

Developments in the Supercritical Fluid Extraction of Chlorophenoxy Acid Herbicides from Soil Samples

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Extraction of chlorophenoxy acid herbicides from soil samples with supercritical carbon dioxide as extractant and tetrabutylammonium hydroxide and methyl iodide as derivatization agents was investigated. The extraction was carried out at 400 atm and 80 °C for 15 min static, followed by 15 min dynamic, at a carbon dioxide flow rate of approximately 1.5 mL/min (as liquid). The use of other derivatization agents (trimethylphenylammonium hydroxide, benzyltrimethylammonium chloride, and benzyltriethylammonium chloride) proved to be less effective than the tetrabutylammonium hydroxide/methyl iodide combination. Attempts to extract other compounds currently listed in the EPA SW-846 Method 8151 using supercritical carbon dioxide and tetrabutylammonium hydroxide/methyl iodide were unsuccessful, either because these compounds did not derivatize (dalapon, dinoseb, and 4-nitrophenol) or because decomposition occurred (DCPA diacid, pentachlorophenol, and picloram), apparently in the injection port of the gas chromatograph. An in-situ derivatization and supercritical fluid extraction of the chlorophenoxy acids with pentafluorobenzyl bromide/triethylamine reagent also proved to be feasible for qualitative determination of these compounds.

INTRODUCTION

Supercritical fluid extraction (SFE) is becoming recognized as an efficient and faster alternative to Soxhlet extraction, but most published studies on sample extraction by SFE address extraction of nonpolar organic compounds, such as polynuclear aromatic hydrocarbons, polychlorinated biphenyls, and polychlorinated dibenzodioxins. Recently, Hawthorne et al. (1992) reported the extraction and methylation of 2,4-D and dicamba from sediment samples using supercritical fluid derivatization/extraction with trimethylphenylammonium hydroxide (TMPA) or BF₃/methanol as derivatizing agents. While the derivatization with TMPA is known to require relatively high temperatures, Hawthorne and co-workers claimed that the derivatization reaction of 2,4-D takes place during the SFE with carbon dioxide at 400 atm and 80 °C. They based their conclusion on findings that increasing the concentration of TMPA during SFE resulted in increased recoveries of 2,4-D, while adding more TMPA to the extracts after SFE did not. The derivatization with BF₃/methanol under supercritical conditions was demonstrated for 2,4-D; however, dicamba was not methylated under these conditions (Hawthorne et al., 1992).

SFE with carbon dioxide under various derivatization conditions [silylation, BF₃/methanol derivatization, ion-pairing with (*m*-trifluoromethylphenyl)trimethylammonium hydroxide] and soil treatment procedures were investigated by Rochette et al. (1993). Ionic displacement of 2,4-acid with calcium chloride was found to be the most promising approach for quantitative SFE.

Other works in which derivatization reactions were combined with SFE were published by Hills et al. (1991), who combined silylation reactions with SFE to extract oxalic acid, dicarboxylic acids, and alcohols from roasted

coffee; Lee and Peart (1992), who investigated the in-situ derivatization of resin and fatty acids from sediments collected at pulp mill sites with α -bromo-2,3,4,5,6-pentafluorotoluene (PFBBR) and triethylamine (TEA); Lee et al. (1992), who developed an SFE method in which phenolic compounds were acetylated under static conditions with acetic anhydride in the presence of TEA; and Field et al. (1992), who reported an ion-pair SFE and derivatization GC/MS technique using tetrabutylammonium hydroxide for quantitative determination of sulfonated aliphatic and aromatic surfactants in sewage sludge.

The purpose of our study was to develop an SFE method for extracting the seven chlorophenoxy acid herbicides listed in Table I from soils using supercritical carbon dioxide. It was desirable that the extraction and the derivatization reaction be occurring simultaneously since the chlorophenoxy acid herbicides must be converted to their corresponding esters prior to analysis by gas chromatography.

The use of several derivatization agents [TMPA, benzyltrimethylammonium chloride (BTMAC), benzyltriethylammonium chloride (BTEAC), and tetrabutylammonium hydroxide/methyl iodide (TBA/MI)] in SFE was investigated. The extractions were carried out with supercritical carbon dioxide or with supercritical carbon dioxide modified with 10% methanol. Attempts were made to extract other compounds currently listed in EPA SW-846 Method 8151 using supercritical carbon dioxide and TBA/MI. An in-situ derivatization and SFE of the chlorophenoxy acids with PFBBR/TEA reagent was also performed.

EXPERIMENTAL PROCEDURES

The seven chlorophenoxy acid herbicides (Table I), except dicamba, and dichlorprop and 2,4,5-TP were purchased from Aldrich Chemical Co. (Milwaukee, WI); dicamba was purchased

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Table I. Retention Times, Internal Standards, and Quantitation Ions of the Methyl Esters

compd no.	compd name	chemical name	RT, min	IS used for quantitation ^a	quantitation ion, ^b <i>m/z</i>	five most intense ions in the mass spectrum, <i>m/z</i> (relative abundance)
1	dicamba	3,6-dichloro-2-methoxybenzoic acid	8.38	IS-1	203	203 (100), 205 (70), 234 (25), 188 (25), 205 (20)
2	MCPPP	2-(4-chloro-2-methylphenoxy)propionic acid	9.14	IS-1	169	169 (100), 142 (95), 228 (88), 107 (58), 141 (42)
3	MCPA	4-chloro-2-methylphenoxyacetic acid	9.54	IS-1	214	214 (100), 141 (90), 155 (56), 125 (56), 77 (53)
4	2,4-D	2,4-dichlorophenoxyacetic acid	10.97	IS-1	199	199 (100), 234 (74), 175 (64), 45 (54), 236 (46)
5	2,4,5-T	2,4,5-trichlorophenoxyacetic acid	14.43	IS-2	233	233 (100), 235 (64), 268 (60), 270 (58), 45 (49)
6	MCPB	4-(4-chloro-2-methylphenoxy)butyric acid	14.23	IS-2	101	101 (100), 59 (98), 77 (19), 41 (16), 69 (16)
7	2,4-DB	4-(2,4-dichlorophenoxy)butyric acid	15.79	IS-2	101	101 (100), 59 (98), 162 (20), 41 (20), 69 (18)
IS-1	acenaphthene- <i>d</i> ₁₀		7.76		164	
IS-1	phenanthrene- <i>d</i> ₁₀		13.49		188	

^a The concentrations of the internal standards (IS) were 20 ng/ μ L. ^b The quantitation ions recommended in this method are those for the corresponding methyl esters of the chlorophenoxy acid herbicides.

from Chem Service, Inc. (West Chester, PA). The corresponding methyl esters were purchased from Crescent Chemical (Hauppauge, NY). All compounds were used as received without further purification (their purities were stated to be at least 98%, except MCPA at 95% and 2,4-DB at 97%). Stock solutions of the individual acids or esters were prepared in methanol at 10 mg/mL and kept at 4 °C in the dark. A spiking solution of the chlorophenoxy acids was made by combining portions of the individual stock solutions and diluting them to 1 mg/mL with methanol.

Derivatizing agents were purchased as solutions in methanol or as neat materials. TMPA (1.5% in methanol) was purchased from Eastman Kodak Co. (Rochester, NY) and Fluka Chemical Co. (Ronkonkoma, NY). To prepare 10% (w/v) TMPA in methanol, ethanol, or 2-propanol, we evaporated 20 mL of 1.5% TMPA in methanol to dryness under a gentle stream of nitrogen and dissolved the residue in 3 mL of methanol, ethanol, or 2-propanol. BTMAC and BTEAC (purity 98%) were purchased as neat materials from Fluka. To prepare 40% solutions, 40 g of each was dissolved separately in 100 mL of methanol. TBA (25% solution in methanol) and MI (neat) were purchased from Fluka. PFBBR and TEA were purchased from Sigma Chemical Co. (St. Louis, MO) and Chem Service, respectively. A fresh 5% PFBBR solution in acetone was prepared weekly by dissolving 1 g of PFBBR into 20 mL of acetone.

SFC/SFE grade carbon dioxide with a helium head pressure of 2000 psi was obtained from Air Products and Chemicals, Inc. (Allentown, PA). SFC grade carbon dioxide modified with 5% methanol with a helium head pressure of 1500 psi was obtained from Scott Specialty Gases (Plumsteadville, PA).

The two soil samples used in this study, which were obtained from Sandoz Crop Protection Division (Gilroy, CA), were a topsoil (58% sand, 22% silt, 20% clay; pH 7.5; moisture content 2.6%; organic carbon content 0.1%) and a clay soil (34% sand, 35% silt, 31% clay; pH 7.4; moisture content 10.6%; organic carbon content 1.8%). The composition of the sand samples was not known.

For spiking the soil and the sand matrices, 2-g matrix portions were weighed into an aluminum cup, and a concentrated stock solution containing the chlorophenoxy acid herbicides in methanol (concentration 1 μ g/ μ L) was added with a syringe; adsorption losses were minimized by ensuring that the solution did not contact the aluminum cup. Mixing was performed with the tip of a disposable glass pipet. After the solvent had evaporated (approximately 15 min), the spiked sample was transferred to the extraction vessel. The derivatizing agent (e.g., TMPA, BTMAC, BTEAC, or TBA/MI) was added to the sample in the extraction vessel; the reagent volume and its concentration varied as noted in the tables. The extracted material was collected in 5 mL of methanol (B&J Brand High Purity Solvent, Burdick & Jackson, Muskegon, MI).

In the case of the PFBBR/TEA experiments, 5-g portions of the topsoil sample were spiked with the chlorophenoxy acids in the aluminum cup as described above. Following spiking, we added either 250 μ L of PFBBR (5% in acetone) and 50 μ L of neat TEA or 1000 μ L of PFBBR (5% in acetone) and 200 μ L of neat TEA. The topsoil was allowed to dry at room temperature and was then loaded into an extraction vessel.

The extractions with TMPA, BTMAC, BTEAC, and TBA/MI were performed using an Isco (Lincoln, NE) SFE System 1100 with an Isco Model 100D syringe pump and an Isco Model SFX 2-10 extraction module with two extraction vessels. The extraction module has shutoff valves at the inlet and outlet of the extraction vessel, allowing static and dynamic extractions to be performed. The SFE system was equipped with two fused-silica restrictors (37-cm length \times 50 μ m i.d.), which resulted in flow rates of approximately 1.5 mL/min (as liquid carbon dioxide) per vessel. All extractions were carried out at 400 atm; the extraction temperature was varied (80, 100 °C), and the extraction times were 15 min static followed by 15 min dynamic. The static step was included to allow longer interaction of TBA solution and MI with the matrix. The extracted analytes were collected in 2 mL of methanol. Blowdown evaporation was not used because earlier blowdown evaporation experiments resulted in low recoveries.

The experiments with PFBBR/TEA were performed on a Hewlett-Packard (Wilmington, DE) SFE system Model 7680T using supercritical carbon dioxide alone. All extractions were carried out at 5400 psi and a chamber temperature of 80 or 120 °C, for 30 min static and 30 min dynamic. The flow rate of the carbon dioxide was 3 mL/min (as liquid). The nozzle temperature was the same as the chamber temperature (80, 120 °C), and the trap temperature was 15 °C. The trap was packed with octadecyl-bonded silica (Hewlett-Packard). The extracted material was removed from the trap with two 1.5-mL acetone rinses. No compounds were detected in the second rinse. The trap temperature during the rinse step was 45 °C.

All extracts except those from the PFBBR/TEA experiments were analyzed with a Hewlett-Packard 5890 Series II gas chromatograph equipped with a Model 5971A mass spectrometer and a Hewlett-Packard DOS Chemstation. The injections were performed in the splitless mode (valve time 1 min) into a 30-m length \times 0.25-mm i.d., 0.25- μ m film thickness, PTE-5 fused-silica capillary column. Helium was used as carrier gas at a flow rate of 0.87 mL/min. The injector temperature was maintained at 250 °C. The oven temperature was programmed from 80 to 140 °C at 20 °C/min and then to 250 °C (5-min hold) at 4 °C/min. The mass spectrometer was scanned from 40 to 600 amu at a scan rate of 1 s. Acenaphthene-*d*₁₀ and phenanthrene-*d*₁₀ were used as internal standards. Figures 1 and 2 show GC/MS chromatograms of a composite standard containing the methyl esters of the chlorophenoxy acid herbicides and of a composite mixture

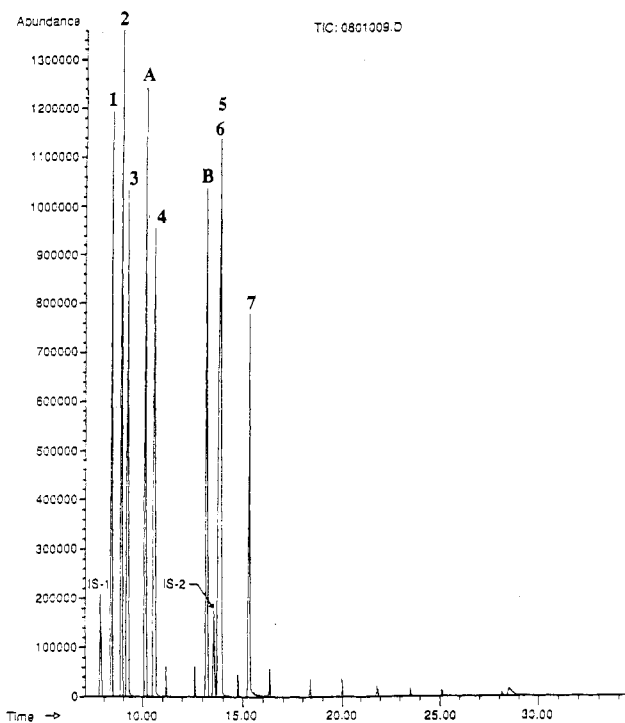


Figure 1. GC/MS chromatogram of the composite standard containing the methyl esters of the chlorophenoxy acid herbicides. The GC/MS operating conditions are given under Experimental Procedures. Peaks A and B are compounds 8 and 9 in Table VII.

of the chlorophenoxy acid herbicides derivatized with TBA/MI, respectively. Attempts were made to analyze the extracts from the TBA/MI experiments by GC with electron capture detection (ECD); however, the presence of excess derivatizing reagent (MI) in the extracts interfered with the GC/ECD determination. Quantification of the analytes was performed by internal standard calibration using acenaphthene-*d*₁₀ and phenanthrene-*d*₁₀ (Table I). The retention times (minutes), quantitation ions, and five most intense ions in the mass spectra of the methyl esters are included in Table I.

The extracts from the PFBBR/TEA experiments were analyzed with a Hewlett-Packard 5890 Series II gas chromatograph equipped with an electron capture detector and a Hewlett-Packard DOS Chemstation. The injections were performed in the splitless mode (valve time 1 min) into a 15-m length \times 0.53-mm i.d., 0.88- μ m film thickness, HP-5 fused-silica open tubular column. Helium at a flow rate of 7.6 mL/min was used as carrier gas; nitrogen at a flow rate of 19.7 mL/min was used as the makeup gas. The injector and detector temperatures were maintained at 250 and 320 $^{\circ}$ C, respectively. The oven temperature was programmed from 60 (1-min hold) to 160 $^{\circ}$ C at 10 $^{\circ}$ C/min and then to 275 $^{\circ}$ C (5-min hold) at 4 $^{\circ}$ C/min. Quantification of the analytes was performed by external standard calibration.

RESULTS AND DISCUSSION

Extraction without Derivatizing Agent. Preliminary SFE work done in our laboratory with supercritical carbon dioxide at 400 atm and 80 $^{\circ}$ C (30 min in the dynamic mode) failed to recover 2,4-D from sand and topsoil samples spiked at low microgram per gram levels. When the experiments were performed at different pressure/temperature conditions and higher spike levels, we recovered some of the seven spiked chlorophenoxy acids, but their recoveries were inconsistent. For example, the recovery of 2,4-DB was 37% in one experiment but only 7.1% in another one; for 2,4,5-TP, the recovery was 54% in one experiment but only 6.7% in another one. The extraction conditions were 150 atm for 10 min, 200 atm for another 10 min, and 250 atm for an additional 10 min; the extraction temperature was 70 $^{\circ}$ C; the extractions were performed

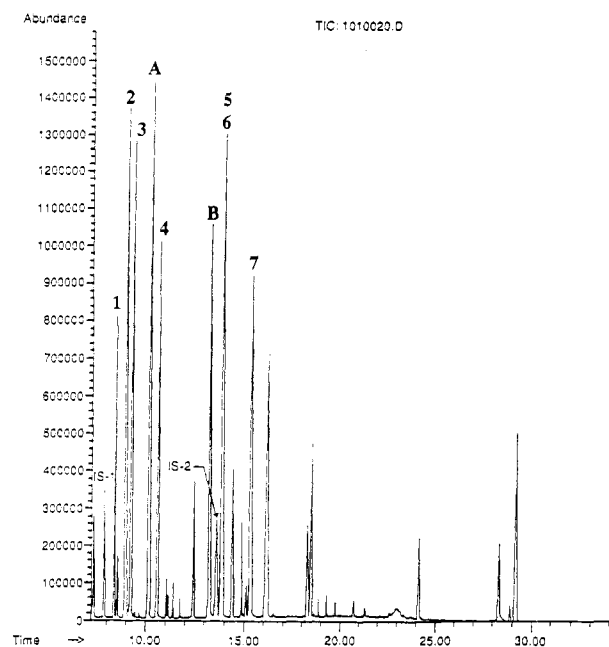


Figure 2. GC/MS chromatogram of the chlorophenoxy acid herbicides derivatized to their corresponding methyl esters upon injection into a GC/MS system (injector temperature 250 $^{\circ}$ C). The GC/MS operating conditions are given under Experimental Procedures. Peaks A and B are compounds 8 and 9 in Table VII.

in the dynamic mode; a 30-cm length \times 50- μ m i.d. fused-silica restrictor heated at 100 $^{\circ}$ C was used, which resulted in a flow rate of approximately 1.5 mL/min as compressed fluid. Another researcher, Richards (1991, Dow Chemical, personal communication), has reported similar results. Thus, we focused our study on establishing conditions under which these chlorophenoxy acid herbicides could be extracted from a solid matrix and, at the same time, derivatized to their corresponding methyl esters.

Extraction in the Presence of Selected Derivatizing Agents (TMPA, BTMAC, BTEAC). Solutions of BTMAC and BTEAC in methanol were selected as methylating reagents on the basis of data published by Li et al. (1991a,b), who reported that the chlorophenoxy acid herbicides can be extracted from soil samples and esterified in situ to their corresponding methyl esters using BTMAC (this work was done using liquid solvents, not supercritical fluids). The authors further stated that the relatively low pH of 4.8 of the BTMAC solution appeared to favor formation and stabilization of the methyl esters.

TMPA in methanol was selected as methylating reagent on the basis of results published by Hawthorne et al. (1992), who reported that 2,4-D and dicamba could be extracted from a spiked soil sample as their methyl esters (the extraction was carried out at 400 atm and 80 $^{\circ}$ C for 5–45 min static, followed by 5–15 min dynamic; the amount of reagent per 2-g sample spiked with the herbicides at 20 μ g/g levels varied from 50 to 1000 μ L).

Our data (Table II) indicate low recoveries of the derivatized chlorophenoxy acid herbicides from sand when BTMAC and BTEAC were used as derivatizing reagents at either 80 or 150 $^{\circ}$ C. In contrast, recoveries between 63 and 96% were achieved with TMPA for six of the compounds investigated. When we used BTMAC or BTEAC, we detected not only the benzyl esters but also the methyl and ethyl esters, respectively. Figure 3 shows a GC/MS chromatogram of a composite acid standard containing BTMAC (which reacted with the acids in the GC inlet at 250 $^{\circ}$ C). Compounds 1–7 in Figure 3 are the methyl esters of the compounds listed in Table I; com-

Table II. Percent Recoveries of the Chlorophenoxy Acid Herbicides (as Methyl Esters) from Spiked Sand by SFE at 80 and 150 °C with Carbon Dioxide Using Selected Derivatizing Agents^a

compd no.	compd name	BTMAC ^b	BTEAC ^c	TMPA ^d
Temperature, 80 °C				
1	dicamba	1.8	ND ^e	89.1
2	MCPP	8.8	11.7	71.5
3	MCPA	14.8	ND	85.6
4	2,4-D	15.1	10.4	71.4
5	2,4,5-T	4.3	8.8	ND
6	MCPB	13.1	4.3	79.7
7	2,4-DB	11.0	7.8	68.9
Temperature, 150 °C				
1	dicamba	6.0	ND	73.5
2	MCPP	12.2	21.8	65.7
3	MCPA	27.3	47.9	73.5
4	2,4-D	29.1	39.4	62.9
5	2,4,5-T	27.8	27.2	13.4
6	MCPB	11.5	9.1	95.7
7	2,4-DB	11.4	10.3	79.1

^a The extractions were performed at 400 atm/80 °C/15 min static, followed by 15 min dynamic, with the reagents added to the sample in the extraction vessel. Extraction vessel volume: 10 mL for BTMAC and BTEAC experiments and 2.5 mL for TMPA experiments. The sample size was 2 g, and the spike level was 250 µg/g per compound. The extracted material was collected in 2 mL of methanol and adjusted to 5 mL for GC/MS analysis. Single determinations. ^b The volume of reagent was 3 mL of BTMAC solution (40% in methanol). ^c The volume of reagent was 3 mL of BTEAC solution (40% in methanol). ^d The volume of reagent was 1 mL of TMPA solution (10% in methanol). ^e Not detected. Approximate detection limit was 10 µg/g.

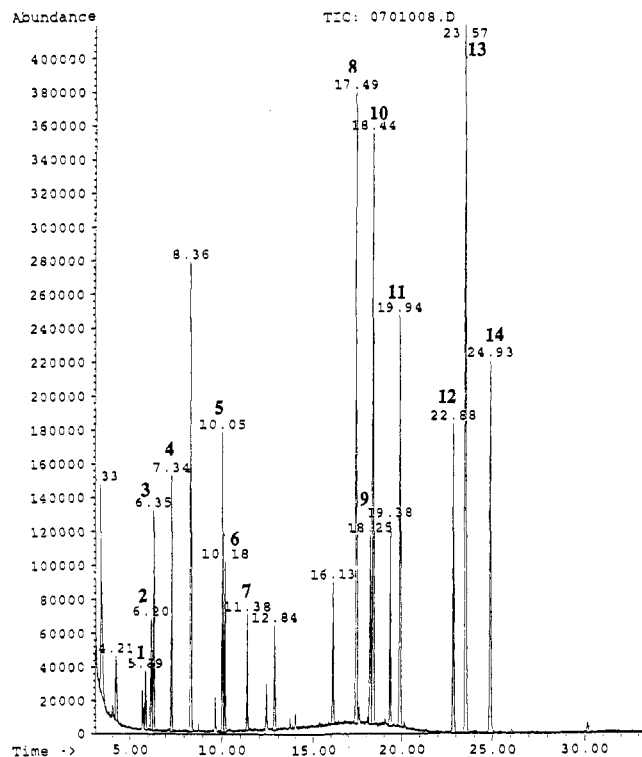


Figure 3. GC/MS chromatogram of acid standard in methanol containing BTMAC and analyzed by GC/MS. Compounds 1-7 are those listed in Table I; compounds 8-14 are the corresponding benzyl esters of the same compounds.

pounds 8-14 are the corresponding benzyl esters of MCPA, dicamba, MCPA, 2,4-D, 2,4,5-T, MCPB, and 2,4-DB, respectively.

Additional experiments were performed with TMPA dissolved in methanol, ethanol, and 2-propanol to determine whether the methylation reagent in the TMPA/

Table III. Percent Recoveries of Seven Chlorophenoxy Acid Herbicides from Spiked Sand by SFE with Carbon Dioxide and 10% TMPA in Methanol, Ethanol, or 2-Propanol^a

compd no.	compd name	TMPA in methanol	TMPA in ethanol	TMPA in 2-propanol
1	dicamba	89.1	102	73.8
2	MCPP	71.5	52.9	61.0
3	MCPA	85.6	11.1	ND ^b
4	2,4-D	71.4	56.1	39.4
5	2,4,5-T	ND	ND	ND
6	MCPB	79.7	68.3	47.9
7	2,4-DB	68.9	38.4	14.5

^a The values given are the percent recoveries as methyl ester. The extractions were performed at 400 atm/80 °C/15 min static, followed by 15 min dynamic, with 1 mL of TMPA solution added to the sample in the extraction vessel (10% in methanol, ethanol, or 2-propanol). Extraction vessel volume: 2.5 mL. The sample size was 2 g, and the spike level was 250 µg/g. The extracted material was collected in 5 mL of solvent (methanol, ethanol, or 2-propanol). Single determinations. ^b Not detected. Approximate detection limit was 10 ng/µL.

methanol combination is indeed TMPA or rather the alcohol in which the TMPA is dissolved. The recoveries are given in Table III. In all cases, we found the methyl esters and only traces of ethyl or isopropyl esters, indicating that the methyl group is derived from the TMPA and not from the alcohol.

The SFE procedure involving ionic displacement of the chlorophenoxy acids with TMPA appears to be attractive. Several researchers have claimed that the derivatization with TMPA is taking place during the static SFE, but our data do not support such a claim. Experiments which we performed at different injection port temperatures (e.g., 80, 200, 300 °C) indicate clearly that the conversion of the chlorophenoxy acids to the corresponding methyl esters is taking place in the injection port of the gas chromatograph. This type of "intrajector derivatization" was reported recently by Brondz and Olsen (1992) for the same types of compounds.

Extraction in the Presence of TBA/MI. TBA and MI were selected as derivatizing agents because they have been successfully used by Hopper (1987) to derivatize three of the chlorophenoxy acid herbicides investigated here (2,4-D; 2,4,5-T; 2,4-DB) and pentachlorophenol in food samples. TBA serves to ionize the compounds, and MI is the alkylating agent (Hopper, 1987). Extraction of linear alkyl sulfonates from sludge samples using SFE and TBA was reported by Field et al. (1992), who isolated the ion pairs and confirmed their presence in the extracts obtained by SFE. We have not investigated the extraction mechanism, but we noted that only in the presence of TBA and MI were we able to extract the acids with supercritical carbon dioxide. Upon injection of the extract into the GC/MS system, the acids are apparently converted to their corresponding methyl esters. The minimum injection temperature at which the methylation reaction occurs is about 100 °C as we have determined in separate experiments.

To optimize the amounts of TBA and MI needed for extraction and derivatization, we first established the minimum amount needed for derivatization. Standards of dicamba and 2,4-D were spiked with various amounts of TBA/MI (Table IV), and the mixtures were injected immediately into a GC/MS system. The results indicate that the amount needed for derivatization is approximately 50 µL of TBA solution (25% in methanol) and 50 µL of neat MI. The average derivatization yields under those conditions were 84% (2.2% RSD) for dicamba and 100% (1.3% RSD) for 2,4-D. When extracting soil samples of

Table IV. Optimization of the Derivatization Yields for Dicamba and 2,4-D with TBA/MI^a

compd name	vol of TBA, ^b μ L	vol of MI, ^c μ L	av derivatization yield, %	% RSD
dicamba	0	0	4.7	
	10	10	47	3.5
	25	25	74	9.2
	50	50	84	2.2
	100	100	80	3.2
2,4-D	0	0	3.2	
	10	10	82	4.7
	25	25	102	3.4
	50	50	100	1.3
	100	100	103	1.2

^a The number of derivatizations was three. The derivatization yield was determined at a concentration of 50 ng/ μ L in solution. The target analyte/TBA/MI solution was injected into the GC/MS system at an injector temperature of 250 °C. ^b 25% solution in methanol. ^c Neat material.

Table V. Derivatization Yields for the Chlorophenoxy Acid Herbicides with Excess TBA/MI^a

compd no.	compd name	derivatization yield, %		% RSD
		range	av	
1	dicamba	44.6–71.7	57.3	16.6
2	MCPP	59.6–77.7	73.1	7.2
3	MCPA	76.8–101	93.6	7.5
4	2,4-D	74.2–94.4	87.1	7.4
5	2,4,5-T	43.6–57.2	46.4	8.6
6	MCPB	79.8–89.2	87.1	3.1
7	2,4-DB	75.6–95.8	86.6	5.9

^a The number of determinations was 10. The derivatization yield was determined at a compound concentration of 100 ng/ μ L in solution. The target analyte/TBA/MI solution was injected into the GC/MS system at an injector temperature of 250 °C.

unknown composition, we chose to spike 10 times the amount needed of TBA/MI to compensate for reagent reacting with other compounds that could be present in the sample.

To determine the reproducibility of the methylation reaction, we prepared 10 standards that contained the seven chlorophenoxy acids in methanol (nominal concentrations of the test compounds were 100 ng/ μ L), added TBA and MI, and injected these solutions into our GC/MS system (injector temperature was held at 250 °C). The derivatization yields (Table V) were determined by comparing the instrument responses for these 10 injections with those obtained by analyzing a composite standard containing the seven methyl esters. The reproducibility (percent RSD) of the methylation reaction was 8.6% or better, except for dicamba at 16.6%. The derivatization yields ranged from 46.4 to 93.6%, with two compounds exhibiting average yields lower than 70% (2,4,5-T at 46.4% and dicamba at 57.3%). The low derivatization yield for dicamba may be explained by the fact that in the presence of excess derivatization reagent (TBA/MI) there was more butyl ester being formed; however, we cannot explain the low derivatization yield for 2,4,5-T.

We then proceeded with the determination of the actual extraction recoveries by SFE using TBA/MI. All experiments were performed with spiked materials because of the lack of certified standard reference materials. Three different matrices (sand, clay soil, and topsoil) were spiked at two concentrations (50 and 250 μ g/g) with the chlorophenoxy acids and immediately extracted. Table VI shows the recoveries for each spike level and matrix for each of the seven compounds. Overall, the average recovery over the three matrices and the two spike levels

was 95.5% (standard deviation was 18.1%). The individual recoveries ranged from 57.4 to 141%.

Attempts to extract other compounds currently listed in Method 8151 by using SFE combined with TBA/MI were unsuccessful, either because the compounds did not derivatize to the methyl or butyl esters (dalapon, dinoseb, and 4-nitrophenol) or because decomposition occurred, as with DCPA diacid, pentachlorophenol, and picloram. We also found that upon injection of the extracts of some of these compounds into the GC/MS system, large peaks appeared that did not match those of the corresponding methyl esters. We identified these peaks as the butyl esters. For some of the compounds (acifluorfen, dicamba, and 3,5-dichlorobenzoic acid), we found that the approximate concentrations of the butyl esters were 4–6 times as high as those of the methyl esters.

Extraction in the Presence of PFBBBr/TEA. Preliminary experiments were carried out with PFBBBr/TEA at 5400 psi, two different temperatures (80, 120 °C), and two different amounts of PFBBBr/TEA reagent. The recovery data are summarized in Table VII. At 80 °C and using 250 μ L of PFBBBr (5% in acetone)/50 μ L of neat TEA, the recoveries were below 10% for most of the compounds (except dicamba and 2,4,5-TP at 12 and 14%, respectively). When the temperature was increased to 120 °C and the amount of reagent was kept constant, the recoveries increased to 78% for dicamba and 28% for MCPP but were little affected for the other compounds. Two other chlorophenoxy acids, dichlorprop and 2,4,5-TP, that were spiked into the soil with the other compounds showed slight improvement in their recoveries. By quadrupling the amount of reagent and extracting at 120 °C, we have achieved recoveries in the 70–80% range for MCPP, dichlorprop, and 2,4,5-TP, and 24–38% for the other compounds investigated. Despite the fact that recoveries were still low for five of the nine compounds investigated, we feel that this in-situ derivatization and extraction technique is promising, especially since it may allow detection of the chlorophenoxy acids at parts per billion levels. Assuming that the derivatization yields are the same at concentrations in the parts per billion range, we estimate a limit of detection of approximately 10 ppb when a 5-g sample is extracted by SFE and the final volume of the extract is 1 mL. Further developmental work of this technique is in progress in our laboratory.

Extraction with Carbon Dioxide Modified with Methanol. Experiments were carried out with carbon dioxide containing 5 and 10% methanol as modifier. To investigate whether the acids could be extracted from the spiked sand by the methanol-modified carbon dioxide, sand samples were spiked with the chlorophenoxy acid herbicides and extracted at 400 atm and 80 °C for 15 min in the static mode and 15 min in the dynamic mode. Since the acids need to be derivatized prior to GC/MS analysis, we added TBA and MI to the collection solvent. Our results indicate that compound recoveries were quantitative, except for MCPB at the 50 μ g/g spike level [we recovered MCPB (average recovery 107%), however, at the 250 μ g/g spike level]. This also demonstrates that the derivatization agents are retained in the collection solvent. When we extracted topsoil with carbon dioxide alone, we were not able to recover the chlorophenoxy acid herbicides. By adding 500 μ L of 25% TBA in methanol and 500 μ L of MI to the spiked topsoil (directly in the extraction vessel) and immediately proceeding with the extraction, we were able to recover the compounds satisfactorily. By switching to carbon dioxide modified with 5% methanol, the recoveries were further improved by as much as 20% for

Table VI. Percent Recoveries of Seven Chlorophenoxy Acid Herbicides (as Methyl Esters) by SFE with Supercritical Carbon Dioxide (In-Situ Methylation)

compd no.	compd name	sand		clay soil				topsoil				
		50 µg/g spike	250 µg/g spike	50 µg/g spike	250 µg/g spike	50 µg/g spike	250 µg/g spike	50 µg/g spike	250 µg/g spike			
1	dicamba	87.5	103	98.4	82.6	94.0	108	90.9	85.4	91.0	111	128
2	MCPP	79.0	76.0	83.4	57.4	92.5	99.0	72.7	70.6	80.5	75.5	85.8
3	MCPA	100	110	102	73.5	121	122	84.5	80.5	90.5	96.5	98.7
4	2,4-D	85.0	103	91.7	68.8	105	97.0	77.2	70.4	80.0	85.0	90.6
5	2,4,5-T	77.0	116	86.3	67.5	106	120	75.2	69.0	83.5	97.5	94.9
6	MCPB	107	108	114	61.8	107	104	91.6	96.4	111	106	100
7	2,4-DB	124	124	126	70.4	128	141	102	105	123	120	109

^a The extractions were performed at 400 atm/80 °C/15 min static, followed by 15 min dynamic, with the reagents (0.5 mL of TBA 25% in methanol and 0.5 mL of MI) added to the sample in the extraction vessel. Extraction vessel volume: 10 mL. The sample size was 2 g. The extracted material was collected in 2 mL of methanol. Duplicate determinations; one set of data for the topsoil/250 µg/g spike was lost.

Table VII. Percent Recoveries of the Chlorophenoxy Acid Herbicides from Spiked Topsoil by SFE with Carbon Dioxide and PFBBR/TEA^a

compd no.	compd name	80 °C	120 °C	120 °C
		250 µL of PFBBR 50 µL of TEA	250 µL of PFBBR 50 µL of TEA	1000 µL of PFBBR 200 µL of TEA
1	dicamba	12.2	78.2	82.9
2	MCPP	8.3	28.2	77.1
3	MCPA	3.7	4.3	36.7
4	2,4-D	3.6	1.9	23.7
5	2,4,5-T	6.5	2.8	33.7
6	MCPB	2.5	12.7	38.0
7	2,4-DB	2.9	8.7	32.5
8	dichlorprop	7.8	22.5	73.7
9	2,4,5-TP	14.3	33.0	80.2

^a The extractions were performed at 5400 psi/30 min static, followed by 30 min dynamic, with a carbon dioxide flow rate of 3 mL/min. Extraction vessel volume: 7 mL. The sample size was 5 g, and the spike levels were 1 µg/g for dicamba, MCPP, dichlorprop, and 2,4,5-TP; 2 µg/g for MCPA, 2,4,5-T, MCPB, and 2,4-DB; and 3 µg/g for 2,4-D. The extracts were diluted 10-fold prior to GC/ECD analysis except for the experiment performed at 120 °C with 1000 µL of PFBBR/50 µL of TEA, for which the dilution factor was 30. Single determinations.

Table VIII. Percent Recoveries of the Chlorophenoxy Acid Herbicides from Spiked Topsoil by SFE with Carbon Dioxide Alone and Carbon Dioxide Modified with 5% Methanol^a

compd no.	compd name	carbon dioxide only		carbon dioxide with 5% methanol	
1	dicamba	61.8	58.4	64.8	65.1
2	MCPP	71.1	69.2	98.6	92.6
3	MCPA	70.9	62.0	95.1	91.8
4	2,4-D	59.6	47.0	74.4	74.1
5	2,4,5-T	51.0	24.8	60.0	56.1
6	MCPB	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>
7	2,4-DB	53.6	48.8	78.1	76.9
8	dichlorprop	71.4	67.7	96.3	90.9
9	2,4,5-TP	59.1	44.6	75.0	73.0

^a The extractions were performed at 400 atm/80 °C/15 min static, followed by 15 min dynamic, with 0.5 mL of TBA solution (25% in methanol) and 0.5 mL of MI added to the sample in the extraction vessel. Extraction vessel volume: 10 mL. The sample size was 2 g, and the spike level was 50 µg/g. The extracted material was collected in 5 mL of methanol. Duplicate determinations. ^b Not available for spiking.

some of the compounds (Table VIII). Two other chlorophenoxy acid herbicides, dichlorprop and 2,4,5-TP, showed similar improvements in recovery when TBA/MI and carbon dioxide modified with 5% methanol was used.

CONCLUSIONS

The SFE procedure with TBA/MI described here is applicable to the qualitative determination of chlorophe-

noxy acid herbicides in soil samples at micrograms per gram levels. Because the extraction is performed with supercritical carbon dioxide (a nontoxic, nonflammable, nonpolluting, and relatively inexpensive material) and is fast (typically 30 min for a 2–5-g sample), this method is an attractive alternative to the conventional Soxhlet extraction procedures specified in EPA SW-846 Methods 8150B and 8151. Furthermore, TBA and MI are less toxic than the reagents used in the diazomethane procedure recommended in Method 8150B (the starting material for diazomethane preparation is carcinogenic, and diazomethane is both toxic and explosive). Extraction of the chlorophenoxy acid herbicides from soil with an aqueous base is an alternative procedure; however, since the base would facilitate the extraction of humic matter from the soil, this procedure would result in increased background. Moreover, liquid extraction is very tedious in the case of solid matrices, requiring first extraction from the solid matrix into the aqueous base, preconcentration from the aqueous base (after pH adjustment) onto a cartridge or disk, subsequent elution with an organic solvent from the cartridge or disk, and finally concentration of the extract. Work is in progress in our laboratory to develop a quantitative method for the chlorophenoxy acid herbicides.

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LITERATURE CITED

- Brondz, I.; Olsen, I. Intra-injector Formation of Methyl Esters from Phenoxy Acid Herbicides. *J. Chromatogr.* **1992**, *598*, 309–312.
- Field, J. A.; Miller, D. J.; Field, T. M.; Hawthorne, S. B.; Giger, W. Quantitative Determination of Sulfonated Aliphatic and Aromatic Surfactants in Sewage Sludge by Ion-Pair/Supercritical Fluid Extraction and Derivatization Gas Chromatography/Mass Spectrometry. *Anal. Chem.* **1992**, *64*, 3161–3167.
- Hawthorne, S. B.; Miller, D. J.; Nivens, D. E.; White, D. C. Supercritical Fluid Extraction of Polar Analytes Using In-Situ Chemical Derivatization. *Anal. Chem.* **1992**, *64*, 405–412.
- Hills, J. W.; Hill, H. H., Jr.; Maeda, T. Simultaneous Supercritical Fluid Derivatization and Extraction. *Anal. Chem.* **1991**, *63*, 2152–2155.
- Hopper, M. L. Methylation of Chlorophenoxy Acid Herbicides and Pentachlorophenol Residues in Food Using Ion-Pair Alkylation. *J. Agric. Food Chem.* **1987**, *35*, 265–269.

- Lee, H.-B.; Peart, T. E. Supercritical Carbon Dioxide Extraction of Resin and Fatty Acids from Sediments at Pulp Mill Sites. *J. Chromatogr.* 1992, 594, 309-315.
- Lee, H.-B.; Peart, T. E.; Hong-You, R. L. In-Situ Extraction and Derivatization of Pentachlorophenol and Related Compounds from Soils Using a Supercritical Fluid Extraction System. *J. Chromatogr.* 1992, 605, 109-113.
- Li, C.; Magee, R. J.; James, B. D. Chromatographic Determination of Chlorinated Phenoxyacetic Acids by Simultaneous Extraction-Methylation and Application to Soil Analysis. *Anal. Chim. Acta* 1991a, 255, 187-196.
- Li, C.; Markovec, L. M.; Magee, R. J.; James, B. D. A Convenient Method Using Methanol/Benzyltrialkylammonium Reagents for Simultaneous Extraction and Methylation of 2,4-Dichlorophenoxyacetic Acid in Soil, with Subsequent Analysis via Gas Chromatography. *J. Agric. Food Chem.* 1991b, 39, 1110-1112.

Rochette, E. A.; Harsh, J. B.; Hill, H. H., Jr. Supercritical Fluid Extraction of 2,4-D from Soils Using Derivatization and Ionic Modifiers. *Talanta* 1993, 40, 147-155.

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