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Supercritical fluid extraction (SFE) of PCBs and
organochlorine pesticides from soil.
Comparison with conventional extraction methods
and optimization for real soil samples.

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CONTENTS

LIST OF TABLES AND FIGURES

Tables

Figures

SUMMARY

This report describes the first results of Supercritical Fluid Extraction (SFE) as technique for the extraction of organic components from soil. SFE is based on the extraction properties of supercritical fluids - in this case CO_2 - having liquidlike as well as gaslike behaviour as their low viscosity and high solute diffusivities.

The optimization of SFE of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) from real soil samples is performed, according to a general stepwise set-up for quantitative SFE. An experimental approach was set up to test the influences of different extraction parameters SUCH as pressure, extraction time, static and dynamic extraction, restictor type and collection solvent for off-line SFE. Conditions obtained from these experiments with spiked samples were applied on real soil and optimized to gain maximum concentrations of components in comparison with conventional solvent extraction.

The optimal conditions achieved for spiked samples did not yield maximum concentrations for field samples. Stronger extraction conditions were necessary to overcome interactions between matrix and analytes. Longer dynamic extraction times were needed and the use of modifiers appeared to be essential for SFE of real samples. An increase in extraction pressure did not have any influence on extraction results. Comparable results were obtained for SFE and solvent extraction with an overall standard deviation between both methods of 25 %.

The stepwise approach is useful in method development to visualize several aspects of SFE, such as the initial conditions, the importance of extraction parameters and the success of SFE for a specific compound-matrix combination. Possibilities in quantitative SFE are discussed, as well as restrictions in the break-through of SFE as technique for future sample pretreatment of solid samples.

SAMENVATTING

Dit rapport beschrijft de eerste resultaten van superkritische vloeistof extraktie (SFE), als techniek voor de extraktie van organische componenten in grond. SFE is een relatief nieuwe analytisch chemische techniek, die gebaseerd is op de extraktie eigenschappen van superkritische vloeistoffen - in dit geval CO_2 - welke zowel vloeistof-achtige als gas-achtige eigenschappen bezitten zoals een lage viskositeit en een hoog oplossend vermogen voor organische verbindingen.

De optimalisatie van SFE voor poly-gechloreerde biphenylen (PCBs) en organochloor pesticiden (OCPs) uit grond is uitgevoerd volgens een algemene, stapsgewijze, procedure voor kwantitatieve SFE. Een experimentele benadering is gekozen voor het testen van de invloed van de verschillende extraktie parameters, zoals de druk, de extraktie duur, de statische en/of dynamische extraktie, het type restrictor en de opvangvloeistof bij off-line SFE. De met deze experimenten verkregen kondities voor gespiked monsters zijn toegepast op reële grondmonsters en verder geoptimaliseerd naar maximale opbrengst voor de diverse componenten in vergelijking met conventionele vloeistofextraktie.

De optimale kondities van de gespiked monsters gaven geen maximale concentraties voor de veldmonsters. Zwaardere extraktie kondities bleken noodzakelijk om interakties tussen de componenten en de grond-matrix te overwinnen. Een langere dynamische extraktie duur was noodzakelijk en het gebruik van modifiers bleek essentieel voor SFE van reële monsters. Een verhoging van de extraktie druk had geen verdere invloed op de resultaten. Uiteindelijk werden vergelijkbare resultaten verkregen voor SFE en vloeistof extrakties met een totale standaard deviatie tussen beide methoden van 25 %.

Een stapsgewijze benadering blijkt een goede procedure te zijn om de verschillende aspekten die een rol spelen bij de methode ontwikkeling van SFE inzichtelijker te maken, zoals de uitgangskondities, het belang van de verschillende extraktie parameters en het eventuele succes van SFE voor een specifieke component - matrix combinatie. Mogelijkheden voor de verdere toepassing van SFE worden bediscussieerd, evenals de beperkingen voor een mogelijke doorbraak van SFE als toekomstige techniek voor de monstervoorbewerking van vaste matrices.

1 INTRODUCTION

Every five year a monitoring program on organochlorine pesticides and PCBs is carried out to observe trends in the levels of these components in the soil in the Netherlands [1]. Several studies have shown that SFE results in good extraction yields for chlorinated contaminants in soil [2-12]. Therefor the application of supercritical fluid extraction as an alternative extraction technique to conventional methods for extraction of soil samples was evaluated in our laboratory. The conventional techniques are laborious, time consuming and require large amounts of high purity solvents, which produce problems of hazardous waste. In addition, especially for the more apolar components (HCB, DDTs and PCBs) solvent extraction often suffers from low extraction yields, when applied on soils with higher contents of organic carbon.

1.1 Supercritical fluid extraction

SFE offers a lot of potential advantages compared to conventional solvent extraction methods. These include reduced extraction times and amounts of extraction solvents, it offers the opportunity to more efficient extractions, increased selectivity and the capability of coupling with other chromatographic techniques. Based on their variable solvating power as a function of density, supercritical fluids have several characteristics that make them ideal extraction solvents to selectively extract and isolate discrete fractions from sample matrices. Rapid mass transfer during extraction is facilitated by the low viscosity and high solute diffusivities due to the liquid- and gaslike behaviour of supercritical fluids. In applying SFE, CO_2 is most often chosen as extraction solvent because of the moderate critical temperature (31 °C) and pressure (73 atm), the nonflammable and non-toxic properties, its low cost and minimized problems of waste.

Several studies have described the use and potentials of SFE for the extraction of organic contaminants from different matrices. Lopez-Avila *et al.* [2] extracted several groups of organochlorine and organophosphorous pesticides spiked on sand with good recoveries (from 80 to 125 %) using either CO_2 or, more advantageously, CO_2 modified with 10 % methanol. Hawthorne and Miller [3-5] studied the extraction of polycyclic aromatic hydrocarbons (PAHs) from reference materials and other matrices and obtained results in good agreement with the certified values, in contrast with results reported by Lopez-Avila *et al.* [2]. The extraction of total PCBs from a certified sediment sample was demonstrated by Onuska and Terry at 20 MPa, 40 °C in 8 min using CO_2 with 2% methanol [6]. SFE of pesticides from soil and sediment has been studied using CO_2 saturated with water or with the direct addition of methanol to the extraction cell to increase recoveries [7-9]. Several studies have reported the

optimization in terms of modifier, pressure, temperature and flow-rate of the extraction efficiencies of diuron and linuron from sand [10], several chlorinated components [11] and 2,3,7,8-TCDD from sediment [12].

In the beginning of SFE, a lot of qualitative studies were reported, showing the effects of extraction at different densities on groups of components or reporting SFE of spiked samples only. Unfortunately, many studies seem to stop after the performance of spiked samples [13-16]. More recent studies emphasize the importance of the differences between the investigations on spiked samples and field samples. In most cases, addition of modifiers or stronger extraction conditions seemed to be necessary to obtain SFE results which can be compared with conventional extraction techniques or with values of certified reference materials. Paschke *et al.* [17] optimized the SFE of PAHs and nitro-PAHs from diesel particulates with different kinds of fluids (CO_2 and $CHClF_2$) and modifiers (methanol and toluene), whereas Dankers *et al.* [18] improved PAH results in soil with the addition of relatively large volumes of dichloromethane. The quantitative extraction of PCBs from river sediment was improved with supercritical $CHClF_2$ or with methanol modified CO2 [19], as was also reported by Onuska and Terry [12]. In most studies, the extraction parameters were optimized varying parameter by parameter, Van der Velde *et al.* [20] showed the application of an experimental design in the extraction of triazines from soil.

1.2   Goal

Hawthorne *et al.* discussed a general set–up for quantitative SFE, based on sequential optimization of extraction parameters [21]. Basic parameters in SFE, the partitioning of the analyte in the fluid, the sweeping from the extraction cell and the collection method, can be best determined by spiking on inert material excluding matrix influences. Only the solubility of the components in the supercritical fluid is tested, as well as the extraction time and the collection device and solvent. Next, recoveries were determined of spiked samples and then SFE was performed on real samples in comparison with conventional extraction techniques. See Figure 1.

In the first part of the study, results are described for the optimization of SFE conditions for the analysis of organochlorine pesticides and PCBs spiked onto soils, with different contents of organic carbon. Results are compared with those obtained by application of conventional extraction techniques. This study was presented at the International Symposium of Hyphenated Techniques 1992 in Antwerpen and was published in the Journal of Chromatography [16].

In the second part, the SFE method is applied and optimised for OCPs in real soil samples. Experiments were started from the earlier investigations of the first part. Several parameters were investigated for optimization of SFE, to equal or to improve the results from solvent extractions. Finally, the possibilities and restrictions of SFE will be discussed as technique for

future pretreatment of soil samples. This study was presented at the International Symposium of Hyphenated Techniques 1994 in Antwerpen and was published in the Journal of Chromatography [23].
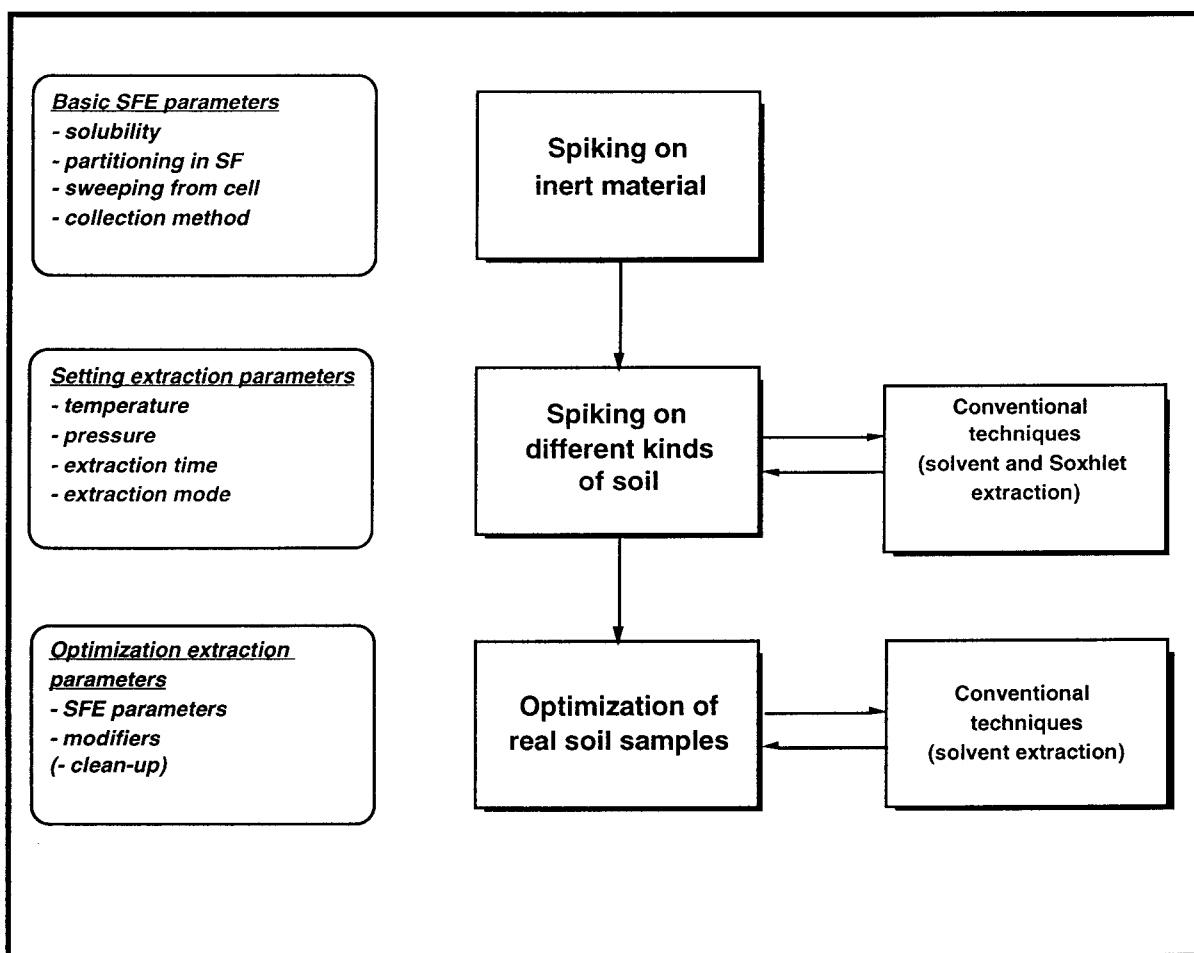

Figure 1. Flow scheme of a general set-up for the optimization procedure of quantitative SFE.

2 MATERIALS AND METHODS

2.1 Samples

Soils were air-dried, allowed to pass through a 2.8 mm sieve and subsequently homogenized in a ball mill. Field samples were obtained from the Dutch monitoring programme on soil, concerning soils with about 5 % organic carbon used for grass land, agriculture land and orchard soil, respectively. Blank soils were characterized as sand and peat soil, with 0.3 and 3.3 % organic carbon respectively. Individual soil samples were spiked just before analysis, waiting for 15 min to 1 h to allow evaporation of the solvents (the evaporation time depended on the amount of solvents used). Based on their occurrence in Dutch soils, the following compounds were selected for this study: α-hexachlorocyclohexane (α-HCH), HCB, β-HCH, γ-HCH, β–heptachloroepoxide (β-HEPO), 2,2-bis(p-chlorophenyl)-1,1-dichloroethene (p,p'–DDE), dieldrin, 2,2-bis(p-chlorophenyl)-1,1-dichloroethane (TDE), o,p'-DDT, p,p'- DDT and and PCB 28, PCB 52, PCB 101, PCB 118, PCB 138 and PCB 153. PCBs were from CIL (Cambridge Isotope Laboratories, Woburn, MA, USA), OCPs from Promochem (Wesel, Germany). Spiking levels were chosen based on levels previously observed and were typically between 1 and 10 ng/g of dry soil.

2.2 Extraction procedures

2.2.1 *Solvent extraction*
Aliquots of 25 g of soil were extracted twice with 40 ml of acetone for 30 min by using a shaking machine. The organic layer was separated by centrifugation. The liquid-fractions were mixed with 800 ml of water and a few ml of saturated sodium chloride, and were then extracted twice with 50 ml of hexane. The combined hexane fractions were dried and, after addition of internal standards (PCBs 44 and 141), concentrated in a Kuderna-Danish apparatus until 10 ml remained. In some cases, a clean–up is performed to remove interferences from sulphur components. All solvents used were pesticide-grade (hexane) or distilled (acetone, petroleum-ether).

2.2.2 *Soxhlet extraction*
Aliquots of 5 g of soil, mixed (1:3, w/w) with quartz sand (Boom, Meppel, The Netherlands) were placed into a modified Soxhlet-extraction unit, consisting of a fritted porosity glass extraction tube and were extracted for 14 hours with 150 ml of acetone/petroleum-ether (b.p. 30-60 °C) (1:1, v/v). After cooling, 600 ml of water and a few ml of saturated sodium chloride were added for solvent extraction, petroleum ether was separated and a second extraction with

50 ml of PE was performed. The combined petroleum ether-fractions were dried and, after addition of internal standards, were concentrated in a Kuderna-Danish apparatus until 5 ml remained.

2.2.3 *Supercritical fluid extraction*

Supercritical fluid extractions were performed on a Carlo Erba SFC 3000 instrument using a double 70-ml syringe pump (SFC 300) and a SFE-30 extraction unit (Carlo Erba Instruments, Milan, Italy). The apparatus can be used in on-line and off-line modes; in the latter the restrictor is disconnected from the transfer tube and solvent sample-collection can be performed. Extractions can be performed using constant pressure (varying from 15 to 50 MPa) or at constant flow and the temperature of the extraction unit can be varied between 40 and 150 °C. The extraction process was pre-programmed using integrated software to perform valve switching and time programmed extractions with combinations of static and dynamic extraction conditions at various pressure settings. The specific extraction conditions are given in the tables. Supercritical pressure was maintained inside the extraction vessel using a deactivated fused-silica 1.5 m x 25 or 50 μm I.D. restrictor (SGE, Austin, Texas, USA), resulting in a liquid CO_2 flow of 160 – 180 μL/min (at 20 MPa).

Optimization experiments were carried out using glass beads (250 μm, acid washed and silanized; Supelco, Bellefonte, PA, USA) as the sample matrix. Accurately weighed soil samples were brought into a 0.5 ml extraction cell, filled up at one side with a thin layer of quartz sand to prevent clogging of the system and stamped to achieve homogeneous packing. Solvent collection was performed into a 2 ml vial containing approximately 1 ml of organic solvent, with a known concentration of internal standard mixture (PCBs 44 and 141). Modifier was added directly to the extraction cell, just before supercritical extraction. CO_2 was SFC grade from Ucar (Union Carbide, Westerlo, Belgium) or Air Products & Chemicals Inc. (Waddinxveen, The Netherlands).

2.3   Analysis

An HP 5890 gas chromatograph, equipped with an HP 7673A autosampler, an electron capture detector (ECD), and an Ultra-2 (50 m x 0.2 mm I.D.; 0.33 μm; HP) or DB–5 (30 m x 0.25 mm I.D.; 0.25 μm; J & W Scientific, Folsom, CA, USA) was used for chromatographic separation and was interfaced with an HP-Chem data system (Hewlett Packard, Palo Alto, California). Helium was used as the carrier gas (2 ml/min) and argon/methane as the purge gas (60 ml/min). After injection of 2-4 μl, the temperature program consisted of an initial temperature of 80 °C, 2 min hold, then an increase to 170 °C at 30 °C/min, then 3 °C/min to the final temperature of 290 °C and held for 5 min. The injector temperature was 250 °C and

detector temperature was 325 ℃. Quantification was performed by comparison with a reference standard mixture using PCB 44 and 141 as internal standards.

Limits of determination for each component were 0.5 ng/g of soil, using the conditions specified above for sample preparation and analysis.

3 RESULTS AND DISCUSSION

3.1 Spiking on inert material

The first step in the optimization experiments have been carried out using glass beads, spiked with a standard mixture of 16 pesticides and PCBs in hexane followed by off-line SFE with solvent sample collection in hexane. After each experiment, a second extraction was performed to check if all components were extracted under the conditions used and to confirm that a clean system was used for the next experiment. Preliminary experiments were performed using different, tapered and linear, restrictors. Tapered restrictors caused a lot of clogging problems, so linear restrictors of 25 and 50 μm I.D. were used, in spite of the disadvantages of decrease in pressure over the whole linear range of the restrictor. The internal diameter of the restrictor in combination with its length (at fixed pressure) determines the flow-rate and the volume of CO_2 passing the extraction cell during a centain time period. Table 1 gives the results for

Table 1. Comparison of extraction recoveries (%) for 25 and 50 μm linear restrictors with different extraction times (in min).

Component	Restrictor diameter (Static/dynamic extraction time (min))		
	25 μm (10/20)	25 μm (10/50)	50 μm (10/20)
a-HCH	26	34	55
HCB	-	-	37
b-HCH	63	90	91
g-HCH	44	-	64
b-HEPO	63	82	91
pp-DDE	69	96	106
dieldrin	67	87	96
TDE	69	91	96
op-DDT	73	116	107
pp-DDT	80	109	106
PCB 28	53	74	78
PCB 52	61	83	88
PCB 101	71	100	97
PCB 118	70	90	99
PCB 153	70	94	105
PCB 138	70	91	101
Mean	63	88	89

SFE conditions: 50 °C, 20 MPa.
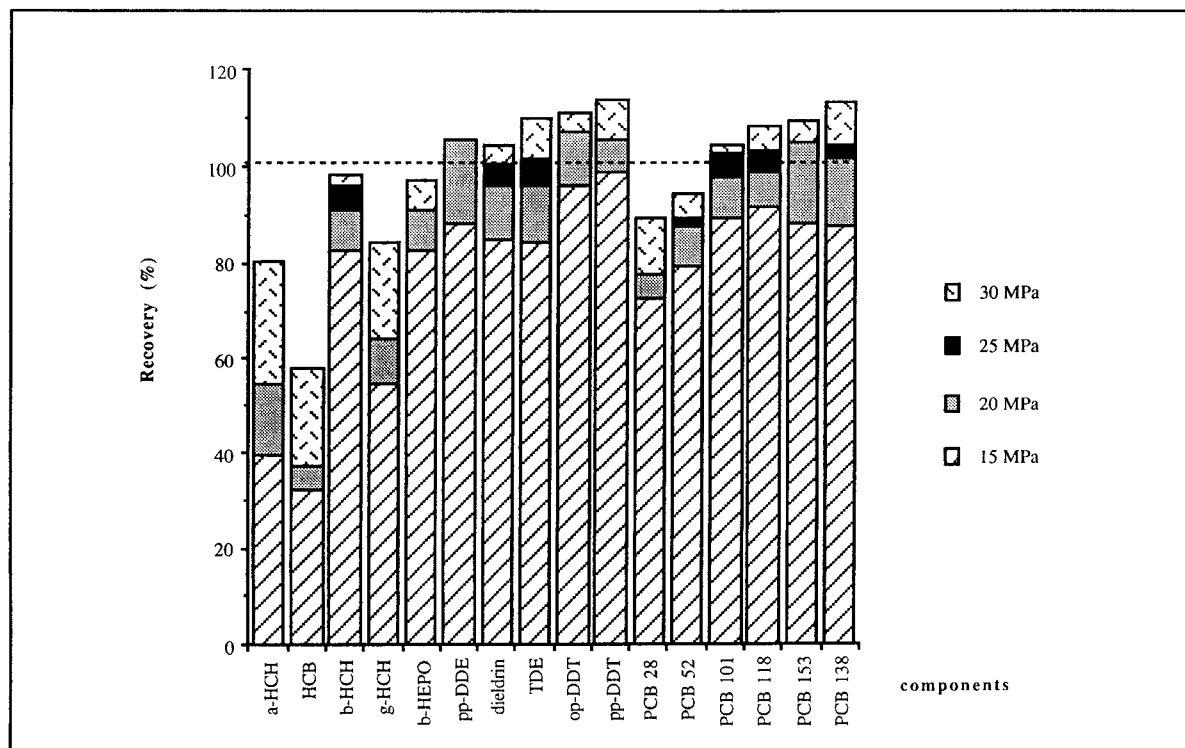
Figure 2. Influence of varations in pressure on extraction recoveries. The largest improvement in recovery can be observed when increasing the pressure from 15 to 20 MPa.

25 and 50 µm restrictors for 20 and 50 min dynamic extractions, respectively. The same extraction yields (89 and 88 %, respectively) can be obtained for a restrictor with a larger internal diameter in a shorter time (50 µm and 20 min), than for restrictors with a smaller I.D. (25 µm and 50 min). Finally, 50 µm restrictors were chosen because of the shorter time of analysis and to diminish clogging problems by extraction of real samples.

Subsequently, extraction pressure, combinations of static and dynamic extractions, extraction times and collection solvents were varied and the reproducibility of the system was tested. The extraction pressure was varied from 15 to 30 MPa at constant temperature of 50 °C and 30 min of extraction. Figure 2 (Table 2 of enclosure) shows the recoveries for the different extraction conditions. A second extraction of the same sample did not gave any yield. A few components, α-HCH, HCB and γ-HCH, show relatively low recoveries compared with the other components, probably as a result of their volatility, which resulted in a less efficient trapping in the solvent used. Excluding the latter components, the largest improvement in recovery can be observed when increasing the pressure from 15 to 20 MPa, whereas only a slight increase of recovery is obtained going to 25 and 30 MPa. As higher pressures increase the risks of co-extractants from the matrix, 20 MPa was chosen as optimal pressure. Several experiments were performed to establish the ideal extraction times at various
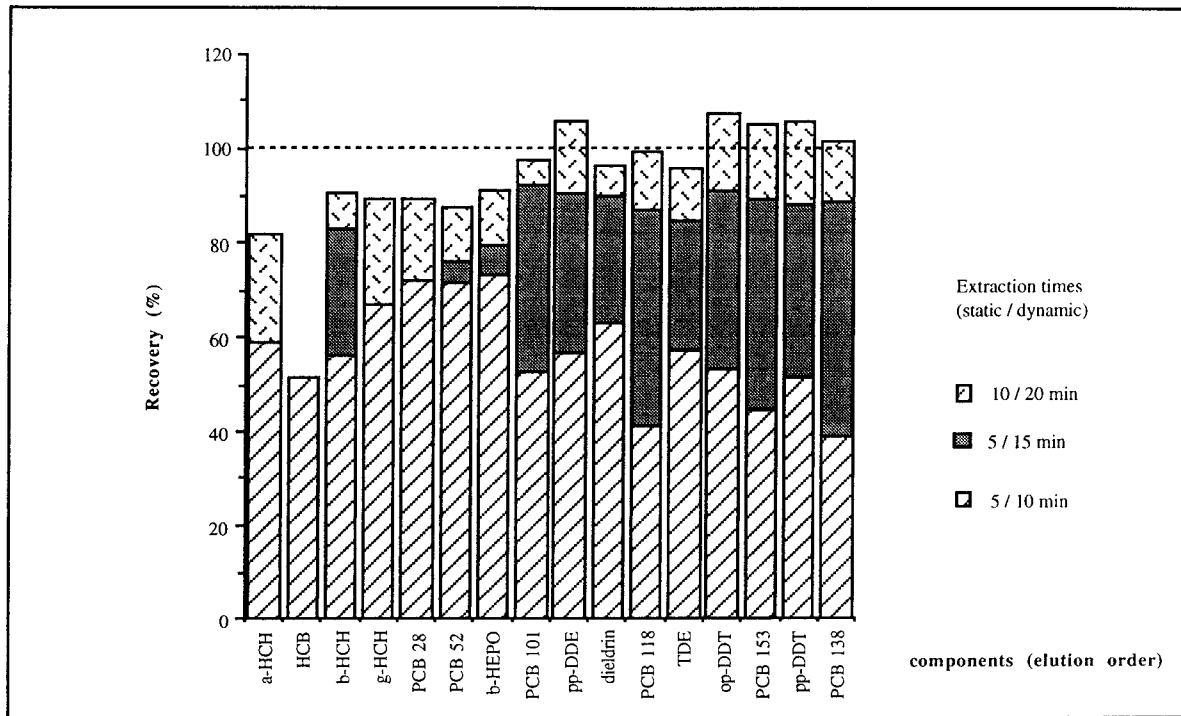
Figure 3. Influence of extraction times (static/dynamic in minutes) on extraction yields. Better extraction yields are reached especially for the less volatile components going to longer extraction times (Note: components in order of elution).

combinations of static and dynamic extraction times. Earlier experiments with long static extractions and shorter dynamic extractions showed that recoveries were lower and second extractions of these samples gave higher yields. Therefor experiments were carried out with 15, 20 and 30 min of extraction (Table 3 of enclosure). From Fig. 3, it can be seen that better extraction yields are reached, especially for the less volatile components, when using longer dynamic extraction times. Using the longest extraction time of 10 min static and 20 min dynamic, for all components, except the earlier mentioned more volatile components, extraction yields between 78 and 107 % were found.

The reproducibility of the extraction procedure was studied by triplicate extractions using the proposed conditions (50 °C, 20 MPa, 10 min static and 20 min dynamic extractions). Table 4 shows that supercritical fluid extraction gives reproducible extractions with good mean recoveries (89 %) and low mean standard deviations (4.2 %), with a range of 90.4 ± 3.2 % to 115.5 ± 12.9 % for all PCBs and chlorinated pesticides, excluding the more volatile pesticides. In order to improve recovery for the volatile pesticides, iso-octane was investigated as collection solvent. Table 5 shows that a considerable increase in recovery is obtained by chosing the most appropriate collection solvent. By changing to iso-octane, acceptable recoveries were also found for α-HCH, HCH and γ-HCH. The mean recovery for all components was 101 %, at a level of 5 ng absolute.

Table 4. Reproducibility of extraction recoveries (%) of SFE procedure.

Component	Reproducibility				
	Experiment			Mean ± S.D. (%)	
	1	2	3		
a-HCH	55	56	54	55 ± 0.5	
HCB	37	37	40	38 ± 1.0	
b-HCH	91	98	90	93 ± 1.6	
g-HCH	64	63	64	64 ± 0.1	
b-HEPO	91	93	87	90 ± 2.0	
pp-DDE	106	103	96	101 ± 2.8	
dieldrin	96	97	92	95 ± 1.5	
TDE	96	98	89	94 ± 2.6	
op-DDT	107	122	102	110 ± 4.2	
pp-DDT	106	137	104	116 ± 5.7	
PCB 28	78	88	80	82 ± 0.9	
PCB 52	88	91	87	89 ± 0.8	
PCB 101	97	101	93	97 ± 2.0	
PCB 118	99	106	96	100 ± 2.3	
PCB 153	105	100	94	100 ± 2.8	
PCB 138	101	102	94	99 ± 2.5	
Mean	89	93	85	89 ± 1.9	

SFE conditions: 20 MPa, 50 ℃, 10 min. static and 20 min. dynamic extraction.

Table 5. Influence of collection solvent on extraction recoveries (%).

Component	Extraction solvent	
	Hexane	Iso-octane
a-HCH	55	95
HCB	38	78
b-HCH	93	105
g-HCH	64	91
b-HEPO	90	103
pp-DDE	101	107
dieldrin	95	105
TDE	94	102
op-DDT	110	105
pp-DDT	116	101
PCB 28	82	105
PCB 52	89	102
PCB 101	97	108
PCB 118	100	110
PCB 153	100	104
PCB 138	100	101
Mean	89	101

SFE conditions : 20 MPa, 50 ℃, 10 min. static and 20 min. dynamic extraction.

3.2   Spiking on different kinds of soil and comparison of SFE with other techniques

The optimized conditions (20 MPa, 50 °C, 10 min static and 20 min dynamic extraction and solvent collection in iso-octane) have been used for the SFE of components from two soil samples spiked with a standard mixture of 16 pesticides and PCBs at a level of 5 ng/g of dry matter. Separate aliquots of the samples were also extracted with two conventional techniques: solvent extraction with acetone/hexane and Soxhlet extraction with acetone/petroleum ether. All samples were extracted directly, after a fixed time to evaporate the solvent after spiking, thus minimizing the influence of the spiking solvent acting as a modifier during extraction. In this way the analytical recovery has been determined.

Table 6 and 7 give the recoveries and standard deviations for the various techniques applied to sand and peat-soils. Solvent extraction of sand gives good recoveries with low standard deviations for all components, varying from 79 ± 3.8 % for p,p'-DDT to 87 ± 4.3 % for α–HCH. For peat-soil, the recoveries show more variation, with fairly low recoveries for p,p'-DDE (32 ± 6.5%), o,p'-DDT (41.6 ± 7.0%), p,p'-DDT (41 ± 9.6%), PCB 138 (18 ± 8.1%) and PCB 153 (12 ± 6.9%). These results are in agreement with values found for these components in the Dutch monitoring program on soil [1]. Obviously, rapid, almost irreversible adsorption of these components takes place in soils with higher contents of organic carbon. Using Soxhlet extraction, very high recoveries are found for both sand (mean value 121 ± 3.7%) and peat-soil (125 ± 7.1%), probably caused by impurities co-extracted during the more intensive Soxhlet extraction procedure (see Figure 4).
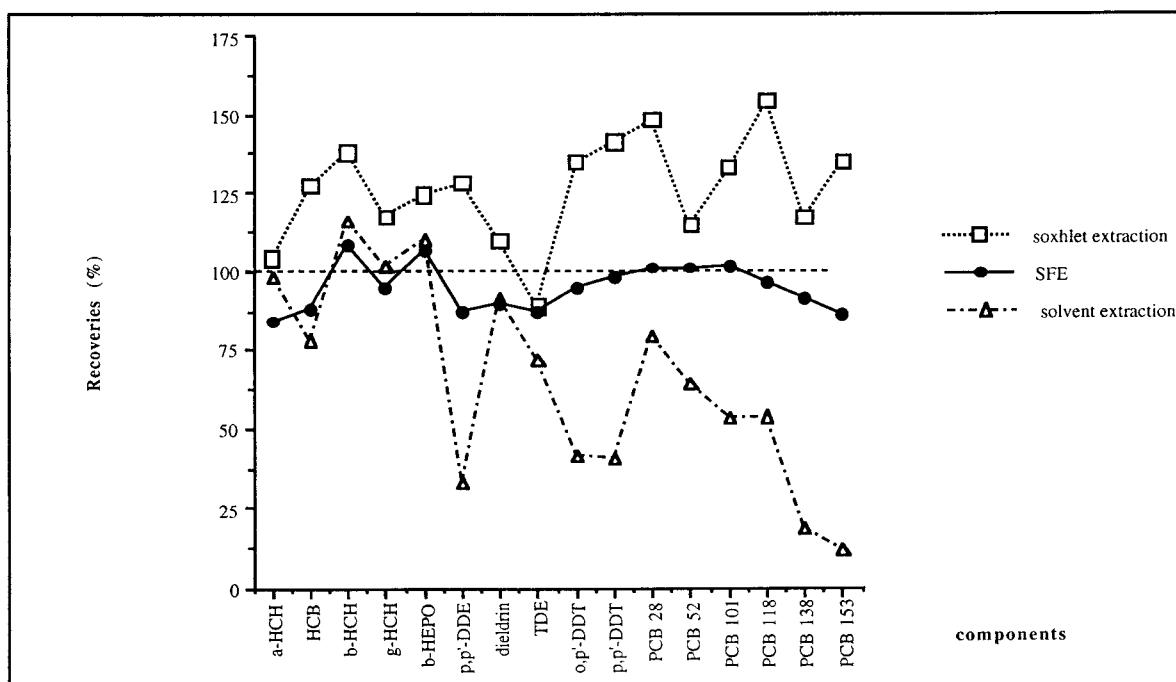

Figure 4. Comparison of extraction recoveries for PCBs and OCPs for three extraction techniques in peat soil.

Table 6 : Comparison extraction recoveries for PCBs and pesticides from sand for different extraction techniques.

Component	Addition (ng/g)	Solvent extraction (n=5)		Soxhlet extraction (n=5)		SFE (n=3)	
		Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)
a-HCH	2.3	86.9	4.3	100.7	1.3	98.8	2.1
HCB	0.9	94.0	5.2	113.0	1.9	88.7	7.2
b-HCH	3.3	93.3	4.0	120.0	4.7	100.5	5.1
g-HCH	2.2	89.6	4.2	120.9	6.6	100.2	5.2
b-HEPO	3.2	88.3	4.2	106.0	2.7	94.2	1.9
p,p'-DDE	5.1	89.0	3.8	116.1	3.6	92.9	9.6
dieldrin	4.8	88.1	3.2	101.3	2.8	92.9	3.5
TDE	8.2	87.8	8.2	95.6	2.8	96.2	5.0
o,p'-DDT	8.9	91.5	3.5	164.0	8.0	89.2	2.0
p,p'-DDT	10.1	96.9	3.8	-	-	91.2	5.4
PCB 28	4.9	96.3	4.8	139.6	3.1	98.0	1.5
PCB 52	4.6	92.0	5.3	121.3	4.4	-	-
PCB 101	2.9	94.9	5.0	125.5	4.8	88.2	1.9
PCB 118	2.3	93.2	4.4	136.8	5.3	90.2	6.9
PCB 138	2.4	94.1	3.8	126.0	4.4	86.5	4.7
PCB 153	2.1	94.6	4.4	120.3	3.5	96.2	3.9
Mean	4.3	91.9	4.5	120.5	3.7	93.6	4.1

SFE conditions: 20 MPa, 50 °C, 10 min. static and 20 min dynamic, collection in iso-octane).

Table 7. Comparison extraction recoveries for PCBs and pesticides from peat for different extraction techniques.

Component	Addition (ng/g)	Solvent extraction (n=5)		Soxhlet extraction (n=5)		SFE (n=3)	
		Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)
a-HCH	2.3	98.2	7.5	103.6	5.2	83.9	1.8
HCB	0.9	78.2	7.7	127.4	7.6	88.3	2.2
b-HCH	3.3	115.6	9.2	137.7	8.4	108.0	1.6
g-HCH	2.2	101.7	8.2	117.0	6.4	94.7	6.4
b-HEPO	3.2	110.3	9.0	124.2	5.8	107.0	3.9
p,p'-DDE	5.1	32.6	6.5	127.6	7.8	86.9	1.1
dieldrin	4.8	90.8	8.3	108.9	5.6	89.2	2.5
TDE	8.2	71.9	8.3	88.9	14.2	86.7	3.1
o,p'-DDT	8.9	41.6	7.0	134.5	0.4	94.6	2.6
p,p'-DDT	10.1	40.9	9.6	-	-	98.0	1.9
PCB 28	4.9	79.1	7.9	148.1	8.3	101.0	6.5
PCB 52	4.6	63.9	6.6	114.1	1.8	-	-
PCB 101	2.9	53.3	6.9	133.2	18.8	101.3	1.9
PCB 118	2.3	53.9	7.3	154.1	6.3	96.0	2.7
PCB 138	2.4	18.4	8.1	116.4	6.5	90.8	1.7
PCB 153	2.1	11.9	6.9	134.6	11.2	86.4	2.3
Mean	4.3	66.4	7.8	124.7	7.1	94.2	2.6

Conditions: 20 MPa, 50 °C, 10 min. static and 20 min dynamic, collection in iso-octane).

In Fig. 5a and b, the chromatograms are shown for peat-soil extracted with solvent extraction and Soxhlet extraction, respectively. The chromatograms show several impurities giving increased baselines. An extra clean-up before analysis, which was not applied here, should be necessary to obtain reliable analytical data.

Supercritical fluid extraction of both sand and peat-soil gives good recoveries for all components varying from $87 \pm 4.7\%$ for PCB 138 to $101 \pm 5.1\%$ for β-HCH in sand, and from $84 \pm 1.8\%$ for α-HCH to $107 \pm 3.9\%$ for β-HEPO in peat-soil. In Fig. 5c, the chromatogram of peat-soil extracted with SFE shows a clean extract giving a straight baseline, better than results obtained for the other techniques. The reproducibility is fairly good (1.9 - 6.9 %), and is comparable with that found in the optimization experiments on glass beads. As can be seen from Fig. 4, SFE is comparable with the other techniques for sand with respect to reproducibility and gives better results for peat-soil, especially for the more apolar components. SFE seems to be more efficient than conventional techniques for spiked samples, giving higher recoveries than solvent extraction and no need for clean-up in comparison with Soxhlet extraction.

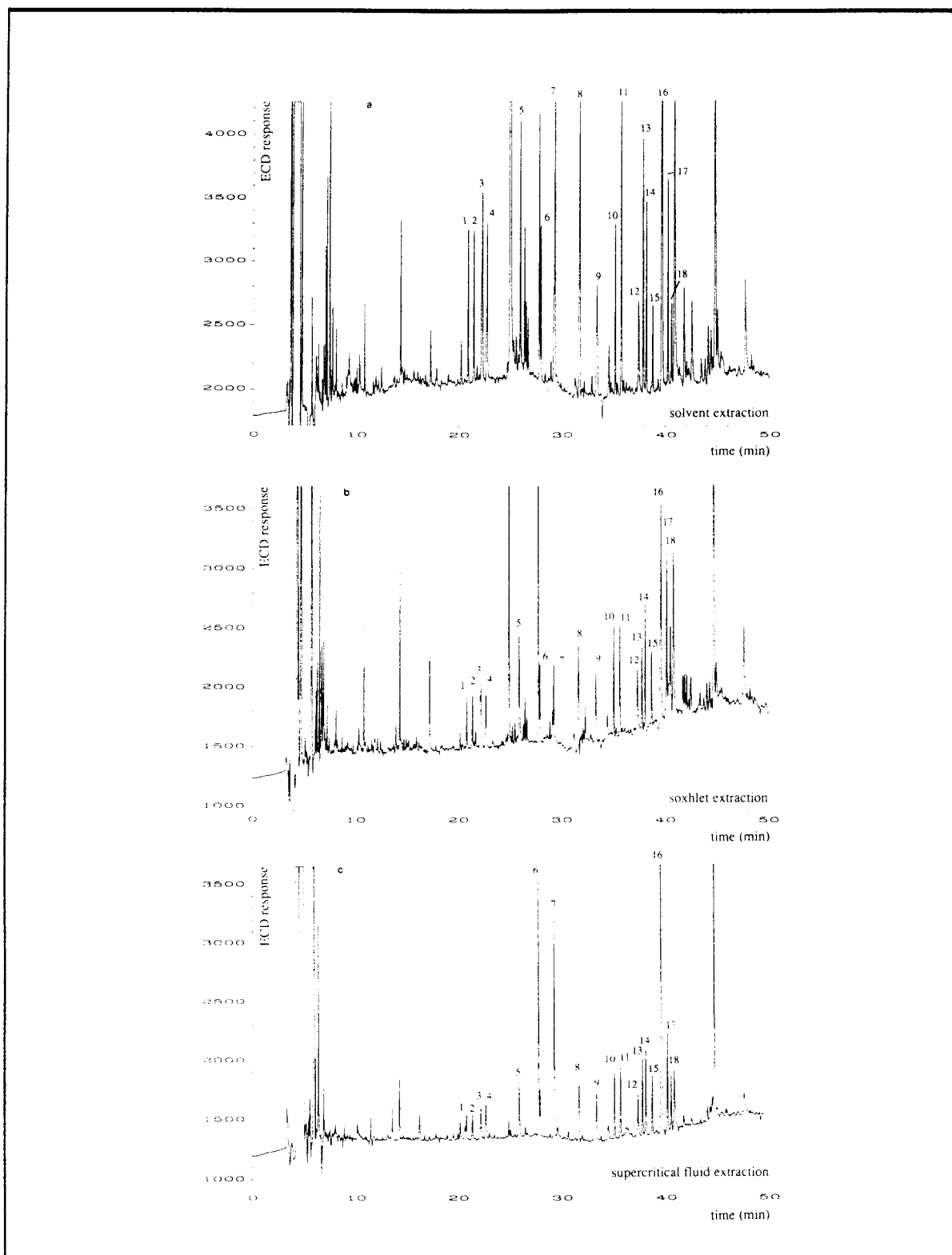Figure 5. Chromatograms of pesticides and PCBs extracted from peat-soil. a) solvent extraction, b) Soxhlet extraction and c) SFE. 1. α-HCH, 2. HCB, 3. β-HCH, 4. γ-HCH, 5. PCB 28, 6. PCB 52, 7. PCB 44 (I.S.), 8. b-HEPO, 9. PCB 101, 10. p,p'-DDE, 11. dieldrin, 12. PCB 118, 13. TDE, 14. o,p'-DDT, 15. PCB 153, 16. PCB 141 (I.S.), 17. p,p'- DDT and 18. PCB 138 (GC-conditions see Materials and methods).

3.3 Optimization of extraction parameters for real soil samples

Because there was a longer time period between the last experiments and the next step of the optimization of extraction parameters for real soil samples, some experiments were repeated and extended. Extraction times were varied from 10 min static and 20 min dynamic (SFE 10/20) to 30 min dynamic extraction (SFE 30) for spiked samples to sand and peat soil, in comparison with solvent extraction (Table 8). For sand no effect of extraction times was found, but for peat soil a decrease in recoveries of 10 to 20 % for all components was found going to longer dynamic extraction times. With respect to the identical results for sand, this cannot be an effect of the collection method. A possible explanation is the difference in kinetics of the partitioning process for components to become available from sand or from peat soil. Starting from these optimized conditions for spiked samples, a first series of extraction experiments with three different field soils was performed.

Table 8. Recovery experiments with solvent extraction (LLE) and SFE of PCBs and OCPs on sand and peat soil (n=3).

| Component | Add. | Sand | | | | | | Peat soil | | | | | |
| | (ng/g) | LLE | | SFE 10/20 | | SFE 30 | | LLE | | SFE 10/20 | | SFE 30 | |
		Rec (%)	S.D. (%)	Rec (%)	S.D. (%)	Rec (%)	S.D. (%)	Rec (%)	S.D. (%)	Rec (%)	S.D. (%)	Rec (%)	S.D. (%)
a-HCH	2.3	98.3	5.3	98.4	6.7	98.6	12.5	101.0	14.0	96.4	6.8	78.3	4.4
HCB	0.9	108.0	3.0	101.6	3.7	100.4	13.3	113.0	19.0	95.9	8.4	89.7	7.5
b-HCH	3.3	110.0	4.0	103.6	0.8	102.8	14.5	157.0	11.0	112.8	14.6	97.3	5.1
g-HCH	2.2	118.0	8.0	103.1	4.7	103.7	12.2	109.0	12.0	91.2	7.7	81.8	5.5
b-HEPO	3.2	98.5	2.9	92.1	19.3	97.4	5.6	*	*	93.9	6.6	79.1	2.1
p,p'-DDE	5.1	86.7	3.8	99.2	1.4	94.2	8.1	75.6	12.6	107.2	3.8	97.6	11.0
dieldrin	4.8	80.5	1.7	98.3	2.4	96.7	8.7	83.1	11.9	95.6	4.6	78.2	5.8
TDE	8.2	75.9	2.8	97.5	6.1	97.8	10.5	78.9	11.3	91.3	6.8	77.1	6.4
o,p'-DDT	8.9	84.5	3.9	97.1	3.4	95.9	8.7	50.7	10.5	98.3	6.4	82.5	7.7
p,p'-DDT	10.2	91.1	6.6	93.5	7.8	93.3	17.6	64.5	10.9	103.4	9.5	111.0	42.0
PCB 28	4.5	107.0	1.0	112.4	2.2	103.5	11.3	103.0	11.0	104.9	9.8	92.5	6.4
PCB 52	3.2	89.9	2.9	96.7	3.0	97.1	11.6	59.0	8.7	94.9	6.0	78.7	5.6
PCB 101	3.9	96.9	3.1	102.7	3.1	97.9	9.6	83.8	8.4	110.5	6.7	91.4	8.1
PCB 118	2.8	90.5	4.2	109.0	4.8	99.6	7.1	98.0	13.4	102.8	8.2	89.5	9.5
PCB 138	2.8	96.5	3.7	102.7	2.5	102.4	11.2	64.2	12.5	106.7	10.2	90.1	10.8
PCB 153	4.0	89.5	6.9	102.2	1.1	94.9	7.0	57.8	13.3	99.6	6.2	85.2	9.9
Mean	4.4	95.1	11.4	100.6	5.2	98.5	3.3	81.2	27.8	100.3	6.7	87.5	9.3

SFE conditions: 20 Mpa; 50 °C; CO2; 10 min static and 20 min dynamic (SFE10/20) or 30 min dynamic (SFE 30); flow ca. 160 µl/min.

In Table 9, the results of the solvent extraction and SFE are given for the organochlorine pesticides (no PCBs were found), using as extraction conditions: 20 MPa, 50 °C, 10 min static and 20 min dynamic extraction times and pure CO_2. In a second series, the extraction time was changed to 30 min dynamic extraction to increase recoveries.

The SFE results of the field soils are in the same order of the concentrations found after solvent extraction. In this regard it must be realized, that concentrations are not corrected for extraction recoveries. For the measured components LLE recoveries ranged from 50 to 80 %, whereas for SFE recoveries were between 91 and 107 % (Table 8). Going to longer dynamic extraction times in SFE, a slight improvement can be seen (a further increase in dynamic extraction time did not give any better extraction results). This is in contradiction with the SFE for spiked samples. Apparently, the binding of the components to the matrix and the extraction process cannot be compared for real samples and spiked samples with regard to diffusion, partitioning and kinetics.

Modifiers with different characteristics were added directly to the extraction cell for the orchard soil to try to improve the SFE results. Table 10 shows concentrations after addition of toluene, acetonitrile and methanol, respectively. Toluene did not gave any improvement, but rather a decrease, whereas Mulcahey and Taylor reported an increase for PCBs [22]. Both acetonitrile and methanol give an increase in concentrations, from which methanol gives the best overall results. This is in agreement with Hawthorne et al. [19] and Onuska and Terry [12]. Obviously, to overcome the interactions between the analyte and the matrix, introduction of modifier is essential. To test the influence of the amount of modifier, a series of 5 to 30 µl methanol was added to the extraction cell. In Table 11, the results are shown for the orchard
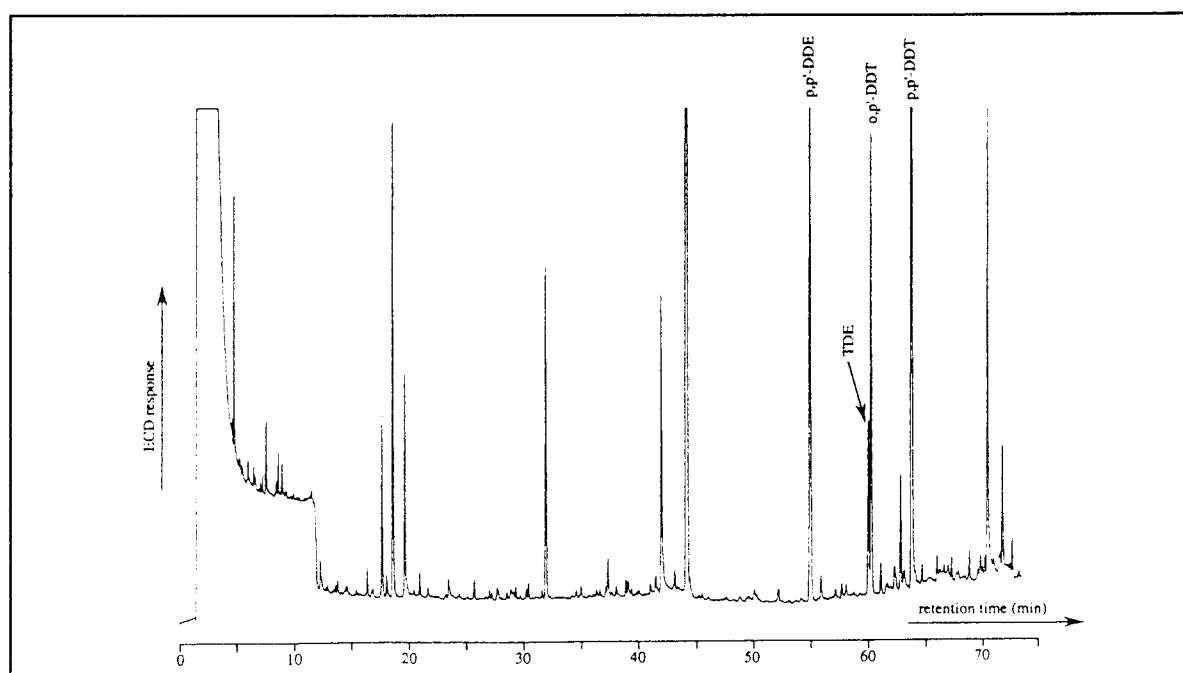

Figure 6 A typical GC-ECD chromatogram of organochlorine pesticides in orchard soil using final SFE conditions (for chromatographic conditions see Materials and Methods).

Table 9 Comparison field samples with liquid extraction and SFE using different extraction times for field samples (concentrations are not corrected for recoveries).

component	grass land						agriculture land						orchard soil					
	LLE (n=4)		SFE 10/20 (n=3)		SFE 30 (n=3)		LLE (n=4)		SFE 10/20 (n=3)		SFE 30 (n=3)		LLE (n=2)		SFE 10/20 (n=2)		SFE 30 (n=2)	
	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD
HCB	-	-	-	-	-	-	6.4	0,1	6.0	0.6	5.8	0.4	-	-	-	-	-	-
p,p'-DDE	4.1	0.3	4.4	0.4	5.5	1.1	1.1	0,0	1.6	0.2	1.5	0.2	63.1	-	50.3	-	56.2	-
TDE	6.1	0.5	0.8	0.2	1.7	0.4	-	-	-	-	-	-	12.2	-	12.8	-	20.1	-
o,p'-DDT	2.3	0.2	3.0	0.5	4.0	1.0	0.6	0,1	1.1	0.2	1.0	0.2	44.8	-	37.7	-	43.7	-
p,p'-DDT	11.4	1.7	21.9	2.4	29.7	4.6	-	-	5.5	0.4	6.1	0.3	260.6	-	236.8	-	296.4	-

SFE conditions: 20 Mpa; 50 °C; CO2; 10 min static and 20 min dynamic (SFE 10/20) or
30 min dynamic (SFE 30) extraction times; flow ca. 160 µl/min.
(-) not detectable (0.5 ng/g)

Table 10 SFE with addition of different modifiers on orchard soil.

component	SFE 30						SFE 10/20	
	toluene (n=3)		acetonitrile (n=3)		methanol (n=6)		methanol (n=3)	
	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD
p,p'-DDE	48.2	2.1	68.4	5.7	72.0	2.9	59.5	0.6
TDE	24.5	0.7	32.9	1.6	20.6	0.8	16.9	0.3
o,p'-DDT	41.3	2.4	57.2	3.9	61.8	1.5	52.5	0.9
p,p'-DDT	224	11	325	19	355	8	353	48

SFE conditions: 20 Mpa; 50 °C; CO2;
20 µL modifier; flow ca. 160 µl/min.

Table 11 Influence of the amount of modifier (MeOH) on orchard soil.

component	5 µl MeOH 2.4 % (#) (n=3)		10 µl MeOH 4.8 5 (n=3)		15 µl MeOH 7.1 % (n=3)		20 µl MeOH 9.5 % (n=6)		25 µl MeOH 11.9 % (n=3)		30 µl MeOH 14.3 % (n=3)	
	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD	ng/g	SD
p,p'-DDE	53.9	1.1	55.9	2.3	63.3	4.7	72.0	2.9	61.3	5.2	65.3	1.0
TDE	17.1	0.5	17.1	0.4	16.6	0.5	20.6	0.8	16.3	0.6	17.3	0.2
o,p'-DDT	42.9	1.0	44.5	1.9	52.3	3.6	61.8	1.5	51.8	5.0	56.6	0.8
p,p'-DDT	250	3	261	9	311	22	355	8	315	23	344	1

SFE conditions: 20 MPa; 50 °C; CO2; 30 min dynamic; flow ca. 160-180 µl/min.
(#) volume % of the modifier with respect to CO2, on basis of free cell volume.

Table 12 Influence of extraction pressure

components	20 MPa density 0,791 g/ml 160-180 µl/min (n=6)		25 MPa density 0,841 g/ml 190-210 µl/min (n=2)		30 MPa density 0,880 g/ml 230-250 µl/min (n=2)	
	ng/g	SD	ng/g	SD	ng/g	SD
p,p'-DDE	72.0	2.9	59.3	-	70.4	-
TDE	20.6	0.8	20.5	-	24.5	-
o,p'-DDT	61.8	1.5	49.7	-	57.6	-
p,p'-DDT	355	8	329	-	383	-

SFE conditions: 50 °C; CO2; 30 min dynamic;
addition of 20 µl MeOH; orchard soil

soil. The amount of 20 µl methanol added to the extraction cell gave the highest concentration of OCPs. This amount corresponds with a volume percentage of about 10 % with respect to CO_2 on basis of the free cell volume (total cell volume minus the sample volume). Probably, concentrations decrease by using higher amounts of modifier because the fluid is no longer supercritical above this value. Addition of modifier and using a combination of static and dynamic extraction times, does not give a better extraction yield, as can be seen in the last column of Table 10. This confirms the idea that the acting of the modifier is primarily the wetting of the matrix to facilitate the accessibility of the analytes and not an increasement of diffusion.

Finally, the influence of pressure was tested. Table 12 shows that an increase in pressure did not had any influence on concentrations and therefore further extractions were performed at 20 MPa to diminish co-extraction of matrix components. Second extractions were performed for several experiments described above, under the same extraction conditions, and no detectable amounts of components were found.

Reproducibilities of SFE experiments are good for p,p'-DDE, TDE and o,p'-DDT with standard deviations of 0.2 to 5.7 %, for p,p'-DDT standard deviations are higher, because of instability on GC-ECD. A typical chromatogram of SFE of OCPs in orchard soil is shown in Figure 6.

Some random soil samples were analysed using SFE with final conditions and solvent extraction. In Figure 7, the results of both methods are plotted. Assuming a concentration independent relative standard deviation between both methods, a pooled standard deviation of
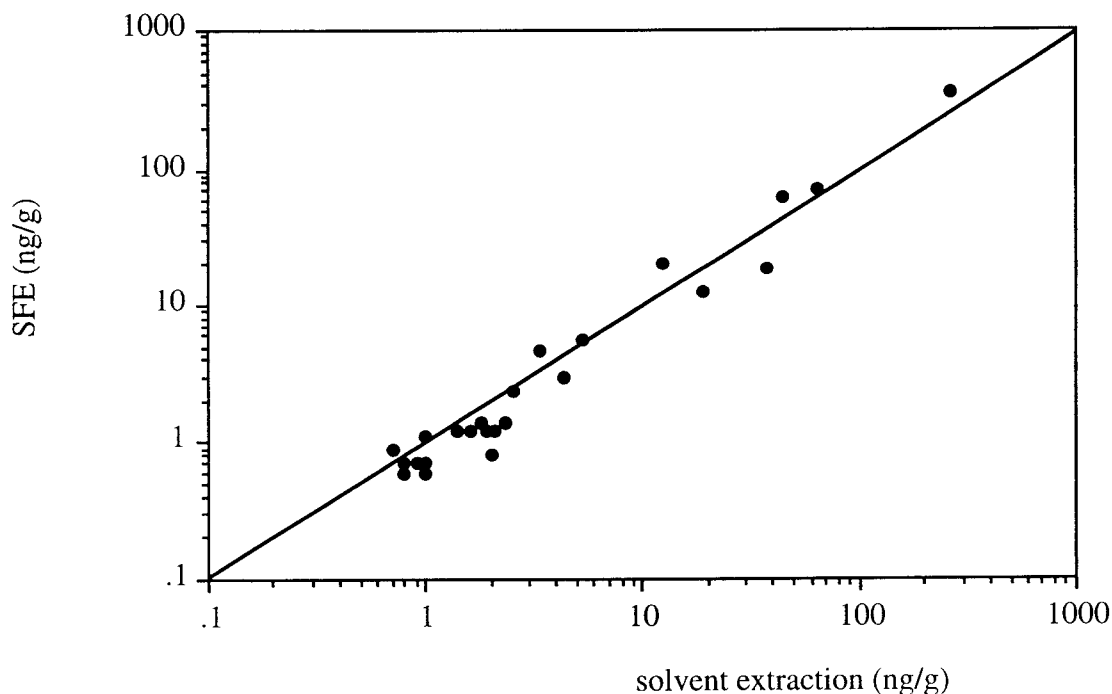

Figure 7. Correlation of SFE with solvent extraction for several field samples and components.

25 % was calculated for all soils, components and concentrations.

So, comparable results were obtained for SFE and solvent extraction. More soils have to be analysed to make a final comparison between LLE and SFE. The reproducibility of both methods is comparable (Table 9), the time needed for sample preparation is not faster using a non-automated SFE instrument, but the use of toxic and environmentally hazardous solvents is highly reduced.

3.4   Possibilities and restrictions in quantitative supercritical fluid extraction

The set-up of this study, using a stepwise approach for the optimization, offers several advantages. Information has been obtained about the relative importance of extraction parameters for these specific compounds, such as the solubility of the compounds by the chosen density, the transport in the SFE system and the collection method. By the next step, the spiking on the soil, the extraction of the compounds from the matrix can be studied. If no satisfactory results have been obtained in these steps, it is in principle useless to continue with SFE for this compound-matrix combination. On the other hand, it is important to avoid endless optimizations, because this process has to be repeated with the extraction of real soil samples, as described in this paper. The SFE of real samples shows that stronger extraction conditions are necessary, because compound-matrix interactions are different for real samples, probably resulting from bound residues.

A sequential as well as a statistical approach can be chosen for the optimization of extraction parameters for either spiked or real samples. The statistical approach is only appropriate, if it is known that SFE will be a suitable method for compounds and matrix. A statistical method offers the opportunity to study the effects of the different parameters with fewer experiments and to distinguish the interactions between parameters [20]. In practice, the sequential approach is applied more often, especially when chemometrical support is not available, and offers the opportunity to adjust parameters during the experiments, as is shown in this study. So, the choice of the approach has to be dependent on the specific SFE problem.

In overlooking our results and other publications in the field of SFE, a major break-through of SFE has not been reached yet, while comparable results with conventional methods have been reported. In our opinion, SFE suffers from the same problems as, for example, solvent and soxhlet extraction. It is difficult to achieve complete extraction from solid matrices like soil, because certain fractions of compounds will irreversibly bind to the matrix. Besides, in the case of soils, every soil sample is different in composition, requiring specific optimizations and determinations of recovery. However, in conventional techniques these aspects have been accepted and endless optimizations for complete extraction yields have been dropped. In using a new technique like SFE, a solution for these problems and also higher extraction efficiencies have been expected. At this moment, SFE is not ready to offer this solution, rather than an approximation of the results of conventional techniques.

3.5   Off-line versus on-line SFE

In continuing with SFE, either with our present instrumentation or regarding the purchase of new equipment, the choice has to be made for off-line versus on-line SFE. On-line SFE offers theoretically the opportunities in less sample handling and direct transportation of the sample to the GC-ECD without losses, offering greater sensitivity. However, good homogenization of samples is necessary because of the small sample sizes, an extra clean-up is not possible and the interfacing also has to be optimized. In practice, problems arise in obtaining clean backgrounds in combination with SFE-GC/ECD.

So, it was decided to continue with off-line SFE, offering a restriction in parameters in comparison with on-line SFE, the possibility of an extra clean-up or second injection and larger sample sizes. Nevertheless, the present instrumentation is not suitable anymore, the oven is too small for the larger samples needed for off-line SFE (upto 10 grams) and the new type of variable restrictors cannot be implemented in the system. Furthermore, automation of SFE - a must in developing applications and more routine analysis - is not possible. The purchase or loan of a new generation of off-line SFE instrumentation with variable restrictors and adsorbent trapping or solvent trapping will be explored for the next developments.

4. CONCLUSIONS

Obtaining of knowledge and practical experience takes a lot of time for a relatively new technique like supercritical fluid extraction. The physical and chemical behaviour of a supercritical fluid and the many extraction parameters makes it a difficult technique to start with and many starting problems concerning initial conditions, clogging of restrictors and instrumental problems have to be sustained. Worldwide - and also in our laboratory - can be established, that in the beginning too much attention is paid to spiked samples. But especially for SFE, difficulties and further optimization of extraction conditions arise by the extraction of real soil samples.

In this study, method development for SFE of OCPs in field samples has been performed using a general stepwise approach for quantitative SFE, starting with spiking on glass beads to establish initial conditions, followed by spiking on different kinds of soil to further adapt SFE parameters. As was expected, the conditions were not directly suitable for real soil samples. The component-matrix interactions in real samples behave different with respect to spiked samples as can be seen in adaptations in extraction times and the effects of modifiers. The stepwise approach gives a deeper understanding in the parameters which are important in development of quantitative SFE.

Finally, comparable results were obtained for SFE and solvent extraction. But in our opinion, SFE has the same difficulties as other extraction techniques in order to achieve complete extraction from solid matrices like soil, because certain fractions of compounds will irreversibly bind to the matrix and because of the large variety between the composition of soil samples. In conventional techniques these aspects have been accepted and endless optimizations for complete extraction yields have been dropped. As technique, SFE is not ready yet to overcome the general problems in the extraction of soil samples, rather than an approximation of the results of conventional techniques.

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Table 2. Influence of varations in pressure on extraction recoveries (%).

Component	Pressure			
	15 MPa	20 MPa	25 MPa	30 MPa
a-HCH	39	55	53	78
HCB	32	37		58
b-HCH	83	91	96	98
g-HCH	54	64	54	75
b-HEPO	83	91	91	97
pp-DDE	89	106	105	105
dieldrin	85	96	101	105
TDE	85	96	102	110
op-DDT	96	107	104	108
pp-DDT	99	106	103	112
PCB 28	72	78	77	88
PCB 52	79	88	89	94
PCB 101	89	97	103	105
PCB 118	92	99	103	109
PCB 153	88	105	105	109
PCB 138	88	101	105	114
Mean	78	89	93	98

SFE conditions: 50 °C, 30 min. extraction.

Table 3. Influence of extraction times (static/dynamic in minutes) on extraction recoveries (%).

Component	Extraction time (static / dynamic) (min)		
	(5 / 10)	(5 / 15)	(10 / 20)
a-HCH	59,3	32,1	54,7
HCB	51,4	-	37,2
b-HCH	56,5	83,2	90,8
g-HCH	67,0	41,4	63,8
b-HEPO	73,6	79,7	91,3
pp-DDE	56,8	90,7	105,5
dieldrin	62,9	90,1	96,2
TDE	57,3	84,9	96,1
op-DDT	53,3	91,6	107,2
pp-DDT	51,7	88,2	105,8
PCB 28	72,4	60,6	77,6
PCB 52	72,0	76,2	87,8
PCB 101	52,6	92,3	97,4
PCB 118	41,2	86,8	99,1
PCB 153	44,4	89,5	105,1
PCB 138	39,0	88,9	101,4
mean	57,0	73,5	88,6

SFE conditions : 50 °C, 20 MPa.