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Optimization of a solid-phase extraction technique for the extraction of pesticides from soil samples

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Abstract

Progress has been made towards optimizing a rapid, accurate method for extracting pesticides from soil for routine analyses. The basis of the method is the formation of a supernatant by sonication of the soil with a water–acetone mixture. After removal from the soil particles, the pesticides are extracted by passing through a disk of octyl-bonded silica sorbent.

To establish the suitable extraction conditions, several variables affecting the performance of the C_8 disk, such as the influence of the use of centrifugation or filtration for the removal of supernatant and soil particles and the effectiveness of the prior sonication step, such as water proportion, water temperature and pH of the extractive solution, have been studied. Maximum yields were obtained by extraction with a mixture of water at 50°C and acetone (1:1), and centrifugation to remove the supernatant prior to the solid-phase extraction (SPE).

In order to evaluate the matrix effect in SPE of the pesticides, recovery experiments were performed for soil samples with different physical and chemical characteristics.

1. Introduction

A proportion of all pesticides used in agriculture reaches the soil. Even when the pesticide is applied to plant foliage, the soil is a major reservoir and site of potential degradation. It is known that when some pesticides, or their degradation products, enter the soil they become bound to the organic matter or the clay mineral fraction of the soil [1]. Because of the difficulties involved in extraction and identification, it has generally only been possible to demonstrate the presence of these soil-bound residues using radiolabeled pesticides [2].

Since pesticides are toxic by design, there is a

natural concern about the impact of their presence in the environment on human health and environmental quality. Moreover, it is well known that several pesticides used for agricultural purposes are associated with surface and groundwater contamination [3,4]. Therefore, it is important to be able to predict the fate of pesticide residues in the environment, and optimization of their extraction is a key step towards this goal.

A wide variety of methods and solvents have been used to extract pesticides from soil, including agitation of the soil and a solvent for various periods of time at various temperatures [5,6], sonication [7] and soxhlet [8] extraction. However, difficulties are encountered when these techniques are used prior to chromatography

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determination, in which the limits of quantification are often in the ng/ml range, because the solvents are non-selective and therefore tend to extract endogenous material from the soil, which produces spurious peaks on the chromatogram. A clean-up process is therefore generally needed in order to attain a satisfactory limit of detection, but the cleaning methods are time-consuming and require the use of large volumes of solvent [6,9].

The use of supercritical fluids in environmental analysis has become increasingly apparent in the last few years. Supercritical fluid extraction (SFE) has been recently used to extract pesticide residues from soil and it has been shown to be a successful analytical technique [10,11]. However, routine SFEs are considered to be expensive and, because of instrument limitations, simultaneous parallel extractions cannot be performed, which is a clear disadvantage [10,12].

The need for more efficient and more economical methods of sample preparation has generated considerable interest in solid-phase extraction (SPE) as an alternative to extracting organic compounds from solid matrices.

In the past few years, the method of combined extraction and preconcentration of organic compounds by adsorption on proper solid materials followed by desorption with a small quantity of an organic solvent has been employed for trace determination of contaminants in environmental waters [13,14].

The recent commercial availability of sorbents prepared for different analytical purposes in small, inexpensive cartridges or in the form of membranes, in which efficient chromatographic particles are enmeshed in a network of polytetrafluoroethylene (PTFE) fibrils (47 or 25 mm disks), has contributed to a great expansion in solid-phase extraction methods, as evidenced by some publications in the field of pesticide analysis in environmental samples [15–18]. Solid-phase methods reported in the literature for the extraction, isolation and quantitation of pesticide residues in soil generally use the stationary phase packed in a cartridge [19–22], although sorbent disks have also been employed recently [23,24].

SPE has already found application in field

studies on the persistence of different pesticides [25,26]. However, the main purpose of the work reported in this paper was to optimize the SPE method for the extraction of soil using commercially available 47 mm C₈ disks, previously reported [24], so as to improve the applicability of the technique for the routine determination of pesticides in soil samples. Different parameters were studied in order to optimize the extraction procedure, and the following report discusses the preliminary results of our optimization effort.

2. Experimental

2.1. Reagents

Molinate, 98% purity, was from Serpiol; carbofuran, 99% purity, was from Otsuka Chemical; prometryn, diazinon, thiobencarb and pirimicarb, 98.7, 98.8, 98.9 and 99.9% purity, respectively, were from Promochem (Wesel, Germany), and tetradifon, 99% purity, was from Afrasa. Stock solutions of the pesticides were prepared in ethyl acetate.

Ethyl acetate and acetone, both of pesticide grade, were from Promochem. Methanol was from Romyl (Leics, UK). Buffer solutions of pH 3 and pH 10 were prepared [27].

2.2. Extraction apparatus

Standard Millipore 47-mm filtration apparatus equipped with a 47-mm disk of octyl-bonded silica Empore (Analytichem International, Harbor City, CA, USA) was used for all extractions.

2.3. Gas chromatographic determinations

Gas chromatography was performed with a Hewlett-Packard 5890 Series II instrument, equipped with a nitrogen-phosphorus detection (NPD) and electron-capture detection (ECD) systems, an automatic injector Model H-P 7673, an H-P 3365 integrator, and a 30-m DB-17 capillary column, film thickness 0.25 μm, supplied by J&W Scientific (Folsom, CA, USA).

The injector and detector temperatures were 285 and 300°C, respectively.

Injection was performed in the splitless mode with the column oven at 50°C. The temperature was kept at 50°C for 1 min and then programmed at 30°C min⁻¹ to 140°C. This was held for 2 min and then increased at 5°C min⁻¹ to 280°C, at which level it was kept for 10 min. Helium, flow-rate 1.6 ml min⁻¹, was used as the carrier gas. The detector gases were: air 70.9 ml min⁻¹, hydrogen 2.7 ml min⁻¹ and helium (make-up) 18.3 ml min⁻¹.

2.4. Soil samples

The main characteristics of the three soils selected for this study are presented in Table 1. The soils used differ with respect to organic matter content, soil pH levels, sand, silt and clay content, and all three are characteristic of the Mediterranean area. Soil samples were collected from the 15–30 cm layer of the soil profile and sieved to pass through a 2-mm sieve.

2.5. Soil spiking procedure

A standard solution was prepared at concentration levels of 5 µg/ml for molinate, carbofuran, diazinon and tiobencarb, and 2 µg/ml for pirimicarb, prometryn and tetradifon.

Fortified samples were prepared by adding 1 ml standard fortification solution to 5 g soil. Additional acetone was added until the solvent completely covered the soil particles. The bulk of the solvent was slowly evaporated at room temperature. The mixture was then thoroughly mixed for 1 h with a mechanical shaker.

2.6. Extraction procedure

A 5-g moist soil sample was added to 5 ml 50°C distilled water and the mixture was shaken by sonication for 15 min. The soil was subsequently shaken with acetone (5 ml) by sonication for 15 min.

Following centrifugation at 3000 g for 15 min, the supernatant was decanted into a reservoir containing approximately 500 ml distilled water and passed through a conditioned 47-mm disk of octyl-bonded silica sorbent. The adsorbed residues were eluted with 10 ml ethyl acetate. The extract was concentrated at 45°C to 1 ml, and 2-µl samples were injected into the gas chromatograph.

3. Results and discussion

3.1. Gas chromatography

Fig. 1 shows the simultaneous determination of the seven pesticides after solid-phase extraction from fortified soil B. The chromatograms obtained from soils A and C are similar.

The optimum detection depends on the chemical structure of the pesticide; therefore, to extend the range of chemical structures used as pesticides that could be determined in a single same sample, an NPD and an ECD system were connected in parallel.

The DB-17 semi-polar capillary column provides excellent resolution of all pesticides, and it can be also noted that the SPE method yields a final elute with few co-extractive interferences. There are no interference peaks, which could

Table 1
Physical and chemical properties of soils

Soil	OM (%) ^a	pH (H ₂ O)	Sand (%)	Silt (%)	Clay (%)	CEC ^b
(A) Sandy loam	2.36	8.2	39.89	37.28	22.84	16.20
(B) Sandy loam	1.44	8.3	61.54	23.63	14.83	12.02
(C) Sandy loam	1.89	8.2	39.29	30.10	30.60	21.65

^a OM = Organic matter.

^b CEC = Cation-exchange capacity (equiv./100 g of soil).

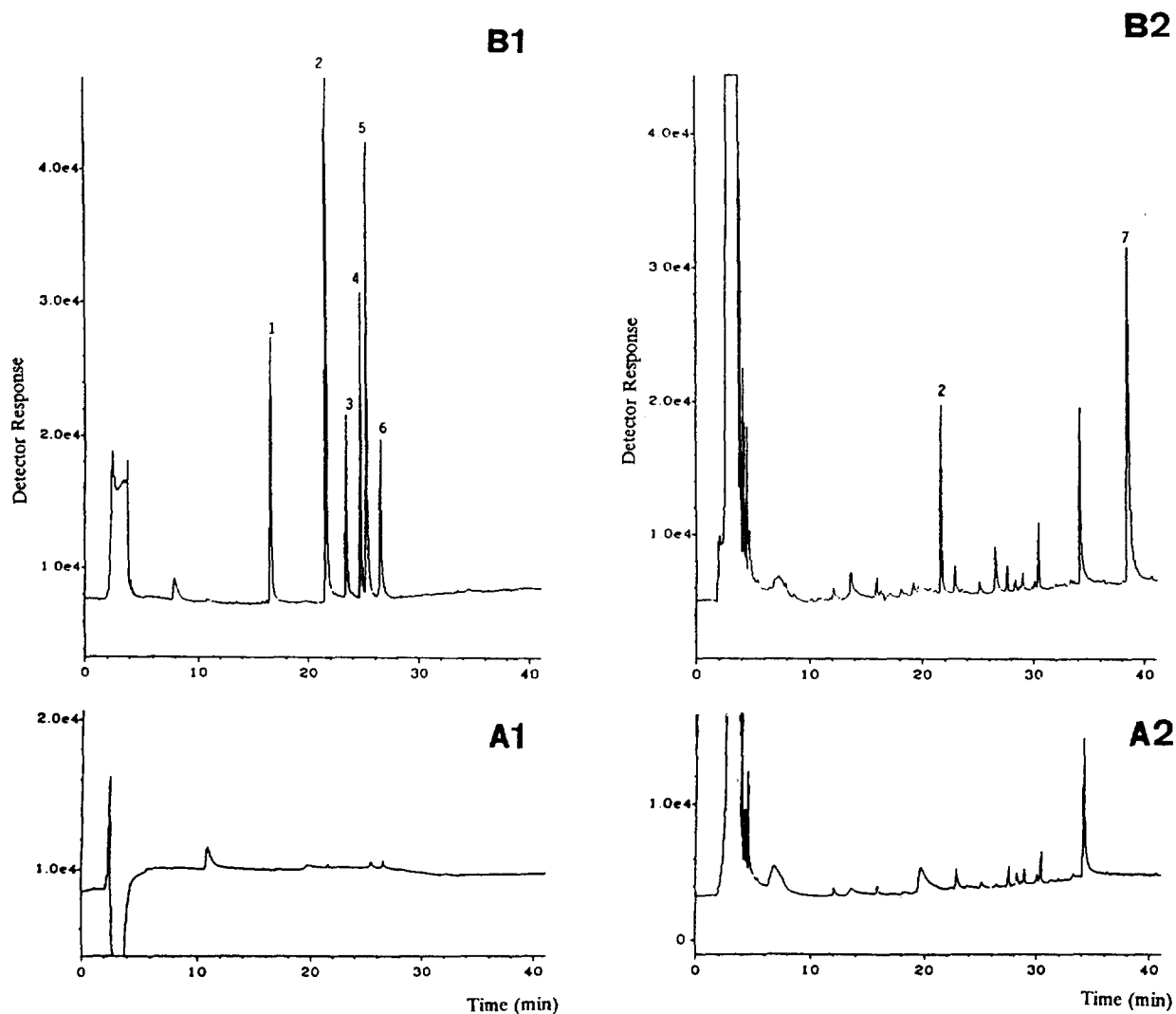


Fig. 1. (A) Chromatogram of a real soil sample prior to fortification. (A1) NPD and (A2) ECD. (B) Gas chromatogram showing the separation of the pesticides in a soil extract obtained by SPE. (B1) NPD and (B2) ECD. Peaks: 1 = molinate; 2 = diazinon; 3 = carbofuran; 4 = pirimicarb; 5 = prometryn; 6 = thiobencarb; 7 = tetradifon.

cause erroneous results in the quantification, in any of the chromatograms from the different types of the soils studied.

3.2. Recovery experiments

Recovery experiments were performed by spiking the soil samples with the pesticides being

studied. The recoveries reported represent the means of three analyses. The pesticide concentrations in the soil were maintained at a constant level through the entire test.

It is known that spiked samples may not always represent the extractability of 'real-world' materials [28]. However, since we spiked the samples in the same way for all the extraction

techniques, the comparison between the techniques should be valid.

To establish the most suitable extraction conditions, several variables affecting either the performance of the octyl-bonded silica sorbent or the prior step, the preparation of a supernatant liquid by adding the soil to a combination of an organic and aqueous solvent, were evaluated.

With a view to assessing the extent to which the matrix effect could affect the extraction of the seven pesticides studied, recovery studies were performed for soil samples with various physical and chemical characteristics, which are listed in Table 1.

All the soil samples were sieved to the same particle size in order to avoid different recoveries for coarse or fine fractions being imputed to the nature of the soil sample.

3.3. Techniques for the removal of supernatant and soil particles

For the first experiment, the influence of the technique employed for removing supernatant and soil particles prior to the SPE was evaluated. Filtration and centrifugation procedures were compared. It can be observed that the Whatman 40 filter does not remove all of the fine clay particles, increasing the time required for the subsequent SPE process. Centrifugation at 4500 rpm in a 'Sigma Laborcentrifugen 2-15' centrifuge provided better separation of the soil particles, reducing the total analysis time, and generally improved recoveries. The problems encountered with the Whatman filter were probably caused by suspensions in the supernatant plugging up the sorbent disk, making difficult the partitioning between the solid and the aqueous phases.

Moreover, centrifugation makes it possible to use the same sorbent disk three times without diminishing the recoveries significant, which means that the cost of the analysis is lower than with filtration.

Fig. 2 shows the centrifugation and filtration recoveries of pesticides from different types of

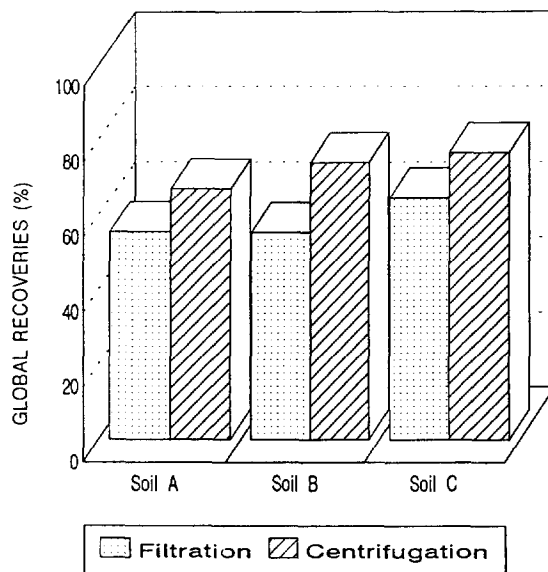


Fig. 2. Total recoveries of pesticides from spiked soils using different techniques for supernatant and soil-particle removal prior to SPE.

spiked soils. There was no difference in the behaviour of the three soils analyzed.

3.4. Water content

It has been clearly established that the presence of water can improve the efficiency of pesticide extraction from a variety of soil types [29]. Because acetone–water is an effective combination solvent for soil extraction [30], the effect of the percentage of water on method recovery was examined. It has been reported that the presence of 20% water in the extractive mixture can improve the efficiency of pesticide extraction from a variety of soil types [6], but other investigators [28,31] suggest that a 1:2 water–acetone ratio is ideal.

The results given in Fig. 3A show the effect of the percentage of water on the recoveries of all seven pesticides at pH 7 and room temperature. In this experiment we observed that there was no difference between the addition of 1 ml water and 5 ml acetone (proportion 1:5), 2.5 ml water

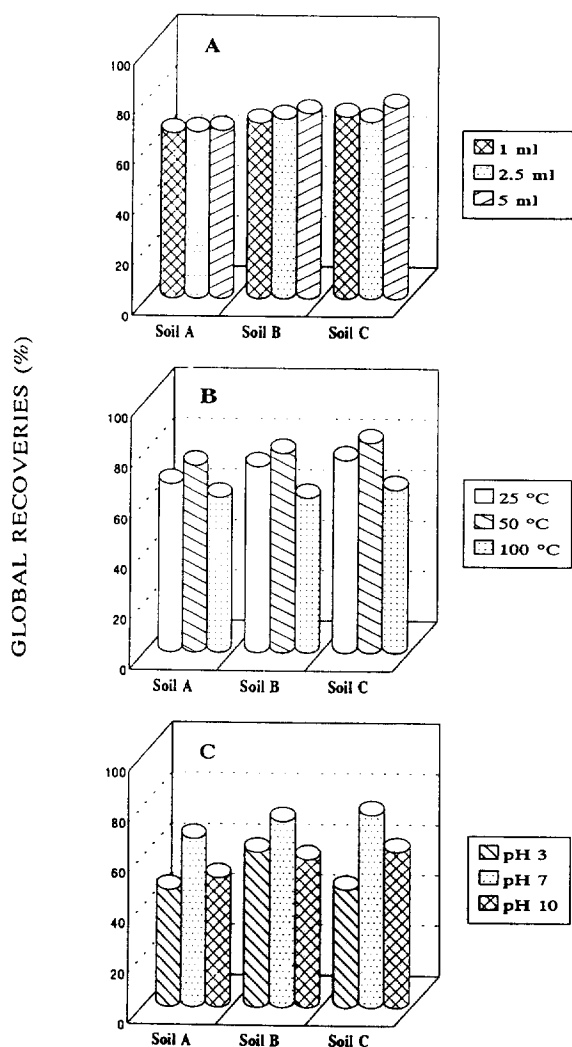


Fig. 3. Effect of analytical parameters in the preparation of the supernatant prior to SPE on total pesticide recoveries. (A) Water content; (B) water temperature; (C) extractive solution pH.

(1:2) or 5 ml water (1:1) in the sonication step, except that the reproducibility was found to be better with the 1:1 water–acetone mixture. This combination was therefore considered optimal for extraction.

3.5. Water temperature

The influence of temperature on the extraction of pesticides from soil is clear because pesticides

are more soluble when the bonds are broken as a result of heat input. Therefore, extraction methods which use a high temperature, like soxhlet, generally yield better recoveries of pesticides than cold extraction procedures.

We examined the effect of the temperature of the water added at the sonication step (room temperature, 50°C and 100°C). The percentage recovery of pesticides was greater after hot extraction with 5 ml water at 50°C, as shown in Fig. 3B. However, at 100°C the recovery apparently declined, probably because of the evaporation losses of the pesticides at this temperature.

Mills and Thurman [22] propose a temperature of 75°C for the extraction of triazines from soil by shaking with methanol–water, while Huang and Pignatello [6], with the same extractive mixture, estimate that 95°C is the optimum temperature for the extraction of atrazine.

3.6. Extractive solution pH

Several experiments were carried out in order to optimize this parameter. The recoveries obtained by adding 5 ml of distilled water (pH 7) or 5 ml buffer solutions at pH 3 (citric–citrate) or pH 10 (carbonate–bicarbonate) in the sonication step, were compared.

It may be necessary to adjust the pH to ensure that the compound is in the appropriate form to achieve both desorption from the soil and the efficient retention by the solid-phase.

When the SPE disks are used several times, the progressive occlusion of the disk by particulate matter constitutes a great problem. Flow reduction is probably caused by small particles present in the supernatant of a soil sample, which may be highly variable in chemical and physical composition. The use of acidification would be effective at least in dissolving the inorganic particles, so several investigators recommend the addition of an acid in order to diminish the occlusion of the disk pores, reducing the time needed for the extraction and making easier and more effective the partitioning between aqueous- and solid-phases. [15,18].

However, the results shown in Fig. 3C indicate that a pH value of 7 gives the best overall

recoveries. Neither a pH value of 3 nor a pH value of 10 improves the results, although theoretically the extraction of weak basic compounds like Prometryn would be better in alkaline conditions. The low stability of the silica phases at extreme pH values (lower than 2.5 or higher than 9) is well known [32], which constitutes a great disadvantage when disks are reutilized. Thus, the use of extreme pH is not advisable.

3.7. Optimal conditions

The optimal conditions for maximum recoveries of the pesticides were established as a 1:1 proportion of acetone–water, water temperature 50°C and pH 7 for the solution added in the sonication step prior to SPE with C_8 extraction disks.

It was observed that the composition of the three soil samples analyzed did not influence the results of the extraction experiments, in spite of the fact that, depending on the complex nature of the soil, the soil sorption characteristics of different soils can vary widely, and therefore affect pesticide extractability.

The accuracy, calculated as the percentage of recovery, and the reproducibility, expressed as relative standard deviations (R.S.D.s) of the proposed method, are shown in Table 2. The general decrease in recoveries implies that proportions of the analytes are strongly bound to the soil, because the spiking method carried out ensures maximum contact between the pesticide

Table 3

Detection limits (LD, ng/g in soil) of pesticides obtained with the SPE method

Pesticide	LD (ng/g)
Carbofuran	24.2
Diazinon	28.2
Molinate	13.9
Pirimicarb	8.5
Prometryn	6.4
Tetradifon	12.3
Thiobencarb	11.5

and the soil surface and makes it possible to reach sorption equilibrium, as takes place with environmental samples. It would be possible to improve the extraction efficiency, but only by adopting much more severe methods such as supercritical or high-temperature extraction.

The limits of detection were calculated by extrapolation and represent amounts that produce a chromatographic peak with a height equal to three times the standard deviation of the baseline noise [33]; they are listed in Table 3. These limits of detection are considered adequate for the subsequent use of this method for environmental analysis.

In contrast to traditional liquid–liquid extraction techniques for the extraction of pesticides from soils, the proposed method has the advantages of being economical and fast in routine analyses.

Table 2

Mean recovery (R) (%) and R.S.D. (%) ($n = 3$) from fortified soil samples using the SPE method

Pesticide	Soil A		Soil B		Soil C	
	R (%)	R.S.D.	R (%)	R.S.D.	R (%)	R.S.D.
Carbofuran	89.0	10.8	93.0	7.9	100.1	10.0
Diazinon	75.4	5.8	81.7	4.7	82.8	3.8
Molinate	63.1	9.0	72.7	6.9	76.9	5.7
Pirimicarb	79.8	12.8	83.2	8.4	84.6	7.3
Prometryn	81.1	7.4	88.1	5.3	85.8	4.1
Tetradifon	55.8	7.9	57.7	5.2	70.0	5.2
Thiobencarb	74.6	4.9	78.4	4.2	81.5	4.7

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