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Rapid Solid Phase Extraction Cleanup for Pesticide Residues in Fresh Fruits and Vegetables

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The multiresidue procedure of Luke et al. (1975) for the determination of pesticides in fresh fruits and vegetables involves using a minimal sample cleanup and gas chromatographic (GC) analysis using element selective detectors. This method was developed and validated for use with packed column GC systems. The nonvolatile sample matrix coextractants resulting from the method had only a minimal effect on these columns. The capillary GC columns that are widely used today are much more susceptible to contamination from sample matrix coextractants. An improved variation of the Luke et al. methodology, which has been referred to as Luke II, has been developed (Cairns et al. 1993). The Luke II procedure uses a pre-partition cleanup with a C_{18} solid phase extraction (SPE) column, an acetone methylene chloride partitioning cleanup, and a post-partition cleanup, using strong and weak anion exchange SPE columns.

The use of tandem graphitized carbon black (GCB) and anion exchange SPE columns for the cleanup of plant extracts has been reported (Selwyn 1995, Nolan 1996, Meola and Sheridan 1997). In the present study, we present a rapid SPE cleanup for Luke extracts using tandem GCB and anion exchange SPE columns.

MATERIALS AND METHODS

Pesticide stock standard solutions (0.25-1.0 mg/mL) were prepared in acetone. Mixed organochlorine pesticide (OCP) and organophosphorus pesticide (OPP) working standard solutions (10.0 µg/mL) were prepared by mixing stock standard solutions as appropriate and diluting to volume with acetone.

SPE columns were Supelclean Envi-Carb graphitized carbon black (GCB), 6-mL 500 mg (Supelco, Bellefonte, PA), Bond Elut primary/secondary amine (PSA), 3-mL, 500 mg (Varian Sample Preparation Products, Harbor City, CA). Centrifuge tubes, stoppered, conical, glass, 15-mL were obtained from Corning Glass (Corning, NY). All other reagents, chemicals, and apparatus were the same as described elsewhere (Luke et al. 1975).

Gas Chromatography was performed with a HP-5890 Series II® (Hewlett Packard

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Corp., Palo Alto, CA) equipped as follows: flame photometric detector (FPD); column 30 m, 1.5 pm, 0.53 mm id DB-5 widebore capillary (J&W Scientific), carrier gas ultra high purity (UHP) Helium, 12 mL/min; make-up gas UHP helium, 10 mL/min. Injector 220°C; detector 225°C; oven temperature 130°C for 1 min, 6°C/min, final temperature 225°C and a Model 540 gas chromatograph (Tremetrics Inc. Austin TX) equipped as follows: Hall electrolytic conductivity detector (halogen mode); column, 30 m, 1.5 pm, 0.53 mm id DB-1701 widebore capillary (J&W Scientific); temperature 195 °C; carrier gas UHP Helium, 20 mL/min; make-up gas UHP helium, 10 mL/min. Injector 225°C; reactor base 250°C; reactor furnace 850°C; reactor gas, UHP hydrogen, 60 mL/min.; solvent, n-propanol 0.3 mL/min.

One hundred grams of sample was weighed into a blender cup, 1.0 mL of working standard was added and the sample was allowed to equilibrate for 15 min. The sample was extracted by the Luke method, complete details of which have been previously described (Luke et al. 1975). Briefly, the sample was blended with acetone, residues were transferred from aqueous acetone to methylene chloride/petroleum ether by partitioning, and the sample was concentrated in the presence of petroleum ether and then acetone. The final volume of the extract was adjusted to 10.0 mL with acetone.

Sodium sulfate (ca. 1 cm) was added to a GCB SPE column and a PSA SPE column was attached below the GCB column. The tandem columns were placed on an SPE vacuum manifold and washed with 5.0 mL of a freshly prepared acetone/toluene (3/1, v/v) mixture. A 15-mL graduated centrifuge tube was placed in the manifold below the tandem SPE columns and 5.0 mL of Luke extract was applied to the GCB column. The SPE columns were eluted at 1-2 drops per second. When all of the acetone had been eluted from the SPE columns, the columns were eluted two times with 5 mL of an acetone/toluene (3/1, v/v) mixture. The combined eluates were evaporated to ca. 1.0 mL with the aid of a stream of nitrogen at 50°C. Acetone (ca. 10 mL) was added and the eluate was again evaporated to ca. 1.0 mL. Acetone was added to the 5.0 mL mark, and the tube was capped and mixed. Luke extracts that had been subjected to SPE cleanup and standards in acetone were all injected (2.0 µL) into the GC.

RESULTS AND DISCUSSION

The surface of GCB particles are contaminated with oxygen complexes that interact very strongly with acidic compounds, to the extent that strong organic solvents such as acetone and acetonitrile are incapable of desorbing them (Andreoloni at al 1987, Di Corcia and Marchetti 1989). The GCB surface also has an affinity for compounds containing aromatic rings (Andreoloni et al. 1987). Most of the aliphatic base-neutral pesticide molecules can be easily eluted through a GCB with polar solvents such as acetone or acetonitrile. Some of the aromatic pesticides (e.g. phosmet, phosalone, lepotophos), which have a greater affinity for the GCB, cannot be eluted with acetone or acetonitrile, but will be rapidly eluted with toluene. Consequently, an

acetonitrile/toluene (3:1) mixture has been used to elute pesticides from GCB SPE columns (Selwyn 1995, Nolan 1996, Meola and Sheridan 1997). These methods use a fairly large volume of this elution solvent (25-40 mL) to elute the pesticides from the SPE column. Work in our laboratory has shown that while most pesticides can be eluted from the GCB SPE column with 5 mL of the acetonitrile/toluene (3:1), a larger volume of solvent is required for a few pesticides. For example, as much as 25 mL of this solvent may be required to elute the polar OPP acephate from a GCB SPE column. We found that acephate and some of the other OPPs that are difficult to elute from the GCB column (e.g. bromophos ethyl, leptophos) can be much more rapidly eluted with an acetone/toluene (3:1) mixture.

Apple, carrot and zucchini squash samples were spiked at 0.10 ppm, and extracted using the Luke method. The visual appearances and colors of these extracts were: turbid tan, transparent deep yellow and opaque dark green, respectively. When portions of these extracts were eluted through a GCB SPE column most of the color in each extract was removed. A further cleanup can be achieved by placing an anion exchange SPE column beneath the GCB SPE column. We evaluated strong and weak anion exchange and primary/secondary amine (PSA) SPE columns and found that the PSA resulted in a better cleanup.

Luke extracts and the Luke extracts that had been subjected to the SPE cleanup were injected on gas chromatographs equipped with flame photometric and Hall electrolytic conductivity (halogen mode) detectors. Recoveries of OCPs and OPPs are shown in Tables 1 and 2, respectively. Certain OPP residues exhibited abnormally high recoveries (ca 140-185%) due to sample matrix enhancement, a phenomenon which has been studied by Erney et al. (1993). The recoveries of the OCPs and most of the OPPs were slightly lower when using the SPE cleanup because the SPE cleanup resulted in a lessening of the matrix enhancement effect. Work in our laboratory that compared matrix standards (standards made up in blank Luke extracts and blank Luke extracts that had been subjected to SPE cleanup) to acetone standards has shown that

Table 1. Recoveries of fortified organochlorine pesticide residues (0.1 ppm) from apples, carrots and zucchini.

	Recovery (%)[a]	
	Luke method	Luke method with SPE cleanup
trans-chlordane	97.7 (14.3)	80.1 (11.5)
p,p'-DDE	105.1 (18.9)	89.8 (9.3)
endrin	103.2 (18.6)	88.1 (7.8)
heptachlor epoxide	95.8 (16.5)	79.7 (10.4)
lindane	95.5 (9.1)	89.7 (8.4)
cis-nonachlor	102.1 (6.5)	89.5 (7.3)

[a] n=3; values in parentheses are coefficients of variation (%).

Table 2. Recoveries of fortified (0.1 ppm) organophosphorus pesticide residues from apples, carrots and zucchini

	Recovery (%)[a]	
	Luke method	Luke method with SPE cleanup
acephate	148.5 (8.3)	109.2 (21.2)
bromophos ethyl	80.3 (11.6)	76.8 (11.8)
carbophenothion	98.7 (1.0)	101.1 (5.6)
β chlorfenvinphos	97.0 (9.1)	104.9 (1.9)
chlorpyrifos	96.7 (4.7)	96.0 (10.3)
chlorthiophos	93.1 (9.4)	101.5 (1.3)
cyanophos	104.2 (1.2)	88.1(15.7)
DEF	91.2 (7.9)	101.2 (0.98
diazinon	96.1 (1.3)	81.9 (14.1)
dicrotophos	112.4 (4.4)	106.5 (14.4)
dimethoate	128.5 (2.4)	116.0(13.2)
disulfoton	74.0 (2.7)	62.1 (7.1)
ethion	98.4 (1.4)	100.7 (6.2)
fenamiphos	104.5 (5.8)	101.5 (8.0)
fenitrothion	99.5 (2.2)	101.4 (0.6)
fenthion	86.0 (12.7)	79.8 (11.6)
fonophos oxygen analog	99.0 (3.6)	101.5 (4.2)
formathion	94.2 (12.3)	82.9 (15.3)
gardona	106.3 (2.4)	103.9 (12.1)
iodenofos	99.6 (3.7)	98.4 (11.0)
leptophos	99.8 (7.5)	107.6 (1.7)
malathion	100.6 (1.7)	97.5 (7.9)
malathion oxygen analog	113.6 (3.6)	107.9 (13.3)
methamidophos	94.0 (3.5)	62.0 (24.9)
methidathion	107.3 (4.8)	103.2 (12.7)
mevinphos-E	97.2 (2.2)	94.9 (8.8)
monocrotophos	185.5 (1.1)	139.3(21.2)
omethoate	165.6 (2.4)	145.0 (16.5)
parathion	103.1 (9.0)	100.1 (7.5)
parathion methyl	95.8 (9.2)	97.7 (5.1)
parathion oxygen analog	104.9 (5.5)	101.9 (12.2)
phosalone	110.1 (2.2)	105.3 (4.9)
phosmet	142.8 (1.6)	135.4 (6.5)
pirimiphos methyl	93.7 (4.1)	91.7 (9.9)
quinalphos	100.1 (1.0)	99.4 (7.2)
ronnel	98.4 (1.8)	86.5 (10.8)
terbufos	122.6 (8.0)	123.0 (6.2)

[a] n=3; values in parentheses are coefficients of variation (%).

the SPE cleanup of Luke extracts will result in a significant reduction of the sample matrix enhancement effect (*unpublished data F. Schenck*). The SPE method will result in an excellent rapid cleanup of sample extracts that will prolong the life of capillary GC columns, while also reducing the effect of sample matrix enhancement. Since only a single post partition SPE cleanup step is used, this method is faster than the Luke II.

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