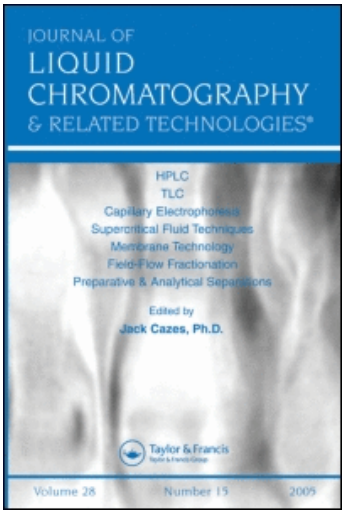

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SEMI-PREPARATIVE REVERSE PHASE HPLC FRACTIONATION OF PESTICIDES FROM EDIBLE FATS AND OILS

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ABSTRACT

A semi-preparative (25 cm x 9.4 mm i.d.) HPLC ODS-bonded silica column was evaluated for the fractionation of pesticides from edible fats and oils of both animal and vegetable origin. Using acetonitrile as a mobile phase, organochlorine and organophosphorous pesticides with a wide range of polarity were eluted in the first 40 ml of eluate. Co-eluted lipid material ranged from 0 to 35 mg for 500 mg injections, depending on sample type. Excessively "dirty" samples (e.g., tallow) were further cleaned up on small Florisil columns. Recoveries of selected pesticides from fortified samples, which were determined by gas chromatography with electron capture and flame photometric detectors, ranged from 80 to 108%.

INTRODUCTION

In a previous publication (1), we described the application of adsorption chromatography using a semi-preparative HPLC silica column for the separation of pesticides and PCB from butterfat for subsequent gas

chromatographic quantitation. This approach, using an isocratic solvent system, provided efficient isolation of relatively nonpolar organochlorine (OC) compounds but was of limited usefulness for the more polar organophosphorus (OP) compounds tested. Subsequent studies (unpublished) in our laboratory, in which stepwise gradient solvent systems were used with the HPLC silica column, showed some improvement over the isocratic system reported earlier (1). However, the apparent similarity in polarity between many OP and organonitrogenous (ON) pesticides and the lipids of both animal and plant origin precluded the development of practical separation schemes for these compounds using this approach.

A method using a silica HPLC column with a stepwise gradient solvent system for the fractionation of OC compounds from lipids and aliphatic and polyaromatic hydrocarbons in environmental samples was recently reported (2). Numerous methods for the separation of pesticides from lipids have been reported over the years, most of which employ various types of adsorption or gel permeation chromatography systems. Several of these were previously discussed (1) and will therefore not be reiterated here.

The AOAC official method of analysis for multipesticide residues in fat (3) employs a form of reverse phase chromatography in the initial liquid-liquid extraction steps. The fat sample is dissolved in petroleum ether (lipophilic phase) and pesticides are extracted into acetonitrile (lipophobic phase) leaving behind the majority of lipid material. Low to medium polarity pesticides are subsequently back-extracted into petroleum ether from the acetonitrile layer by adding water and salt to the acetonitrile layer, thereby reducing the solubility of these pesticides in the acetonitrile layer and effecting their partitioning

into petroleum ether. Pesticides of relatively high polarity are not readily partitioned into petroleum ether in the latter step, however. In addition, considerable lipid material (~10% of original sample weight (4)) is carried over at this point and the method therefore includes adsorption chromatography on Florisil to further isolate the pesticides. However, only those compounds of relatively low polarity are effectively separated from lipids by Florisil. Those fractions containing compounds with intermediate to high polarity also contain high concentrations of lipids which rapidly contaminate GC columns and detectors. Hence, there is a need for practical multi-pesticide residue extraction/cleanup methodology applicable to medium to high polarity compounds in fatty foods.

In the present study, we investigated the potential of reverse phase semi-preparative HPLC using a C-18 bonded (lipophilic) silica packing (6 um spherical particles) with polar (lipophobic) mobile phase for multi-pesticide extraction from edible fats and oils prior to gas chromatographic analysis. It was postulated that the lipids, consisting primarily of long chain fatty acids and esters, would be highly retained in this system. Pesticides, having relatively little alkyl content, would be expected to elute rapidly and thus be separated from the bulk of the sample. It was further anticipated that this microparticulate column would offer the same advantages as the semi-preparative silica column evaluated previously, i.e., low solvent consumption, high efficiency and reproducibility.

EXPERIMENTAL

Reagents

All solvents used were suitable for spectrophotometry, liquid and gas chromatography (EM Science,

Cherry Hill, NJ). Standard materials were obtained from the Pesticides and Industrial Chemicals Repository of the U.S. Environmental Protection Agency. Standard solutions for GC quantitations were prepared in either isooctane (low to medium polarity compounds) or acetone (higher polarity compounds not readily soluble in isooctane). Standard solutions for HPLC experiments were prepared in 1:3 methylene chloride-acetonitrile.

HPLC

HPLC instrumentation consisted of a Waters 590 programmable pump with attached Autochrom Solvent Selector and automated switching valves, a Waters U6K injector equipped with a 10 ml sample loop, a Tracor 970A variable wavelength detector (or Waters 440 absorbance detector) at 254 nm, and a Shimadzu C-R6A data processor. The column used was a 9.4 mm i.d. x 25 cm ZORBAX ODS (DuPont Co., Wilmington, DE). The pump and switching valve system was set up such that the column could be automatically back-flushed with methylene chloride to remove retained lipids following elution of the analytes with acetonitrile mobile phase. A precolumn filter (Upchurch No. A-316 with 0.5 µm frit) was installed between the injector and column and the frit was replaced as necessary.

GLC

GLC instrumentation consisted of a Hewlett-Packard 5880A gas chromatograph equipped with a ^{63}Ni electron capture (EC) detector and a 1.8 m x 2 mm i.d. glass column packed with 5% OV-101 on Chromosorb WHP (80-100 mesh), and a Hewlett-Packard 5890 gas chromatograph equipped with a flame photometric detector (FPD) in the phosphorous mode and a 1.2 m x 2 mm i.d. glass column

packed with 2% DEGS on Chromosorb W AW (80-100 mesh). The GC-EC system was generally operated at column, injector and detector temperatures of 200°C, 220°C and 340°C, respectively, using 5% methane in argon carrier gas at 30 ml/min. Sensitivity was set to give about 50% FSD for 0.2 ng heptachlor epoxide. The GC-FPD system was operated with column, injector and detector temperatures of 180°C, 220°C and 220°C, respectively. Nitrogen carrier gas at 20 ml/min was used and sensitivity was set to give about 50% FSD for 0.8 ng chlorpyrifos.

METHODS

HPLC Retention Characteristics of Standards

Elution patterns of standards were determined by injecting individual solutions prepared in relatively high concentration in acetonitrile (e.g., ≥ 100 ug/ml) such that their peaks could be readily monitored with the UV detector. Verification of HPLC peak identities was made by trapping the individual peaks, evaporating and redissolving in appropriate solvent (isooctane or acetone), and injecting into the appropriate gas chromatograph for comparison with GC standard retention times.

Preparation of Samples

Direct injection technique. - Samples were liquefied by warming and 0.5-1.0 g portions were weighed into conical tubes. (Butterfat was first separated from whole butter by warming at 50°C until the fat separated). Each weighed portion was dissolved in about 5 ml of 1:3 methylene chloride-acetonitrile and placed in a 50°C water bath to decrease viscosity. The

entire volume was loaded in the HPLC injector with a Hamilton #1005 5 ml syringe, using small volumes of acetonitrile to rinse the sample container and syringe.

Pre-extraction technique. - Alternatively, a 0.5-1.0 g sample portion was mixed with 0.5 ml petroleum ether in a conical tube and extracted 4 times with 2-ml portions of petroleum ether-saturated acetonitrile by placing on a vortex mixer for about 30 sec. each time. Each extraction mixture was centrifuged for about 30 sec. and the upper acetonitrile layer was transferred to another tube with a Pasteur capillary pipet. The combined acetonitrile extracts were then loaded into the HPLC injector.

HPLC FRACTIONATION

Because of the rather high viscosity of sample solutions prepared for the direct injection technique, the flow rate was held at 2-3 ml/min after the injection was made until the sample was distributed at the head of the column. The flow rate was then increased to 4-5 ml/min to complete the fractionation. The first 10 ml of acetonitrile eluent (slightly less than one column volume) were discarded and the next 40 ml (analyte fraction) were collected for analysis. The column was back-washed with methylene chloride to remove retained lipids (at least 100 ml at 5 ml/min were used to assure thorough cleaning) and then re-equilibrated with acetonitrile for the next injection. The analyte fraction was evaporated just to dryness using a rotary vacuum evaporator (Büchi, Flawil, Switzerland) and a 55°C water bath. A stream of nitrogen was then applied to assure removal of trace acetonitrile which would interfere in subsequent GC quantitation.

Lipid Breakthrough Studies

A selection of fats and oils of both plant and animal origin were prepared and subjected to HPLC fractionation using the procedures previously described. For the sake of comparison, sample size was limited to about 0.5 g because the high viscosity of some of the commodities tested made it difficult to load larger portions into the 10 ml injection loop. The 40-ml analyte fraction was collected, evaporated to dryness in a tared flask and weighed to measure the amount of co-eluted lipid material.

Standard Fortification Studies

Mixtures of selected standard solutions were prepared and aliquots equivalent to sub-ppm levels were added to 0.5 g portions of selected fat and oil samples. Solvent was evaporated by warming under a stream of nitrogen. Samples were then subjected to the sample preparation and HPLC fractionation procedures described. Recoveries of added standards were quantified by GC analysis.

RESULTS AND DISCUSSION

HPLC retention volumes for a selection of compounds with a wide range of polarity are shown in Table 1. As expected with this system, the smaller and most polar OP and ON compounds eluted rapidly whereas the relatively nonpolar OC compounds were retained the longest. However, later recovery experiments indicated that the polar compounds tailed extensively on this column. Though not evident from the UV detector chromatograms, GC analysis of collected HPLC fractions found 5-10% of highly polar OP compounds

TABLE 1

Retention Volumes for Standards on Semi-Preparative HPLC ODS Column using Acetonitrile Mobile Phase.

Compound	Rv, ml	Compound	Rv, ml
Methamidophos	12.1	Lindane	17.5
Cyanophos	12.2	Mirex	17.5
Acephate	12.4	p,p'-DDD	17.5
Malathion	12.5	Ronnel	17.5
Omethoate	12.8	Diazinon	18.0
Parathion	13.0	Heptachlor epoxide	18.0
Thiometon	13.2		
Parathion methyl	13.5	Endrin	19.0
Tsumacide	13.6	Monocrotophos	19.9
Ethion	15.5	p,p'-DDT	20.5
Nitrothal isopropyl	15.9	Heptachor	21.0
		o,p'-DDT	23.0
p,p'-Methoxychlor	16.0	p,p'-DDE	24.5
Dieldrin	16.5	Aldrin	28.0
Chlorpyrifos	17.5	Aroclor 1254	27.0, 31.0

(e.g., methamidophos and omethoate) remaining in an additional 10 ml elution after eluting with 30 ml acetonitrile. It was presumed that these compounds were highly adsorbed on free silanol sites left unbonded on the silica particles. The addition of reagents (e.g., acetic acid, triethylamine) to counter this activity may have alleviated adsorption, but the possible adverse effects of these reagents on lipid retention and on the analytes in subsequent concentration steps were of concern and remain to be investigated. Thus, a 40-ml fraction of acetonitrile eluent (between 10 and 50 ml after injection) was collected which provided essentially complete recovery of the analytes tested.

Results of lipid breakthrough studies are shown in Table 2. The negligible amounts of co-eluted lipids

TABLE 2

Co-eluted Lipids in Analyte Fractions Collected from 0.5 g Injections of Selected Fats and Oils

Sample	Weight of lipids, mg (duplicate runs)
Lard	0, 0.6
Butterfat	2.5, 2.5
Tallow	32.8, 35.8
Cottonseed oil	0, 0
Safflower oil	0.2, 0
Soybean oil	1.0, 2.0
Corn oil	1.1, 2.8
Olive oil	6.2, 4.6
Sesame oil	8.1, 7.2
Coconut oil	15.3, 13.2
Peanut oil	16.3, 15.7

found for lard, butterfat and several of the vegetable oils can be reasonably tolerated by GC systems, thus permitting quantitative analysis of a goodly number of these samples so prepared before cleaning or replacement of GC injection ports and column would be required. The lipids present in the fractions collected from tallow, coconut oil and peanut oil would generally preclude satisfactory GC quantitation of sub-ppm residue levels without further treatment, however.

Poor solubility of lipids in acetonitrile precluded dissolving samples in this solvent for direct HPLC injection. The use of lipophilic solvents (hexane or methylene chloride) for this purpose resulted in excessive lipid breakthrough in the collected analyte fraction. Ultimately, a 1:3 methylene chloride-

acetonitrile mixture was found to dissolve the sample without inducing significant breakthrough.

The pre-extraction technique described under Sample Preparation is a miniaturization of the initial step in the AOAC official method (3). This procedure added an additional step to the proposed method, but simplified the HPLC fractionation by significantly reducing the lipid concentration and allowing direct injection of the sample in acetonitrile. For example, a 1 g sample of soybean oil was reduced to about 40 mg and a 0.5 g sample of butterfat was reduced to about 96 mg when preextracted from petroleum ether into acetonitrile. However, the amount of lipid "breakthrough" in the HPLC analyte fraction was not significantly reduced by the pre-extraction technique when compared with the direct injection technique for equivalent weights of samples taken (comparisons were made for soybean oil, butterfat and tallow). Presumably, the fat and oil constituents eluting in the analyte fraction are compounds with relatively little alkyl content which are readily partitioned from petroleum ether into acetonitrile and are poorly retained on the ODS column. It therefore appears that the co-eluted lipid material from 0.5 - 1 g of sample injected directly is not a result of breakthrough of heavier lipids; the latter would be anticipated only if the column were overloaded.

Results of recovery studies on samples fortified with selected pesticides and analyzed by the "direct injection technique" are listed in Table 3. Recoveries are generally well above 80%, which is considered to be "complete" for the purpose of residue analysis at these levels. Problems with loss of low polarity analytes (particularly aldrin) during the solvent evaporation step were encountered in our previous work with silica

TABLE 3

Recovery of Selected Pesticides Fractionated from Fortified Samples Using Direct Injection Technique

Pesticide	Added, ppm	No. of Trials	Recovery, % Average	Range
		SOYBEAN OIL, 1g*		
Diazinon	0.86	3	86.8	84.5- 90.0
Malathion	0.52	3	94.7	91.8- 98.8
Parathion methyl	0.40	3	104.1	99.0-107.1
Dieldrin	0.21	3	97.4	93.1- 99.6
Aldrin	0.19	3	85.4	79.7- 90.6
		BUTTERFAT, 0.5g*		
Diazinon	0.86	2	93.8	92.8- 94.9
Malathion	0.52	2	108.2	107.6-108.9
Parathion methyl	0.40	2	93.8	83.0-104.6
Dieldrin	0.32	2	105.6	105.6-105.6
Aldrin	0.31	2	89.4	89.1- 89.7
		COTTONSEED OIL, 1g**		
Methamidophos	0.20	2	88.5	85.5- 91.5
	0.10	4	85.0	78.0- 91.0
Acephate	0.20	2	99.2	93.9-104.6
	0.10	4	89.8	81.2- 98.5
Omethoate	0.32	2	92.4	91.9- 92.8
	0.16	4	87.4	79.5- 96.6
Monocrotophos	0.23	2	101.8	97.0-106.5
	0.12	4	103.2	100.0-107.8

*Eluates were evaporated using alternative isooctane addition technique described in DISCUSSION section.
**Eluates were evaporated without isooctane addition.

column fractionation (1) in which methylene chloride-hexane eluates were evaporated in Kuderna-Danish (K-D) concentrators. The major loss occurred during the initial evaporation stage in which the more polar methylene chloride was driven off. Recoveries were

improved by reducing the temperature of the initial methylene chloride evaporation stage. In the present situation, evaporation in a K-D concentrator required more heat and time because of the lower volatility of the acetonitrile eluate. Rotary evaporation under reduced pressure was more efficient, allowing reasonably rapid (~20 min) elimination of acetonitrile at temperatures of 50-55°C. However, significant losses (~40-50%) of aldrin occurred under these conditions also. Since it had been previously found that volatility loss of low polarity analytes was not a serious problem when concentrating solutions in the nonpolar solvent hexane, an alternative procedure was developed in which an excess of isooctane (also nonpolar but with a higher boiling point than acetonitrile) was added prior to evaporating acetonitrile solutions. The isooctane formed a layer on top of the acetonitrile which effectively retained aldrin during the evaporation step. A 2.5:1 isooctane:acetonitrile volume ratio was required to consistently achieve aldrin recoveries of >80%. As a result, the evaporation of a larger volume of solvent requiring an additional 10-15 minutes was necessary for the sake of aldrin recovery. (Temperature was raised to speed up isooctane evaporation after eliminating acetonitrile). The recoveries listed in Table 3 for analyte mixtures added to soybean oil and butterfat were obtained using this alternative evaporation step, thereby achieving the adequate aldrin recoveries shown.

Typical gas chromatograms of collected fractions of soybean and cottonseed oils fortified with pesticide mixtures and injected directly into the HPLC column are shown in Figure 1. The less polar OC and OP compounds (chromatogram A) were added to the samples as a single mixture in isooctane, and were therefore quantified concurrently in the same GC-EC chromatogram for the

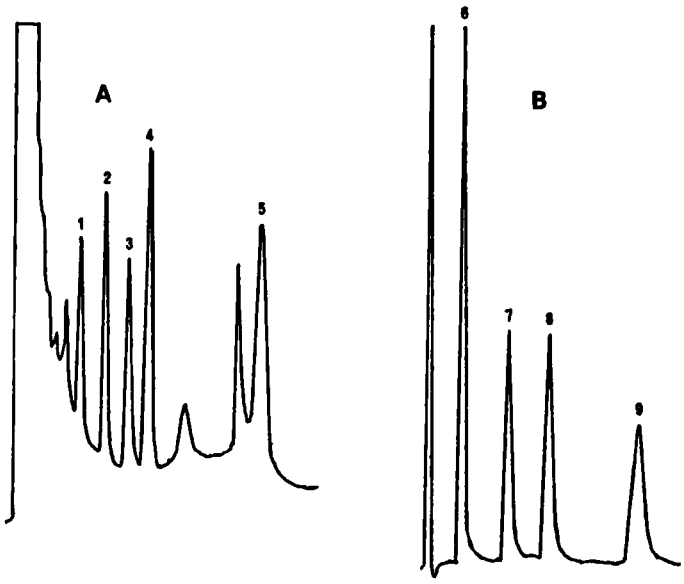

FIGURE 1. Gas chromatograms of HPLC analyte fractions collected from direct injections of 1g samples of pesticide-fortified oils. (A) GC-EC (5% OV-101) of soybean oil fraction dissolved in isooctane. (B) GC-FPD (2% DEGS) of cottonseed oil fraction dissolved in acetone. Peaks: 1, diazinon; 2, malathion; 3, methyl parathion; 4, aldrin; 5, dieldrin; 6, methamidophos; 7, acephate; 8, omethoate; 9, monocrotophos.

sake of convenience. Though the OP compounds are more typically determined by the more selective GC-FPD, the collected fractions were sufficiently clean to allow quantitation of these compounds by GC-EC. The polar OP compounds (chromatogram B) were added as a mixture in acetone, and required the more polar DEGS column for satisfactory chromatography. No peaks with retention times matching the added pesticides were seen in the chromatograms of control samples. The unlabeled peaks in the GC-EC chromatogram were present in the soybean control and were not identified.

TABLE 4

Recovery of Selected Pesticides Fractionated from Fortified Samples Using Pre-Extraction Technique.

Pesticide	Added, ppm	No. of trials	Recovery, %* Average	Range
		SOYBEAN OIL, 1g		
Diazinon	0.86	2	79.8	79.5- 80.0
Malathion	0.52	2	98.4	96.4-100.3
Parathion methyl	0.40	2	100.6	96.3-104.8
Dieldrin	0.21	2	88.6	81.8- 95.4
Aldrin	0.19	2	61.8	61.5- 62.0
		BUTTERFAT, 0.5g		
Diazinon	0.86	3	96.4	91.0-103.1
Malathion	0.52	3	96.6	88.7-101.5
Parathion methyl	0.40	3	96.0	88.5-104.1
Dieldrin	0.21	3	91.0	84.7- 94.7
Aldrin	0.19	3	55.7	50.0- 60.9

*Acetonitrile eluates were evaporated without isooctane addition.

Recoveries listed in Table 4 for several pesticides fractionated from soybean oil and butterfat after pre-extraction from petroleum ether into acetonitrile were generally "complete" with the exception of aldrin. Aldrin was lost during evaporation in these runs because the acetonitrile eluates were concentrated without adding the isooctane trapping solvent. Though no significant differences in recoveries related to the evaporation technique used were apparent for the other pesticides tested in this study, volatility losses from acetonitrile might be anticipated for other low polarity compounds.

Further cleanup of "dirty" samples for OC analysis was achieved by adsorption chromatography on "minia-

TABLE 5

Recovery of OC Pesticides from Fortified Tallow Using HPLC Fractionation with Direct Injection Technique and Miniature Florisil Column Cleanup.

Pesticide	Added, ppm	No. of trials	Recovery, % Mean	Range
Dieldrin	0.21	2	86.8	85.6-87.9
p,p'-Methoxychlor	1.01	2	88.0	85.3-90.7

ture" Florisil columns per the procedure described in section 29.034 of the official AOAC methods (3) as follows. The evaporated HPLC analyte fraction was dissolved in petroleum ether, placed on the miniature (4 g) column, eluted with 35 mL each of 6+94 and 15+85 ethyl ether-petroleum ether and concentrated for GC-EC analysis. Recoveries of dieldrin and p,p'-methoxychlor from fortified tallow so analyzed are shown in Table 5. A cheese fat sample, which had been previously analyzed by the official AOAC method (sections 29.014-29.015) and found to contain 0.61 ppm bioincurred dieldrin, was also analyzed by proposed direct injection HPLC fractionation followed by miniature Florisil cleanup. The result of 0.63 ppm was in excellent agreement with the official method result, thus supporting the reliability of the proposed method for authentic residues. The final extracts from both tallow and cheese fat were adequately cleaned up for GC-EC analysis of both the 6+94 and 15+85 ethyl ether-petroleum ether fractions.

As was previously observed for the semi-preparative silica column (1), the ODS column proved to be resilient and reproducible after numerous injections of fat and reverse flow purgings with methylene chloride (i.e., no loss of efficiency or capacity was apparent).

In conclusion, a semi-preparative ODS column with acetonitrile mobile phase has been found to provide a rapid and effective separation of polar OP pesticides as well as relatively nonpolar OC pesticides from fats and oils in a single 40 ml fraction. In most cases, samples are sufficiently clean for sub-ppm residue analysis without additional treatment. The high selectivity of GC-FPD for the OP pesticides minimized matrix interferences after ODS-HPLC fractionation. Additional cleanup on miniature Florisil columns virtually eliminated co-eluting polar, low molecular weight lipid constituents from "dirtier" samples, thereby permitting GC-EC analysis of OC pesticides without difficulty.

Disadvantages of the proposed method are the cost and complexity of equipment and the limited sample throughput with a single column and HPLC system. The programmable pump and automated switching valves used simplified the process of back-flushing and re-equilibrating the column. Total automation of this system could be achieved with the addition of an automatic sampler and fraction collector, but at even greater expense. Alternatively, we have initiated a study of ODS solid phase extraction (SPE) cartridges which require relatively simple equipment and permit multiple sample processing in less time. An obvious significant disadvantage of SPE cartridges when compared with semi-preparative HPLC is the relatively small sample capacity. However, when combined with the

"pre-extraction technique" described herein, a multiple SPE cartridge fractionation yielding extracts sufficiently clean for GC analysis appears feasible and is the subject of a forthcoming report.

REFERENCES

1. Gillespie, A.M. and Walters, S.M., HPLC Silica Column Fractionation of Pesticides and PCB from Butterfat, J. Liq. Chromatogr., 9, 2111 (1986).

2. Petrick, G., Schulz, D.E. and Duinker, J.C., Cleanup of Environmental Samples by High-Performance Liquid Chromatography with Electron-Capture Detection, J. Chromatogr., 435, 241 (1988).

3. Williams, S., ed., Official Methods of Analysis of the Association of Official Analytical Chemists, 14th edition, Association of Official Analytical Chemists, Inc., Arlington, VA USA, 1984. Chapter 29.

4. Mills, P.A., Bong, B.A., Kamps, L.R. and Burke, J.A., Elution Solvent System for Florisil Column Cleanup in Organochlorine Pesticide Residue Analyses, J. Assoc. Off. Anal. Chem., 55, 39 (1972).