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Multiresidue Method for the Analysis of Pesticide Residues in Fruits and Vegetables by Accelerated Solvent Extraction and Capillary Gas Chromatography

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An analytical procedure using accelerated solvent extraction and capillary gas chromatography with electron capture and flame photometric detections was developed to simultaneously determine residues of different pesticides in fruits and vegetables. Single laboratory validation of the method was carried out for 28 compounds selected from eight pesticide classes, in blank and fortified samples of fresh pear, cantaloupe, white potato, and cabbage. The method had to meet specific established validation criteria for regulatory purposes applicable to our laboratory. At each of the two fortification levels studied, 24 of the 28 pesticides gave recoveries of more than 70% with a coefficient of variation of less than 10%. With respect to existing procedures, the method showed acceptable limits of detection (from 0.0019 to 0.14 μg/g depending on the pesticide and matrix) while minimizing environmental concerns, time, and labor.

Keywords: *Analysis; accelerated solvent extraction; gas chromatography; fresh produce; pesticide; validation*

INTRODUCTION

Pesticide residues in food have potential detrimental effects on human health. In an effort to monitor the levels of these residues, many governmental and industrial programs have been implemented for the regulatory analysis of pesticide residues in food through multiresidue methods. The most commonly used of these procedures present the drawback of requiring too much labor and time, and using large amounts of hazardous solvents (*1*). In recent years, the analysis of pesticide residues in food has incorporated new technologies to develop and use procedures which minimize environmental concerns, time, labor, and exposure of laboratory technicians to toxic chemicals.

Accelerated solvent extraction (ASE), also known as pressurized liquid extraction (PLE), is one of these analytical techniques and has been described elsewhere (*2*). Its use in the analysis of residual pesticides in food has been reported by a few researchers. Lehotay and Lee (*2*) successfully used ASE to extract a broad range of pesticide residues from food matrixes for gas chromatography/mass spectrometry (GC/MS) analysis. Obana et al. (*3*) combined ASE, gel-permeation chromatography, and gas chromatography/flame photometric detection (GC/FPD) to analyze residues of selected organophosphorus pesticides from food. In 1998, Nemoto and Lehotay (*4*) analyzed multiple herbicides in soybeans using ASE and capillary electrophoresis.

The purpose of this study was to simultaneously analyze residues of varied classes of pesticides in fruits and vegetables using accelerated solvent extraction, solid-phase extraction, and capillary gas chromatography with electron capture and flame photometric detections. Here we present the analytical procedure resulted from this investigation as well as an internal process used to validate this method.

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Table 1. Fortification and Calibration Solutions

pesticide	class	spike solution (μg/mL)	Cal 1 (μg/mL)	Cal 2 (μg/mL)	Cal 3 (μg/mL)	Cal 4 (μg/mL)	Cal 5 (μg/mL)	Cal 6 (μg/mL)
Alachlor	acetanilide	49.8	0.208	0.415	0.830	1.66	2.49	3.32
Azinphos-methyl	organophosphate	29.7	0.124	0.248	0.495	0.990	1.49	1.98
Chlorothalonil	organochlorine	9.84	0.0410	0.0820	0.164	0.328	0.492	0.656
Chlorpyrifos	organophosphate	18.0	0.0750	0.150	0.300	0.600	0.900	1.20
DDD-p,p′	organochlorine	6.57	0.0274	0.0548	0.110	0.219	0.329	0.438
DDE-p,p′	organochlorine	4.42	0.0184	0.0368	0.0737	0.147	0.221	0.295
DDT-p,p′	organochlorine	8.76	0.0365	0.0730	0.146	0.292	0.438	0.584
Diazinon	organophosphate	11.6	0.0483	0.0967	0.193	0.387	0.580	0.773
Dicloran	nitroanoline	7.83	0.0326	0.0653	0.131	0.261	0.392	0.522
Dichlorvos	organophosphate	10.0	0.0417	0.0833	0.167	0.333	0.500	0.667
Diclofop-methyl	organophosphate	26.1	0.109	0.218	0.435	0.870	1.31	1.74
Endosulfan 1	organochlorine	4.16	0.0173	0.0347	0.0693	0.139	0.208	0.277
Endosulfan 2	organochlorine	6.56	0.0273	0.0547	0.109	0.219	0.328	0.437
Endosulfan sulfate	organochlorine	7.91	0.0330	0.0659	0.132	0.264	0.396	0.527
Ethion	organophosphate	10.6	0.0442	0.0883	0.177	0.353	0.530	0.707
Fenthion	organophosphate	23.5	0.0979	0.196	0.392	0.783	1.18	1.57
Lindane	organochlorine	6.55	0.0273	0.0546	0.109	0.218	0.328	0.437
Malathion	organophosphate	22.3	0.0929	0.186	0.372	0.743	1.12	1.49
Methoxychlor	organochlorine	24.3	0.101	0.203	0.405	0.810	1.22	1.62
Metolachlor	chloracetanilide	60.3	0.251	0.503	1.01	2.01	3.02	4.02
Monocrotofos	organophosphate	40.0	0.167	0.333	0.667	1.33	2.00	2.67
Myclobutanil	triazole	51.9	0.216	0.433	0.865	1.73	2.60	3.46
Parathion-methyl	organophosphate	14.8	0.0617	0.123	0.247	0.493	0.740	0.987
Pendimethalin	dinitroaniline	16.0	0.0667	0.133	0.267	0.533	0.800	1.07
Permethrins	pyrethroid	36.2	0.151	0.302	0.603	1.21	1.81	2.41
Phosmet	organophosphate	24.1	0.100	0.201	0.402	0.803	1.21	1.61
Terbufos	organophosphate	8.06	0.0336	0.0672	0.134	0.269	0.403	0.537
Trifluralin	dinitroaniline	12.5	0.0521	0.104	0.208	0.417	0.625	0.833

Table 2. Recovery Results at Low Spike Levels (SP1) in Four Commodities

pesticide	spike level (μg/g)	average recovery (%CV), $n = 4$ cantaloupe	potato	pear	cabbage
Alachlor	0.311	96.5 (2.5)	95.9 (0.38)	103 (2.4)	100 (1.4)
Azinphos-methyl	0.186	147 (3.8)	130 (7.2)	117 (3.4)	142 (4.2)
Chlorothalonil	0.0615	74.8 (9.2)	88.3 (3.8)	101 (7.2)	75.9 (3.2)
Chlorpyrifos	0.113	106 (4.0)	90.8 (3.8)	106 (1.6)	92.2 (2.9)
DDD-p,p′	0.0411	89.6 (2.0)	93.5 (1.5)	94.9 (2.7)	99.8 (1.6)
DDE-p,p′	0.0276	90.5 (3.1)	95.1 (2.1)	93.5 (2.9)	93.3 (1.8)
DDT-p,p′	0.0548	93.3 (2.9)	106 (1.1)	124 (1.9)	104 (1.4)
Diazinon	0.0727	107 (4.3)	87.6 (3.5)	108 (2.6)	82.3 (1.6)
Dicloran	0.0489	96.5 (2.8)	98.4 (1.8)	108 (1.4)	118 (0.75)
Dichlorvos	0.0626	105 (7.0)	33.7 (13)	92.4 (10.7)	
Diclofop-methyl	0.163	93.6 (4.4)	38.0 (6.3)	103 (2.6)	105 (1.5)
Endosulfan 1	0.0260	88.3 (3.1)	87.7 (2.2)	97.9 (1.3)	89.3 (1.5)
Endosulfan 2	0.0410	91.9(3.8)	95.1 (0.76)	99.0 (2.9)	107 (2.6)
Endosulfan sulfate	0.0495	74.5 (4.2)	115 (0.74)	128 (1.5)	127 (1.4)
Ethion	0.0662	105 (3.9)	93.1 (2.8)	107 (1.4)	95.7 (2.9)
Fenthion	0.147	105 (3.3)	81.6 (3.7)	77.5 (4.9)	85.4 (2.0)
Lindane	0.0409	87.4 (3.4)	86.2 (2.5)	103 (1.5)	96.2 (1.8)
Malathion	0.140	112 (3.4)	57.8 (11)	107 (2.6)	96.4 (4.9)
Methoxychlor	0.152	99.8 (4.6)	106 (0.73)	121 (2.3)	107 (2.4)
Metolachlor	0.377	93.0 (2.0)	95.9 (1.6)	114 (3.3)	97.6 (2.5)
Monocrotofos	0.250	106 (3.6)	82.9 (4.9)	89.8 (30)	81.2 (3.1)
Myclobutanil	0.325	99.7 (2.4)	100 (0.99)	103 (1.7)	106 (2.8)
Parathion-methyl	0.0926	111 (5.2)	93.3 (3.1)	112 (1.8)	98.3 (3.6)
Pendi-methalin	0.0998	92.3 (2.9)	91.5 (0.92)	96.5 (2.5)	105 (1.5)
Permethrins	0.226	96.9 (2.0)	76.5 (2.3)	101 (3.6)	99.0 (6.9)
Phosmet	0.150	136 (3.4)	112 (2.0)	112 (7.5)	127 (3.3)
Terbufos	0.0504	102 (5.9)	73.6 (4.6)	90.9 (2.5)	72.0 (1.1)
Trifluralin	0.0783	83.5 (3.2)	76.3 (3.9)	84.4 (1.4)	70.9 (17)

Table 3. Recovery Results at High Spike Levels (SP2) in Four Commodities

pesticide	spike level (μg/g)	average recovery (%CV), $n = 4$ cantaloupe	potato	pear	cabbage
Alachlor	0.622	90.7 (5.4)	96.7 (2.3)	100 (1.4)	101 (5.7)
Azinphos-methyl	0.372	132 (2.1)	128 (4.7)	116 (5.7)	136 (1.9)
Chlorothalonil	0.123	43.7 (11)	83.2 (4.8)	99.6 (3.0)	39.9 (2.3)
Chlorpyrifos	0.226	97.7 (5.8)	94.9 (3.7)	104 (2.1)	92.5 (1.7)
DDD-p,p′	0.0819	86.2 (5.7)	93.3 (0.88)	91.7 (1.6)	101 (5.2)
DDE-p,p′	0.0553	88.9 (6.6)	96.0 (1.2)	94.8 (2.2)	98.2 (6.1)
DDT-p,p′	0.110	84.4 (7.6)	99.1 (5.9)	108 (3.4)	93.3 (4.4)
Diazinon	0.145	96.2 (6.1)	89.7 (6.5)	104 (2.6)	84.6 (2.8)
Dicloran	0.098	89.9 (6.1)	94.1 (4.5)	103 (1.7)	117 (5.7)
Dichlorvos	0.125	86.8 (11)	28.1 (23)	86.8 (5.4)	
Diclofop-methyl	0.326	84.5 (5.8)	28.4 (1.3)	98.44 (2.6)	98.5 (4.0)
Endosulfan 1	0.0519	86.9 (7.1)	88.9 (1.3)	95.5 (2.0)	96.9 (6.4)
Endosulfan 2	0.0819	88.7 (6.4)	94.6 (1.1)	95.5 (1.6)	104 (6.1)
Endosulfan sulfate	0.0989	78.8 (7.6)	105 (1.2)	110 (1.4)	114 (4.6)
Ethion	0.132	97.2 (6.2)	97.4 (2.5)	106 (3.0)	96.0 (1.4)
Fenthion	0.294	95.7 (5.9)	83.4 (3.5)	68.3 (5.5)	85.3 (0.92)
Lindane	0.08219	83.4 (7.9)	84.5 (8.3)	96.8 (1.9)	96.8 (4.8)
Malathion	0.280	101 (4.5)	49.8 (6.9)	103 (1.8)	95.0 (2.6)
Methoxychlor	0.304	94.0 (7.7)	105 (0.89)	106 (2.0)	97.7 (5.3)
Metolachlor	0.754	88.9 5.2)	94.4 (1.9)	104 (0.99)	97.69(5.6)
Monocrotofos	0.500	61.8 (55)	85.2 (7.5)	80.3 (51)	83.4 (9.4)
Myclobutanil	0.650	90.7 (4.5)	97.0 (1.2)	99.1 (2.0)	104 (4.5)
Parathion-methyl	0.185	99.8 (4.0)	97.4 (4.4)	108 (1.6)	96.9 (1.6)
Pendimethalin	0.200	83.9 (6.2)	87.7 (2.0)	93.0 (1.5)	100 (5.1)
Permethrins	0.452	89.0(5.5)	70.9 (1.0)	101 (4.0)	98.5 (5.2)
Phosmet	0.300	119.3 (3.6)	115 (3.6)	121 (9.2)	122 (1.2)
Terbufos	0.101	92.3 (8.4)	75.2 (12)	86.2 (3.7)	75.4 (2.2)
Trifluralin	0.157	80.1 (8.8)	74.6 (9.8)	87.8 (2.3)	81.7 (12)

MATERIALS AND METHODS

Accelerated Solvent Extraction. Accelerated solvent extraction was performed using a Dionex (Sunnyvale, CA) ASE 200 accelerated solvent extractor equipped with 33-mL stainless steel cells and 60-mL collection vials. ASE conditions were as follows: extraction solvent, acetone/dichloromethane (3/1, v/v); temperature, 110 °C; pressure, 1500 psi; 2 cycles; flush volume, 60%. Hydromatrix was used as drying agent and was provided by Varian (Harbor City, CA).

Solid-Phase Extraction. ASE extracts (0.5 to 1 mL of water for approximately 40 mL of extract) were cleaned up by solid-phase extraction using a Supelco (Belfonte, PA) SPE manifold, 1 g-florisil cartridges obtained from Fisher Scientific (Pittsburgh, PA), 250 mg-Supelclean ENVI−CARB carbon SPE columns provided by Supelco, and hexane/dichloromethane/acetone (10:60:30) (v/v) as eluant.

Analytical Standards and Miscellaneous Materials. Pesticide reference standards were provided by the U.S. Environmental Protection Agency (Fort Meade, MD). Individual stock solutions were prepared by weighing appropriate amounts of active ingredients in a 40-mL brown bottle with a Teflon-lined screw cup and dissolving the weighed standard with 30 mL of pesticide-residue-grade acetone. The resulting concentration was then corrected for the stated purity. Appropriate aliquots of the obtained solutions were subsequently

Table 4. Recovery Results at Low and High Spike Levels

pesticide	average recovery at SP1 and SP2 (%CV), $n = 8$			
	cantaloupe	potato	pear	cabbage
Alachlor	93.6 (5.1)	96.3 (1.8)	102 (2.5)	101 (1.4)
Azinphos-methyl	140 (6.2)	129 (6.0)	116 (4.4)	139 (4.3)
Chlorothalonil	59.3 (30)	85.7 (5.4)	101 (5.2)	57.9 (4.2)
Chlorpyrifos	102 (6.2)	92.8 (4.5)	105 (1.9)	92.4 (2.9)
DDD-p,p′	87.9 (4.4)	93.4 (1.2)	93.3 (2.7)	100 (1.6)
DDE-p,p′	89.7 (4.8)	95.7 (1.7)	94.1 (2.5)	95.7 (1.8)
DDT-p,p′	88.4 (7.3)	103 (5.7)	116 (7.5)	98.6 (1.5)
Diazinon	102 (7.4)	88.6 (5.6)	106 (3.4)	83.5 (1.5)
Dicloran	93.2 (5.7)	96.3 (4.3)	106 (3.3)	117 (0.76)
Dichlorvos	96.0 (13)	30.9 (21)	89.6 (8.7)	
Diclofop-methyl	89.3 (7.0)	33.2 (16)	101 (3.5)	102 (1.6)
Endosulfan 1	87.6 (5.1)	88.3 (1.9)	96.7 (2.0)	93.1 (1.4)
Endosulfan 2	90.3 (5.2)	94.8 (1.0)	97.2 (2.9)	106 (2.7)
Endosulfan sulfate	76.7 (7.2)	110 (4.7)	119 (8.2)	120 (1.5)
Ethion	101 (6.3)	95.3 (3.6)	106 (2.2)	95.8 (2.9)
Fenthion	100 (6.5)	82.5 (3.8)	72.9 (8.3)	85.4 (2.0)
Lindane	85.4 (6.1)	85.3 (6.5)	100 (3.7)	96.5 (1.8)
Malathion	107 (6.5)	53.8 (12)	105 (2.9)	95.7 (4.9)
Methoxychlor	96.9 (6.6)	106 (0.96)	118 (3.0)	102 (2.6)
Metolachlor	91.0 (4.3)	95.2 (2.0)	109 (5.2)	97.7 (2.5)
Monocrotofos	83.8 (39)	84.1 (6.7)	85.0 (38)	82.3 (3.0)
Myclobutanil	95.2 (6.0)	98.6 (2.0)	101 (2.5)	93.4 (3.2)
Parathion-methyl	105 (7.0)	95.4 (4.6)	110 (2.4)	97.6 (3.7)
Pendimethalin	88.1 (6.7)	89.6 (2.8)	94.7 (2.8)	103 (1.5)
Permethrins	93.0 (5.9)	73.7 (4.4)	101 (3.6)	98.8 (6.9)
Phosmet	127 (7.6)	113 (3.2)	117 (8.9)	124 (3.4)
Terbufos	97.4 (8.6)	74.4 (9.4)	88.5 (4.0)	73.7 (1.1)
Trifluralin	81.8 (6.4)	75.5 (7.9)	86.1 (2.8)	76.3 (16)

mixed into a 50-mL volumetric flask which was completed to volume with acetone. This mixture was used for spiking purposes and serial-diluted to obtain working calibration solutions.

Anhydrous and granular sodium sulfate, sodium chloride, and pesticide-residue-grade organic solvents (acetone, hexane, and dichloromethane) were obtained from Fisher Scientific.

Sample Preparation. Samples were first homogenized using a Hobart food homogenizer model 84142 (Hobart Manufacturing Company, Troy, OH). The chopped sample was blended using a blender equipped with a stainless steel cut unit, a glass jar of approximately 40 oz, and pulse options. Approximately 8 g of homogenized sample was placed into a 250-mL beaker. After fortification of any spike with the appropriate amounts of fortification solution, the contents of the beaker were allowed to stand at room temperature for 15−20 min. Hydromatrix (5 g) was added to the beaker and the contents were mixed with a spatula to obtain a free-flowing powder. Two cellulose micro-filters were placed at one end (bottom) of a Dionex 33-mL cell; 5 g of Ottawa sand standard (Fisher Scientific) was then introduced into the cell, followed by the mixture. After gentle tapping of the cell to settle the contents, the empty space (above the mixture) was filled with sand. One micro-filter was then placed on top the sand. The cell was tightly closed and extraction was performed using the ASE conditions listed above. Using a 250-mL separatory funnel, the top organic layer of the ASE extract was separated from the aqueous portion (bottom) and transferred to a 300-mL flask containing approximately 10 g of anhydrous sodium sulfate. The aqueous layer was mixed with 15 mL of a 5% sodium chloride solution, and liquid−liquid partitioned (about 30 s of shaking) with 20 mL, then 10 mL, of dichloromethane. The two organic portions resulting from the partition were combined with the ASE extract in the 300-mL flask, which was then capped and left to stand undisturbed for 30−40 min. The contents of the flask were transferred to a 300−500-mL pear-shaped flask containing a glass funnel with approximately 60 g of sodium sulfate. The flask was rinsed twice with two 20-mL portions of fresh dichloromethane (with any caked portion of sodium sulfate crushed), and the rinsates were added to the sodium sulfate funnel. In a concentration process where dryness was avoided, the dried extract was exchanged with about 2 mL of hexane on a rotary evaporator set at 40 °C. A 1-g SPE florisil column containing 2 g of sodium sulfate was placed on top of a 250-mg Supelclean ENVI-CARB carbon SPE cartridge, and the tandem columns were placed on an SPE manifold. After washing the columns with 12 mL of hexane, a 15-mL conical glass tube was introduced underneath the tandem. The hexane extract was transferred to the florisil cartridge and solid phase extraction was performed, under moderate vacuum, using two 6-mL portions of eluant (hexane/dichloromethane/acetone (10:60:30) (v/v)). During this step, the pear-shaped flask was rinsed with each 6-mL portion of eluant, and dryness was allowed only after the second portion of eluant. The collected eluate was evaporated to about 0.5 mL on a nitrogen evaporator set at 40 °C. Finally, the extract volume was adjusted to 3 mL with hexane for fresh pear, white potato, and cantaloupe or to 4 mL with hexane for fresh cabbage. All extracts were kept at no more than 4 °C when instrumental analysis by GC could not be performed immediately.

Instrumental Analysis. Non-phosphorus-containing compounds with an appropriate electron affinity were analyzed with an HP GC model 6890 series (Hewlett-Packard Corp., Palo Alto, CA) equipped with a μECD set at 300 °C and a Phenomenex ZB-5 widebore capillary column of dimensions 30 m × 0.53 mm × 1.25 μm (Phenomenex, Torrance, CA). The carrier gas was UHP helium at a rate of 1.1 mL/min and a constant pressure of 6.5 psi. The makeup gas, 5% argon/methane, was supplied at such a rate that the total column plus makeup flow was constantly equal to 60 mL/min.

4 μL of the final extract was injected in a splitless mode, at a temperature of 280 °C. The following oven temperature program was used: from 120 to 200 °C at a rate of 5 °C/min with a hold time of 5 min, then from 200 to 240 °C at a rate of 2 °C/min with a final time of 1 min, and finally from 240 to 290 °C at a rate of 5 °C/min. The total run length was 56 min.

Peak identification was performed by the HP ChemStation calibration table set up with a relative retention time window of 0.65%.

Organophosphorus pesticides were analyzed using an HP GC model 5890 Series II equipped with an HP FPD, model 19256A, maintained at 290 °C and a 30 m × 0.53 mm × 0.83 μm DB 608 widebore capillary column (J&W Scientific, Folsom, CA). UHP helium was used as carrier and auxiliary (aux) gas and supplied at a rate of 6.6 mL/min through the column (the total column plus aux flow was 33.3 mL/min). Hydrogen and air flows were respectively 75 and 95 mL/min. Extract (2 μL) was injected in a splitless mode, at a temperature of 220 °C. The oven temperature was programmed to rise from 120 to 190 °C at a rate of 6 °C/min with a hold time of 4 min, then from 190 to 280 °C at a rate of 10 °C/min with a final time of 4 min. The total run length was 28.7 min. Peak identification was performed by the HP ChemStation calibration table set up with a relative retention time window of 0.65%.

Confirmation and Quantification. Confirmation of analyte identities was performed by dual-column analysis or by GC/MS. Quantification was performed using a least-squares linear regression line based on a minimum of 5 external calibration solutions. When applicable, analyte concentrations were corrected for any amount of pesticide incurred in the blank (nonfortified matrix).

Limits of Detection. For each analyte in each sample, the limit of detection was estimated as:

$$\mathrm{LOD} = \frac{\mathrm{Cal}_{\min} \times v}{W} \times \frac{1}{R}$$

where LOD is the limit of detection in μg/g, v is the extract final volume in mL, W is the sample weight in g, R is the average recovery on a minimum of four laboratory fortified replicates with an RSD of no more than 15, and $\mathrm{Cal}_{\min}$ is the minimum standard concentration detectable by the analytical instrument as a peak. To determine $\mathrm{Cal}_{\min}$, extracts of nonfortified subs of each commodity (referred to as blanks in this study) were analyzed. Around each retention time of interest, signals below a certain response were considered noise, and the average noise, in terms of Hz or counts, was manually calculated. To qualify as a peak, a signal's response had to be equal to, or higher than, three times the average noise. Under the GC conditions used in this study, a chlorpyrifos calibration solution of 0.0751 μg/mL could cause a μECD response of at

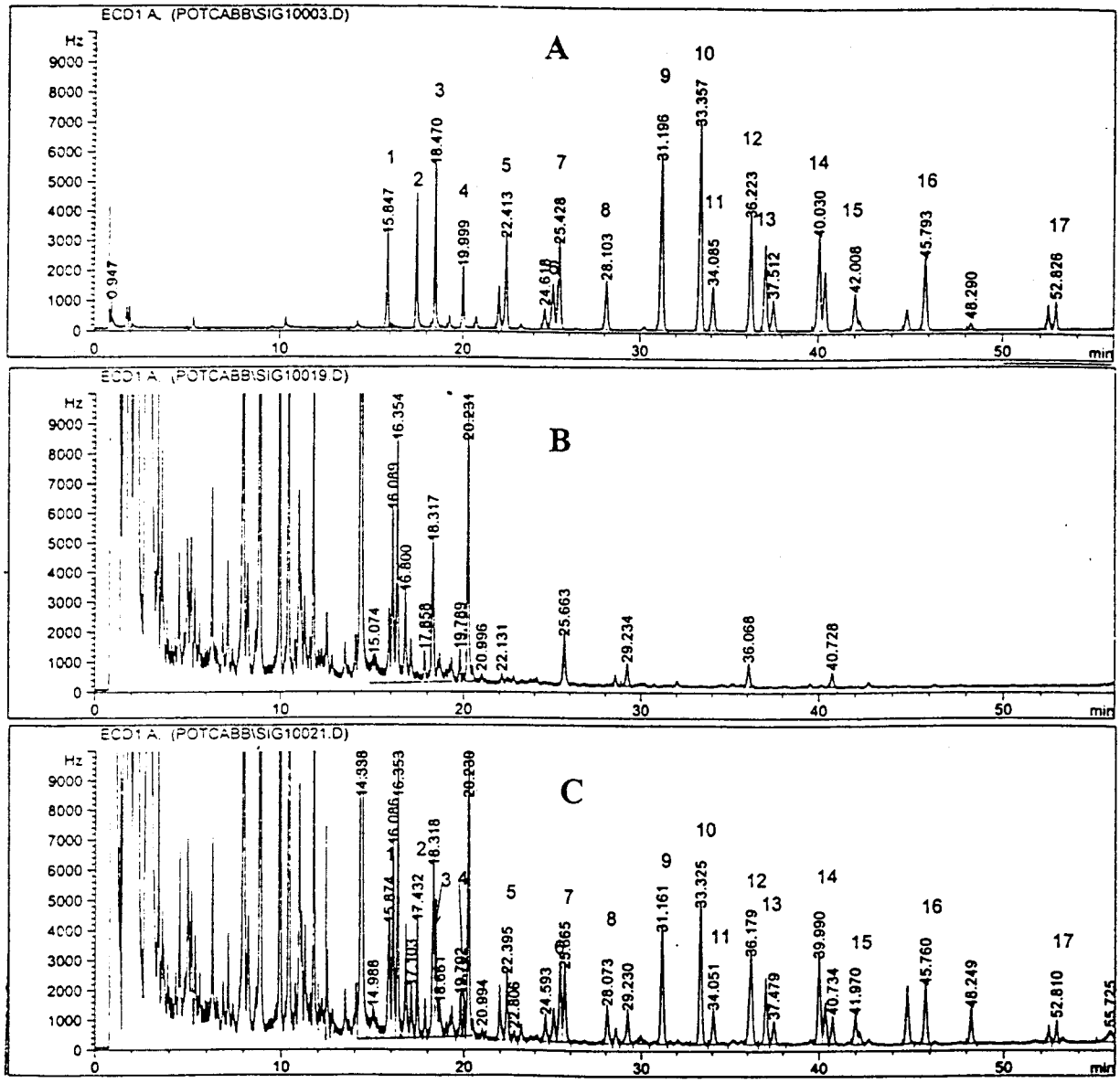

Figure 1. GC/ECD Chromatograms of (A) calibration level 3, (B) blank cabbage, and (C) cabbage fortified at level 1. Peaks: 1, Trifluralin; 2, Dicloran; 3, Lindane; 4, Chlorothalonil; 5, Alachlor; 6, Metolachlor; 7, Pendimethalin; 8, Endosulfan 1; 9, DDE; 10, Myclobutanil; 11, Endosulfan 2; 12, DDD; 13, Endosulfan sulfate; 14, DDT; 15, Diclofop-methyl; 16, Methoxychlor; 17, Permethrins.

least 600 Hz (peak height) and a FPD response of at least 55000 counts (peak height).

Validation Acceptance Criteria. The above procedure was validated using a scheme adapted from Bauer and Cristy (*5*) and comprising the following criteria: matrixes and replicates, linearity, accuracy and precision, selectivity, limit of detection (LOD), organic solvents usage, and analysis time and labor.

Matrixes and Replicates. To be acceptable for our purposes, the method had to satisfy the conditions described below on a minimum of three different vegetables or fruits. A minimum of nine samples had to be analyzed per matrix, of which one blank sample and eight fortified samples were subdivided into two groups of four replicates. One group was fortified at a relatively low level and the other group was fortified at a high level.

Linearity. For each analyte, the external calibration procedure had to generate a least-squares linear regression line with a correlation coefficient of 0.995 or higher.

Accuracy and Precision (Repeatability). Recovery values from fortified blanks had to be within the range 70−130% and 70−140% for μECD and FPD compounds, respectively. For each compound, the method could be considered valid if the coefficient of variation (CV) was less than or equal to 15% at each fortification level and less than or equal to 10% for all levels, on each matrix.

Selectivity. The method had to meet each of the following conditions for interferences: (a) confirmation of the analyte could not be inhibited by the presence of the interferant; (b) the analyte concentration and recovery had to be corrected for the interfering peak; and (c) the method had to satisfy the above accuracy and precision criteria.

Limits of Detection (LOD). The LOD of each analyte was to compare well with previously reported LODs.

Organic Solvents Usage. The goal was to use similar amounts, or less, of organic solvents than used for existing methods for pesticide residues analysis in fruits and vegetables.

Analysis Time and Labor. An analysis set had to require less or similar time and labor than existing procedures.

Experimental Procedures. Standard solutions were prepared as described above. Detailed information on these solutions is given in Table 1, where Calx means calibration level *x*.

Matrixes analyzed include fresh cantaloupe, pear, white potato, and cabbage. These commodities were purchased at

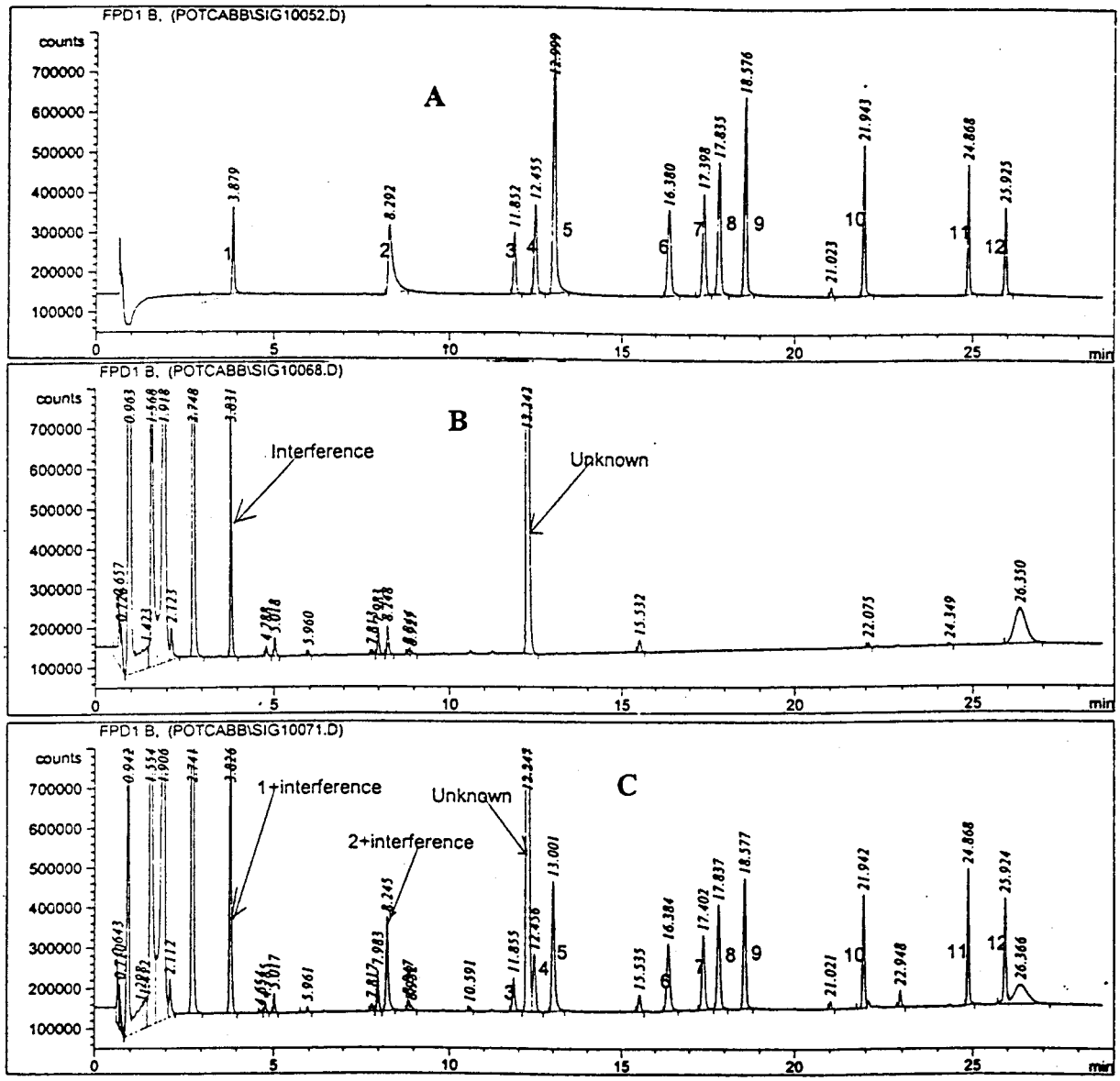

Figure 2. GC/FPD Chromatograms of (A) calibration level 3, (B) blank cabbage, and (C) cabbage fortified at level 1. Peaks: 1, Dichlorvos; 2, Acephate; 3, Terbufos; 4, Diazinon; 5, Monocrotophos; 6, Parathion-methyl; 7, Chlorpyrifos; 8, Malathion; 9, Fenthion; 10, Ethion; 11, Phosmet; and 12, Azinphos-methyl.

key market places in the vicinity of Glen Burnie, Maryland, and are representative of some of the fresh produce consumed in the Baltimore metropolitan area. Control samples of the above-referred matrixes were fortified with known amounts of a fortification solution containing 28 pesticides selected from 8 pesticide families. Sample preparation and instrumental analysis were carried out according to the developed analytical method. For each matrix, an analysis set included a total of nine samples: one blank sample, four blanks fortified at low level with 50 μL of fortification solution (spike level 1-SP1), and another set of four blank samples fortified at high level with 100 μL of fortification solution (spike level 2-SP2). Instrumentation and operating conditions were identical to those listed in the procedure. Peak identification and quantification were performed as outlined in the method. For each group, confirmation by mass spectrometry was based on retention time, ion ratios, and spectral library matches and was performed by an HP 6890 Series GC system equipped with HP 5973 mass selective detector.

RESULTS AND DISCUSSION

The method's accuracy and precision data are listed in Tables 2, 3, and 4.

All standard curves were within the acceptance limits of the linearity criterion, with the exception of chlorothalonil which showed a correlation coefficient of 0.994 in the validation set for cantaloupe.

Eight of the 28 compounds studied did not fit the method acceptance limits in terms of accuracy and/or precision: azinphos-methyl and chlorothalonil in cantaloupe and cabbage; dichlorvos in cantaloupe, white potato, and cabbage; fenthion in pear; diclofop-methyl and malathion in white potato; monocrotophos in cantaloupe and pear; and trifluralin in cabbage. Of these eight analytes, only azinphos-methyl, chlorothalonil, dichlorvos, and monocrofos did not give acceptable data on more than two matrixes. They were therefore excluded from our validated compounds list with respect to criterion 1.

In cabbage, trifluralin coeluted with an unknown peak, which could explain the relatively low precision obtained for this compound. In potato, the method showed a particularly poor accuracy for dichlorvos, malathion, and diclofop-methyl and a relatively low

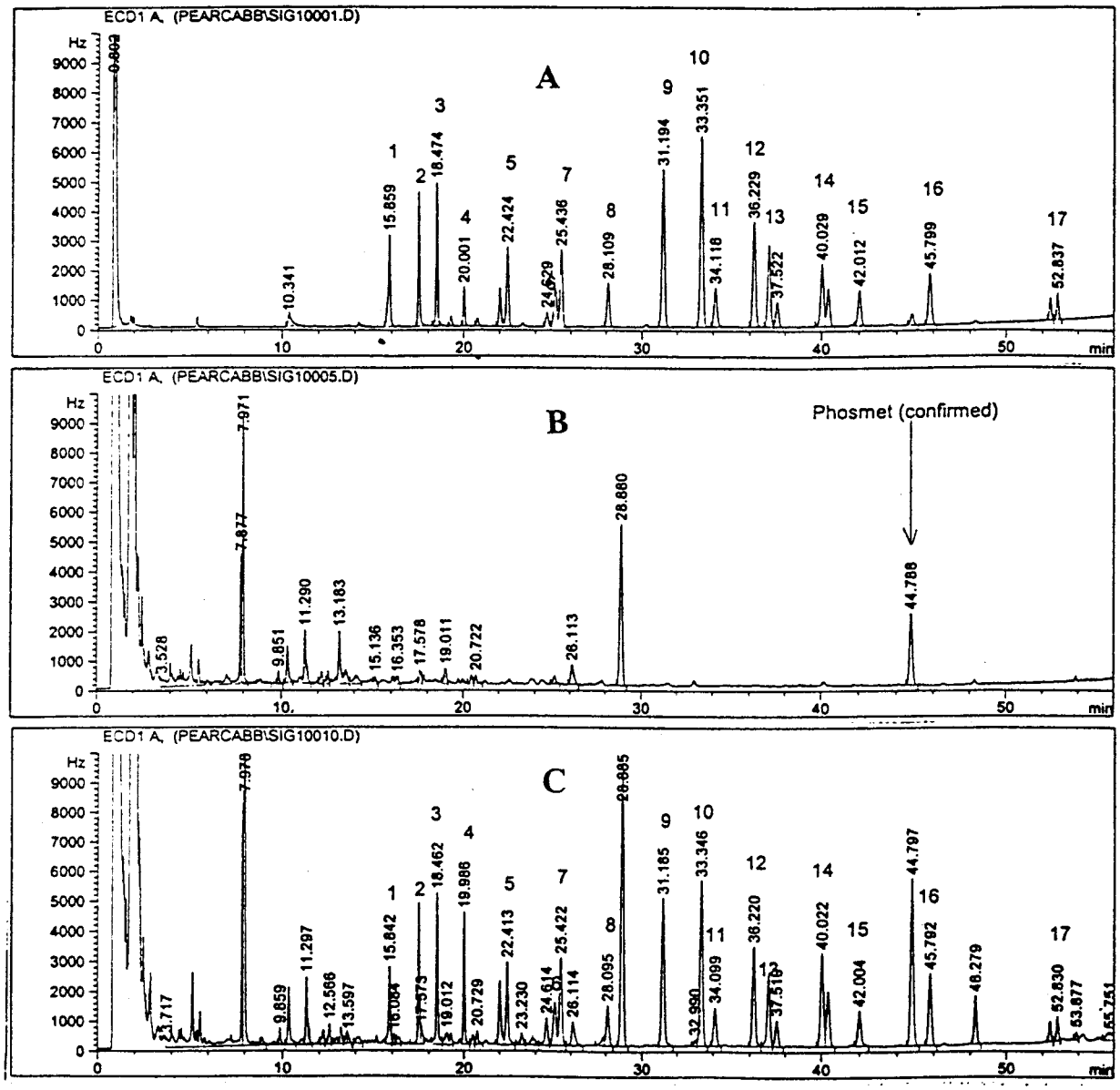

Figure 3. GC/ECD Chromatograms of (A) calibration level 3, (B) blank pear, and (C) pear fortified at level 1. Peaks: 1, Trifluralin; 2, Dicloran; 3, Lindane; 4, Chlorothalonil; 5, Alachlor; 6, Metolachlor; 7, Pendimethalin; 8, Endosulfan 1; 9, DDE; 10, Myclobutanil; 11, Endosulfan 2; 12, DDD; 13, Endosulfan sulfate; 14, DDT; 15, Diclofop-methyl; 16, Methoxychlor; 17, Permethrins.

recovery for permethrins, although excellent recoveries were obtained with these analytes on other matrixes. An examination of all 28 chemical structures shows that only malathion, diclofop-methyl, and permethrins contain ester groups in their structures. This fact tends to indicate that in white potato, ASE operating at 110 °C may be causing a breakdown of certain pesticides. In general, this problem is observed with compounds containing ester groups.

Significant matrix enhancement was obtained with a few analytes: azinphos-methyl in cantaloupe, cabbage, and potato; phosmet in cantaloupe; DDT-p,p′ in pear; and endosulfan sulfate in pear and cabbage. Similar cases have been observed elsewhere (*1*, *6*). According to Erney et al. (*7*), organophosphate pesticide recoveries higher than 100% are caused by the sample matrix, which acts as a shield for the analyte molecules against loss in hot injectors, "ensuring a more complete transfer from injector to column, compared to results" obtained with sample-free standard solutions. On the basis of this explanation, they suggested the use of calibration curves generated by standard solutions prepared in blank matrixes. Because we used standards prepared in acetone, the choice of our acceptance limits for the accuracy criterion was heavily based on these previous findings.

The method demonstrated an acceptable selectivity for most of the analyzed pesticides in the selected matrixes as shown in the sample chromatograms of Figures 1 through 4. Very minor interferences were observed in the elution area of the analyzed pesticides, except for trifluralin, dicloran, lindane, and chlorothalonil in cabbage. This problem was solved for the last three analytes by increasing the extract final volume to 4 mL. Under these conditions, the blank cabbage chromatogram did not show any detectable peak at the elution time of dicloran, lindane, or chlorothalonil. On the other hand, an unknown peak still coeluted with trifluralin. Although the two peaks were well resolved on a different column, the precision criterion was not met, possibly because of this interference. Chromatograms obtained under GC/FPD conditions showed fewer

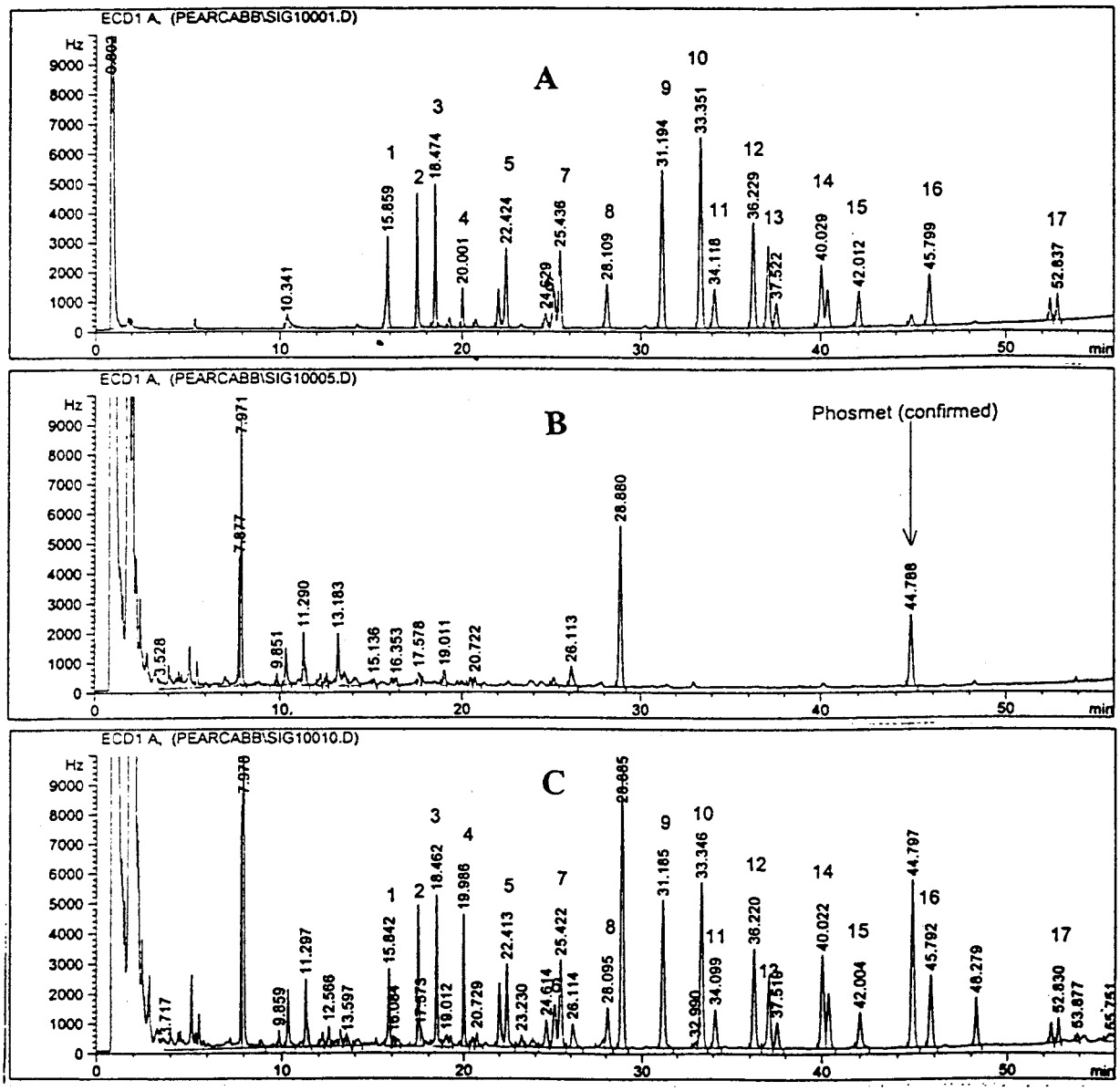

Figure 4. GC/FPD Chromatograms of (A) calibration level 3, (B) blank pear, and (C) pear fortified at level 1. Peaks: 1, Dichlorvos; 2, Acephate; 3, Terbufos; 4, Diazinon; 5, Monocrotophos; 6, Parathion-methyl; 7, Chlorpyrifos; 8, Malathion; 9, Fenthion; 10, Ethion; 11, Phosmet; and 12, Azinphos-methyl.

interferences. The only interference encountered was in blank cabbage where a giant peak coeluted with dichlorvos. Although this interference was eliminated on a different column (Phenomenex ZB-5 widebore capillary column of dimensions 30 m × 0.53 mm × 1.25 μm), proper identification and quantification of this compound were not possible, which caused dichlorvos to fail validation in cabbage. Two phosphorus-containing compounds, phosmet and azinphos-methyl, were detected in blank pear (Figure 4) and confirmed by GC/MSD. In Tables 2 and 3, the recoveries given for these two compounds were corrected for the amount of pesticide incurred in the blank and they still satisfied all acceptance criteria. In the GC/MSD confirmation process, thiabendazole, a nontarget analyte, was also detected with a spectral library match quality of 98 and strong ion ratios and retention time matches.

LODs are shown in Table 5, along with LODs reported in the USDA Pesticide Data Program (PDP). In general, the method's LODs were within acceptable ranges with regard to those in the PDP. They also compare with LODs reported by several published papers such as that by Fillion and al. (*10*). A somewhat different procedure for the determination of detection limits has been suggested by several published materials (*11*). In particular, these materials recommend the estimation of the LOD, the subsequent analysis of seven field samples fortified at 1 to 5 times the estimated LOD, and the determination of the LOD using the standard deviation of the seven results in μg/g and the value of the one-sided *t*-distribution table for a degree of freedom of $n - 1$. Although the calculated limit is generally stated at the confidence level of 99%, the procedure outlined above was found simpler and more suitable to this analytical procedure. Most major parameters that affect the detection of an analyte were taken into consideration in the above LOD formula: the instrument response, the sample matrix, the amount of sample analyzed, and the extraction efficiency.

A typical Luke method for nonfatty foods (*12*) uses approximately 900 mL of organic solvents per sample, of which 375 mL is dichloromethane, for extraction and

Table 5. Pesticide Data Program (PDP) LODs and LODs Estimated in Four Commodities

pesticide	pear LOD, μg/g	cana-loupe LOD, μg/g	white potato LOD, μg/g	cabbage LOD, μg/g	PDP[a] LOD, μg/g
Alachlor	0.041	0.044	0.045	0.043	NA
Azinphos-methyl	0.025	0.020	0.023	0.025	0.006−0.024
Chlorothalonil	0.020	0.027	0.023	0.027	0.005−0.030
Chlorpyrifos	0.013	0.013	0.015	0.014	0.003−0.011
DDD, p−p′	0.0068	0.0072	0.0069	0.0065	0.003−0.008
DDE, p−p′	0.0019	0.0019	0.0019	0.0019	0.003−0.007
DDT, p−p′	0.012	0.016	0.014	0.015	0.003−0.008
Diazinon	0.0095	0.0096	0.012	0.013	0.002−0.010
Dicloran	0.0056	0.0063	0.0062	0.0052	0.006−0.010
Dichlorvos	0.011	0.0095	0.030	0.014	0.003−0.010
Diclofop-methyl	0.064	0.058	0.14	0.052	NA
Endosulfan 1	0.0020	0.0023	0.0023	0.0026	0.001−0.007
Endosulfan 2	0.0049	0.0053	0.0051	0.0045	0.002−0.007
Endosulfan sulfate	0.0070	0.0078	0.0078	0.0071	0.003−0.010
Ethion	0.0048	0.0049	0.0055	0.0040	0.001−0.006
Fenthion	0.012	0.0087	0.011	0.0096	0.003
Lindane	0.0042	0.0041	0.0041	0.0037	0.002−0.006
Malathion	0.013	0.012	0.023	0.016	0.002−0.018
Methoxychlor, p−p′	0.026	0.031	0.029	0.029	0.009−0.026
Metolachlor	0.099	0.12	0.12	0.12	NA
Monocrotofos	0.085	0.072	0.092	0.078	NA
Myclobutanil	0.11	0.11	0.11	0.10	0.008−0.057
Parathion-methyl	0.013	0.014	0.016	0.016	0.002−0.013
Pendimethalin	0.029	0.030	0.031	0.027	NA
Permethrins	0.056	0.059	0.074	0.058	0.005−0.032
Phosmet	0.018	0.015	0.018	0.014	0.005−0.024
Terbufos	0.011	0.010	0.014	0.012	0.002−0.025
Trifluralin	0.023	0.023	0.026	0.11	0.002−0.068

[a] Range of LODs in the PDP Summaries of calendar years 1994 and 1998 (refs *8*, *9*).

clean up. Since the original Luke methods were developed, significant efforts to develop new procedures involving less organic solvent have been made by several researchers. In 1996, Casanova (*13*) developed a solid-phase extraction method which uses just about 170 mL of solvent. With respect to these existing methods, this procedure was quite within the acceptance limits of our criterion on the use of organic solvents. Indeed, the extraction and clean up of a sample using the newly developed method involves approximately 160 mL of solvent, of which about 90 mL is dichloromethane. The solid-phase extraction method used for cleanup was adapted from Schenck and Howard-King (*6*).

In terms of speed and labor, this method did not show an advantage over existing procedures, despite the semi-automation feature of ASE. Significant time and labor were required for each of the sample preparation steps (cell preparation, partitioning, and drying steps). In addition, occasional leaks of extracts were observed during the extraction process, which was found as a disadvantage of ASE. However, the overall amounts of time and labor involved were acceptable compared to most existing procedures. A minimum of seven samples could be extracted and cleaned up daily by working at an efficient, unhurried pace.

CONCLUSION

This study lead us to three major findings. First, it was a strong confirmation of the fact that accelerated solvent extraction may be used to extract a wide range of pesticide residues from a variety of fresh vegetables and fruits. Second, the task of analyzing these food extracts by gas chromatography with electron capture detection while minimizing labor, time, and environmental concerns prompted us to develop a cleanup procedure capable of collecting all target compounds with 12 mL of a single eluant. Finally, this work proved that, in addition to techniques such as mass spectrometry and capillary electrophoresis (reported by other researchers), accelerated solvent extraction may be combined with one of the most popular and sensitive chromatographic detectors, ECD, to analyze pesticide residues in fresh produce. The method was validated for the analysis, in fruits and vegetables, of 25 of the 28 target compounds: alachlor, chlorpyrifos, DDD-p,p′, DDE-p,p′, DDT-p,p′, diazinon, dicloran, diclofop-methyl, endosulfan 1, endosulfan 2, endosulfan sulfate, ethion, fenthion, lindane, malathion, methoxychlor, metolachlor, myclobutanil, parathion-methyl, pendimethalin, permethrins, phosmet, terbufos, and trifluralin.

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