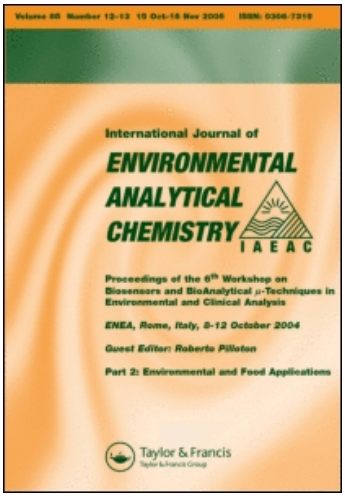

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Seidel, V. and Lindner, W.(1995) 'Evaluation of a Supercritical Fluid Extraction Method for Hexachlorobenzene from Artificially Spiked and Naturally Contaminated Oil Seeds and Soil Samples', International Journal of Environmental Analytical Chemistry, 59: 1, 1 — 13
To link to this Article: DOI: 10.1080/03067319508027631
URL: http://dx.doi.org/10.1080/03067319508027631

Intern. J. Environ. Anal. Chem., Vol. 59, pp. 1–13
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EVALUATION OF A SUPERCRITICAL FLUID EXTRACTION METHOD FOR HEXACHLOROBENZENE FROM ARTIFICIALLY SPIKED AND NATURALLY CONTAMINATED OIL SEEDS AND SOIL SAMPLES

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(Received, 25 April 1994; in final form, 1 July 1994)

Great differences in the supercritical fluid extraction (SFE) yield of hexachlorobenzene (HCB) in artificially spiked and naturally contaminated soil samples have been found. Extraction methods optimized for organochlorine pesticides (OCP's) in spiked samples yielding 98% recovery for HCB, failed in terms of sufficient recovery from real samples. The extraction yield dropped to 50% for HCB and, consequently, resulted in an unacceptable high variance of the results obtained (R.S.D. > 10%). Therefore, a procedure for the optimization of SFE parameters including carbon dioxide (CO_2) density, extraction temperature and addition of organic modifiers for reliable HCB determination in real samples is reported. A nearly complete recovery of HCB (98%) was obtained for 0.80 g/ml CO_2, 80°C, 15 min and the addition of 5% methanol.

Direct injection of SFE-extracts from soil into GC-ECD could be achieved after selectively trapping the analytes on minicolumns filled with C18 coated silica material and elution with petroleum ether.

For reliable GC-ECD determination of OCP's in oil seeds an additional cleanup step via solid phase extraction (SPE) and matrix degradation with sulfuric acid using newly developed "sandwich"-type adsorption columns was performed off-line or in-line to the SFE procedure.

KEY WORDS: Supercritical fluid extraction, hexachlorobenzene, organochlorine pesticides, soil, oil seeds.

INTRODUCTION

Hexachlorobenzene (HCB), a fungicide and industrial waste product in the manufacture of pesticides[1], has been found among other organochlorine pesticides (OCP's) in all compartiments of the Austrian terrestrial ecological system and can be a major contaminant in Austrian food[2–5]. Because of their lipophilic properties and bioaccumulative persistence, there is a strong demand for elucidation of the environmental fate and the possible sources of OCP uptake into the biological food chain. For monitoring trace amounts of OCP's in single compartiments such as agricultural soil, fatty plant material, oil seeds, animal tissues or human milk, effective extraction and sample pretreatment techniques have been applied, including soxhlet extraction (SE),

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sonication extraction[6], clean-up and concentration by solid phase extraction (SPE)[7], gel permeation chromatography (GPC)[8] and other chromatographic methods [9].

Breaking of the strong OCP adsorption on sites within the sample matrix and the accurate separation of the lipid-soluble analytes from the co-extracted fatty matrix material have been recognized as crucial parameters for effective extraction and clean up. Often, compromises are made for these two parameters, which has to be paid for by either low recovery or various chromatographic interferences in the course of gas chromatographic (GC) separation and final electron capture detection (ECD) of the OCP's.

Further drawbacks of some conventional techniques are: they are laborious, time consuming, create problems with hazardous waste disposal and involve purification steps that might be degrading not only towards the matrix compounds but also to the analytes[10,11].

To overcome these problems "advanced" enrichment and purification techniques have been developed for OCP determination, such as simultaneous steam distillation-solvent extraction (SDE)[12–14] and supercritical fluid extraction (SFE)[15–18].

Especially, SFE has been reported to be superior in extraction efficiency of OCP's[19], PCB's[20,21], dioxins[22] and pesticides[23,24], compared to the classical sonication extraction and soxhlet extraction methods. Most of the time, SFE was reported to be faster, more convenient and to possess several benefits in terms of costs and hazardous waste disposal due to the small amounts of solvents which are necessary. However, SFE of naturally contaminated samples[24] or SFE of more complex predominatly fat containing matrices[25,26] has only been rarely reported.

In this study we report on the optimization of SFE parameters for extraction of HCB from a highly adsorptive soil matrix by comparing also the extraction yields for artificially spiked and real samples. Secondly, a new and effective clean-up technique for the GC-ECD determination of HCB in pumpkin seeds (as a peculiar complex oleaginous matrix), which can be performed off-line by a recently developed "sandwich"-type solid phase extraction (SPE) column or in-line within the SFE chamber, is reported.

EXPERIMENTAL

Instrumentation

GC-apparatus. All analyses were performed using a Hewlett Packard HP 5890A gas chromatograph equipped with a Ni^{63}-ECD and a fused-silica column (30 m x 0.25 mm I.D.) coated with 0.25 µm crossbonded™, 65% dimethyl-35% diphenyl-polysiloxane (RTX-35, Restec Corp.). The carrier-and make-up gas was nitrogen at 18 p.s.i. (125 kPa) column headpressure. A 1-µl volume of the sample was injected using an HP 7673 A autosampler into a glass-lined capillary inlet in the splitless mode, with a split delay of 60 s. The temperatures of the injector and detector were 290°C and 350°C, respectively. The oven temperature was held at 60°C for 1 min followed by temperature programming to 220°C at 20°/min, and then to 230°C at 3°/min and to 290°C at 6°/min, with a final hold at 290°C for 2 min. A HP Chem-Station 5895 A was employed for data storage and integration. All quantitations were achieved using 100 ng/ml pentachlorobenzene (PCB) as an internal standard in order to compensate for variations in precision of injection.

SFE-apparatus. All supercritical fluid extractions were performed using a HP 7680A SFE unit (Hewlett Packard GmbH, Vienna, Austria) equipped with a 7 ml extraction

chamber, a heatable nozzle for CO_2 depressurization and an analyte trap (total volume 1.0 ml) filled with C18 coated silica (Hypersil ODS, 30 µm) material.

SDE device. SDE was accomplished in the mode of simultaneous steam distillation-solvent extraction rendering analyte extraction with water steam combined with an on-line organic solvent extraction in the gas phase. All technical details concerning the design of the SDE apparatus are reported elsewhere[14].

Soxhlet. For SE a conventional soxhlet device was used equipped with a 100 ml cartridge and a 250 ml extraction-solvent vessel.

Chemicals

Standards and reagents. The carbon dioxide used for SFE was of "SFE-grade" quality obtained from a special "middle fraction" form Linde GmbH (Vienna, Austria). CO_2 used as coolant for the nozzle was of technical grade from Linde GmbH (Vienna, Austria). All solvents (methanol, ethanol, acetonitrile) were of Pestanal® quality from Merck (Darmstadt, FRG). Petroleum ether (40 – 60°C) was of Pestanal® quality from Riedel de Haen (Seelze, FRG). Pesticide standards, mix IV (α-HCH, β-HCH, γ-HCH = lindane, HCB, heptachlor, heptachlor epoxide, endosulfan I) and mix V (2.4'-DDE, 2.4'-DDT, 4.4'-DDE, 4.4'-DDT, dieldrin), 1 ng/µl each in cyclohexane; PCB and all single standard compounds (10 ng/µl each in cyclohexane) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, FRG).

Adsorption materials used for SPE clean up were C18-coated silica from Hewlett Packard, Extrelut (a polar macroporous magnesia-silicate material from Merck) and Florisil (15% MgO, 84% SiO_2, < 1% Na_2SO_4, surface area 298 m^2/g, 0.15–0.25 mm, 60–100 mesh) from Serva (Heidelberg, FRG).

Sample material

Soil samples: About 1 kg of soil representing a mixture of at least 30 randomly taken subsamples from each agricultural field was collected. The sample was dried at 35°C to 2% humidity, then finely ground and sieved to obtain a grain size below 2 mm. The content of organic carbon, generally regarded as an index for the adsorptive properties of a soil matrix, was determined to be between 3 and 6.5%.

Spiked soil samples were prepared by adding a 1-ml volume of an accordingly diluted pesticide standard mixture in cyclohexane to a 100-g amount of ground soil. To provide good homogenity the spiked soil was then thumbled for 2 hours and dried at 35°C to evaporate the cyclohexane.

Pumpkin seeds, representing a typical oil seed sample, were investigated as a peculiar oleaginous matrix due to their economical importance in Austria. Pumpkin seeds also contain a high content of various wax-alcohols, -esters, fatty acids, glycerides and chlorophyll.

Methods

Soxhlet extraction procedure (SE): A 10 g aliquot of the previously described ground soil sample or 10 g of pumpkin seed granulate was placed in a Soxhlet extraction cartridge, spiked with 10 µl PCB solution in cyclohexane as internal standard (final concentration 10 ppb in soil), mixed with 10 g anhydrous sodium sulfate, and extracted with 180 ml petrolbenzine by a 4 h Soxhlet-extraction. After cooling, the extract was concentrated to 1 ml by gentle rotary evaporation at ambient temperature. Oleaginous extracts were concentrated to about 8 ml, transferred to a calibrated vial and filled up to 10 ml with petrolbenzine. This solution can be stored at 5°C for several days [11].

Solid phase extraction (SPE) clean up: A 1 ml aliquot of the obtained soil extract was subjected to a further clean up by solid phase extraction (SPE) on Florisil minicolumns[11]. Oleaginous plant extracts (1 ml aliquot or 1 ml of vegetable oils) were purified on a new SPE sandwich-type extraction column filled with different adsorption layers of 4 g Na_2SO_4, 1.5 g Extrelut soaked with 1.0 ml concentrated sulfuric acid as a triglyceride decomposing additive and at the top 1.5 g Florisil[11]. SPE and SPE sandwich-type columns, respectively, were rinsed with 2 x 10 ml of the upper phase of a two layer system of petroleum ether : acetonitrile : ethanol = 100 : 25 : 5, whereby only the adsorbed OCP's were eluted. The total eluate was collected in a conical vial and concentrated by a gentle stream of nitrogen at room temperature to 1 ml.

Steam distillation-solvent extraction: An aliquot of 100 g of the finely ground soil samples was weighed into the water steam extraction chamber (1 l round bottom flask), spiked with 100 µl PCB solution in cyclohexane as internal standard (final concentration 10 ng/g in soil) and wetted with 20 ml tap water and 10 ml pure ethanol followed by ultrasonication for 1 min. After filling the U-shape separation chamber in the center part of the SDE-device with tap water, and the small conical-tapered vessel (50 ml) with the extraction solvent (e.g. petroleum ether, b.p. 40–60°C), the apparatus was initially filled with organic vapour by heating the vessel in a water bath at 70°C. Subsequently the steam, generated and flow controlled by a separate steam generator, was blown through the soil sample until the sample flask was filled with about 700 ml of condensed water (using a 1000 ml flask A, the effective extraction time was 1 hour). For trace analysis the final petroleum ether extract (20 ml) was concentrated down to 1 ml by means of a Kuderna-Danish type concentrator, which enabled an effective solvent evaporation with minimum loss of highly volatile compounds[27].

Optimized parameters for supercritical fluid extraction (SFE) of HCB from soil: Five grams of finely ground and dry soil (or 0.5 g pumkin seed granulate) was spiked with 5 µl of a PCB solution in cyclohexane as internal standard (final concentration 10 ppb in soil) and mixed with 2.5 g anhydrous sodium sulfate, 350 µl methanol was added as a modifier by injecting this amount directly into the sample matrix previously put into the 7 ml extraction chamber of the SFE apparatus. The extraction procedure was performed at 80°C and 365 bar (CO_2 density of 0.8 g/ml) in a static mode for 15 min, followed by dynamical extraction over 5 min at a flow rate of CO_2 of 2.4 ml/min. The temperature setting of the restrictor nozzle was set to 80°C to prevent from plugging. The extract was automatically trapped on a reversed phase (C18) minicolumn at 45°C. After evaporation of the carbon dioxide, the temperature of the nozzle and the trap were set to 30°C and 15°C, respectively, and the adsorbed pesticides were eluted with 1 ml petroleum ether.

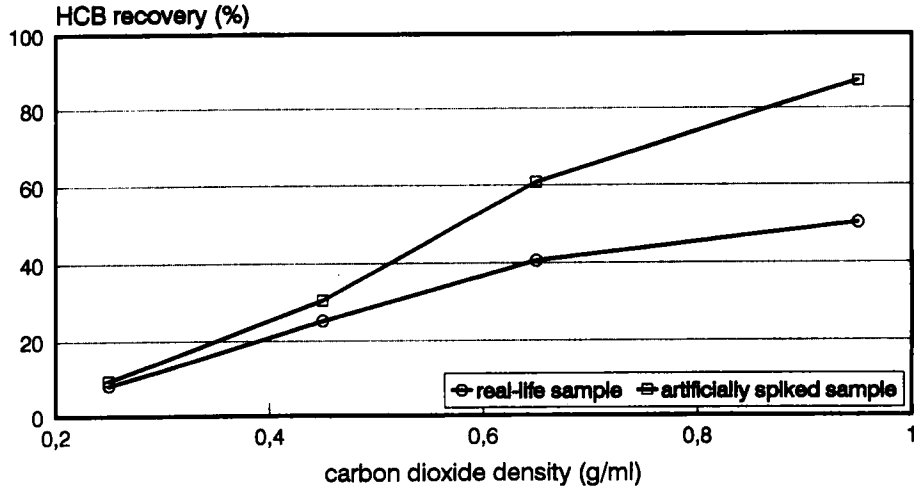

Figure 1 Comparison of HCB recovery with SFE from 5 g soil (≤ 2% water content) artificially spiked with 10 ppb HCB and naturally contaminated soil also containing 10 ng/g HCB refered to a SDE method. This value was arbitrary set to 100%. Experimental parameters: as given in the methods section in the text except extraction temp. 40°C; nozzle temp. 45°C; variable CO_2 density (0.25–0.95 g/ml); variable pressure (77–383 bar); without organic modifier.

RESULTS AND DISCUSSION

Extraction efficiency of SFE for HCB in soil compared to conventional techniques (SE, SDE).

The main intention of this work was to overcome the unacceptable low recovery values of HCB using SFE for real samples and setting SFE parameters initially optimized for spiked and therefore "artificial" samples. This was especially evaluated for the recovery rates of HCB from soil samples.

HCB showed a great tendency for adsorption onto lipophilic sites in agricultural soil with a high carbon content. Therefore, efficient HCB extraction was only possible by increasing the solubility strength of the supercritical fluid. This was performed in a first attempt by increasing the CO_2 density up to 0.95 g/ml at 40°C by using elevated pressure settings of nearly 400 bar (which was also the limit of the used extraction device). The results from SFE-extraction followed by the GC-ECD analysis of soil samples spiked with 100 ng/g HCB revealed a nearly complete recovery of 98.5% with a good relative standard deviation (R.S.D.) of 2.3%, compared to external standard solutions injected separately into the GC-ECD system. But, using this parameter settings, the SFE-GC-ECD determinations of a real soil sample contaminated with 100 ng/g HCB yielded only 50% of the results obtained by the alternatively used SDE-GC-ECD method. The R.S.D. of 12.6% was also unacceptably high. Figure 1 demonstrates the great difference in recovery of HCB from spiked and naturally contaminated soil in relation with the CO_2 density.

For more efficient HCB extraction further SFE parameters, besides the CO_2 density, had to be optimized. It should be stressed that all subsequent results presented in this work have been obtained by employing naturally contaminated sample material. The improvement of extraction efficiency up to 75% compared to the SDE determination by using elevated temperatures up to 80°C is depicted in Figure 2. The positive effect of temperature rise can be explained by the higher volatility and the enhanced diffusion

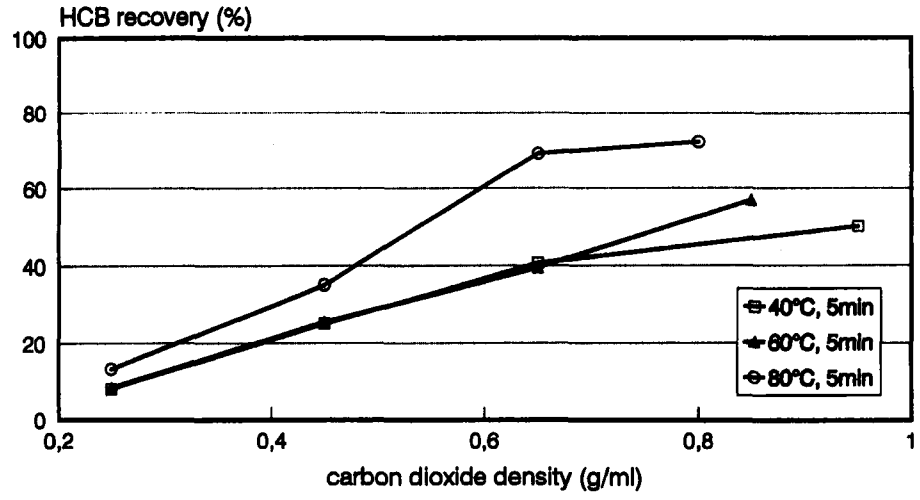

Figure 2 HCB recovery with SFE from 5 g soil (≤ 2% water content) naturally contaminated with 10 ppb as a function of the carbon dioxide density (g/ml) at various extraction temperatures: □ 40°C extraction temp., pressure 77–383 bar, CO_2 density (0.25–0.95 g/ml); Δ 60°C extraction temp., pressure 93–329 bar, CO_2 density (0.25–0.85 g/ml); ○ 80°C extraction temp., pressure 108–365 bar, CO_2 density (0.25–0.80 g/ml). Further experimental parameters: as given in the methods section in the text. The nozzle temperature was set 5° above the extraction temperature.

velocity of HCB and CO_2 at these conditions. In order to maintain the supercritical status of the CO_2, the temperature could not be further increased without exceeding the pressure limit of the SFE apparatus.

When working at high temperatures (80°C) within the whole SFE process, there is a potential risk of substance loss by insufficient trapping of the highly volatile analytes (e.g. HCB) onto the SPE column after depressurization of the extraction solvent. Therefore, an efficient cooling of the subsequent SPE-type analyte-trap down to 65°C by depressurization of technical grade CO_2 should be provided. At a trap temperature of 80°C a decrease of HCB recovery of 25% has been observed. But, the temperature setting for the restrictor should not be below 65°C to prevent first from plugging of the nozzle and second from prematurely elution of HCB from the trap with the condensed methanol. The final elution of HCB from the trap with petroleum ether (b.p. 40–60°C), however, requires low temperature settings of the nozzle and trap to 30°C and 15°C, respectively. This has become evident by a 15% decrease in HCB recovery using a trap temperature of 30°C.

The time interval of the static extraction process, studied in the range from 2 to 60 min., showed to be optimal at 15 min., revealing a HCB recovery of 82%. By a further prolongation of extraction time a slight improvement of the R.S.D. from 3.5 to 2.9% was observed, but HCB recovery leveled off. In addition, a prolonged extraction time would demolish the strong argument of the speed of analysis propagated as one of the greatest advantages of SFE.

Any changes in the SFE parameter settings during the dynamic extraction period,when the extraction chamber is swept with CO_2 and the extracted analytes are transported to the restrictor, revealed to be irrelevant for HCB extractions. It was shown that a total extraction volume of 14 ml (twice the volume of the extraction chamber) and a flow rate of 2.4 ml/min gave satisfactory HCB recovery results.

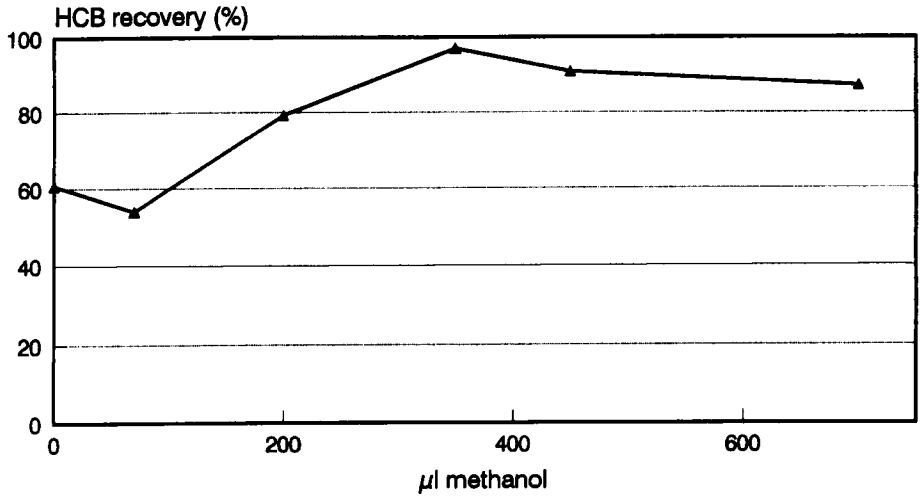

Figure 3 HCB recovery with SFE from 5 g soil (≤ 2% water content) naturally contaminated with 10 ng/g using different amounts of methanol as a polarity modifier added at once into the sample matrix in the extraction chamber (7 ml volume). Experimental parameters: as given in the methods section in the text except extraction temp. 60°C; nozzle temp. 65°C; pressure 329 bar; CO_2 density 0.85 g/ml.

Finally, the influence of the addition of organic modifiers to the extraction fluid has been studied. The effect of different µl-volumes of methanol added directly into the extraction chamber on the HCB finding is demonstrated in Figure 3. This effect was recognized to be twofold; first the organic modifier reduced the analyte affinity for sorptive sites of the soil matrix and secondly increased the analyte solubility in the supercritical CO_2. However, the slight change of the CO_2 density caused by the modifier content was not corrected throughout this work. By the addition of 5% methanol (corresponding to a 350 µl absolute volume when using an extraction chamber of 7 ml) the extraction efficiency could be finally increased up to 98% HCB, compared to the reference SDE determination. Exceeding a modifier content of 10% lead to a dramatic loss of HCB recovery due to a cancellation of the supercritical status. The application of modifiers also allowed to obtain similar results for less drastic conditions (95% HCB for CO_2 with 5% methanol at 40°C and 365 bar).

Table 1 shows the SFE recovery and precision data in relation to efficiency and overall process time compared to the classical sample preparation techniques such as soxhlet extraction (SE) and steam distillation-solvent extraction (SDE), revealing better performance for SDE and SFE than for the most commonly used SE method. SFE, however, seemed to be superior in terms of potential for automation.

For reasons of completeness the extraction yields of selected OCP's (EPA methods 608 and 612[28]) using variable CO_2 densities at 80°C is given in Figure 4. The authors wish to emphasize that the results given in % of maximum recovery acquired at highest density are derived from an agricultural soil sample artificially spiked with 100 ng/g of each pesticide. However, from the results obtained with spiked and real-life HCB samples the relevance of recovery data from artificial samples has to be relativized.

The supercritical fluid extracts of naturally contaminated soils obtained at conditions optimized for a maximum HCB recovery followed by adsorption and desorption from a C18-SPE-trap (see experimental section) were suitable for direct injection into GC-ECD

Table 1 Comparison of general processing parameters and the corresponding extraction / purification efficiency in terms of recovery and precision of HCB as a natural contaminant in agricultural soil and oil seeds using SE, SDE and SFE.

Process parameter	*SE*		*SDE*		*SFE*	
	soil	*oil seed*	*soil*	*oil seed*	*soil*	*oil seed*
Recovery (%)[a] ± RSD	55 ± 7.5	92 ± 5.5	100 ± 3.0	85 ± 8.0	98 ± 2.9	90 ± 5.8
Process time	4 h	4 h	60 min	60 min	25 min	25 min
Clean up	SPE/ Florisil	SPE/ sandwich/[b]	not required	not required	not required	SPE/ sandwich
Clean up time (min)	45	45				45
Solvent evapn. time (min)	20	20	30	30	5	5
Overall analysis time (h)	6	6	1.5	1.5	0.5	1.3
Max sample amount (g)	100	10	100	1	5	0.5
LOD[c] (ng/g)	0.5	2	0.05	2	0.1	2

a: naturally contaminated soil: the result of the SDE determination revealing 10 ppb HCB was arbitrary set to 100%. oil seeds: a sample spiked with 100 ppb HCB was used.
b: Florisil, Extrelut soaked with conc. sulfuric acid, sodium sulfate (see experimental).
c: limit of determination for a signal to noise ratio (S/N) of 6:1

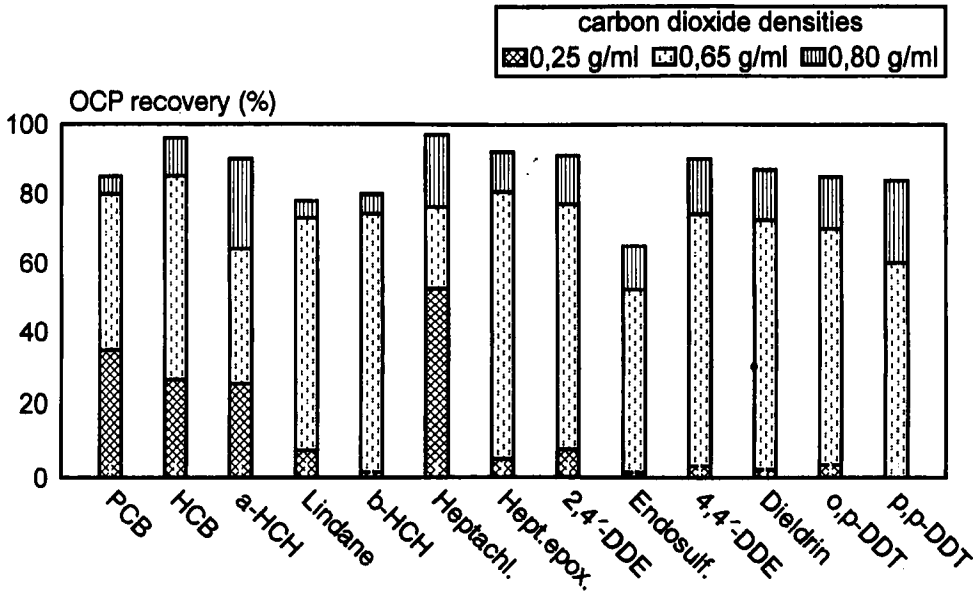

Figure 4 Recovery of OCP's with SFE from 1 g pumpkin seed granulate, spiked with 100 ng/g of each pesticide, using different carbon dioxide densities. Experimental parameters: as given in the methods section in the text at variable CO_2 densities (0.25–0.08 g/ml), pressure 108–365 bar, extraction temp. 80°C, nozzle temp. 80°C, without modifier.

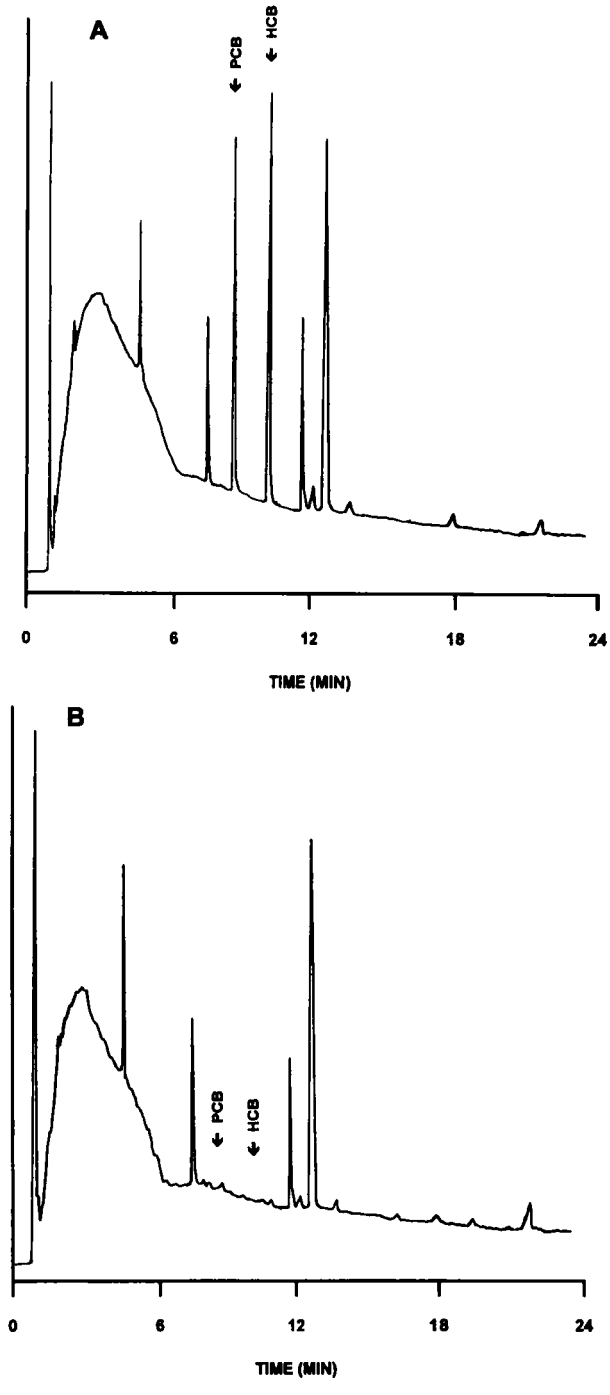

Figure 5 (A) GC-ECD chromatogram of an SFE extract of 5 g soil naturally contaminated with 10 ppb HCB. 10 ng/g PCB were added as internal standard. (B) GC-ECD chromatogram of a blank SFE-extract. SFE-parameters as given in the methods section (extraction temp. 80°C, pressure 365 bar, CO_2 density 0.80 g/ml, addition of 350 µl methanol) GC-conditions see instrumentation section in the text.

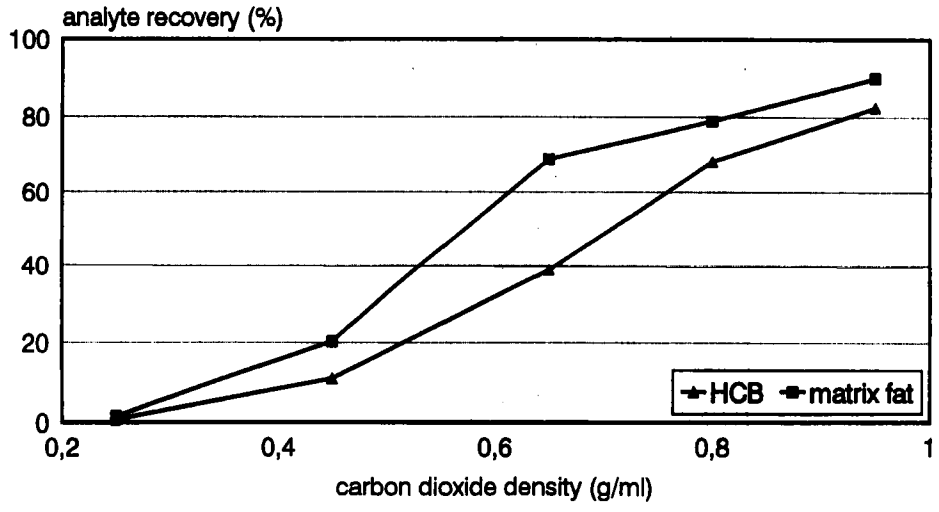

Figure 6 Comparison of recovery of HCB and co-extracted matrix compounds after SFE of 0.5 g pumpkin seed granulate contaminated with 100 ng/g HCB using different CO_2 densities (0.25–0.95 g/ml); pressure 77–383 bar; extraction temp. 40 °C; without organic modifier. Futher experimental parameters: as given in the methods section in the text.

(see chromatogram in Figure 5). The lack of any disturbing interferences in the retention time window of HCB and the internal standard PCB allowed a determination limit of 0.1 ng/g HCB in soil. However, this was only true when the analyte trap was rinsed with petroleum ether instead of more polar methanol. Thus, a selective elution of the relatively unpolar organochlorine pesticides was performed, whereas the co-extracted but slightly more polar matrix interferences were sufficiently retained. It has to be emphasized that for reliable HCB analysis via SFE sample pretreatment it was essential to compensate the lack of selectivity in recovery-optimized SFE by the introduction of an additional selective clean-up step performed on the incorporated trap filled with C18 material.

SFE and sample pretreatment of fatty samples

A second aim of this work was to evaluate recovery and selectivity properties of SFE for HCB as a "natural" contaminant in predominantly fat containing matrices as e.g. oil seeds, in particular pumpkin seeds. The results obtained indicate that maximum recovery for HCB and maximum selectivity of HCB extraction towards disturbing matrix interferences seem always to run counterpart to each other. Figure 6 illustrates the correlation of HCB recovery and the content of matrix compounds (triglycerides, fatty acids, wax alcohols etc.) versus the applied CO_2 density. The overall recovery of all extractable but undefined matrix compounds was determined by simply weighing the dry residue after complete evaporation of the petroleum ether eluent from the ODS trap column. It can be clearly derived from Figure 6 that a high HCB recovery always has to be paid for by a high accompanying content of oleaginous matrix components, which can deteriorate sensitive GC detectors and capillaries and/or lead to false positive results by various chromatographic interferences.

For SFE of the particular pumpkin seed matrix the selectivity introduced by selective adsorption of the CO_2 extract and selective desorption of the analytes from the C18-SPE-

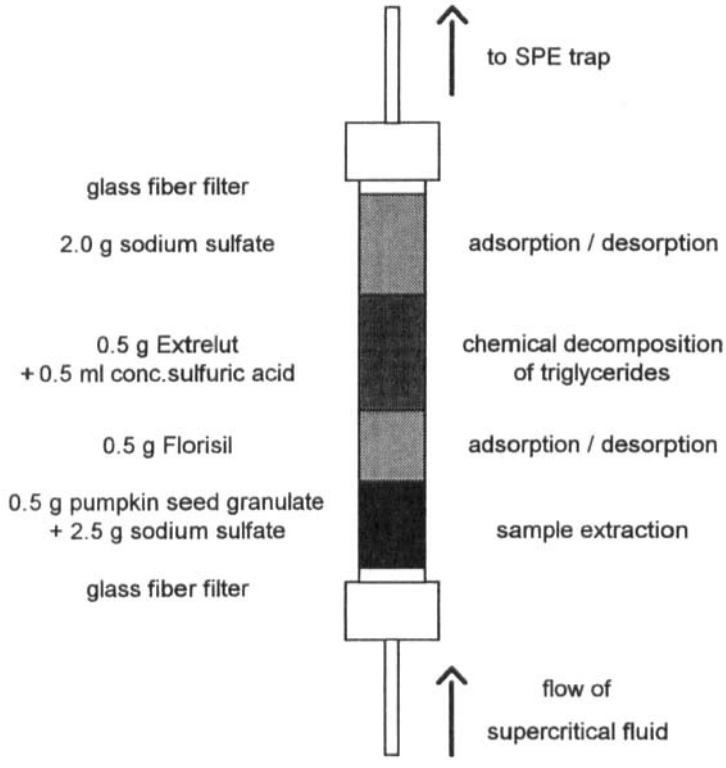

Figure 7 "Sandwich"-type extraction, adsorption and decomposition layers in the SFE-chamber performing SPE with supercritical CO_2 and matrix degradation with concentrated sulfuric acid on-line to the analyte extraction process. The total volume of the extraction chamber was 7 ml.

trap was sufficient up to maximum CO_2 density of 0.45 g/ml revealing "clean" solutions, ready for direct GC-ECD injection. However, the resulting R.S.D. for HCB determinations at a contamination level of 100 ng/g was relatively high (13.2%) due to the poor extraction yield of 45%.

Extracts obtained by the use of CO_2 of higher densities required an additional clean up step using SPE minicolumns prior to GC-ECD. We used tailor-made "sandwich"-type clean-up columns filled with different adsorption layers of 4 g Na_2SO_4, 1.5 g Extrelut soaked with 1.0 ml H_2SO_4 and at the top 1.5 g Florisil (see experimental section). This off-line combination of the superior extraction efficiency using supercritical CO_2 and a highly OCP-selective SPE clean-up step accomplished reliable trace analysis of HCB in GC-ECD routine analysis of oil seeds[11].

Since it would be beneficial to completely eliminate all error-prone manual manipulations and sample transfer steps during the course of sample pretreatment prior to GC-ECD, we also developed an in-line sample extraction-purification process in a closed system, preventing contamination and substance loss (recovery) problems. Therefore, we built up a sandwich-type adsorption system similar to the one used for off-line SPE directly in the SFE-chamber (Figure 7). Consequently, we obtained high extraction yield for HCB (90% at a 100 ng/g contamination level) and selectively decomposed and / or adsorbed all disturbing interferences in a single step. The efficiency of matrix degradation is demonstrated by the GC-ECD chromatogram (see Figure 8) obtained after SFE

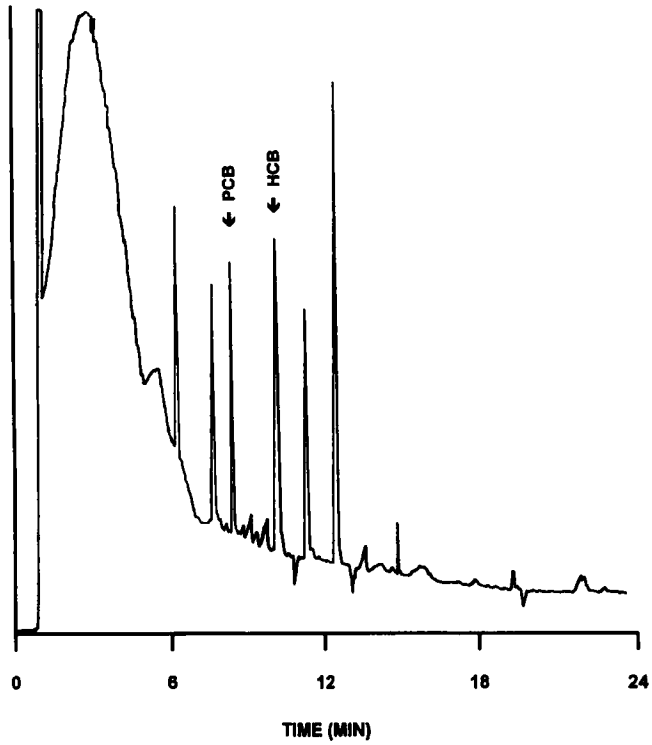

Figure 8 GC-ECD chromatogram of an extract from 0.5 g pumpkin seed granulate spiked with 50 ppb HCB after SFE and in-line "sandwich"-type SPE clean-up. 50 ppb PCB were added as internal standard. SFE-parameters as given in the methods section (extraction temp. 80°C, pressure 365 bar, CO_2 density 0.80 g/ml, addition of 350 µl methanol) GC-conditions see instrumentation section in the text.

and in-line SPE purification. Sample extraction and purification for GC-ECD analysis were performed within 30 min, but the reproducibility was slightly decreased (R.S.D. = 7.9%) compared to the one reported for off-line SPE clean-up (R.S.D. = 5.8%). However, these encouraging results could not be evaluated with respect to the long term reliability of this method, due to our limited access to the SFE apparatus. Anyhow, the long term stability of the SFE equipment (extraction chamber is made of stainless steel) towards the use of highly degradative concentrated sulfuric acid used together and in-line with supercritical fluid CO_2 should not be a problem but needs more testing.

CONCLUSIONS

From the results obtained in this work we can conclude that there should be a strong demand for adaptation of all methodical performance (recovery, precision and reproducibility) data derived from artificially spiked samples to real-life samples. The extraction efficiency of a well established SFE method for HCB determination in soil had to be adapted for reliable HCB determination in highly adsorptive soil matrices, without losing efficiency of sample purification. This could be achieved by combination of SFE and SPE on a reversed phase trap. However, the limit of this on-line SPE clean-up incorporated into the SFE device was clearly demonstrated for the application of

oleaginous matrix extracts e.g. of pumpkin seeds. Reliable GC-ECD determinations of OCP's in oil seeds were accomplished by the introduction of an additional clean-up procedure using the recently developed "sandwich"-type extraction columns. The performance of HCB extraction by SFE and efficient elimination of matrix interferences by the off-line combination with "sandwich"-type SPE clean-up has been evaluated and the potential for fully automation by in-line extraction-purification in a closed SFE-system could be demonstrated. In addition, the preliminary results reported for the new in-line combination of SFE, SPE and sulfuric acid treatment within the extraction chamber should also elucidate the possibility for an introduction of the dimension of "specific chemistry" into the SFE technique.

Acknowledgements

We gratefully acknowledge Hewlett Packard Ges.m.b.H. (Vienna, Austria) for the instalment of their SFE apparatus HP 7680A in our laboratories for a limited time.

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