Jitka Zrostlíková[a], Steven J. Lehotay[b], Jana Hajšlová[a]

[a] Institute of Chemical Technology, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic
[b] United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center; 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

Simultaneous analysis of organophosphorus and organochlorine pesticides in animal fat by gas chromatography with pulsed flame photometric and micro-electron capture detectors

A method using simultaneous pulsed flame photometric (PFPD) and micro-electron capture detection (μECD) in gas chromatography (GC) was developed and validated for the analysis of 23 organophosphorus (OP) and 17 organochlorine (OC) pesticides in animal fat. The method entailed the extraction of animal tissue (mixed with twice the sample weight of sodium sulfate) with 7 mL ethyl acetate per 1 g tissue. After the blending step, the extract was centrifuged and 3 mL cyclopentane was added to a 7 mL portion of the extract. A 2.5 mL portion was injected into a 2 cm ID × 22.5 cm Biobeads S-X3 gel permeation chromatography column (4.5 mL/min flow rate of 70/30 ethyl acetate/cyclopentane). A 36 mL fraction (from 8 to 16 min) was collected, evaporated, and solvent-exchanged to 1 mL final volume in iso-octane. The GC/PFPD+μECD system used a single injector and column, but the flow was split after the chromatographic separation to the two detectors. The final extract was injected (2 μL) into the GC/PFPD + μECD system for simultaneous analysis of the OP and OC analytes. The PFPD was used in the phosphorus-only mode to detect OPs and the μECD mainly detected halogenated pesticides but a few N-containing OPs could be sensitively detected with it as well. Recoveries were 60–70% for the bulk majority of pesticides except for methamidophos, acephate, and omethoate which are more difficult in GC analysis due to their more polar nature. Fenthion and phorate also gave more variable recoveries, presumably due to their degradation to sulfones and sulfoxides. In fortification recovery experiments at several different concentrations over multiple days, reproducibilities of 10–20% relative standard deviation were achieved, and limits of quantitation were typically 10–20 ng/g.

Key Words: Multiresidue; Pesticide; Analysis; Fats; Gas chromatography

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1 Introduction

Regulations designed to limit the presence of chemical contaminants in foods exist in nearly every country of the world. In the U.S., the EPA sets the tolerance limits for pesticides in food, and the U.S. Department of Agriculture FSIS is responsible for the regulatory enforcement monitoring of pesticides in meat, poultry, and unshelled eggs. Since the implementation of the Food Quality Protection Act in 1996, the EPA must re-assess the tolerances of many pesticides in order to comply with the new law. The highest priority pesticides to be re-evaluated consist of OP insecticides, which all share the same cholinesterase-inhibiting mode of action.

FSIS last conducted a survey of OP insecticides in 1986 and reported no findings in the liver samples analyzed [1]. Since then, FSIS has monitored only chlorinated hydrocarbons including several OC pesticides and chlorinated OPs in fat samples using GC/ECD [2]. However, liver was not necessarily the most appropriate matrix for analysis of many OPs, and the pesticides often reside in fat and/or muscle tissue at higher concentrations than in the liver [3]. Thus, analysis of both OP and OC pesticides in fatty sam-

Correspondence: Steven J. Lehotay, USDA ARS ERRC, 600 East Mermaid Ln., Wyndmoor, PA 19038, USA
E-mail: slehotay@arserrc.gov
Fax: +1 215 233–6642

Acronyms
GPC = gel permeation chromatography; NPD = nitrogen-phosphorus detector; PFPD = pulsed flame; photometric detector; AED = atomic emission detector; μECD = micro-electron capture detector; ECD = electron capture detector; ELCD = electrolytic conductivity detector; GC = gas chromatography; FSIS = Food Safety Inspection Service; XSD = halogen specific detector; OC = organochlorine; pesticide; OP = organophosphorus pesticide; EPA = Environmental Protection Agency; MS = mass spectrometry; RSD = relative standard deviation; OCP = organochlorine/phosphorus pesticide; LCL = lowest calibration level; SPE = solid phase extraction.

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ples is more appropriate, but laboratories do not necessarily have additional instruments, space, or means to handle the increased workload involved in expanding the number of analytes in a monitoring program. Ideally, the OPs can be incorporated into current analytical methods for OC analysis and be detected simultaneously while minimizing the need for additional resources or effort.

One approach is to utilize GC/MS for analysis which enables simultaneous detection and confirmation of chemical analytes independent of their elemental composition [4]. However, GC/MS instruments are more expensive than GC instruments with selective detectors, greater analyst skill is required, and hydrocarbon background interferences sometimes cause more problems in MS detection than selective detectors, even in the case of MS-MS [5]. Also, selective detectors can often achieve lower detection limits.

The GC analysis of OC contaminants can be accomplished with an ELCD [6], halogen specific detector (XSD) [7], ECD, or μECD [8, 9]. Any one of these detectors is likely to meet the regulatory monitoring needs of this application with some differences in advantages and disadvantages. For example, the XSD is noted for its high degree of selectivity to halogenated compounds, but the μECD is a very sensitive and rugged GC detector.

The analysis of OPs can be accomplished with the NPD [10], FPD [11], or more recently, the PFPD [12–15]. For the detection of phosphorus, the NPD and FPD have interferences from nitrogen and sulfur, respectively, whereas the PFPD in phosphorus-mode gives no interferences from nitrogen, and sulfur interferences can be distinguished and removed from the signal. The PFPD also possesses the versatility to selectively detect 28 elements (at individually-optimized conditions). The AED is able to detect multiple elements simultaneously [16], but it is more expensive than GC/MS and lacks the sensitivity of the μECD or PFPD for OC and OP pesticides, respectively, and it is no longer commercially available.

The use of ECD enables the feasibility to stack an NPD or FPD on top of one [17], but this was not possible with the commercial μECD and PFPD devices in this study. Therefore, 4 options remained in using the selective detectors to analyze the wide range of OP and OC pesticides: 1) use 2 GC systems; 2) use 2 inlets and 2 columns with 1 GC; 3) use 1 inlet and split flow to 2 columns; or 4) use 1 column and split flow to both detectors. The cost of option 1 is essentially twice as expensive and takes up twice the space as the other options. Option 2 also was more expensive and/or time consuming in routine applications because either 2 autosamplers or a type of autosampler that can inject at different injection ports would be needed (or 2 sequential analyses could be performed which would essentially take twice as long). Option 4 was chosen over option 3 because the post-separation split-flow approach avoids potential differences between columns and simplifies correlation of analyte peaks arising at the same retention time on the two detectors.

Several different analytical methods for extraction and clean-up have been devised for the analysis of pesticides in fatty samples [18–24]. A nonpolar solvent is needed for extraction from fat, and GPC is a very useful approach to separate the co-extracted lipids from the smaller pesticide molecules. The use of Florisil or silica sorbents for SPE cleanup is also possible, but then different types of pesticides elute from the sorbents under different solvent conditions and in different fractions. This is not convenient in the simultaneous analysis of both OPs and OCs. All types of pesticides can be recovered from GPC in a relatively narrow fraction, and although GPC generally uses more solvent volume than SPE methods, it is more easily automated and a single column can be re-used many times. In this study, a smaller than typical GPC column was used to minimize solvent consumption. This is a growing trend in GPC and newer approaches often use smaller diameter or in-line columns [25–26].

The goal of this study was to optimize and validate a rapid and easy analytical method for both OP and OC pesticides in fatty animal tissue in a low-cost and effective approach utilizing GC/PFPD + μECD.

2 Experimental

2.1 Chromatographic instrumentation and conditions

An Agilent (Little Falls, DE, USA) 6890 GC fitted with a 6890 enhanced parameters autosampler, standard split/splitless injector, micro-cell ECD (Agilent) and Model 5380 PFPD from OI Analytical (College Station, TX, USA) was used for analysis. A 30 m, 0.32 mm ID, 0.25 μm film thickness HP-5 (Agilent) analytical column was used for GC. Two 20 cm pieces of the column were taken to split the flow 1 : 1 to each detector using a Restek (Bellefonte, PA, USA) press-fit deactivated glass splitter. Splitless injection (2 μL) at 250°C in a 4 mm ID double tapered deactivated glass liner was used with a pressure pulse of 60 psi (6,895 Pa = 1 psi) for 2 min followed by 2 mL/min constant flow of He carrier gas. The oven temperature program was 60°C for 2 min, 7°/min to 150°C, 2.5°/min to 210°C, 30°/min to 300°C and held for 5 min (total run time of 46.86 min).

The computerized instrument control and data collection used version A.06.01 Chemstation software (Agilent), and an Agilent Model 35900E analog/digital converter was required for the PFPD signal to be analyzed on the Chemstation. The PFPD was controlled by WinPulse software

(OI Analytical) and the PFPD controller which required manual adjustment of gas flows. H_2 flow was ≈9 mL/min and air flow was ≈25 mL/min through the PFPD (signal was optimized by small adjustments). The optimized conditions for phosphorus response was used with the PFPD which entailed a 3 mm combustor, 300°C temperature, GG-495 filter, and 490 V Model R1924 photomultiplier tube. PFPD pulse frequency was 2.94 Hz according to the WinPulse readout, and phosphorus signal was collected from 4.5–15 ms after the pulse. The μECD was also set at 300°C and N_2 make-up gas was 60 mL/min flow rate. The Chemstation data collection rate was 20 Hz on both detectors.

For GPC, a Hewlett-Packard (Agilent) Model 1050 HPLC was used with an OI Analytical Optima column (2 cm ID × 22.5 cm packed with 24 g Biobeads S-X3 styrene divinylbenzene copolymer beads in 70/30 ethyl acetate/cyclopentane). Ethyl acetate and cyclopentane were stored in separate bottles and flow rate of the 70/30 ethyl acetate/cyclopentane mobile phase was 4.5 mL/min; an Alltech (Cincinnati, OH, USA) on-line degassing system was used to remove air from the solvents. A 2.5 mL sample loop was used for injection, and a LKB Bromma Model 2112 fraction collector was used to collect the extracts. Four fractions per sample were collected in which each fraction was 4 min long (18 mL volume). The first two fractions were waste and the 3rd and 4th tubes (36–72 mL fraction) were combined for analysis.

2.2 Materials

The pesticide standards in this study were obtained from Chemservice (West Chester, PA, USA), EPA National Pesticide Repository (Fort Meade, MD, USA), or Dr. Ehrenstorfer GmbH (Augsburg, Germany). Four standard solutions of OPs and OCs were prepared of 10–40 ng/μL (depending on the pesticide) in iso-octane. The ground beef, beef fat, and pork fat samples were obtained from a local grocery store. Ethyl acetate (EtAc) and iso-octane were pesticide residue grade from Burdick & Jackson (Muskegon, MI, USA), and cyclopentane was HPLC grade from Aldrich (Milwaukee, WI, USA). Analytical reagent grade anhydrous Na_2SO_4 (annealed 5 h at 450°C) was from Mallinckrodt (Paris, KY, USA).

2.3 Method

A modified approach of FSIS [2] and the USDA Agricultural Marketing Service [24] was used for sample preparation in this study. A fat or meat sample was mixed with twice as much Na_2SO_4 (*w/w*) and sufficient dry ice to disperse the sample during chopping with a Robotcoupe (Ridgeland, MS, USA) Model RSI 2Y1 chopper. In a humid environment, the analyst must work especially fast to avoid water condensation. The powdery sample was given enough time (≈30 min) for the dry ice to sublime (this was done in a freezer to further avoid moisture condensation). Then, 10.71 g of this homogenized powdery sample (3.57 g tissue) was blended with 25 mL EtAc in a 40 mL Teflon centrifuge tube using an Ultra-Turrax probe blender. EtAc has moderately nonpolar and polar properties and thus serves as a good solvent for extraction, and the extract can be taken immediately to the GPC cleanup step. The blender was used in several ≈10 s bursts or the sample would get too hot. The tube was centrifuged for 10 min at 2000 rpm in a Sorvall (Newtown, CT, USA) RT6000B centrifuge at 25°C. A 7 mL portion of the extract was mixed with 3 mL cyclopentane in a vial, and ≈5 mL was taken up in a 10 mL gas-tight syringe, a 0.45 μm Teflon filter disk was placed on the syringe, and the extract was filtered as the 2.5 mL sample loop was filled in the GPC setup. GPC was conducted, and the 8–16 min fraction (3rd and 4th tubes) was transferred to a 100 mL round-bottom flask (3 × 2 mL iso-octane was used to rinse each tube). A roto-evaporator at 40°C bath temperature was used to concentrate the extracts to ≈3 mL, and the extract was transferred to 15 mL volumetric centrifuge tubes (3 × 2 mL iso-octane was used to rinse the flask). A stream of N_2 and 40°C bath was used to evaporate the temperature extracts to ≈0.5 mL then adding iso-octane to make a final volume of 1 mL. The extract was transferred to an autosampler vial and 2 μL was injected in the GC/PFPD+μECD setup for analysis. Calibration standards were prepared from blank beef or pork fat extracts by adding appropriate volumes of spiking solution in iso-octane prior to bringing the final volume up to 1 mL.

3 Results and discussion

3.1 Setting of GC conditions

Table 1 lists the pesticides included in this study, their average retention time in the final method, pesticide class, quantitative detector used, molecular formula, and U.S. tolerance level in beef fat. The variability of the retention times within an analytical sequence were <0.01% RSD with either detector, and among runs on different days, RSD was <0.08%. This consistency was very important in assigning analytes and distinguishing analyte peaks from background peaks, especially without an MS detector. Also, for those pesticide analytes detected with both detectors, the μECD peak occurred 0.025–0.037 min (1.5–2.2 s) prior to the PFPD peak, presumably due to differences in the electronics, but possibly due to slight flow rate or separation differences in the 20 cm pieces of capillary column leading to the detectors.

Several chromatographic conditions were evaluated to provide the maximum signal and adequate separation

Table 1. Pesticides included in the study and other pertinent information.

#	Pesticide	Retention time (min)	Type/ detector(s)	Molecular formula	Tolerance in cattle fat (ng/g)
1	Methamidophos	9.139	OP/PFPD	$C_2H_8NO_2PS$	100[a)]
2	Dichlorvos	9.648	OCP/PFPD	$C_4H_7Cl_2O_4P$	20
3	Acephate	13.307	OP/PFPD	$C_4H_{10}NO_3PS$	100[a)]
4	Omethoate	16.089	OP/PFPD	$C_5H_{12}NO_4PS$	20[b)]
5	Phorate	18.219	OP/PFPD	$C_7H_{17}O_2PS_3$	50
6	Hexachlorobenzene	18.396	OC/μECD	C_6Cl_6	500
7	Dimethoate	18.938	OP/both	$C_8H_{12}NO_3PS_2$	20[b)]
8	Lindane	19.613	OC/μECD	$C_6H_6Cl_6$	7,000
9	Diazinon oxon	20.465	OP/PFPD	$C_{12}H_{21}N_2O_4P$	–
10	Diazinon	21.182	OP/PFPD	$C_{12}H_{21}N_2O_3PS$	700
11	Parathion-methyl	23.112	OP/PFPD	$C_8H_{10}NO_5PS$	–
12	Aldrin	24.917	OC/μECD	$C_{12}H_8Cl_6$	300
13	Pirimiphos-methyl	25.111	OP/PFPD	$C_{11}H_{20}N_3O_3PS$	200
14	Malathion	25.678	OP/PFPD	$C_{10}H_{19}O_6PS_2$	4,000
15	Fenthion	25.891	OP/PFPD	$C_{10}H_{15}O_3PS_2$	100
16	Chlorpyrifos	26.024	OCP/both	$C_9H_{11}Cl_3NO_3PS$	300
17	Chlorfenvinphos	28.582	OCP/both	$C_{12}H_{14}Cl_3O_4P$	200
18	γ-Chlordane	28.741	OC/μECD	$C_{10}H_6Cl_8$	300
19	Methidathion	29.227	OP/PFPD	$C_6H_{11}N_2O_4PS_3$	50
20	Endosulfan I	29.381	OC/μECD	$C_9H_6Cl_6O_3S$	200[c)]
21	*trans*-Nonachlor	30.124	OC/μECD	$C_{10}H_5Cl_9$	–
22	Tetrachlorvinphos	30.150	OCP/both	$C_{10}H_9Cl_4O_4P$	1,500
23	*p*,*p*′-DDE	30.984	OC/μECD	$C_{14}H_{10}Cl_4$	5,000[d)]
24	Profenofos	31.377	OCP/both	$C_{11}H_{15}BrClO_3PS$	50
25	Dieldrin	31.459	OC/μECD	$C_{12}H_8Cl_6O$	300
26	Endrin	32.231	OC/μECD	$C_{12}H_8Cl_6O$	300
27	Endosulfan II	32.895	OC/μECD	$C_9H_6Cl_6O_3S$	200[c)]
28	Ethion	34.815	OP/PFPD	$C_9H_{22}O_4P_2S_4$	2,500
29	Endosulfan sulfate	35.694	OC/μECD	$C_9H_6Cl_6O_4S$	200[c)]
30	Famphur	35.895	OP/both	$C_{10}H_{16}NO_5PS_2$	100
31	*p*,*p*′-DDT	36.396	OC/μECD	$C_{14}H_9Cl_5$	5,000[d)]
32	Phosmet	39.314	OP/PFPD	$C_{11}H_{12}NO_4PS_2$	200
33	Dicofol	39.675	OC/μECD	$C_{14}H_9Cl_5O$	–
34	*p*,*p*′-Methoxychlor	39.876	OC/μECD	$C_{16}H_{15}Cl_3O_2$	3,000
35	Mirex	40.357	OC/μECD	$C_{10}Cl_{12}$	100
36	Azinphos-methyl	40.414	OP/PFPD	$C_{10}H_{12}N_3O_3PS_2$	100
37	Phosalone	40.487	OCP/both	$C_{12}H_{15}ClNO_4PS_2$	250
38	*cis*-Permethrin	41.547	pyrethroid/μECD	$C_{21}H_{20}Cl_2O_3$	3,000
39	Coumaphos	41.594	OCP/both	$C_{14}H_{16}ClO_5PS$	1,000
40	Deltamethrin	42.986	pyrethroid/μECD	$C_{22}H_{19}Br_2NO_3$	–

[a),b),c),d)] Combined tolerances or regulatory action levels.

using the given column and 2 mL/min He flow rate (1 mL/min to each detector). The final conditions were not devised to be as rapid as possible, but only to separate the pesticides in less than 45 min. Originally, 4 more OPs (parathion, isofenphos, carbophenothion, and chlorpyrifos-methyl) and two other OCs (heptachlor and *trans*-permethrin) were included in the study, but they co-eluted with other analytes and made their individual quantitation impossible. Of course, these pesticides (and many others) would still be detected by this method, but confirmation of analyte identity would have to be conducted separately. This stresses how independent confirmation of analyte identity on GC/MS should be done for positive responses in real samples.

Analyte co-elution problems and the lack of identification are the main disadvantages of GC with selective detectors, but one of the possibilities gained by using the split flow dual detector approach was to provide more and better information about the peaks than from using a single detector. Retention time locking is a commercial product that correlates retention times with multiple detectors on separate instruments, but as much as 20 s differences occur in the retention times of some analytes with the

approach [27]. With the single column approach using two detectors, a nearly exact assignment of peaks can be made [14].

For example, tetrachlorvinphos co-eluted with *trans*-nonachlor in this study. If an actual sample contained *trans*-nonachlor and not tetrachlorvinphos, only the μECD would respond. Tetrachlorvinphos by itself would lead to a response on both detectors, and the ratio of the responses would indicate whether *trans*-nonachlor (or another substance) was also present. Thus, co-elutions could be tolerated between the PFPD and μECD which enabled a faster run time than if a single detector were used.

Pulsed splitless injection is a technique in which the initial flow of carrier gas is increased to better focus the solvent during injection and more quickly sweep the analytes out of the injection liner [28, 29]. An experiment was conducted to compare peak responses of the analytes under different injection conditions and at different initial column temperatures. **Figure 1** shows how pulsed splitless injection at 60 psi improved the injection efficiencies for the most volatile analytes versus splitless injection at 15 psi inlet pressure, and how the initial oven temperature should be kept at 60°C rather than 90°C to maintain better focusing of methamidophos and dichlorvos. In contrast to a previous report [15], the PFPD was unaffected by this initial surge in flow rate. The lower initial oven temperature slightly increased the chromatographic separation time and re-equilibration time between injections, but the increased response was a more important consideration.

Figure 2 gives PFPD and μECD chromatograms with the final method from an injection of a mid-range calibration standard of the pesticides in beef fat extract.

3.2 Sample preparation

The first step in the current FSIS CHC3 method is to place a 40 g fat sample in an oven at 100°C for 3 h and collect the liquid drippings. Then, merely 0.5 g of this melted fat is simply dissolved in 5 mL cyclopentane and injected in the same GPC approach as used in this study [2]. Volatile and thermally labile analytes, some of which include important OPs and OCs, are not recovered through this approach, as demonstrated by Albritton [30]. Also, the adequacy of the small subsample size taken to sufficiently represent an animal carcass is questionable.

In this study, a larger sample size of 150–200 g fat or meat was thoroughly homogenized, and a larger, representative subsample was taken for analysis. The use of a frozen sample and dry ice was essential to help break up the tissue and better disperse the sample [24, 31]. It was important that sample size be appropriate to the volume of the chopper to obtain best sample homogeneity [32]. A flowable powdery substance was formed in this sampling approach which gave greater confidence in the representativeness of the subsample. Analytes are not volatilized or thermally degraded in this frozen sample approach, and the chopper used has been demonstrated to yield very reproducible subsamples of meat tissue [33].

3.3 GPC

A typical S-X3 GPC column in pesticide analysis is 2.5 cm ID, 45 cm long, and utilizes 1:1 EtAc:cylcohexane or dichloromethane mixtures as the mobile phase [21, 22]. In the late 1980's, a manufacturer met an FSIS competitive bid to develop a new GPC method for the CHC3 method

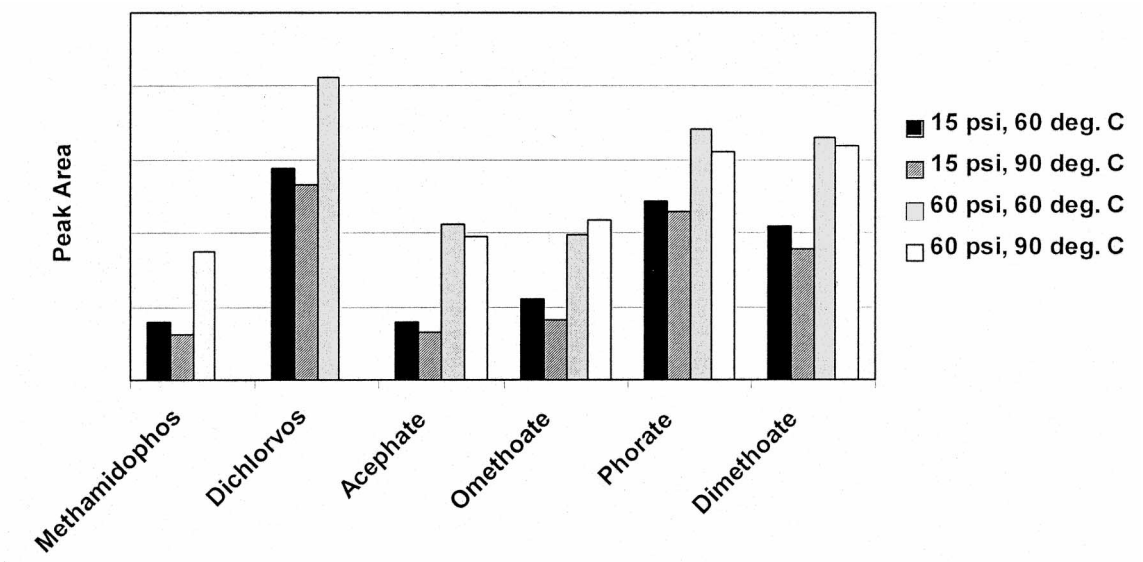

Figure 1. Effect of pulsed splitless injection at different inlet pressures and initial column oven conditions for the early eluting analytes in the method.

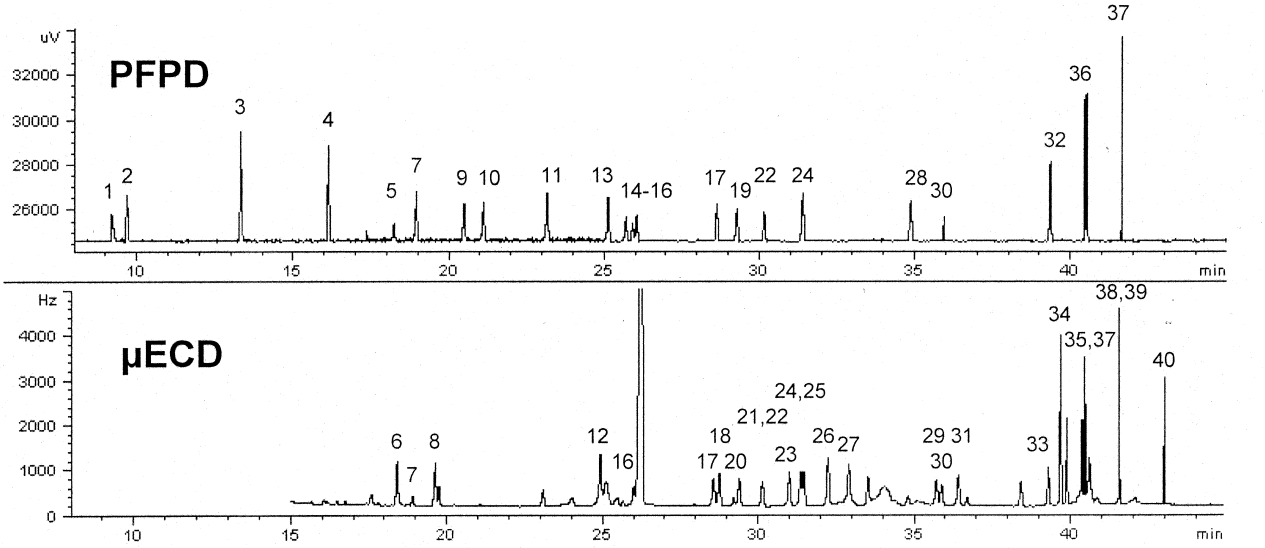

Figure 2. PFPD and μECD chromatograms from a pesticide calibration standard in fat extract. The numbered peaks refer to the pesticides in Table 1 and concentrations are given in Table 2.

that eliminated the use of chlorinated solvents and reduced solvent consumption overall. Cyclopentane : EtAc (3 : 7) was found to give better separation than cyclohexane : EtAc (1 : 1), and since adequate detection limits were still maintained, a smaller column could be used. This GPC method was used in this study as well.

Other than its chemical properties, EtAc was chosen as the extraction solvent out of convenience and compatibility with the GPC step. It is more polar than traditional extraction solvents of fats, such as hexane, but it is not as polar as acetonitrile or acetone which are completely miscible with water.

The amount of EtAc used for extraction was designed to give adequate recoveries of the analytes while not overloading the GPC column when 2.5 mL of extract was injected. An experiment was conducted to determine the satisfactory amount of EtAc extraction solvent per g fat in the sample needed to achieve high pesticide recovery. The amount of co-extracted fat was also measured gravimetrically by taking the extracts to dryness. The pesticide recoveries of fortified samples were satisfactory at the conditions tested, and the sample : EtAc ratio was chosen mainly to avoid overloading the GPC column with fat.

Figure 3 shows the amount of co-extracted fat in experiments with beef fat. Although a single point at 7.5 mL EtAc per g beef fat gave an outlying result, the trend was clear that the amount of fat dissolved by EtAc slightly increased as more EtAc was added to a given amount of fat. Similar results were obtained for pork fat as for beef fat, and the amount of co-extracted matrix from ground beef also correlated with the fat content of the sample. Thus, much more concentrated meat extracts could be injected into the GPC without overloading the column, but this was not thoroughly tested.

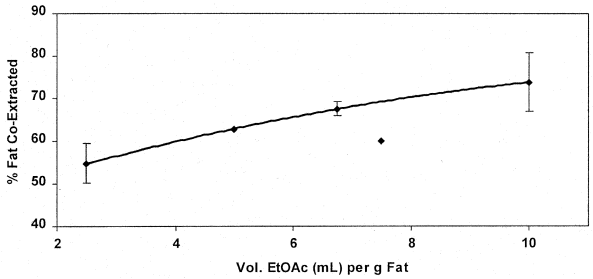

Figure 3. Co-extracted lipid material from pork and cattle fat tissue with different ratios of sample:EtAc.

Figure 4 displays the elution profile (smoothed data) from an experiment in which 300 mg of beef fat was injected into the GPC. Also shown in the figure are the elution profiles for the first pesticide to elute from the GPC column (deltamethrin), the last to elute (hexachlorobenzene), and a third pesticide (phosmet) to represent the 36 pesticides that gave >90% recoveries in 36–54 mL fraction. If the 36–54 mL fraction were taken for analysis, losses would have been 15% for deltamethrin (appearing in the 27–36 mL fraction) 21% endrin, 23% mirex, and 87% hexachlorobenzene (appearing in the 54–63 mL fraction). Ideally, the 36–63 mL fraction would have been collected in this method, but the 36–72 mL fraction was taken because it was more convenient to use 4 min intervals rather than 2 min intervals with the fraction collector. In an automated GPC system, this would not be an issue, and a more careful and selective choice of the optimal collection volume could be made.

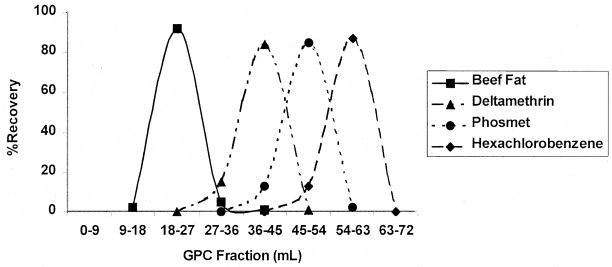

Figure 4. Elution profiles (smoothed lines) of beef fat, deltamethrin (the first analyte to elute), phosmet (a representative typical pesticide), and hexachlorobenzene (the last analyte to clute).

The fat eluted prior to the elution of the pesticides except for a 1% fat co-elution in the 36–45 mL fraction. No differences were observed in the elution profile between injection of 200–300 mg fat (either pork or beef). However, the 300 mg injection of fat (3 mg co-elution with the pesticide fraction) was felt to be too much, and the method was designed to inject the lower amount. As Figure 3 shows, a 7:1 EtAc:fat (mL:g) ratio yields an average amount of co-extracted fat of 68% according to the best-fit trend line. Thus, 170 mg fat (equivalent to 250 mg original sample) was injected into the GPC column in the final method, and accounting for the 1% fat co-elution in GPC, this corresponded to 3.4 μg lipid injected per sample into the GC.

3.4 Minimizing dichlorvos, phorate, and fenthion losses

Dichlorvos is a volatile pesticide that is easily lost during evaporation steps in analytical methods. In this study, we found that the use of a rotary evaporator does not lose as much dichlorvos as evaporation in a tube under nitrogen flow. The reason for this was probably due to a combination of 2 effects. In the rotary evaporator, the glass surfaces in the round bottom flask are continually coated with solvent and there is little gas flow to sweep analytes away. In the case of the nitrogen flow evaporator, the solvent surface recedes as it evaporates, leaving the dissolved solutes behind on the glass surface, and the flowing N_2 gas probably carries the volatile analytes away. If a vortex is created in the tube with N_2, the walls are coated with solvent that serves as a keeper for the analytes.

During the course of this study, losses of phorate and fenthion (and other OPs after long storage times) were found to occur in EtAc solutions and during the GPC step, especially when fat or other protecting components were not present. Losses did not occur in the iso-octane solutions constituting the final extracts. In the PFPD chromatograms, 4 extra peaks appeared in the standard solution when phorate and fenthion were lost, and these conceivably were the sulfone and sulfoxide metabolites. These phosphorus-containing chemicals occurred at retention times of 26.84, 32.75, 34.02, and 36.52 min. We did not have standards for these compounds to confirm their retention times, and all the relative retention time data we checked in the literature for the sulfones and sulfoxides were not specific to our stationary phase. That they showed significantly longer retention times than their parents agreed with our results [34].

We hypothesized that the cause of the losses of fenthion and phorate was the appearance of acetic acid in the EtAc solutions when water was present in the solvent. To address this problem, the amount of Na_2SO_4 mixed with the samples was increased in the final method to minimize co-extraction of water. An experiment showed that as the GPC mobile phase aged, pesticide losses became greater. Thus, precautions were taken to minimize the time 7:3 EtAc:cyclopentane was stored or extracts were contained in the mobile phase. In GPC, the solvents were kept in individual bottles and mixed in the HPLC mixing chamber as the instrument was running (this also served to reduce the effect of changing solvent ratio over time as the more volatile cyclopentane evaporated preferentially over EtAc in solution). The cause for the fenthion and phorate losses were unknown, however, and recoveries were still more variable than other analytes, despite the attempts made to minimize degradation.

3.5 Validation

The final method was carried out in a series of validation experiments that involved many replicate fortifications of beef fat at several different concentrations on multiple days. **Table 2** shows the results from the recovery validation experiment for mid-concentration spikes for each pesticide on 3 different days ≈1 month apart from each other. More than 60 injections were made and no chromatographic maintenance was conducted between analyses during these experiments. The reproducible results among the different data sets indicated that the method gives consistent results over time and is quite rugged. Also, the similar results between the PFPD and μECD for those pesticides detected on both detectors (e.g. dimethoate, chlorpyrifos, phosalone) indicated that both detectors were operating in agreement.

Figure 5 shows how similar recoveries were obtained for pesticides independent of spiking concentration. Again, these experiments were conducted on different days over an extended period of time. Error bars represent 1 standard deviation from triplicate spikes at each concentration.

Table 3 summarizes the results from the different pesticides in the validation experiments. The limit of detection was not calculated, but the LCL and concentration range

Table 2. Pesticide recoveries (and RSD) from triplicate fortifications per day at the designated concentrations in beef fat on 3 different days several weeks apart ([a] PFPD results, [b] μECD results).

Pesticide	Conc. (ng/g)	Experiment 1		Experiment 2		Experiment 3		Overall		*n*
		%Recov.	%RSD	%Recov.	%RSD	%Recov.	%RSD	%Recov.	%RSD	
Acephate[a]	279	54	14	40	28	34	5	43	26	8
Aldrin[b]	72	50	4	75	17	63	7	62	21	9
Azinphos-methyl[a]	146	73	6	71	2	65	10	70	8	8
γ-Chlordane[b]	71	54	6	65	8	68	12	62	14	9
Chlorfenvinphos[a]	137	67	5	65	9	65	10	66	8	9
Chlorfenvinphos[b]	137	66	9	67	7	56	6	64	11	8
Chlorpyrifos[a]	65	78	12	70	13	66	13	71	14	9
Chlorpyrifos[b]	65	63	8	76	5	–	–	70	11	6
Coumaphos[a]	133	72	2	70	3	67	4	70	4	8
p,p′-DDE[b]	69	60	5	69	16	70	8	66	13	8
p,p,′-DDT[b]	78	66	7	69	8	71	11	68	9	8
Deltamethrin[b]	204	54	7	54	9	52	9	54	8	8
Diazinon[a]	76	67	4	55	1	66	11	64	11	8
Diazinon Oxon[a]	145	54	1	44	2	64	12	55	16	8
Dichlorvos[a]	64	60	6	49	2	64	4	58	12	8
Dicofol[b]	204	65	5	65	6	–	–	65	5	6
Dieldrin[b]	74	59	11	66	14	65	16	63	15	8
Dimethoate[a]	71	67	4	57	3	70	16	66	13	8
Dimethoate[b]	71	70	8	69	14	49	7	64	18	8
Endosulfan I[b]	66	64	7	67	8	65	4	66	7	9
Endosulfan II[2]	68	66	9	79	11	67	3	71	13	8
Endosulfan sulfate[b]	77	53	9	54	12	62	7	55	12	8
Endrin[b]	208	62	6	66	8	80	11	68	13	8
Ethion[a]	66	71	9	67	3	66	10	68	9	9
Famphur[a]	71	73	10	66	9	86	5	74	14	8
Famphur[b]	71	59	8	66	7	59	7	62	9	8
Fenthion[a]	78	78	6	75	30	76	16	76	20	9
Hexachlorobenzene[b]	68	60	3	72	7	60	11	64	12	8
Lindane[b]	65	56	5	68	5	66	12	63	12	9
Malathion[a]	64	80	19	61	24	67	20	69	24	9
Methamidophos[a]	281	52	14	45	–	27	27	39	34	6
Methidathion[a]	70	71	5	75	6	73	7	73	7	9
Methoxychlor[b]	212	74	3	74	7	59	8	70	11	8
Mirex[b]	64	78	8	67	8	70	13	72	12	9
trans-Nonachlor[b]	65	65	6	71	8	68	10	68	9	9
Omethoate[a]	247	69	8	38	6	40	15	51	31	8
Parathion-methyl[a]	73	68	5	53	5	72	11	63	15	8
cis-Permethrin[b]	612	67	5	–	–	72	11	69	9	6
Phorate[a]	64	48	30	53	49	70	8	58	33	8
Phosalone[a]	156	69	5	67	1	68	10	68	6	8
Phosalone[b]	156	68	7	63	5	57	9	63	9	8
Phosmet[a]	144	70	6	70	3	78	11	73	10	9
Phosmet[b]	144	72	5	74	7	83	14	77	12	9
Pirimiphos-methyl[a]	69	65	13	61	8	65	15	64	12	8
Profenofos[a]	150	69	1	70	4	–	–	70	3	6
Profenofos[b]	150	69	7	73	12	–	–	71	10	6
Tetrachlorvinphos[a]	82	65	4	61	5	78	3	68	12	9

for recovered spikes gives an indication of the detection limits for the individual analytes. LCL is the lowest concentration used in a calibration curve that is measured (peak area or peak height) reliably. The use of LCL has emerged as a practical way to avoid controversial definitions of the limit of detection [35]. For any given analysis, the LCL serves as the practical limit of quantitation, and avoids variable definitions among chemists of stated limits of detection. Detectable concentrations were nearly always less than the tolerance levels for the different pesticides in fat, even accounting for the less than ideal recoveries.

For most compounds, recoveries ranged from 60–70% with 10–20% RSD, except for the polar OPs, methamido-

Table 3. Lowest calibrated level (LCL) and pesticide recoveries (and RSD) from multiple fortifications at different concentrations in beef fat ([a] PFPD results, [b] μECD results).

Pesticide	LCL (ng/g)	Spike Conc. Range (ng/g)	Overall %Recov.	Overall %RSD	*n*
Acephate[a]	18	37–1863	43	33	22
Aldrin[b]	5	10–482	61	20	23
Azinphos-methyl[a]	9	19–973	75	15	26
γ-Chlordane[b]	4	9–477	66	15	26
Chlorfenvinphos[a]	9	18–913	70	14	24
Chlorfenvinphos[b]	9	18–913	70	18	25
Chlorpyrifos[a]	9	9–435	72	17	24
Chlorpyrifos[b]	22	22–435	73	12	16
Coumaphos[a]	9	18–889	73	12	26
p,p′-DDE [b]	4	9–459	73	17	25
p,p,′-DDT [b]	5	10–519	74	10	20
Deltamethrin[b]	13	27–1367	61	12	26
Diazinon[a]	5	10–505	65	12	24
Diazinon Oxon[a]	9	19–969	54	19	24
Dichlorvos[a]	4	8–426	57	16	25
Dicofol[b]	13	27–1362	80	18	23
Dieldrin[b]	5	10–496	74	17	22
Dimethoate[a]	4	9–473	65	13	23
Dimethoate[b]	9	22–473	67	21	18
Endosulfan I[b]	4	9–440	70	10	24
Endosulfan II[b]	4	9–454	72	20	22
Endosulfan sulfate[b]	5	10–515	61	15	26
Endrin[b]	13	27–1390	69	12	23
Ethion[a]	9	9–445	68	13	26
Famphur[a]	4	9–473	73	20	26
Famphur[b]	4	9–473	63	20	26
Fenthion[a]	23	23–524	73	20	19
Hexachlorobenzene[b]	4	9–454	69	17	25
Lindane[b]	4	9–435	66	15	26
Malathion[a]	9	9–431	70	20	24
Methamidophos[a]	37	74–1881	41	45	16
Methidathion[a]	9	9–468	72	12	23
Methoxychlor[b]	14	28–1418	76	21	23
Mirex[b]	8	8–426	69	11	21
trans-Nonachlor[b]	4	9–435	73	15	25
Omethoate[a]	16	33–1652	48	30	19
Parathion-methyl[a]	5	10–487	66	14	25
cis-Permethrin[b]	40	81–4090	67	8	18
Phorate[a]	22	22–431	63	25	18
Phosalone[a]	10	21–1044	72	13	26
Phosalone[b]	10	21–1044	70	14	26
Phosmet[a]	10	19–964	75	12	27
Phosmet[b]	10	19–964	77	13	25
Pirimiphos-methyl[a]	4	9–463	63	17	22
Profenofos[a]	20	20–1002	77	18	19
Profenofos[b]	20	20–1002	72	22	19
Tetrachlorvinphos[a]	5	11–548	68	16	26

phos, acephate, and omethoate. The reason for the systematically lower but consistent recoveries is unknown. If the recoveries were dependent upon fat content and analyte polarity, then recoveries of the nonpolar OCs would be lower than those of semi-polar OPs. In retrospect, an internal standard should have been used which may have corrected for the systematic differences in the recoveries. Simonds et al. applied heat during the extraction which served to improve recoveries for OP and OC pesticides [24].

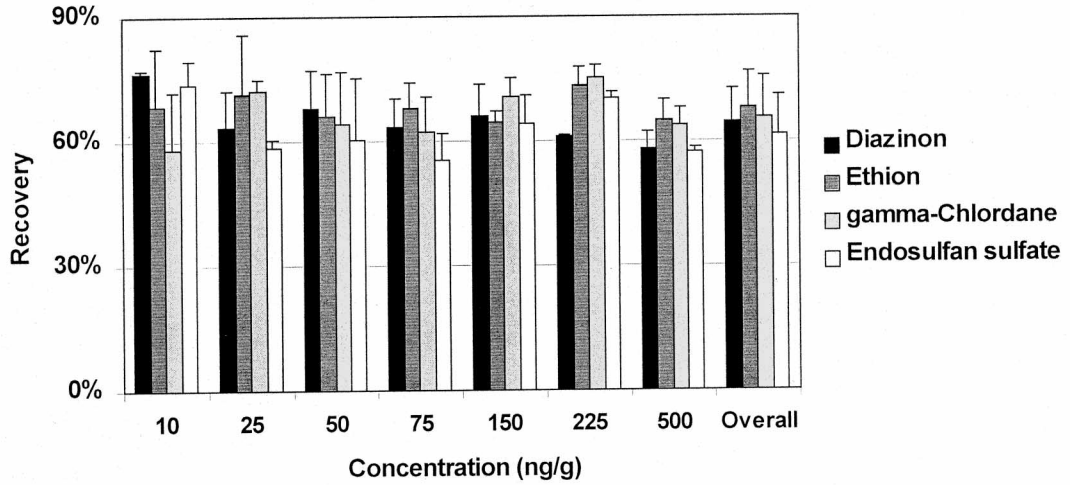

Figure 5. Recoveries of typical pesticides al different concentrations in fortified beef fat (error bars represent standard deviation of results).

4 Conclusions

An analytical method for 40 OP and OC insecticides in animal fat was developed and validated in fortification experiments. The GC method relied upon simultaneous PFPD and μECD to save time, instrumentation needs, labor, and lab space. The method used GPC to separate fats from the pesticide analytes, and no further clean-up was needed before conducting the rugged analytical step. Analyte recoveries in the method were typically 60–70% with 10–20% RSDs and 10–20 ng/g limits of quantitation except for certain problematic OPs.

Many other OP and OC contaminants are also likely to be detected by the method which would be very useful for screening applications. Indeed, organochlorinated compounds or small molecules with both phosphorus and sulfur atoms present are not synthesized naturally [14], thus this approach utilizing the PFPD and μECD provides the possibility to screen for any GC-amenable synthetic chlorinated or phosphorus compound above the detection limits of the detectors. In this particular setup, the detectors were not specific to these types of human-made chemicals, but the possibility exists after some modifications of the approach and we hope to pursue this in the future.

Disclaimer

Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

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