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Multiresidue Analysis of Pesticides in Fruits and Vegetables Using Disposable Pipette Extraction (DPX) and Micro-Luke Method†

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Simple and rapid methods for the analysis of pesticides in produce samples have been developed. The current study involves analysis of nonpolar and polar pesticides using GC-ECD and GC-NPD, respectively. The nonpolar pesticides were extracted using disposable pipette extraction (DPX) following a preliminary acetone extraction based on the Luke method. Recoveries of the fortified pesticides ranged between 70.5 and 110.0%, with relative standard deviations (% RSD) below 10% for most target analytes. From calibration plots, the limits of detection (LOD) were determined to be <0.05 ppm for most studied pesticides with coefficients of determination (r^2) >0.99. A micro-Luke method was adapted and used for the analysis of polar pesticides, which greatly reduces the volumes of solvent and time for sample preparation. This new method of analysis, combining DPX and micro-Luke, was compared directly with the modified Luke method for analysis of pesticides in produce samples over a 9 week period. This study validates the suitability for routine analysis of pesticides in acetone extract of fruits and vegetables using the proposed DPX and micro-Luke method.

KEYWORDS: Nonpolar pesticides; GC; DPX; Luke method

INTRODUCTION

Regulatory and public concern over pesticide residues in fruits and vegetables has been increasing due to potential health hazards. Measuring trace levels of pesticide residues in the presence of large amounts of sample matrix components that occur naturally is a challenging task. There is growing interest in developing simple, rapid, cost-effective, and reliable analytical methods to ensure that levels of toxic pesticides incurred in produce are below tolerance levels.

Sample preparation is the first step in the analysis. Achieving accurate and precise results for the analysis of pesticides in complex matrices such as food depends largely on the extraction and cleanup methods that are employed. Many methods have been reported for the extraction of pesticides from fruits and vegetables (*1*−*8*). Perhaps the two most widely employed organic solvents used for sample extraction are acetone, which is used in the Luke method (*9*), and acetonitrile, which is used in the QuEChERS method (which stands for quick, easy, cheap, effective, rugged, and safe) (*10*−*13*).

Due to its reliability, the Luke method has been employed for monitoring of pesticides in fruits and vegetables in approximately 80% of the FDA pesticide residue analyses in the 1980s (*9*). The Luke method is still the accepted residue analysis method in many federal and state laboratories. This method uses an acetone extract that can be used in conjugation with various GC systems with element-selective and element-specific detectors for the determination of essentially all nonionic pesticides in the organochlorine (OC), organophosphate (OP), organonitrogen (ON), and hydrocarbon (HC) classes. The major advantage of the Luke method is that it provides quantitative recovery of pesticides and, after Florisil cleanup, the final extracts have greatly reduced matrix interference.

The biggest disadvantage of the Luke method is that it requires multiple exhaustive solvent concentration steps and is therefore time-consuming. The method also uses over 375 mL of organic solvent for each sample including methylene chloride and petroleum ether, and these solvents are subsequently evaporated for concentration steps prior to analysis. The most time-consuming part of the Luke method is the use of column chromatography (Florisil) to prepare samples for GC-ECD (electron capture detector) analysis of OC pesticides. The analysis of OP and ON pesticides is not nearly as time-consuming because there is not a separate "cleanup" method due to the use of highly selective GC-NPD (nitrogen−phosphorus detector), and this procedure can be scaled down to minimize the time and volume of solvents for analysis.

Although QuEChERS has shown great results for comprehensive screening of pesticides by GC-MS and HPLC-MS/MS, the extracts are generally found to be too dirty for use with selective detectors such as ECD. Hence, this sample preparation

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method is not preferred for laboratories that want to utilize the high sensitivity that ECD provides for analysis of chlorinated compounds.

Disposable pipette extraction (DPX) is a new solid phase extraction (SPE) method used to rapidly extract sample solutions. Unlike traditional SPE devices, DPX mixes solutions with the sorbent in a dispersive manner to provide rapid equilibration and adsorption of analyte with the solid phase sorbent. In a previous study, we developed a DPX method using reversed phase (RP) mechanisms that provided high recoveries of nonpolar pesticides (*14*). We have found this method to be ideal for analysis of acetonitrile extracts for organochlorine and other nonpolar pesticides. To evaluate the suitability of DPX extraction using acetone extraction, the present research was performed to compare the DPX method directly to the Luke method for extraction of nonpolar pesticides using GC-ECD.

This study emphasizes the analysis of the nonpolar pesticides by DPX using GC-ECD. The objective is to replace the time-consuming portion of the Luke method for analysis of OC pesticides and to improve the efficiency of polar pesticides by using a micro-Luke procedure. This study also compares results of the DPX/micro-Luke to the Luke method of analysis of pesticide residues in fruits and vegetables over a 9 week period.

MATERIALS AND METHODS

Standards, Reagents, and Materials. A mixed OC stock solution (1000 ppm) was purchased from ULTRA Scientific (N. Kingstown, RI). Individual standards of 100 ppm of bifenthrin, chlorothalonil, captan, and malathion were supplied by the South Carolina Department of Agriculture (SCDA).

GC grade acetone, methylene chloride, petroleum ether, iso-octane, hexane, and ethyl ether were purchased from Burdick & Jackson (Muskegon, MI). HPLC grade sodium chloride crystals were obtained from J. T. Baker (Phillipsburg, NJ). GC grade Florisil (60−110 mesh) was purchased from EM Science (Gibbstown, NJ). Glass microfiber filters (grade 691, size 9.0 cm) were obtained from VWR (West Chester, PA).

DPX-RP tips were supplied by DPX Laboratories, LLC (Columbia, SC). A food processor from Hamilton Beach (Washington, NC) was employed to blend fruits and vegetables with acetone.

Sampling, Transport, Processing, and Storage of Samples. The SCDA chemical residue laboratory analyzes fruits and vegetables obtained from supermarkets and farmers' markets statewide to detect chemical adulterants or pesticide residues that might be present. To comply with food safety laws, a market basket survey is conducted, which involves the collecting of samples of individual lots of domestically produced and imported foods as close as possible to their point of entry into the distribution system. Fresh fruits and vegetables were collected and transported to the laboratory in clean polyethylene bags within 1 day. Each sample was identified clearly and indelibly by allocating a unique identification number. Chopping, followed by blending and mixing, was performed to ensure that the samples were homogeneous enough so that subsampling variability would be acceptable and the extracts were truly representative of the corresponding samples as a whole. All analyses were undertaken within the shortest time possible (within 2 days) to minimize potential analyte losses.

Method 1: Extraction of Pesticides in Fruits and Vegetables Using the Modified Luke Method. Fruits and vegetables were analyzed at the SCDA using a modified Luke method (*9*). After removal of stalks, caps, and stems, the initial extract was obtained as follows: 100 g of chopped sample was weighed out into a blender, and 200 mL of acetone was added and blended for 2 min. After filtering through a glass microbore filter (9.0 cm), 50 mL of the acetone extract was transferred into a separatory funnel. For initial extraction, 50 mL of methylene chloride, 50 mL of petroleum ether, and 10 mL of saturated sodium chloride solution were added. The separatory funnel was shaken vigorously for 1 min and left to stand for 2−3 min to permit immiscible layers to separate. The upper layer was transferred through 300 g of sodium sulfate, and the lower layer was extracted twice using methylene chloride. All organic layers were combined after passing through sodium sulfate. An additional

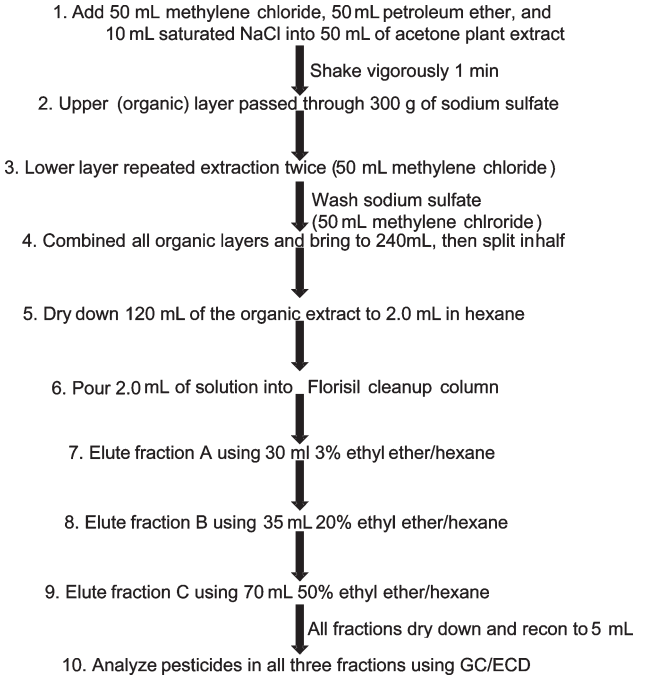

Figure 1. Schematic flow diagram of modified Luke method for extraction of multiresidue pesticides in fruits and vegetables for GC-ECD analysis (part of method 1). For analysis of OP pesticides, the other half of the 240 mL (120 mL) in step 4 is solvent evaporated and concentrated for direct analysis using GC-NPD.

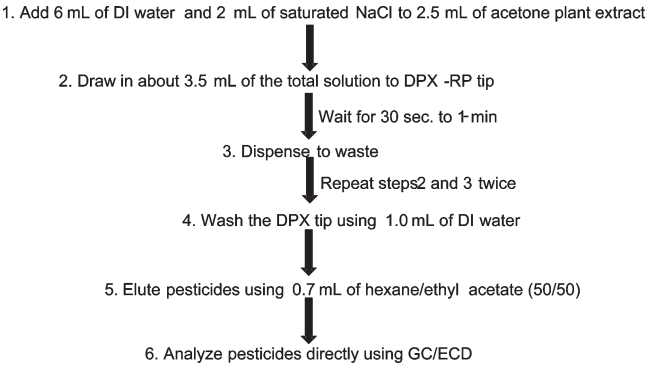

Figure 2. Schematic flow diagram of the DPX method for extraction of multiresidue pesticides in fruits and vegetables for GC-ECD analysis (method 2).

50 mL of methylene chloride was used to wash the residual sodium sulfate and combined with the sample extract. A final volume of sample extract was brought up to 240 mL by adding acetone. This acetone solution was split into two portions, one for OC pesticides and the other for OP pesticides.

A 120 mL volume of the total acetone extract (one portion of the original solution) was used for extraction of OC pesticides and pyrethroids and analysis using GC-ECD. The solution was evaporated to about 2.0 mL on a steam bath. To prevent losses of analyte during solvent exchange, 10 mL of hexane was added, and the solution was concentrated again to 2.0 mL. This procedure was repeated twice (to remove the chlorinated solvent prior to ECD analysis), and the concentrated extract was transferred through 5 g of Florisil for cleanup using a 2 cm × 45 cm glass column with a 3.0 mm layer of sodium sulfate below the Florisil. Three fractions were collected separately: fraction A was eluted using 30 mL of 3% ethyl ether/hexane; fraction B was eluted with 35 mL of 20% ethyl ether/hexane; and fraction C was collected by adding 70 mL of 50% ethyl ether/hexane. All fractions were concentrated to approximately 2.0 mL on the steam bath, reconstituted to 5.0 mL by adding hexane, and only 1 μL

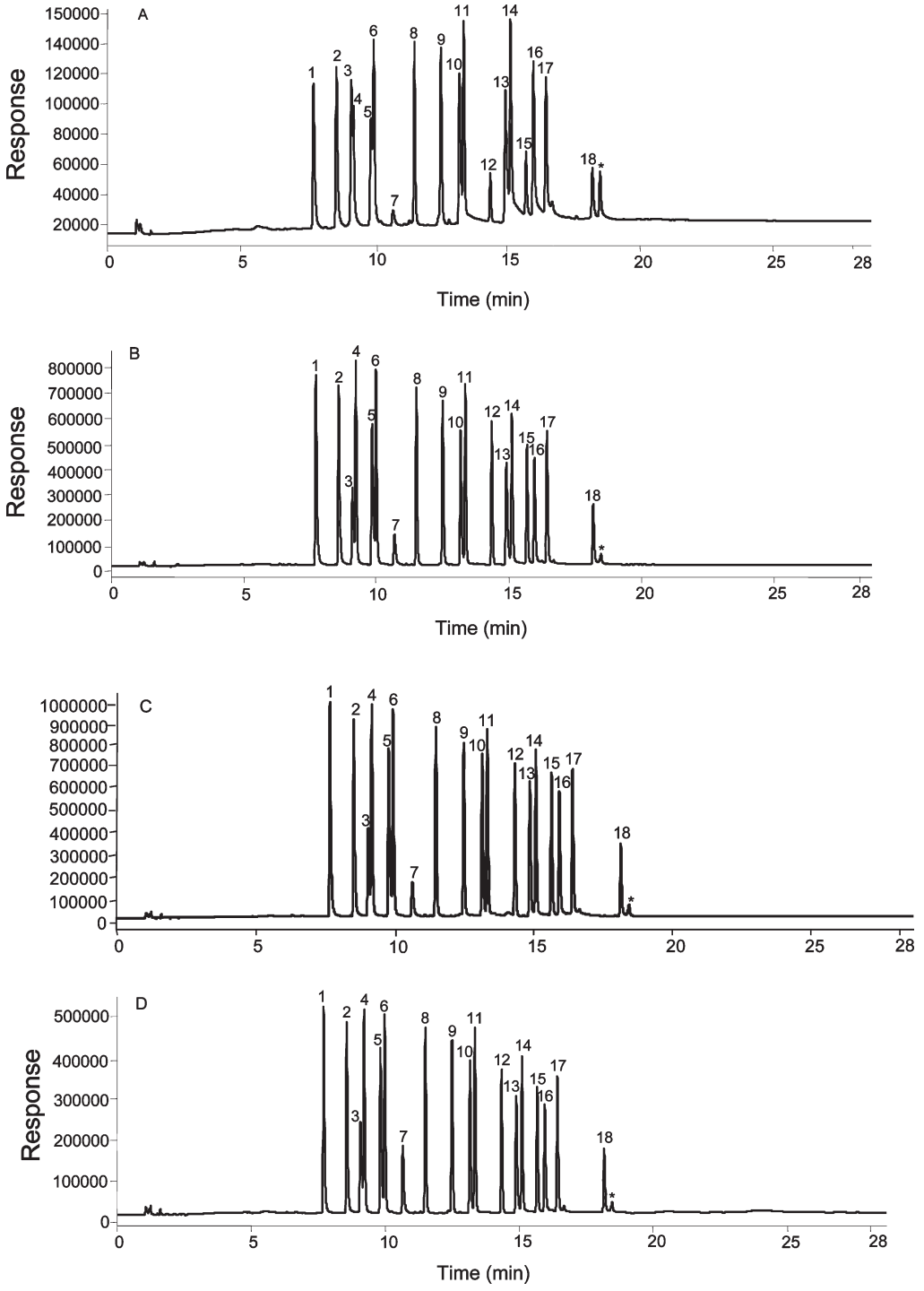

Figure 3. GC-ECD chromatograms of various matrices spiked with 0.5 ppm organochlorine pesticides following DPX extraction: (**A**) yellow squash; (**B**) tomato; (**C**) carrot; (**D**) tangerine. Separation was performed using a DB-17 column. Peak identification: 1, α-BHC; 2, γ-BHC; 3, β-BHC; 4, δ-BHC; 5, heptachlor; 6, aldrin; 7, chlorpyrifos-ethyl; 8, heptachlor epoxide; 9, endosulfan I; 10, *p,p′*-DDE; 11, dieldrin; 12, endrin; 13, *p,p′*-DDD; 14, endosulfan II; 15, *p,p′*-DDT; 16, endrin aldehyde; 17, endosulfan sulfate; 18, methoxychlor. Peaks marked with an asterisk (∗) are matrix derived.

of each fraction was injected into the GC-ECD for analysis. **Figure 1** shows a flow diagram of the extraction procedure of the modified Luke method for GC-ECD.

For analysis of polar pesticides, such as OP pesticides, a 120 mL volume of the total acetone extract (the other portion of the original solution of step 4 in **Figure 1**) was evaporated to 2.0 mL on a steam bath and transferred to a C-tube (*9*). The concentrate was brought to 5.0 mL with the addition of acetone before injection into the GC-NPD.

Method 2: Extraction of Pesticides in Fruits and Vegetables Using DPX. DPX-RP tips obtained from DPX Laboratories, LLC (Columbia, SC), contained styrene divinylbenzene (SDVB) sorbent for reversed phase mechanisms of extraction (*14*). Matrix extracts were initially prepared according to the Luke method delineated above, which used 100 g of chopped sample blended with 200 mL of acetone and filtered.

An aliquot of 2.5 mL of initial acetone extract was used with the DPX extraction method for OC pesticides. A 6.0 mL volume of deionized (DI) water and 2.0 mL of saturated sodium chloride were added (to increase the ionic strength of the solution) and mixed with the acetone solution. Approximately 3.5 mL of solution was aspirated into a DPX-RP tip by using an attached 10 mL syringe as a pipettor. By aspirating approximately 5 mL of air, small bubbles created by air passing through the pores of the screen of the DPX tip create a perturbation of the solution and result in

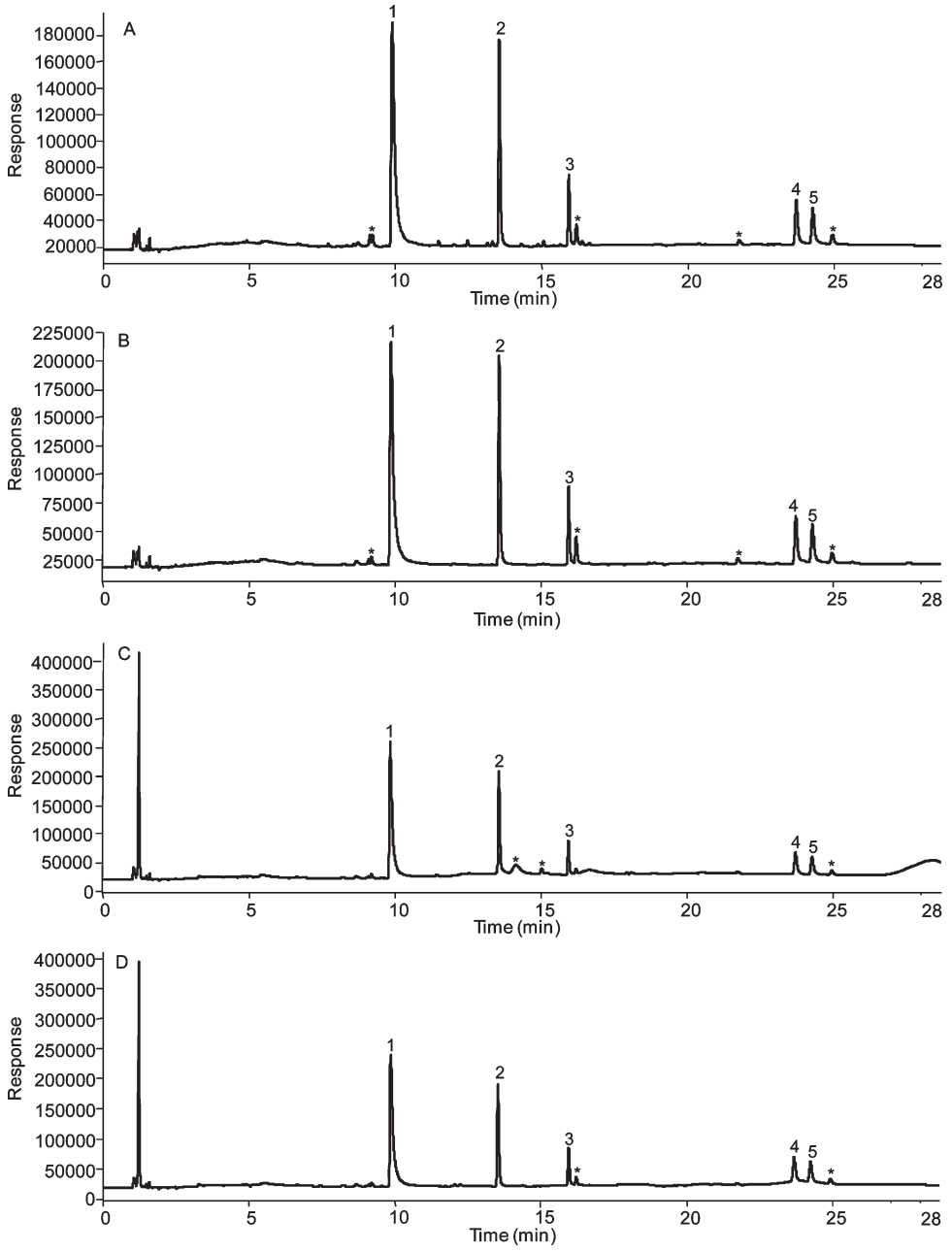

Figure 4. Representative GC-ECD chromatograms of DPX extracted 0.5 ppm bifenthrin, captan, chlorothalonil, and esfenvalerate in (**A**) yellow squash, (**B**) tomato, (**C**) carrot, and (**D**) tangerine. Separation was performed using a DB-17 column. Peak identification: 1, chlorothalonil; 2, captan; 3, bifenthrin; 4, esfenvalerate 1; 5, esfenvalerate 2. Peaks marked with an asterisk (∗) are matrix derived.

thorough mixing of the RP sorbent with the sample solution. The solution was allowed to stand for at least 30 s, allowing the analyte to partition into or adsorb onto the SDVB sorbent. The solution was dispensed to waste, and this extraction procedure was repeated twice to extract the entire sample solution (approximately 10.5 mL). To remove salt and water-soluble matrix interferences, 1.0 mL of DI water was added to the top of the DPX tip and dispensed through the SDVB sorbent using positive pressure from the attached syringe. Finally, elution of the pesticides was performed using 0.7 mL of hexane/ethyl acetate (50:50, v/v), which was added to the top of the DPX tip and passed through the SDVB sorbent into a labeled GC vial using positive pressure.

This elution volume was found to result in a final volume of eluate of 0.5 mL due to solvent exchange of the organic solvent into the SDVB sorbent, replacing and removing a small volume (approximately 200 μL) of water. It should be noted that this final volume was found to be very repeatable and consistent with all extractions, and a graduated GC vial was used to confirm this final volume prior to analysis. This solvent exchange during the elution step occurs very readily, and there is no need for time-consuming drying steps that are often required with many SPE methods to obtain high recoveries. The bottom water layer was removed from the vial using a Pasteur pipette, and the vial was capped and placed on the GC autosampler, which subsequently injected 1 μL into a GC-ECD for analysis. **Figure 2** shows a flow diagram of the pesticide extraction using the DPX method.

Fruits and vegetables were analyzed over a 9 week period in the SCDA pesticide residue laboratory by both the Luke (method 1) and DPX methods (using the same solutions from the initial extraction in acetone). Comparable sensitivity is achieved because the concentration factor is 5 for both the DPX method (2.5 mL concentrated to 0.5 mL) and the Luke method (25 mL concentrated to 5 mL).

Method 3: Extraction of Pesticides in Fruits and Vegetables Using the Micro-Luke Method. An aliquot of 5.0 mL of initial acetone extract was added to a 50 mL centrifuge tube and used for polar pesticides analysis by GC-NPD. Five milliliters of saturated sodium chloride, 5.0 mL of petroleum ether, and 15 mL of methylene chloride were added and mixed with the acetone solution. Then 14−15 g of anhydrous sodium sulfate was added to the same centrifuge tube. After vortex mixing and

Table 1. Calibration, Linearity, Limit of Detection (LOD), and Limit of Qauntification (LOQ) of the Studied Pesticides in Different Matrices

	method validation results											
	yellow squash			tomato			carrot			tangerine		
pesticide	r^{2a}	LOD (ppm)	LOQ (ppm)	r^2	LOD (ppm)	LOQ (ppm)	r^2	LOD (ppm)	LOQ (ppm)	r^2	LOD (ppm)	LOQ (ppm)
α-BHC	0.9996	0.002	0.006	0.9968	0.016	0.050	0.9970	0.016	0.047	0.9987	0.038	0.114
γ-BHC	0.9979	0.003	0.010	0.9959	0.019	0.057	0.9958	0.012	0.036	0.9989	0.042	0.127
bifenthrin	0.9924	0.020	0.060	0.9968	0.031	0.093	0.9937	0.024	0.073	0.9969	0.076	0.230
captan	0.9981	0.011	0.032	0.9966	0.012	0.037	0.9985	0.009	0.027	0.9962	0.006	0.018
chlorothalonil	0.9981	0.025	0.076	0.9965	0.025	0.077	0.9963	0.019	0.057	0.9980	0.024	0.072
chlorpyrifos-ethyl	0.9953	0.036	0.109	0.9914	0.011	0.034	0.9958	0.012	0.036	0.9929	0.077	0.233
p,p′-DDD	0.9980	0.014	0.043	0.9956	0.032	0.097	0.9947	0.008	0.024	0.9981	0.025	0.075
p,p′-DDE	0.9950	0.005	0.015	0.9922	0.032	0.096	0.9905	0.017	0.050	0.9990	0.025	0.075
p,p′-DDT	0.9971	0.011	0.033	0.9940	0.030	0.092	0.9912	0.014	0.042	0.9977	0.029	0.088
dieldrin	0.9985	0.008	0.024	0.9937	0.024	0.074	0.9938	0.013	0.039	0.9984	0.040	0.121
endosulfan I	0.9977	0.008	0.025	0.9954	0.027	0.082	0.9924	0.011	0.035	0.9991	0.050	0.152
endosulfan II	0.9919	0.027	0.082	0.9934	0.026	0.079	0.9930	0.024	0.072	0.9977	0.042	0.128
endosulfan sulfate	0.9907	0.023	0.069	0.9958	0.022	0.067	0.9967	0.017	0.053	0.9974	0.026	0.080
endrin	0.9952	0.010	0.031	0.9954	0.024	0.074	0.9964	0.011	0.032	0.9983	0.048	0.145
endrin aldehyde	0.9975	0.025	0.077	0.9960	0.026	0.079	0.9960	0.010	0.030	0.9985	0.037	0.113
esfenvalerate 1	0.9958	0.029	0.089	0.9921	0.044	0.132	0.9954	0.012	0.037	0.9959	0.045	0.135
esfenvalerate 2	0.9940	0.031	0.093	0.9950	0.034	0.104	0.9914	0.015	0.046	0.9921	0.062	0.186
heptachlor epoxide	0.9972	0.007	0.022	0.9913	0.017	0.052	0.9933	0.011	0.033	0.9991	0.066	0.199
methoxychlor	0.9942	0.020	0.059	0.9964	0.043	0.131	0.9968	0.011	0.033	0.9982	0.054	0.164

[a] Coefficient of determination (r^2).

Table 2. Recovery (Percent) and Precision (% RSD) of the Studied Pesticides in Different Matrices

	average recovery (mean ± SD; %RSD)			
pesticide	yellow squash	tomato	carrot	tangerine
α-BHC	96.9 ± 1.5 (1.5)	97.2 ± 4.0 (4.1)	99.5 ± 4.3 (4.3)	103.0 ± 4.6 (4.4)
γ-BHC	94.5 ± 7.4 (7.9)	97.6 ± 4.1 (4.2)	98.9 ± 4.4 (4.4)	102.4 ± 4.4 (4.3)
bifenthrin	87.7 ± 5.6 (6.4)	79.33 ± 10.6 (13.4)	70.5 ± 5.5 (7.8)	77.5 ± 4.1 (5.3)
captan	110.0 ± 3.0 (2.7)	82.4 ± 10.0 (12.2)	98.6 ± 6.2 (6.3)	94.9 ± 4.2 (4.5)
chlorothalonil	105.7 ± 3.4 (3.2)	88.3 ± 6.8 (7.7)	101.1 ± 4.8 (4.8)	97.2 ± 3.0 (3.1)
chlorpyrifos-ethyl	108.4 ± 13.5 (12.4)	103.4 ± 3.2 (3.1)	91.5 ± 3.9 (4.0)	99.7 ± 3.4 (3.4)
p,p′-DDD	94.1 ± 2.1 (2.2)	94.2 ± 5.0 (5.3)	94.5 ± 3.2 (3.4)	101.4 ± 3.5 (3.4)
p,p′-DDE	91.1 ± 0.4 (0.4)	88.7 ± 7.5 (8.5)	91.5 ± 2.8 (3.1)	98.2 ± 3.4 (3.4)
p,p′-DDT	98.6 ± 0.4 (0.4)	92.7 ± 6.2 (6.7)	93.0 ± 2.9(3.1)	100.2 ± 2.9 (2.9)
dieldrin	91.5 ± 4.2 (4.5)	96.7 ± 4.1 (4.2)	95.7 ± 3.6 (3.8)	106.8 ± 4.7 (4.4)
endosulfan I	91.3 ± 3.40 (3.7)	97.2 ± 4.4 (4.5)	97.0 ± 3.8 (3.9)	102.6 ± 3.0 (3.0)
endosulfan II	106.1 ± 2.48 (2.3)	99.3 ± 3.1(3.1)	95.6 ± 4.0 (4.2)	102.9 ± 3.8 (3.6)
endrin	99.9 ± 5.2 (5.2)	98.1 ± 3.6 (3.7)	97.1 ± 3.4 (3.5)	101.9 ± 3.6 (3.6)
endrin aldehyde	107.1 ± 7.6 (7.1)	95.9 ± 3.4 (3.5)	96.6 ± 3.3 (3.4)	99.1 ± 3.6 (3.6)
esfenvalerate 1	87.9 ± 8.7 (9.9)	79.4 ± 2.5 (3.2)	87.7 ± 7.1 (8.1)	82.7 ± 8.6 (10.4)
esfenvalerate 2	82.2 ± 4.6 (5.6)	82.8 ± 4.3 (5.2)	80.3 ± 5.8 (7.2)	81.0 ± 8.0 (9.9)
heptachlor epoxide	90.8 ± 5.0 (5.5)	96.0 ± 4.1 (4.3)	96.7 ± 3.9 (4.0)	102.1 ± 3.2 (3.2)
methoxychlor	92.1 ± 2.3 (2.5)	102.2 ± 3.5 (3.4)	95.0 ± 3.9 (4.1)	91.4 ± 4.6 (5.0)

centrifugation at 3500 rpm for 5 min, the upper layer was decanted into an accurate graduated glass 15 mL C-tube and dried to 1.0 mL for analysis.

Method Validation of the DPX Method. Analysis of calibration data of nonpolar pesticides was performed to determine coefficients of determination (r^2), limits of detection (LOD), and limits of quantitation (LOQ) for accuracy and precision. It is noted that just 1.0 mL of acetone solution was used in this portion of the study, rather than 2.5 mL that was used for produce, to simplify the extraction method (one mixing step with the sample solution rather than three mixing steps used for produce).

External calibration was performed with pesticides fortified in extracts of samples from four different matrices (yellow squash, tomato, carrot, and tangerine). All matrices were previously confirmed for the absence of pesticide residues using the Luke method. Pesticide standard working solutions (20 ppm) were spiked into 1.0 mL of acetone extracts of different matrices at five levels ranging from 0.1 to 2.0 ppm. Extraction of 1.0 mL of sample was carried out by the addition of 2.4 mL of DI water and 0.8 mL of saturated NaCl followed by DPX extraction as described in the procedure above. Calibration data were generated from six replicate samples at 0.1 ppm, two replicate samples at 0.2 ppm, six replicate samples at 0.5 ppm, two replicate samples at 1.0 ppm, and six replicate samples at 2.0 ppm. LOD and LOQ were determined as (eqs **1** and **2**) (*15*)

$$\mathrm{LOD} = \frac{3.3 \times s_{\mathrm{bl}}}{m} \quad (1)$$

$$\mathrm{LOQ} = \frac{10 \times s_{\mathrm{bl}}}{m} \quad (2)$$

where m is the slope of the calibration line and the standard deviation of the blank (s_{bl}) was estimated by calculating the standard deviation of the replicate results at the lowest fortification level (100 ppb). (It should be mentioned that these equations result in very conservative calculations of LODs and LOQs, and considerably lower levels than calculated from these equations have been detected.) Five samples of each matrix spiked at 0.5 ppm with each pesticide were used to determine the recovery and precision for the DPX method. To reduce or eliminate matrix interferences, a matrix-matched sample was processed by spiking the same amount of pesticides into a blank extract following the same procedures. The recovery

was calculated by comparing the peak area of the pesticides in extracted samples to the matrix-matched sample (eq 3). Precision was represented as the relative standard deviation (RSD = SD/mean × 100%).

Instrumentation. An Agilent 6890 GC-ECD/NPD (Agilent Technologies, Little Falls, DE) was used for the study of the comparison between the Luke with DPX (ECD) and the Luke with the micro-Luke (NPD) methods at SCDA (using incurred samples). A DB-608 column (30 m × 0.32 mm i.d., 0.50 μm film thickness) was employed for the separation and determination of OC pesticides using GC-ECD. Ultrapure helium was used as carrier gas at a constant flow rate of 1.0 mL/min. A splitless injection of 2 μL was made with the inlet temperature set at 200 °C and the ECD temperature set at 300 °C. The oven temperature program was the same as described above for fortified samples. A DB-35MS340 column (30 m × 0.32 mm i.d., 0.25 μm film thickness) was used for the analysis of OP pesticides using GC-NPD. The inlet temperature was set at 190 °C, and the NPD temperature was 325 °C. The oven temperature program was the same as described above for fortified samples.

Positive pesticide residues were confirmed by GC-MS or a second column of the GC-ECD, and quantitation was performed using single-point calibration using matrix-matched standards (from spiking standard pesticides into a blank sample extract). Calibration plots and retention times of chlorpyrifos-ethyl were verified periodically for the performance of the GC-ECD.

For fortified extracts of samples (used for method validation), an Agilent 5890 gas chromatograph equipped with a DB-17 column (30 m × 0.25 mm i.d., 0.250 μm film thickness), an Agilent autosampler, and an electron-capture detector (ECD) (Agilent Technologies) were used. Ultrapure helium was used as carrier gas at a constant flow rate of 1.0 mL/min. The inlet temperature was set at 200 °C, and a splitless injection was made of 2 μL. The ECD temperature was 300 °C. The total GC analysis time was 28.67 min with the oven programmed to hold for 1 min at 80 °C, ramp at 30 °C/min to 190 °C and hold for 1 min, followed by ramp at 6 °C/min to a final temperature of 280 °C and hold for 8 min.

RESULTS AND DISCUSSION

Matrix Effect and Matrix Matched Calibration. DPX has been found to provide good cleanup for pesticide residue determination in fruits and vegetables (*14*). Matrix effects were investigated through analyses of sample blanks, and matrix peaks and possible interferences were noted in the chromatograms. Matrix-matched calibrations were used and performed by spiking extracts prepared from the original food samples with different levels of pesticides.

Figure 3 shows GC-ECD chromatograms of (**A**) yellow squash, (**B**) tomato, (**C**) carrot, and (**D**) tangerine spiked with 0.5 ppm OCs and extracted by the DPX method. As can be seen, only one peak was identified as a matrix component at this spiked level, and the peak is completely separated from any pesticide peaks. This result indicates excellent selective extraction of pesticides using DPX-RP. **Figure 4** shows GC-ECD chromatograms of those samples spiked with 0.5 ppm bifenthrin, captan, chlorthalonil, and esfenvalerate after DPX extraction. Again, low background of matrix peaks verified satisfactory cleanup of food samples with the DPX method.

Table 1 presents coefficients of determination (r^2), LOD, and LOQ from calibration plots using DPX methodology for pesticides in yellow squash, tomato, carrot, and tangerine. The coefficients of determination were all above 0.99. LODs were determined to be below 0.1 ppm for all studied pesticides in the 1.0 mL of acetone extract (for spiked studies, which extracted 1 mL and concentrated to 0.5 mL). LODs and LOQs can be assumed to be much lower for the analyses of produce samples because more acetone extract (2.5 mL of acetone extract, which was extracted and concentrated to 0.5 mL) was employed than used in the method validation study (1.0 mL of acetone extract, which was extracted and concentrated to 0.5 mL).

Accuracy and Precision of the DPX Method. The accuracy and precision of the DPX method were evaluated by study of recoveries and relative standard deviations (% RSDs) by spiking pesticides in yellow squash, carrot, tomato, and tangerine at 0.5 ppm. These particular food matrices were selected to represent basic (yellow squash), neutral (carrot), and acidic (tomato and tangerine) samples. **Table 2** shows the average recoveries and

Table 3. Pesticide Concentrations Determined Using the Luke and DPX Methods in Produce Samples Using GC-ECD

sample ID	matrix	pesticide	Luke method 1 (ppm)	DPX method 2 (ppm)	EPA tolerance (ppm)
63818	celery	dicloran	0.15	0.42	15.0
63820	grape tomatoes	chlorothalonil	0.03	0.06	5.0
63820	grape tomatoes	endo I	0.04	0.05	2.0
63820	grape tomatoes	endo II	0.07	0.08	2.0
63820	grape tomatoes	endo sulfate	0.02	0.02	2.0
63820	grape tomatoes	bifenthrin	0.04	0*	0.15
64447	strawberries	captan	3.04	0*	25.0
65885	strawberries	bifenthrin	0.27	0.41	3.0
65497	apple bites	captan	0.22	0.29	25.0
65498	red apples	captan	0.42	0.45	25.0
65498	red apples	endosulfan	0.02	0.03	2.0
65881	celery	dicloran	0.28	0.38	15.0
65883	pears	captan	0.75	0.99	25.0
65883	pears	bifenthrin	0.03	0.05	0.5
65883	pears	L. cyhalothrin	0.03	0.04	2.5
62875	red grapes	chlorpyrifos-ethyl	0.14	0.20	1.5
65579	yellow squash	endo I	0.015	0.024	2.0
65579	yellow squash	endo sulfate	0.011	0.033	2.0
65579	yellow squash	bifentrhin	0.190	0*	0.4
65579	yellow squash	chlorothalonil	0.100	0*	5.0
65521	bell peppers	chlorpyrifos-ethyl	0.06	0.08	1.0
47323	strawberries	captan	4.80	8.39	25.0
47323	strawberries	endo I	0. 007	0.014	2.0
47323	strawberries	endo II	0. 032	0.067	2.0
47323	strawberries	endo sulfate	0.104	0.022	2.0
64288	tomatoes	chlorothalonil	0.02	0.06	5.0
65525	grape tomatoes	chlorothalonil	0.06	0.22	5.0
66006	strawberries	captan	0.60	0.85	25.0
66126	blueberries	esfenvalerate	0.08	0.15	3.0
66129	baking potatoes	*p,p′*-DDE	0.006	0.005	1.0
63645	peaches	phosmet	0.17	0.32	10.0
65198	apricots	phosmet	0.09	0.20	5.0
62931	celery	dicloran	4.202	5.329	15.0
66560	tomatoes	bifenthrin	0.473	0.437	0.2
66560	tomatoes	endo I	0.183	0.191	2.0
66560	tomatoes	endo II	0.110	0.131	2.0
66561	spinach	permethrin (c and t)	2.792	2.572	20.0
66564	blueberries	esfenvalerate	0.079	0.071	3.0
66564	blueberries	malathion	0.075	0.122	8.0
60081	Gala apples	dursban	0.010	0.013	1.5
60081	Gala apples	azinphos-methyl	nr	0.2243	2.0
66251	peaches	phosmet	0.246	0.441	10.0
66638	snap beans	cypermethrin (c and t)	0.123	0.158	0.5
64891	yellow squash	chlorothalonil	0.05	0*	5
64891	yellow squash	dieldrin	0.01	0.01	0.1
64891	yellow squash	endosulfan sulfate	0.02	0.04	1.00
65270	peaches	captan	0.01	0.01	25.00
65270	peaches	phosmet	0.09	0.28	10.00
65270	peaches	cyfluthrin (c and t)	nr	0.05	0.05
67479	tomatoes	chlorothalonil	0.99	2.71	5.0
67483	strawberries	malathion	nr	0.056	8.0
65286	peaches	captan	0.03	0*	25.00
65286	peaches	phosmet	0.12	0.18	10.00
65286	peaches	cyfluthrin	0.17	0.13	0.05
65286	peaches	esfenvalerate	0.08	0.07	10.00
65287	tomatoes	chlorothalonil	0.02	0.13	5.00
66476	zucchini	endosulfan I	0.02	0.02	1.00
66473	yellow squash	dieldrin	0.03	0.04	0.1

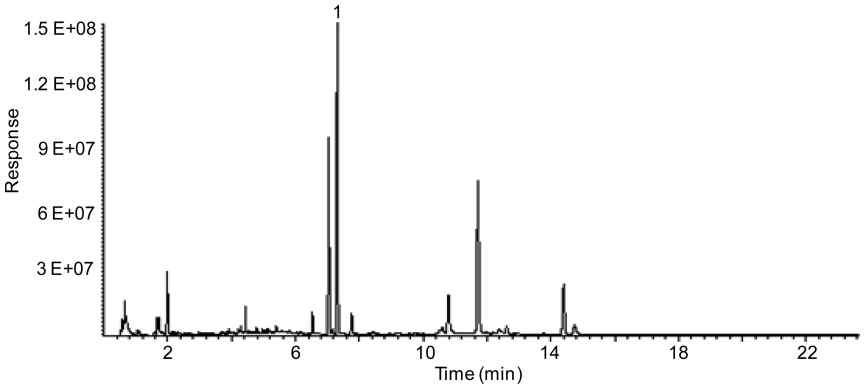

Figure 5. GC-ECD chromatograms of DPX extracted from celery from produce. Separation was performed using a DB-608 column. Pesticides identified correspond to (peak 1) dicloran.

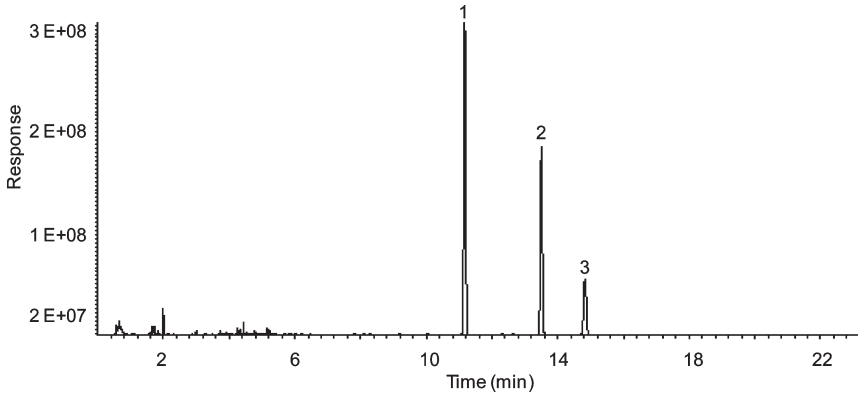

Figure 6. GC-ECD chromatograms of DPX extracted from tomatoes from produce. Separation was performed using a DB-608 column. Pesticides identified correspond to (peak 1) endosulfan 1, (peak 2) endosulfan II, and (peak 3) endosulfan sulfate and bifenthrin.

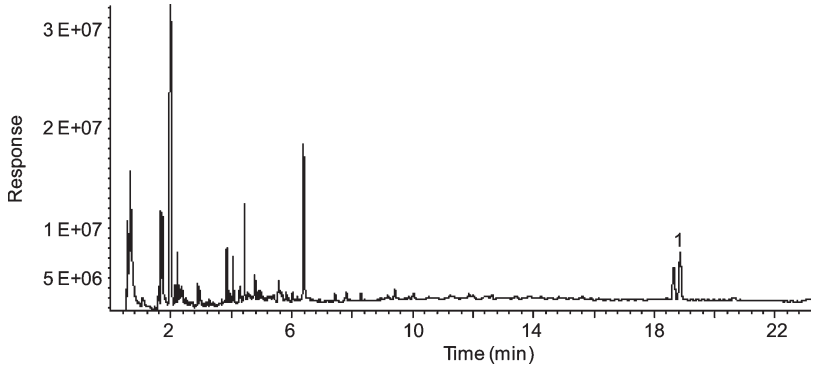

Figure 7. GC-ECD chromatograms of DPX extracted from spinach from produce. Separation was performed using a DB-608 column. Pesticides identified correspond to (peak 1) permethrin.

RSDs obtained for the studied pesticides using the DPX method. Recoveries range from 70.5 to 110.0%, indicating sufficient recoveries. RSD values for all studied pesticides were below 10% except for bifenthrin (10.6% in tomato) and chlorpyrifos-ethyl (13.5% in yellow squash). Note that this DPX method provides efficient and reproducible recoveries of chlorothalonil and captan, which are apparently difficult to recover using the Florisil cleanup procedure of the Luke method (*16*).

Application of the DPX Method for the Analysis of Produce Samples Using GC-ECD. Following the present method, DPX was able to detect pesticides in produce samples that were determined to be positive using the Luke method. **Table 3** summarizes pesticide concentrations determined using the Luke and DPX methods in the 9 week study. The DPX results were very comparable to those obtained by the Luke method with a few exceptions. Bifenthrin, captan. and chlorothalonil were found to be easily degraded in acetone solution. Because the DPX method was performed the day following the Luke method, these analytes were not detected in these samples (even though the extracts were stored overnight at −20 °C). The negative results of the three pesticides using the DPX method is most likely due to degradation, which is supported by the fact that these same pesticides gave good recoveries with DPX from spiked and freshly processed samples.

Figures 5−**7** show GC-ECD chromatograms of produce samples (celery, tomatoes, and spinach) with unknown pesticide application history after DPX extraction. As can be seen, DPX is able to provide clean extracts for different produce sample matrices including celery and spinach.

Figure 8 shows plots of correlation between the Luke and DPX methods for analysis of samples during the 9 week study. Correlation coefficients were 0.9868 and 0.9565 for all pesticides (**A**) and for pesticide concentrations below 1.0 ppm (**B**), respectively, which indicates a good agreement in results from using the DPX and Luke methods. The slopes of correlation plots are slightly higher than 1, and paired *t* tests provided *p* values below 0.05 (0.01 and 0.000025 for all pesticides and pesticide concentrations below 1.0 ppm, respectively), indicating that the DPX extraction produced higher recoveries than the modified Luke method for the analysis of produce samples.

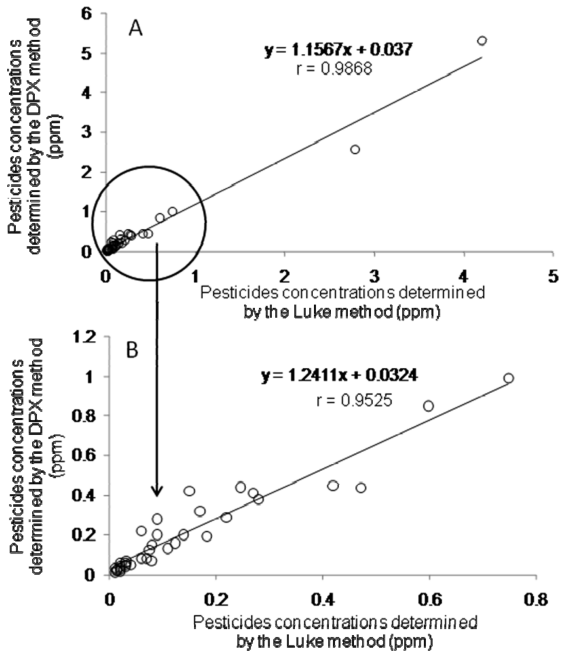

Figure 8. (**A**) Correlation between the Luke and DPX methods for determination of pesticides in fruits and vegetables during the 9 week study (full graph). (**B**) Correlation between the Luke and DPX methods for determination of pesticides below 1.0 ppm in fruits and vegetables during the 9 week study.

Application of the Micro-Luke Method for the Analysis of Produce Samples Using GC-NPD. Our previous study demonstrated that a DPX method using reversed phase sorbent SDVB is not suitable for analysis of polar pesticides due to its hydrophobicity nature (*14*). A "micro-Luke" method, which was modified from a previous Luke method (*9*), was employed for the analysis of polar pesticides using GC-NPD in the same study. **Table 4** is for comparison of pesticide concentrations in produce samples determined using the Luke and micro-Luke methods during the 9 week period field study, and **Figure 9** shows the correlation of the two methods. The correlation coefficient was determined to be 0.9827, indicating good agreement between the micro-Luke and Luke methods. Moreover, statistical analysis (paired *t* test) shows that there is no significant difference between the micro-Luke and Luke methods ($p = 0.047$).

The aim of the current work was to develop a simple, rapid, and reliable sample preparation method, suitable for the extraction of

Table 4. Pesticide Concentrations Determined Using the Luke and Micro-Luke Methods in Produce Samples Using GC-NPD

sample ID	matrix	pesticide	Luke method (ppm)	micro-Luke method 3 (ppm)	EPA tolerance (ppm)
63643	yellow squash	carbofuran	10.90	16.03	*
61824	cucumbers	chlorpyrifos-ethyl	0.079	1.070	*
66563	apple slices	DPA	0.493	0.732	10.0
66563	apple slices	thiabendazole	0.564	0.788	10.0
66564	blueberries	malathion	0.136	0.188	8.0
66560	tomato-SPK	DEF	0.760	1.068	*
60081	Gala apples	dursban	0.0274	0.0471	1.5
60082	Gala apples	thiabendazole	0.7869	1.1844	10.0
60083	Gala apples	azinphos-methyl	0	0.3652	2.0
66251	peaches	phosmet	0.0758	0.0858	10.0
60082	cucumbers-SPK	demeton	0.4318	2.0617	*
65871	acephate	acephate	2.25	2.46	*
65871	thiabendazole	thiabendazole	5.56	6.99	*
64891	yellow squash	dichlorovos	1.00	1.13	*
65270	peaches	carbaryl	0.29	0.63	10.00
65285	apples	DPA	0.74	0.61	10.00
66473	yellow squash	diazinon-O	4.22	4.04	*

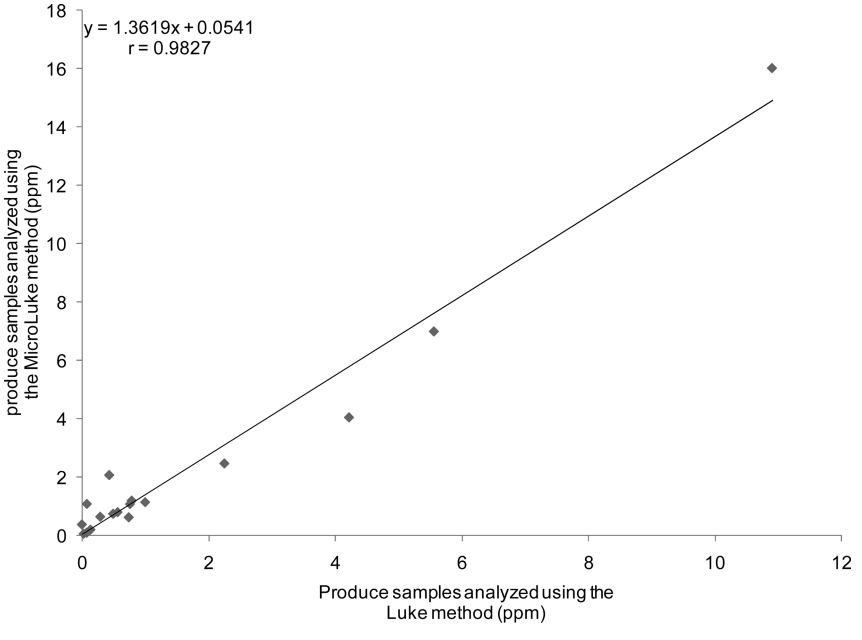

Figure 9. Correlation between the Luke and micro-Luke methods for determination of pesticides in fruits and vegetables during the 9 week study.

pesticides from fruits and vegetables using acetone. Due to the large number of samples submitted for analysis, the speed of analysis and high throughput are important issues in real world sample analysis. DPX alleviates the tedious nature of sample preparation, especially compared to the traditional Luke method. Using the combined micro-Luke method with DPX, multiresidue pesticide analysis is possible that is both fast and efficient using acetone extraction.

ABBREVIATIONS USED

DPX, disposable pipette extraction; HC, hydrocarbon; LOD, limit of detection; LOQ, limit of quantitation; OC, organochlorine; OP, organophosphate; ON, organonitrogen; PSA, primary−secondary amine; % RSD, relative standard deviation; SCDA, South Carolina Department of Agriculture; SDVB, styrene−divinylbenzene; s_{bl}, standard deviation of the blank; SPE, solid phase extration.

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