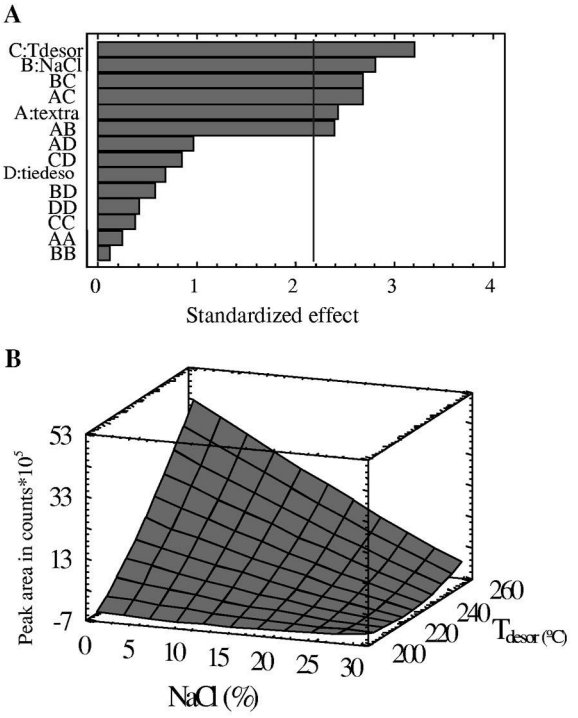

Fig. 3. Carbophenothion (A) pareto chart for the standardised main effects in the central composite design, and (B) response surface estimated from the factorial design by plotting temperature of desorption versus NaCl% with an extraction time of 22.5 min and a desorption time of 3.5 min.

obtained for the SPME of the different compounds studied, a consensus solution was adopted for subsequent experiments. The final proposed method for simultaneous screening of the pesticides selected consisted on a direct immersion of the SPME fiber placed off-centre in a vial containing a 3-ml water sample at room temperature for 30 min followed by desorption at 260°C (5 min). Because of the opposite trends observed for pesticides with different polarity when adding NaCl to the water, an intermediate percentage of NaCl (15%, w/v) could be initially considered as a convenient solution. However, due to the fast degradation of the PDMS SPME fiber previously reported when using percentages of NaCl higher than 15% [4], a 10% NaCl was selected for subsequent experiments.

Finally, as the rate-limiting step in SPME is the diffusion of the compounds throughout the solution and this diffusion rate can be speeded up by increasing the agitation, this agitation speed was set at the maximum for subsequent analysis.

The feasibility of the SPME method developed was evaluated by analysing water spiked at different concentration levels (in the range 0.1–10 ng/ml, except for chlorpyriphos, 0.03–3.3 ng/ml). The recoveries of the pesticides investigated at the 0.1 ng/ml level were 74% for atrazine, 22% for methyl parathion, 100% for chlorpyriphos, 98% for methidathion and 54% for carbophenothion. These values were in the range [5,14] or even better [4] than those previously published for similar applications involving a PDMS SPME fiber, except for carbophenothion for which no previous data were found in the literature. The repeatability of the whole SPME procedure, evaluated as the relative standard deviation (RSD, $n=3$) at the 0.1-ng/ml level, was better than 6%, except for atrazine (20%) because the corresponding chromatographic peak showed some tailing. It is important to note that, for obvious reasons, the absolute recoveries tended to decrease with the spiking level for concentrations higher than

5 ng/ml. However, the amount versus area response was linear over the whole range tested for all the compounds studied with coefficient of correlation in the range 0.91–0.97. The experimentally calculated limits of detection in water were in the range 0.01–0.04 ng/ml which proves the feasibility of the proposed SPME method for environmental applications.

3.2. SPME of pesticides from soil

The SPME method developed was tested for pesticides analysis in the aqueous soil extracts obtained by either ultrasonic extraction with methanol or surfactant mediated extraction with SDS of a spiked sandy soil. Fig. 4 summarises the results of these experiments.

In all instances, ultrasonic extraction was found to be more efficient than surfactant mediated extraction for the extraction of the target compounds from the spiked soil studied. The recoveries obtained with the ultrasonic method ranged from 72 to 90%, except for atrazine (47%). These results can be also read out from Fig. 5 which shows that the chromatogram corresponding to the ultrasonic extract was cleaner than that obtained with the procedure involving surfactants. Therefore, the former extraction method was used in subsequent experiments.

Table 1 summarises the recoveries obtained when analysing four different soils spiked at the 100ng/g level (chlorpyriphos, 33 ng/g). The recoveries ranged from 72 to 123% with SD lower than 16% for the organophosphorus pesticides studied and were somewhat lower, but still in the range of those previously published for SPME methods [4], for atrazine (43–62% with SD below 11%). The high polarity of atrazine compared with the rest of the pesticides included in the study and the dependence of its adsorption on this particular fiber on the percent of NaCl added to the solution (see Fig. 1) could be proposed as possible factors related to the relatively low recoveries obtained for this pesticide under the experimental conditions used in this study. The typical experimentally calculated limits of detection in real soil samples were in the range 0.6–7 ng/g soil (except for atrazine, 30 ng/g soil, and methyl parathion, 24 ng/g soil).

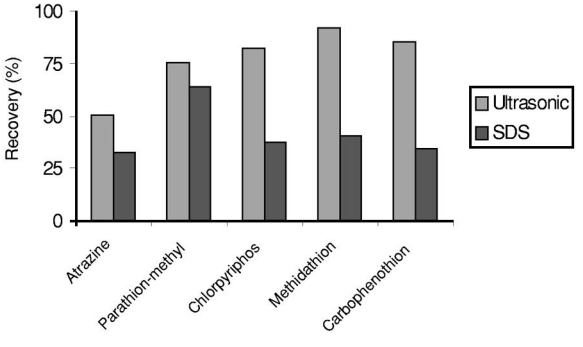

Fig. 4. Comparison of results obtained after ultrasonic and surfactant mediated extraction of the pesticides studied from a soil spiked at the 100-ng/g soil level (chlorpyriphos, 33 ng/g soil) followed by SPME of the analytes from the aqueous extracts. Results as average of two separate analyses.

The method proposed was also applied to the determination of the endogenous levels of the pesticides studied in non-spiked soils. Pesticides were quantified by GC–ECD while GC–MS was used for confirmation purposes. As a typical example of the results obtained, Fig. 6 shows the MS chromatogram obtained when applying the ultrasonic extraction plus SPME method developed to the determination of the endogenous pesticides in a soil as well as the fragmentograms of chlorpyriphos and carbophenothion. Only these two pesticides were found at quantifiable levels in the samples investigated (levels in the range 3.7–4.8 ng/g soil for chlorpyriphos and 13–16 ng/g soil for carbophenothion). Atrazine and methyl parathion were never detected. Analysis of the soil extracts by GC–MS solved the interference affecting the GC–ECD determination of methidathion (see Fig. 5) and proved that the levels of this pesticide were below the limit of detection in the four soils studied.

4. Conclusions

A fast screening method based on a 15-min ultrasonic extraction of a 0.5-g soil sample with methanol, dilution of the methanolic extract and subsequent SPME of the analytes from the aqueous solution has been developed for the determination of atrazine and four organophosphorus pesticides, methyl parathion, chlorpyriphos, methidathion and carbophenothion. The main experimental parameters

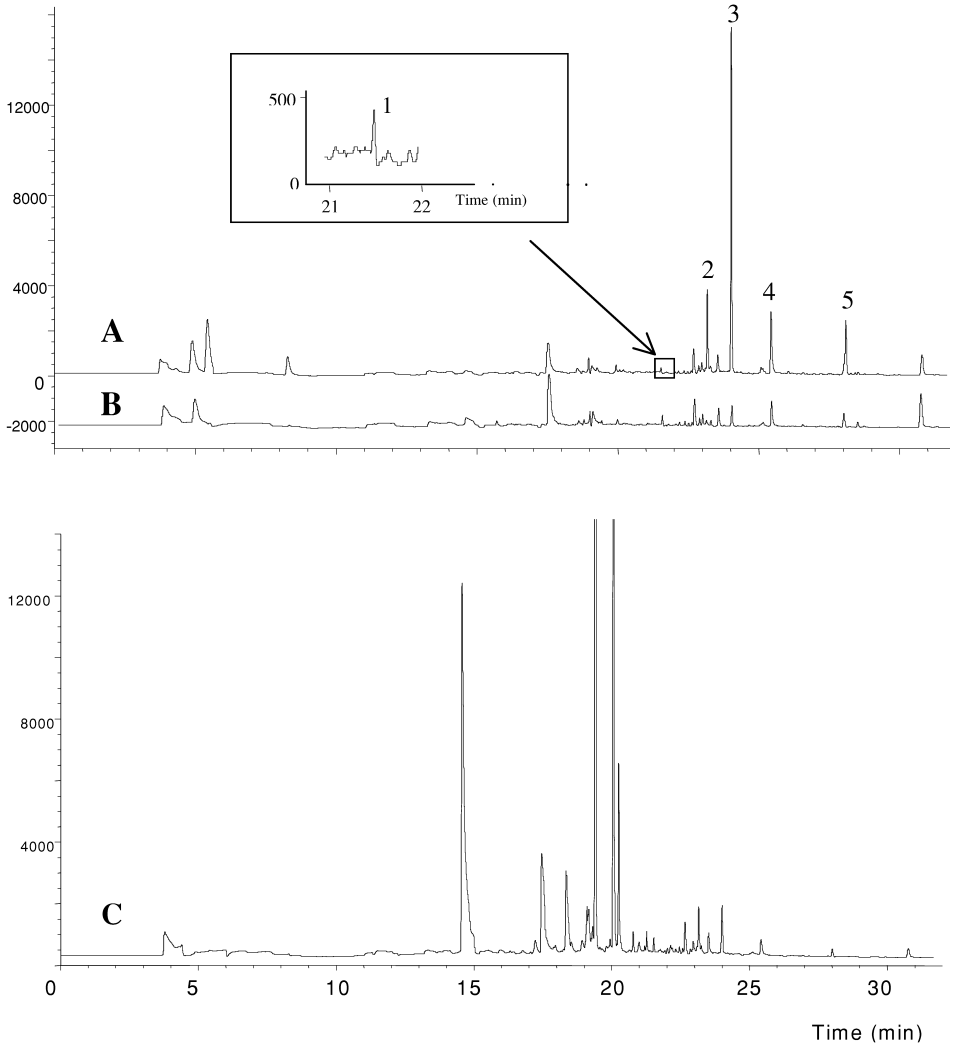

Fig. 5. GC–ECD chromatograms obtained after ultrasonic extraction of a soil with (A) and without (B) spiking, and (C) surfactant mediated extraction of the same spiked soil followed by SPME of the analytes from the aqueous extracts. Spiking level 100 ng/g (chlorpyriphos, 33 ng/g). The same scale has been used in all cases. Pesticides: 1. atrazine, 2. parathion-methyl, 3. chlorpyriphos, 4. methidathion and 5. carbophenothion.

affecting the SPME step were optimised by applying an experimental design which allowed us to obtain maximum information with a minimum number of assays. The method developed is simple, as the ultrasonic extracts can be subjected to SPME without any additional clean-up, fast (around 50 min) and involves a minimum of organic solvent (5 ml of methanol). The similar recovery and SD values obtained for the target compounds when the procedure developed was used for the analysis of different soils support its feasibility for the fast screening of the pesticides selected irrespective of the properties of the soil investigated.

Acknowledgements

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Table 1
Recoveries (%, $n=3$) and SD obtained after ultrasonic extraction and SPME of the aqueous extracts of four different soils spiked at the 100 ng/g level (chlorpyriphos, 33 ng/g)

Pesticides	Soil 1	Soil 2	Soil 3	Soil 4
Atrazine	47±7	62±9	43±11	60±7
Methyl parathion	79±6	107±6	97±15	105±10
Chlorpyriphos	86±1	105±16	105±14	107±5
Methidathion	90±11	ND [a]	ND [a]	ND [a]
Carbophenothion	72±7	112±5	123±7	106±12

[a] Not determined because of coelution with an impurity.

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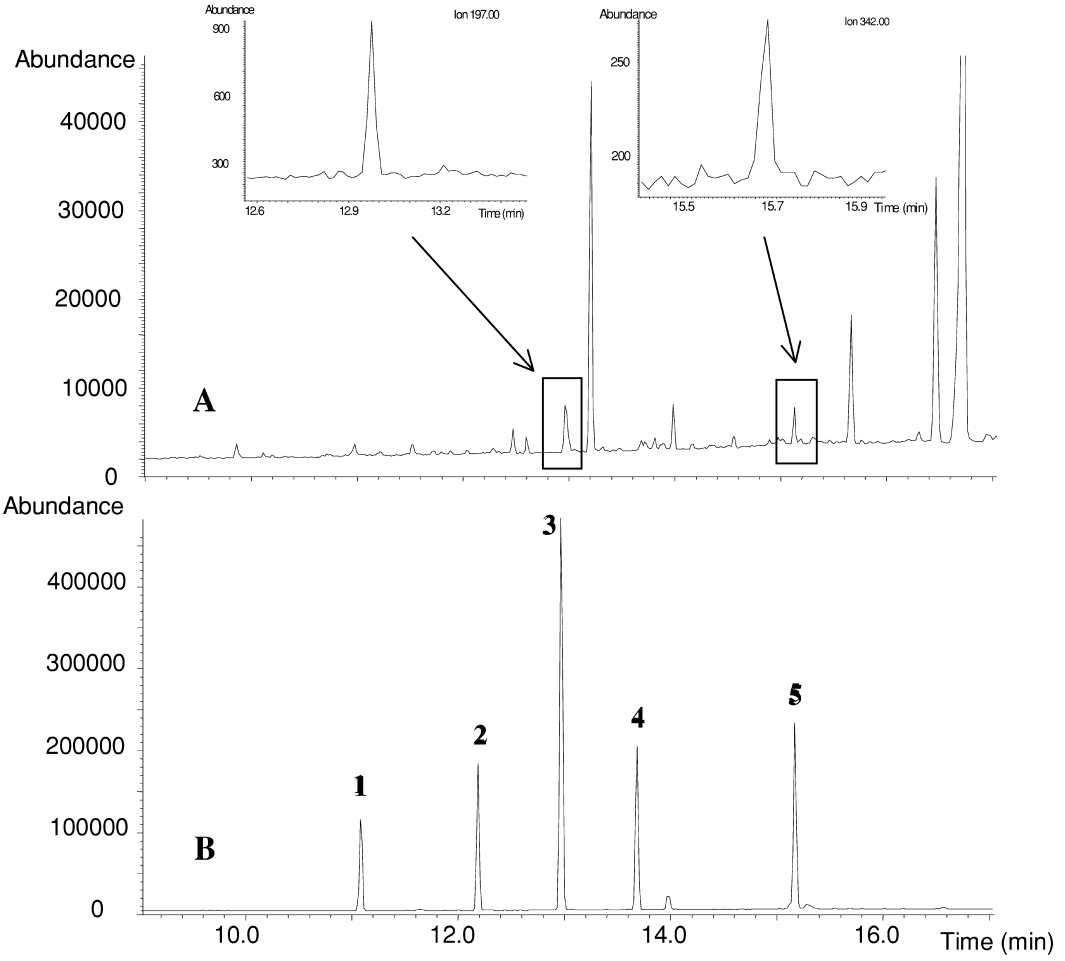

Fig. 6. GC–MS chromatogram obtained (A) after ultrasonic extraction plus SPME of the aqueous extract for a non-spiked soil and (B) direct injection of a standard solution containing the pesticides investigated at the 10-ng/ml level. Inserts correspond to the fragmentograms of chlorpyriphos (ion 197) and carbophenothion (ion 342). See Fig. 5 legend for peak numbering.

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