Development of a Method for Extraction of Organochlorine Pesticides from Rendered Chicken Fat via Supercritical Fluoroform

Mehdi Ashraf-Khorassani and Larry T. Taylor*

Department of Chemistry, Virginia Tech, Blacksburg, Virginia 24061

Frank K. Schweighardt

Air Products and Chemicals Inc., Allentown, Pennsylvania 19158

Quantitative recoveries relative to liquid−solid extraction of organochlorine pesticides (OCPs) from rendered chicken fat were obtained using pure fluoroform as an extraction fluid. No cleanup procedure of the extract was employed prior to analysis via gas chromatography/electron capture detection (GC/ECD). Extraction of OCPs from rendered chicken fat was also performed with methanol-modified fluoroform. However, coextracted fat components were trapped along with the OCPs such that the GC/ECD trace of the extract showed a high background level which caused quantification to be less reliable. While no cleanup step nor activated alumina to retain fat in the extraction vessel was necessary, it was demonstrated that a longer extraction time was required to extract OCPs from fat using pure fluoroform than with methanol-modified fluoroform. Since little or no fat was extracted by fluoroform, the longer extraction time is believed to be caused by partitioning of OCPs between unextracted fat and fluoroform.

Keywords: *Supercritical fluid; fluoroform; rendered fat; organochlorine*

INTRODUCTION

Analysis of organochlorine pesticides (OCPs) and other chlorinated organic contaminants such as polychlorinated biphenyls and chlorinated benzodioxins which are of toxicological concern in food matrices requires extensive sample preparation. After the analyte has been extracted and concentrated, the analytical sample must then undergo an extensive cleanup step which is designed to remove interfering coextractive material. The effectiveness of the required cleanup operation will depend on the analyte concentration. For example, analyte at parts-per-billion levels will necessitate minimal background contaminants especially if the assay is via electron capture detection (ECD). Fatty samples are of particular concern in this regard because organochlorine compounds exhibit appreciable bioaccumulation and solubility in the fatty tissue. Traditional sample preparation methods invoke organic solvents which are not very selective for extraction. Consequently, removal of the organochlorine components is accompanied by removal of the fat components. Isolation of the organochlorine moieties (e.g., cleanup step) is accomplished via size exclusion or normal phase chromatography with a mobile phase such as isooctane and via liquid−liquid partitioning. The resulting organochlorine fraction is then usually analyzed by gas chromatography (GC) coupled with either ECD or mass spectrometry (MS).

A number of specific sample preparation methods have been proposed for isolating OCPs from matrices with a high fat content. Walters (1990) has reviewed the cleanup techniques that have been employed with fatty food matrices over the past 20 years. Table 1 lists the major techniques which have been reported. The current approved analytical methodology relies on tedious, time-consuming protocols which use large volumes of expensive solvents which are toxic and must be disposed of as hazardous waste. The procedures also may involve several solvent exchange/concentration steps which afford ample opportunity for both introduction of contaminants and loss of target compounds.

Table 1. Cleanup Procedures Used for the Analysis of OCPs in Fatty Matrices[a]

technique	reference
liquid-liquid partitioning	Jones and Riddick, 1952
adsorption chromatography	
magnesia	Klein et al., 1956
Florisil	Mills, 1959; McKinley and Mahon, 1959
alumina	Steinwandter, 1982
saponification	Klein et al., 1956; Mills, 1959; McKinley and Mahon, 1959; Niemann et al., 1983
acid digestion	Stanley and LeFavoure, 1965
sweep codistillation	Storherr and Watts, 1965; Ott and Gunther, 1964; Luke et al., 1984
low-temperature precipitation	McLeod and Wales, 1972
gel-permeation chromatography	Stalling et al., 1972; Tindle and Stalling, 1972; Hopper, 1981
semipreparative HPLC	
normal phase (silica)	Gillespie and Walters, 1986
reversed phase (ODS)	Gillespie and Walters, 1989
supercritical fluid extraction	King, 1989; Wheeler and McNally, 1989

[a] Taken from Walters, 1990.

One of the techniques which Walters briefly reviewed is supercritical fluid extraction (SFE). SFE offers a potential solution to some of the aforementioned experimental problems since greater selectivity is afforded by a supercritical fluid (SF) such as carbon dioxide (CO_2) than a conventional organic solvent. More specifically, the solvating power of a SF can be varied by small changes in pressure and/or temperature. SFE for determination of pesticides in fatty matrices has received relatively little attention. In spite of its greater selectivity, supercritical CO_2, in like fashion to organic solvents (although to a lesser extent), extracts not only OCPs but also a significant amount of fatty material.

S0021-8561(96)00037-4 CCC: $12.00

Table 2. Extraction Conditions Used for Analysis of OCPs in Rendered Chicken Fat

	extraction run						
	A	B	C	D	E	F	G
pressure, atm	450	450	450	450	450	450	450
temp, °C	60	60	60	60	60	60	60
liq flow, mL/min	2	2	2	2	2	2	2
fluid (CHF_3/MeOH)	95/5	90/10	90/10	90/10	90/10	100/0	100/0
sample size	0.5	0.5	0.5	0.5	0.5	0.2	0.2
sample mixed with	Alu[a]	Alu	Alu	Alu	Alu	GB[a]	GB
ext vessel size, mL	10	10	10	10	10	10	10
static ext period, min	5	5	5	5	5	5	5
dynamic ext period, min	45	45	45	60	45	45	75
trap temp during collection, °C	30	30	10	15	10	10	10
trap temp during rinse, °C	25	25	25	25	25	25	25
rinse solvent, MeOH/CH_2Cl_2	100/0	100/0	100/0	100/0	80/20	80/20	80/20
trap adsorbent	Alu	Alu	Alu	Alu	Alu	C_{18}[a]	C_{18}
rinse volume, mL	5	5	5	5	5	5	5
cleanup step	yes	yes	yes	yes	no	no	no

[a] Alu, alumina; GB, glass beads; C_{18}, octadecyl silica.

Inclusion of activated alumina, silica, or diatomaceous earth inside the extraction vessel (e.g., intimately mixed with the matrix) coupled with high-density supercritical CO_2 has been reported to eliminate the need for any additional off-line purification. For example, France et al. (1991) used alumina or silica with CO_2 and methanol-modified CO_2 as a cleanup technique for analysis of three OCPs at low parts-per-million levels in chicken fat. Recoveries (93−111%) and precision (% RSD < 10) with the SF technique compared favorably with data obtained via conventional methodology. The same laboratory (Hopper and King, 1991) used pelletized diatomaceous earth (Hydromatrix) as an extraction enhancer for analysis of pesticides in both fatty and nonfatty foods. The research effort yielded analyte recoveries greater than 85% for more than 30 pesticides at incurred levels ranging from 0.005 to 2 ppm. A mixed sorbent composed of bonded silicas (e.g., 93% octadecyl/7% diol) had been employed to immobilize supercritical fluid extractable lipid-like material from tissue containing carbamate pesticides. This procedure removed fatty acids/sterols and enabled recoveries of 71−96% of the pesticides from chicken muscle to be obtained (Murugaverl et al., 1993).

More recently, Nam and King (1994a) have utilized coupled SFE/SFC/GC for trace analysis of pesticide residues in chicken fat, ground beef, and lard (SFC = supercritical fluid chromatography). The sample preparation and assay techniques were instrumentally integrated for efficient and automated on-line analysis wherein minimal sample handling would be required. The incorporation of packed column SFC allowed the fractionation of relatively small sized, nonpolar pesticides from coextracted fatty material. The final separation of pesticides was obtained by high-resolution capillary gas chromatography with ECD detection. Stallings et al. (1992) used on-line SFE and cleanup by gel permeation chromatography for analysis of pesticides in corn oil. The on-line cleanup of the SF extract enhanced the efficiency of sample processing. A number of other researchers have published reports regarding the SFE of pesticides from fatty samples using either an in-line or off-line cleanup procedure (Nam and King, 1994b; France and King, 1991; Kopec et al., 1993; King et al., 1993; Paquet and Khan, 1995; Penwell and Comber, 1994).

The use of alternate fluids to overcome the limitations of CO_2 such as inadequate solvating power and reactivity has been in part achieved. Ashraf-Khorassani et al. (1990) compared the extraction efficiency of different amines from several matrices using SF N_2O and CO_2. It was shown that primary aliphatic amines could be extracted with N_2O but not with CO_2. Hawthorne et al. (1992) extracted PCBs from a standard reference material and PAHs from both a petroleum waste sludge and railroad bed soil using SF, N_2O, CO_2, and $CHClF_2$. Highest analyte extraction efficiencies were obtained with N_2O. Stahl and co-workers (1981) determined the solubilities of different alkaloids in SF, N_2O, CO_2, and fluoroform (CHF_3). In general alkaloid solubility was found to be greatest in CHF_3. Sulfonylurea herbicides and PAHs have been used as probes to establish the extraction effacacy of CHF_3 (Howard et al., 1993). A 30% increase in extraction efficiency was realized when CHF_3 was used as the extraction fluid over CO_2. On the other hand, under equivalent conditions recoveries of the less polar PAHs were higher with CO_2 than with CHF_3. Recently, Hillmann et al. (1995) showed that a 15% increase in extraction efficiency of pesticides could be realized when CHF_3 was used as the extraction medium rather than CO_2.

We wish to report here on the use of SF CHF_3 for extraction of OCPs from rendered chicken fat. CHF_3 has milder critical parameters than CO_2, it is not currently scheduled for phase out as are chlorofluorocarbons, and it exhibits a dipole moment of 1.6 D. It will be demonstrated that with CHF_3 coextractive fat components are minimal and no cleanup steps are necessary. The object of the study was to obtain quantitative extraction of OCPs from rendered chicken fat which had been fortified at the low parts-per-billion level.

EXPERIMENTAL PROCEDURES

A Suprex (Pittsburgh, PA) automated Prepmaster 44 (AP-44) system with variable flow restrictor was used for all extractions. A complete description of the system has been described elsewhere (Ashraf-Khorassani, 1995). Extraction of organochlorine pesticides (OCPs) from rendered fat was obtained under a variety of conditions. Table 2 shows extraction and collection conditions which were used. Sample weight for extractions A−E was 0.5 g of fat mixed with 6 g of alumina, while for F and G, 0.2 g was used and, instead of alumina, glass beads were mixed with the fat.

Extracts from experiments A−D were subjected to a single step cleanup procedure prior to GC/ECD analysis. Extracts from experiments E−G were analyzed without a cleanup procedure. The cleanup procedure consisted of evaporation of the external alumina trap rinse solvent (5 mL of methanol) to dryness followed by reconstitution with 1.0 mL of isooctane.

Table 3. Percent Recoveries[a] of OCPs from Rendered Chicken Fat Employing Methanol-Modified Fluoroform

analyte	retention time, min	A	B	C	D
HCB	19.38	61 (9)	57 (14)	71 (18)	73 (16)
α-BHC	20.82		38 (4)	48 (8)	23 (6)
lindane	23.46	40 (26)	61 (5)	45 (43)	42 (8)
heptachlor	25.43	41 (32)	51 (19)	51 (11)	59 (4)
aldrin	27.38	60 (5)	58 (5)	62 (12)	62 (5)
chlorpyrifos	29.20	22 (32)	43 (1)	33 (39)	14 (20)
HEP EP B	30.68	75 (1)	78 (6)	71 (10)	59 (1)
HEP EP A	31.27	48 (18)	50 (12)	49 (15)	35 (13)
trans-nonchlor	31.68	63 (3)	66 (6)	59 (10)	63 (7)
CVP	32.15				
A-chlordane	32.41	67 (2)	68 (6)	62 (9)	64 (5)
dieldrin and *p,p′*-DDE	33.85	97 (99)	102 (14)	106 (14)	117 (5)
stirofos tetrachlorvinphos	34.11	15 (5)	14 (7)	12 (2)	13 (4)
endrin	36.11	79 (1)	86 (9)	76 (16)	61 (12)
endosulfan	36.76	65 (5)	70 (9)	61 (14)	64 (11)
p,p′-DDD	37.07	28 (19)	55 (13)	30 (27)	41 (12)
p,p′-DDT	38.28	70 (1)	67 (5)	58 (9)	60 (8)
DBC and mirex	42.66	81 (6)	84 (8)	75 (8)	77 (3)
methoxychlor	43.11	55 (3)	55 (2)	52 (6)	58 (8)
phosalone	43.77				
coumaphos-S	48.70				

[a] $n = 3$. Numbers in parentheses are percent relative standard deviations.

The resulting solution was passed through a 6 mL Florisil cartridge which had been preconditioned with 3 mL of ethyl acetate and 6 mL of isooctane, respectively. The solution was eluted from the Florisil with 10 mL of 90/10 isooctane/ethyl acetate. Next, the Florisil eluent was evaporated again to dryness and redissolved in 1.0 mL of isooctane. The solution was then analyzed via gas chromatography/electron capture detection (GC/ECD). For the extracts from experiments E−G, rinse solvent was evaporated to dryness. The analyte was then redissolved in 1 mL of isooctane and analyzed directly by GC/ECD.

A Hewlett Packard (Wilmington, DE) 5890 Series II gas chromatograph equipped with split−splitless injection and a Hewlett Packard ECD were used for separation and detection of extracted analyte. A DB-608 (30 m × 0.32 mm i.d., d_f = 0.5 μm) column was used for all separations. Other experimental conditions appear in the figure legends.

All extractions were performed with SFE/SFC grade supercritical CO_2 and fluoroform (CHF_3) both with helium head pressure from Air Products and Chemical Inc. (Allentown, PA). The fortified rendered fat samples, appropriate standards, and recoveries obtained after liquid−solid extraction were provided by Leon P. Ilnicki of USDA/FSIS, Western Research Laboratory, Alameda, CA. All solvents were purchased from EM Science (Gibbstown, NJ) as HPLC grade.

RESULTS AND DISCUSSION

The objective of this study was to obtain quantitative extraction of OCPs at low part-per-billion levels from rendered fat using either supercritical CO_2 or CHF_3 without any further cleanup procedure. Initially, extraction of OCPs from rendered fat was attempted using pure CO_2 and activated alumina intimately mixed with the fat matrix. Unfortunately, alumina failed to retain the rendered fat in the extraction vessel. In fact, most of the fat could be readily seen in the solid phase trap rinse solvent after termination of the extraction. Attempts to clean up the extract were not successful. The failure of the alumina to immobilize the fatty material is difficult to explain. Even though the alumina had been activated by heating it to 150 °C for 6 h, the activity may have been insufficient (France et al., 1991). On the other hand, more alumina per sample weight may have been required in the vessel. The size of our fatty sample may also have been too large such that when the vessel was pressurized the fat moved enmass from the vessel to the trap without being fully extracted. Alternately, thermally rendered fat may be of such low molecular weight that activated alumina is not capable of immobilization. At this point, our attention turned from CO_2 to CHF_3 because Taylor and King (1994) had previously reported in a very preliminary fashion (e.g., industrial application note) the failure of CHF_3 to extract relatively large quantities of fatty material from poultry tissue, but at the same time CHF_3 adequately extracted four pesticides. Although this work has been also cited at several scientific meetings, it has never appeared in a refereed publication to our knowledge.

Different percentages of methanol-modified CHF_3 were used for extractions A−D. It was believed that at this point, methanol-modified CHF_3 rather than pure CHF_3 would provide the necessary solvating power to extract the OCPs. Also, our previous experience of extracting sulfa drugs from chicken liver with methanol-modified CHF_3 (Ashraf-Khorassani and Taylor, 1996) suggested that minimal fat would be extracted even under these conditions. However, in this study with rendered chicken fat and methanol-modified CHF_3, a small amount of fat could, unexpectedly, be seen in the trap rinse solution. This was the case even when activated alumina was used as both an external and an internal trap. In other words, we were unable to selectively rinse the external alumina trap with various organic solvents to isolate an exclusively OCP fraction.

Table 3 shows percent recovery of OCPs relative to liquid−solid extraction from rendered fat using extraction conditions A−D (Table 2). These numbers should be viewed with skepticism since the GC/ECD trace of the rendered fat extract (Figure 1) did not match very well the GC/ECD trace of the standard solution (Figure 2). This discrepancy is believed to be due to the high background level of the methanol-modified CHF_3 extract. As evidence for this notion, SFE of the nonfortified rendered (0.5 g) fat was performed employing methanol-modified CHF_3. The SFE extract of the blank was subjected to the same cleanup procedure with Florisil. Figure 3 shows the resulting GC/ECD trace which is quite noisy. No doubt, coextractive ECD active components that escape the cleanup operation give rise to the excessive noise. Extraction efficiencies of most analytes for experiments A−D were less than 80%. Extraction pressure and temperature were 450 atm and

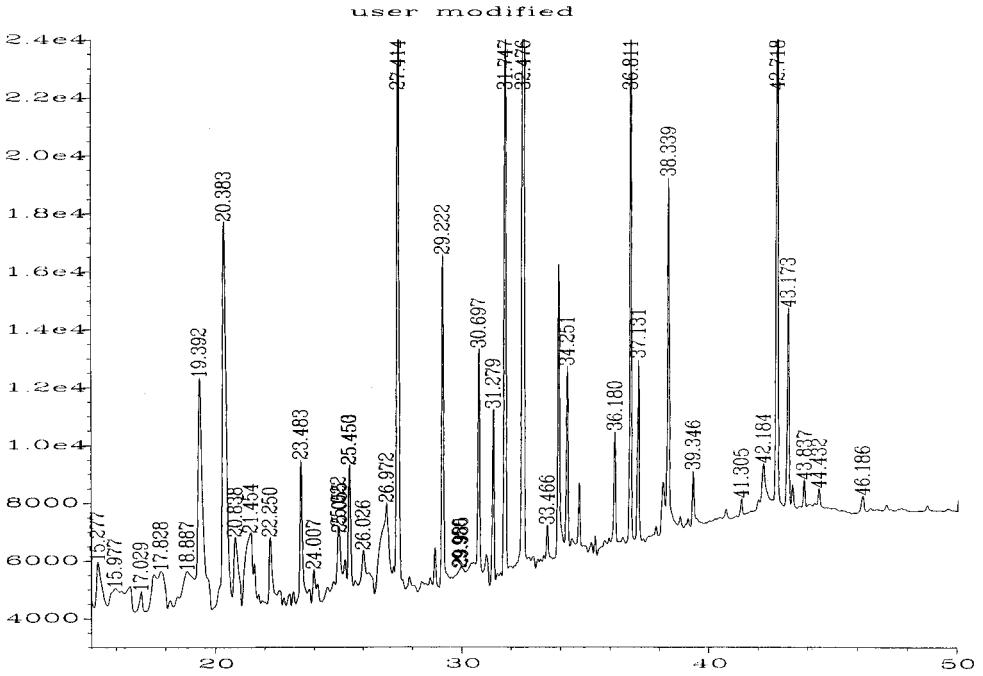

Figure 1. Gas chromatogram trace of organochlorine pesticides extracted from rendered fat via SFE. SFE conditions are the same as the conditions in Table 2. GC conditions: DB-608 column (30 m × 0.320 mm, 0.5 μm d_f); temperature program, 150 °C (hold 10 min), ramp to 280 °C (4 °C/min), 280 °C (hold for 10 min); detector, ECD; injection, 1 mL splitless; carrier gas, helium; head pressure, 18 psi.

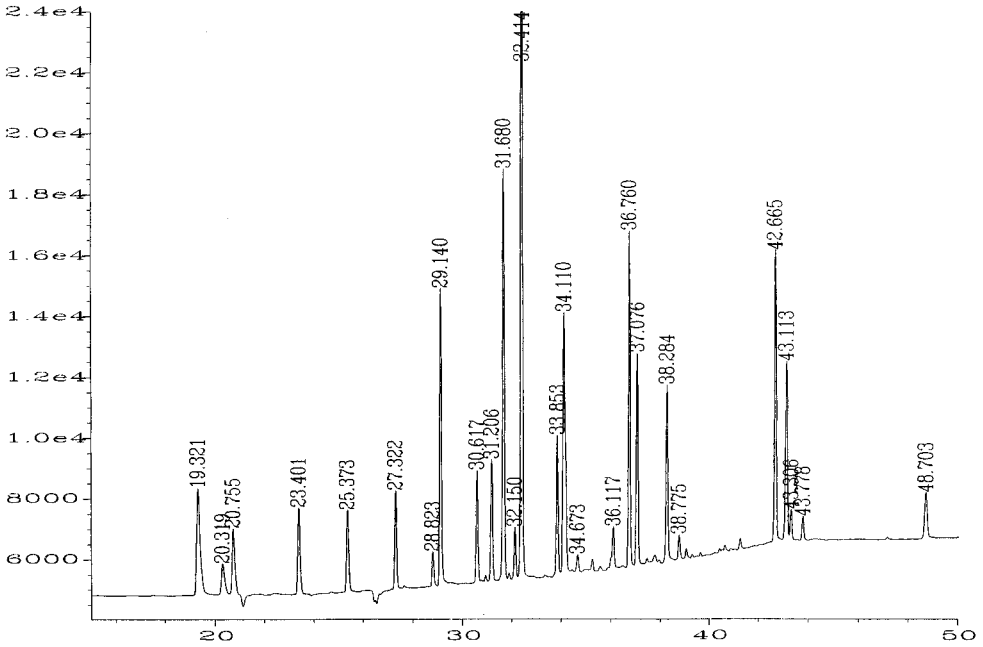

Figure 2. Gas chromatogram trace of organochlorine pesticides standard. GC conditions are the same as in Figure 1.

60 °C for the four runs. These conditions were deemed necessary in order to exhaustively extract the pesticides even though under the conditions a lot of fat was expected to be brought over. Changes in dynamic extraction time, percent modifier, and trap temperature seemed to have little effect on recoveries except for a few analytes. In retrospect, the GC/ECD traces of the extracts were so different from the standards that peak area integration was quite subjective. In fact, certain OCPs could not even be identified because of the apparent high background and low analyte level.

At this point, we began to speculate as to why the extraction efficiencies of OCPs from the rendered fat were not successful. The following points are noteworthy. One of the major problems which caused GC/ECD quantitation to be less reliable and difficult was the high background level. Second, it is known that many OCPs are fat soluble analytes. Therefore, OCPs partitioning with CHF_3 and fat may be significant, which, if true, might require a larger quantity of CHF_3 to obtain exhaustive extraction. In this regard, one might wonder whether OCPs can be removed without removing the fat. Third, alumina is known to catalyze the decomposition and/or interconversion of certain OCPs under supercritical conditions (e.g., DDT to DDE) (Cochrane and Chau, 1971). This has been demonstrated in our laboratory for the extraction of DDT from mussel tissue. This may also account for the fact that certain OCPs are not quantitatively recovered. Fourth, it is known that with certain analytes and high modifier levels, loss

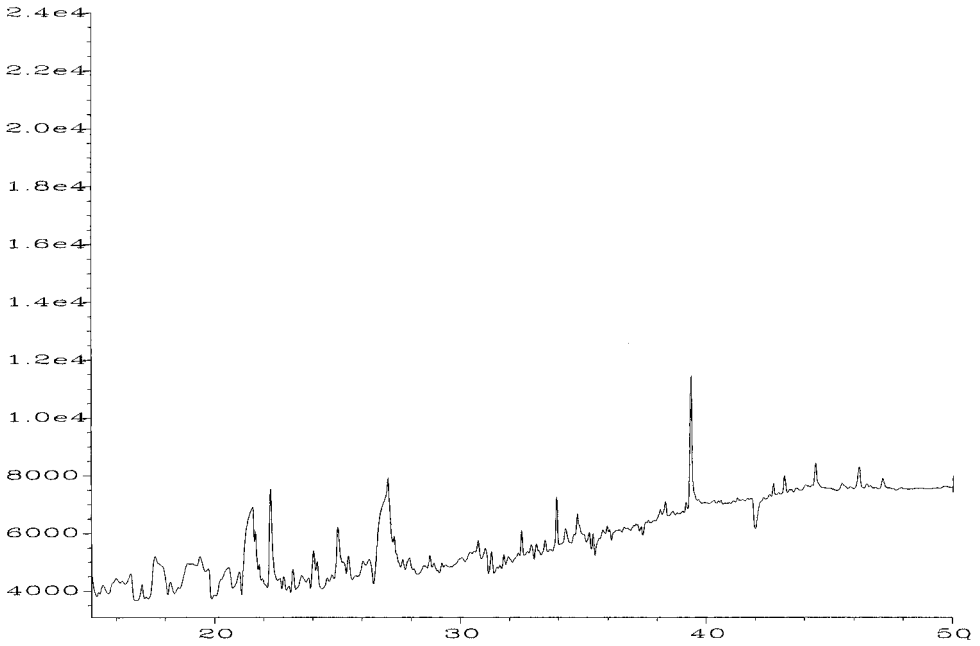

Figure 3. GC/ECD trace of blank rendered fat via SFE. SFE and GC conditions are the same as in Figure 1.

Table 4. Percent Recoveries of OCPs from Rendered Chicken Fat Employing No Cleanup Procedure

analyte	retention time, min	E[a]	F[b]	G[c]	analyte	retention time, min	E[a]	F[b]	G[c]
HCB	19.38	91	32 (15)	94	dieldrin and *p,p′*-DDE	33.85	107	84 (13)	87
α-BHC	20.82	54	41 (2)	80	stirofos tetrachlorvinphos	34.11	140	125 (7)	96
lindane	23.46	134	50 (25)	80	endrin	36.11	77	86 (12)	146
heptachlor	25.43	77	49 (13)	73	endosulfan	36.76	75	83 (22)	70
aldrin	27.38		73 (19)	99	*p,p′*-DDD	37.07	14	75 (12)	76
chlorpyrifos	29.20	60	69 (29)	122	*p,p′*-DDT	38.28	75	79 (16)	92
HEP EP B	30.68	83	70 (14)	76	DBC and mirex	42.66	91	115 (20)	114
HEP EP A	31.27	75	74 (29)	91	methoxychlor	43.11	50	53 (22)	75
trans-nonchlor	31.68	74	113 (16)	101	phosalone	43.77		112 (22)	113
CVP	32.15	40	139 (11)	117	coumaphos-S	48.70	120	178 (20)	146
A-chlordane	32.41	80	63 (14)	114					

[a] single run. [b] $n = 3$. [c] Average of duplicate runs.

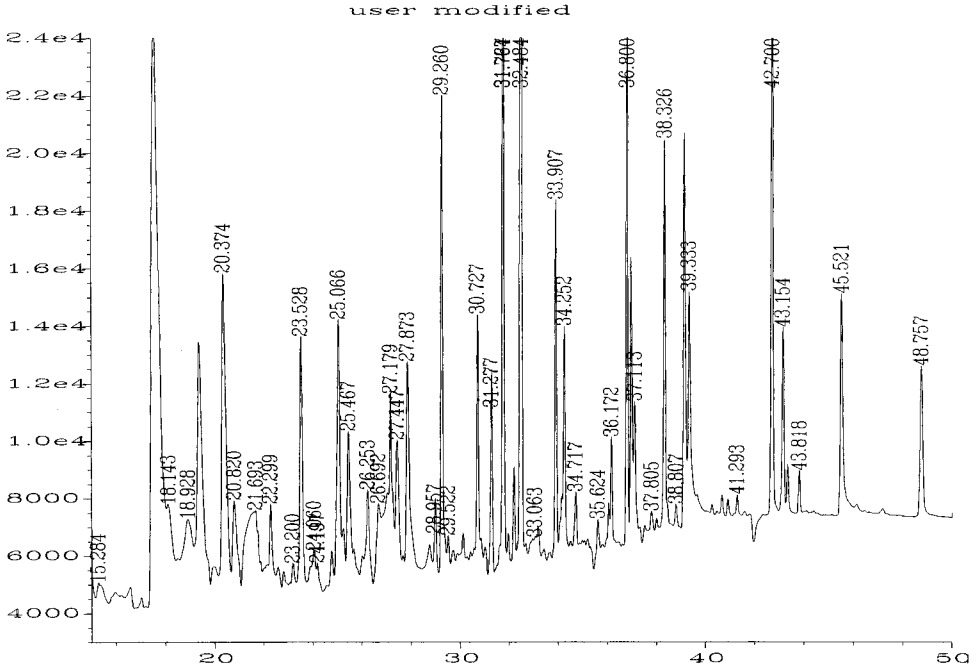

Figure 4. GC/ECD trace of organochlorine pesticides from rendered fat via SFE. SFE conditions are the same as E conditions in Table 2. GC conditions are the same as in Figure 1.

may occur due to poor trapping after decompression. Previous work (Mulcahey and Taylor, 1992) has demonstrated that methanol-modified CO_2 at concentrations greater than 4% required a heated trap or tandem trap in order to yield quantitative recoveries. Last, it was believed that analyte loss may occur during the sample

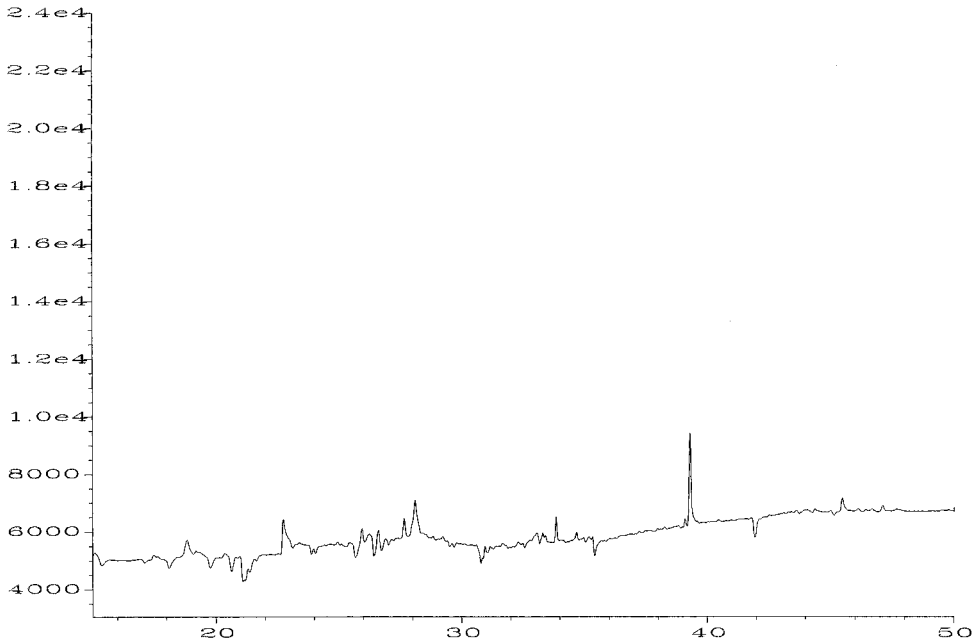

Figure 5. GC/ECD trace of blank rendered fat via SFE. Conditions are the same as G conditions in Table 2. GC conditions are same as in Figure 1.

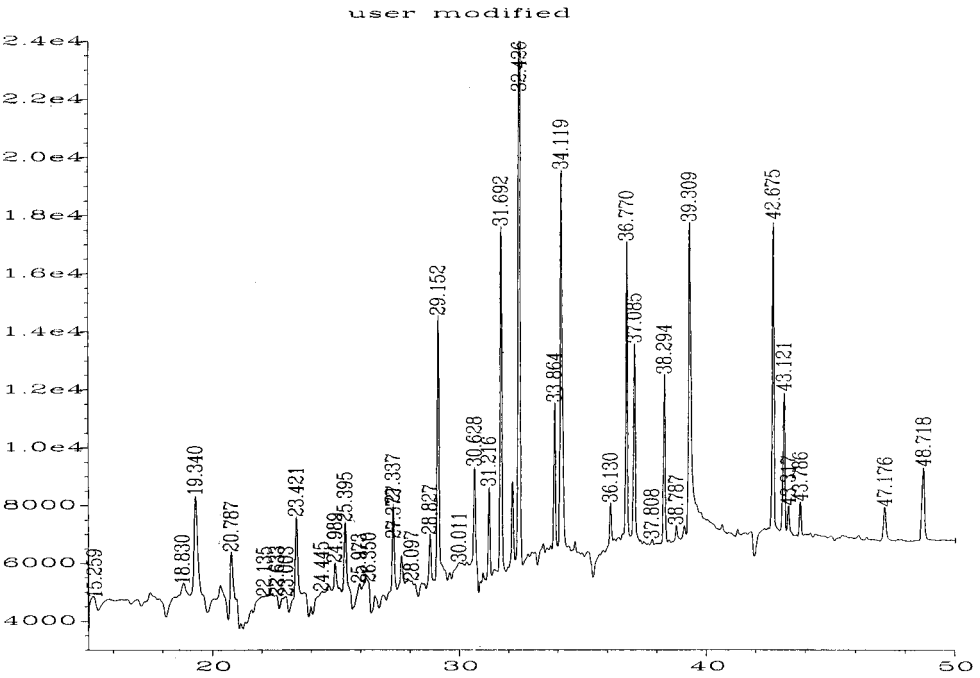

Figure 6. GC/ECD trace of organochlorine pesticides from rendered fat via SFE. SFE conditions are the same as G conditions in Table 2. GC conditions are same as in Figure 1.

cleanup procedure. Consequently, no cleanup procedure was used for experiment E. The trap rinse solvent was slightly altered to 80/20 MeOH/CH_2Cl_2. Extraction efficiency of most analytes relative to liquid-solid extraction increased (Table 4) to more than 70% employing these conditions. Figure 4 shows the GC/ECD trace of the OCPs extract from rendered fat using the E conditions. Eliminating the cleanup procedure had a positive effect on recovery, while altering the rinse solvent did not affect recovery. The GC/ECD trace of the extract matched more closely that of the standards that were used to fortify the matrix. Quantitative results, however, were not obtained because the extraction was thought to not be exhaustive.

In light of these findings, a decision was made to use the simplest conditions possible (e.g., no modifier, no alumina, no extract cleanup) for extraction of OCPs from rendered fat. Also, sample size was reduced from 0.5 to 0.2 g in hopes of minimizing any mechanical removal of fat during extraction. Glass beads were used to disperse the fat in the extraction vessel. Octadecylsilica rather than alumina served as the external trap. All other extraction parameters were similar to conditions E. Figure 5 shows the GC/ECD trace of a 0.2 g fat extract which was not spiked with OCPs. As can be observed, the background level was much lower when pure CHF_3 was used as an extraction fluid rather than methanol-modified CHF_3. Table 4 shows the extraction efficiency and % RSD of OCPs extracted from rendered fat using conditions F. In general, recoveries for some of the analytes were similar to extractions A−D; however, quantitative results (75−125%) for triplicate mea-

surements were obtained for some of the analytes. In several cases recoveries relative to liquid−solid extraction were quite high (e.g., CVP, 139%; coumaphos-S, 178%). It is also important to note that all 21 GC peaks in our standard trace could be accounted for in this extract GC/ECD trace (Figure 6). In other words, background was now minimal. Reflecting on the notion that OCPs may strongly partition with the fat that is either not extracted nor immobilized and thereby remains in the matrix, a longer dynamic extraction step (75 vs 45 min) was instituted (extraction G). The results obtained in duplicate (Table 4) were much improved with only four analytes falling out of the 75−125% range relative to liquid−solid extraction. Unfortunately, we were not able to obtain more than two extractions on this matrix because our supply of sample was exhausted.

In conclusion, the extraction of OCPs from rendered fat using CHF_3 yielded quantitative recoveries without a further cleanup procedure. Due to the low solubility of fat in CHF_3, the OCPs were removed, while fat remained in the extraction vessel. Extraction of rendered fat with methanol-modified CHF_3 demonstrated that low percentages of fat are moved (even in the presence of activated alumina) from the extraction vessel into the collection vial which can yield a high background GC/ECD signal. Also it was demonstrated that glass beads can be a good candidate for dispersing the fat in these type applications. The preliminary work reported here sets the stage for a tremendous breakthrough in pesticide residue analysis in terms of both time and also organic solvent useage. The technique has already been demonstrated with sulfa drug analysis in chicken liver (Ashraf-Khorassani and Taylor, 1996), and we currently are exploring the feasibility of CHF_3 for sample preparation in the determination of other analyte classes in fatty matrices.

LITERATURE CITED

Ashraf-Khorassani, M.; Taylor, L. T. Comparison of Supercritical CHF_3 and CO_2 for Extraction of Sulfonamides from Chicken Liver. *J. AOAC Int.* **1996**, submitted for publication.

Ashraf-Khorassani, M.; Taylor, L. T.; Zimmerman, P. Nitrous Oxide Versus Carbon Dioxide for Supercritical-Fluid Extraction and Chromatography of Amines. *Anal. Chem.* **1990**, *62*, 1177.

Ashraf-Khorassani, M.; Combs, M. T.; Taylor, L. T. Effect of Moisture on Supercritical Fluid Extraction of PAHs and Phenols From Soil Using Automated Extractor. *High Resolut. Chromatogr*, **1995**, *18*, 709.

France, J. E.; King, J. W. Analysis of Organophosphorus Pesticides in Rice by Supercritical Fluid Extraction and Quantitation Using an Atomic Emission Detector. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 1013.

France, J. E.; King, J. W.; Snyder, J. M. Supercritical Fluid-Based Cleanup Technique for the Separation of Organochlorine Pesticides from Fats. *J. Agric. Food Chem.* **1991**, *39*, 1871.

Gillespie, A. M.; Walters, S. M. HPLC Silica Column Fractionation of Pesticides and PCB From Butter Fat. *J. Liq. Chromatogr.* **1986**, *9*, 2111.

Gillespie, A. M.; Walters, S. M. Semi-Preparative Reverse Phase HPLC Fractionation of Pesticides From Edible Fats and Oils. *J. Liq. Chromatogr.* **1989**, *12*, 1687.

Hawthorne, S. B.; Langenfeld, J. J.; Miller, D. J.; Burford, M. D. Comparison of Supercritical Chlorodifluoromethane, Nitrous Oxide, and Carbon Dioxide for the Extraction of Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons. *Anal. Chem.* **1992**, *64*, 1614.

Hillmann, R.; Baechmann, K. Extraction of Pesticides Using Supercritical Trifluoromethane and Carbon Dioxide. *J. Chromatogr. A* **1995**, *695*, 149.

Hopper, M. L. Gel Permeation System for Removal of Fats During Analysis of Foods for Residues of Pesticides and Herbicides. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 720.

Hopper, M. L.; King, J. W. Enhanced Supercritical Fluid Carbon Dioxide Extraction of Pesticides from Foods Using Pelletized Diatomaceous Earth. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 661.

Howard, A. L.; Yoo, W. J.; Taylor, L. T.; Schweighardt, F. K.; Emery, A. P.; Chesler, S. N.; MacCrehan, W. A. Supercritical Fluid Extraction of Environmental Analytes Using Trifluoromethane. *J. Chromatogr. Sci.* **1993**, *40*, 401.

Jones, L. R.; Riddick, J. A. Separation of Organic Insecticides from Plant and Animal Tissues. *Anal. Chem.* **1952**, *24*, 569.

King, J. W. Fundamentals and Applications of Supercritical Fluid Extraction in Chromatographic Science. *J. Chromatogr. Sci.* **1989**, *27*, 355.

King, J. W.; Hopper, M. L.; Luchtefeld, R. G.; Taylor, S. L.; Orton, W. L. Optimization of Experimental Conditions for the Supercritical Carbon Dioxide Extraction of Pesticide Residues from Grains. *J. AOAC Int.* **1993**, *76*, 857.

Klein, A. K.; Laug, E. P.; Tighe, J. F.; Ramsey, L. L.; Mitchell, L. C.; Kunze, F. M. Biological Assay of Endrin in Leafy Vegetables and Its Confirmation by Paper Chromatography. *J. Assoc. Off. Anal. Chem.* **1956**, *39*, 242.

Lee, H.; Chau, A. S. Y.; Kawahara, F. In *Analysis of Pesticides in Water*; Afghan, B. K., Robinson, J. W., Eds.; CRC Press: Boca Raton, FL, 1982.

Luke, B. G.; Richards, J. C.; Dawes, E. F. Recent Advances in Cleanup of Fats by Sweep Co-Distillation. Part I: Organochlorine Residues. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 295.

McKinley, W. P.; Mahon, J. H. Identification of Pesticide Residue in Extracts of Fruits, Vegetables, and Animal Fats. I. Chromatography. *J. Assoc. Off. Anal. Chem.* **1959**, *42*, 725.

McLeod, H. A.; Wales, P. J. A Low Temperature Cleanup Procedure for Pesticides and Their Metabolites in Biological Samples. *J. Agric. Food Chem.* **1972**, *20*, 624.

Mills, P. A. Detection and Semiquantitative Estimation of Chlorinated Organic Pesticides Residues in Foods by Paper Chromatography. *J. Assoc. Off. Anal. Chem.* **1959**, *42*, 734.

Mulcahey, L. J.; Taylor, L. T. Collection Efficiency of Solid Surface and Sorbent Traps in Supercritical Fluid Extraction with Modified Carbon Dioxide. *Anal. Chem.* **1992**, *64*, 2352.

Murugaverl, B.; Gharaibeh, A.; Voorhees, K. J. Mixed Adsorbent for Cleanup During Supercritical Fluid Extraction of Three Carbamate Pesticides in Tissues. *J. Chromatogr.* **1993**, *657*, 223.

Nam, K.; King, J. W. Coupled SFE/SFC/GC for the Trace Analysis of Pesticide Residues in Fatty Food Samples. *J. High Resolut. Chromatogr.* **1994a**, *17*, 577.

Nam, K.; King, J. W. Supercritical Fluid Extraction and Enzyme Immunoassay for Pesticide Detection in Meat Products. *J. Agric. Food Chem.* **1994b**, *42*, 1469.

Niemann, R. A.; Brumley, W. C.; Firestone, D.; Sphon, J. A. Analysis of Fish for 2,3,7,8-Tetrachlorodibenzo-p-dioxin by Electron Capture Capillary Gas Chromatography. *Anal. Chem.* **1983**, *55*, 1497.

Ott, D. E.; Gunther, F. A. Forced Volatilization Cleanup of Butter Fat for Gas Chromatography Evaluation of Organochlorine Insecticide Residues. *J. Agric. Food Chem.* **1964**, *12*, 239.

Paquet, A.; Khan, S. U. Release of Covalently Bound Metabolites of Organophosphate Pesticides from Synthetic Dialkylphosphosryl Peptides by Supercritical Fluid Extraction. *J. Agric. Food Chem.* **1995**, *43*, 843.

Penwell, A. J.; Comber, M. H. I. A Note on Supercritical Fluid Extraction (SFE) - An Alternative to Traditional Techniques in Regulatory Environmental Analysis? *Sample Prep. Biomed. Environ. Anal.* **1994**, 203.

Skopec, Z. V.; Clark, R.; Harvey, P. M.; Wells, R. J. Supercritical Fluid Extraction/Enzyme Assay: A Novel Technique to Screen for Pesticide Residues in Meat Products. *J. Chromatogr. Sci.* **1993**, *31*, 445.

Stahl, E.; Willing, E. Quantitative Determination of the Solubility of Opium Alkaloids. *Mikrochim. Acta (Wien) II* **1981**, 465.

Stalling, D. L.; Tindle, R. C.; Johnson, J. J. Cleanup of Pesticides and PCBs Residues in Fish Extracts by Gel Permeation Chromatography. *J. Assoc. Off. Anal. Chem.* **1972**, *55*, 32.

Stalling, D. L.; Saim, S.; Kuo, K. C.; Stunkel, J. J. Integrated Sample Procesing by On-Line Supercritical Fluid Extraction-Gel Permeation Chromatography. *J. Chromatogr. Sci.* **1992**, *30*, 486.

Stanley, R. L.; LeFavoure, H. T. Rapid Digestion and Cleanup of Animal Tissue for Pesticides Residue Analysis. *J. Assoc. Off. Anal. Chem.* **1965**, *48*, 666.

Steinwandter, H. An On-Line Method for Extracting and Isolating Chlorinated Hydrocarbons, Pesticides, and PCBs From Milk and Diary Products. *Fresenius' Z. Anal. Chem.* **1982**, *342*, 312.

Storherr, R. W.; Watts, R. R. A Sweep Co-Distillation Cleanup Method for Organophosphate Pesticides. *J. Assoc. Off. Anal. Chem.* **1965**, *48*, 1154.

Taylor, S. L.; King, J. W. Supercritical Fluid Extraction of Organochlorine Pesticides Using Trifluoromethane. *Air Products Performance Bulletin*; Air Products & Chemicals: Allentown, PA, 1994.

Tindle, R. C.; Stalling, D. L. Apparatus for Automated Gel Permeation Cleanup for Pesticides Residue Analysis. *Anal. Chem.* **1972**, *44*, 1768.

Walters, S. M. Clean-up Techniques for Pesticides in Fatty Foods. *Anal. Chim. Acta* **1990**, *236*, 77.

Wheeler, J. R.; McNally, M. E. Supercritical Fluid Extraction and Chromatography of Representative Agricultural Products with Capillary and Microbore Columns. *J. Chromatogr. Sci.* **1989**, *27*, 534.

Received for review January 20, 1996. Revised manuscript received July 12, 1996. Accepted August 12, 1996.[⊗] Support for this project was graciously provided by the Suprex Corp. and Air Products and Chemical Inc.

JF960037W

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1996.