Automated Gel Permeation Chromatographic Preparation of Vegetables, Fruits, and Crops for Organophosphate Residue Determination Utilizing Flame Photometric Detection

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A sensitive, flame photometric procedure for organophosphate residue determination using automated gel permeation chromatographic cleanup is described. Elution volumes of 26 parent and major metabolites of four representative organophosphates were determined. Fortification of 12 sample types with six parent compounds and one metabolite at 0.05–0.10 ppm yielded average recoveries of 83–103%. To demonstrate applicability of the method to multiresidue screening, three samples were fortified with eight organophosphates and analyzed simultaneously.

A gel permeation chromatographic (GPC) technique for pesticide residue determination was first introduced by Stalling et al. (1972). Numerous publications have since appeared describing the use of GPC for the cleanup of samples containing chlorinated pesticides (Tindle and Stalling, 1972; Stalling, 1974; Brookhart et al., 1976; Johnson et al., 1976), carbamates (Krause, 1976; Leicht et al., 1977), PCB's, PBB's (Feringer, 1975), chlorinated styrenes (Kuehl et al., 1976), triazines, dioxins, industrial chemicals, polychlorinated terphenyls (Wright et al., 1978), and organophosphates.

The flexibility of the gel permeation method allows the detection of many organophosphates and their metabolites in various sample matrices. The recovery of the compounds from all sample matrices tested are comparable to other methods reviewed to date (Official Methods of Analysis, 1975 Aoki et al., 1975). Time and solvent savings are also realized utilizing GPC since no liquid–liquid extractions are necessary and only one eluant is used.

Sample composition does not alter the method markedly, as long as quantitative extractions are obtained prior to sample cleanup. Excessive loading (>1.0 g) of oils or sugars onto the gel permeation column should be avoided, however, as elution of the matrix may be extended into the pesticide fraction.

Metabolites and analogues of organophosphorus compounds are becoming more important in residue analysis. Hydrolysis of organophosphate and organothiophosphate compounds may be assumed to destroy the actual or potential toxicity of organophosphate compounds (McMahan and Sawyer, 1977), even though little is known about the byproducts potential toxicity (Doughton et al., 1976). Getz (1962) pointed out that the determination of metabolites or degraded esters resulting from the use of organophosphate ester pesticides is necessary for either qualitative or quantitative determination of the residues. As an added incentive for methods capable of total residue determination, metabolite levels in weathered samples have been requested more frequently by government and private agencies.

The sulfur–phosphorus emission detector (SPED) for gas–liquid chromatography has increased the specificity and sensitivity of organophosphate analysis. Interferences from compounds other than bound, organic phosphorus is almost nonexistent (Bowman and Beroza, 1966, 1967, 1968; Brody and Chaney, 1966). Sample cleanup is needed, not because of chromatographic interference, but rather to eliminate GLC column deterioration through repeated sample injections.

The gel permeation cleanup of samples for organophosphate residue analysis presents a simplified and quantitative method which should improve the efficiency and effectiveness of laboratory operations.

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EXPERIMENTAL SECTION

Apparatus. *Gel Permeation Chromatograph (GPC).* An AutoPrep 1001 GPC (Analytical Biochemistry Laboratories, Inc., Columbia, MO) equipped with a 60.0 cm × 2.5 cm i.d. column packed with 50 g of BioBeads SX-3 resin, 200–400 mesh (Bio-Rad Laboratories, Richmond, CA) compressed to a bed length of approximately 30 cm. The eluting solvent was methylene chloride/cyclohexane (15:85, v/v) pumped at a constant flow rate of 5.0 mL/min with an operating pressure of approximately 8 psig.

Gas Chromatographs. A Model 2500 Bendix gas chromatograph was equipped with a flame photometric detector (SPED) utilizing a phosphorus specific filter (526 nm). The 6 ft × 4 mm i.d. glass column was packed with 1.5% OV-17, 2% OV-210, on 100–120 mesh Gas-Chrom Q support.

A RAC gas chromatograph was equipped with a Varian alkali flame ionization detector, CsCl pellet. A 2 ft × 2 mm i.d. glass column was packed with either 1% Reoplex 400 on 100–120 mesh Gas-Chrom Q support, to analyze acephate and monocrotophos, or 5% SE-30 on 100–120 mesh Gas-Chrom Q support to analyze fenthion.

Reagents. Acetone, chloroform, methylene chloride, and cyclohexane were obtained in pesticide grade (J. T. Baker Chemical Co., Phillipsburg, NJ, or Mallinckrodt Chemical Works, St. Louis, MO).

Procedure. Before samples could be processed with the gel permeation system, it was necessary to determine the elution volume of each compound. The AutoPrep 1001 GPC autofractionates a sample into twenty-three 10-mL fractions for elution profile determination. This is accomplished by collecting the gel column eluant for 2 min from each of the 23 sample collection tubes at a constant flow rate of 5.0 mL/min. The fractions were injected onto the gas chromatograph and detected by the SPED, except for acephate, fenthion, and monocrotophos which were detected by alkali flame ionization. Table I reflects the elution volumes of 33 organophosphate compounds from the 50-g Biobeads SX-3 column eluted with methylene chloride/cyclohexane (15:85). Since time would not permit the analysis of samples for 33 compounds, representative

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Table I

compound (common name)	elution vol., mL
acephate (Orthene)	200-320
carbophenothion (Trithion)	140-180
chlorpyrifos (Dursban)	120-210
crufomate (Ruelene)	120-210
Demeton-S (DiSyston POS, Systox)	110-160
diazinon	80-160
dichlorvos (DDVP, Vapona)	100-200
dimethoate	140-240
dioxathion (Delnav)	140-190
disulfoton (DiSyston)	110-160
disulfoton, sulfoxide metabolite (DiSyston-S)	100-160
disulfoton, sulfone metabolite	160-210
EPN	150-220
ethion	110-160
fensulfothion (DaSanit)	140-230
fensulfothion, oxygen analogue	140-220
fensulfothion, sulfone	160-250
fensulfothion, oxygen analogue sulfone	160-250
fention (Baytex)	130-200
fonofos (Dyfonate)	130-210
malathion	120-200
methidathion (Supracide)	120-240
methyl parathion	190-250
methyl paraoxon	200-270
mevinphos (Phosdrin)	120-190
monocrotophos (Azodrin)	150-240
naled (DiBrom)	120-190
paraoxon	155-235
parathion	120-230
phorate (Thimet)	110-170
pirimiphos-methyl (Actellic)	100-160
ronnel (Fenchlorphos)	130-210
tetrachlorvinphos (Gardona)	120-180

compounds were chosen for analysis. These seven compounds were selected because of their differing elution characteristics, i.e., diazinon is the earliest eluting and methyl parathion is one of the latest eluting.

To 20 g of sample in a Sorval blender cup was added the spiking solution and 80 mL of chloroform. After blending for approximately 3 min, the sample was filtered through a Buchner funnel fitted with filter paper (Whatman no. 42 ashless or equivalent) into a 250-mL separatory funnel. The blender cup and filter cake were rinsed twice with 20-mL portions of chloroform which was combined with the filtrate.

The sample extract was allowed to stand 5 min to permit any water/chloroform phase separation and the organic phase drained through 10 g of Na_2SO_4 (prewashed with hexane) into a 250-mL round-bottom flask. The separatory funnel and Na_2SO_4 and the aqueous layer, if present, were rinsed with two 10-mL portions of chloroform which were combined with the original extract. Ten drops of decanol were added to the flask to retard volatilization losses of the pesticide residues, and the sample was rotary-vacuum evaporated in a 30 °C water bath.

The sample residue was dissolved in methylene chloride/cyclohexane (15:85) and transferred to a culture tube, and the volume was adjusted to 10 mL. A 5-mL aliquot was injected onto the GPC and processed by the parameters of Table I depending upon the compound to be analyzed. The GPC eluant was collected in a 250-mL round-bottom flask and evaporated to dryness. The sample residue was transferred to a culture tube with acetone and the volume was adjusted to 1 mL. The extract was injected onto the GLC-SPED for quantitation by peak height comparison of standard solutions.

The sample matrices chosen for this experiment were broccoli stalks, lettuce leaves, cabbage leaves, green bean pods and seeds, celery stalks, tomato, pear, apple, grape, wheat forage, wheat straw, and soybean vines. A control for each matrix was extracted and processed using the GPC by collecting the eluant over a 200-mL range which included the collect fraction of each organophosphate analyzed. Thus one control from each crop served for all subsequent fortified sample analyses.

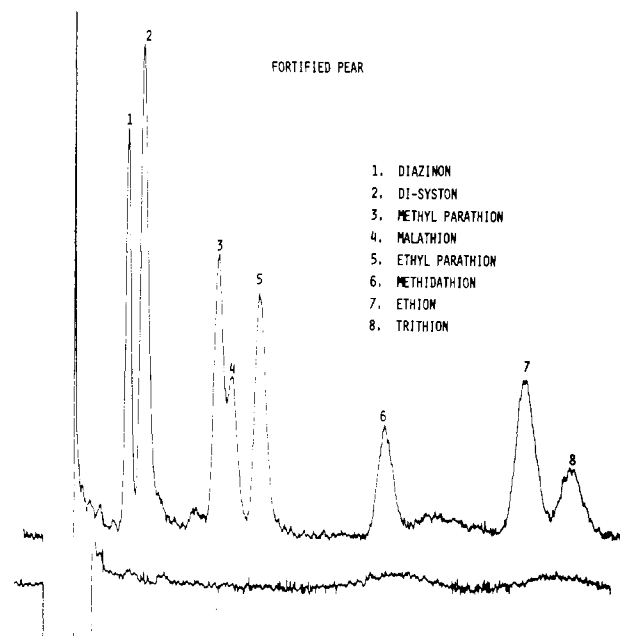

Figure 1. GLC chromatogram of fortified pear sample. Conditions: 6 ft × 4 mm i.d. glass column packed with 1.5% OV-17, 2.0% OV-210 on Gas-Chrom Q, 100–120 mesh; detector: sulfur–phosphorus emission with a 526-nm filter; Temperatures: inlet, 215 °C; oven 215 °C; detector, 220 °C; nitrogen carrier gas approximately 40 mL/min. GPC parameters: dump, 20 min; collect, 36 min; wash, 0 min. (The sample control is shown below the chromatogram.)

RESULTS AND DISCUSSIONS

Samples were extracted with chloroform and the bulk of coextracted water removed by partitioning. Subsequent drying of the extract with anhydrous sodium sulfate resulted in an extract compatible with gel permeation cleanup on BioBeads SX-3, 15% methylene chloride/85% cyclohexane. The use of a chloroform/methanol extraction solvent can also be used for extracting weathered samples (McMahon and Sawyer, 1975, Sec. 253.102). The methanol would have to be removed by a water wash-partition before the sodium sulfate drying step described in this procedure. Many other solvent systems which are compound-specific could also be used in conjunction with this gel permeation procedure by dissolving the residue in 15% methylene chloride/85% cyclohexane before injection into the AutoPrep 1001.

The results of the analysis are presented in Tables II–IV. The recoveries are comparable to other methods so far reviewed. The reproducibility of the data was calculated by averaging the percent recovery of each compound from all sample types. These data are presented with the standard deviation and relative standard deviation in Table V.

To demonstrate the flexibility of this procedure for multiresidue determination, broccoli, wheat forage, and pear samples were fortified with eight organophosphates, extracted and processed simultaneously. Figures 1–3 are chromatograms of the results. The control chromatogram

Table II. Fortified Vegetable Sample Analysis

compound	μg/g added	percent recovered				
		broccoli	lettuce	cabbage	celery	green beans
diazinon	0.050	84	88	84	100	88
dichlorovos	0.055	96	95	100	98	80
disulfoton	0.063	76	86	83	102	78
malathion	0.048	104	96	100	96	92
methyl parathion	0.055	93	90	100	98	100
paraoxon	0.095	101	99	100	108	104
parathion	0.051	94	86	116	112	106

Table III. Fortified Fruit Sample Analysis

compound	μg/g added	percent recovered			
		tomato	apple	pear	grape
diazinon	0.050	98	90	96	82
dichlorovos	0.055	95	84	76	84
disulfoton	0.063	89	87	87	75
malathion	0.048	90	106	90	100
methyl parathion	0.055	96	98	93	100
paraoxon	0.095	105	98	100	103
parathion	0.051	96	104	98	112

Table IV. Fortified Crop Sample Analysis

compound	μg/g added	percent recovered		
		wheat straw	wheat forage	soybean vine
diazinon	0.050	96	72	88
dichlorovos	0.055	100	80	78
disulfoton	0.063	83	71	84
malathion	0.048	104	79	102
methyl parathion	0.055	100	100	96
paraoxon	0.095	76	94	98
parathion	0.051	118	102	90

Table V. Data Evaluation

compound	no. of samples	mean % recov.	SD	rel. SD
diazinon	12	89	7.9	0.09
dichlorovos	12	89	9.3	0.10
disulfoton	12	83	8.1	0.10
malathion	12	97	7.8	0.08
methyl parathion	12	97	3.4	0.04
paraoxon	12	99	8.1	0.08
parathion	12	103	10.4	0.10

Table VI. Fortified Multiresidue Sample Analysis

compound	μg/g added	percent recovered		
		broccoli	pears	wheat forage
carbophenothion	0.050	107	100	126
diazinon	0.050	90	97	94
disulfoton	0.050	91	91	91
ethion	0.050	103	109	120
malathion	0.050	103	120	109
methidathion	0.050	122	105	122
methyl parathion	0.050	97	105	102
parathion	0.050	101	107	121

appears below each figure. Table VI reflects the recovery of each compound from the screening experiment.

The GLC column used for this analysis showed no deterioration in resolution or retention time shift further displaying the cleanup provided by gel permeation. The automated GPC was capable of handling 23 samples simultaneously and most operation time was overnight and unattended. At a time when solvent and labor costs are soaring, gel permeation chromatography should prove to be an invaluable aid to analytical laboratories.

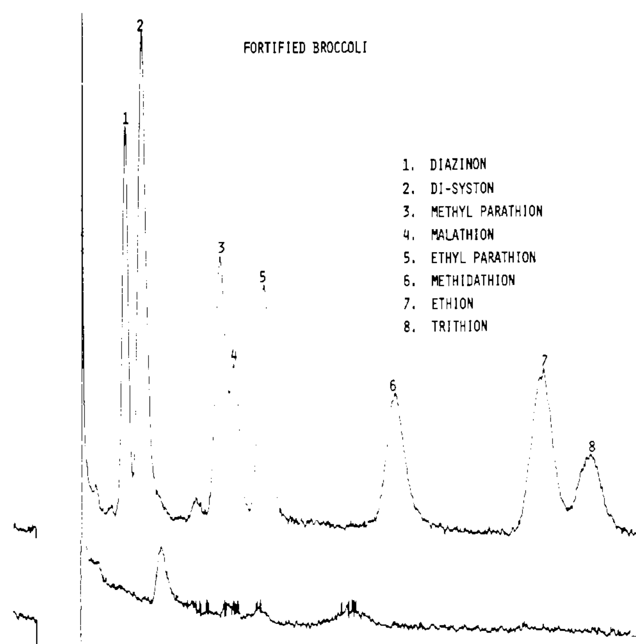

Figure 2. GLC chromatogram of fortified broccoli sample. Conditions: same as Figure 1. (The sample control is shown below the chromatogram.)

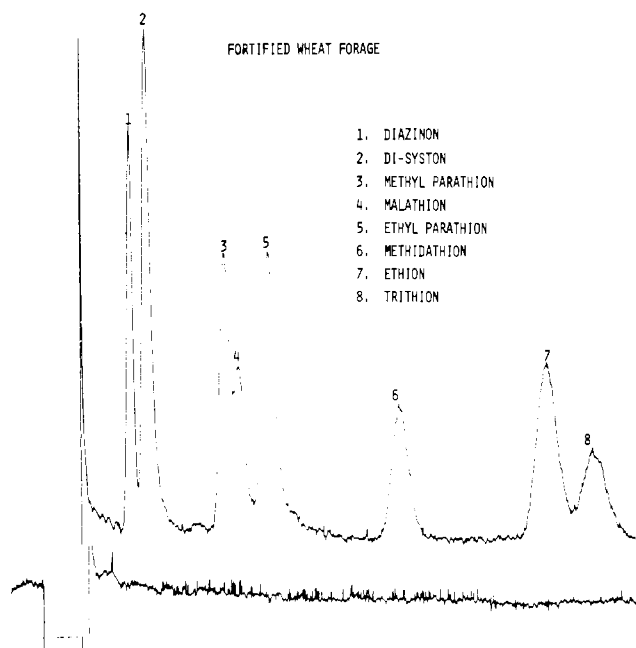

Figure 3. GLC chromatogram of fortified wheat forage sample. Conditions: same as Figure 1. (The sample control is shown below the chromatogram.)

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Screening of Nonanal and Related Volatile Flavor Compounds on the Germination of 18 Species of Weed Seed

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Eighteen species of weed seed were tested in light and dark for germination responses to 28 volatile compounds that have been shown previously to stimulate fungal spore germination. Most of these chemicals occur naturally as components of flavors and fragrances. Seeds of curly dock (*Rumex crispus*) and red sorrel (*Rumex acetosella*) were stimulated by nonanenitrile, octyl thiocyanate, 2-nonanol, 2-nonanone, and other compounds. Inhibition was the most common response observed and was expressed by two or more compounds on nine species. Formative effects such as inhibition of radicle, swelling of radicle, swelling and splitting of seed, and production of exudate were observed. The swelling of smartweed seed, forming a turgid spherical body, induced by nonanal and other aldehydes, and the excretion of a gel, induced by citral and related compounds in morningglory, were particularly noteworthy.

Nonanal and 6-methyl-5-hepten-2-one were identified in uredospores of *Puccinia graminis* f. sp. *tritici* and other rusts (French and Weintraub, 1957; Rines et al., 1974). Nonanal or β-ionone, and related flavor components including terpenes, stimulated germination of spores in 24 fungal species, belonging to seven genera: *Puccinia*, *Uromyces*, *Coleosporium*, *Melampsora*, *Ustilago*, *Urocystis*, and *Penicillium* (French, 1961; French et al., 1975b, 1978). Stimulation of germination of pine pollen was also observed (French et al., 1979). The broad spectrum of activity of these volatile, naturally occurring flavor compounds, some of which are also insect pheromones, suggested testing other propagules for stimulation. This report summarizes the results from testing 28 compounds on the germination of 18 species of weed seed.

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MATERIALS AND METHODS

Seed used in this study were obtained from commercial sources. Because of space limitations in the desiccators used to contain the volatile chemicals, seed were tested in two groups as follows: group I—velvetleaf (*Abutilon theophrasti* Medic.), redroot pigweed (*Amaranthus retroflexus* L.), johnsongrass [*Sorghum halepense* (L.) Pers.], curly dock (*Rumex crispus* L.), wild mustard [*Brassica kaber* (DC.) L.C. Wheeler var.], giant foxtail (*Setaria faberi* Herrm.), jimsonweed (*Datura stramonium* L.), Pennsylvania smartweed (*Polygonum pensylvanicum* L.); group II—common morningglory [*Ipomoea purpurea* (L.) Roth], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], green foxtail [*Setaria viridis* (L.) Beauv.], lambsquarters (*Chenopodium album* L.), quackgrass [*Agropyron repens*

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