

# Selectivity in Supercritical Fluid Extraction: Recovery of Pesticides from Model Matrices

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## Abstract

Selected pesticides are spiked in an empty extraction thimble for use as a model system to study the solvating power of supercritical carbon dioxide and spiked in solid-phase sorbent materials to study the influences of matrix effects on the recoveries. Results from this study show that it is possible to identify supercritical fluid extraction conditions where the molecular structure of the analytes affect the extraction efficiency. The results also indicate that parameters other than solubility (e.g., volatility) have a marked influence on the extraction process, even under simplistic conditions (extraction from empty thimble).

## Introduction

The monitoring of residues of pesticides and other substances of environmental interest is costly, time consuming, and in itself an activity of environmental concern. Large amounts of "ultrapure" organic solvents and other reagents are used in the extraction, cleanup, and detection of pollutants in environmental and food samples, exposing the environment and laboratory staff to hazardous chemicals. Thus, the urgent improvement of analytical procedures in order to decrease the use of organic solvents, reduce analysis time, and cut analytical costs is of top priority. To meet these goals, all types of new techniques are pursued in the pesticide residue laboratories.

Soxhlet and other conventional solvent extraction methods are time-consuming, labor-intensive, and require large volumes of organic solvents. An alternative technique is supercritical fluid extraction (SFE). Its main advantages are the replacement of organic solvents with environmentally acceptable supercritical fluids (such as carbon dioxide), high recovery, short extraction time, and selectivity in the extraction process. The SFE technique has been exploited for the quantitative extraction of organic compounds, such as pesticides from a variety of environmental matrices (1–3).

The selectivity in the SFE process offers considerable advantages, such as cleaner extracts than liquid solvent extractions that frequently contain large amounts of matrix organics. This reduces the need for cleanup and facilitates the quantitation of

target analytes. Such selective extractions of atrazine and three metabolites from soils, sediment, and plants were recently demonstrated by Papilloud et al. (4). Excluding a cleanup step minimizes both the analysis time and the number of error sources. The efficient extraction of an analyte by SFE has been described to be dependent on solubility in the extraction medium, transfer of analyte from the matrix to the supercritical phase (diffusion), and interactions with the matrix (5). High solubility in the supercritical phase by itself is thus no guarantee for efficient extraction. Some analytes with a high solubility in supercritical carbon dioxide may also be difficult to extract at trace levels.

Another, and perhaps less obvious, advantage of high selectivity in the extraction is the possibility of collecting information about the different binding mechanisms and interactions between analyte and matrix components. This relation between a successful extraction and matrix interaction was noted by Cheng (6), who suggested the use of combinations of solvents to exhaustively extract pesticides from environmental matrices. It is suggested that SFE offers better opportunities to study matrix interactions, because a large number of parameters can be varied continuously to achieve the necessary extraction conditions. SFE performed at moderate extraction conditions, in combination with NIR and multivariate methods, was used for the determination of pesticide sorption in soils (7). These results support the hypothesis that nonquantitative extraction conditions can be used to access the relative strength of pesticide–soil interactions. In addition, SFE has also been used to extract bound pesticide residues from aged soil samples (8,9). In these studies, harsh conditions involving supercritical methanol (8) and modified carbon dioxide (9) at high temperatures were used to extract the strongly matrix-bound pesticides, which was not accessible by the use of liquid solvent extraction.

To determine the influence of basic parameters (such as density/pressure and temperature) and instrumental characteristics on analyte recoveries, initial studies with supercritical carbon dioxide were performed (10). In order to facilitate studies of the influence of specific interactions between pesticides and matrix components, sets of analytes with a strong correlation between extraction conditions and recovery for matrices with known properties are desired. The addition of a standard solution directly to an empty extraction thimble has been used to compare extraction

performance for materials such as sand and filter paper (11) and for trapping studies of efficiency in order to eliminate matrix effects (12).

By studying the adsorption of a pesticide on a model matrix with well known properties (such as silica), information of different possible retention mechanisms for particular pesticides on soil can be determined (13). Hsieh and Chang (14) studied matrix influence (silica and XAD sorbents) on SFE efficiencies for PAH. They showed that chemical properties of the matrix significantly influenced the analyte–matrix interactions. In the present paper, a mixture of selected pesticides with different physical/chemical properties was used as a model system to study the influence of different SFE parameters and identify SFE conditions where sample matrix and molecular structure of the analyte affect the extraction efficiency. Initial studies were performed with supercritical CO<sub>2</sub> in the absence of a sample matrix (empty thimble), making the extraction success simply due to the interplay between analyte structure and the solvating power of the supercritical fluid. The influence of the interaction of pesticides with model matrices was then investigated.

## Experimental

### SFE Instrumentation

An ISCO (Lincoln, NE,) SFE System 2200 was used for all the extractions presented in this paper. The system included an SFX 2-10 dual-chamber extraction module equipped with two 2.5-mL volume extraction thimbles, two 260D 260-mL capacity syringe pumps, and a pump controller. At the outlet of the extractor, stainless steel tubing producing a flow of 1 mL/min at 60°C and 350 atm was used as a restrictor. The instrument was used in both static mode, during which a valve between the extraction thimble and the restrictor was closed, and in dynamic mode, during which the valve was opened.

### Chemicals and standards

Pesticide standards (Figure 1) were purchased as stock solutions from the National Food Administration (Uppsala, Sweden). A standard mixture solution containing 10–400 µg/mL of each of the 11 pesticides (bitertanol, 200 µg/mL; chloridazone, 200 µg/mL; p,p'-DDE, 10 µg/mL; dichlobenil, 10 µg/mL; dimethoate, 40 µg/mL; fenpropimorph, 400 µg/mL; hexazinone, 160 µg/mL; lindane, 10 µg/mL; linuron, 160 µg/mL; parathion-methyl, 90 µg/mL; triadimenol, 160 µg/mL) was prepared in acetone (p.a., LabScan Analytical Sciences). Cyclohexane (p.a., LabScan Analytical Sciences) and ethylacetate (p.a., LabScan Analytical Sciences) were used as collecting solvents. Methanol (p.a., Merck, Darmstadt, Germany) was used as modifier. Carbon dioxide (quality 4.8, AGA Specialty Gases, Maumee, OH) was used as the supercritical fluid. Seasand and silica were obtained from Merck. All chemicals and standards were used as purchased without further purification.

### Sample preparation

#### Empty thimble

A pesticide standard mixture (10 µL) was spiked directly onto the walls of an empty extraction thimble to avoid matrix effects, and the solvent was carefully evaporated prior to extraction to avoid modifier effects.

#### Model matrices

Sand and silica were spiked with pesticide standards by adding a standard mixture solution (as with the empty thimble) to each sorbent (100 µL/g). The sample was mixed on a shaker for 5 h, and the solvent was evaporated at room temperature for 12 h.

### SFE extraction

The restrictor was heated to 80°C with a restrictor heater to prevent plugging. Analytes were collected in 10-mL screw-cap vials containing 2 mL cyclohexane fortified with parathion-ethyl as an internal standard. The

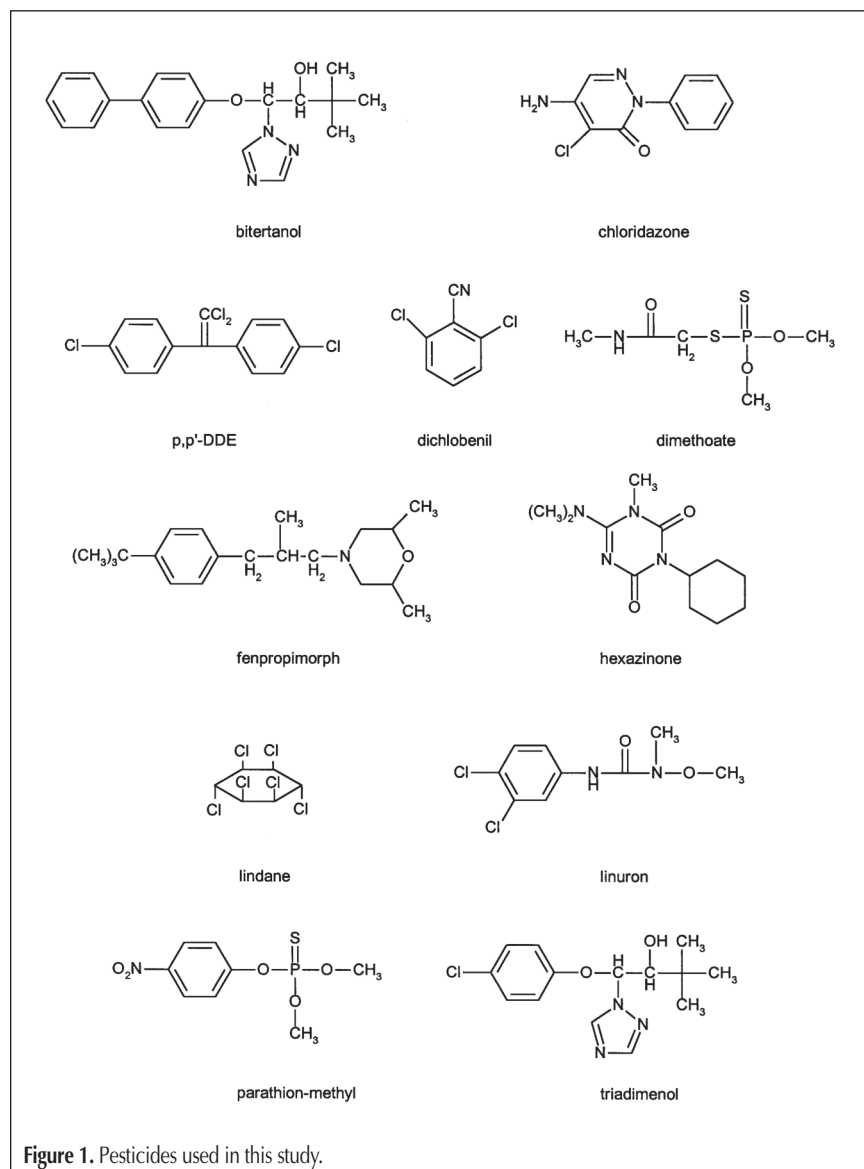


Figure 1. Pesticides used in this study.

vial was placed in a water bath at room temperature during the extraction to avoid ice formation.

#### Extraction from empty thimble

The SFE apparatus was used for extraction with pure carbon dioxide using the combinations of 5 densities (0.25, 0.40, 0.55, 0.70, and 0.85 g/mL) and 3 temperatures (40, 55, and 70°C). One minute of static extraction was followed by extraction with 10 mL CO<sub>2</sub> in the dynamic mode.

#### Extraction from model matrices

Fortified sand (1.0 g) and silica (0.6 g) were extracted with and without the addition of methanol as modifier (200 µL, added directly to the thimble prior to extraction) at 5 densities (0.25, 0.40, 0.55, 0.70, and 0.85 g/mL) and 55°C.

Sorbents were sandwiched between plugs of silanized glass wool to reduce the void volume. Five minutes of static extraction was followed by extraction with 20 mL CO<sub>2</sub> in the dynamic mode.

#### Gas chromatographic analysis

Off-line gas chromatographic (GC) analyses of the extracts were performed on 3 instruments. Nitrogen- or phosphorus-containing pesticides were analyzed on a Hewlett-Packard (Wilmington, DE) 5890 GC or a Varian (Varian Associates, Walnut Creek, CA) 3400 GC, both equipped with a split/splitless injector connected to two Chrompack open-tubular capillary columns (25-m × 0.32-mm-i.d. CP-Sil 5CB and CP-Sil 19CB with 0.25-µm and 0.2-µm film thicknesses, respectively) and two NP detectors; helium was used as the carrier gas (at 1 mL/min) and detector make up gas (at gas flows of 30 and 25 mL/min, respectively), and hydrogen (4 mL/min) and air (at gas flows of 80 and 175 mL/min, respectively) were used as detector gases.

Organochlorinated pesticides were analyzed on a Hewlett-Packard 5890 GC equipped with a split/splitless injector connected to two open-tubular capillary columns (SE-30, Quadrex, and DB-1701, J&W Scientific, Folsom, CA, both 25 m × 0.25-mm i.d., 0.25-µm film thickness); two <sup>63</sup>Ni electron capture detectors (ECDs) were used with nitrogen as the carrier and detector make up gas (at gas flows of 1 and 50 mL/min, respectively).

The injector was 250°C; the detector was 290°C (NPD) and 300°C (ECD). For oven temperature, the temperature was held at 90°C for 1 min, ramped at 30°C/min to 180°C, then ramped at 4°C/min to 260°C, and the temperature was held isothermic for 6–12 min. Samples of 1–2 µL were injected in the splitless mode (60 s) by an autosampler.

A chromatography data system (GynkoSoft) was used for data collection and quantitation. Densities for supercritical CO<sub>2</sub> at different pressures and temperatures were calculated with SF-Solver software (Isco, Lincoln, NE).

## Results and Discussion

SFE efficiency was studied by determining the influence of the following variables: density, temperature, modifier addition, collection solvent, and volume of extraction fluid.

#### Empty thimble

A set of 11 pesticides normally detected by GC representing different classes of compounds was selected. Care was taken to include compounds with a large variation in polarity (water and organic solvent solubility) and volatility (Figure 1). These 11 pesticides were initially spiked into and extracted from an empty thimble in order to study the influence of instrumental parameters.

Influences of the temperature and density of the supercritical CO<sub>2</sub> on extraction efficiency were studied at 3 temperatures (40, 55, and 70°C) and 5 densities (0.25, 0.40, 0.55, 0.70,

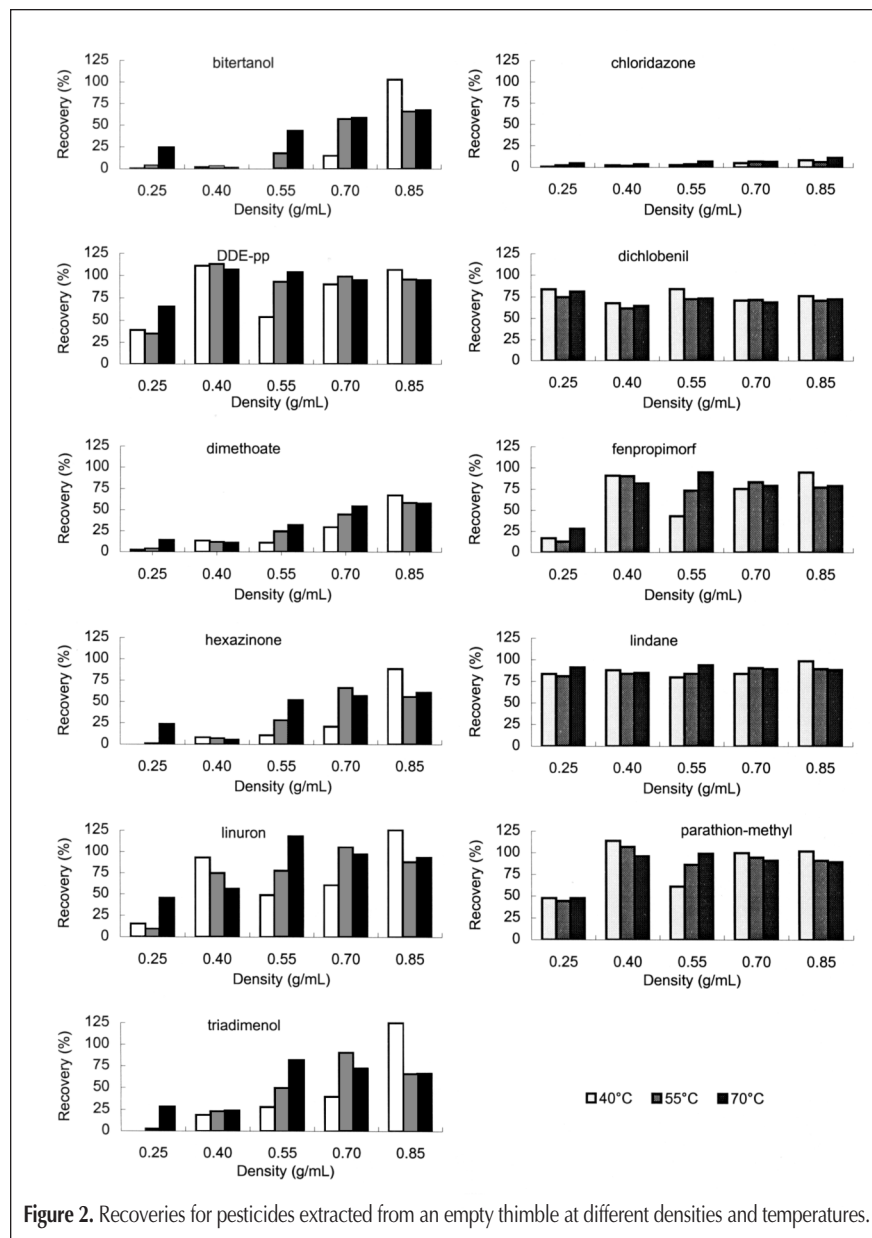
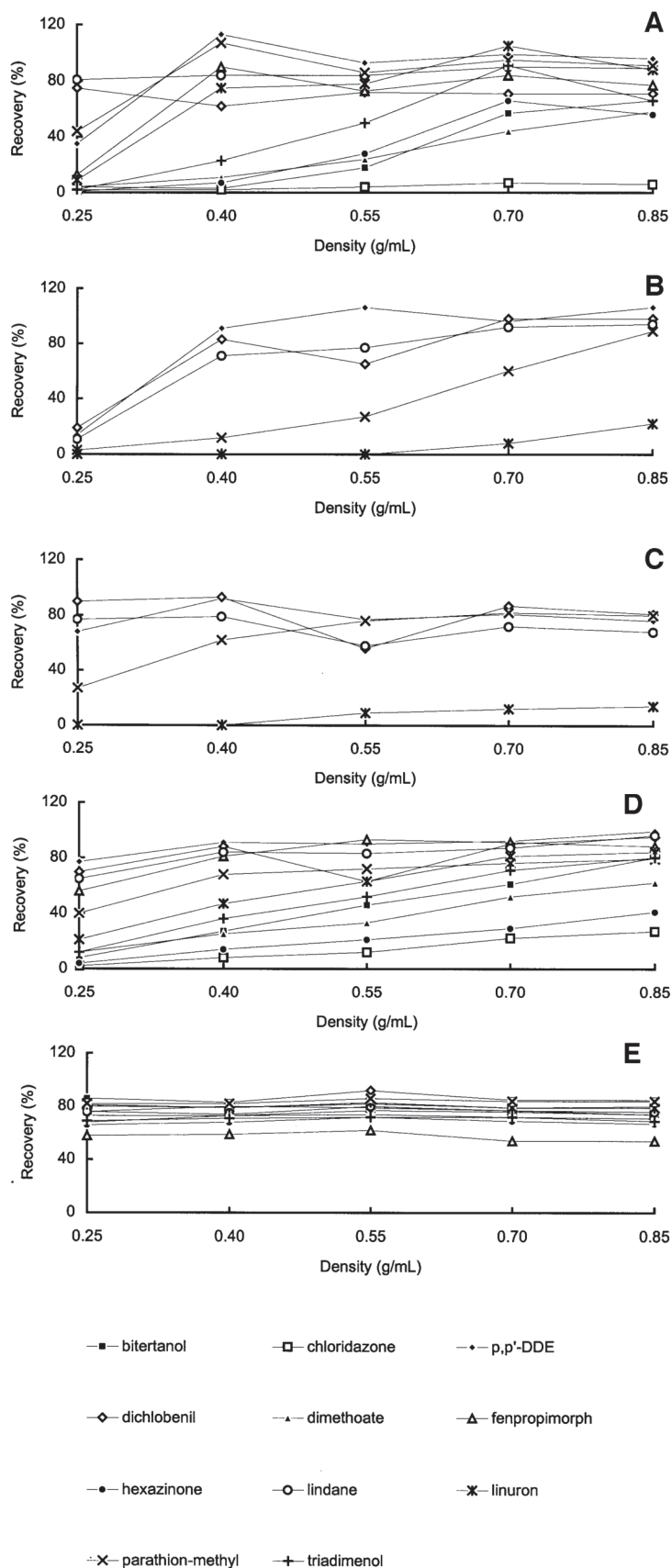


Figure 2. Recoveries for pesticides extracted from an empty thimble at different densities and temperatures.



**Figure 3.** Solubility profiles of pesticides, varying the density of supercritical carbon dioxide at a temperature of 55°C. Extraction from an empty thimble (A), silica (B), sand (C), silica modified with 200  $\mu$ L methanol (D), and sand modified with 200  $\mu$ L methanol (E).

and 0.85 g/mL) (Figure 2). Increasing the temperature at a given density should increase the diffusion coefficient in the fluid and increase the volatilization of the analyte whenever possible (15). The results indicated that extraction temperature had less influence than density on the recovery when pesticides were extracted from an empty thimble. A medium extraction temperature of 55°C was therefore selected as suitable for the remaining experiments.

Results showing the influence of CO<sub>2</sub> density at 55°C on analyte recovery are summarized in Figure 3A. Recoveries are, as expected, generally found to increase with the density of the supercritical fluid and be dependent on the structure/properties of the extracted pesticides. Two compounds are almost quantitatively recovered, even at the lowest density investigated (0.25 g/mL). These compounds (dichlobenil and lindane) are both volatile and nonpolar with high solubility in the supercritical fluid. Four other compounds of low polarity (p,p'-DDE, fenpropimorph, linuron, and parathion-methyl) are extracted quantitatively when density has reached a threshold value at 0.4 g/mL. For 4 other compounds of higher polarity (bitertanol, dimethoate, hexazinone, and triadimenol), the extraction efficiency increases more or less linearly, or at least continuously, with increasing density. Only chloridazone differs in behavior from the other pesticides studied. The extraction of chloridazone from the empty thimble is a special case with consistently low recoveries at all densities investigated.

In an attempt to recover the lost chloridazone, the thimble was opened, and 200  $\mu$ L methanol was added directly to the thimble. Thereafter, the thimble was closed and the extraction continued at a temperature of 55°C and a density of 0.85 g/mL with 1 min static extraction followed by extraction with 10 mL CO<sub>2</sub> in the dynamic mode. It was more likely that the analytes were dissolved in the modifier prior to extraction and then rinsed from the thimble by the supercritical fluid. This experiment recovered approximately an extra 60% of chloridazone (and extra 20% of bitertanol and dimethoate).

Maximum selectivity occurs at medium densities (e.g., 0.55 g/mL) with recoveries spread over the full range from insignificant to quantitative (5–95%). There is a negative correlation ( $R^2 = 0.80$ , excluding bitertanol and chloridazone from the calculation) between achieved recoveries and corresponding water solubility (Figure 4). The results could thus be expected strictly due to solubility reasons, using a predominantly nonpolar extraction medium such as unmodified CO<sub>2</sub> at medium density. However, there are two noticeable exceptions from this rule (chloridazone and



bitertanol) that both show recoveries well below those expected for polarity reasons alone. For these two compounds, some specific retention mechanism can be suspected. Because both compounds are aromatic, contain high amounts of nitrogen, and heterocyclic, an interaction with the metal surface of the extraction thimble is possible. These compounds are also rather non-volatile, and a combination of high polarity and low volatility could influence the recovery. Further studies would also be

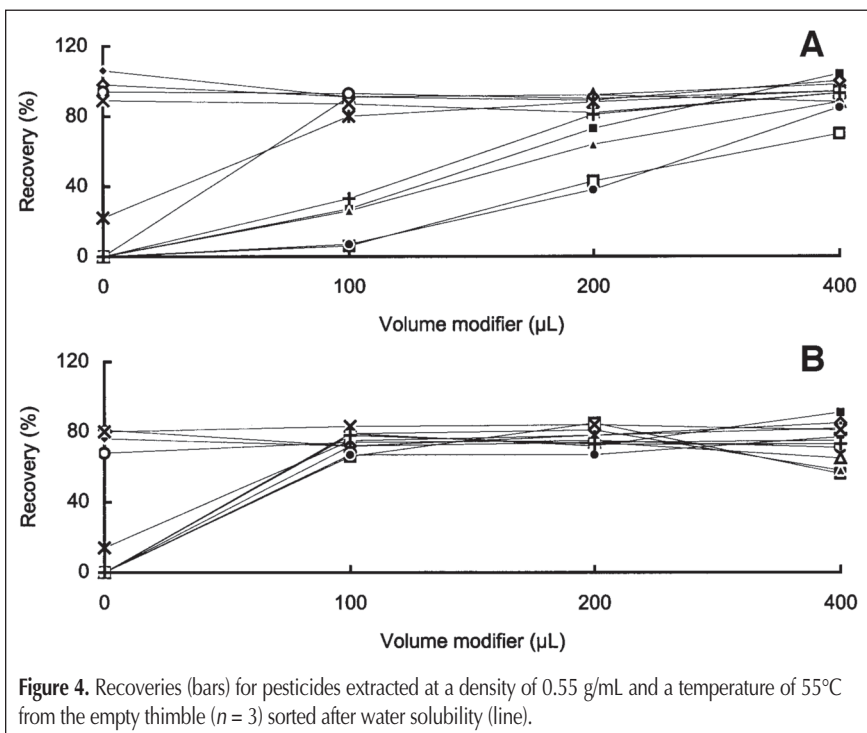
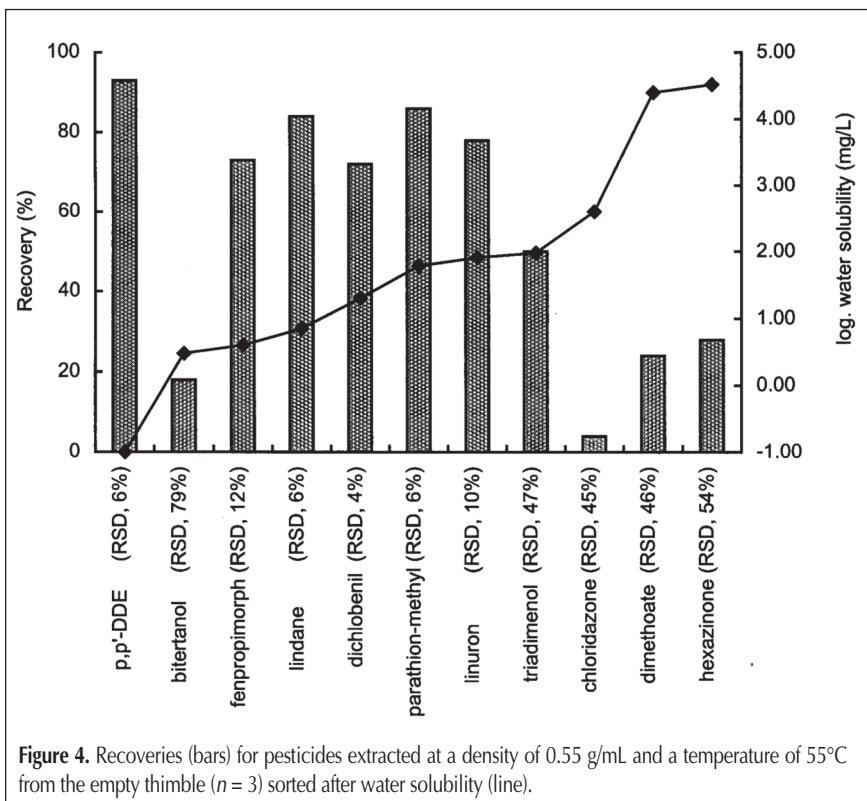
needed to investigate why closely related compounds such as hexazinone and triadimenol (similar to chloridazone and bitertanol, respectively) behave differently in this respect.

#### Matrix effects

The influence of the interaction of pesticides with model matrices was subsequently investigated. Initially, glass wool spiked with pesticides was used in a preliminary study on matrix

influence on recovery and extraction performed at a temperature of 55°C and a density of 0.85 g/mL, resulting in recoveries similar to extraction from an empty thimble. The addition of 200  $\mu$ L methanol to the glass wool in the thimble increased the absolute recoveries for chloridazone, dimethoate, and bitertanol 60, 20, and 20%, respectively.

Seasand and silica were used as model sorbents/supports for the initial spiking experiments. Figures 3B and 3C show plots of recovery versus density for pesticides extracted at a temperature of 55°C with pure carbon dioxide from silica and sand matrices. The graphs appear rather similar, despite the fact that large difference in surface area between silica and sand exist. Only 5 compounds representing the low polarity range (p,p'-DDE, dichlobenil, lindane, parathion-methyl, and linuron) eluted with linuron as a borderline case. Compounds of higher polarity were impossible to extract, even at the highest density (0.85 g/mL), without enhancing polarity with the addition of a modifier. Although the recovery profiles differ completely in comparison with a plot from the empty thimble study (Figure 3A), none of the pesticides could be extracted quantitatively from silica at the lowest density (0.25 g/mL). One explanation is that the hydrogen bonds between proton-accepting groups on the analytes and silanol groups on the matrix are stronger and more pronounced in silica than in sand. It is not surprising that silica, with its proton-donating surface, retains proton-accepting molecules more strongly (16). Another explanation is that there is a more efficient sorption on silica due to a higher surface area. It has been suggested that organic contaminants can exist in two states on solid sorbents, deposited and sorbed (17). Contaminants that are deposited as a separate phase on the solid surface can simply be removed by dissolution (solubility limitation) in the supercritical phase, whereas extraction of the sorbed material requires harsher extraction conditions. These results indicate that a very small amount of the pesticides are deposited, and the rest are sorbed on the sorbents, thus requiring harsher extraction con-



ditions. From these results, it was obvious that the addition of a modifier was necessary to break the hydrogen bonds. Methanol was therefore selected because of its ability to deactivate silica and break the hydrogen bonds between the analytes and the sand or silica surfaces. (18,19).

The effect of modifier addition at a density of 0.85 g/mL was studied as a function of different volumes of methanol (100–400  $\mu$ L) added directly to the silica and sand samples in the thimble (Figure 5). From this result, 200  $\mu$ L methanol was chosen as a suitable average modifier volume according to the extraction profiles. Recoveries of pesticides extracted from sand and silica with carbon dioxide modified with 200  $\mu$ L methanol are thus shown in Figures 3D and 3E. The addition of 200  $\mu$ L modifier to silica reestablishes a selective density dependent recovery similar to the results obtained from the empty thimble (Figure 3A). The methanol volume seems sufficient to just break the bonds between analyte and matrix. The observed recovery is clearly solubility dependent, just as it was for extraction of pesticides from an empty thimble. It could also be concluded that the added volume of 200  $\mu$ L methanol was in excess for the sand sample. Thus, all analytes were extracted with high recoveries, completely independent of density.

Average relative standard deviations (RSDs) for SFE performed at a density of 0.85 g/mL were 3% for sand and 7% for silica ( $n = 3$ ). The reproducibility for extraction from empty thimbles was poorer with an average RSD of 23% at a density of 0.85 g/mL ( $n = 3$ ). Extraction from empty thimbles performed at lower densities resulted in greater RSDs. Under such conditions (low density), there is a tendency for an "on/off" effect with a few higher values interspersed among the majority of low (or zero) recoveries. The origin of this behavior is unclear, but among the possibilities to be considered are traces of remaining spiking solvent acting as a modifier, local variations in the flow dynamics of CO<sub>2</sub> due to the inexact assembly of the thimble parts, or dead volumes/mixing of the sample in the thimble. Another explanation could be that pressures at lower densities are below or close to the analytes' threshold pressures (i.e., the pressure where the solubility starts) (20). Therefore, small variations in extraction conditions might affect the extraction behavior.

### Collection solvents

A study by Wuchner et al. (21) suggested that polar pesticides extracted from sand with unmodified carbon dioxide are not effectively trapped in nonpolar solvents such as cyclohexane, giving preference to collection in ethyl acetate. The present study shows that only the extraction efficiency of chloridazone (polar compound) from silica performed at a density of 0.85 g/mL and a temperature of 55°C was slightly improved by changing collection solvent from cyclohexane (not collected) to ethyl acetate (14% recovery), although the precision was poor (RSD, 94%). However, collection in cyclohexane gave comparable or higher recoveries and better precision (average RSD, 4%) than collection in ethyl acetate (average RSD, 25%) for 5 low-polar pesticides (p,p'-DDE, dichlobenil, lindane, linuron, parathion-methyl) extractable from silica ( $n = 3$ ).

### Fluid volume

The influence of the volume of supercritical carbon dioxide

used in the recovery was studied during the extraction of silica. Extractions were performed at a density of 0.85 g/mL, a temperature of 55°C, and a modifier addition of 200  $\mu$ L methanol. It was found that the main fraction of the pesticides elutes during the first two mL, and no detectable increase in recovery was found after 5 mL. Thus, the fluid volume of 20 mL used in this investigation could be reduced with preserved recovery.

## Conclusion

The results obtained in this study show that it is possible to identify extraction conditions where the analytes' molecular structure affect the extraction efficiency. The results also indicate that parameters other than solubility (e.g., volatility) has a marked influence on the extraction process, even under simplistic conditions (extraction from empty thimble). This investigation gave valuable information for further studies on binding mechanisms of other model matrices with well known properties, the purpose of which would be to characterize different interactions that determine the behavior of pesticides in complex matrices (soils).

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## References

1. I.J. Barnabas, J.R. Dean, and S.P. Owen. Supercritical fluid extraction of analytes from environmental samples. *Analyst* **119**: 2381–94 (1994).
2. S. Bowadt and S.B. Hawthorne. Supercritical fluid extraction in environmental analysis. *J. Chromatogr. A* **703**: 549–71 (1995).
3. J.R. Dean. Effect of soil–pesticide interactions on the efficiency of supercritical fluid extraction. *J. Chromatogr. A* **754**: 221–33 (1996).
4. S. Papilloud and W. Haerdi. Supercritical fluid extraction of triazine herbicides: a powerful selective analytical method. *Chromatographia* **40**: 705–711 (1995).
5. M.E.P. McNally. Advances in environmental SFE. *Anal. Chem.* **67**: 308A–315A (1995).
6. H. H. Cheng. Organic residues in soils: mechanisms of retention and extractability. *Intl. J. Environ. Anal. Chem.* **39**: 165–71 (1990).
7. S. Bengtsson, T. Berglöf, and T. Sjöqvist. Predicting the leachability of pesticides from soils using near infrared reflectance. *J. Agric. Food Chem.* **44**: 2260–65 (1996).
8. P. Capriel, A. Haisch, and S.U. Khan. Supercritical methanol: an efficacious technique for the extraction of bound pesticide residues from soil and plant samples. *J. Agric. Food Chem.* **34**: 70–73 (1986).
9. S.U. Khan. Supercritical fluid extraction of bound residues from soil and food commodities. *J. Agric. Food Chem.* **43**: 1718–23 (1995).
10. T. Berglöf and G. Jonsäll. SLL Rapport, nr 70, Uppsala, Sweden, 1993.
11. J. Poustka, K. Holadova, and J. Hajslova. Application of supercritical fluid extraction for analysis of organophosphates in cereals. *Intl. J. Environ. Anal. Chem.* **60**: 139–44 (1995).

12. G. Maio, C. von Holst, B.W. Wenclawiak, and R. Darskus. Supercritical fluid extraction of some chlorinated benzenes and cyclohexanes from soil: optimization with fractional factorial design and simplex. *Anal. Chem.* **69**: 601–606 (1997).
13. W.C. Koskinen and S.S. Harper. The retention process: mechanisms. In *Pesticides in the Soil Environment: Processes, Impact, and Modelling*, H.H. Cheng, Ed. Soil Science Society of America, book series 2, Madison, WI, 1990, pp 51–77.
14. Y.Z. Hsieh and A.C. Chang. Effects of analyte-matrix interactions on supercritical fluid extraction efficiencies of polycyclic aromatic hydrocarbons. *Anal. Chim. Acta* **342**: 41–50 (1997).
15. J.R. Wheeler and M.E. McNally. Supercritical fluid extraction and chromatography of representative agricultural products with capillary and microbore columns. *J. Chromatogr. Sci.* **27**: 534–39 (1989).
16. S.P. Boudreau and W.T. Cooper. Determination of surface polarity by heterogeneous gas–solid chromatography. *Anal. Chem.* **59**: 353–58 (1987).
17. C. Erkey, G. Madras, M. Orejuela, and A. Akgerman. Supercritical carbon dioxide extraction of organics from soil. *Environ. Sci. Technol.* **27**: 1225–31 (1993).
18. J.G.M. Janssen, P.J. Schoenmakers, and C.A. Cramers. A fundamental study of the effects of modifiers in supercritical fluid chromatography. *J. High. Resolute. Chromatogr.* **12**: 645–51 (1989).
19. C.H. Lochmüller and L.P. Mink. Adsorption isotherms on silica for methanol and 1-hexanol modifiers from supercritical carbon dioxide. *J. Chromatogr.* **471**: 357–66 (1989).
20. J.W. King. Fundamentals and applications of supercritical fluid extraction in chromatographic science. *J. Chromatogr. Sci.* **27**: 355–64 (1989).
21. K. Wuchner, R.T. Ghijsen, U.A.T. Brinkman, R. Grob, and J. Mathieu. Extraction of organophosphorous pesticides from soil by off-line supercritical fluid extraction. *Analyst.* **118**: 11–16 (1993).

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