Effect of Extraction Conditions and Modifiers on the Supercritical Fluid Extraction of 88 Pesticides

Satoru Nemoto*, Kumiko Sasaki, Masatake Toyoda, and **Yukio Saito**
Division of Foods, National Institute of Health Sciences, 18-1, Kamiyoga 1-Chome, Setagaya-ku, Tokyo 158, Japan

Abstract

Supercritical fluid extraction (SFE) conditions for multiresidue analysis of pesticides are evaluated using diatomaceous earth (Celite) spiked with 88 pesticides (16 organochlorine, 33 organophosphorus, 8 pyrethroid, 12 carbamate, and 19 other pesticides). The SFE parameters considered are CO_2 density, CO_2 flow rate, extraction temperature, static and dynamic extraction times, trap temperature, and addition of modifier. SFE without modifier is insufficient to extract polar pesticides from fortified Celite. The addition of water to Celite is most effective in enhancing the recoveries of pesticides. Methanol is also an effective modifier, but the recovery of captafol, captan, phosmet, and chinomethionat decreases as time goes on after the addition of methanol. The best obtained conditions of SFE (2.0 g sample) are as follows: 0.40 mL of water as a modifier, 0.70 g/mL CO_2 density, 50°C extraction temperature, 2.0 mL/min CO_2 flow rate, 3.0 min of static extraction, and 20.0 min of dynamic extraction. The extracted pesticides are collected on an octadecylsilane trap at 30°C. Quantitative analysis of the 88 pesticides is performed by gas chromatography–mass spectrometry using the selected ion monitoring mode. Recoveries from fortified Celite are greater than 90% for 79 pesticides and greater than 70% for the other pesticides, except acephate, methamidophos, and propamocarb. The relative standard deviations of the recoveries are less than 5% for almost all of the pesticides.

Introduction

In recent years there has been increasing interest in supercritical fluid extraction (SFE), which offers an alternative to traditional methods of solvent-based extraction (1–4). Supercritical fluids have unique physicochemical properties that give them advantages as extraction solvents. The densities of supercritical fluids are greater than those of gases and are close to those of liquids. Therefore, the solvation properties of supercritical fluids are similar to those of liquid solvents. Moreover, because supercritical fluids have lower viscosities and solutes have higher diffusion coefficients in supercritical fluids than in liquid solvents, SFE often provides faster extraction compared with traditional methods of extraction. Other advantages of SFE compared with solvent-based extraction are smaller amounts of organic solvents, smaller space requirements, and minimal sample handling. In SFE, CO_2 is most widely used as a supercritical fluid because of its low toxicity and reactivity, moderate critical temperature and pressure, availability, low cost, and nonflammability.

The application of SFE to pesticide residue analysis has been demonstrated for some pesticides in sediment (5), soil (6–9), and agricultural products (10–15). However, in many of the previous studies, the number of pesticides studied was rather limited. Several applications of SFE to multiresidue analysis of pesticides has also been attempted in recent years (16–19). However, the effects of SFE parameters on the extraction of pesticides are not completely understood because pesticides have various polar and chemical properties and because pesticide–matrix interactions are complex. In order to improve the analyte extractability, modifiers (e.g., methanol) are usually added to the sample matrix or extraction fluids (7–9,20,21). Because the effects of modifiers are highly dependent on analyte and matrix, the use of modifiers sometimes complicates the optimization of SFE conditions.

The objective of this study was to evaluate the feasibility of SFE for multiresidue analysis of pesticides and to investigate the SFE conditions that enhance the recovery of the pesticides. For this purpose, 88 pesticides with various polarities and structures were used. In addition, Celite, a diatomaceous earth, was used as a sample matrix to simplify the pesticide–matrix interaction. The 88 pesticides consisted of 16 organochlorine, 33 organophosphorus, 8 pyrethroid, 12 carbamate, and 19 other pesticides. We isolated the following SFE parameters: density and flow rate of CO_2 fluid, extraction temperature, static and dynamic extraction times, and trap temperature. The influence of modifiers on pesticide recovery was also studied.

Experimental

Reagents

All organic solvents were of high quality for pesticide residue analysis (Wako Pure Chemical Industries, Osaka, Japan). A 99.999% purity of CO_2 (Showatansan, Kanagawa, Japan) was used for all extractions. Celite (no. 545, Wako Pure Chemical

* Author to whom correspondence should be addressed.

Industries), a diatomaceous earth, was used as the sample matrix. All pesticides were of ultra-pure quality and were obtained either from Riedel-de Haën (Seelze, Germany) or from Wako Pure Chemical Industries. Table I lists the pesticides, arranged by classification, included in this study. Individual stock standard solutions (1 mg/mL) were prepared by dissolving each standard in *n*-hexane and/or acetone. Working standard mixtures in *n*-hexane contained 10 µg/mL (50 µg/mL for acephate, captafol, methamidophos, and propamocarb) for each pesticide and were used for spiking samples and preparing calibration standards.

Table I. Pesticides Included in the Study and Their Monitor Ions (*m/z*) for GC–MS (SIM) Analysis

Pesticide	Monitor ion (*m/z*)	Pesticides	Monitor ion (*m/z*)
Organochlorine (16)		Organophosphate (33)	
Aldrin	264.8	Acephate	136.0
α-BHC	218.9	Azinphos-ethyl	160.0
β-BHC	218.9	Azinphos-methyl	160.0
γ-BHC	218.9	Bromophos-ethyl	358.8
δ-BHC	218.9	(E)-Chlorfenvinphos	268.9
Captafol	79.0	(Z)-Chlorfenvinphos	268.9
Captan	79.0	Chlorpyrifos	313.9
Chlorobenzilate	250.9	Chlorpyrifos-methyl	285.9
p,p'-DDD	235.0	Diazinon	179.1
p,p'-DDE	246.0	Dichlorvos	109.0
o,p'-DDT	235.0	Dimethoate	87.0
p,p'-DDT	235.0	Dioxabenzofos	215.9
Dieldrin	262.8	Disulfoton	88.0
Endrin	262.8	Edifenphos	310.0
Heptachlor	271.8	EPN	157.0
Heptachlor epoxide	352.8	Ethoprophos	157.9
		Etrimfos	292.1
Pyrethroid (8)		Fenitrothion	277.0
Cyfluthrin	226.1	Fensulfothion	292.0
Cyhalothrin	181.0	Fenthion	278.0
Cypermethrin	163.0	Malathion	173.1
Deltamethrin	181.0	Methamidophos	94.0
Fenvalerate	167.0	Methidathion	145.0
Flucythrinate	199.1	Parathion	291.0
Fluvalinate	250.0	Parathion-methyl	262.9
Permethrin	183.0	Phenthoate	273.9
		Phosalone	181.9
Other (19)		Phosmet	160.0
Amitraz	293.2	Pirimiphos-methyl	290.0
Benalaxyl	148.1	Prothiofos	309.0
Bitertanol	170.1	Quinalphos	146.0
Chinomethionat	234.0	Terbufos	231.0
Dichlofluanid	123.0	Thiometon	88.0
Dimethipin	118.0		
Flutolanil	173.0	Carbamate (12)	
Lenacil	153.0	Bendiocarb	151.0
Mefenacet	192.0	Chlorpropham	126.9
Mepronil	269.1	Diethofencarb	124.0
Methoprene	73.1	Esprocarb	222.1
Metribuzin	198.1	Ethiofencarb	107.1
Myclobutanil	179.0	Fenobucarb	121.0
Pendimethalin	252.1	Isoprocarb	121.0
Pretilachlor	238.1	Methiocarb	168.0
Propiconazole	259.1	Pirimicarb	166.1
Pyridaben	147.1	Propamocarb	58.1
Triadimefon	208.0	Propoxur	110.0
Triadimenol	112.0	Thiobencarb	100.1

Apparatus

A Hewlett-Packard (Little Falls, DE) model 7680T supercritical fluid extractor equipped with a 7-mL stainless steel extraction vessel and a 30-µm Hypersil octadecylsilane (ODS) analyte trap was used. Optimal SFE conditions for 2.0 g Celite were as follows: 0.40 mL of water was added as a modifier, the CO_2 density was 0.70 g/mL (pressure, 151 bar), the extraction chamber temperature was 50°C, the CO_2 flow rate was 2.0 mL/min, the nozzle temperature was 50°C, the trap temperature was 30°C, and the static extraction was 3.0 min followed by a 20.0-min dynamic extraction. After extraction, the pesticides were eluted from the ODS trap with 1.5 mL of acetone at 1.0 mL/min and 30°C. The trap was rinsed with 2.0 mL acetone at 1.0 mL/min to clean the ODS between extractions.

A Hewlett-Packard model 5890 Series II gas chromatograph equipped with a Hewlett-Packard model 7673 autosampler and coupled with a model 5971A mass selective detector was used. A DB-5ms (J&W Scientific, Folsom, CA) capillary column (30 m × 0.25-mm i.d., 0.25-µm film) connected to a 1-m × 0.25-mm-i.d. deactivated fused-silica guard column (J&W Scientific) was used with helium carrier gas at 35 cm/s. The injector temperature was 240°C. The column oven temperature was maintained at 50°C for 1 min, then increased at 25°C/min to 125°C, followed by a 10°C/min increase to 300°C and was finally held isothermally at 300°C for 3.5 min. The transfer line temperature was 310°C. The electron energy and electron multiplier voltage were 70 eV and 2800 V, respectively. The injection volume was 1 µL (splitless). The monitor ions for the determination of the pesticides are listed in Table I.

Sample preparation and analysis

For fortified samples, a 2.0-g portion of Celite was weighed into an extraction vessel and 100 µL of the 10 µg/mL (50 µg/mL for acephate, captafol, methamidophos, and propamocarb) spiking standard solution was added to the Celite. The spiking level was 0.5 µg/g (or 2.5 µg/g for acephate, captafol, methamidophos, and propamocarb) in the sample. A few minutes were allowed for the solvent to evaporate, and then the samples were extracted by SFE. To study the modifier, modifiers were added directly onto the sample in the extraction vessel before extraction. The pesticides in the SFE extracts were determined by gas chromatography–mass spectrometry (GC–MS) in the selected ion monitoring (SIM) mode. The SFE extracts were diluted to

5 mL with acetone before injection for quantitation by GC–MS. The same solution as the spiking standard solution was diluted to make the calibration standards. Calibration standards at concentrations of 0.05, 0.1, 0.2, and 0.4 µg/mL were analyzed with each set of samples. Calibration curves for all pesticides were linear in the range considered (correlation coefficients were between 0.985 and 1.000).

Results and Discussion

Celite, a diatomaceous earth, was used as a sample matrix. Celite is not completely inert, but it represents a more well-defined surface than real samples with fewer or less active sites of interaction. Examination of extraction from Celite, therefore, enables a rapid determination of whether potential extraction difficulties with real samples are related to the pesticide property or matrix–analyte interactions (22).

SFE without modifiers

To determine the effects of CO_2 density and extraction temperature on the extractability of pesticides by SFE without modifier, the pesticides were extracted from fortified Celite using pure CO_2.

Effect of CO_2 density

Figure 1 shows typical plots of recovery versus CO_2 density for five organophosphorus pesticides. The experiments were performed in duplicate by extracting the pesticides from fortified Celite at CO_2 densities of 0.30, 0.50, 0.70, and 0.85 g/mL and a constant extraction temperature of 40°C. These corresponded to pressures of 81, 91, 115, and 211 bar, respectively. Thiometon gave a good recovery (86.2%) at a density of 0.30 g/mL; over the remainder of the density range, the recovery of thiometon remained constant. At a density of 0.30 g/mL, the recoveries of quinalphos, malathion, and (E)-chlorfenvinphos were 49.2, 24.3, and 5.7%, respectively. However, significant increases in their recoveries occurred at a density of 0.50 g/mL. Good recoveries (≥ 80%) were obtained for quinalphos and malathion at densities of 0.50 and 0.70 g/mL, respectively. The recovery of (E)-chlorfenvinphos increased with increasing density and achieved more than 80% at 0.85 g/mL. Azinphos-methyl was not recovered at densities of 0.30 and 0.50 g/mL, and its recovery also increased with increasing density; however, it gave a poor recovery (51.0%), even at a density of 0.85 g/mL.

The results for all investigated pesticides versus density are summarized in Table II. The pesticides were classified into six groups on the basis of recovery and CO_2 density. The pesticides listed in groups A, B, C, and D showed recoveries of 80% or greater at densities of 0.30, 0.50, 0.70, and 0.85 g/mL, respectively. Thiometon, quinalphos, malathion, and (E)-chlorfenvinphos in Figure 1 were classified in groups A, B, C, and D, respectively. The pesticides listed in group E showed low recoveries (< 80%) at a density of 0.85 g/mL (e.g., azinphos-methyl in Figure 1). Organophosphorus and carbamate pesticides exhibited various extractabilities with respect to CO_2 density and appeared in every group in Table II. Most of the organochlorine pesticides exhibited a similar behavior to thiometon in Figure 1, except captafol, captan, and chlorobenzilate. Pyrethroids, except permethrin and cyhalothrin, mainly appeared in group C; they showed a similar behavior to malathion in Figure 1. For almost all of the pesticides in this study, increasing CO_2 density increased the recoveries. However, even at the maximum density of 0.85 g/mL, 11 pesticides listed in group F were not recovered at all without the addition of modifier.

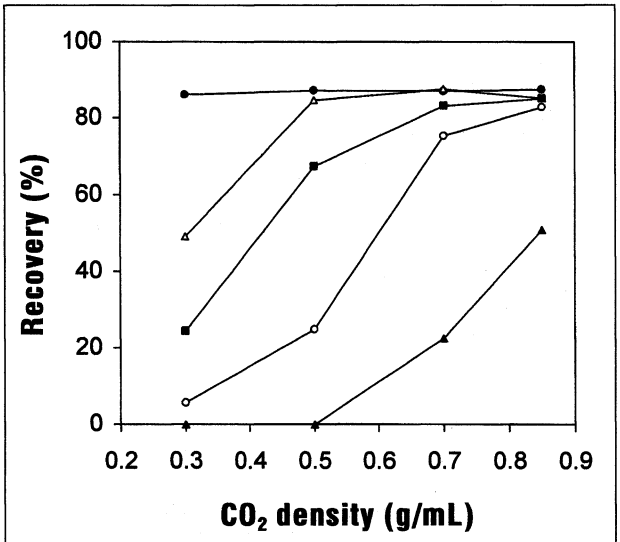

Figure 1. Effect of CO_2 density on the recoveries of five pesticides from fortified Celite by SFE without modifier: thiometon (●), quinalphos (△), malathion (■), (E)-chlorfenvinphos (O), and azinphos-methyl (▲). SFE conditions: extraction temperature, 40°C; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min.

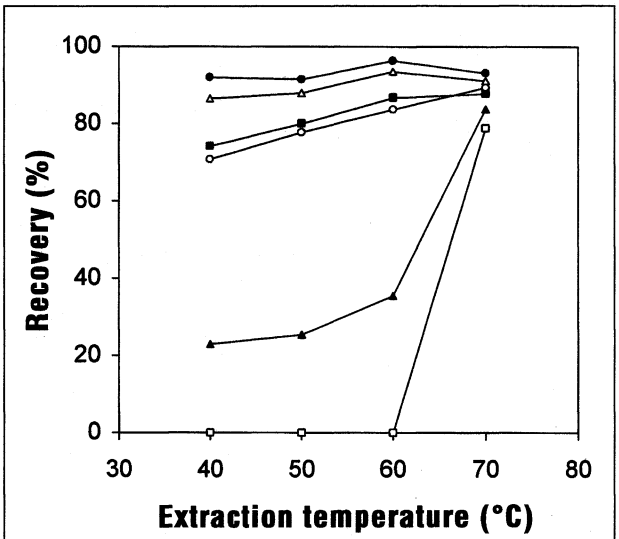

Figure 2. Effect of extraction temperature on the recoveries of six pesticides from fortified Celite by SFE without modifier: thiometon (●), quinalphos (△), malathion (■), (E)-chlorfenvinphos (O), azinphos-methyl (▲), and dimethoate (□). SFE conditions: CO_2 density, 0.70 g/mL; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min.

Table II. Effect of CO_2 Density on Pesticide Recoveries from Fortified Celite by SFE Without Modifier

Group A: Recovery ≥ 80%, CO_2 density ≥ 0.30 g/mL
OC: aldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, *p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, dieldrin, endrin, heptachlor, heptachlor epoxide OP: chlorpyrifos, chlorpyrifos-methyl, dioxabenzofos, disulfoton, etrimfos, fenitrothion, fenthion, parathion, parathion-methyl, prothiofos, terbufos, thiometon CA: esprocarb OT: pendimethalin
Group B: Recovery ≥ 80%, CO_2 density ≥ 0.50 g/mL
OP: bromophos-ethyl, diazinon, ethoprophos, phenthoate, pirimiphos-methyl, quinalphos PY: permethrin CA: chlorpropham, thiobencarb OT: methoprene
Group C: Recovery ≥ 80%, CO_2 density ≥ 0.70 g/mL
OP: EPN, malathion, methidathion, phosalone PY: cyfluthrin, cypermethrin, deltamethrin, fenvalerate, flucythrinate, fluvalinate CA: fenobucarb, isoprocarb, methiocarb OT: chinomethionat, dichlofluanid, pyridaben
Group D: Recovery ≥ 80%, CO_2 density = 0.85 g/mL
OP: (E)-chlorfenvinphos PY: cyhalothrin CA: bendiocarb OT: mepronil
Group E: 0 < Recovery < 80%, CO_2 density = 0.85 g/mL
OC: captafol, captan, chlorobenzilate OP: azinphos-ethyl, azinphos-methyl, (Z)-chlorfenvinphos, dichlorvos, edifenphos, phosmet CA: diethofencarb, ethiofencarb, pirimicarb, propoxur OT: amitraz, benalaxyl, flutolanil, mefenacet, metribuzin, pretilachlor, triadimefon
Group F: Not recovered, CO_2 density = 0.3–0.85 g/mL
OP: acephate, dimethoate, fensulfothion, methamidophos CA: propamocarb OT: bitertanol, dimethipin, lenacil, myclobutanil, propiconazole, triadimenol
SFE conditions: extraction temperature, 40°C; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min. OC = organochlorine; OP = organophosphate; PY = pyrethroid; CA = carbamate; OT = other.

Table III. Physicochemical Properties of Modifiers*

Modifier	Formula	Boiling point (°C)	Snyder polarity index (*P'*)	Dielectric constant
n-Hexane	$CH_3(CH_2)_4CH_3$	68.7	0.1	1.9
Dichloromethane	CH_2Cl_2	39.8	3.1	7.8
Ethyl acetate	$CH_3COOC_2H_5$	77.1	4.4	6.1
Acetone	CH_3COCH_3	56.3	5.1	21
Acetonitrile	CH_3CN	81.6	5.8	38
2-Propanol	$CH_3CH(OH)CH_3$	82.3	3.9	20
Ethanol	C_2H_5OH	79.0	4.3	25
Methanol	CH_3OH	64.7	5.1	33
Water	H_2O	100.0	10.2	80

* References 28 and 29.

Effect of extraction temperature

In general, solvent strength and diffusivity can both be increased by raising the extraction temperature. If the extraction temperature is increased at a constant pressure, the density of supercritical CO_2 will decrease. In this study, the effect of extraction temperature was examined while keeping CO_2 density constant. Duplicate extractions were performed at extraction temperatures of 40, 50, 60, and 70°C and a constant CO_2 density of 0.70 g/mL. These corresponded to pressures of 115, 150, 187, and 223 bar, respectively. Figure 2 shows the typical plots of recovery versus extraction temperature for dimethoate and the same organophosphorus pesticides appearing in Figure 1. For thiometon and quinalphos, varying extraction temperature had little influence on recoveries. The recoveries of malathion and (E)-chlorfenvinphos increased slightly from 50 to 70°C. The effect of extraction temperature on the recoveries of the pesticides listed in groups A, B, C, and D in Table II was not very large at these SFE conditions.

The recoveries of pesticides (except mefenacet) listed in group E increased with increasing extraction temperature and achieved at least 80% at 70°C, with the exceptions of captafol, chlorobenzilate, and amitraz. For azinphos-methyl, which yielded poor recovery (23.1%) at an extraction temperature of 40°C, the recovery increased slightly from 50 to 60°C, and then a greater than twofold increase in recovery was observed at 70°C. Similar trends were observed for azinphos-ethyl, pirimicarb, and benalaxyl. Dimethoate was not recovered at 40–60°C but showed a significant increase in recovery at 70°C. Similar results were obtained for mefenacet (group E pesticides) and group F pesticides except acephate and propamocarb. In particular, the recoveries of fensulfothion, dimethoate, mefenacet, propiconazole, and dimethipin were dramatically increased at 70°C; their recoveries at 70°C were 63.8, 78.9, 83.8, 88.4, and 93.8%, respectively. Thus, extraction temperature was an important parameter in improving the recovery of some pesticides. The recoveries of almost all of the pesticides investigated were at least 80% at 70°C. However, even at 70°C, myclobutanil, triadimenol, lenacil, bitertanol, and methamidophos showed poor recoveries (17–48%), and acephate and propamocarb were not recovered at all without the addition of modifier.

Effect of modifiers

The low polarity of CO_2 limits the range of analytes that can be extracted by SFE. Therefore, polar solvents are often added to modify the CO_2 fluid or sample matrices for sufficient extraction

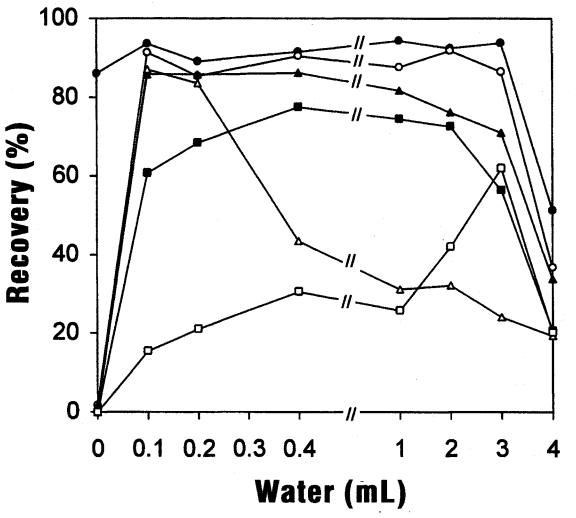

Figure 3. Effect of water on the recoveries of six pesticides from fortified Celite by SFE: aldrin (●), azinphos-methyl (O), propoxur (▲), dimethoate (△), triadimenol (■), and bitertanol (□). SFE conditions: CO_2 density, 0.50 g/mL; extraction temperature, 40°C; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min.

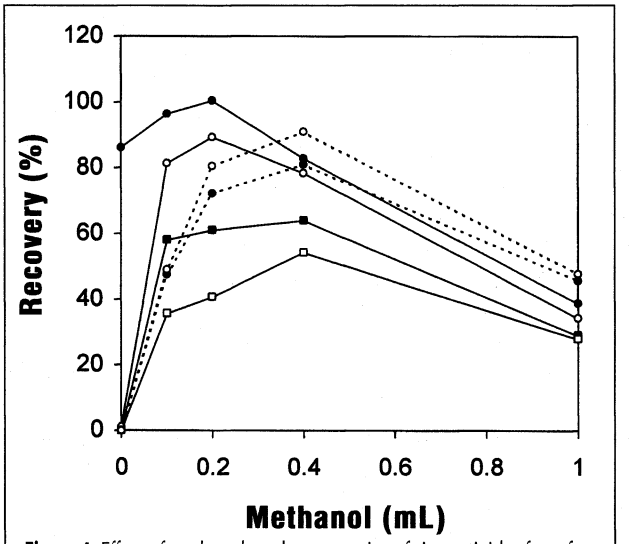

Figure 4. Effect of methanol on the recoveries of six pesticides from fortified Celite by SFE: aldrin (-●-), azinphos-methyl (-O-), triadimenol (-■-), bitertanol (-□-), acephate (·●·), and methamidophos (·O·). SFE conditions: CO_2 density, 0.50 g/mL; extraction temperature, 40°C; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min.

of polar analytes.

For the effect of modifiers, nine solvents (water, methanol, ethanol, 2-propanol, acetonitrile, acetone, ethyl acetate, dichloromethane, and *n*-hexane) were evaluated in duplicate extractions. The physicochemical properties of the solvents are shown in Table III. Prior to extraction, 0.40 mL of each solvent was added as a modifier directly onto the 2.0 g of Celite in the extraction vessel. There are a number of ways to add modifier in SFE. Taylor's group found direct addition of modifier to the sample to be superior to the use of modifier pumps in some cases (23). The fortified Celite was extracted at a density of 0.50 g/mL and an extraction temperature of 40°C.

Table IV shows the average recoveries of pesticides listed in Table II by SFE with or without various modifiers. For group A and B pesticides, the modifiers had little influence on recoveries, whereas the recoveries of group C–F pesticides improved with the addition of modifiers, with the exception of dichloromethane. For group E and F pesticides, water and the alcohols (methanol, ethanol, and 2-propanol) gave higher average recoveries of pesticides than the other modifiers. In particular, for group F pesticides, water and the alcohols increased the average recoveries more than twice as much as the other modifiers.

Although mechanisms of the effect of modifiers on extraction efficiencies in SFE are not completely understood, several hypotheses have been proposed (24,25): (*a*) the addition of modifiers to supercritical CO_2 increases the polarity and solvent strength of supercritical CO_2, resulting in an increased solubility of the analytes; (*b*) the modifier can cover active sites on the surface of the sample matrix and prevents readsorption or partitioning of the analytes back onto the matrix active sites; (*c*) the modifier can alter the sample matrix to allow the supercritical CO_2 to access remote sites in the matrix and allow the transport of the analytes to the bulk fluid; and (*d*) the modifier can interact with the analyte–matrix complex and lower the activation energy barrier of desorption.

Water and the alcohols were the effective modifiers for improvement in recovery of pesticides. Therefore, the increased solubility of the pesticides in CO_2 by modifiers (hypothesis *a*) is probably the main mechanism of the effect of modifiers for Celite. *n*-Hexane gave similar average recoveries of pesticides to more polar modifiers such as ethyl acetate, acetone, and acetonitrile. Because *n*-hexane is a nonpolar solvent and has no functional groups that can interact with active sites on the surface of the sample matrix, dispersion interactions are the most likely mode of action (24). Thus, for Celite, dispersion interactions by the modifier may also have a certain effect on the extraction efficiency in SFE. Dichloromethane gave the lowest average recoveries of pesticides in all of the modifiers studied, though it is more polar than *n*-hexane. The likely cause of this discrepancy was the high volatility of dichloromethane, which was rapidly purged from the extraction vessel with CO_2 flow.

As shown in Table IV, water gave the highest average recoveries in all of the modifiers examined except for group F pesticides. For group F pesticides, methanol gave the highest average recovery. In particular, for poorly recovered pesticides (myclobutanil, triadimenol, lenacil, bitertanol, methamidophos, and acephate), methanol gave higher recoveries than ethanol and 2-propanol, except for bitertanol, as shown in Table V. Based on these results, further experiments were carried out for water and methanol.

Water

Duplicate experiments were performed on 2.0 g of Celite fortified with pesticides at a CO_2 density of 0.50 g/mL and an extraction temperature of 40°C; 0.10–4.0 mL of water was added

onto the Celite in the extraction vessel prior to extraction.

As shown in Figure 3, the relationship between recovery and the amount of water showed several patterns. The recovery of aldrin increased slightly with the addition of 0.10 mL of water and remained constant up to 3.0 mL, but the recovery dropped off significantly with the addition of 4.0 mL. The recovery of azinphos-methyl increased dramatically with the addition of 0.10 mL of water, remained constant up to 3.0 mL, and then decreased at 4.0 mL. Of the 88 pesticides studied, 70 showed similar trends to aldrin and azinphos-methyl. The optimum amount of water for these pesticides was 0.10–3.0 mL. In particular, for the pesticides that showed low recoveries when extracted without modifier, the recoveries were dramatically improved with the addition of 0.10 mL of water. The significantly lower recoveries observed at 4.0 mL of water were probably caused by the addition of excess water that could not be held in Celite (18).

The recoveries of propoxur and dimethoate maximized at 0.10 mL of water and then began to decrease with less than 4.0 mL of water. Similar trends were observed for bendiocarb, captafol, captan, dimethipin, fensulfothion, lenacil, metribuzin, and pirimicarb. The recovery of triadimenol increased with the addition of 0.10–0.40 mL and then decreased with the further addition of water. The pesticides that showed a similar trend to triadimenol were ethiofencarb, myclobutanil, and propiconazole. For bitertanol, the recovery increased moderately with the addition of 0.10–3.0 mL of water and decreased at 4.0 mL. Acephate, methamidophos, and propamocarb were not recovered when water was used as a modifier.

Methanol

The effect of methanol as a modifier was investigated similarly to the study for water, except the addition range of methanol was 0.10–1.0 mL. The results of acephate, methamidophos, and a few of the same pesticides that appeared in Figure 3 are shown in Figure 4. The recoveries of aldrin and azinphos-methyl maximized with the addition of 0.20 mL of methanol. Almost all of the pesticides investigated showed maximum recoveries with the addition of 0.10–0.20 mL of methanol. For triadimenol, bitertanol, acephate, and methamidophos, the highest recoveries were achieved with 0.40 mL of methanol. Similar trends were observed for fensulfothion, metribuzin, and myclobutanil. Propamocarb was not recovered by SFE using methanol as a modifier. The addition of 1.0 mL methanol caused reduced recoveries in all cases. Similar results were obtained for other pesticides and matrices using methanol-modified CO_2 (20,21). The decreases in recovery

Table IV. Effect of Modifiers on the Average Recoveries of Group A–F Pesticides in Table II from Fortified Celite by SFE*

	Average percent recovery (standard deviation)					
Modifier	**Group A**	**Group B**	**Group C**	**Group D**	**Group E**	**Group F**
None	86.1 (2.9)	85.2 (2.7)	45.4 (27)	20.5 (36)	4.2 (5.8)	undetected
n-Hexane	85.3 (2.3)	82.0 (3.6)	80.7 (4.7)	75.1 (7.5)	61.8 (10)	8.5 (16)
Dichloromethane	82.4 (3.3)	74.6 (12)	56.2 (18)	38.0 (26)	14.6 (10)	undetected
Ethyl acetate	84.8 (4.3)	80.9 (4.8)	74.9 (8.8)	66.2 (13)	48.4 (11)	2.7 (4.6)
Acetone	85.6 (2.3)	82.0 (4.0)	76.8 (4.5)	71.1 (10)	59.0 (7.4)	12.7 (12)
Acetonitrile	78.0 (5.5)	76.3 (4.9)	74.4 (6.5)	69.8 (9.2)	61.2 (10)	13.7 (14)
2-Propanol	81.0 (4.7)	82.0 (2.9)	79.4 (4.0)	77.5 (5.4)	72.9 (6.7)	39.1 (20)
Ethanol	80.5 (3.7)	81.6 (2.8)	79.9 (3.9)	78.7 (5.3)	73.1 (9.6)	52.3 (20)
Methanol	79.6 (5.7)	78.6 (4.9)	79.0 (6.6)	79.1 (3.1)	74.3 (8.4)	63.2 (22)
Water	87.7 (1.9)	87.1 (2.8)	87.3 (4.0)	86.4 (4.2)	84.5 (7.2)	47.1 (35)

* SFE conditions: CO_2 density, 0.50 g/mL; extraction temperature, 40°C; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min; 0.40 mL of each solvent was added as a modifier.

Table V. Effect of the Alcohols on Pesticide Recoveries from Fortified Celite*

	Methanol (4 replicates)		**Ethanol (3 replicates)**		**2-Propanol (3 replicates)**	
Pesticides	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Myclobutanil	64.9	19.6	50.0	8.1	31.8	23.1
Triadimenol	62.3	15.6	55.6	12.0	40.4	28.0
Lenacil	74.1	11.8	61.4	1.2	55.5	19.3
Bitertanol	54.6	21.0	60.9	15.7	31.2	24.3
Methamidophos	77.7	10.2	42.4	7.8	30.0	14.0
Acephate	74.5	9.2	38.1	16.5	13.2	87.2

* SFE conditions: CO_2 density, 0.50 g/mL; extraction temperature, 40°C; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min; 0.40 mL of each solvent was added as a modifier.

with the addition of more than 0.4 mL methanol were probably caused by the decrease in trapping efficiency due to the saturation of the ODS trap with methanol (22,26,27).

In the case of methanol, acephate and methamidophos were recovered more than 80% with the addition of 0.40 mL. Water is more polar than methanol; however, acephate and methamidophos were not recovered when water was used as a modifier. Because acephate and methamidophos are very soluble in water, they could strongly partition into the water absorbed by the Celite and could not be extracted. Moreover, because methanol is miscible with supercritical CO_2 and water is not, acephate and methamidophos could partition into methanol mixed in supercritical CO_2 and could be extracted from the Celite with CO_2 flow.

Table VI shows the recoveries of four pesticides before and after storage of fortified Celite in the extraction vessel at room temperature for 45 min with either water or methanol as a modifier. The recoveries of captafol, captan, phosmet, and chinomethionat decreased after storage with methanol, but they did not decrease when water was used as a modifier. In particular, the recoveries of captafol and captan fell below half those before storage with methanol.

As mentioned above, water showed a wide range of the optimum amount as a modifier compared with methanol, and the decrease in pesticide recovery after storage shown in methanol was not observed for water. Therefore, water was chosen as a modifier in further study despite its lower recovery of acephate and methamidophos. Moreover, 0.40 mL was chosen for the amount of water added to 2.0 g Celite because of the result for dimethoate and bitertanol (Figure 3).

Effect of CO_2 density and extraction temperature

To determine if water modifier altered the effect of CO_2 density on recoveries, experiments were performed by extracting in triplicate the pesticides from fortified Celite with 0.40 mL of water added as a modifier at variable CO_2 densities. The extraction temperature was held constant at 40°C, the CO_2 flow rate was 2.0 mL/min, the static extraction was 3.0 min, and the dynamic extraction was 20.0 min. The recoveries of all pesticides studied reached a plateau at the CO_2 density of 0.30–0.50 g/mL except dimethipin, dimethoate, lenacil, bitertanol, acephate, methamidophos, and propamocarb. The recoveries of dimethipin, dimethoate, lenacil, and bitertanol increased with increasing CO_2 density and maximized at 0.70 g/mL. Therefore, a CO_2 density of 0.70 g/mL was chosen for subsequent extractions. Acephate, methamidophos, and propamocarb were not recovered, even at 0.85 g/mL.

Similarly, to determine the effect of extraction temperature on recoveries with the addition of water as a modifier, experiments were performed by extracting the pesticides from fortified Celite with 0.40 mL of water added as a modifier in triplicate at variable extraction temperatures and a CO_2 density of 0.70 g/mL. In the SFE without modifier, the extraction temperature influenced pesticide recoveries (e.g., azinphos-methyl and dimethoate in Figure 2); however, temperature had little influence when water was added as a modifier. All pesticides studied showed nearly constant recovery (> 90%) at temperatures of 40–70°C except dimethoate, dichlorvos, triadimenol, bitertanol, acephate, methamidophos, and propamocarb. As shown in Figure 5, the recovery of dimethoate reached a plateau at an extraction temperature of at least 50°C, whereas the recovery of dichlorvos decreased slightly at 70°C, and the recoveries of triadimenol and bitertanol decreased at temperatures at and above 60°C. Therefore, an extraction temperature of 50°C was used for subsequent extractions.

Although the recoveries of acephate and methamidophos slightly increased with increasing extraction temperature, the maximum recoveries were less than 10%. Propamocarb was not recovered, even at an extraction temperature of 70°C.

Effect of static and dynamic extraction time

The SFE system used in this study can accommodate two extraction modes: static extraction mode (in which the sample is allowed to steep in CO_2 fluid) and dynamic extraction mode (in which CO_2 fluid continuously flows through the sample).

Four static extraction times (0, 3.0, 6.0, or 12.0 min) followed by 10.0 min of dynamic extraction were evaluated in triplicate. Pesticides were extracted from fortified Celite with 0.40 mL of water added as a modifier at a CO_2 density of 0.70 g/mL, 50°C extraction temperature, and 2.0 mL/min CO_2 flow rate. The length of static extraction time made no significant difference in pesticide recoveries. Similar results were obtained for the recoveries of pesticides extracted from soil (18).

The static mode, however, is often used when modifiers and derivatizing reagents are employed, especially when a modifier or derivatizing reagent is directly added to the extraction vessel prior to pressurization. Moreover, a static extraction is often done before dynamic extraction for the supercritical fluid extraction of pesticides from other matrices such as soil or agricultural products. Therefore, we used 3.0 min of static extraction time for other experiments in our study, except the experiment for the effect of CO_2 flow rate.

For dynamic extraction, four times (5.0, 10.0, 20.0, or 30.0 min) were evaluated in triplicate after 3.0 min of static extraction. These corresponded to vessel volumes swept of 1.9, 3.8, 7.5, and 11.3, respectively. Pesticides were extracted from fortified Celite with 0.40 mL of water added as a modifier, the CO_2 density was 0.70 g/mL, the

Table VI. Effect of Modifiers on the Recoveries of Captafol, Captan, Phosmet, and Chinomethionat During Storage*

Modifier	Time (min)	Recovery (%)			
		Captafol	Captan	Phosmet	Chinomethionat
Water (0.40 mL)	0	93.5	87.2	88.9	88.2
Water (0.40 mL)	45	99.0	84.2	87.4	86.6
Methanol (0.20 mL)	0	69.8	81.8	90.2	90.4
Methanol (0.20 mL)	45	24.6	39.3	62.0	77.0

* Samples were kept at room temperature for 45 min in an extraction vessel before performing SFE. SFE conditions: CO_2 density, 0.50 g/mL; extraction temperature, 40°C; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min.

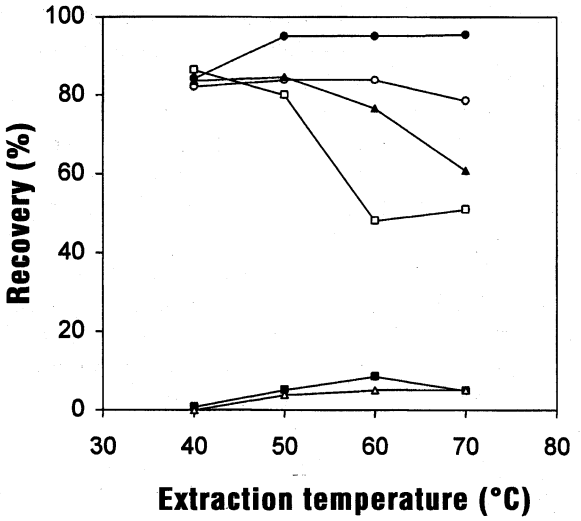

Figure 5. Effect of extraction temperature on the recoveries of six pesticides from fortified Celite by SFE using 0.40 mL of water as a modifier: dimethoate (●), dichlorvos (O), triadimenol (▲), bitertanol (□), methamidiphos (■), and acephate (△). SFE conditions: CO_2 density, 0.70 g/mL; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min.

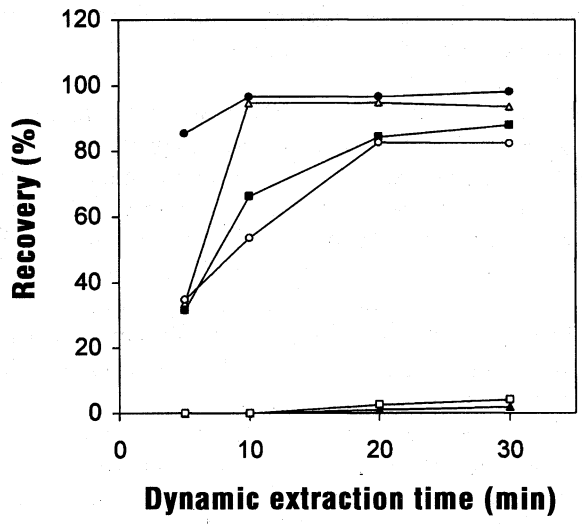

Figure 6. Effect of dynamic extraction time on the recoveries of six pesticides from fortified Celite by SFE using 0.40 mL of water as a modifier: aldrin (●), bitertanol (△), dimethoate (■), dichlorvos (O), acephate (▲), and methamidophos (□). SFE conditions: CO_2 density, 0.70 g/mL; extraction temperature, 50°C; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min.

extraction temperature was 50°C, and the CO_2 flow rate was 2.0 mL/min. The results for some of the pesticides are shown in Figure 6. The recoveries of aldrin and bitertanol reached a plateau at a dynamic extraction time of 10.0 min. All other pesticides studied gave similar results except dimethoate, dichlorvos, acephate, methamidophos, and propamocarb. Dimethoate and dichlorvos required 20.0 min; however, the recoveries of acephate and methamidophos were very low (< 5%), even at 30.0 min, and propamocarb was not recovered. Therefore, a dynamic extraction time of 20.0 min was chosen.

Effect of CO_2 flow rate

Experiments were performed in triplicate at CO_2 flow rates of 1.0, 2.0, 3.0, and 4.0 mL/min. In order to keep the total amount of CO_2 (6.8 vessel volumes swept) pumped through the extraction vessel equal at each flow rate, the dynamic extraction times were varied to be 36.0, 18.0, 12.0, and 9.0 min, respectively. Pesticides were extracted from fortified Celite added with 0.40 mL of water as a modifier at a CO_2 density of 0.70 g/mL and an extraction temperature of 50°C. In these experiments, no static extraction was used. The overall average recovery of the 88 pesticides at CO_2 flow rates of 1.0, 2.0, 3,0, and 4.0 mL/min were 93.0% (relative standard deviation [RSD], 2.6%), 92.5% (RSD, 2.3%), 93.1% (RSD, 2.9%), and 92.3% (RSD, 3.4%), respectively. A CO_2 flow rate of 2.0 mL/min gave the smallest RSD value, whereas 4.0 mL/min gave a slightly higher RSD value. However, there were no significant differences in the overall average recovery and precision of the 88 pesticides at each CO_2 flow rate evaluated. Similarly, no significant differences in recovery and precision were shown for each pesticide.

In general, a lower flow rate results in a lower linear velocity and usually increases the extraction efficiency as a result of an extended contact between the supercritical fluid and the analytes. Moreover, a lower flow rate increases the trapping efficiency of analytes at the analyte trap. Therefore, we selected 2.0 mL/min as a CO_2 flow rate. From these results and the previous study, we selected 3.0 min of static extraction and 20.0 min of dynamic extraction at a CO_2 flow rate of 2.0 mL. If one used these conditions, total extraction time would be 23.0 min, and total time for extraction–elution per sample, including the time to achieve proper pressure and temperature conditions and to elute the pesticides from the analyte trap, would be approximately 45 min.

Effect of trap temperature

Four temperatures (10, 20, 30, and 40°C) were evaluated to optimize the trap temperature. Pesticides were extracted from fortified Celite added with 0.40 mL of water as a modifier at a CO_2 density of 0.70 g/mL, 50°C extraction temperature, 2.0 mL/min CO_2 flow rate, 3.0 min of static extraction, and 20.0 min of dynamic extraction. No significant differences in recoveries were observed at each trap temperature evaluated. The recovery of dichlorvos, which is the most volatile pesticide in this study, was not affected by trap temperatures. Therefore, a trap temperature of 30°C was chosen because it required less CO_2 to cool the trap.

Recovery from Celite

Recoveries of 88 pesticides from fortified Celite by the optimized SFE method and by the same SFE method without modifier are shown in Table VII. Pesticides that were not recovered without modifier (bitertanol, dimethipin, lenacil, mefenacet, myclobutanil, propiconazole, triadimenol, dimethoate, and fensulfothion) had recoveries greater than 90% (88.9% for bitertanol) when water was added as a modifier. Acephate,

Table VII. Recoveries of 88 Pesticides from Fortified Celite using the SFE and GC–MS (SIM) Method*

Pesticide	$-H_2O$† (2 replicates) Recovery (%)	$+H_2O$‡ (3 replicates) Recovery (%) (RSD [%])	Pesticide	$-H_2O$† (2 replicates) Recovery (%)	$+H_2O$‡ (3 replicates) Recovery (%) (RSD [%])
Organochlorine			Organophosphate		
Aldrin	91.7	98.9 (4.1)	Acephate	undetected	1.0 (15)
α-BHC	91.8	96.3 (3.3)	Azinphos-ethyl	21.9	100.1 (2.2)
β-BHC	90.0	96.1 (1.9)	Azinphos-methyl	25.5	100.3 (4.4)
γ-BHC	89.3	96.5 (1.7)	Bromophos-ethyl	90.9	97.8 (4.3)
δ-BHC	91.7	98.3 (3.8)	(E)-Chlorfenvinphos	77.9	99.4 (2.1)
Captafol	34.9	85.3 (9.8)	(Z)-Chlorfenvinphos	70.7	95.9 (2.2)
Captan	55.6	82.4 (14)	Chlorpyrifos	97.2	97.4 (3.5)
Chlorobenzilate	47.4	100.3 (2.7)	Chlorpyrifos-methyl	85.5	96.5 (3.2)
p,p'-DDD	86.0	101.5 (2.4)	Diazinon	89.2	96.2 (3.0)
p,p'-DDE	93.6	96.9 (3.3)	Dichlorvos	66.9	81.9 (4.0)
o,p'-DDT	77.1	96.3 (3.3)	Dimethoate	undetected	92.2 (4.4)
p,p'-DDT	72.4	96.2 (3.3)	Dioxabenzofos	86.1	94.1 (1.1)
Dieldrin	92.2	98.6 (4.6)	Disulfoton	90.7	95.3 (5.0)
Endrin	84.3	98.9 (3.2)	Edifenphos	39.3	94.0 (4.3)
Heptachlor	84.4	97.4 (4.0)	EPN	78.8	97.0 (2.8)
Heptachlor epoxide	95.0	98.8 (2.8)	Ethoprophos	81.0	95.1 (1.5)
			Etrimfos	90.2	98.1 (1.5)
Pyrethroid			Fenitrothion	78.6	98.8 (1.1)
Cyfluthrin	71.7	94.1 (2.8)	Fensulfothion	undetected	100.7 (1.7)
Cyhalothrin	74.6	96.5 (2.3)	Fenthion	91.1	99.4 (0.8)
Cypermethrin	79.0	94.3 (1.9)	Malathion	78.9	97.0 (4.2)
Deltamethrin	82.3	98.5 (1.5)	Methamidophos	undetected	2.0 (32)
Fenvalerate	83.3	94.4 (1.7)	Methidathion	78.6	95.9 (3.3)
Flucythrinate	80.3	96.3 (2.9)	Parathion	80.6	95.8 (3.3)
Fluvalinate	76.4	94.7 (1.5)	Parathion-methyl	79.8	99.6 (4.1)
Permethrin	85.5	95.0 (1.9)	Phenthoate	80.1	98.8 (2.2)
			Phosalone	75.9	100.5 (2.1)
Other			Phosmet	38.8	98.2 (3.3)
Amitraz	49.8	74.0 (2.5)	Pirimiphos-methyl	89.2	97.2 (2.0)
Benalaxyl	32.4	96.3 (2.5)	Prothiofos	88.0	95.1 (2.5)
Bitertanol	undetected	88.9 (9.6)	Quinalphos	88.0	98.2 (1.5)
Chinomethionat	87.9	93.4 (3.2)	Terbufos	82.5	97.7 (3.1)
Dichlofluanid	95.1	97.5 (4.3)	Thiometon	91.5	94.4 (3.6)
Dimethipin	undetected	97.9 (0.3)			
Flutolanil	40.8	100.4 (2.5)	Carbamate		
Lenacil	undetected	99.5 (1.0)	Bendiocarb	77.8	93.5 (4.5)
Mefenacet	undetected	98.4 (0.5)	Chlorpropham	89.6	93.4 (1.8)
Mepronil	63.3	89.8 (9.3)	Diethofencarb	55.0	98.2 (2.0)
Methoprene	87.3	94.1 (2.0)	Esprocarb	91.8	97.8 (0.6)
Metribuzin	38.6	95.4 (4.2)	Ethiofencarb	45.7	91.9 (4.7)
Myclobutanil	undetected	99.6 (1.2)	Fenobucarb	93.0	98.2 (3.0)
Pendimethalin	82.0	95.0 (2.8)	Isoprocarb	94.6	95.7 (1.0)
Pretilachlor	53.7	95.2 (3.2)	Methiocarb	81.0	97.8 (1.5)
Propiconazole	undetected	97.7 (2.9)	Pirimicarb	20.2	98.3 (1.2)
Pyridaben	84.6	97.8 (1.4)	Propamocarb	undetected	undetected
Triadimefon	42.2	100.5 (4.6)	Propoxur	63.1	95.9 (3.2)
Triadimenol	undetected	92.5 (3.0)	Thiobencarb	91.6	94.1 (1.0)

* SFE conditions: CO_2 density, 0.70 g/mL; extraction temperature, 50°C; CO_2 flow rate, 2.0 mL/min; static extraction, 3.0 min; dynamic extraction, 20.0 min; trap temperature, 30°C. Spiking level was 0.5 µg/g (2.5 µg/g for acephate, captafol, methamidophos, and propamocarb).
† Without modifier.
‡ 0.40 mL of water was directly added to Celite as a modifier.

methamidophos, and propamocarb were not extracted, even in the presence of modifier. The optimized method gave recoveries greater than 90% for 79 pesticides; 80–90% recoveries for captafol, captan, bitertanol, mepronil, and dichlorvos; 74.0% recovery for amitraz; and 1–2% recoveries for acephate and methamidophos. RSDs of the recoveries of almost all of the pesticides were very small (< 5%). The large RSDs of captafol (9.8%) and captan (13.9%) were probably caused by their thermal decomposition in GC–MS.

Conclusion

The effects of SFE parameters on the extractability of pesticides were studied for multiresidue analysis of pesticides. Celite was used as a matrix in order to simplify the interaction between the pesticides and the matrix. The evaluated parameters were the type and amount of modifier, the density and flow rate of CO_2 fluid, extraction temperature, static and dynamic extraction time, and trap temperature. In general, the recoveries of pesticides increased with increasing CO_2 density and/or extraction temperature. SFE without modifier was insufficient to extract all 88 pesticides of various polarities. The addition of modifier was useful in improving the recovery. In particular, the modifiers that had –OH groups such as water, methanol, ethanol, and 2-propanol were effective. Water was chosen as a modifier because of its wide range of optimum amount added to Celite and lack of recovery loss with captafol, captan, phosmet, and chinomethionat, as shown in methanol.

The optimum SFE conditions to extract the pesticides from Celite (2.0 g) were as follows: 0.70 g/mL CO_2 density, 50°C extraction temperature, 2.0 mL/min CO_2 flow rate, 0.4 mL of water as a modifier, 3.0 min of static extraction, 20.0 min of dynamic extraction, and 30°C trap temperature. Total time for extraction per sample was approximately 45 min.

Recoveries of pesticides from fortified Celite by the optimized SFE method were greater than 90% for 79 pesticides and 70–90% for 6 pesticides; RSDs of the recoveries for almost all of the pesticides were less than 5%. These results demonstrate that SFE gives a promising method of extraction for the multiresidue analysis of pesticides. This study for the extractability of pesticides from Celite is helpful in considering SFE of pesticides from other matrices.

Acknowledgment

The authors are grateful to Steven J. Lehotay of the U.S. Department of Agriculture for reviewing the manuscript. We also thank Hewlett-Packard for the use of the 7680T SFE instrument.

References

1. B.E. Richter. *Emerging Strategies for Pesticide Analysis,* T. Cairns & J. Sherma, Eds., CRC Press, Boca Raton, FL, 1992, pp. 51–68.
2. J.W. King and M.L. Hopper. Analytical supercritical fluid extraction: Current trends and future vistas. *J. AOAC Int.* **75:** 375–78 (1992).
3. V. Camel, A. Tambuté, and M. Caude. Analytical-scale supercritical fluid extraction: A promising technique for the determination of pollutants in environmental matrices. *J. Chromatogr.* **642:** 263–81 (1993).
4. V. Janda, K.D. Bartle, and A.A. Clifford. Supercritical fluid extraction in environmental analysis. *J. Chromatogr.* **642:** 283–99 (1993).
5. V. Janda, G. Steenbeke, and P. Sandra. Supercritical fluid extraction of s-triazine herbicides from sediment. *J. Chromatogr.* **479:** 200–205 (1989).
6. E.G. van der Velde, W. de Haan, and A.K.D. Liem. Supercritical fluid extraction of polychlorinated biphenyls and pesticides from soil. *J. Chromatogr.* **626:** 135–43 (1992).
7. M.A. Locke. Supercritical CO_2 fluid extraction of fluometuron herbicides from soil. *J. Agric. Food Chem.* **41:** 1081–84 (1993).
8. K. Wuchner, R.T. Ghijsen, U.A.T. Brinkman, R. Grob, and J. Mathieu. Extraction of organophosphorus pesticides from soil by off-line supercritical fluid extraction. *Analyst* **118:** 11–16 (1993).
9. T.M. Fahmy, M.E. Paulaitis, D.M. Johnson, and M.E.P. McNally. Modifier effects in the supercritical fluid extraction of solutes from clay, soil, and plant materials. *Anal. Chem.* **65:** 1462–69 (1993).
10. J.W. King, M.L. Hopper, R.G. Luchtefeld, S.L. Taylor, and W.L. Orton. Optimization of experimental conditions for the supercritical carbon dioxide extraction of pesticide residues from grains. *J. AOAC Int.* **76:** 857–64 (1993).
11. J.W. King, J.M. Snyder, S.L. Taylor, J.H. Johnson, and L.D. Rowe. Translation and optimization of supercritical fluid extraction methods to commercial instrumentation. *J. Chromatogr. Sci.* **31:** 1–5 (1993).
12. A.L. Howard, C. Braue, and L.T. Taylor. Feasibility of thiocarbamate pesticide analysis in apples by supercritical fluid extraction and high-performance liquid chromatography. *J. Chromatogr. Sci.* **31:** 323–29 (1993).
13. Z.V. Skopec, R. Clark, P.M.A. Harvey, and R.J. Wells. Analysis of organophosphorus pesticides in rice by supercritical fluid extraction and quantitation using an atomic emission detector. *J. Chromatogr. Sci.* **31:** 445–49 (1993).
14. S.J. Lehotay and M.A. Ibrahim. Supercritical fluid extraction and gas chromatography/ion trap mass spectrometry of pentachloronitrobenzene pesticides in vegetable. *J. AOAC Int.* **78:** 445–52 (1995).
15. A. Valverde-García, A.R. Fernández-Alba, A. Agüera, and M. Contreras. Extraction methamidophos residues from vegetables with supercritical fluid carbon dioxide. *J. AOAC Int.* **78:** 867–73 (1995).
16. V. Lopez-Avila, N.S. Dodhiwala, and W.F. Beckert. Supercritical fluid extraction and its application to environmental analysis. *J. Chromatogr. Sci.* **28:** 468–76 (1990).
17. M.L. Hopper and J.W. King. Enhanced supercritical fluid carbon dioxide extraction of pesticides from foods using pelletized diatomaceous earth. *J. Assoc. Off. Anal. Chem.* **74:** 661–66 (1991).
18. J.L. Snyder, R.L. Grob, M.E. McNally, and T.S. Oostdyk. The effect of instrumental parameters and soil matrix on the recovery of organochlorine and organophosphate pesticides from soils using supercritical fluid extraction. *J. Chromatogr. Sci.* **31:** 183–91 (1993).
19. S.J. Lehotay and K.I. Eller. Development of a method of analysis for 46 pesticides in fruits and vegetables by supercritical fluid extraction and gas chromatography/ion trap mass spectrometry. *J. AOAC Int.* **78:** 821–30 (1995).
20. N. Aharonson, S.J. Lehotay, and M.A. Ibrahim. Supercritical fluid extraction and HPLC analysis of benzimidazole fungicides in potato, apple, and banana. *J. Agric. Food Chem.* **42:** 2817–23 (1994).

21. R. Alzaga, J.M. Bayona, and D. Barceló. Use of supercritical fluid extraction for pirimicarb determination in soil. *J. Agric. Food Chem.* **43:** 395–400 (1995).
22. A.L. Howard and L.T. Taylor. Considerations for analytical supercritical fluid extraction of sulfonyl ureas employing a modified fluid. *J. High Res. Chromatogr.* **16:** 39–45 (1993).
23. Ashraf-Khorassani and L.T. Taylor. Comparison of modifier addition to the matrix versus modifier addition to the fluid in the supercritical fluid extraction of PCBs from river sediment. *Am. Lab.* **December:** 23–28 (1995).
24. J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, and J. Pawliszyn. Role of modifiers for analytical-scale supercritical fluid extraction of environmental samples. *Anal. Chem.* **66:** 909–916 (1994).
25. T.S. Oostdyk, R.L. Grob, J.L. Snyder, and M.E. McNally. Optimization of the supercritical fluid extraction of primary aromatic amines. *J. Chromatogr. Sci.* **31:** 177–82 (1993).
26. L.J. Mulcahey and L.T. Taylor. Collection efficiency of solid surface and sorbent traps in supercritical fluid extraction with modified carbon dioxide. *Anal. Chem.* **64:** 2352–58 (1992).
27. L.T. Taylor. *Supercritical Fluid Extraction.* John Wiley & Sons, New York, NY, 1996, pp. 84–100.
28. L.R. Snyder. Classification of the solvent properties of common liquids. *J. Chromatogr. Sci.* **16:** 223–34 (1978).
29. H. Honma. *High Performance Liquid Chromatography Handbook.* Maruzen, Tokyo, Japan, 1985, pp. 103–108.

Manuscript accepted April 29, 1997.