Development and validation of a novel method for the analysis of chlorinated pesticides in soils using microwave-assisted extraction–headspace solid phase microextraction and gas chromatography–tandem mass spectrometry

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Abstract

A new procedure for determining eleven organo- chlorine pesticides in soils using microwave-assisted ex- traction (MAE) and headspace solid phase microextraction (HS-SPME) is described. The studied pesticides consisted of mirex, α- and γ-chlordane, p,p'-DDT, heptachlor, heptachlor epoxide isomer A, γ-hexachlorocyclohexane, dieldrin, en-drin, aldrine and hexachlorobenzene. The HS-SPME was optimized for the most important parameters such as ex-traction time, sample volume and temperature. The present analytical procedure requires a reduced volume of organic solvents and avoids the need for extract clean-up steps. For optimized conditions the limits of detection for the method ranged from 0.02 to 3.6 ng/g, intermediate precision ranged from 14 to 36% (as CV%), and the recovery from 8 up to 51%. The proposed methodology can be used in the rapid screening of soil for the presence of the selected pesticides, and was applied to landfill soil samples.

Introduction

Pesticides play an important role in increasing agricultural productivity. Most of them constitute serious environmental threats due to their high toxicity and persistence, leading to the complete banning or restriction on the use of some organochlorine pesticides (OCPs) during the 1970s [1]. However, despite their prohibition in industrialized countries, they still exist in the environment and were described in the United Nations Environmental Program—UNEP—as persistent organic pollutants. Most OCPs were banned in Portugal in the late 1980s, although they still can be detected in sera taken from the general population [2], in plankton along the coast [3], and in surface and ground waters [4].

The analysis of OCPs in environmental matrices remains a challenging task and requires an extraction and enrich-ment step prior to instrumental analysis, due to the low levels present and to the complexity of the matrices. This step can be performed by liquid-liquid [5], solid-phase [6, 7], liquid-solid or Soxhlet extraction [8, 9] prior to solvent evaporation. These routine methods are time-consuming and require the use of considerable amounts of toxic sol-vents. Recent and anticipated changes in environmental regulations will severely restrict the amount of solvent usage in laboratories worldwide. For example, in the United States, a recent executive order has called for a 50–90% reduction in solvent usage in all federal laboratories. The popularity of microwave-assisted extraction (MAE) has risen rapidly over the last decade and it has proven to be effective (compared to traditional extraction techniques) at extracting OCPs, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, pesticides, phenols, and neutral and basic priority pollutants from environmental samples. The major benefits of MAE are decreased extraction times, reduced solvent consumption and increased sample throughput [10–18]. However, further sample clean-up and purification is often needed, due to the coextraction of interfering compounds in addition to the target analytes,

resulting in a multistep analytical procedure. Coupling solid phase microextraction (SPME) with high-resolution gas chromatography and tandem mass spectrometry minimizes sample handling and solvent consumption, thus providing a useful way to sidestep the need for purification/concentration steps after MAE, and it also reduces analysis time.

SPME eliminates the need for solvents or complicated apparatus, is useful for concentrating volatile or nonvolatile compounds in liquid samples or its headspace, and is compatible with analyte separation and detection by high-performance liquid chromatography and gas chromatography, providing linear results for wide concentrations of analytes [19]. However, some difficulties arise with SPME when there are strong interactions between the analytes and the matrix, such as those that occur in soils/sediments with chlorinated pesticides.

To overcome this difficulty, the sequential or simultaneous application of MAE with SPME has been successfully used to determine the chlorinated compounds in water [20, 21], ash [22] or plants [23, 24].

To our knowledge, there are no reports on the application of MAE combined with HS-SPME to the analysis of OCPs in sediments and/or soils. This technique provides efficient enrichment and clean-up, as well as good selectivity, sensitivity and speed, with only minor production of laboratorial residues containing organic solvents.

This paper describes a procedure based on the MAE of eleven organochlorine pesticides from soil samples, using a mixture of hexane and acetone as the extraction solvent, followed by HS-SPME and GC-MS/MS analysis using a PDMS-coated SPME fiber. The analytical methodology was validated and applied to real samples collected in a Portuguese landfill.

Experimental

Materials

Hexane and acetone (ENVISOLV) and ethanol were analytical grade and from Riedel de Häen (Seelze Germany). Mirex (MIR), α- and γ-chlordane (α-CHLOR and γ-CHLOR), 4,4′-DDT (DDT), endrin (END), heptachlor (HPTC) and heptachlor epoxide isomer A (HEE) were from Riedel-de Haën; γ-hexachlorocyclohexane (LIN) was from Sigma (Steinheim, Germany); dieldrin (DIE) was from Supelco (Bellefonte, USA). The chlorinated pesticides included in this study were used to prepare a "working standard" in ethanol with a concentration of 300 μg/l on average, except in the case of chlordanes (5 μg/l). This solution was used to obtain dilute standard solutions and to spike soil samples to the required concentration. In the solid phase microextraction step, all dilute standard solutions contained 1.8% of ethanol and so 720 μl of ethanol was added before the extraction of each sample (40 ml).

Water was distilled and deionized. Helium carrier gas (99.9999% purity) was supplied by Praxair (Madrid, Spain).

To minimize adsorption and loss as well as desorption of the studied compounds during handling and analysis, all glass material was silanized prior to utilization. Silanization was performed by soaking glassware overnight in a 10% dichlorodimethylsilane solution in toluene, and then rinsed with toluene and methanol and finally dried thoroughly for 4 h at 400 °C.

Preparation of the spiked soil samples

Two different soils (type I: pH 7.8, organic matter content 2.2%, water content 0.17%; type II: pH 5.8, organic matter content 8.4%, water content 1.8%) were collected from two fields in the Chaves region (in the north of Portugal) and were thoroughly mixed to ensure homogeneity. After air-drying and sieving to a grain size of 2 mm, the soils samples were stored at 4 °C. For pH measurements, the soils were shaken with demineralized water (soil:water ratio 1:1) for ~30 min [25].

Spiked soil samples were prepared by adding appropriate volumes of the OCP concentrated stock solution to a 5.0±0.1 g portion of soil (for blanks, pure ethanol was added). The spiked and blank soil samples as well as the unknown samples were allowed to stand for 24 h to air-dry and were extracted by MAE thereafter.

The sandy soil sample was used to obtain the MAE–HS-SPME–GC-MS/MS calibration curve.

Microwave-assisted extraction of sediments and soil samples and SPME conditions

Microwave-assisted extractions were performed with a MARS-X 1,500 W Microwave Accelerated Reaction System for Extraction (CEM, Mathews, NC, USA) configured with a 14-position carousel. The spiked soil samples (used to obtain the MAE-HS-SPME-GC-MS/MS calibration curve and for recovery assays) or portions of samples were transferred quantitatively to the glass extraction vessels. After adding 20 ml of the n-hexane-acetone (1:1) solvent to each sample, the extraction vessels were closed. The operational parameters of the MARS-X apparatus applied were: magnetron power 100%; time to reach settings 10 min; extraction temperature 115 °C; extraction duration 10 min; medium speed stirring; maximum vessel pressure cut-off 200 psi. During operation, both temperature and pressure were monitored in a single vessel, magnetic stirring was used in each extraction vessel and a sensor monitored for solvent leaks in the interior of the microwave oven. After the extraction, the vessels were allowed to cool to room temperature before they were opened. Fifteen milliliters of the supernatant were filtered through a Whatman N°42 filter paper and evaporated to dryness under a gentle stream of nitrogen. Immediately before the GC-MS analysis, the residue was redissolved by the addition of 720 μL of ethanol and 40 ml of water and subjected to HS-SPME using the procedure described below.

The SPME device (fiber and holder) was purchased from Supelco (Bellefonte, PA, USA). The fiber used was coated with 100 µm polydimethylsiloxane (PDMS). Magnetic stirring bars, PTFE-coated, 20×7.5 mm, were used during SPME. After each extraction the stirring bars were rinsed consecutively with acetone, n-hexane, acetone, and finally with water, to prevent significant carry-over between samples.

For HS-SPME extraction, 50 ml (nominal size) of crimp-top HS vials (actual capacity about 55 ml), 20 mm black Viton septa and aluminum seals were used, all from Supelco (Oakville, Ontario, Canada). During extraction, the SPME fiber assembly was extended so that the end of the fiber was about 1 cm above the surface of the liquid. Agitation used was appropriate to give a vortex depth of 0.5 cm. The optimized HS-SPME conditions were: head-space sampling of 40 ml of sample (1.8% of ethanol) at 65 °C for 60 minutes, with 100 µm PDMS-coated fiber.

Chromatographic and MS/MS conditions

For the chromatographic separation and detection of the studied compounds, a Varian (Walnut Creek, CA, USA) CP-3800 gas chromatograph equipped with a split/splitless injector (model 1079) and a Varian Saturn 2000 ion trap detector were used. The analytical column was a Varian 60 m × 0.25 mm CP-Sil 8 CB lowbleed/MS (0.24 µm film thickness). Helium(0.9 ml/min, constant flow) was used as carrier gas.

The analytes were desorbed from the SPME fiber into the injector at 260 °C, in splitless mode. After 10 min the split valve was opened. The SPME fibers remained in the injector for at least 15 min to minimize carryover.

The chromatographic oven temperature program was as follows: the initial temperature of 80 °C was held for 10 min after injection; it was then ramped up at a rate of 20 °C/min to 170 °C and to 260°C at 3°C/min; then to 300°C at 5 °C/min; then, after holding for 2 min at 300 °C, the temperature was returned to its initial value. Total run time was 55 minutes.

Tandem mass spectrometry was carried out under the following fixed conditions: ionization with electron impact at 70 eV in MS/MS mode with multiple reaction monitoring (MRM).

Transfer line, manifold and trap temperatures were 290 °C, 50 °C, and 210 °C, respectively. The emission current was set to 60 µA for all MS segments and the axial modulation voltage to 4.0 volts.

Detection was made by resonant collision-induced dissociation (CID) MS/MS, with the CID frequency offset kept at zero and the excitation time set at 40 ms for all compounds. The most critical parameters were set to obtain maximum sensitivity, and are summarized in Table 1.

The compounds were identified and quantified by extracting the characteristic ions of each studied compounds, monitored at the specific retention time, within a peak window of ±0.2 min.

Table 1 Ion preparation method parameters for each segment of the GC-MS/MS method

Segment	Compounds	Start time (min)	End time (min)	Precursor ions (m/z)	Quantification ions
Solvent delay	-	0.00	10.0	-	-
2	HCB	10	28.2	284	249
	LIN			181	145
3	HPTC	28.2	35.5	272	237
	ALD			263	220+228
4	HEE	35.5	36.2	289	219+253
5	γ-CHLOR	36.2	39.2	373	266+301+337
	α-CHLOR				
6	DIE	39.2	43.0	277	241
	END			281	245
7	DDT	43.0	47.9	235	165+200
8	MIR	47.9	55.0	272	237

Note: Prescan time was set to 1,500 µs, target TIC to 2,000, and the maximum ionization time employed was 25 ms for all segments. The collision-induced dissociation frequency offset was set to 0 Hz in all segments

Quantification was achieved via an external standard, using the most prominent ion(s) obtained in each case.

Results and discussion

The MAE procedure used to extract the OCPs from soil samples was based on U.S. EPA Method 3546 [26] and was adapted to the features of the equipment that existed in the laboratory of our research team (one of the latest models, which allows mechanical stirring at different speed during extraction, uses glass vessels, and has higher power output and sample throughput than mentioned in [26] was used).

Optimization of the HS-SPME procedure

Several commercially available SPME fiber coatings, such as carbowax-divinylbenzene, carboxen-polydimethylsilox-ane; divinylbenzene-carboxen-polydimethylsiloxane, poly dimethylsiloxane and polyacrylate, can be used to extract the OCPs from different matrices [27–29]. PDMS-coated fibers are probably the most studied and have been described as more efficient in the extraction of some of the studied OCPs [28]. Therefore, PDMS-coated fibers were selected in the present work.

The HS-SPME procedure was optimized by studying the effect of several parameters on the peak areas of each of the studied compounds: extraction temperature, extraction time, flask/sample volume ratio and desorption time. These experiments were conducted on a standard solution containing, on average, 540 ng/l of the studied OCPs, except for the case of chlordanes (~36 ng/l).

The effect of three different temperatures (45, 65 and 85 °C) on the HS-SPME was studied. The influence of temperature on the peak area varied depending on the compound. For the more volatile OCPs, such as HCB, LIN and HPTC, a higher extraction temperature decreased the peak area, while for the less volatile OCPs, such as DIE, END, DDT and MIR, the opposite result was observed; see Fig. 1. The remaining compounds, ALD, HEE and γ- and α-chlordanes, showed higher peak areas for temperatures of around 65 °C. Raising the temperature caused the vapor pressures of these compounds to increase. However, at higher temperatures, the partition coefficient from the gas phase in the headspace into the fiber was reduced. This could explain the decrease in sensitivity for those more volatile compounds. For the less volatile OCPs (which are therefore more difficult to extract into the headspace),

Fig. 1 Effect of HS-SPME extraction temperature on the normalized peak areas of the organochlorine pesticides studied
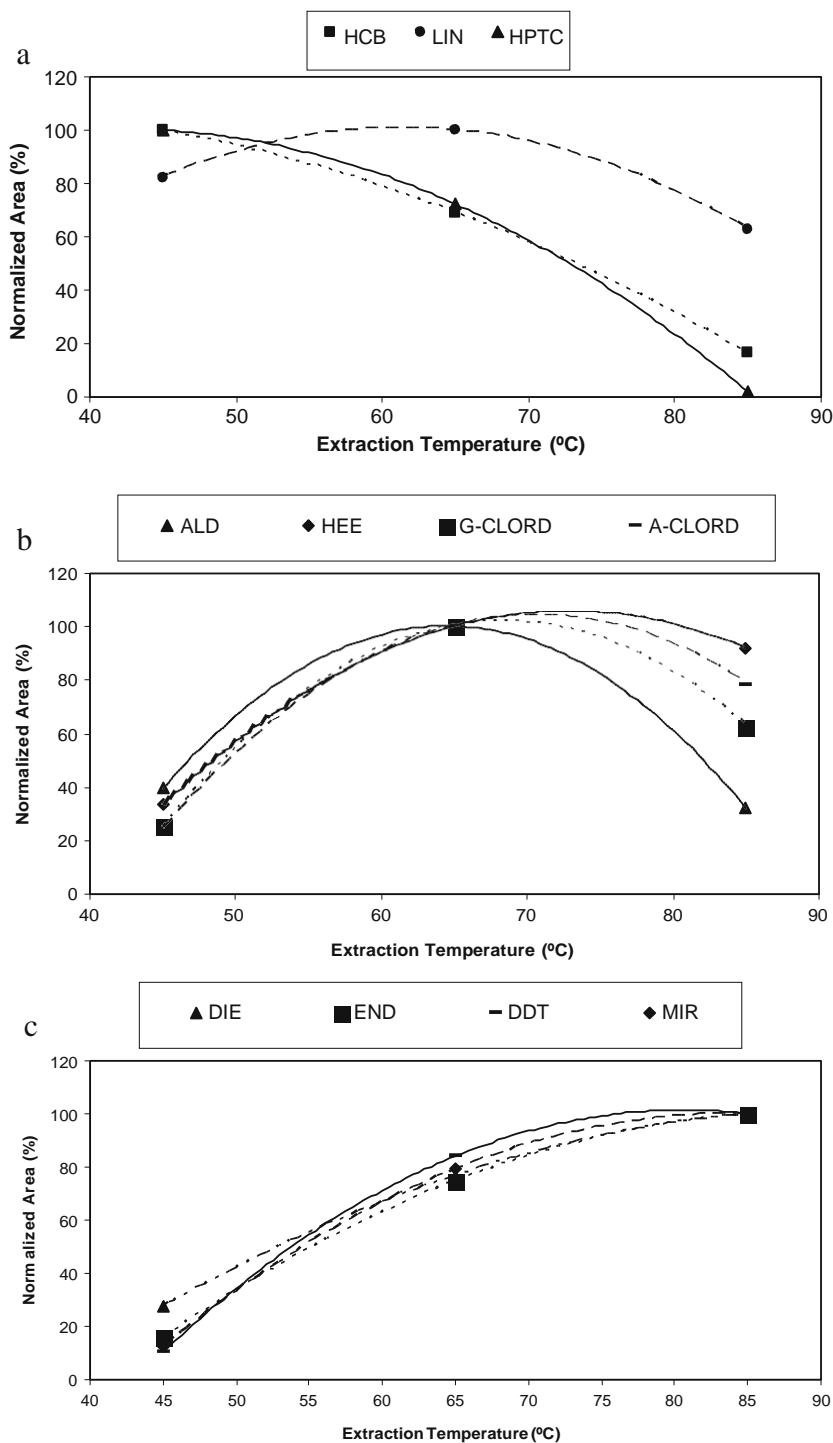
Table 2 Efficiency of the MAE–HS-SPME method

Compound	Correlation coefficient (R^2) for direct injection; $n=3$ (0.054–32 ng)*	Intermediate precision (%) R.S.D., $n=6$	Correlation coefficient (R^2) for MAE–HS-SPME (14–269 ng)**	MAE–HS-SPME efficiency (%)
HCB	0.9948	15	0.9971	13
LIN	0.9878	16	0.9869	1
HPTC	0.9999	22	0.9953	30
ALD	0.9999	20	0.9939	37
HEE	0.9999	21	0.9980	11
γ-CHLOR	0.9992	23	0.9860	26
α-CHLOR	0.9994	26	0.9927	20
DIE	0.9996	25	0.9879	11
END	0.9980	25	0.9973	8
DDT	0.9993	37	0.9972	6
MIR	0.9999	32	0.9763	3

* Average injected mass for all OCPs, ranging from 0.045 to 27 ng for LIN and END, and 0.067 to 41 ng for DIE
** Average extracted mass for all OCPs, ranging from 2 to 23 ng for γ-CHLOR, and 21 to 404 ng for DIE

increasing the extraction temperature enhances sensitivity. To achieve an acceptable sensitivity for all compounds, the temperature was set to 65 °C.

Except for HCB and HPTC, whose responses slightly decreased, the peak areas increased for all of the compounds with the duration of extraction within the time range studied (40 to 120 min; data not shown). In order to maximize the sample output, the extraction time was set to 60 minutes (approximately the same time was used for the chromatographic separation).

Using vials with different capacities (16, 40 and 55 ml), the effect of sampling at different volume ratios (total vial volume to aqueous volume) of 4, 3, 2.7 and 1.4 was studied (data not shown). Other authors that have performed headspace sampling of OCPs in aqueous matrices [30]

reported that the response to these compounds increases as the headspace/sample volume ratio decreases because of the greater concentration of the volatilized compounds in the gaseous fraction. In the present situation, and because the soil extracts are first evaporated to dryness and then redissolved with water, an increase in the sample volume dilutes the analytes present in the extracts. Therefore, in theory, better detection limits should be achieved if smaller extraction vessels are used while maintaining a lower headspace/sample volume ratio. Nevertheless, a smaller water volume could also produce a lack of linearity in those more concentrated samples due to the low water solubilities of some of the selected pesticides. In order to maximize the method sensitivity and the linearity range, a sample volume of 40 ml (volume ratio of 1.4) in the 55 ml vials was chosen.

It was observed that, at least 15 minutes were needed to avoid carryover on fiber redesorption, and so each fiber stood in the injector for longer than this time before further use.

Performance of the MAE–HS-SPME

The linearity of the response of the mass spectrometric detector was tested using OCP standards prepared in *n*-hexane, injected in the splitless mode. Linearity was studied between 0.054 ng and 32 ng (injected mass), on average, for all the selected OCPs. Quadratic correlation coefficients of 0.9878 (LIN) and 0.9999 (HPTC, ALD, HEE and MIR) were obtained. The efficiency of the whole extraction procedure (MAE-HS-SPME) was evaluated by comparing the amount of each compound concentrated on the PDMS fiber (measured against the calibration obtained by direct injection) with the initial amount present on the type I spiked soil sample mass; see Table 2.

Although the efficiency of MAE–HS-SPME efficiency is quite low in some cases (Table 2), such as for LIN, MIR, DDT and END, the limits of detection reached (Table 3) are sufficiently low to make it possible to use this methodology as a screening method for environmental contamination with the selected pesticides.

Table 3 Analytical method validation parameters

Compound	Retention time (min)	Limits of detection* (ng/g)	Intermediate precision (RSD; $n=6$)	Recovery (%) Soil type I** (±SD; $n=12$)	Recovery (%) Soil type II** (±SD; $n=6$)
HCB	26.4	0.2	14	112 ± 17	51 ± 13
LIN	27.8	3.6	25	115 ± 31	45 ± 17
HPTC	31.5	2.4	17	79 ± 17	43 ± 12
ALD	33.6	1.4	22	84 ± 19	22 ± 6
HEE	36.0	1.8	23	85 ± 20	35 ± 7
γ-CHLOR	37.1	0.02	28	75 ± 17	14 ± 3
α-CHLOR	37.8	0.03	28	72 ± 16	13 ± 4
DIE	39.6	1.9	21	100 ± 22	35 ± 5
END	40.4	0.3	21	94 ± 22	38 ± 10
DDT	43.6	1.7	36	83 ± 28	42 ± 24
MIR	49.6	0.4	34	86 ± 35	8 ± 2

* Signal-to-noise = 5; ** two spiking levels were averaged (5.6 and 54 ng/g, and 8.2 and 54 ng/g for soil types I and II, respectively)

Method validation

Six standards were used to calibrate selected OCPs. Appropriate volumes of standard solutions containing the OCPs were added to 5 g of model soil in order to facilitate the construction of a calibration curve from 3 to 54 ng/g, on average. Because different amounts of extracted sample could be used afterwards, the calibration curve was constructed based on the mass of each OCP present in each standard. The calibration functions were linear within the concentration range considered for each compound.

The limits of detection were defined as the concentration of a sample that gives rise to a peak with signal-to-noise ratio of 5. Detection limits ranged from 0.02 ng/g (γ-CHLOR) to 3.6 ng/g (LIN) when 5 g of samples were extracted; see Table 3.

Method precision was evaluated using six replicate determinations distributed over three days (intermediate precision) of a 5.6 ng/g (on average) type II spiked soil sample, expressed as the relative standard deviation (R.S. D.). Intermediate precision values ranged from 14% to 36% (Table 3) and can be considered to be slightly higher than those usually reported for MAE or SPME methods. Nevertheless, the variability observed in the present method comes mainly from the chromatographic step, as can be seen by comparing the precision obtained in the direct injection (Table 2) with that of the whole MAE–HS-SPME–GC-MS/MS process. The chromatographic variability is mainly due to the tandem mass spectrometry. Nevertheless, this can be possibly reduced using deuterated pesticide surrogates.

Accuracy was assessed by the spiking method. The values presented in Table 3 are the mean values obtained from two sets of six replicate standard additions to type I soil samples (5.6 ng/g and 54 ng/g, on average) and two sets of three replicate standard additions (8.2 ng/g and 54 ng/g, on average) to type II soil samples. Recoveries from type I spiked samples ranged from 72 to 115% and from 35 to 51% for type II soil, with four exceptions (aldrin, the chlordanes and mirex). Type I soil was used to build the calibration curve, and this had a low content of organics (2.2%), while type II soil had a high organic

Fig. 2 Extracted ion chromatograms of two landfill soil samples (*upper* and *middle*) and a type I spiked soil sample (*lower*). (*1*) HCB, (*2*) LIN, (*3*) HPTC, (*4*) ALD, (*5*) HEE, (*6*) γ-CHLOR, (*7*) α-CHLOR, (*8*) DIE, (*9*) END, (*10*) DDT, (*11*) MIR
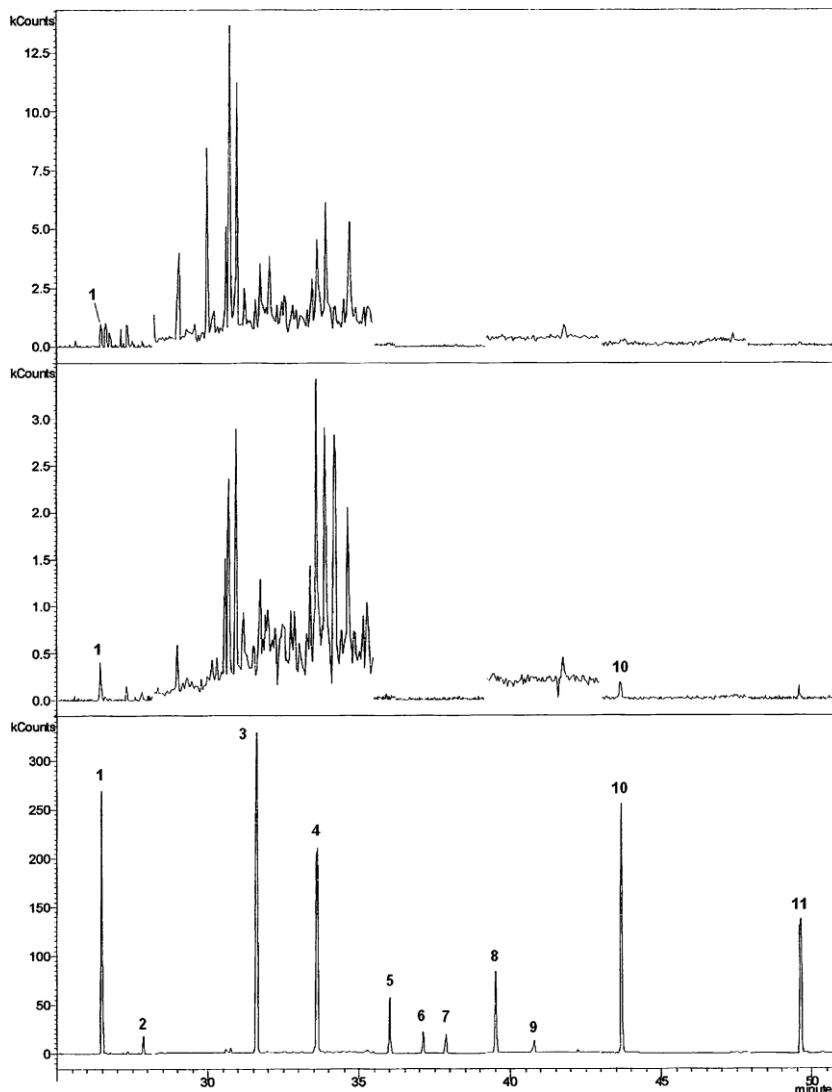
content (8.4%). The different recovery values obtained for the two types of soil indicate that the recoveries are dependent on the organic content of the samples. The low recovery values obtained need accounting for in the calculations of the final concentrations. It is well known that SPME is affected by matrix effects, as with other extraction methods, and the results obtained here also indicate that the accuracy may be improved through the use of deuterated pesticide surrogates or other internal standards.

Analysis of real samples

During the development of the present methodology, several landfill soil samples were screened for the presence of the selected pesticides. Figure 2 shows the extracted ion chromatograms of two soil samples and a type I spiked soil sample extracted under the same conditions (5 g extracted). In the lower chromatogram (the standard), the baseline separation of all of the compounds studied is evident. Although the separation time could be significantly reduced in this case, the presence of several coextracted compounds in the other landfill soil sample, as observed in the upper and middle chromatograms (especially in the first portion of the chromatogram), makes it necessary to use an extended separation time in order to achieve adequate selectivity and identification capacity. The selected landfill soil samples, obtained from an uncontrolled (and now closed) landfill, were contaminated with hexachlorobenzene (both samples) and 4,4′-DDT (middle). Further studies will be needed in order to characterize the distributions of these compounds in the landfill soil, in terms of the contamination of the different soil layers.

Conclusions

This study demonstrated that the extraction of soil samples by MAE (using a small volume of *n*-hexane–acetone), followed by evaporation of the solvent and headspace SPME of the redissolved extracts, and finally by GC-MS/MS, allowed eleven organochlorine pesticides to be determined in very complex matrices such as landfill soil samples. The method reduced sample preparation time and only consumed very small amounts of organic solvents, avoiding the need for extract purification and clean-up steps. The method gave limits of detection ranging from 0.02 to 3.6 ng/g, intermediate precisions of 14 to 36%, and recoveries of up to 51% using 5 g of sample.

The method could be further improved by including other organochlorine pesticides and/or appropriate internal standard (s) in order to minimize matrix effects during the extraction steps. The method will be applied to a larger number of landfill soil samples as a part of an ongoing project.

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