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Extraction of DDT [1,1,1,-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and its metabolites DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene] and DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane]) from aged contaminated soil

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Abstract

Pressurised liquid extraction (PLE) was used to extract DDT [1,1,1,-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and its metabolites, DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] from an aged, contaminated soil. Using three sequential static phases, PLE removed an equivalent quantity of DDT and its metabolites as Soxhlet extraction, in less time and with less solvent. Recovery was almost quantitative, implying appropriate sample work-up and manipulation.

Keywords: Soil; Extraction methods; Pressurised liquid extraction; Environmental analysis; DDT; DDE; DDD; Pesticides; Organochlorine compounds

1. Introduction

DDT and its metabolites do not occur naturally in the environment, DDT is classed as a persistent organic pollutant. It is an organochlorine pesticide that was banned in most western countries in the early 1970s, due to health concerns for humans and animals. Before then it had been widely used for the control of disease carrying insects, such as Tsetse fly and mosquitoes, as well as to control insects on agricultural crops. It is a nerve poison and attacks the central nervous system of insects [1]. DDT is metabolised by the body to DDE, which, along with DDT can be stored in the body fat [1].

Pressurised liquid extraction (PLE; also known under the Dionex trade name ASE, accelerated solvent extraction) was introduced in 1995, and quickly gained importance in an increasingly environmentally conscious world [2]. PLE not only reduces sample preparation time, but also reduces the

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amount of solvent required to complete the extraction. PLE typically uses 10–20 ml of solvent as compared to traditional methods of extraction, such as Soxhlet which can use in excess of 200 ml of (chlorinated) solvent.

PLE has been used on various occasions to extract organic contaminants from soils, sediments and sludge's [3–6]. Comparisons between PLE and conventional extraction techniques for the removal of organophosphorous herbicides and pesticides [7,8], yielded similar results, indicating PLE is a potential replacement for older techniques such as Soxhlet extraction. PLE has been used successfully to extract hexaconazole residues from three weathered soils [9]. Frost et al. [9] compared their results with microwave-assisted extraction, supercritical fluid extraction and Soxhlet extraction. The results obtained by PLE were comparable with those obtained by Soxhlet extraction and significantly better than those obtained by the other two methods.

Schantz et al. [6] evaluated PLE for the extraction of organics from environmental reference materials. They reported the extraction of DDT and its metabolites from urban dust (SRM 1649a), waterway sediment (SRM 1944), freeze-dried mussel tissue (SRM 2974), and ground whole carp (CARP-1/2) using a range of solvents [dichloromethane (DCM), acetone and hexane–acetone]. In all cases, agreement with certified values and/or Soxhlet values were in agreement. In addition, Popp et al. [10] used a 2×5 min static extraction time for the extraction of DDT and its metabolites from two soils. For each soil three solvent systems were used: acetone–hexane (1:1, v/v), DCM–acetone (1:1, v/v) and toluene). Comparable results were obtained in each case for the different solvents and soils. The aim of this work is to evaluate the effectiveness of PLE for the extraction of DDT and its metabolites from soil.

2. Experimental

2.1. Instrumentation

An ASE 200 Accelerated Solvent Extractor (Dionex (UK), Camberley, UK) with 11 ml extraction cells was used to perform the extractions. The extracts were analysed on a GC–MSD system (Hewlett-Packard) in the selected ion monitoring (SIM) mode.

2.2. Soil

Soil contaminated with DDT and its metabolites (DDE and DDD) was provided by Zeneca Environmental Labs., Brixham, UK. After being air-dried and sieved (<2 mm) it was characterised as follows: pH 2.5, organic matter content 7.2% and cation-exchange capacity, 12 mequiv. per 100 g. It was shipped and stored at −18°C.

2.3. Chemicals

The solvents used in this study were certified analytical reagents (Fisher Scientific, Loughborough, UK). Hydromatrix (Varian, Surrey, UK) was used to fill the head space of the PLE extraction cells (Dionex). Anhydrous sodium sulphate (BDH, Poole, UK) was mixed with the soil sample during Soxhlet extraction. A pesticide standard comprising of twenty organochlorine pesticides was purchased from Supelco, Walton-on-Thames, UK.

2.4. GC–MS analysis

The GC–MS (HP G1800A GCD system, Hewlett-Packard, Palo Alto, USA) was operated in the SIM mode with a splitless injection volume of 0.5 μl. The column used was a DB-5ms (J&W Scientific, Folsom, CA, USA), with dimensions of 30 m×0.25 mm I.D., 0.25 μm film thickness. The temperature program used for the analysis was: 120°C, held for 2 min to 290°C at a rate of 5°C/min, with a final hold time of 2 min. The injection port and detector temperature were set at 280°C.

GC–MS-SIM mode was used to determine the presence of each of the analytes. Table 1 shows the

Table 1
Ions chosen for SIM

Compound	Quantifying ion	Qualifying ion
DDE	246	248
DDD	235	237
DDT	235	237

ions that were selected for monitoring. Quantification was achieved by the use of a seven point calibration curve from 0 μg ml^{-1} to 5 μg ml^{-1}. Correlation coefficient (R^2) values for each of the analytes were in excess of 0.99. Selected standards were run every day to assess analytical performance.

2.5. Procedures

2.5.1. PLE extraction

Soil (1–2 g, accurately weighed) was placed in a stainless steel extraction cell (11 ml capacity) on top of a filter to prevent cell frit blockage. Hydromatrix was used to fill the head space to reduce solvent consumption. The cell was placed in the carousel and extracted using the following conditions: pressure; 2000 p.s.i. [1 p.s.i.=6894.76 Pa], temperature, 100°C, with a static extraction time of 10 min (preceded by a 5 min heat-up time). Initial work was done with a single static flush cycle. With additional time required for rinsing with fresh solvent and N_2, the total extraction time was approximately 17 min per sample.

2.5.2. Soxhlet extraction

Soil (1–2 g, accurately weighed) was placed in a cellulose extraction thimble with an equivalent quantity of anhydrous sodium sulphate which had been previously dried at 60°C for 48 h. A round bottomed flask was filled with 40 ml of solvent (dichloromethane). The extraction was performed for either 6 or 24 h. The liquid extract was quantitatively transferred to a volumetric flask. DDE content was determined prior to dilution using GC–MS. A 1/20 dilution of the extract was prepared for determination of DDD and DDT content by GC–MS. In addition, the soil was also extracted, according to the above scheme, with a 1:1, v/v mixture of dichloromethane–acetone (1:1, v/v) in accordance with solvent choice as recommended in US Environmental Protection Agency (EPA) method 3540C [11].

2.6. Recovery experiments by pressurised liquid extraction

2.6.1. Spot spike

Hydromatrix (1–2 g, accurately weighed) was placed in a stainless steel extraction cell (11 ml capacity). Stock solution (100 μl of a 2000 μg/ml standard) was added directly to the hydromatrix to give a final concentration of 8 μg/ml. The solvent (dichloromethane) was allowed to evaporate before additional hydromatrix was used to fill the head space of the cell. The cell was capped and placed in the carousel prior to extraction. The spiked hydromatrix was extracted as follows: temperature, 100°C; pressure, 2000 p.s.i.; and a static extraction time of 10 min with one static flush cycle.

2.6.2. Slurry spike

Hydromatrix (6 g, accurately weighed) was placed in a glass beaker. Stock solution (600 μl of 2000 μg/ml) of the target analytes were added to a 25 ml volumetric flask, and made up to the mark with dichloromethane. This was added to the hydromatrix. The slurry was stirred and the solvent was allowed to evaporate. Forty eight hours after solvent evaporation, portions of the spiked hydromatrix (~1 g) were placed in the stainless steel extraction cell and the head space was filled with hydromatrix. The cell was placed in the carousel and extracted under the same conditions as the spot spiked samples.

3. Results and discussion

3.1. Recovery experiments

Initial experiments were based on recoveries from spiked (spot and slurry spiked) hydromatrix, an inert support material. This was done to investigate the effectiveness of PLE and to assess the sample work-up procedure. Results from recovery experiments from spot and slurry spiked hydromatrix are shown in Table 2. Average recoveries of 91% for spot spiking and 85% for slurry spiked for the three analytes, coupled with precisions of <5.1% RSD indicated appropriate extraction/sample work-up based on a single static flush cycle.

3.2. Optimisation of pressurised liquid extraction

Initial studies were undertaken to assess the influence of temperature on the recovery of DDT, DDD and DDE from aged, contaminated soil. Seven temperatures were chosen to investigate in the range

Table 2
Results of PLE recovery extractions (n=6)

	DDE		DDD		DDT	
	Spot	Slurry	Spot	Slurry	Spot	Slurry
Mean μg/g (Recovery, %)	6.7 (84)	7.1 (88)	7.4 (93)	7.4 (92)	7.3 (91)	7.3 (91)
RSD (%)	3	1.7	4.3	4.8	5.1	2.5

80–200°C in 20°C increments. Pressure was maintained at 2000 p.s.i. and a static extraction time of 10 min. Duplicate extraction/analyses were performed at each temperature. The results are shown in Fig. 1. The average extraction efficiencies (n=14) for DDT, DDD and DDE were 205 (8.6% RSD), 42.9 (10.2% RSD) and 10.8 (14.9% RSD) μg g^{-1}, respectively over the temperature range. It was concluded that an increase in temperature does not significantly alter the amount of analyte extracted. A temperature of 100°C was chosen as the extraction temperature for further work.

Another important variable in PLE is solvent choice. The ability to select an appropriate solvent for extraction is often neglected, in favour of instrumental variables. Little attempt is often made to select the most appropriate solvent for the analyte. In an attempt to select the most appropriate solvent for extraction of DDT, DDD and DDE from aged, contaminated soil a range of solvents with different properties were chosen. In order to simplify the choice single solvents were chosen, with two exceptions. Acetone–DCM (1:1, v/v) was included as a solvent mixture as it is recommended in EPA method 3545A [12] for the extraction of organochlorine pesticides by PLE. The solvents selected are shown in Table 3. The solvents were chosen to provide a range of polarities and solvent types e.g. an alcohol, ketone, linear hydrocarbon, aromatic etc. PLE was performed on ~1 g of aged, contaminated soil under the conditions previously outlined. Fig. 2A–C show the results of this study (each determinand was extracted six times with each solvent or solvent combination; error bars represent one standard devia-

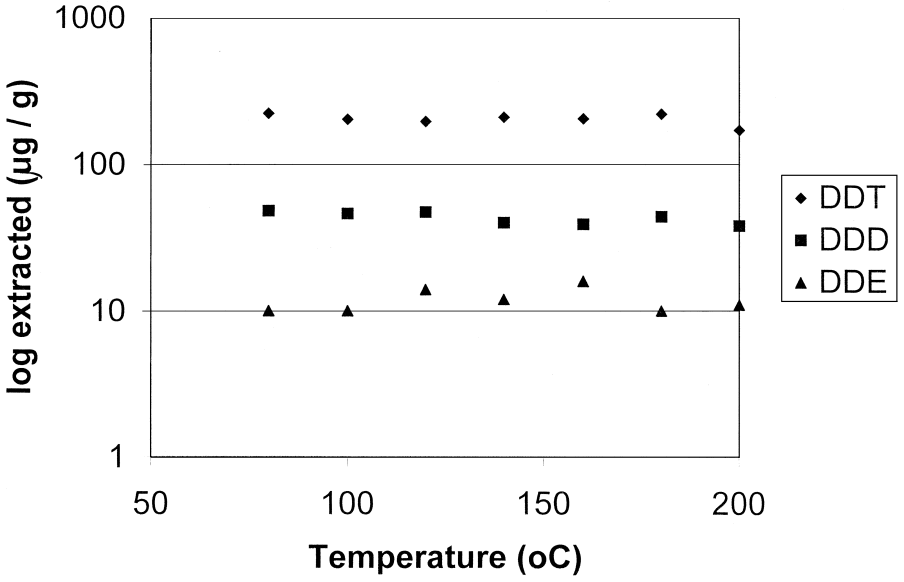

Fig. 1. Influence of extraction temperature on the recovery of DDT and its metabolites.

Table 3
Solvents used in solvent study

Acetonitrile	Organic cyanide
Dichloromethane	Chlorinated hydrocarbon
Acetone	Ketone
Methanol	Alcohol
2-Chlorotoluene	Chlorinated aromatic
Toluene	Aromatic non polar
Isohexane	Non-polar hydrocarbon
Acetone–DCM (1:1)	Ketone–chlorinated hydrocarbon
Toluene–DCM (1:1)	Chlorinated hydrocarbon–aromatic non polar mixture

tion of the mean). It is observed (Fig. 2A and B) that dichloromethane gave the highest recovery for DDT and its major metabolite DDD. It was also interesting to note that another chlorinated solvent (2-chlorotoluene) gave a high recovery for DDD. This situation was not as clear with the recovery of DDE however (Fig. 2C). In this case, while chlorinated solvents gave comparable recoveries, so also did non-chlorinated and non-polar solvents, such as isohexane and toluene. While these latter results were unexpected it identifies that a simple model of ''like extracts like'' is not always possible. Further work in modelling extraction efficiencies is therefore required. Based on these experimental data however, dichloromethane was selected as the solvent of choice for further work.

3.3. Soil extractions: Soxhlet versus PLE

It is common, in this type of work to compare the traditional approach of Soxhlet extraction with the newer extraction technique. Using dichloromethane as the extraction solvent, Soxhlet was performed over two different extraction times, 6 and 24 h. The nature of the Soxhlet extraction process allows ''clean'' solvent to pass through the sample at a rate of four times per hour. Thus for extraction of DDT, DDD and DDE it would be expected that the solvent passed through the aged, contaminated soil sample either 24 or 192 times at a temperature less than that of the solvent (dichloromethane, b.p. 40°C). The results are shown in Table 4. The data shows that 24 h with dichloromethane is required for maximum extraction of DDT, DDD and DDE from aged soil. By way of a comparison, the results are also compared with a 24 h Soxhlet extraction using a solvent mixture dichloromethane–acetone (1:1, v/v) [11]; comparable results are obtained. The results for the 24 h Soxhlet extraction are compared to the results obtained by PLE (both using dichloromethane) in Table 5. The surprising results show that Soxhlet for 24 h is more efficient at removing DDT, DDD and DDE from the aged, contaminated soil. This is contrary to findings previously reported in the scientific literature. Further investigation of PLE was required.

The fundamental difference between Soxhlet extraction and PLE is the fact that in Soxhlet, a large volume of fresh organic solvent is re-cycled through the sample. However, this is not strictly the case in PLE, but the volume of fresh solvent involved is minimal. During the heat-up period in PLE, solvent in the extraction cell expands causing the pressure to increase. To prevent overpressurisation of the cell, the static valve opens and closes automatically allowing a small volume of solvent to vent. To maintain pressure fresh solvent is pumped in to the cell. It is estimated that the volume of solvent vented is of the order of 0.1–0.2 ml/cycle of the static valve [2]. In order to determine if solvent re-cycling was the reason for the apparent poorer recovery of DDT, DDD and DDE from the aged, contaminated soil in PLE, an assessment of the static flush cycles was undertaken. The results of this study are shown in Tables 6. In each case a new soil sub-sample was extracted and analysed. The data shows that three static flushes are required to quantitatively remove DDT, DDD and DDE from aged, contaminated soil using a chlorinated solvent. Results from the cumulative total via PLE are compared with 24 h Soxhlet extraction in Fig. 3. Good agreement is achievable between the two techniques. It has clearly been shown that the number of static flush cycles required for quantitative recovery from aged, contaminated soil samples is three. A similar study, with similar findings, was reported by Popp et al. [10] for a series of chlorinated pesticides from contaminated soil. For future work by PLE it is therefore recommended that the number of static flush cycles be investigated for unspiked, aged samples.

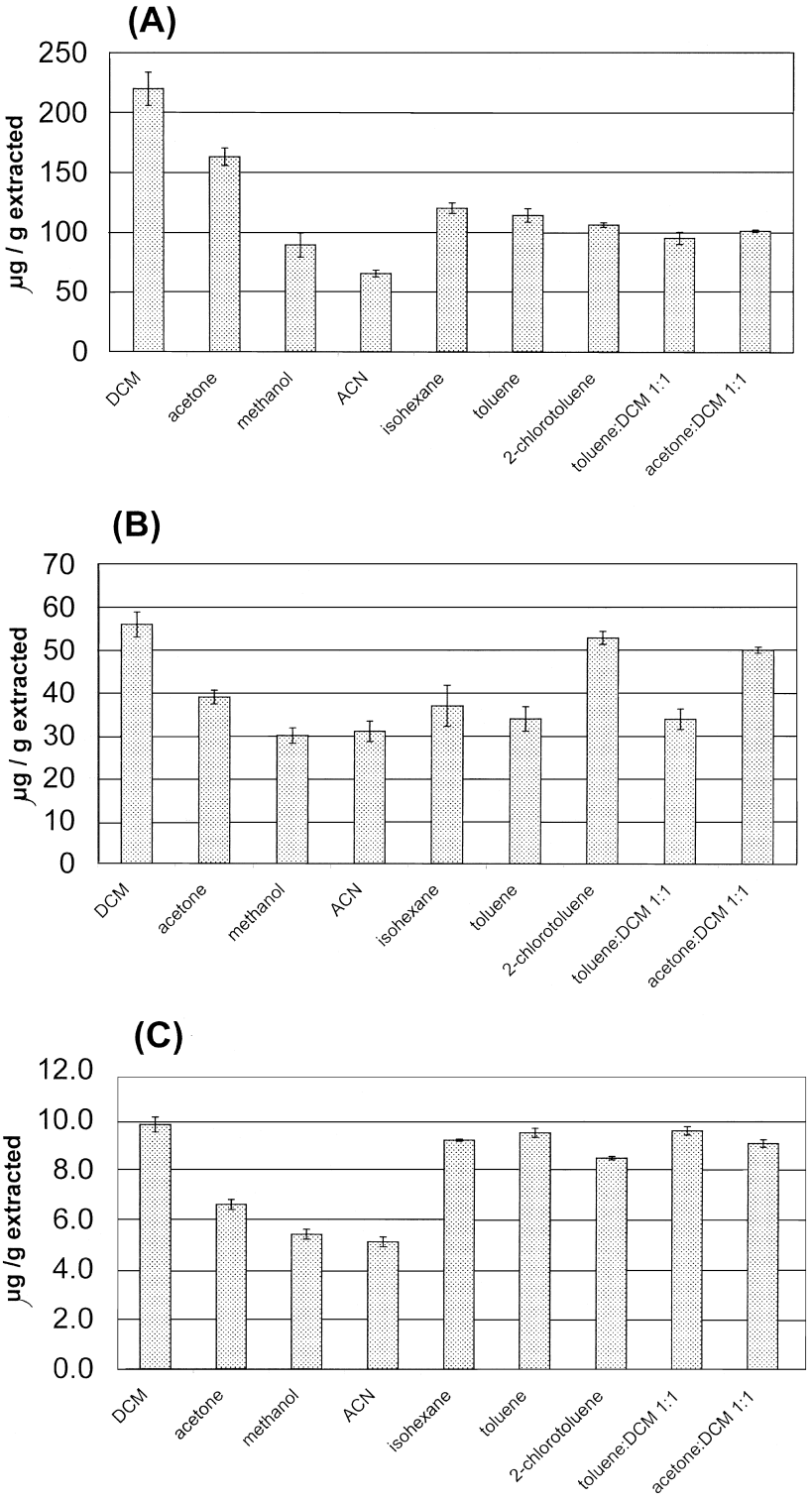

Fig. 2. Effect of solvent on the extraction of DDT and its metabolites from aged soil using PFE. (A) DDT, (B) DDD, and (C) DDE.

Table 4
Soxhlet extraction of contaminated soil (n=6)

	DDE			DDD			DDT		
	DCM		DCM–acetone (1:1, v/v)	DCM		DCM–acetone (1:1, v/v)	DCM		DCM–acetone (1:1, v/v)
	6 h	24 h	24 h	6 h	24 h	24 h	6 h	24 h	24 h
Mean μg/g	4.8	13.1	12.5	79.5	96.1	90.5	196.6	468.4	457.5
RSD (%)	9.7	5.9	4.5	23.5	3.9	1.4	25.5	3.1	3.3

Table 5
Comparison of Soxhlet for 24 h with PLE (1 static flush) of aged contaminated soil using dichloromethane

	NODE		DDD		DDT	
	PFE	Soxhlet	PFE	Soxhlet	PFE	Soxhlet
Mean (μg/g)	10.9	13.1	40.0	96	226	421
RSD (%)	5.7	5.9	5.0	3.9	8.1	10.7

Table 6
Influence of static flush cycles on the recovery of DDT and its metabolites from aged, contaminated soil

Sample	Number of PLE flush cycles	DDT			DDD			DDE		
		Mean[a] (μg g^{-1})	Re-extraction (μg g^{-1})	Cumulative total[a] (μg g^{-1})	Mean[a] (μg g^{-1})	Re-extraction (μg g^{-1})	Cumulative total[a] (μg g^{-1})	Mean[a] (μg g^{-1})	Re-extraction (μg g^{-1})	Cumulative total[a] (μg g^{-1})
1	1	225.78	122.59	348.37	39.91	37.79	77.7	10.87	1.02	11.89
2	2	336.06	63.55	399.61	59.20	30.08	89.28	11.86	0.43	12.29
3	3	404.82	0.00	404.82	79.97	0.00	79.97	13.23	0.00	13.23

[a] n=6.

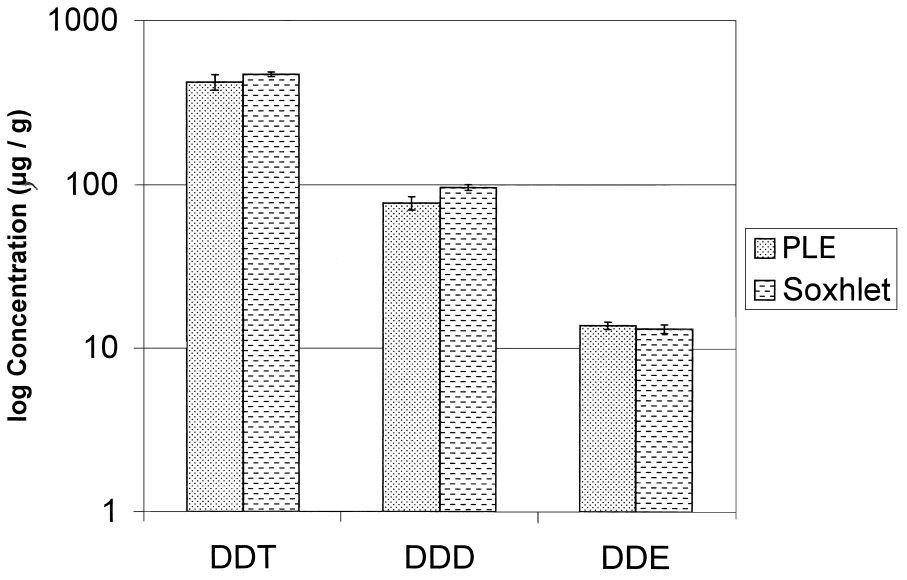

Fig. 3. Extraction of DDT and its metabolites from aged, contaminated soil.

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