RESIDUES AND TRACE ELEMENTS

Multiresidue Analysis of Pesticides in Fresh Fruits and Vegetables Using Procedures Developed by the Florida Department of Agriculture and Consumer Services

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Improved quality and efficiency of pesticide residue analysis were achieved by examining all aspects of the laboratory process. In an effort to eliminate methylene chloride hazardous waste, an acetonitrile extraction method, originally developed by the California Department of Agriculture, was modified and adopted. Sample size and solvent consumption were reduced with the new method. Custom glassware racks and disposable supplies reduced overall analysis time. Gravity-fed, solid-phase extraction simplified sample preparation and provided cleaner extracts for gas chromatographic analyses. Modifications to the method were made to achieve the ruggedness needed to maintain quality objectives during routine analysis. Instrumental improvements, including new selective detectors, retention time locking, and mass spectrometry screening for all samples, provided the laboratory with efficient, reliable, and confirmed analytical results.

Pesticide residue analyses at the Florida Department of Agriculture and Consumer Services (FDOACS) are performed to meet 2 distinctly different objectives: risk exposure assessment and residue tolerance enforcement. The U.S. Environmental Protection Agency (EPA) uses risk assessment data in its determination of acceptable pesticide tolerances for raw agricultural commodities (1). EPA needs current residue data to comply with the mandates of the Food Quality Protection Act of 1996 (FQPA; 2). The FQPA directs the EPA to reassess the risks due to pesticides from all sources, including foods. Special attention must be paid to effects on infants and children. Pesticides that may interfere with hormonal activity also must be studied.

Surveillance and compliance monitoring of pesticide residues ensure the public that domestic, imported, exported, and organic foods meet established tolerances for pesticide residues. Import monitoring is important because of the increase in foreign-grown produce in the United States since passage of the North American Free Trade Agreement (3). Other countries are seeking pesticide residue analysis to certify that U.S.-exported commodities meet their country's tolerances. In 1999, Congress will reconsider passage of the National Organic Food Program (4) to ensure that products labeled "organic" are organically grown.

Residue analyses for risk assessment, which target specific pesticides and commodities, require extensive analytical method validation, low detection limits, and the best available precision and accuracy. Residue monitoring programs must analyze an increasingly complex array of agricultural chemicals and commodities in a short time to prevent distribution of harmful products. The integrity and effectiveness of surveillance and compliance monitoring relies on timeliness, legally defensible confirmation, and good laboratory quality assurance practices. A pesticide residue method must meet the challenges of risk assessment and compliance monitoring.

Since the early 1960s, pesticide residue analyses at the FDOACS are performed for Florida surveillance and compliance monitoring. Since 1991, Florida and other states also analyze residues for the U.S. Department of Agriculture Pesticide Data Program (PDP; 5). PDP results are used by EPA for risk assessment. Previous FDOACS pesticide residue methods used acetone extraction, methylene chloride partitioning, and dual-column gas chromatography (GC) or liquid chromatography (LC) as described by Luke et al. (6, 7) and the U.S. Food and Drug Administration's (FDA) *Pesticide Analytical Manual* (PAM; 8). The development of the method described in this paper relied heavily on acetonitrile extraction methods used by the California Department of Agriculture (9) and the Washington Department of Agriculture (10). Other multiresidue pesticide methods suggesting acetonitrile and other alternative solvents and detector systems have also been published (11–16). Quality assurance and method validation procedures were developed to comply with the guidelines of PDP (17) and the Good Laboratory Practices of the EPA and FDA (18, 19).

The entire pesticide residue analysis process is briefly described in this paper, including collection, sample extraction, instrumental analysis, and interpretation of results. A detailed description of pesticide residue programs, including extrac-

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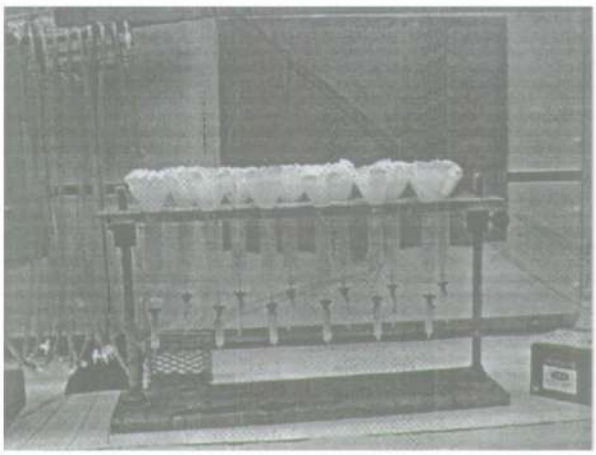

Figure 1. Extraction rack was set up by placing 70 mL reservoirs in wooden rack, attaching preconditioned C_{18} cartridges to the bottom with column-connecting adaptors, and adding funnels and fluted filter paper to the top.

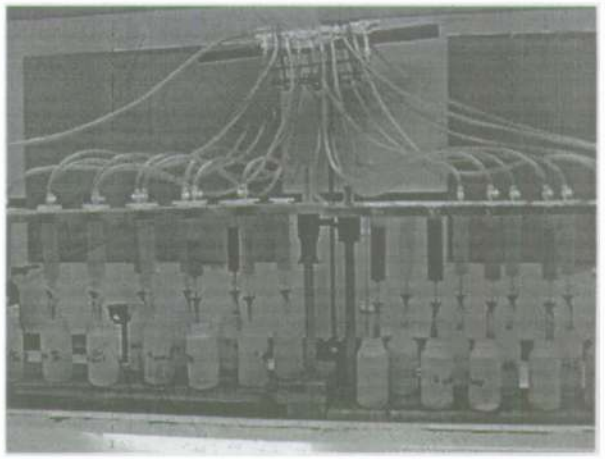

Figure 2. Sample extracts were eluted through C_{18} cartridges into polyethylene bottles by using positive nitrogen pressure.

tion, instrumental analysis, and regulatory significance, is described by Fong et al. (20). A new extraction method was adopted to avoid the use of hazardous solvents such as methylene chloride and ethyl ether. This method uses an acetonitrile extraction with a gravity-fed, C_{18} solid-phase extraction (SPE) cleanup. This extract is concentrated, and the solvent is changed to acetone for analysis of phosphorus, sulfur, and nitrogen pesticides. Florisil and aminopropyl SPE fractions are prepared for halogen and carbamate analysis, respectively. All samples are quantitated using GC or LC detectors and qualitatively identified by GC/mass spectrometry (MS) in selected-ion monitoring (SIM) mode whenever possible.

Experimental

Instrumentation

(a) *GC selective detector systems.*—Multiresidue screens were performed by using Hewlett-Packard (HP) 5890 Series II gas chromatographs equipped with splitless injectors, electronic pressure control (EPC), 7673 autosamplers, HP ChemStation V. 3.34, and several different selective detectors: electron capture detector (ECD), HP Model 19223 (Hewelett Packard, Avondale, PA); electrolytic conductivity detector (ELCD), Model 4420, OI Analytical (OI; College Station, TX); nitrogen–phosphorus detector (NPD), HP Model 19234; flame photometric detector (FPD), HP Model 19256A; halogen specific detector (XSD), OI Model 5360; atomic emission detector (AED), HP Model 5921. Method validation and routine dual-column confirmations were made by using DB-5 column (30 m × 0.53 mm × 1.5 μm) and DB-17 column (30 m × 0.53 mm × 1.0 μm); oven program: 150°C (3.00 min), rate 15°C/min to 240°C (1 min), rate 10°C/min to 260°C (3.5 min). Screening was performed with the HP pesticide database (21, 22) as well as dual-column confirmation by using alternative columns; HP5MS (30 m × 0.25 mm × 0.25 μm) or (30 m × 0.53 mm × 0.53 μm) and HP35MS (30 m × 0.25 mm × 0.25 μm) or (30 m × 0.53 mm × 0.53 μm); oven program: 50°C (1.00 min), rate 60°C/min to 150°C (1 min), rate 6°C/min to 205°C (0 min), rate 20°C/min to 250°C (10 min). Capillary columns provide improved resolution, but megabore (0.53 mm) columns are more rugged for the analysis of fruit and vegetable extracts. All GC analysis were performed by using retention time locking (RTL; 21, 22).

(b) *GC/MS system.*—HP 5890 Series II equipped with EPC, HP Model 5972 GC/MS detector (HP) equipped with autosampler, HP 7673 autosampler, and ChemStation G1036 rev. C software. GC operating conditions: splitless injector, 280°C; septum purge flow, 1 mL/min; inlet purge flow, 50 mL/min; injector purge time, 0.5 min; He carrier gas in constant flow mode at ca 1.0 mL/min. MS operating conditions: electron impact mode, transfer line, 280°C; ion source

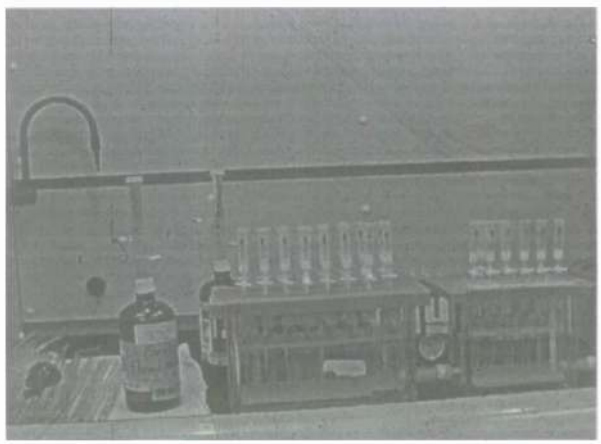

Figure 3. Extracts were gravity fed through preconditioned Florisil solid-phase extraction (SPE) cartridges.

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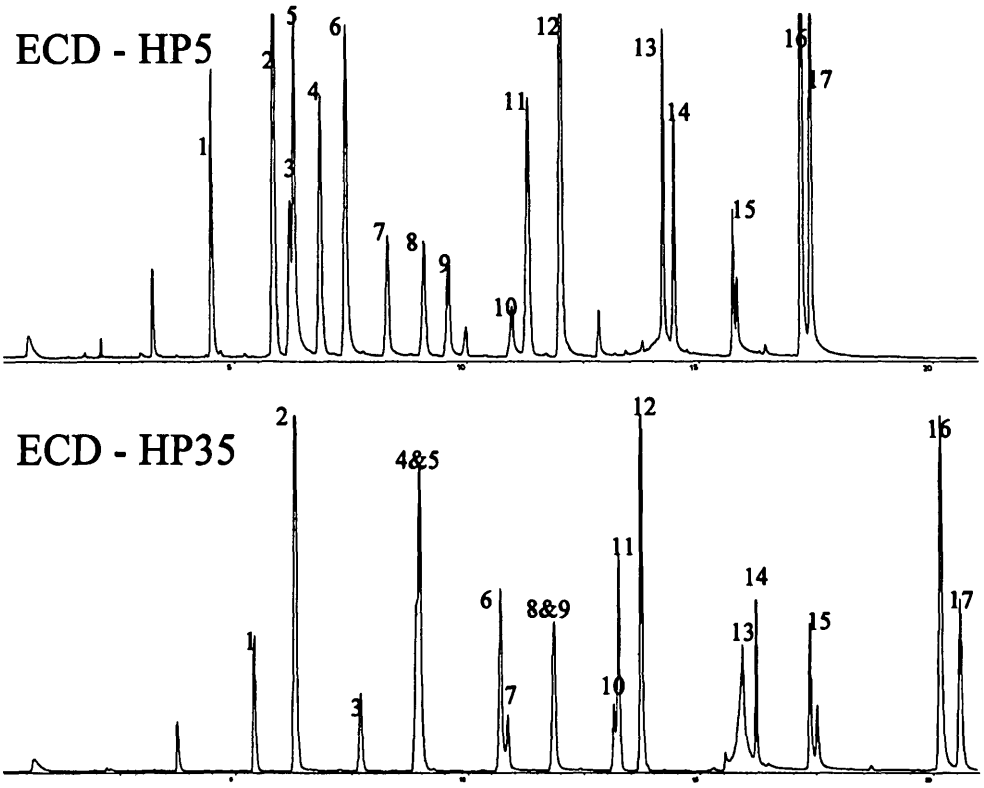

Figure 4. Dual column confirmation is shown. Pesticides eluted with different retention times on 2 different column phases but the same oven temperature program. (Electron capture detector [ECD]; top: HP5MS 30 m × 0.25 mm × 0.25 μm, bottom: HP35MS 30 m × 0.25 mm × 0.25 μm). 1 = Pentachlorobenzene, 2 = trifluralin, 3 = hexachlorobenzene, 4 = pentachloronitrobenzene, 5 = dicloran, 6 = chlorothalonil, 7 = chlorpyrifos-methyl, 8 = linuron, 9 = chlorpyrifos, 10 = procymidone, 11 = endosulfan I, 12 = DDE-*pp'*, 13 = iprodione, 14 = methoxychlor, 15 = permethrin, *cis* and *trans*, 16 = fenvalerate, 17 = es fenvalerate.

temperature, 280°C; electron energy, 70 eV; mass calibration, peak widths (typically 0.5 mass unit), and electron multiplier voltage (typically 2400 V, offset 400 V) were set during the instrument tuning to meet EPA Method 625 decafluorotriphenylphosphine criteria (23). Data acquisition was in the selected ion mode. HP5MS column, 30 m × 0.25 mm id × 0.25 μm film thickness. Oven temperature program: 40°C (1 min), rate 30°C/min to 150°C, rate 6°C/min to 280°C (10 min). Or HP35MS, 30 m × 0.25 mm × 0.25 μm film thickness. Oven temperature program: 70°C (1 min), rate 23°C/min to 150°C, rate 4°C/min to 280°C (5 min).

(c) *LC system.*—HP carbamate analysis system with 1050 pump and autosampler and fluorescence detector (330 nm excitation; 460 nm emission), HP ChemStation Data Station, Pickering (Mountain View, CA) postcolumn derivatization detector (24); (*1*) *System 1.*—Waters (Milford, MA) C_{18} guard column, Pickering carbamate analysis column, C_{18} (4.6 × 250 mm, 5 μm), 1.5 mL/min, gradient, acetonitrile in water 10–70% in 20 min; (*2*) *System 2.*—Waters CN guard column, Zorbax (Part No. 820950-905, Hewlett Packard) CN analytical column (4.6 × 250 mm × 5 μm), 1.5 mL/min, gradient, acetonitrile in water 10–50% in 12 min, ambient column temperature. Pickering reactor, 100°C, Pickering hydrolysis reagent flow, 0.3 mL/min, Pickering OPA reagent (*o*-phthalaldehyde) 100 mg OPA/10 mL methanol with 2 g Thiofluor, flow, 0.3 mL/min.

Materials and Apparatus

(a) *Homogenizer.*—Robot Coupe Model RSI6Y-1 scientific industrial blender (Robot Coupe, Inc., Ridgeland, MS).

(b) *Balance.*—Top loading, 3000 g capacity, 0.01 g accuracy, Model Basic (Sartorius Corp., Bohemia, NY).

(c) *Shaker.*—Reciprocating, platform type, variable speed, Model 6000 with Model 6050 adaptor to hold 2 utility box carriers (Model 6044; Eberbach Corp., Ann Arbor, MI). Wrist action shakers may also be used.

(d) *Evaporator.*—Model 112 N-EVAP (Organomation Associates, Inc., Berlin, MA).

(e) *Centrifuge.*—IECC-6000, floor type with 4-station head, Model 276 (International Equipment Co., Needham Heights, MA).

(f) *Water (steam) bath.*—Capability of 100°C, Cat. No. 66738 (Precision Scientific, Chicago, IL).

(g) *Solvent dispensers.*—Analog, 10–100 mL, Cat. No. 13-688-232; 1–10 mL digital, Cat. No. 13-688-222 (Fisher Scientific, Pittsburgh, PA).

(h) *SPE holding rack.*—Wooden support rack designed to hold a "tower" consisting of the extraction funnel and filter

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paper, 70 mL reservoir, stopcock, C_{18} cartridge and 250 mL jar for C_{18} cleanup is shown in Figure 1.

(**i**) *SPE vacuum station.*—Preptorr, 10 port (Fisher Scientific).

(**j**) *SPE cartridges.*—C_{18}, 500 mg, end capped, 6 mL reservoir capacity, Part No. 221-0050-C; Florisil, 500 mg, PR grade, 10 mL (XL) reservoir capacity, Part No. 712-0050-H; NH_2, 500 mg, 10 mL (XL) reservoir capacity, Part No. 470-0050-H (Jones Chromatography, Inc., Lakewood, CO). Equivalent SPE cartridges may be used providing they are tested for elution patterns and recoveries.

(**k**) *SPE accessories.*—70 mL reservoirs, Part No. 120-1008-F; adaptors for 70 mL reservoir, Part No. 120-1103; adaptors for 6 mL capacity reservoir, Part No. 120-1101; Universal PTFE stopcocks, Part No. 121-0009 (Jones Chromatography, Inc.).

(**l**) *Funnels.*—Heavy duty, LDPE, Cat. No. 4260-0030 (Fisher Scientific).

(**m**) *Filter paper.*—15 cm id, coarse, quantitative P8 fluted, Cat. No. 09-790-14E (Fisher Scientific).

(**n**) *Sample extraction containers.*—High-density polyethylene, 250 mL with screw-cap tops (Nalge International, Cat. No. 02-893-5D, Fisher Scientific).

(**o**) *Adjustable pipet and tips.*—0.5–5.0 mL Wheaton, Socorex pipet, Part No. 13-707-61; 0.5–5.0 mL capacity disposable tips, Part No. 21-375-3 (Fisher Scientific).

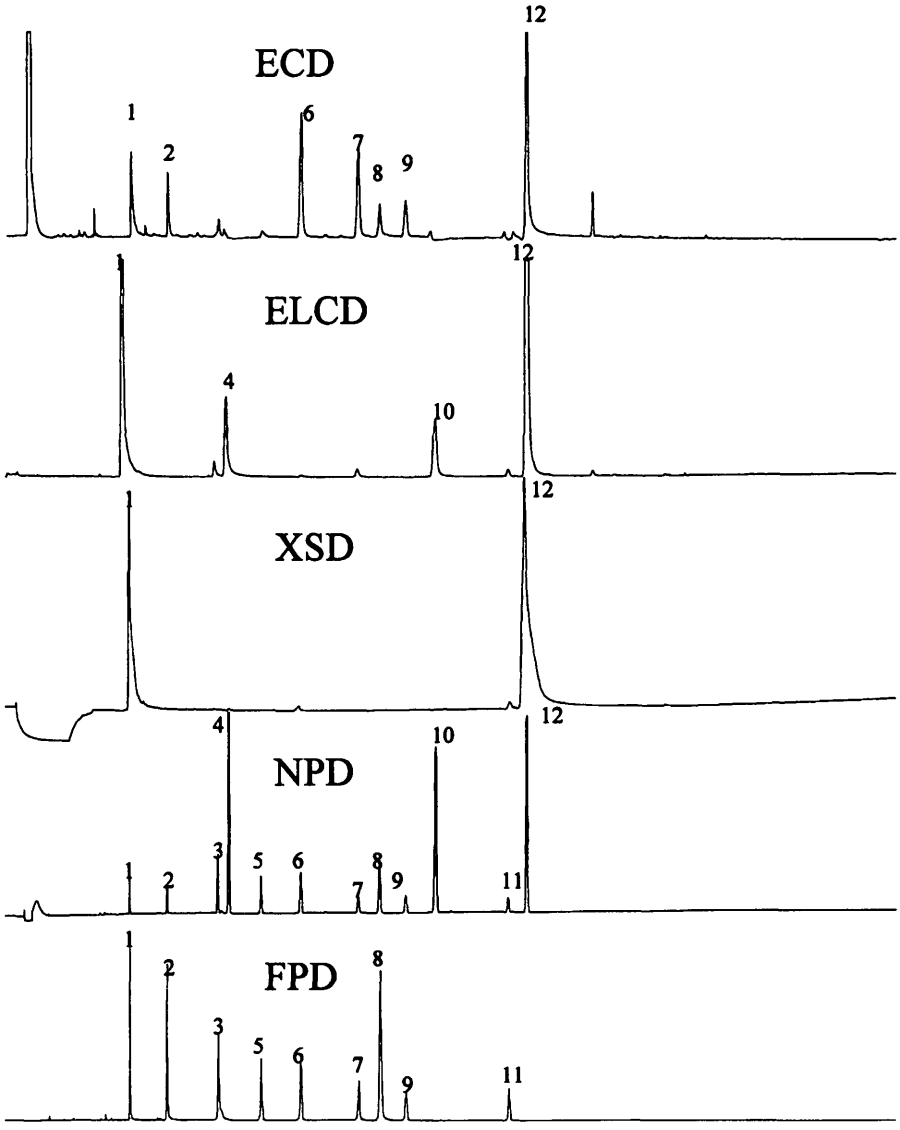

Figure 5. Retention times remain constant from detector to detector. Analysis of the same pesticides on 5 different GC selective detectors by using the same column, oven temperature program, and retention time locking. (HP5MS, 30 m × 0.25 mm × 0.25 μm). ELCD, electroylic conductivity detector; XSD, halogen-specific detector; NPD, nitrogen–phosphorus detector; FPD, flame photometric detector. 1 = Dichlorvos, 2 = mevinphos, 3 = omethoate, 4 = diphenylamine, 5 = fonofos oxygen analog, 6 = fonofos, 7 = parathion methyl, 8 = oxydemeton methyl sulfone, 9 = malathion, 10 = diphenamid, 11 = fenamiphos, 12 = myclobutanil.

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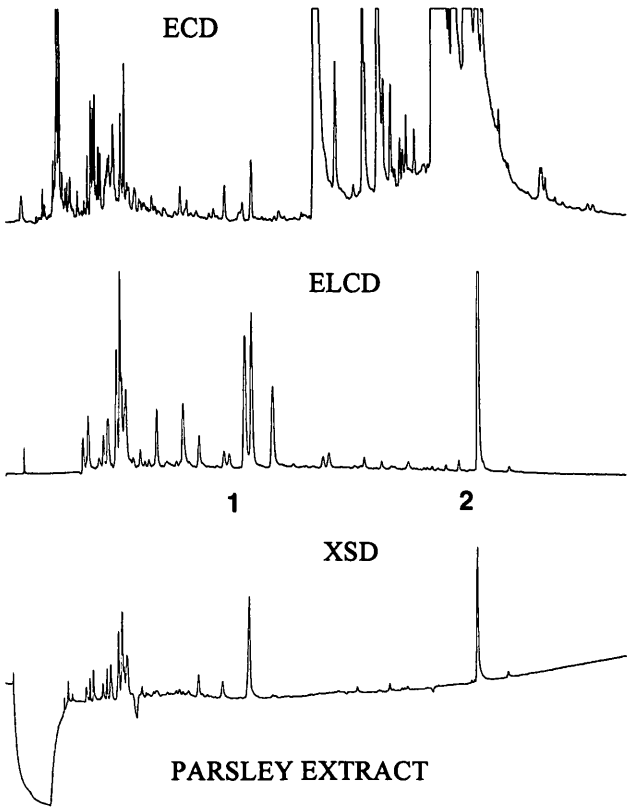

Figure 6. Hydrocarbon interference on ECD and nitrogen interference on ELCD was reduced on XSD. Parsley extract was analyzed on 3 different detectors by using the same column, oven temperature program, and retention time locking sample contains process control (1) and hydrocarbon (2; HP5MS, 30 m × 0.25 mm × 0.25 μm).

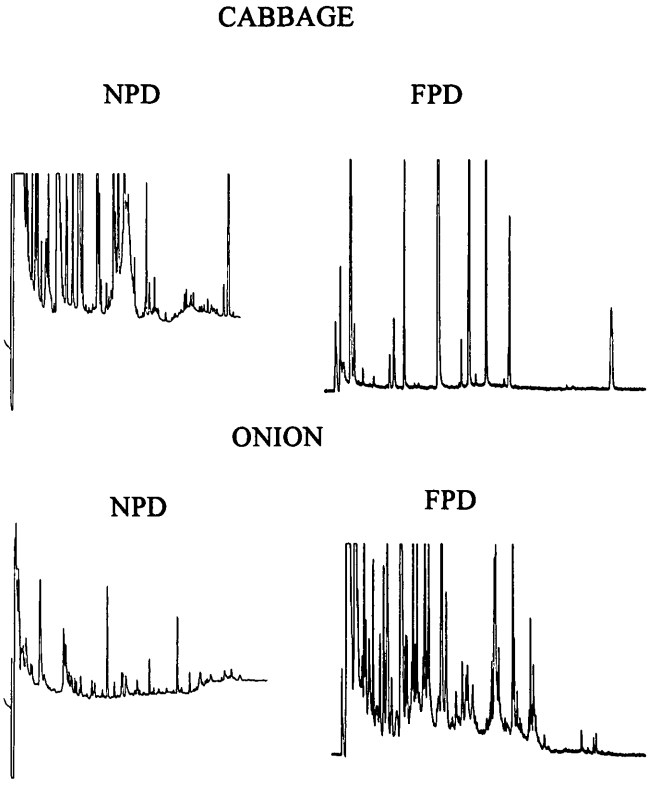

Figure 7. Cabbage and onion extracts were analyzed on the same instrument with 2 different selective detectors. Matrix interference in these commodities is shown. These extracts did not contain any pesticides. (Nitrogen detector: DB-5, 30 m × 0.53 mm × 1.5 μm; FPD: DB-17, 30 m × 0.53 mm × 1.0 μm.)

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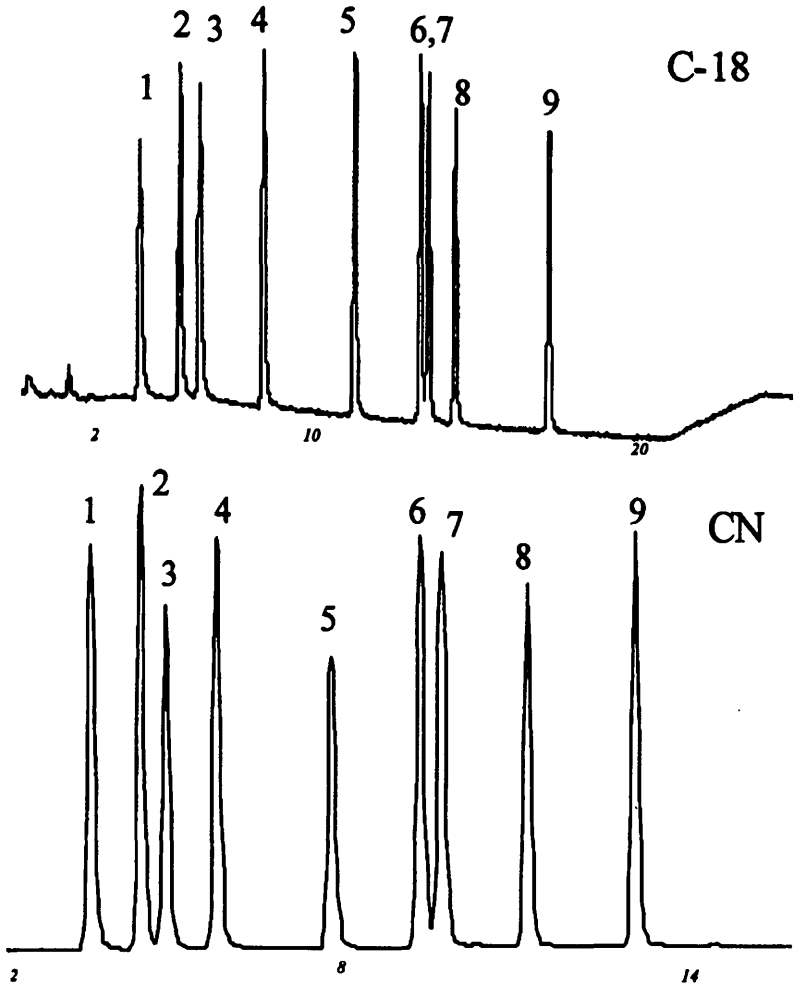

Figure 8. Carbamate pesticides eluted in 27 min on the primary screening C_{18} column (top) and in 19 min on the cyano phase confirmation column (bottom). Compounds shown from left to right on both columns are aldicarb sulfoxide (1), oxamyl (2), methomyl (3), 3-hydroxycarbofuran (4), aldicarb (5), propoxur (6), carbofuran (7), carbaryl (8), and methiocarb (9).

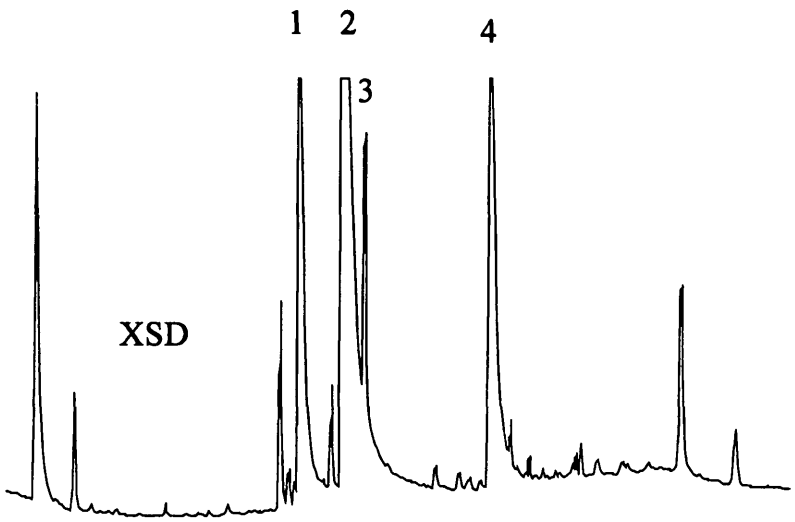

Figure 9. Onion extract contained 2 unknown analytical responses (UAR). Folpet was identified by using atomic emission detector (AED) and pesticide database. Chlorothalonil metabolite was identified later by using retention time locking. Multiple halogens in green onion: 1 = UAR (chlorothalonil metabolite), 2 = chlorothalonil, 3 = methyl chlorpyrifos (process control), 4 = folpet.

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(**p**) *Assorted glassware and supplies.*—Volumetric pipets, 10 mL repipet, 100 mL beakers, spatulas, spoons. Disposable pasteur pipets; borosilicate glass, 5¾ in. length, Part No. 13-678-20B (Fisher Scientific). Tygon tubing; 5/16 in. id, 1/16 in. wall thickness, 7/16 in. od, Cat. No. 14-169-1M (Fisher Scientific). Test tubes, disposable; borosilicate glass, 16 × 125 mm screw-cap culture tubes, Part No. 14-959-35A; borosilicate glass, 16 × 125 mm nonscrew-cap culture tubes, Part No. 14-961-30; GPI-15-415, black-molded W/T, PTFE-faced, screw-type caps, Part No. 14-930-15E. Nondisposable, 13 mL, graduated with ground glass stops, Cat. No. 05-538-40A (Fisher Scientific).

(**q**) *Chemicals.*—NaCl crystals, American Chemical Society (ACS) certified, Cat. No. 271-500; Na_2SO_4 anhydrous granules 10–60 mesh, ACS certified, tested for pesticide residues, Cat. No. S415-500 (Fisher Scientific).

(**r**) *Solvents.*—All solvents were of pesticide grade or better (Optima grade, Fisher Scientific).

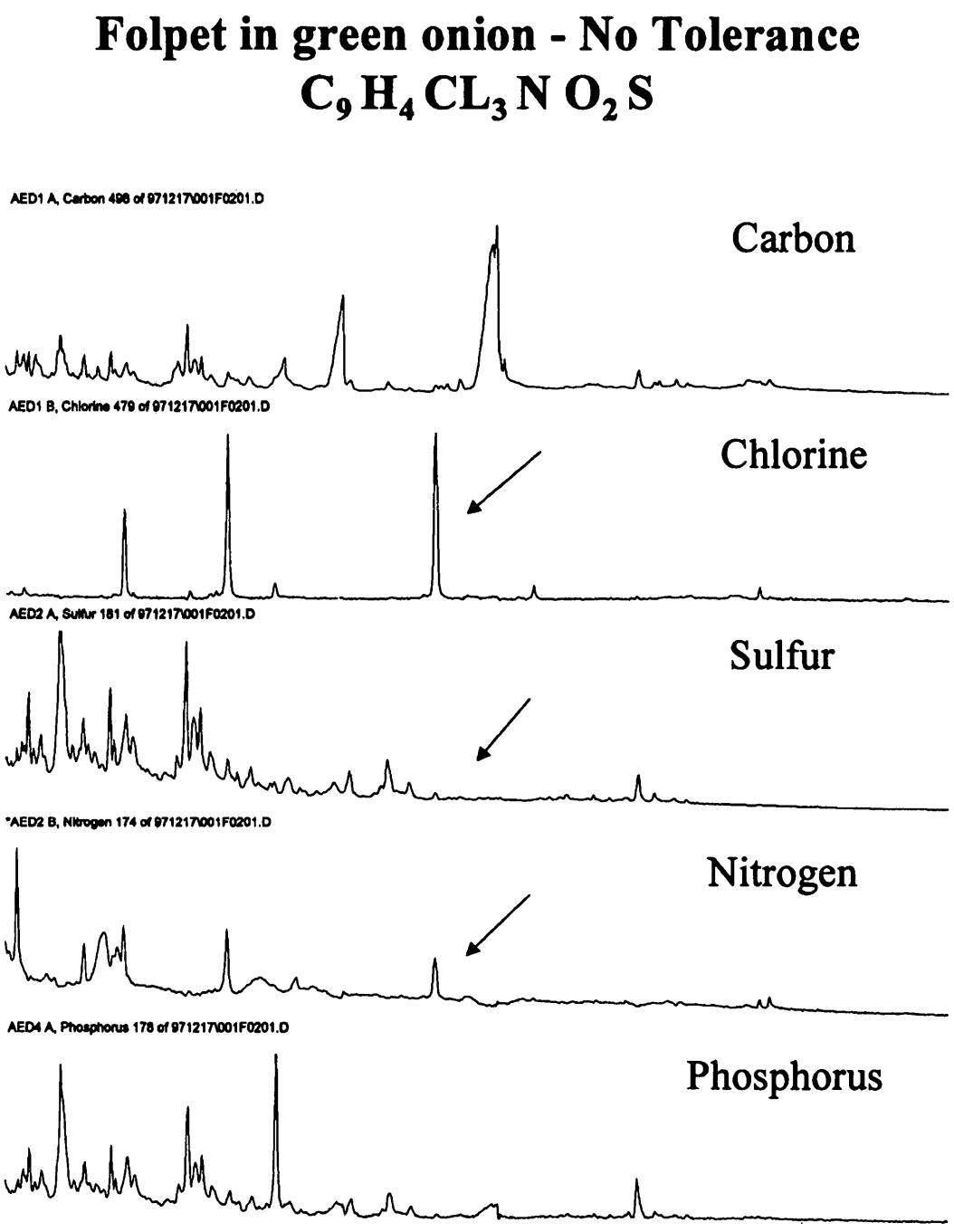

Figure 10. AED analysis of onion extract indicated that the UAR contained a large chlorine, moderate nitrogen, and small sulfur response as indicated by the arrows. Pesticide database indicated that folpet had a similar retention time. Identification was confirmed by dual column and GC/MS to a folpet standard.

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UAR Identified as Breakdown of Chlorothalonil

	DB-5 RTL	DB-17 RTL	Chlorothalonil
snap beans	11.22	12.22	+
bok choy	11.18	12.15	+
bok choy	11.17	12.26	+
cabbage	11.16	12.16	ND
cabbage	11.18	12.19	+
cabbage	11.16	12.18	+
cabbage	11.22	12.22	ND
cabbage	11.15	12.21	+
cabbage	11.15	12.22	ND
collards	11.15	12.17	+
tomatos	11.15	12.21	+
turnip roots	11.13	12.17	+
AVERAGE	**11.17**	**12.20**	
MAX-MIN	**0.09**	**0.11**	

Figure 11. Records of UAR RTL retention times indicated that the same compound was detected in several commodities and many of these commodities also contained chlorothalonil. A comparison with metabolite standards confirmed the identification. Max, maximum; min, minimum.

(s) *Standards.*—Stock standard solutions were prepared from certified, neat materials (ChemService, West Chester, PA) in either isooctane or acetone. Working standard mixes were prepared from dilutions of the stock solutions in the same solvent as the respective extracts. In-matrix standards were prepared for GC analysis by drying blank sample extract under a stream of nitrogen and reconstituting with 4 times the volume of working standard.

Reagents

(a) *Water–acetonitrile for C_{18} column conditioning.*—Prepared at least monthly. This recipe is enough for 3 sets of 24 samples (ca 18 mL/sample). Deionized water (360 mL) was measured into a 1000 mL graduated cylinder; 900 mL acetonitrile was measured into a 1000 mL graduated cylinder. Acetonitrile and water were combined into a clean, 4 L bottle and mixed well by shaking. The mixture was kept in a sealed bottle and transferred to Teflon squeeze bottle just before sample analysis.

(b) *Acetone–hexane reagent for Florisil SPE.*—Prepared fresh for each set. This recipe is enough for one set of 24 samples (ca 20 mL/sample). Hexane (425 mL) was measured into a 500 mL graduated cylinder; 75 mL acetone was measured into a 100 mL graduated cylinder. Hexane and acetone were combined into a 1 L repipette bottle, mixed well by shaking, and kept in a sealed bottle.

(c) *Methanol–acetone reagent for carbamate SPE.*—Prepared fresh each week. This recipe is enough for 3 sets of 24 samples (ca 14 mL/sample). Methanol (30 mL) was pipetted into a 1000 mL volumetric flask and made up to the mark with acetone. The solution was mixed well by shaking and transferred to a clean 1 L sealed bottle.

Sample Collection and Preparation

(a) *Collection, Florida Residue Tolerance Enforcement Program (20).*—Field inspectors, located throughout the state, surveyed vegetable fields, interviewed farmers, and collected a variety of fresh fruits and vegetables from fields, packing houses, central warehouses, wholesalers, retailers, and importing facilities. The laboratory kept in touch with the FDA and other state regulatory agencies to obtain pesticide usage information on products shipped into Florida. Depending on the time of the year, samples to be tested were either Florida grown or shipped in from other states or foreign countries. The selection of commodities for testing is based on a Surveillance Index procedure described by Magness et al. (25), which combines the propensity of the crop to accumulate significant residues with the toxicity and other characteristics of the pesticides applied to the crop. Magness et al. grouped commodities into 7 classifications ranging from low to highest residue potential. Although a wide variety of commodities are collected, Florida's sampling is targeted to monitor potentially high residue classifications and suspected usage problems. Samples may be collected any day of the week to enable inspectors to sample on the day of harvest. Samples are shipped

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in brown paper bags and cardboard boxes overnight by bus to the laboratory.

(**b**) *Collection, PDP (17).*—Each PDP analysis set consisted of a specific number of samples of the same commodity. The PDP national office coordinated the collection sites, commodity, and number of samples collected by participating states so that the data collected statistically represent the consumption patterns of the nation as a whole. Each participating state may collect various commodity sets monthly on specific, randomly chosen collection days. Samples were shipped overnight in coolers with ice packs to one of the participating state laboratories.

(**c**) *Sample preparation, Florida Residue Tolerance Enforcement Program.*—EPA regulations specify that samples "shall consist of the whole raw agricultural commodity" with a few exceptions for the removal of stems, hulls, etc. (1). The PAM guide clarifies these instructions (8). The whole commodity was homogenized, as specified in the regulations, and analyzed immediately. One hundred grams or more of homogenate were frozen at –40°C for reanalysis of violative samples. Occasionally, frozen homogenates may be stored for less than 1 week before analysis.

(**d**) *Sample preparation, PDP.*—PDP samples were washed, cored, and peeled so that only the edible portion was analyzed. Refer to specific PDP instructions for each commodity (17). From each sample, 5-6 lb were homogenized. Five 100 g containers were frozen at –40°C for analysis within 3 months.

Extraction Procedure

(**a**) *Safety precautions.*—All work with organic solvents must be done in a hood. Personal protective gear required includes safety glasses, solvent-resistant gloves, and laboratory coat.

(**b**) *Labeling.*—Some pieces were reused within the procedure. To prevent cross contamination, apparatus used in the method was labeled appropriately (sample containers, extraction containers for each fraction, etc.).

(**c**) *SPE C_{18} column conditioning.*—The columns were conditioned just before sample cleanup. A 500 mg C_{18} cartridge with a stopcock attached to the bottom was placed in a holder such as a vacuum manifold port. The cartridge was filled with methanol and allowed to drip completely from the cartridge by gravity feed. Immediately after the methanol had dripped through, the procedure was repeated by using C_{18} column conditioning reagent (**a**; *see Reagents*), and allowing it to drip completely through. Immediately after the acetonitrile–water had dripped through, the procedure was repeated again with deionized water. The water was allowed to drip through until the level was ca 0.5 in. above the packing, and then the flow was stopped with the stopcock.

(**d**) *Sample extraction.*—50 ± 0.50 g homogenized sample was weighed into a 250 mL polyethylene bottle. Where applicable, spiking solution was added to the matrix. By using a pipet or a 100 mL graduated cylinder, 100 mL acetonitrile was added to the bottle and the bottle was capped. The bottle was agitated for a few seconds by hand to premix the contents. The bottles were clamped in the shaker and agitated for 3 min with shaker set on an aggressive action to ensure pesticide extraction. Samples may be shaken vigorously by hand for 3 min; however, a shaker is preferable because it provides uniformity.

(**e**) *Rack setup (Figure 1).*—A 70 mL reservoir was inserted in the hole of the wooden rack. A stopcock was firmly attached to the bottom of the reservoir. A column-connecting adaptor was placed on top of a preconditioned C_{18} cartridge and snaped into place to maintain vacuum. The C_{18} SPE cartridge was connected to the 70 mL reservoir by inserting the stopcock attached to the bottom of the reservoir into the adaptor on top of the C_{18} cartridge, pressing tightly. The stopcock was removed from the bottom of the C_{18} cartridge. The cartridge should maintain a liquid head. NaCl (10 ± 0.2 g) was weighted into a 250 mL polyethylene screw-cap bottle. The bottle was placed under the C_{18} reservoir, allowing the cartridge to protrude down into the container. A funnel was placed onto the reservoir with the stem of the funnel protruding down into the reservoir. A 5 ¾ in. id fluted filter paper was placed into the funnel.

(**f**) *C_{18} cleanup (Figure 2).*—The sample extract was poured through the filter paper and into the reservoir, filling the reservoir to ca 0.5 in. from the top. Lifting the funnel as the liquid is draining into the reservoir may make this task easier. The stopcock was opened to begin gravity flow through the C_{18} cartridge. The remaining sample can be discarded at this time. An adaptor was pressed onto the top of the 70 mL reservoir. A stopcock was pressed into the adaptor. One end of a Tygon tubing line was connected to the top of the stopcock. The other end of the tubing was connected to a nitrogen source ensuring that all of the connections were airtight. The stopcock on the reservoir was opened. Flow was supplied to the reservoir, forcing the sample through the C_{18} cartridge at a rate approximate to that of a very fast drip. The sample was drained through the cartridge, with the effluent collected in the 250 mL (salt-containing) polyethylene bottle. The C_{18} cartridge and the air supply lines were removed from the reservoirs. The adaptor was pried off the C_{18} cartridge, and the cartridge was discarded. The empty 70 mL reservoir was rinsed with a generous amount of C_{18} column conditioning reagent, discarding the rinse. The stopcock to the reservoir was closed after the rinse had drained completely. The 250 mL container was securely capped and shaken for 2 min in the shaker, set at an aggressive setting. Shaking can be performed by hand; however, a shaker will provide uniformity and should be the first choice. After the containers were fully shaken, they were placed in a centrifuge and centrifuged for ca 2 min at ca 1000 rpm (total centrifuge run time can be 2 min).

(**g**) *Stopping point.*—If the samples were not processed through the remainder of the procedure on the same day, they were stored in a freezer or refrigerator overnight. When the samples were removed from cold storage, they were allowed to warm to room temperature before the next step. Samples were not stored for more than 24 h.

(**h**) *Organophosphosrus (P) and chlorinated (C) fractions.*—The liquids were decanted into their associated 70 mL reservoirs or into a new reservoir and allowed to stand at least

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Table 1. Method validation limits of detection and spike recoveries in spinach, oranges, tomatoes, and peaches

Compound	Fraction[a]	LOD, µg/g[b]	MV[c]	Mean recovery, %	SD[d]	RSD, %[e]	n[f]
Acephate	P	0.010	L	76	9.3	12.2	44
Aldicarb	Carb	0.020	L	91	8.1	8.9	38
Aldicarb sulfoxide	Carb	0.020	L	78	6.0	7.7	38
Aldrin	CL	0.003	P	93	11.1	12.0	36
Anilazine	CL	0.083	P	99	22.8	23.0	29
Atrazine	P	0.033	P	110	9.9	9.0	36
Azinphos-methyl	P	0.025	L	97	13.8	14.2	44
Bifenthrin	CL	0.025	P	100	19.0	19.0	30
Captofol	CL	0.033	P	123	30.0	24.4	27
Captan (OG, TO, PC)[g]	CL	0.017	P	128	6.7	5.2	24
Captan (SP)	CL	0.017	P	51	34.2	66.7	16
Carbaryl	Carb	0.010	L	94	9.7	10.3	38
Carbofuran	Carb	0.015	L	94	8.1	8.6	38
Carbofuran, 3-OH	Carb	0.020	L	95	9.6	10.2	38
Chlorpropham	P	0.167	P	104	5.1	4.9	36
Chlorpyrifos	P	0.008	L	100	9.1	9.1	44
Chlorpyrifos	CL	0.007	L	104	10.6	10.2	36
Chlorpyrifos-methyl	P	0.010	L	100	11.3	11.3	44
Chlorpyrifos-methyl	CL	0.008	L	102	7.7	7.6	36
Chlorothalonil (OG, TO, PC)	CL	0.005	L	104	12.7	12.2	27
Chlorothalonil (SP)	CL	0.005	L	60	17.5	29.3	9
Cypermethrin (total)	CL	0.050	P	109	4.9	4.5	40
DCPA	CL	0.005	P	99	5.5	5.6	40
DDD-*p,p'* (TDE-*p,p'*)	CL	0.003	P	107	16.7	15.6	36
DDE-*p,p'*	CL	0.003	P	112	15.6	14.0	24
DDT-*p,p'*	CL	0.003	P	117	16.9	14.5	32
Diazinon	P	0.010	L	103	9.5	9.2	44
Dichloran	CL	0.008	P	113	6.4	5.7	36
Dichlorvos	P	0.017	P	83	10.3	12.4	36
Dicofol	CL	0.033	P	133	16.5	12.4	36
Dieldrin	CL	0.003	P	119	25.9	21.8	28
Dimethoate	P	0.010	L	104	11.0	10.5	44
Diphenamide	P	0.170	P	82	14.6	17.8	21
Diphenylamine	P	0.167	P	100	6.3	6.3	36
Disulfoton	P	0.008	P	96	5.3	5.5	36
Endosulfan I	CL	0.005	L	100	9.5	9.5	36
Endosulfan II	CL	0.008	P	103	6.9	6.8	40
Endosulfan sulfate	CL	0.008	P	102	4.8	4.8	40
Endrin	CL	0.003	P	96	17.6	18.3	36
Ethion	P	0.010	P	101	8.7	8.6	44
Fenamiphos	P	0.008	P	108	9.9	9.1	36
Fenamiphos sulfone	P	0.020	P	112	13.0	11.6	36
Fenvalerate (total)	CL	0.083	L	105	6.0	5.8	36
Folpet	CL	0.033	P	141	13.4	9.5	21
Fonofos	P	0.010	P	92	11.0	12.0	21
Fonofos oxygen analog	P	0.010	P	111	13.5	12.2	30

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Table 1. (*continued*)

Compound	Fraction[a]	LOD, μg/g[b]	MV[c]	Mean recovery, %	SD[d]	RSD, %[e]	n[f]
Gardona	CL	0.017	P	124	23.5	19.0	30
HCB	CL	0.002	L	77	12.7	16.5	36
Heptachlor	CL	0.002	P	99	7.6	7.6	36
Heptachlor epoxide	CL	0.003	P	100	11.6	11.6	36
Imazalil	P	0.167	L	97	11.1	11.4	44
Iprodione	CL	0.050	L	116	11.3	9.8	36
Lindane	CL	0.003	P	109	6.4	5.9	40
Linuron	CL	0.042	P	117	22.0	18.8	40
Malathion	P	0.008	P	100	6.7	6.7	36
Methamidophos	P	0.017	L	78	8.9	11.5	44
Methidathion	P	0.008	P	100	8.2	8.1	36
Methiocarb	Carb	0.015	L	94	9.3	9.8	38
Methomyl	Carb	0.015	L	93	6.6	7.1	38
Methoxychlor	CL	0.005	L	106	12.1	11.5	36
Mevinphos	P	0.017	P	100	11.3	11.4	36
Myclobutanil	P	0.083	P	108	5.2	4.8	36
Norplurazon	P	0.042	P	108	17.4	16.1	21
Norflurazon desmethyl	P	0.042	P	113	24.4	21.6	21
Omethoate	P	0.017	P	75	18.3	24.5	36
Oxydemeton methyl sulfone	P	0.050	P	89	12.9	14.5	21
Oxamyl	Carb	0.020	L	90	7.8	8.7	38
Parathion-ethyl	P	0.017	P	106	6.2	5.9	36
Parathion-methyl	P	0.008	P	104	7.9	7.6	36
Pentachlorobenzene	CL	0.003	L	69	11.5	16.6	36
Pentachloronitrobenzene	CL	0.003	L	92	9.3	10.1	36
Permethrin (total)	CL	0.083	L	102	9.2	9.0	36
Phorate	P	0.008	P	97	9.6	9.9	36
Phorate sulfone	P	0.027	P	104	5.1	4.9	36
Phorate sulfoxide	P	0.067	P	101	5.6	5.6	36
Phosalone	CL	0.033	P	114	5.0	4.4	40
Phosmet	P	0.017	L	104	11.3	10.9	44
Phosphamidon	P	0.033	P	102	10.4	10.2	36
Pirimiphos-methyl	P	0.008	P	102	10.8	10.6	31
Procymidon	CL	0.010	P	112	23.0	20.5	32
Profenofos	P	0.025	P	99	12.2	12.3	21
Pronamide	CL	0.017	P	111	7.4	6.7	22
Propargite	CL	0.100	L	104	12.9	12.4	36
Propoxur	Carb	0.015	L	95	11.0	11.6	38
Simazine	CL	0.025	P	111	11.0	9.9	21
Terbacil	CL	0.033	P	122	11.0	9.0	21
Terbufos	P	0.010	P	98	7.5	7.6	36
Terbufos sulfone	P	0.010	P	104	5.4	5.2	36
Tetradifon	CL	0.008	P	109	8.7	8.0	21
Thiabendazole	P	0.083	L	101	14.2	14.0	44
Triadimefon	CL	0.025	P	106	9.7	9.2	21
Trifluralin	CL	0.033	L	104	9.0	8.7	36

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Table 1. (*continued*)

Compound	Fraction[a]	LOD, μg/g[b]	MV[c]	Mean recovery, %	SD[d]	RSD, %[e]	*n*[f]
Vinclozalin	CL	0.007	P	106	4.7	4.5	40

[a] Extract fractions: CL, halogens; P, organophosphorus; Carb, carbamates.
[b] LOD, limits of detection.
[c] MV, method validation. L, linearity spikes: 1, 5, and 10 limit of quantitation (LOQ) in triplicate. P, precision spikes: 7 or more spikes at 2 LOQ.
[d] SD, standard deviation.
[e] RSD, relative standard deviation.
[f] *n*, number of samples analyzed.
[g] OG, oranges; TO, tomatoes; PC, peaches; SP, spinach.

5 min. The lower aqueous layer was drained into a waste container by using the stopcock as a control and allowed to stand at least 3 min. Again, the lower aqueous layer was drained into a waste container. A small amount of the acetonitrile layer was included to ensure that all the water had been removed. By using the stopcock as a flow regulator, 15 mL was collected from the 70 mL reservoir in a 25 mL graduated cylinder and transferred to a 100 mL beaker. A second 15 mL aliquot was collected using a separate 100 mL beaker. One beaker was labeled as the P fraction and the other as the C fraction. The remaining amount was collected in a test tube and secured with a stopper. Proceed to *Extraction Procedure* (**j**) for aminopropyl SPE cleanup. (Alternately, all of the acetonitrile extract can be collected in a glass collection vessel such as a beaker or flask and the aliquots removed with volumetric pipets by the analyst completing the cleanup and instrumental analysis. However, aliquots must be removed immediately or the collection vessel must be closed to prevent evaporation.) The beakers containing the P and C fractions were placed on a steam bath and the fractions were evaporated until ca 2 mL remained, but not to dryness. The beakers were removed from the heat and placed at the front of the hood, under the hood sash. The sash height was adjusted to obtain a moderate airflow over the beakers. The remaining liquid in the beakers was allowed to evaporate just to dryness. It is very important not to overdry the samples at any stage of removing solvent, because volatile pesticides may be evaporated to the atmosphere. It is also equally important, in the case of acetonitrile, to remove all the solvent (particularly for the P fraction) because small residues will produce a large solvent front when injected into certain GC detectors such as NPD. When drying acetonitrile from a beaker, it may be helpful to know that when this solvent evaporates it draws heat out of the atmosphere and in effect cools the container from which it is being evaporated. If one can feel that the bottom of the beaker is no longer cooling, it is usually a sign that all the acetonitrile has evaporated and any remaining liquid is water or oil. A 2.0 mL aliquot of acetone was pipetted into the P fraction beaker. The contents were swirled for ca 30 s to thoroughly dissolve any residue in the beaker and then the contents were poured into a prelabeled 16 × 125 mm screw-cap disposable test tube and capped securely. The samples were placed in a refrigerator or freezer for 15 min or longer to help precipitate salt from the solution before GC analysis. The P fraction was now ready for GC analysis using nitrogen-, phosphorus-, and sulfur-selective detectors such as NPD, FPD, and AED. The P fraction was also analyzed by GC/MS for piperonyl butoxide, orthophenylphenol, all GC compounds, and some *N*-methyl carbamate compounds. The C fraction was ready for the Florisil SPE cleanup.

(**i**) *Florisil SPE cleanup of C fraction (Figure 3).*—Florisil is subject to moisture absorption. This absorption can produce erratic effects on column chromatography. The Florisil cartridge was always conditioned within a short time after it was removed from its sealed container. Any remaining cartridges were resealed. Treating all types of SPE cartridges in this manner is highly recommended. A Teflon stopcock was attached to the bottom of a 500 mg Florisil cartridge. The stopcock was opened, and the cartridge was filled ca ⅔ full with reagent (**b**; *see Reagents*) for Florisil SPE. *Note*: if water was suspected in the sample after hood sash evaporation, sodium sulfate was placed into the SPE cartridge, up to the point where the cartridge taper straightens out and the cartridge was conditioned through the sodium sulfate. Some of the reagent was allowed to drip through the cartridge by gravity (ca 2 mL), then the stopcock was closed. By using a precalibrated repipet or other means of accurate measurement, 2 mL reagent (**b**; *see Reagents*) was added to the beaker that contained the C fraction residue. The contents were swirled in preparation for loading onto the Florisil cartridge. The stopcock at the bottom of the Florisil cartridge was removed or opened, allowing the remaining conditioning reagent to drip to waste. The cartridge was inserted into a 15 mL labeled graduated test tube. It is very important to have the sample ready to load onto the Florisil once the conditioning reagent has dripped from the cartridge so that the cartridge packing will not have time to go dry. The contents of the beaker were poured into the cartridge. By using a pipet or another accurate measuring device, 5 mL reagent (**b**; *see Reagents*) was added to the same beaker and swirled. The 2 mL sample aliquot was allowed to just completely elute through the packing, then 5 mL was added from the beaker to the cartridge. A second 5 mL aliquot of reagent (**b**; *see Reagents*) was added to the sample beaker, swirled, and then transferred to the cartridge after the first 5 mL load has passed through the cartridge. The test tubes containing the collected eluate were placed in the evaporator test tube-holding carousel. The tray was submerged into the water bath. By using a gentle stream of nitrogen, the solvent was evaporated until ca 2–3 mL remained, not near dryness. *Note*:

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Table 2. Ongoing method performance 2 LOQ level spike recoveries in compliance and PDP[a] commodities

Compound[b]	Fraction[c]	LOD, µg/g[d]	n[e]	Mean recovery, %	SD[f]	RSD, %[g]
Acephate	P	0.010	135	79	18.0	22.8
Aldicarb	Carb	0.020	106	94	11.2	11.9
Aldiarb sulfoxide	Carb	0.020	106	78	9.6	12.3
Aldrin	CL	0.003	3	108	10.0	9.3
Atrazine	P	0.033	8	113	14.0	12.4
Azinphos-methyl	P	0.025	135	96	24.1	25.1
Captan	CL	0.017	16	150	31.9	21.3
Carbaryl	Carb	0.010	105	97	14.8	15.3
Carbofuran	Carb	0.015	107	98	11.9	12.1
Carbofuran, 3-OH	Carb	0.020	106	97	11.9	12.3
Chlorpropham	P	0.167	16	115	11.6	10.1
Chlorpyrifos	CL, P	0.007	269	104	12.2	11.7
Chlorpyrifos-methyl	CL, P	0.010	271	104	12.6	12.1
Chlorothalonil	CL	0.005	140	97	44.7	46.1
Cypermethrin (total)	CL	0.050	18	119	15.5	13.0
DCPA	CL	0.005	18	114	15.1	13.2
DDD-*p,p'* (TDE-*p,p'*)	CL	0.003	3	114	18.0	15.8
DDE-*p,p'*	CL	0.003	108	97	10.5	10.8
DDT-*p,p'*	CL	0.003	3	111	17.0	15.3
Diazinon	P	0.010	138	102	15.0	14.7
Dichloran	CL	0.008	134	118	17.8	15.1
Dichlorvos	P	0.017	13	95	19.8	20.8
Dicofol	CL	0.033	2	130	4.0	3.1
Dieldrin	CL	0.003	3	127	18.0	14.2
Dimethoate	P	0.010	136	111	16.5	14.9
Diphenylamine	P	0.167	13	107	9.4	8.8
Disulfoton	P	0.008	11	113	17.8	15.8
Disulfoton sulfone	P	0.008	67	109	29.3	26.9
Endosulfan I	CL	0.005	136	106	12.9	12.2
Endosulfan II	CL	0.008	19	111	8.3	7.5
Endosulfan sulfate	CL	0.008	21	114	10.8	9.5
Endrin	CL	0.003	3	123	12.6	10.2
Ethion	P	0.010	135	102	11.2	11.0
Fenamiphos	P	0.008	13	121	14.0	11.6
Fenamiphos sulfone	P	0.020	4	121	34.0	28.1
Fenvalerate (total)	CL	0.083	136	107	15.1	14.1
HCB	CL	0.002	134	80	15.2	19.0
Heptachlor	CL	0.002	3	125	20.4	16.3
Heptachlor epoxide	CL	0.003	2	110	14.0	12.7
Imazalil	P	0.167	137	98	25.3	25.8
Iprodione	CL	0.050	134	111	13.4	12.1
Lindane	CL	0.003	17	114	11.6	10.2
Malathion	P	0.008	13	110	8.7	7.9
Methamidophos	P	0.017	136	78	17.0	21.8
Methidathion	P	0.008	13	115	8.9	7.7
Methiocarb	Carb	0.015	106	97	13.8	14.2

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Table 2. (*continued*)

Compound[b]	Fraction[c]	LOD, μg/g[d]	*n*[e]	Mean recovery, %	SD[f]	RSD, %[g]
Methomyl	Carb	0.015	110	95	11.8	12.4
Methoxychlor	CL	0.005	134	124	23.8	19.2
Mevinphos	P	0.017	14	110	7.2	6.5
Myclobutanil	P	0.083	13	110	10.0	9.1
Omethoate	P	0.017	14	81	12.4	15.3
Oxamyl	Carb	0.020	109	93	11.1	11.9
Parathion-ethyl	P	0.017	5	112	20.7	18.5
Parathion-methyl	P	0.008	13	116	6.7	5.8
Pentachlorobenzene	CL	0.003	134	71	24.0	33.8
Pentachloronitrobenzene	CL	0.003	135	98	14.0	14.3
Permethrin (total)	CL	0.083	137	96	10.5	10.9
Phorate	P	0.008	5	107	21.9	20.5
Phorate sulfone	P	0.027	5	113	22.0	19.5
Phosalone	CL	0.033	17	124	10.0	8.1
Phosmet	P	0.017	134	103	17.5	17.0
Phosphamidon	P	0.033	5	113	22.4	19.8
Procymidon	CL	0.010	12	120	15.0	12.5
Propargite	CL	0.100	68	101	27.4	27.1
Propoxur	Carb	0.015	106	96	11.8	12.3
Terbufos	P	0.010	5	109	21.3	19.5
Terbufos sulfone	P	0.010	2	95	26.0	27.4
Thiabendazole	P	0.083	138	109	28.0	25.7
Trifluralin	CL	0.033	120	100	18.0	18.0
Vinclozalin	CL	0.007	17	121	12.9	10.7

[a] PDP, pesticide data program.
[b] OG, oranges; TO, tomatoes; PC, peaches; SP, spinach.
[c] Extract fractions: CL, halogens; P, organophosphorus; Carb, carbamates.
[d] LOD, limits of detection.
[e] *n*, number of samples analyzed.
[f] SD, standard deviation.
[g] RSD, relative standard deviation.

the water bath temperature should be kept below the boiling point of the solvent (ca 50°C). The test tube was removed from the bath and allowed to reach room temperature. The volume was adjusted to 4.0 mL with isooctane and the C fraction was ready for GC analysis on halogen-specific detectors such as ECD, ELCD, XSD, or AED.

(j) *Aminopropyl SPE analysis of carbamate.*—A stopcock was attached to the bottom of a 500 mg, aminopropyl (NH_2) SPE cartridge and placed on a vacuum manifold port. The stopcock was opened, and the cartridge was filled with ca 7 mL reagent (c; *see Reagents*) for carbamate SPE. The conditioning reagent was dripped through the column, by using vacuum if needed, until the solvent was ca ⅛ in. above the packing material. The stopcock was closed. The column was now fully conditioned and ready for use. A prelabeled disposable (nonscrew-cap-type) or graduated test tube was placed under each cartridge. A 4.0 mL aliquot of sample extract was loaded into the cartridge. The sample was allowed to elute through the cartridge and collect in the test tube, preferably by gravity. Slight vacuum (only if necessary) was used to achieve a 2–3 drop/5 s flow rate. Using the pipet on the solvent bottle or equivalent means to pipet, 4 mL reagent (c; *see Reagents*) was added to the column, and the contents were allowed to elute through the cartridge into the test tube at a rate of 2–3 drops/5 s. The evaporator water bath was turned to low, and the temperature was allowed to rise to 40°C. Just before the test tube was placed on the evaporator, the heat was turned off. The test tube containing the collected eluate was placed in the evaporator carousel. The solvent was evaporated under a gentle stream of nitrogen. When the solvent level reached 0.5 mL, the tube was removed from the water bath, watched constantly, and evaporated just to dryness. *Note*: leaving the samples at dryness for even 30 s can result in severe loss of pesticides. A 1.0 mL aliquot of methanol was added to the test

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tube with a volumetric pipet, the tube was mixed on a Vortex mixer for 15 s, and the solution was filtered through a 0.20 μm solvent-resistant filter into an autosampler vial. The carbamate fraction was now ready for HPLC analysis with florescence detection and postcolumn derivitization or diode array.

Method Validation and Quality Control Procedures

(a) *Limit of detection (LOD).*—LOD was estimated at 3× the method noise, which included the instrument noise and the peaks contributed by naturally occurring compounds in typical fruit or vegetable extracts (26). Limit of quantitation (LOQ) was defined as 10× the method noise. LODs were verified by spiking at the estimated level in duplicate.

(b) *Instrument linearity.*—Linear regression measures of slope, intercept, and correlation coefficients at 1, 5, and 10 LOQ were determined for each detector–pesticide combination. All pesticides passed correlations of 0.990 or better on 3 separate days (R^2 values were better than 0.995 for most pesticides).

(c) *Linearity and accuracy.*—Recoveries were determined for 3 replicates of 1, 5, and 10 LOQ, respectively, in 4 different commodities. Select pesticides representing all the chemical classes, sometimes called marker pesticides, were analyzed for linearity.

(d) *Precision and accuracy.*—Recoveries for nonmarker pesticides were determined for 7 replicates at 2 LOQ in 4 different commodities.

(e) *Demonstration of ongoing method performance.*—Each set of 20 samples or fewer also included a reagent blank, a matrix blank, and 2 or more matrix spikes of pesticide mixes at the 2 LOQ level. All reported pesticides were spiked at least 4 times per year.

Instrumental Analysis and Data Interpretation

When sample sets of fresh fruits and vegetables were analyzed, each of the 3 fractions were analyzed on the appropriate instrument in batches bracketed at beginning and end with pesticide standards. Pesticides are identified by GC or LC retention time. Pesticide identifications were confirmed by retention time on a different column phase and/or by GC/MS. Retention time of the same compounds on 2 different GC column phases is shown in Figure 4. Dual-column GC ovens and dual-tower autosamplers enabled all samples to be routinely screened and quantitated on 2 different columns. To selectively detect all the pesticides of interest, different selective detectors were used. Figure 5 shows the response of different GC detectors to the same pesticide mixture. Use of selective detectors for pesticide heteroatoms in the presence of naturally occurring hydrocarbons resulted in parts-per-billion detection levels in complex fruit and vegetable matrixes. The ELCD detected both halogens and nitrogen compounds like diphenylamine while the XSD detected halogens and was most sensitive to chlorine. The lack of nitrogen sensitivity on the FPD eliminated matrix interferences when most vegetable samples were analyzed. ELCD and the new XSD have replaced ECD GC detectors for halogen analysis because of reduced matrix interference as shown in Figure 6. While the FPD is selective for sulfur and phosphorus and can be used for the analysis of most commodities like cabbage in Figure 7, the NPD remains the best selective detector for the analysis of nitrogen pesticides as shown in Figure 5 and sulfur-containing commodities like onion as seen in Figure 7. The chromatography of both the cabbage and the onion samples shows how complicated it can be to identify pesticides in a vegetable matrix. No pesticides were identified in these fractions.

Carbamates and orthophenylphenol were detected by LC using C_{18} columns and confirmed on a cyanocolumn phase. A chromatograph of carbamate standards on the 2 columns is shown in Figure 8. Some carbamates were also confirmed by GC/MS.

All samples were routinely screened for qualitative identification by GC/MS SIM analysis. GC/MS confirmations provided the compliance laboratory with a higher degree of confidence in identifications and with legally defensible results.

By using a common oven program and RTL (21), the same compound elutes at nearly the same retention time on several different GC instruments and detectors as shown in Figure 5. This enables the analyst to interpret chromatographic results with the benefit of responses from several different detectors. These reproducible retention times can be stored and searched by using pesticide database applications without conversions to relative retention time. Despite screening for over 100 compounds, there are still samples that contain halogens or other unidentified analytical responses (UARs) that can not be identified by routine analysis. AED, while not widely used for routine analysis because of its complexity and cost, can selectively detect all the pesticide heteroatoms but lacks the sensitivity needed for some trace level analyses (22). Figures 9 and 10 show an onion sample in which folpet was identified with the assistance of AED elemental analysis and database search. Another UAR, seen in this same sample, was not identified initially. RTL records of UARs (Figure 11) and comparison to standards provided by Putnam (27) eventually led to the identification of this compound as a metabolite of chlorothalonil.

Regulatory Significance

Tolerances and exemptions from tolerances for pesticide chemicals in or on raw agricultural commodities are set by the EPA (1). The EPA regulations specify acceptable residue levels for commodities or groups of commodities. If a pesticide is found on a commodity for which no tolerance has been established, Florida has established a Regulatory Action Limit (RAL; 28). The RAL includes a safety factor of 10 to be applied to the tolerance on a similar crop when one exists or to the lowest tolerance when there is no similar crop for comparative purposes. If pesticide levels that exceed the tolerance or RAL are detected and confirmed by resampling and reanalysis, the produce product may not be sold. The crop may be destroyed if measures to reduce pesticide levels are unsuccessful. A pesticide usage investigation is conducted to determine why the violation occurred and to prevent future occurrences.

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Results and Discussion

The new method differs from the previous method used in the Florida laboratory in several ways. The method reduces the use of hazardous chemicals, eliminating methylene chloride and ethyl ether. Acetone extraction in a blender was replaced by acetonitrile extraction in a capped bottle on a shaker. Solvent use and waste disposal was minimized. Some modifications to the CDFA method were made. Centrifugation was used to complete the acetonitrile–water separation. The method also took advantage of SPE technology but minimized complicated elution techniques and vacuum apparatus that take time and can lead to errors. SPE C_{18} cleanup was conducted by using a tandem connection of filtration funnel and SPE cartridge fed by positive pressure. Specialized glassware such as seperatory funnels are not needed, but care must be taken to avoid losses in the beaker concentration steps.

The method was developed and tested by a method development team whose experiments showed that GC and LC recoveries of 75–130% were possible. Initial attempts to validate this method by using a sample preparation team resulted in erratic recoveries of halogen and organophosphorus compounds ranging from 0 to 180%. To determine the sources of error and develop a reliable and rugged method, a team consisting of a method development chemist, an analytical chemist, and a sample preparation technician repeated the GC method validation. During revalidation, sources of error were identified, method modifications were made, and the written procedure was revised to detail the exact steps needed to successfully perform the method. Care must be taken with specific conditioning and elution procedures for each of the 3 new SPE cartridges. Elimination of water by centrifugation and completing evaporation by air drying were added to provide ruggedness. As many as 48 beakers were dried on a steam bath at one time during the evaporation step which could lead to confusion.

The final results of method validation are reported in Table 1. Validation recoveries were good except in the case of captan and chlorothalonil in spinach. These 2 pesticides continued to be problematic for some commodities. Transfer of the method to the sample preparation team for routine analysis was completed when the team demonstrated their proficiency by reproducing the method validation performance with several sets of precision spikes. Quality control spikes were carefully reviewed to assure acceptable on-going method performance. A summary of spike recoveries obtained during routine analysis for the past year are presented in Table 2. These recoveries include spikes made in 30 different commodities analyzed in the state tolerance enforcement program. Although a few pesticides continue to be problematic, the majority of analytes were recovered with good precision and accuracy by using this procedure.

Conclusion

The method is useful for the analysis of 89 pesticides representing halogen, nitrogen, phosphorus, sulfur, and carbamate chemistries. New pesticides are being added to the screen annually. Method sample size was reduced from 100 to 50 g. Solvent usage was reduced from 200 mL acetone to 100 mL acetononitrile. More hazardous chemicals like methylene chloride and petroleum ether were eliminated. Glassware racks for tandem filtering and SPE cleanup in one step made the analysis of 24 samples more efficient. Automatic shakers, positive pressure C_{18} SPE cleanup, phase separation by centrifugation, and final solvent evaporation at ambient temperature helped prevent pesticide losses and made the method rugged and reliable in routine use.

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