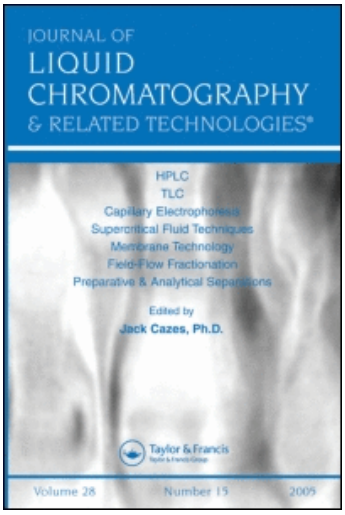

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HPLC SILICA COLUMN FRACTIONATION OF PESTICIDES AND PCB FROM BUTTERFAT

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ABSTRACT

Separation of trace levels of toxic pesticides and other chemical contaminants (e.g., PCB) from edible fats and oils in preparation for quantitation by gas chromatography (i.e., GC-EC, GC-MS, etc.) continues to be an important analytical concern. Commercial HPLC silica columns were evaluated for practical application to the fractionation of selected compounds of different polarity from butterfat. Samples were injected in hexane solution and fractionated using isocratic hexane-methylene chloride mobile phases. Fat was readily purged from the columns with methylene chloride. A 254-nm UV detector was used to monitor elution patterns of concentrated standard solutions and of lipid material. Analyte fractions subsequently collected from samples fortified at sub-ppm levels were analyzed by GC-EC after concentration into hexane. A semi-preparative HPLC silica column (25 cm x 9.4 mm i.d., 6 um spheres) provided complete separation of organochlorine compounds and partial separation of organophosphorous pesticides tested from at least 500 mg of fat. This column was shown to be superior to the "official" (AOAC) Florisil column with regard to resolution, speed of elution, solvent volume required, and loading capacity. The column, which operated at mod-

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erate pressures of <1000 psi, proved to be resilient and reproducible to repeated sample injections, fractionations and purgings made from either end.

INTRODUCTION

Cleanup of samples with high fat or oil content for subsequent determination of pesticides and other chemical contaminants has been the object of many studies over the years. A variety of techniques for separating lipids from pesticides have been reported, including low-temperature precipitation of fat (1), pesticide volatilization or "sweep co-distillation" (2,3), gel permeation chromatographic separation (4,5) and adsorption chromatography. Of these, adsorption chromatography using various types of sorbents has probably been the most widely used.

Sorbents commonly applied to lipid sample cleanup include alumina (6,7), silica gel (8) and Florisil (9-12). The materials traditionally used consist of large particle sizes (e.g., 150 um or larger) which are usually dry-packed in glass columns and eluted with solvents by gravity flow or with slight pressure. Though generally effective in adsorbing the bulk of lipids from fatty sample extracts, these materials suffer several disadvantages such as lot-to-lot variation, poor column efficiency (HETP), slow elution with high solvent volume consumption, and relatively low sample loading capacity.

State-of-the-art HPLC columns packed with microparticles (i.e., 3 to 10 um average diameter) of uniform shape, diameter, pore size and surface area offer significant advantages, e.g., reproducibility, efficiency, speed, lower solvent consumption and higher sample loading capacity (13). Commercial columns commonly available (excluding "microbore") range from "analytical" scale (3-6 mm i.d.) to "semi-preparative" (9-10 mm i.d.) to "preparative" (20 mm or larger i.d.), and are usually 15-30 cm in length.

Though many applications of microparticulate HPLC columns to the separation of structurally similar analytes or to compound "purification" have been made, very little has been reported with regard to their use for the primary isolation of trace contaminants from the bulk of fats and oils. Majors and Johnson (14) demonstrated the effective separation of p,p'-DDT from butterfat on a small pore-size HPLC exclusion (gel permeation) packing consisting of a cross-linked polystyrene gel (8-10 um particle diameter) in a 60 cm x 8 mm i.d. column. That experiment was limited to the separation of the DDT at a relatively high concentration (100 ppm) from a butterfat sample loading of only 5 mg, but it did demonstrate the advantages of speed and small solvent volume required compared to large particle size gels.

The AOAC official method of analysis for several organochlorine and organophosphorous pesticide residues in fat-containing foods (15) uses conventional gravity-flow Florisil columns to remove residual fat from sample extracts after partial cleanup by partitioning between aqueous acetonitrile and petroleum ether. Pesticides are eluted from the Florisil columns and fractionated into groups using solvents of differing polarity. The Florisil cleanup is not entirely adequate in that the more polar solvent fractions usually contain appreciable amounts of residual lipid material. This material must be further removed prior to determination of pesticides by gas-liquid chromatography (GLC) to prevent severe contamination and/or interferences in GLC columns and detection systems (e.g., electron capture or mass spectrometry). AOAC official procedures for this purpose (i.e., "supplemental" cleanups) consist of additional gravity-flow adsorption chromatography on specially prepared magnesium oxide-Celite columns and fat saponification by hot alkali treatment. These procedures are time-consuming and/or detrimental to some pesticides. Alternative cleanup techniques are therefore desirable.

The intent of the work reported here was to evaluate the potential suitability of commercial, state-of-the-art, HPLC silica columns for the isolation of

selected pesticides and PCBs from butterfat. Factors of primary concern included resolution from fat, sample loading capacity, recovery of pesticides from fortified samples, and column durability to repetitive fat injections with subsequent polar solvent strippings. GLC with electron capture detection (GC-EC) was used to quantitate recoveries of the analytes fractionated and collected from samples fortified at levels of 0.1-2.0 ppm.

EXPERIMENTAL

Reagents

All solvents used were "high purity" for use in HPLC, gas chromatography, pesticide residue analysis and spectrophotometry (Burdick & Jackson Laboratories, Muskegon, MI U.S.A.). Pesticide and PCB standards were obtained from the Pesticides & Industrial Chemicals Repository of the U.S. Environmental Protection Agency, and were dissolved in hexane.

HPLC

HPLC instrumentation consisted of a Spectra-Physics Model SP8700 solvent delivery system, a Waters Associates Model U6K syringe loading injection valve, a Waters Associates Model 440 adsorbance detector with 254 nm filter, and a Spectra-Physics Model SP4100 computing integrator. Columns investigated included:

(1) a 6.2 mm i.d. x 25 cm ZORBAX PSM 60-S high performance size exclusion (HPSEC) column (silanized, porous silica spheres of 6 um nominal particle diameter designed for size exclusion separations of compounds in the molecular weight range of 2×10^2 to 10^4); (2) a 4.6 mm i.d. x 25 cm ZORBAX SIL column (6 um, porous spherical silica particles designed for adsorption chromatography); and (3) a 9.4 mm i.d. x 25 cm ZORBAX SIL "semi-preparative" column. All columns were obtained from Dupont Co., Wilmington, DE U.S.A. Mobile phases used consisted of mixtures of methylene chloride and hexane which were sparged with helium before use.

GLC

GLC instrumentation consisted of a Varian Model 3740 gas chromatograph equipped with a ^{63}Ni electron capture detector and a Varian Model 9176 recorder (Varian Instrument Group, Walnut Creek, CA U.S.A.). The column used was 2 mm i.d. x 1.8 m glass, packed with 5% OV-101 on Chromosorb WHP (80-100 mesh). Injector, column, and detector temperatures were 220, 200 and 350°C, respectively. Nitrogen at 30 ml/min was used as carrier gas.

Methods

Butterfat was isolated from whole butter by warming until the fat separated and then decanting the fat

layer (15). A solution for HPLC injection was prepared by dissolving the fat in hexane at a concentration of 0.4 g/ml and filtering through a 0.45 um pore-size nylon membrane. Aliquots of this solution were fortified with pesticide standards and chromatographed on the HPLC systems.

Compounds used in this study represent varying degrees of polarity and were selected from those which have been previously tested with regard to elution patterns on Florisil columns per the AOAC official method (15). These compounds are also representative of those likely to be found as contaminants in fatty samples.

HPLC elution patterns of the pesticide and PCB standards were determined using relatively high concentrations (i.e. $\geq$ 100 ug/ml) of these compounds such that their peaks could be readily monitored with the 254-nm UV detector, since the trace levels of concern are not generally detectable by LC-UV. The elution of the bulk of the lipid material from fat sample injections was also monitored with the UV detector. The amounts of fat sample injected were varied to test the loading capacity of the columns, i.e., the amount which could be injected before significant fat breakthrough into the collected pesticide fraction occurred. The mobile phase composition, in terms of percent methylene chloride in hexane, was also varied and evaluated with respect to resolution of pesticides from fat.

Recoveries of pesticides from fat fortified at trace levels (ppm and lower) were determined by GLC. Fractions of HPLC eluates of standards and fortified fat were collected through the exit tubing from the UV detector into 500-ml Kuderna-Danish (K-D) concentrators (Kontes, Vineland, NJ U.S.A.) and evaporated on a steambath to <10 ml. Hexane (50 ml) was added and the evaporation was repeated to <10 ml to completely eliminate methylene chloride. The hexane solution was then evaporated under a stream of nitrogen at room temperature to 1.0 ml for GLC-EC quantitation.

Though the elution of the bulk of the fat from the column appeared evident as a huge peak in the UV chromatogram, fractions of the eluate preceding this "fat peak" were collected in tared flasks, evaporated and weighed to ascertain whether or not any measurable fat residue (not detected by UV) would occur in the analyte elution region.

RESULTS AND DISCUSSION

HPSEC Column

Using methylene chloride-hexane mobile phases, the HPSEC column behaved more like an adsorption chromatography column in that the lipid material (i.e., "large" molecules) was retained at least as long as pesticide standards; this was apparently due to adsorption of the

fat by active silanol groups left uncovered by incomplete silanization. With the addition of a little methanol to the mobile phase to compete for these active sites, the column behaved more as a size exclusion column with the larger fat molecules eluting first. However, complete separation of pesticides from any appreciable quantity of fat did not appear promising under the latter conditions, as considerable peak overlap occurred between p,p'-DDT and only 100 mg of fat. Further work on this system was therefore abandoned in favor of microparticulate adsorption chromatography using uncoated, porous silica, which appeared to have greater potential for this application.

Silica Adsorption Columns

4.6 mm i.d. column

Prior to purchasing a relatively expensive "semi-preparative" column for this study, a 4.6 mm i.d x 25 cm "analytical" column already available in the authors' laboratory was selected for preliminary evaluation. The preliminary testing demonstrated that complete resolution of all of the organochlorine compounds selected for study from at least 100 mg of butterfat was readily feasible on the 4.6 mm i.d. column. The retention volume of p,p'-methoxychlor, which was the last organochlorine compound to elute from the column, was about 8 ml in 40+60 methylene chloride-hexane.

Using this mobile phase, the p,p'-methoxychlor eluted just ahead of any significant breakthrough of lipid material from a 100 mg injection of fortified fat.

Recoveries of six different standards fractionated from 100 mg fortified fat on the 4.6 mm i.d. column are shown in Table 1. The entire eluate from the point of injection through the elution of p,p'-methoxychlor was collected and evaporated for GC-EC quantitation. The fraction so collected was virtually free of lipid material. Small analyte losses which occurred were attributable primarily to volatility during evaporation, and will be discussed later in this report.

The fat was readily and completely washed off the column with 100% methylene chloride. The column showed

TABLE 1

Recovery of Selected Pesticides Fractionated from 100 mg Fortified Butterfat on 4.6 mm i.d. x 25 cm HPLC Silica Column using 40+60 Methylene Chloride-Hexane Mobile Phase at 1.6 ml/min.

Pesticide	Fortification level, ppm	No. of trials	Recovery, % Mean	Range
Lindane	0.2	5	92.0	83.0- 99.0
Chlorpyrifos	0.6	5	94.7	85.5-103.8
Heptachlor epoxide	0.4	5	92.0	83.2- 99.5
p,p'-DDE	0.6	5	102.9	89.6-111.0
p,p'-DDT	0.6	5	95.5	82.7-104.0
p,p'-methoyxchlor	2.0	2	91.8	89.0- 94.5

no loss of efficiency nor of retentivity with repetitive injections of fortified fat samples and subsequent purgings. Reversal of the column prior to methylene chloride washing permitted more rapid removal of the fat, which was still largely concentrated near the head of the column after fractionation of the analytes. Injections were made from either end with similar results.

9.4 mm i.d. column

Having determined the utility of HPLC silica for repetitive fat sample cleanup and pesticide recovery with the "analytical" column, the "semi-preparative" column was purchased for evaluation. Presumably, the larger column would provide separation of the analytes from a significantly larger sample load, thereby enhancing the analytical sensitivity of the procedure. Indeed, in a comparison of retention data between the two columns for p,p'-methoxychlor in 100 mg of butterfat, the time between the pesticide elution and the onset of visible fat breakthrough in 40+60 methylene chloride-hexane was increased by >30 min (48 ml at a flow rate of 1.6 ml/min) with the use of the larger column. (See Table 2).

LC-UV chromatograms of a mixture of four pesticide standards (Note: This is a concentrated solution) and of these same standards in 100 mg of fortified butter-

TABLE 2

Comparison of Retention Data for Selected Pesticides in 100 mg Fortified Butterfat on 4.6 mm i.d. x 25 cm and 9.4 mm i.d. x 25 cm HPLC Silica Columns using 40+60 Methylene Chloride-Hexane Mobile Phase at 1.6 ml/min.

	Retention time, min	
	4.6 mm i.d.	9.4 mm i.d.
p,p'-DDE	2.2	8.6
p,p'-DDT	2.2	8.7
Chlorpyrifos	3.0	11.6
p,p'-methoxychlor	4.7	22.4

Fat breakthrough point, min	
4.6 mm i.d.	9.4 mm i.d.
~5	~57

fat on the 9.4 mm i.d. column are shown in Figure 1. In this case, a "stronger" mobile phase (50+50 methylene chloride-hexane) was used at a faster flow rate of 2 ml/min for the sake of illustration. (Increased methylene chloride and flow rate resulted in faster elution of both pesticides and fat). A small amount of UV detectable background, presumably attributable to the sample matrix, is seen in the fortified sample chromatogram. This background was negligible, as no "measurable" weight of material (i.e., residue weighed on analytical balance) was found nor were any significant GC-EC peaks other than the added pesticides seen in the eluate collected prior to the onset of the off-scale "fat peak". As could be anticipated, the pesti-

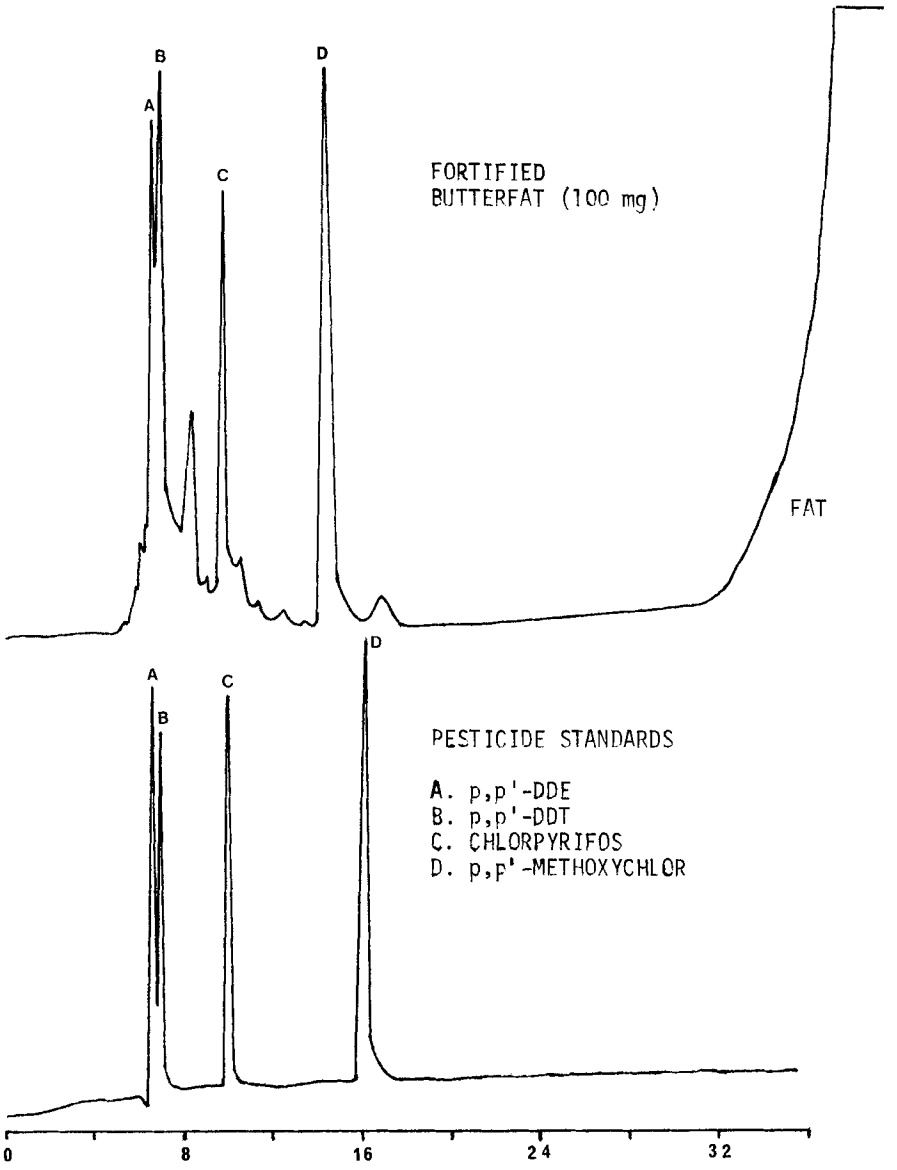

FIGURE 1. LC-UV chromatogram of pesticide standards and of fortified butterfat using 9.4 mm i.d. x 25 cm semi-preparative silica column with 50+50 methylene chloride-hexane mobile phase at 2 ml/min. (NOTE: Concentrated standard solutions used here).

cide retention times were slightly decreased and the peaks were slightly broadened, especially for the longer retained compounds, when co-injected with appreciable fat. As the amount of fat was increased, the analyte retention times decreased; thus, the analyte fraction collection times were also dependent on the sample size.

The resolution of analytes from fat, and consequently the sample load which the column would retain without breakthrough of fat into the analyte fraction (i.e., "overload"), was largely dependent on the methylene chloride strength of the mobile phase. As shown in Figure 2, the separation of p,p'-methoxychlor from 100 mg of fat (i.e., time between elution of pesticide and onset of "fat peak") increased rapidly with reduction of methylene chloride strength below 60% (v/v). Hence, the sample capacity was increased by lowering the methylene chloride strength, but at significant expense of mobile phase consumption. For example, 300 mg of fat was well resolved from p,p'-methoxychlor using 20+80 methylene chloride-hexane at 4 ml/min, but elution of the pesticide required about 37 min or 148 ml of mobile phase. Using 10+90 methylene chloride-hexane, this compound was well resolved from 500 mg fat but required >400 ml of mobile phase for elution.

The compounds for which retention and separation properties were evaluated on the semi-preparative sili-

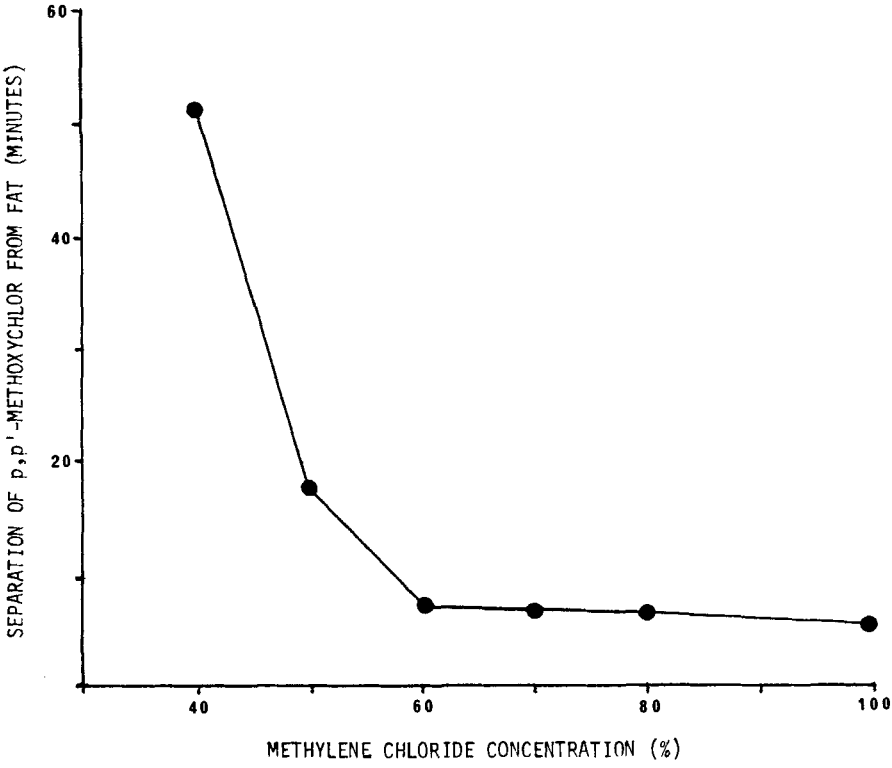

FIGURE 2. Effect of methylene chloride concentration (mobile phase polarity) on separation of p,p'-methoxychlor from 100 mg of butterfat using 9.4 mm i.d. x 25 cm semi-preparative silica column and 2 ml/min flow rate.

ca system are listed in Table 3 along with the retention times of the standards alone (i.e., no fat present) in 40+60 methylene chloride-hexane at 2 ml/min flow rate. The PCB mixture (Aroclor 1254) eluted as a single peak. As expected, the more polar organophosphorous pesticides eluted later than the organochlorine

compounds. (Ronnel, being a less polar chlorophenyl-substituted phosphorothioate, was an exception). Malathion and diazinon were so highly retained that no peaks appeared after extensive elution with this mobile phase.

Figure 3 illustrates the relative fractionation patterns for all of the compounds tested in the presence of 250 mg of butterfat, using the same mobile phase and flow rate as in Table 1. The eluates were

TABLE 3

Retention Data for Standards of all Compounds Tested on 9.4 mm i.d. x 25 cm HPLC Silica Column using 40+60 Methylene Chloride-Hexane Mobile Phase at 2 ml/min.

Compound	Retention	
	Time, min	Volume, ml
Aldrin	6.8	13.6
p,p'-DDE	6.9	13.8
o,p'-DDT	7.1	14.2
Heptachlor	7.1	14.2
Aroclor 1254	7.2	14.4
p,p'-DDT	7.2	14.4
p,p'-DDD	7.8	15.6
Ronnel	10.7	21.4
Heptachlor epoxide	11.1	22.2
Chlorpyrifos	12.0	24.0
Lindane	13.5	27.0
Mirex	14.3	28.6
Dieldrin	17.0	34.0
Endrin	17.7	35.4
p,p'-methoxychlor	21.6	43.2
Ethion	24.4	48.8
Parathion	31.5	63.0
Methyl parathion	37.1	74.2
Malathion, diazinon	Highly retained; elution with 40+60 methylene chloride-hexane is impractical.	

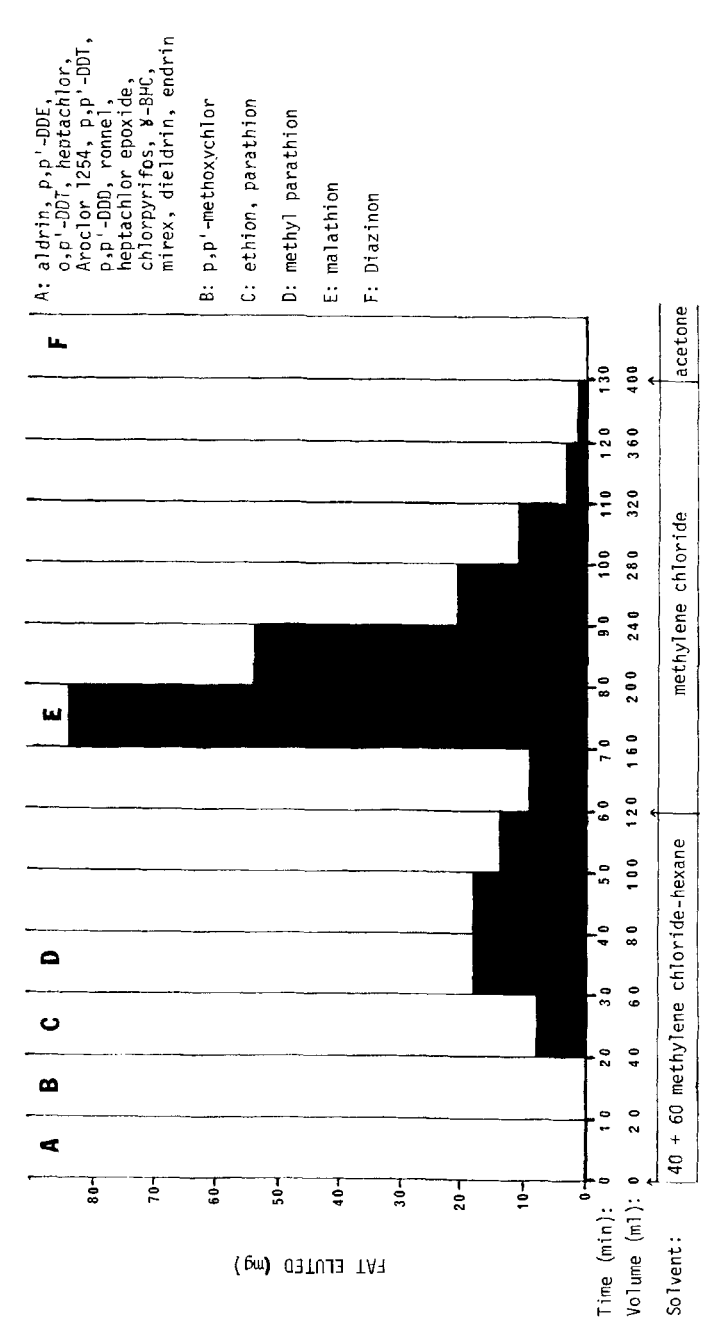

FIGURE 3. Fractionation pattern for pesticides and Aroclor 1254 in 250 mg of fortified butterfat on 9.4 mm i.d. x 25 cm semi-preparative silica column eluted with 120 ml of 40+60 methylene chloride-hexane at 2 ml/min, 280 ml methylene chloride at 4 ml/min, and a few ml of acetone. (NOTE: Diazinon elutes immediately with acetone).

collected every 10 min, evaporated and weighed to determine the amount of fat present. All fifteen organochlorine compounds (and ronnel) eluted fat-free in the first 20 min (40 ml). Ethion and parathion eluted between 20-30 min (40-60 ml) and methyl parathion eluted between 30-40 min (60-80 ml). These latter organophosphorous compounds appeared in the leading edge of the "fat peak" and were thus co-eluted with a small amount of lipid material. After 60 min (120 ml), the mobile phase was changed to 100% methylene chloride at 4 ml/min which subsequently eluted the fat much more rapidly. Though malathion was not discernible in the UV chromatogram in the presence of the fortified lipid background, it was estimated to be in the 160-200 ml total volume fraction (40-80 ml methylene chloride) based on the "standard alone" chromatogram run under the same conditions. Diazinon did not appear even after 440 ml of methylene chloride and all measurable fat had eluted, but was found to elute rapidly when a little acetone was introduced into the column.

Clearly, the more polar organophosphorous compounds, especially malathion, are more difficult to separate from fat using the specified system. Diazinon, being very highly retained, could presumably be eluted and collected for subsequent analysis in a more polar solvent such as acetone after washing off all fat

with methylene chloride. If only those compounds eluting ahead of the bulk of the lipid material were desired for GC-EC determination, the column was reversed after collecting the analyte fraction and the fat was washed off more rapidly with methylene chloride (e.g., 250 mg butterfat was completely eliminated in about 25 min with 100 ml at 4 ml/min). Injections were subsequently made from either end of the column with similar results; this was successfully done numerous times during the course of this study.

The amount of butterfat injected (i.e., 250 mg) in the aforementioned study (Figure 3) is the approximate amount which is carried over from the acetonitrile-petroleum ether partitioning of 2-3 g of sample prior to Florisil chromatography cleanup by the AOAC official method (15). A comparison of fractionation patterns for the compounds tested in the presence of 250 mg butterfat is shown in Table 4. The analyte elution patterns from Florisil shown are as reported in the AOAC method (15) and the amounts of butterfat co-eluted from Florisil are as reported by Mills et al. (16). Using the AOAC "standardized" column of 20 g of activated Florisil, a total eluant volume of 400 ml (200 ml of 6+94 ethyl ether-petroleum ether and 200 ml of 15+85 ethyl ether-petroleum ether) is required to elute all of the compounds which are eluted from the HPLC silica

TABLE 4

Comparison of HPLC Semi-Preparative Silica and Official Florisil Column Fractionation Patterns for Selected Compounds in 250 mg Butterfat

	HPLC Semi-prep. Silica		Florisil, 20 g Column	
Compound	Fraction*	Butterfat co-eluted, mg**	Fraction*	Butterfat co-eluted, mg
Aldrin, p,p'-DDE, p,p'-DDT, heptachlor, Aroclor 1254, p,p'-DDT, p,p'-DDD, ronnel, heptachlor epoxide, chlorpyrifos, lindane, mirex	A	0	1	0
Dieldrin, endrin	A	0	2	180
p,p'-methoxychlor	B	0	1	0
Ethion	C	8	1	0
Parathion	C	8	2	180
Methyl parathion	D	18	2	180
Malathion	E	83	3	70
Diazinon	F	0	2	180

*Fractions (volumes shown are for total solvent added from start of column elution):
A: 0-20 ml (40+60 MeCl-hexane)
B: 20-40 ml (40+60 MeCl-hexane)
C: 40-60 ml (40+60 MeCl-hexane)
D: 60-80 ml (40+60 MeCl-hexane)
E: 160-200 ml (MeCl)
F: Acetone
1: 0-200 ml (6+94 ethyl-petr. ether)
2: 200-400 ml (15+85 ethyl-petr. ether)
3: 400-600 ml (50+50 ethyl-petr. ether)

**Based on a 10-minute collection time for each fraction.

column in 80 ml of 40+60 methylene chloride-hexane (i.e., aldrin through methyl parathion). At the 2 ml/min flow rate used, the HPLC silica column required only 40 min elution time; the Florisil column requires at least 80 min at the specified (15) flow rate of ⩽5 ml/min for this fractionation.

While both solvent volume and elution time are reduced using the HPLC silica column, of even more importance is the improved cleanup of analyte fractions for quantitation. This is especially valuable for dieldrin and endrin, which are fairly common bioincurred contaminants of fatty foods such as dairy products. The latter compounds are well separated from 250 mg butterfat by the HPLC silica column, while they co-elute with >70% of that sample amount from the AOAC Florisil column. The more highly polar organophosphorous compounds, on the other hand, are of less importance here (16) since they are less likely to concentrate in animal fats. The relatively high efficiency of the HPLC silica column serves to significantly reduce analyte peak broadening during elution; however, as previously shown (Figure 1), some broadening does occur due to the fat loading and increases with sample amount (e.g., p,p'-methoxychlor was estimated from the UV chromatogram to be distributed over about 8 ml of eluate when fractionated from 250 mg butterfat). In comparison,

individual analytes eluted in the presence of 250 mg butterfat on Florisil were distributed over eluate volumes of 100-150 ml. The relative band "tightening" of the HPLC column permits complete collection of an analyte or group of analytes in a much smaller volume, thereby also reducing significantly the amount of co-eluted fat in those analyte fractions which are not completely separated from fat.

Mills et al. (16) proposed an alternate solvent elution system for the AOAC Florisil column cleanup procedure. Three solvent mixtures, each 200 ml in volume and consisting of hexane mixed with different strengths of methylene chloride and acetonitrile, were used in that system. The alternate system significantly reduced the amount of butterfat and corn oil eluted from the Florisil column in the second (intermediate) fraction of that system as compared to the AOAC second fraction (i.e., 15+85 ethyl ether-petroleum ether mixture). However, 10-28% of the 250 mg fat or oil sample was still co-eluted in that fraction along with heptachlor epoxide, dieldrin, endrin, p,p'-methoxychlor, and ronnel, all of which were found in the present study to elute fat-free from the HPLC silica column in <40 ml (20 min) of solvent. In addition, incomplete or irreproducible recoveries were noted by the authors of that report (16) for some compounds when using their alter-

nate Florisil elution system. Malathion and diazinon partially eluted in the third fraction of that system along with at least 75% of the fat sample.

Recoveries of several organochlorine compounds collected fat-free from 300-mg fortified butterfat injections on the semi-preparative silica column are shown in Table 5. Since the amount of butterfat was increased to 300 mg in this case, the mobile phase strength was decreased to 20+80 methylene chloride-hexane to assure complete resolution of p,p'-methoxychlor from fat. Flow rate used was 4 ml/min, which eluted all of these compounds in 40 min (160 ml). These recoveries are well above 80%, which is generally considered to be "complete" for the purpose of pesticide residue analysis. However, since the procedure used here is relatively simple requiring few manipulations, the cause for some apparent loss in recovery was investigated. It was determined that significant loss could occur during the solvent evaporation step in the Kuderna-Danish (K-D) concentrator after fraction collection. This loss was found to be highly dependent on the temperature and rate of evaporation, and was apparently due to volatility of the analytes. In particular, the relatively large volume of methylene chloride present was found to be significant. Recoveries shown in Table 1 for the analytical scale column (which

TABLE 5

Recovery of Selected Pesticides Fractionated from 300 mg Butterfat on 9.4 mm i.d. x 25 cm HPLC Silica Column using 20+80 Methylene Chloride-Hexane Mobile Phase at 4 ml/min.

Pesticide	Fortification level, ppm	No. of trials	Recovery, % Mean	Recovery, % Range
Heptachlor epoxide	0.13	2	87.4	86.2-88.5
p,p'-DDE	0.2	2	88.8	87.2-90.3
p,p'-DDT	0.2	2	97.7	96.9-98.5
p,p'-methoxychlor	0.65	2	92.1	89.4-94.8

involved much less solvent) indicated some loss and considerable variability, presumably due to volatility. Fairly large losses (>20%) occurred when the K-D concentrators containing the relatively large analyte fractions collected from the semi-preparative column were placed directly over a "live" steambath at a temperature exceeding 100°C. In subsequent simulated trials with standard solutions alone, losses were greatly decreased but not entirely eliminated by the following procedure: methylene chloride was evaporated more carefully from the solvent mixture by placing the K-D concentrator tube in a 60°C water bath, then slowly raising the temperature over a 30 min period to 95-100°C and holding at the latter temperature to evaporate hexane. The recovery data in Table 5 were obtained using the latter evaporation procedure; these

recoveries show relatively little variability and are equivalent to those obtained from the K-D evaporation of 160 ml of 20+80 methylene chloride-hexane to which measured volumes of standard solutions had been added. Thus, the loss is attributable to evaporation and the recoveries of these analytes in the fractions collected from the column are assumed to be closer to 100% prior to the solvent evaporation step.

The problem of pesticide losses due to volatility at trace levels, especially in very "clean" extracts, has been reported previously (3,17). The presence of some lipid material is presumed to reduce loss of analytes during evaporation. The addition of a few drops of a "keeper solution" consisting of 0.2% Florisil-purified paraffin oil in hexane has been suggested for use as a fixative during solvent concentration. This was tried but was found to be of little help; in addition, some GC-EC background interference was seen in the packed column system used in the present work as a result of adding this solution.

A sample of cheese fat, previously analyzed by the official AOAC method and found to contain 0.61 ppm bio-incurred dieldrin, was analyzed using only the proposed semi-preparative silica column for cleanup. A 200-mg portion of the fat was injected and fractionated using 20+80 methylene chloride-hexane mobile phase at 4 ml/

min. The entire eluate through the elution time of p,p'-methoxychlor was collected so as to check for the presence of other organochlorine compounds in addition to dieldrin. Only dieldrin was found at a level of 0.59 ppm (average of duplicate runs which gave results of 0.61 and 0.57 ppm, respectively). The collected HPLC silica column eluate was fat-free. The AOAC Florisil column fraction containing dieldrin typically contains appreciable fat as well which, without supplemental cleanup, will cause serious deterioration of the GC column. No deterioration of the GC-EC chromatography was noted in the course of the present study with HPLC silica eluates. The good agreement between the dieldrin results supports the reliability of the proposed technique for the analysis of authentic residues in dairy product samples.

The semi-preparative silica column was also found to be resilient and reproducible after numerous injections of fat, reversals of flow, and methylene chloride purgings. The operating pressure was moderate throughout this work, generally well below 1000 psi. Conventional macroparticulate columns, including the AOAC Florisil column, are normally discarded after each sample elution. This practice is clearly not economically feasible with HPLC columns, and thus the durability of the packing to the repetitive treatment des-

cribed is very important in routine applications. The solvents used must be dry and of high purity since moisture and other contaminants will deactivate the silica and reduce the capacity of the column.

CONCLUSIONS

A 9.4 mm i.d. x 25 cm semi-preparative HPLC silica column with simple isocratic binary mobile phases will effectively and rapidly separate a variety of organochlorine compounds from at least 300 mg of butterfat, thereby providing "clean" extracts for subsequent GC-EC quantitation. Complete separation of these compounds from larger sample loads (i.e., at least 500 mg) in a single run through a single column is possible, but at the expense of time and solvent volume. Organophosphorous compounds, due to their higher polarity, are more difficult to separate from fat on this column. The column is durable and reproducible with repetitive use, and provides faster and more complete isolation of important contaminants from fat when compared with the official AOAC method Florisil column.

A plausible means of improving the resolution of the proposed HPLC silica cleanup is to further scale up the chromatographic system, thereby increasing the sample capacity. However, as recently pointed out by Majors (18), this would be done at the expense of loss

in speed, not to mention the increased cost of chromatographic columns, significantly greater solvent consumption, and perhaps the need for higher capacity pumps than commonly available in most laboratories equipped with liquid chromatography instrumentation. Recycling through the semi-preparative system may be a more practical alternative for fractions partially resolved from fat. Yet another option may be the use of different solvent systems to enhance resolution. Hexane and methylene chloride were selected for initial studies based on their past use in column cleanup systems for fats, and because they are easily evaporated in the eluate concentration step. Gradient elution via solvent programming may also improve separation but the additional cost of instrumentation needed for this technique must be considered. As suggested by Verzele and Geeraert in their discussion of gradient elution with preparative silica columns (19), a simple stepwise gradient system may be preferable. Further develment of such a system may warrant additional study and optimization for the application described here.

The HPLC equipment used in the present work is more sophisticated than is necessary to accomplish the task completed. A very basic isocratic HPLC system should suffice to duplicate this work. A refractive index detector might be substituted for the UV detec-

tor, but the chromatograms may be more difficult to interpret when used with fat samples.

One disadvantage of the proposed HPLC cleanup system is the restricted sample throughput with a single column and HPLC system. Ideally, several systems operating simultaneously are needed to increase throughput, which is expensive. However, the potential reduction in materials (sorbent and solvents), time, and manipulations required when compared to conventional macroparticulate chromatographic cleanups (in addition to the demonstrated improvement in results achievable) warrant further consideration of the proposed technique.

The work presented here is admittedly limited in scope with regard to the many chromatographic parameters which could be investigated further. For example, Majors (18) reports that shorter, fatter columns offer advantages of speed and less localized overload. Particle size, shape and porosity are factors that affect sample capacity as well as column cost, and may therefore warrant further evaluation as well.

Future plans include further study of the semi-preparative HPLC technique and its suitability for cleanup of other sample types, particularly oils of vegetable origin which are at least as difficult as animal fats to prepare for pesticide residue determinations (11).

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REFERENCES

(1) McLeod, H.A., and Wales, P.J., J. Agr. Food Chem., 20, 624 (1972).

(2) Watts, R.R., and Storherr, R.W., J. Assoc. Off. Anal. Chem., 50, 581 (1967).

(3) Luke, B.G., Richards, J.C., and Dawes, E.F., J. Assoc. Off. Anal. Chem., 67, 295 (1984).

(4) Stalling, D.L., Tindle, R.C., and Johnson, J.L., J. Assoc. Off. Anal. Chem., 55, 32 (1972).

(5) Hopper, M.L., J. Assoc. Off. Anal. Chem., 64, 720 (1981).

(6) Holden, A.V., and Marsden, K., J. Chromatogr., 44, 481 (1969).

(7) Gillespie, A.M., and Walters, S.M., J. Assoc. Off. Anal. Chem., 67, 290 (1984).

(8) Steinwandter, H., Fresnius Z. Anal. Chem., 312, 342 (1982).

(9) Mills, P.A., J. Assoc. Off. Anal. Chem., 42, 734 (1959).

(10) Porter, M.L., Young, S.J.V., and Burke, J.A., J. Assoc. Off. Anal. Chem., 53, 1300 (1970).

(11) Sawyer, L.D., J. Assoc. Off. Anal. Chem., 65, 1122 (1982).

(12) Moddes, R., J. Assoc. Off. Anal. Chem., 44, 169 (1961).

(13) Majors, R.E., Anal. Chem., 44, 1722 (1972).

(14) Majors, R.E., and Johnson, E.L., J. Chromatogr., 167, 17 (1978).

(15) Williams, S., ed., Official Methods of Analysis of the Association of Official Analytical Chemists, Association of Official Analytical Chemists, Inc., Arlington, VA USA, 1984. Chapter 29.

(16) Mills, P.A., Bong, B.A., Kamps, L.R., and Burke, J.A., J. Assoc. Off. Anal. Chem., 55, 39 (1972).

(17) Chiba, M., and Morley, H.V., J. Assoc. Off. Anal. Chem., 51, 55 (1968).

(18) Majors, R.E., LC, 3, 862 (1985).

(19) Verzele, M., and Geeraert, E., J. Chromatogr. Sci., 18, 559 (1980).