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Disposable pipette extraction for the analysis of pesticides in fruit and vegetables using gas chromatography/mass spectrometry

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ABSTRACT

Organochlorine, organophosphate pesticides and fungicides in fruits and vegetables were analyzed using disposable pipette extraction (DPX) followed by gas chromatography–mass spectrometry-selective ion monitoring (GC/MS-SIM). The intrinsic rapid mixing capabilities of DPX result in fast and efficient extractions, and eluates are concentrated by using minimal elution solvent volumes rather than solvent evaporation methods. Matrix-matched calibrations were performed with reversed phase mechanisms (DPX-RP), and the limits of detection (LOD) were determined to be lower than 0.1 μg/mL for all targeted pesticides in carrot and orange sample matrices. Coefficients of determination (r^2) were greater than 0.995 for most studied pesticides. DPX-RP exhibited recoveries between 72 and 116% for nonpolar and slightly polar pesticides ($\log P > 2$) with most of the recoveries over 88%. Only very polar pesticides (e.g., acephate, mathamidophos) were not extracted well using DPX-RP.

1. Introduction

Pesticides have been widely used to prevent or destroy agricultural pests and thereby improve food production throughout the world. However, extensive use of pesticides may pose potential health risks to humans if harmful residues appear in foods. The healthy food pyramid recommends eating fruits and vegetables for several reasons, including vitamins, cancer prevention, and low calories. Routine and comprehensive testing of multiresidue pesticides in fruits and vegetables is important for regulatory agencies to ensure that concentrations of toxic pesticides are below tolerance levels.

Development of simple and reliable methods for the analysis of trace contaminants in fruits and vegetables is a particularly challenging task. After initial extraction using organic solvent (typically acetonitrile or acetone) [1], cleanup must be performed to avoid false positive results due to matrix effects. Conventional liquid–liquid extraction (LLE) is time-consuming, laborious, and usually involves significant glassware usage and disposal of large volumes of hazardous organic waste [2]. Solid phase extraction (SPE) techniques have gained increasing interest because of their selectivity and because large volumes of organic solvents are not necessary. Almost all adsorbent types can be packed into the SPE column format, and the use of molecularly imprinted polymers expands the range of binding mechanisms [3]. SPE cartridges have been widely employed for extraction and concentration of pesticides from a broad range of sample matrices prior to chromatographic analysis [4–6]. Solid phase microextraction (SPME) has also been used because of its solvent-free nature [7–10]. However, SPME fibers can be somewhat expensive and fragile. Another technique, matrix solid phase dispersion (MSPD), is based on dispersion of solid or liquid sample on an adsorbent, such as florisil, C_{18}, alumina, or silica. The elution of target pesticides is achieved by transferring the sorbent and sample mixture to an extraction column and eluting the target analytes with organic solvents. MSPD combines sample extraction and cleanup, but it requires large amounts of adsorbent and solvent [11,12]. A newer approach, stir-bar sorptive extraction (SBSE), adsorbs pesticide residues on the polymer coating of a magnetic rod during stirring of the sample solution. Pesticides are thermally desorbed in the GC inlet for analysis [13], much like SPME. However, at this time, commercially available coatings for SBSE seem to be limited to polydimethylsiloxane (PDMS) [3,14] which does not provide good recoveries for polar pesticides. Finally, supercritical fluid extraction (SFE) has been used to pre-concentrate pesticides from food samples for chromatographic analysis [15]. However, SFE often requires separate optimization for different analyte types, and may not extract different classes of pesticides in foods with the same efficiency [16].

The QuEChERS method (quick, easy, cheap, effective, rugged, and safe) has recently attracted attention for pesticide analysis [17–19]. This approach removes fatty acid components and pigments from acetonitrile extracts rather than extract-

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Table 1
Pesticides and their properties.

Pesticide	Use[a]	Class[b]	Formula	MW[c] (g/mol)	Vp[d] (mmHg)	Solubility in water (g/kg)[e]	log *P*[f]
Acephate	I	OP	$C_4H_{10}NO_3PS$	183	1.7×10^{-6}	818,000	−0.85
Aldrin	I	OC	$C_{12}H_8Cl_6$	365	6.6×10^{-6}	0.027	6.5
Alpha-BHC	I	OC	$C_6H_6Cl_6$	291	4.5×10^{-5}	2	3.8
Beta-BHC	I	OC	$C_6H_6Cl_6$	291	3.6×10^{-7}	0.24	3.78
Delta-BHC	I	OC	$C_6H_6Cl_6$	291	3.3×10^{-5}	7	4.14
Gamma-BHC	I	OC	$C_6H_6Cl_6$	291	4.20×10^{-5}	7.3	3.72
Bolstar	I	OP	$C_{12}H_{19}O_2PS_3$	322	6.3×10^{-7}	0.31	5.48
Captan	F	OC	$C_9H_8Cl_3NO_2S$	300.5	9.0×10^{-8}	5.1	2.8
Chlorpyrifos	I	OP	$C_9H_{11}C_{l3}NO_3PS$	350	1.7×10^{-5}	0.4	4.98
Chlorothalonil	F	OC	$C_8Cl_4N_2$	266	5.7×10^{-7}	0.6	3.05
Coumaphos	I	OP	$C_{14}H_{16}ClO_5PS$	362	9.7×10^{-8}	1.5	4.13
4,4′-DDD	I	OC	$C_{14}H_{10}Cl_4$	320	1.0×10^{-6}	0.02	6.02
4,4′-DDE	I	OC	$C_{14}H_8Cl_4$	318	6.5×10^{-6}	0.1	6.51
4,4′-DDT	I	OC	$C_{14}H_9Cl_5$	354	1.9×10^{-7}[d]	0.0055	6.91
Demeton-S	I	OP	$C_8H_{19}O_3PS_2$	258	1.0×10^{-3}	60	2.09
Diazinon	I	OP	$C_{12}H_{21}N_2O_3PS$	304	6×10^{-5}	60	3.81
Dichlorvos	I	OP	$C_4H_7Cl_2O_4P$	221	0.02	10,000	1.47
Dieldrin	I	OC	$C_{12}H_8Cl_6O$	381	3.0×10^{-6}	0.2	5.4
Disulfoton	I	OP	$C_8H_{19}O_2PS_3$	274	1.5×10^{-4}	2	4.02
Endosulfan i	I	OC	$C_9H_6Cl_6O_3S$	407	1.7×10^{-7}	0.32	3.83
Endosulfan ii	I	OC	$C_9H_6Cl_6O_3S$	407	1.7×10^{-7}	0.32	3.52
Endosulfan sulfate	I	OC	$C_9H_6Cl_6O_4S$	423	1.0×10^{-5}	0.22	3.66
Endrin	I	OC	$C_{12}H_8Cl_6O$	381	2×10^{-7}	0.23	5.2
Endrin aldehyde	I	OC	$C_{12}H_8Cl_6O$	381	2×10^{-7}	0.024	4.80
Ethoprophos	I	OP	$C_8H_{19}O_2PS_2$	242	3.8×10^{-4}	750	3.59
Fenthion	H	OP	$C_{11}H_{17}O_4PS_2$	308	2.78×10^{-5}	4.2	4.09
Fensulfothion	I	OP	$C_{10}H_{15}O_3PS_2$	278	5.0×10^{-5}	1540	2.23
Heptachlor	I	OC	$C_{10}H_5Cl_7$	374	4×10^{-4}	0.056	6.1
Heptachlor epoxide	I	OC	$C_{10}H_5Cl_7O$	390	1.95×10^{-5}	0.2	4.98
Merphos	I	OP	$C_{12}H_{27}PS_3$	298	2×10^{-5}	0.0035	7.67
Methoxychlor	I	OC	$C_{16}H_{15}Cl_3O_2$	346	2.58×10^{-6}	0.1	5.08
Methyl parathion	I	OP	$C_8H_{10}NO_5PS$	263	1.5×10^{-5}	60	2.86
Mevinphos	I	OP	$C_7H_{13}O_6P$	224	1.3×10^{-4}	600,000	0.13
Methamidophos	I	OP	$C_2H_8NO_2PS$	141	3.5×10^{-5}	1,000,000	−0.80
Phorate	I	OP	$C_7H_{17}O_2PS_3$	260	6.4×10^{-4}	22	3.56
Ronnel	I	OP	$C_8H_8Cl_3O_3PS$	321	7.5×10^{-5}	1	4.88
Stirofos	I	OP	$C_{10}H_9Cl_4O_4P$	366	4.2×10^{-8}	11	3.53
Tokuthion	I	OP	$C_{11}H_{15}C_{l2}O_2PS_2$	345	9.4×10^{-6}	0.07	5.67
Trichloronat	I	OP	$C_{10}H_{12}Cl_3O_2PS$	333	1.5×10^{-5}	50	5.23

[a] I: insecticide; F: fungicide; H: herbicide.
[b] OC: organochlorine pesticides; OP: organophosphate pesticides.
[c] MW: molecular weight.
[d] Vp: vapor pressure [22,23].
[e] Solubility in water [22,23].
[f] log *P*: octanol–water partition coefficient of a neutral compound at equilibrium [21–23].

ing and isolating pesticides. The QuEChERS methods utilize primary–secondary amine (PSA) or aminopropyl sorbent to bind fatty acid compounds and $MgSO_4$ to remove water. Some modifications of QuEChERS include other sorbent material such as C18 or graphitized carbon black to further remove sample matrix components. The main advantage of QuEChERS is that it is comprehensive, being useful for the analysis of pesticides of varying polarities, by virtue of the fact that the sorbent used focuses on binding sample matrix compounds without interacting with the target analytes. The QuEChERS methods can be performed using cartridges (like most SPE cartridges) or used in a "dispersive" manner where the sorbent is mixed with the sample solution and subsequently separated through centrifugation. Much of the literature with QuEChERS suggests that better results are obtained using the dispersive procedure, where mixing with the loose sorbent provides efficient removal of matrix compounds and provides higher recoveries of pesticides with minimal solvent.

The focus of the present research is development and validation of rapid multiresidue methods for the analysis of pesticides in fruits and vegetables using disposable pipette extraction (DPX) followed by GC/MS analysis. In DPX, the solid phase sorbent is contained inside a disposable pipette tip and is thoroughly mixed with sample solutions. Dynamic mixing uses less sorbent and provides faster extractions compared to classical SPE. Analytes are concentrated on the sorbent and can be dispensed in concentrated solution, thus reducing the need for solvent evaporation. In this paper, the use of reversed phase (RP) mechanisms using DPX-RP for extraction of pesticides is shown to be useful for the rapid analysis of nonpolar and slightly polar pesticides. The DPX method is shown to be very rapid, taking just a few minutes to perform without any solvent evaporation. In addition, a few compounds that are problematic with QuEChERS are shown to be easily detected and analyzed using DPX-RP, which suggests that this method can be complimentary to QuEChERS without substantially extending extraction times.

2. Materials and methods

2.1. Reagents

Hexane (ACS grade, Fisher scientific, Fairlawn, NJ), acetonitrile (analytical grade, Mallinckrodt Baker, Phillipsburg, NJ), and ethyl acetate (analytical grade, Mallinckrodt Chemical, Paris, Kentucky) were employed. Sodium chloride (analytical reagent grade) was purchased from Fisher Scientific (Fairlawn, NJ). Mixed sorbents containing graphitized carbon black (GCB), primary–secondary amine (PSA), and magnesium sulfate ($MgSO_4$) were provided by the South Carolina Department of Agriculture (Columbia, SC).

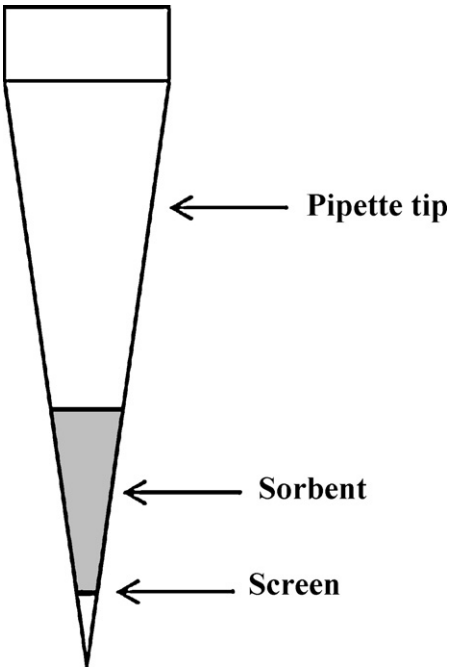

Fig. 1. Schematic diagram of a DPX tip.

2.2. Pesticide standards

All pesticides were purchased from ULTRA Scientific (N. Kingstown, RI), except captan and chlorothalonil, which were provided by the South Carolina Department of Agriculture (Columbia, SC). Working solutions of standards were prepared by dissolving original stock solutions in acetonitrile and diluting to 20 μg/mL. Table 1 summarizes properties of the pesticides used in this work.

External standards were prepared by dissolving 10 mg of D_{10}-parathion-diethyl (Sigma–Aldrich, St. Louis, MO, USA) in acetone, and diluting to 20 μg/mL with hexane/ethyl acetate (50/50, v/v) or acetonitrile. All working solutions were stored in glass vials at −20 °C prior to use.

2.3. Fruit and vegetable samples

Carrots and oranges used as matrices were provided by the South Carolina Department of Agriculture and were stored in the freezer prior to use to prevent spoilage.

2.4. Sample preparation

Initial sample preparation was identical to that used for QuEChERS [17]. An amount (15.0 ± 0.1 g) of ground organic carrots or oranges was weighed into a 50 mL centrifuge tube, and 15 mL of acetonitrile was added. The resulting solution was shaken for 1 min followed by the addition of sodium chloride (1.5 ± 0.1 g) and anhydrous magnesium sulfate (6.0 ± 0.3 g). The centrifuge tube was shaken vigorously for 1–2 min to prevent salt agglomeration before centrifugation at 3000 rpm for 10 min. The supernatant was used for further DPX extraction.

2.4.1. DPX procedures

DPX-RP tips (5 mL) containing styrene divinylbenzene (SDVB, 60 mg) were obtained from DPX Labs, LLC (Columbia, SC, USA). A schematic diagram of a DPX tip is shown in Fig. 1. A 1 mL aliquot of the initial extract of fruit or vegetable was used for extraction. Deionized (DI) water (2.4 mL) and saturated sodium chloride (0.8 mL) were added and mixed. The total solution was then aspirated into the DPX-RP tip twice from the bottom (to ensure a good mix of SDVB with sample solution) followed by an equilibration time of 30–60 s. The solution was dispensed to waste, and 0.5 mL of DI water was added to the top of DPX tip and dispensed to remove salt and water soluble matrix interferences. Pesticides were eluted by adding 0.7 mL of hexane/ethyl acetate (50/50, v/v) to the top of DPX tip, and dispensing the organic solvent through the sorbent and screen of the DPX tip into a GC vial. A small volume of immiscible water at the bottom of the vial was removed with a disposable Pasteur pipette, and 25 μL of external standard solution was added before injection. The use of 0.7 mL elution volume results in a final volume of approximately 0.5 mL due to solvent exchange of the solvent with water in the sorbent. No further solvent evaporation steps were performed. This procedure gives a final concentration factor of 2.

2.4.2. DPX study of polar pesticides

In a separate study, 8 mL of DI water was added to the original extract to further reduce the percentage of organic solvent in hopes to improve recoveries of polar pesticides. The DPX-RP tip was mixed with the solution 3 separate times with approximately 4 mL each time, and the pesticides were eluted with 0.7 mL of hexane/ethyl acetate (50/50, v/v).

2.5. GC/MS system and parameters

Analysis of pesticides was performed on a model 6890 gas chromatograph with a model 5972A mass selective detector (Agilent Technologies, Little Falls, DE). The instrument was equipped with a DB-17 column (50%-phenyl-methylpolysiloxane coated column, 30 m × 0.25 mm ID, 0.25 μm film thickness, Agilent Technologies). The carrier gas was ultra-pure helium at constant flow of 1.0 mL/min. The inlet temperature was set at 250 °C. The total GC analysis time was 19 min with the oven programmed to hold 1 min at 80 °C, ramp at 20 °C/min to final temperature 280 °C, and then held at 280 °C for 8 min. Injections of 2 μL were made in splitless mode with an Agilent 6890 Series Injector.

The mass spectrometer (MS) was operated in electron ionization (EI) mode at 70 eV. The source temperature was 230 °C, and the MS transfer line temperature was set at 290 °C. Detection was accomplished in selected ion monitoring (SIM) mode. The identification of pesticide peaks was confirmed by matching retention times of standards (within ±0.02 min), and by the presence of major ions. MS information for the pesticides is summarized in Table 2.

2.6. Spiked recovery study

Recovery studies of DPX-RP were conducted using 15.0 g of fruit and vegetables spiked with pesticides, followed by initial extraction as described above. To reduce or eliminate matrix interferences, a matrix-matched sample was obtained by spiking the same amount of pesticides to a blank extract following the DPX procedures. To compensate for variations in the final volume, 25 μL of external standards was added to all final extracts before injection. Unless otherwise noted, all calculations reported in this study were based on peak area ratios of analytes to external standard. Recoveries were calculated by the following equation:

$$\%\ \text{recovery} = \frac{\text{peak area of pesticide in sample/peak area of external standard in sample}}{\text{peak area of pesticide in matrix standard/peak area of external standard in matrix standard}} \times 100 \quad (1)$$

Table 2
MS information for the targeted pesticides.

Pesticide	Major ions (*m*/*z*)	Identification ions for SIM method (*m*/*z*)	Quantitation ion (*m*/*z*)
Acephate	94, 136	94, 136	136
Aldrin	66, 79, 91, 101, 263, 293	66, 263, 293	293
Alpha-BHC	109,111, 181, 183, 219	111, 181, 219	219
Beta-BHC	109,111, 181, 183, 219	109, 181, 219	219
Delta-BHC	109,111, 181, 183, 219	109, 181, 219	219
Gamma-BHC	109,111, 181, 183, 219	109, 181, 219	219
Bolstar	125, 139, 140, 156, 322	139, 156, 322	139
Captan	79, 151	79, 151	151
Chlorpyrifos	97, 197, 199, 258, 286, 314	97, 197, 314	314
Chlorothalonil	268, 266, 264	266, 268, 264	268
Coumaphos	97, 109, 210, 226, 362	109, 226, 362	226
4,4′-DDD	75, 165, 235, 237	165, 235, 237	235
4,4′-DDE	176, 246, 248, 316, 318	176, 246, 318	246
4,4′-DDT	75, 165, 199, 235, 237	165, 199, 235	235
Demeton-S	60, 81, 88, 170	60, 88, 170	88
Diazinon	137, 152, 179, 199, 304	137, 152, 179	137
Dichlorvos	79, 109, 185	79, 109, 185	185
Dieldrin	79, 81, 263	79, 81, 263	263
Disulfoton	88, 89, 97, 125, 142, 274	88, 97, 274	88
Endosulfan i	195, 237, 241, 265, 339	195, 241, 339	195
Endosulfan ii	109, 159, 170, 195, 237	170, 195, 237	195
Endosulfan sulfate	170, 229, 237, 272, 387	237, 272, 387	237
Endrin	67, 79, 81, 263, 345	81, 263, 345	345
Endrin aldehyde	67, 250, 345	67, 250, 345	345
Ethoprophos	97, 126, 139, 158, 242	97, 139, 158	158
Fenthion	79, 109, 125, 153, 169, 278	109, 125, 278	278
Fensulfothion	97, 125, 141, 153, 293, 308	141, 293, 308	293
Heptachlor	100, 237, 272, 274, 270, 331, 374	100, 237, 272	100
Heptachlor epoxide	81, 237, 263, 351, 353, 355	81, 263, 353	81
Merphos	57, 113, 169, 202, 314	57, 169, 314	169
Methoxychlor	227, 288, 346	227, 288, 346	227
Methamidophos	94, 141	94, 141	141
Methyl parathion	63, 79, 93, 109, 125, 263	109, 125, 263	263
Mevinphos	67, 109, 127, 192	109, 127, 192	127
Phorate	75, 97, 121, 260	75, 121, 260	75
Ronnel	79, 109, 125, 285, 287	125, 285, 287	285
Stirofos	79, 109, 329, 331	109, 329, 331	329
Tokuthion	43, 113, 162, 267, 309	113, 162, 267	267
Trichloronat	109, 267, 297	109, 267, 297	297

2.7. Method validation

Matrix-matched calibration was performed to account for potential matrix effects. Organochlorine and organophosphate pesticide standard working solutions were spiked into acetonitrile extracts of organic carrot and orange at five levels ranging from 0.1 to 2.0 μg/mL. Calibration data were generated from 6 replicate samples at 0.1 μg/mL, 2 replicate samples at 0.2 μg/mL, 2 replicate samples at 0.5 μg/mL, 2 replicate samples at 1.0 μg/mL, and 6 replicate samples at 2.0 μg/mL. Calibration plots were generated using programs written in MATLAB 7.0 (The Mathworks, Natick, MA). The LOD was determined as the concentration of analyte giving a signal to noise ratio (S/N) of 5 for the target ion; the limit of quantification (LOQ) was determined as the concentration of analyte giving a signal to noise ratio (S/N) of 10 for the target ion.

3. Results and discussion

3.1. Adsorption of pesticides to the DPX-RP sorbent

The retention mechanism for DPX-RP involves hydrophobic (similar to reversed phase LC) and π–π interactions with the styrene divinyl benzene sorbent. The combinations of these mechanisms provide a high potential for selective enrichment of most pesticides and removal of polar matrix interferences. Due to its hydrophobic nature, the sorbent interacts with the analytes through Van der Waals forces, and the aromatic character of pesticides enhances retention by π–π interactions with the sorbent. The logarithm of n-octanol/water partition coefficient (log *P*) is a measure of the hydrophobicity of a molecule [20]. Because retention of the target pesticides depends on the hydrophilic solvent/water ratio in the solution, recoveries of pesticides decreased as the solvent ratio (acetonitrile/water) increased in the sample solution. Water was added to the acetonitrile solution to decrease the percentage of organic solvent and increase retention of pesticides. Fig. 2A and B plot the extraction efficiency using DPX-RP versus log *P* value for each pesticide in carrots and oranges, respectively. Medium polar pesticides whose log *P* values were 2 or lower had recoveries below 60%. Recoveries of 90–100% were obtained for pesticides that have log *P* of 2.86 or above. The correlation between the retention for pesticide with DPX-RP is related to its log *P*, which enables estimation of the expected recovery of pesticides based on its hydrophobicity.

3.2. Elution mode for DPX-RP

Preliminary recovery studies showed that elution of pesticides from the top of DPX-RP tips (add solvent to the top of the upper barrier of the tip and then elute) yielded aproximately15% higher recoveries than from the bottom (aspirate solvent from the bottom of the tip and then elute). To achieve similar recoveries by bottom elution, at least two elutions from the bottom were required, and the samples were therefore diluted. Top elution was employed for all further work. By using hexane/ethyl acetate, water is separated from the eluate due to immiscibility. Hexane/ethyl acetate is an excellent "keeper" solvent that minimizes sample degradation, and is ideally suited for GC analysis with a variety of detectors. Extraction, solvent exchange, and concentration are accomplished in one

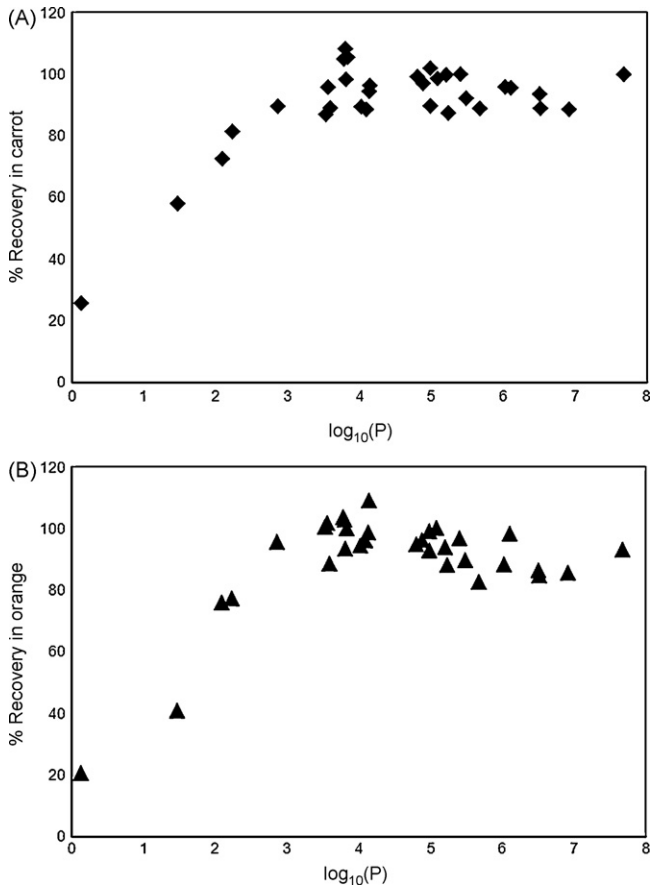

Fig. 2. (A) Relation between the percent recoveries of pesticides in carrot using DPX-RP and log *P*. (B) Relation between the percent recoveries of pesticides in orange using DPX-RP and log *P*.

Table 3
Percent recoveries and %RSD (in parentheses) based on 4 replicate experiments using DPX-RP for the analysis of pesticides in carrots and oranges.

Pesticides	Carrot	Orange
Aldrin	93.4 (4.8)	86.4 (1.6)
Alpha-BHC	108.0 (6.6)	102.8 (0.6)
Beta-BHC	104.7 (3.4)	103.6 (1.3)
Delta-BHC	96.2 (1.4)	109.0 (2.1)
Gamma-BHC	103.3 (3.8)	102.0 (0.2)
Bolstar	92.0 (2.5)	89.7 (0.2)
Captan	107.2 (7.3)	86. 8 (8.8)
Chlorpyrifos	89.6 (7.6)	92.9 (0.7)
Chlorothalonil	98.1 (5.3)	115.7 (9.3)
Coumaphos	94.3 (5.0)	98.7 (1.5)
4,4′-DDD	95.7 (3.0)	88.3 (0.8)
4,4′-DDE	88.8 (5.8)	84.8 (4.0)
4,4′-DDT	88.4 (4.9)	85.6 (0. 7)
Demeton-S	72.4 (3.0)	76.0 (1.2)
Diazinon	98.1 (3.5)	93.4 (1.4)
Dichlorvos	57.9 (3.8)	41.0 (1.8)
Dieldrin	99.8 (2. 8)	96.8 (1.0)
Disulfoton	89.3 (2.0)	94.4 (1.5)
Endosulfan i	105.3 (5.8)	100.0 (0.9)
Endosulfan ii	99.1 (6.6)	102.2 (2.4)
Endosulfan sulfate	98.6 (8.3)	107.6 (0.7)
Endrin	99.6 (1.1)	93.9 (0.5)
Endrin aldehyde	99.0 (3.2)	94.8 (1.5)
Ethoprophos	88.8 (2.6)	88.6 (2.0)
Fenthion	88.4 (6.2)	96.1 (0.7)
Fensulfothion	81.3 (5.0)	77.3 (1.9)
Heptachlor	95.4 (1.2)	98.3 (2.1)
Heptachlor epoxide	101.7 (2.9)	99.1 (0.3)
Merphos	99.7 (3.7)	93.1 (5.1)
Methoxychlor	98.4 (1.4)	100.1 (0.2)
Methyl parathion	89.4 (3.4)	95.6 (1.2)
Mevinphos	25.7 (6.5)	20.7(2.2)
Phorate	95.6 (1.5)	101.7 (1.5)
Ronnel	96.8 (2.0)	96.2 (1.2)
Stirofos	86.8 (5.0)	100.6 (2.4)
Tokuthion	88.7 (3.4)	82.6 (0.9)
Trichloronat	87.2 (6.3)	88.2 (0.6)

step. Although 0.7 mL of elution solvent was utilized, the final volume of eluate was approximately 0.5 mL due to solvent exchange as mentioned in Section 2. The use of external standard compensates for variations in the final volume of solvent.

3.3. Analysis of nonpolar pesticides

Pesticides were spiked in carrot and orange matrices at a final concentration of 0.5 μg/mL and extracted by DPX-RP. Table 3 lists the recoveries of the studied pesticides. Recoveries using DPX-RP were above 72% with relative standard deviations (RSD) less than 10% for all of the studied nonpolar pesticides (log $P > 2$). Recoveries were greater than 81% for 34 out of 36 studied pesticides in both carrots and oranges.

3.4. Analysis of slightly polar pesticides

As shown in Table 3, low recoveries (<60%) were obtained with the slightly polar pesticides dichlorvos and mevinphos (log $P < 2$) using DPX-RP. The polar nature of these compounds is readily suggested by their chemical structures (Fig. 3). For nonpolar to slightly polar pesticides, GC/MS-SIM chromatograms for targeted OPs and OCs using DPX-RP are shown in Fig. 4A and B. Extracted ion chromatograms for peak identification using major ions for each analyte are shown in the insets.

By adding additional water to the original acetonitrile extract (study described in Section 2 under the heading DPX study of polar pesticides), the DPX-RP recoveries of dichlorvos and mevinphos increased from 58 and 26% to 91 and 74%, respectively. However, this dilution did not improve recoveries for several very polar pesticides.

3.5. Analysis of very polar pesticides

Reversed phase sorbent is also not suitable for the analysis of the very polar pesticides, such as acephate and methamidophos. Poor results were obtained for these analytes. It is possible to incorporate a polar sorbent to extract these compounds using DPX technology.

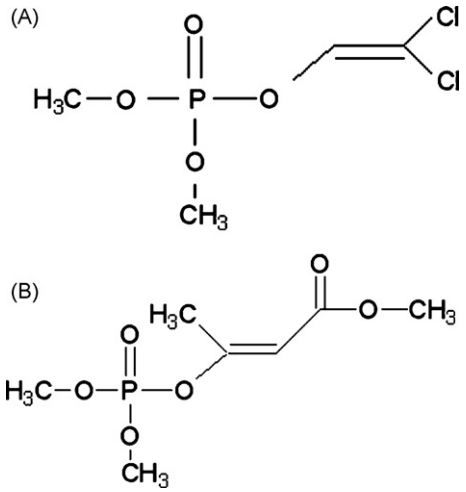

Fig. 3. Chemical structures of dichlorvos and mevinphos.

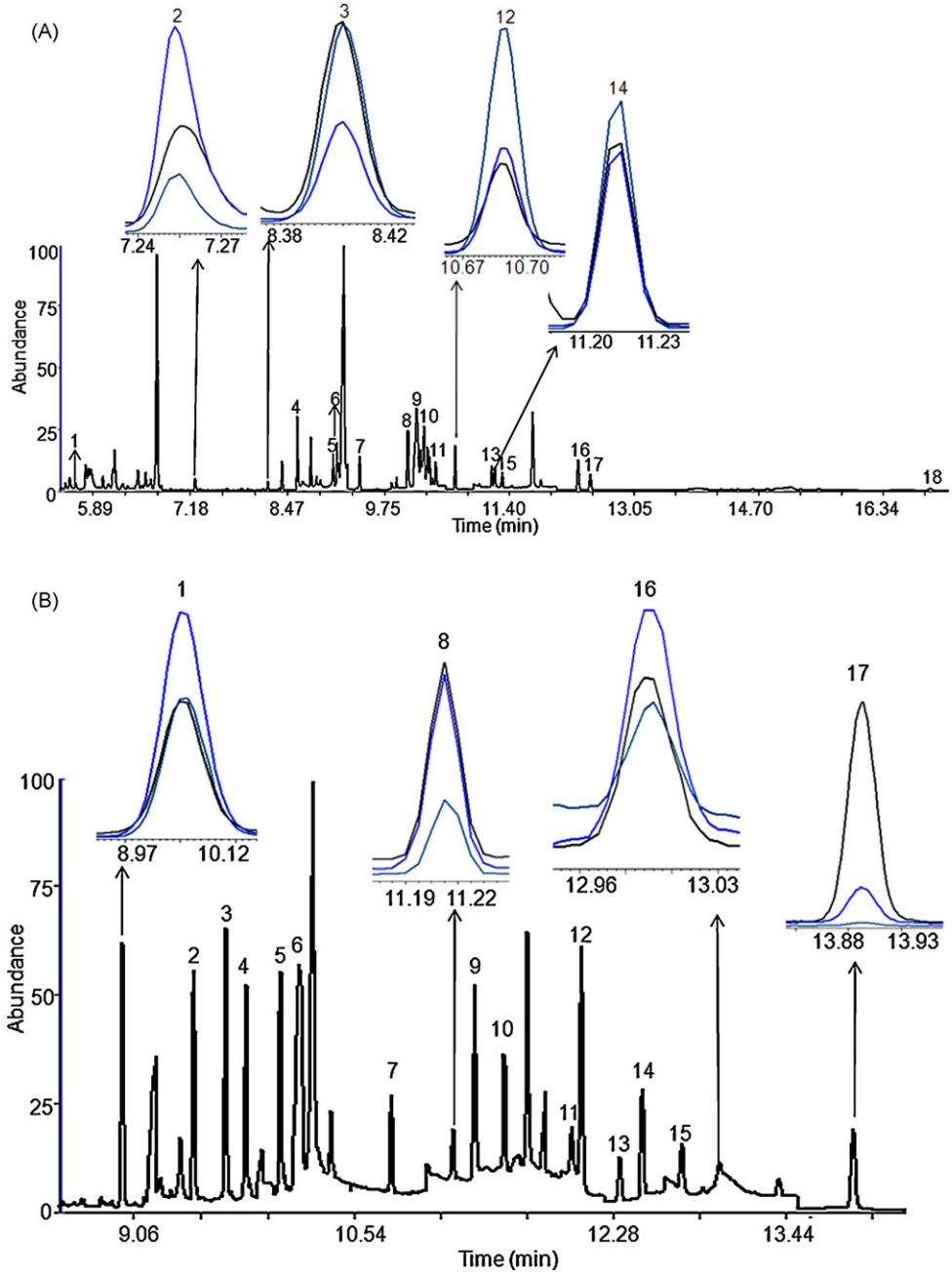

Fig. 4. (A) SIM chromatogram of organophosphate pesticides spiked at 0.5 ppm in orange after DPX-RP extraction. Peak identification in the order of increasing retention time is as follows—1: dichlorvos; 2: mevinphos; 3: ethoprophos; 4: phorate; 5: demeton-S; 6: diazinon; 7: disulfoton; 8: ronnel; 9: methyl parathion; 10: trichloronat; 11: chlorpyrifos; 12: fenthion; 13: merphos; 14: tokuthion; 15: stirofos; 16: bolster; 17: fensulfothion; 18: coumaphos. Examples of peak identification using major identification ions are shown in the insets. (B) SIM chromatogram of organochlorine pesticides spiked at 0.5 ppm in orange after DPX-RP extraction. Peak identification in the order of increasing retention time is as follows—1: alpha-BHC; 2: gamma-BHC; 3: beta-BHC; 4: delta-BHC; 5: heptachlor; 6: aldrin; 7: heptachlor epoxide; 8: endosulfan i; 9: 4,4′-DDE; 10: dieldrin; 11: endrin; 12: 4,4′-DDD; 13: endosulfan ii; 14: 4,4′-DDT; 15: endrin aldehyde; 16: endosulfan SO_4; 17: methoxychlor. Examples of peak identification using major identification ions are shown in the insets.

We have found that it is actually easier to target these very polar pesticides by trying to extract the sample matrix rather than bind these compounds (i.e., QuEChERS method using QuEChERS "tips"), and this is a subject of a separate study.

3.6. Calibration, linearity, LOD, and LOQ

Table 4 summarizes the matrix-matched calibration results, along with LOD and LOQ values for the pesticides studied. The calibration plots exhibit good linearity for pesticides ranging from 0.1 to 2.0 μg/mL. Average coefficients of determination were greater than 0.995. For all 35 studied pesticides, the LODs were less than 0.01 μg/mL. Because LODs and LOQs are matrix dependent, it is recommended to perform matrix-matched calibration for quantitative analysis for unknown samples in complex matrices such as fruit and vegetables. It should be noted that lower LODs and LOQs are achievable by injecting larger volumes of eluate (using large volume injection) or by performing solvent evaporation prior to analysis. In this study, only 1 mL of the acetonitrile extract was used to give a concentration factor of 2.

Matrix interferences may be noted for some qualifier ions, especially those of low masses, at low concentrations below 100 ppb.

Table 4
Calibration, statistics, LOD, and LOQ for the studied pesticides using DPX-RP method.

Pesticide	r^2[a]		LOD (μg/g)[b]		LOQ (μg/g)[c]		Tol.[d] (μg/g)	
	Carrot	Orange	Carrot	Orange	Carrot	Orange	Carrot	Orange
Aldrin	0.998	0.998	0.0033	0.0040	0.0065	0.0080	NT[e]	NT
Alpha-BHC	0.999	0.999	0.0023	0.0025	0.0045	0.0050	NT	NT
Beta-BHC	0.999	0.999	0.0029	0.0031	0.0058	0.0063	NT	NT
Delta-BHC	0.999	0.998	0.0133	0.0115	0.0267	0.0231	NT	NT
Gamma-BHC	0.999	0.999	0.0029	0.0050	0.0058	0.0100	NT	NT
Bolstar	0.998	0.998	0.0036	0.0022	0.0071	0.0043	NT	NT
Chlorpyrifos	0.999	1.000	0.0018	0.0020	0.0036	0.0040	0.1	1.0
Coumaphos	0.998	0.998	0.0139	0.0139	0.0278	0.0278	NT	NT
4,4′-DDD	0.999	0.997	0.0004	0.0013	0.0008	0.0025	NT	NT
4,4′-DDE	0.996	0.998	0.0008	0.0020	0.0017	0.0040	NT	NT
4,4′-DDT	0.997	0.996	0.0007	0.0017	0.0015	0.0033	NT	NT
Demeton-S	0.997	1.000	0.0016	0.0013	0.0031	0.0026	NT	NT
Diazinon	1.000	1.000	0.0036	0.0025	0.0071	0.0050	0.75	NT
Dichlorvos	0.998	0.997	0.0088	0.0083	0.0177	0.0167	NT	NT
Dieldrin	0.999	0.999	0.0083	0.0100	0.0167	0.0200	NT	NT
Disulfoton	1.000	0.998	0.0019	0.0025	0.0038	0.0050	NT	NT
Endosulfan i	0.999	0.999	0.0100	0.0481	0.0200	0.0962	0.2	NT
Endosulfan ii	0.997	0.998	0.0094	0.0174	0.0188	0.0348	0.2	NT
Endosulfan sulfate	0.999	0.995	0.0083	0.0273	0.0167	0.0545	0.2	NT
Endrin	0.999	0.999	0.0129	0.0200	0.0258	0.0400	NT	NT
Endrin aldehyde	0.998	0.999	0.0031	0.0050	0.0062	0.0100	NT	NT
Ethoprophos	1.000	0.999	0.0010	0.0028	0.0021	0.0056	NT	NT
Fenthion	0.999	1.000	0.0002	0.0005	0.0004	0.0011	NT	NT
Fensulfothion	0.998	0.999	0.0009	0.0025	0.0018	0.0050	NT	NT
Heptachlor	0.998	0.998	0.0010	0.0029	0.0020	0.0059	NT	NT
Heptachlor epoxide	0.998	0.999	0.0100	0.0286	0.0200	0.0571	NT	NT
Merphos	0.999	0.996	0.0035	0.0034	0.0069	0.0068	NT	NT
Methoxychlor	0.998	0.996	0.0006	0.0017	0.0013	0.0033	NT	NT
Methyl parathion	0.999	0.998	0.0021	0.0100	0.0043	0.0200	1.0	NT
Mevinphos	0.994	0.999	0.0059	0.0052	0.0118	0.0104	NT	NT
Phorate	1.000	0.999	0.0009	0.0008	0.0017	0.0017	NT	NT
Ronnel	0.999	1.000	0.0005	0.0003	0.0010	0.0006	NT	NT
Stirofos	0.999	0.996	0.0014	0.0021	0.0029	0.0042	NT	NT
Tokuthion	0.999	0.998	0.0017	0.0009	0.0033	0.0018	NT	NT
Trichloronate	0.999	0.999	0.0010	0.0009	0.0020	0.0018	NT	NT

[a] Coefficient of determination.
[b] Limit of detection (LOD) is based on a S/N ratio of 5 (where S is the signal of the target ion and N is the noise intensity).
[c] Limit of quantitation (LOQ) is based on a S/N ratio of 10 (where S is the signal of the target ion and N is the noise intensity).
[d] Tolerance levels from the U.S. Code of Federal Regulations, vol. 40.
[e] NT stands for no tolerance listed in reference.

Despite these limitations, the results indicate that the limits of detection and quantitation are sufficient for food safety purposes. There are only a few tolerance levels in carrots and oranges provided for these pesticides studied. According to the U.S. Code of Federal Regulations (vol. 40, Table 4), chlorpyrifos has a tolerance level of 0.1 and 1.0 μg/g in carrots and oranges, respectively. The DPX method in this study obtained LODs of 0.0018 and 0.0020 for carrots and oranges, respectively. Likewise with diazinon, endosulfan i, endosulfan ii, endosulfan sulfate, and methyl parathion, the DPX method obtained LODs significantly lower than listed tolerance levels.

It is also noteworthy that larger volumes of extracts of fruit and vegetables (such as 2.5 mL of acetonitrile solution) can be extracted with the DPX-RP tips used in this study (1 mL) [21]. In this case, 2.5 mL of acetonitrile solution can be diluted to the same ratio as used in this study to make a final volume of 10.5 mL. Using a 5 mL DPX-RP tip, the extraction can be performed with 3 separate extractions of approximately 3.5 mL of the diluted solution. These 2 additional DPX extractions take only a couple of minutes longer to perform the extraction, and the pesticides are concentrated onto the sorbent without additional wash or elution steps. Using just 1 wash step and elution with just 0.7 mL of hexane/ethyl acetate (to give approximately 0.5 mL of final eluate), a concentration factor of 5 can be obtained for the pesticides without any solvent evaporation. It is most likely that the LODs and LOQs for most of these pesticides studied could have been 2.5 times lower by incorporating this change in the procedure. The point is that this DPX-RP method can be optimized to provide high sensitivity for the analysis of nonpolar pesticides. The use of GC/MS/MS may be recommended for obtaining LODs below 100 ppb in order to provide greater selectivity and confidence in pesticide identification and quantitation. This may be especially true for other complex sample matrices besides carrots and oranges.

3.7. Advantages and disadvantages of DPX-RP

The main advantage of DPX-RP is that it has a built-in concentration step in a suitable solvent without requiring solvent evaporation. The extraction of slightly polar and nonpolar pesticides from fruit and vegetables has been demonstrated to be efficient due to the hydrophobic nature of the DPX-RP sorbent. In addition, better reproducibility is generally observed because the extracts have less background from sample matrix components. However, the disadvantage of this method is that it is not suitable for the analysis of very polar pesticides. An alternative sorbent with increased polarity will be required for DPX in order to target extraction of very polar pesticides.

Another advantage of the DPX method is that automation is readily achievable. The extraction is so fast that the samples can be processed one sample at a time during the chromatographic analysis of a previous sample, providing high throughput analysis.

The analyst will only have to perform the initial sample preparation of extracting a representative sample with acetonitrile. The acetonitrile extract from each sample can then be placed on an autosampler for automated DPX extraction and injection for GC or HPLC/MS analysis. This is the focus of future research.

4. Conclusions

A rapid and sensitive method for the analysis of nonpolar and slightly polar pesticides in carrots and oranges has been demonstrated using DPX-RP. For compounds with log *P* values greater than 2, high recoveries of over 72% were obtained with relative standard deviations of less than 10%. These analyses were achieved with no solvent evaporation procedures. This demonstrates the feasibility of rapid, high throughput analysis of nonpolar pesticides by performing DPX extractions of acetonitrile extracts of fruit and vegetables in a few minutes with no additional concentration and solvent exchange steps.

Analysis of polar pesticides using DPX-RP was not successful. For the analysis of many of these pesticides, reversed phase mechanisms of extraction are not feasible. A polar sorbent will be required for direct extraction of polar pesticides using DPX.

Disclosure

William E. Brewer is a part owner of DPX Labs, which makes the DPX products used in this study.

Conflict of interest

Hongxia Guan and Stephen Morgan do not have any conflicts of interest to disclose.

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