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New Approach to Multiresidue Pesticide Determination in Foods with High Fat Content Using Disposable Pipette Extraction (DPX) and Gas Chromatography−Mass spectrometry (GC-MS)

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Organochlorine and organophosphate pesticides in corn muffin mix and cocoa beans were analyzed using disposable pipette extraction (DPX) for rapid cleanup followed by gas chromatography−mass spectrometry (GC-MS). The DPX method in this study used weak anion exchange (WAX) mechanisms to remove the major sample matrix interferences, fatty acids, from the chromatographic analyses. The limits of detection (LOD) were determined to be <10 ppb for all studied pesticides in corn muffin. DPX-WAX exhibited average recoveries reaching 100% for most targeted pesticides, with relative standard deviations below 10%. These results indicate that DPX with weak anion exchange sorbent is effective at eliminating fatty acid interferences in foods of high fat content prior to multiresidue pesticide analysis. Furthermore, the DPX cleanup method takes approximately 2 min to perform. In addition, removal of fatty acids from cocoa beans demonstrates the high capacity of this extraction method for samples containing up to 50% fat.

KEYWORDS: Disposable pipette extraction (DPX); weak anion exchange; corn muffin mix; cocoa beans; gas chromatography−mass spectrometry (GC-MS)

INTRODUCTION

Pesticides continue to contribute significantly to controlling and destroying various types of agricultural pests and thereby improving food production throughout the world. However, uncontrolled pesticide use has led to the deaths of animals and humans (*1*). The publication of Rachel Carson's book *Silent Spring* (*2*) is often acknowledged for increasing awareness of the potential health hazards posed by pesticides. Routine and comprehensive testing of multiresidue pesticides in food is important for regulatory agencies to ensure that concentrations of toxic pesticides are below tolerance levels.

Analysis of target pesticides at low concentrations, particularly in grain products and other food products of high fat content, requires cleanup or elimination of matrix interferences prior to chromatographic and mass spectrometric analysis. Although liquid−liquid extraction and gel permeation chromatography (GPC) have been commonly employed for cleanup with multiresidue pesticide analysis (*3*, *4*), solid-phase extraction (SPE) largely replaced these traditional methods due to its selectivity and elimination of large volumes of organic solvents (*5*−*9*). The presence of fatty acids produces severe interferences with gas chromatography of pesticides, and their removal is necessary prior to analysis. In recent years, there has been a great amount of interest in using SPE to remove sample matrix components from extracts, with most research focused on reducing or eliminating fatty acids (*3*−*5*, *10*, *11*). Florisil, strong anion-exchange sorbents (SAX) (*5*, *12*), and weak anion-exchange sorbents including primary−secondary amine (PSA) (*13*, *14*), aminopropyl (−NH_2) (*15*, *16*), and diethylaminopropyl (DEA) (*17*) have been investigated for removal of fatty acids naturally occurring in food samples. Of the sorbents involved in previous studies, Florisil was found to be unsuitable for multiresidue pesticide analysis because it strongly adsorbs polar pesticides, such as organophosphate pesticides (*18*). SAX was also reported to have little effect on the removal of fatty acids (*5*, *19*). The aminopropyl and PSA sorbents were found to provide the most effective cleanup (*5*), with PSA being more efficient due to its higher capacity compared to aminopropyl (*14*, *20*).

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was developed for the rapid analysis of multiresidue pesticides in fruits and vegetables (*14*, *21*−*23*). This method removes fatty acids using a PSA sorbent. However, the QuEChERS method is designed for fatty acid removal from fruit and vegetable extracts, and the PSA sorbent lacks capacity for removing fatty acids from samples high in fat content, such as grains and beans. The combination of PSA and octadecylsilyl (C-18) has been reported to provide better results than PSA alone for removal of fatty acid in grain products (corn, oat, rice, wheat), possibly due to combining the hydrophobicity of the C-18 sorbent with PSA. However, the use of C-18 has the potential of losing lipophilic analytes such as organochlorine pesticides (*24*).

In our current study, we examined the use of disposable pipette extraction (DPX) with different sorbents to remove fatty acids from acetonitrile extracts of foods of high fat content. DPX is a dispersive SPE method that uses loosely contained sorbent that is mixed with sample solutions in a pipette tip. Dynamic mixing of DPX sorbent with solutions provides rapid equilibration,

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Table 1. MS Information for Targeted Organochlorine Pesticides

pesticide	major ions (*m*/*z*)	identification ions for SIM method (*m*/*z*)	target ion (*m*/*z*)
aldrin	66, 79, 91, 101, 237, 263, 293	237, 263, 293	353
α-BHC	109, 111, 181, 183, 219	111, 181, 219	181
β-BHC	109, 111, 181, 183, 219	109, 181, 219	181
δ-BHC	109, 111, 181, 183, 219	109, 181, 219	181
γ-BHC	109, 111, 181, 183, 219	109, 181, 219	181
p,*p*′-DDD	75, 165, 235, 237	165, 235, 237	235
p,*p*′-DDE	176, 246, 248, 316, 318	176, 246, 318	246
p,*p*′-DDT	75, 165, 199, 235, 237	165, 199, 235	235
dieldrin	79, 81, 263, 277	79, 263, 277	263
endosulfan I	195, 237, 241, 265, 339	195, 241, 339	195
endrin	67, 79, 81, 263, 345	81, 263, 345	263
heptachlor	100, 237, 272, 274, 270,	100, 237, 272	272
heptachlor epoxide	81, 237, 263, 351, 353, 355	81, 263, 353	353
methoxychlor	227, 288, 346	227, 346	227

Table 2. MS Information for Targeted Organophosphate Pesticides

pesticide	major ions (*m*/*z*)	identification ions for SIM method (*m*/*z*)	target ion (*m*/*z*)
bolstar	125, 139, 140, 156, 322	139, 156, 322	322
chlorpyrifos	97, 197, 199, 258, 314	97, 197, 314	197
demeton-S	60, 81, 88, 170, 258	88, 170, 258	88
diazinon	137, 152, 179, 199, 304	137, 179, 304	137
dichlorvos	79, 109, 185	109, 185	109
disulfoton	88, 89, 97, 125, 186, 274	88, 186, 274	88
ethoprophos	97, 126, 139, 158, 242	139, 158, 242	158
fensulfothion	97, 125, 141, 293, 308	141, 293, 308	293
fenthion	109, 125, 153, 278, 301	125, 278, 301	278
merphos	57, 113, 169, 202, 314	169, 202, 314	169
mevinphos	67, 109, 127, 192	109, 127, 192	127
parathion-methyl	79, 93, 109, 125, 263	109, 125, 263	263
phorate	75, 97, 121, 260	75, 121, 260	75
ronnel	79, 109, 125, 285, 287	125, 285, 287	285
tokuthion	43, 113, 162, 267, 309	113, 267, 309	267
trichloronat	109, 269, 297	109, 269, 297	109

partitioning, and enhanced contact between analytes and solid-phase sorbent (*25*−*27*). In this application, the sorbent was chosen to selectively bind sample matrix interferences such as fatty acids from acetonitrile solutions without adsorption of pesticides, thereby providing a very rapid cleanup procedure. By combining DPX with large-volume injection (LVI) and gas chromatography−mass spectrometry (GC-MS), the analysis of pesticides in these difficult sample matrices is both rapid and sensitive.

MATERIALS AND METHODS

Standards, Reagents, and Materials. Organochlorine (OC) and organophosphorous (OP) pesticides were purchased from ULTRA Scientific (North Kingstown, RI). Working solutions of standards were prepared by dissolving original stock solutions in acetonitrile and diluting to 10 ppm. An external standard solution was prepared by dissolving 10 mg of D_{10}-parathion-diethyl (Sigma-Aldrich, St. Louis, MO) in acetone and diluting to 10 ppm with acetonitrile. The use of this standard was to correct for any differences in the final low volume of eluate analyzed. All working solutions were stored in glass vials at −20 °C.

Acetonitrile (analytical grade) was purchased from Mallinckrodt Baker (Phillipsburg, NJ). Sodium chloride (analytical reagent grade) was purchased from Fisher Scientific (Fair Lawn, NJ). Corn muffin mix was purchased from a local supermarket in Columbia, SC. Cocoa beans were obtained from the Hershey Co. (Hershey, PA).

DPX tips were obtained from DPX Laboratories, LLC (Columbia, SC). Three types of sorbents were studied: styrene−divinylbenzene (SDVB) for reversed phase (DPX-RP), polyamino (DPX-PA), and weak anion exchange (DPX-WAX).

Preliminary Sample Preparation. A 50 g quantity of sample (corn muffin mix or cocoa beans) was blended with 100 mL of 85% acetonitrile in deionized (DI) water. The solutions were subsequently filtered under vacuum using a glass microfiber filter 691 from VWR Scientific (West Chester, PA).

For the blended corn muffin mix sample, 0.5 mL of the resulting acetonitrile solution was transferred into a clean test tube and spiked with OC and OP pesticides. Also, 10 μL of external standard and a 0.25 mL volume of saturated NaCl were then added, and the solution was vortex mixed. Following centrifugation, the upper organic layer was transferred to a clean labeled tube and used for DPX extraction (cleanup).

For the blended cocoa bean sample, 0.2 mL of the resulting acetonitrile solution was transferred to a clean test tube and spiked with the pesticides. An additional 0.1 mL of acetonitrile and 10 μL of external standard were added to the solution, and 0.5 mL of saturated NaCl was then added, and the solution was vortex mixed. Following centrifugation, the upper organic layer was transferred to a clean labeled tube and used for DPX cleanup.

DPX Cleanup. The DPX extraction was performed by adding the acetonitrile extracts to the top of the DPX tip, which means introducing the solution through the large opening of the pipette tip. In this manner, the screen of the DPX tip acts as a filter, and it also ensures that all of the solution is thoroughly mixed with the sorbent to provide the most efficient mixing and extraction of the fatty acids. By adding the solutions to the top of the DPX tip, the extractions can be performed by mixing the solution with the sorbent only one time, and then the solution is dispensed into the vial for analysis. This extraction method takes only about 60 s to perform.

It is also possible to aspirate the solution from the narrow bottom end of the pipette tip, using the pipette tip as it is commonly employed. However, to obtain clean extracts, the aspiration of the solution in and out of the DPX tip must be repeated at least one time. This additional step increases the DPX extraction time to approximately 2 min, so the extraction is still very rapid. However, to obtain the fastest and most reproducible results using this manual extraction method, all of the experiments in this study added the solutions to the top of the DPX tip.

The mixing of the sample solution was accomplished by aspirating air, which creates small bubbles that agitate the solution. This mixing with the WAX sorbent in the tip creates a kind of gel with high surface area. After a short equilibration time of approximately 5 s, the solution was dispensed directly into a GC vial for subsequent analysis.

To recover residual analytes and obtain the highest recoveries, an additional 0.2 mL of acetonitrile was added to the top of the DPX tip and eluted into the same GC vial. The resulting eluate was injected into the GC-MS without further processing. The total DPX extraction time was approximately 2 min.

Fast GC-FID Analysis. Fast GC chromatograms were recorded with an Agilent 6890 GC (Agilent Technologies, Little Falls, DE) equipped with a MACH system (Gerstel, Linthicum, MD) and a flame ionization detector (FID). Analysis of corn muffin blank samples by fast GC was carried out on an RTX-5 column (5 m × 0.18 mm i.d., 0.2 μm of film thickness, adapted for the MACH) from Restek (Bellefonte, PA). The temperature program was controlled by the MACH system (Gerstel) as follows: held at 70 °C for 0.5 min, increased to 280 °C at a rate of 70 °C/min, and held at this final temperature for 0.5 min. The total analysis time was 5 min.

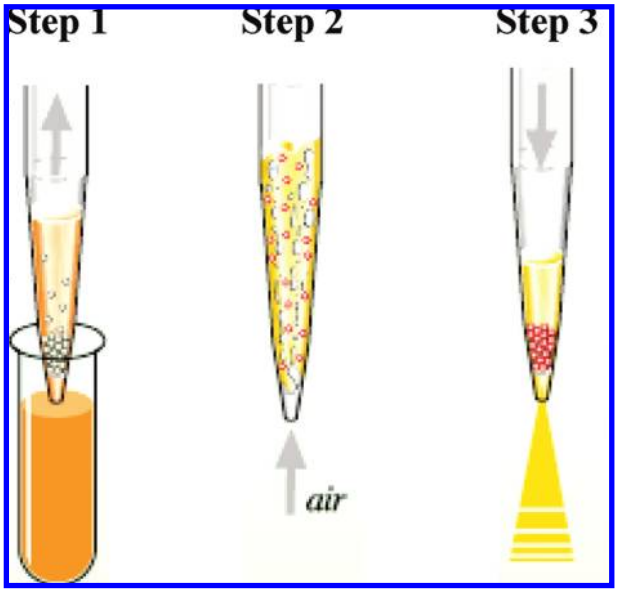

Figure 1. Schematic of the rapid DPX cleanup extraction method. The sample solution (after blending and filtering) is aspirated into the DPX tip (step 1), and then air is aspirated into the tip to create air bubbles that result in mixing (step 2); then the solution is dispensed after a short time (approximately 5 s) to the GC vial (step 3) for analysis. Optional additional steps include adding a small volume of acetonitrile to the top of the DPX tip (200 μL) and then dispensing this solvent through the sorbent into the GC vial from step 3.

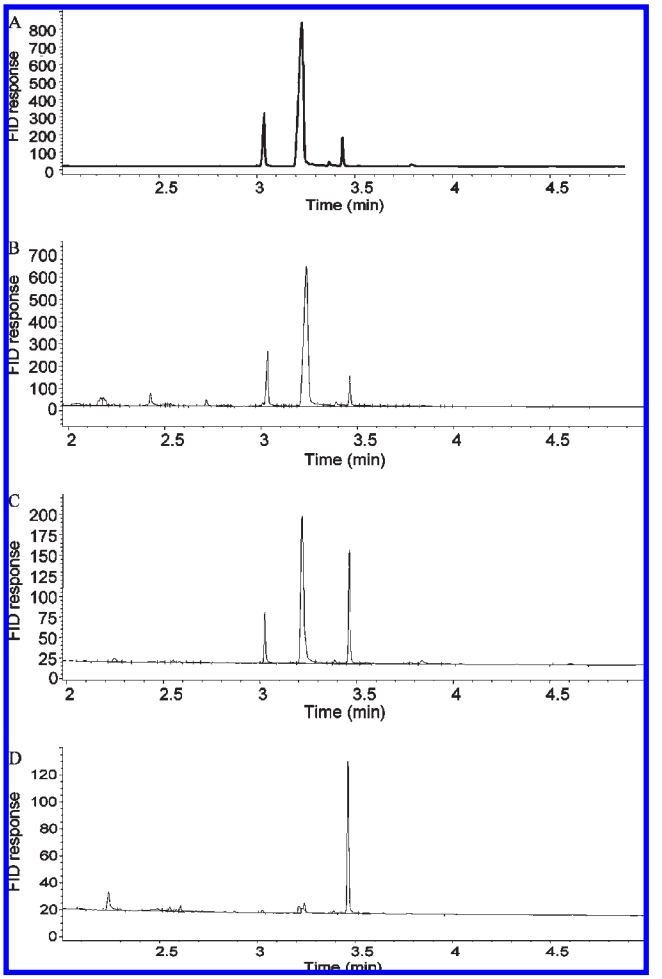

Figure 2. Fast GC-FID chromatograms of corn muffin extract (**A**) without cleanup, (**B**) with reversed phase (SDVB) cleanup, (**C**) with polyamino (PA) cleanup, and (**D**) with high-capacity weak anion exchange (WAX) cleanup.

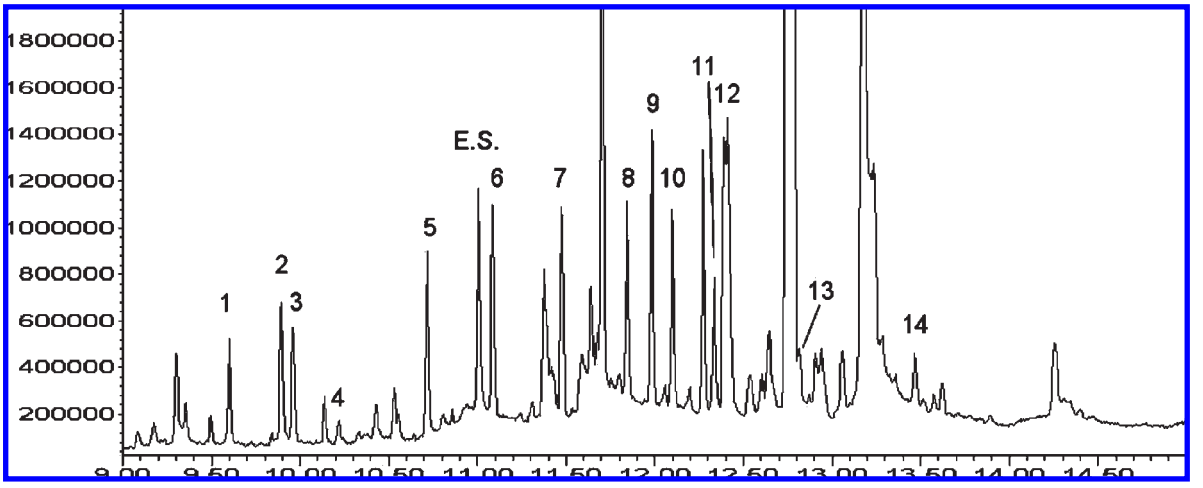

Figure 3. GC-MS full scan chromatogram for an acetonitrile extract of corn muffin sample spiked with 0.5 ppm OCs extracted using DPX-WAX. Peaks: 1, α-BHC; 2, β-BHC; 3, γ-BHC; 4, δ-BHC; 5, heptachlor; 6, aldrin; 7, heptachlor epoxide; 8, endosulfan I; 9, *p,p′*-DDE; 10, diedrin; 11, endrin; 12, *p,p′*-DDD; 13, *p,p′*-DDT; 14, methoxychlor.

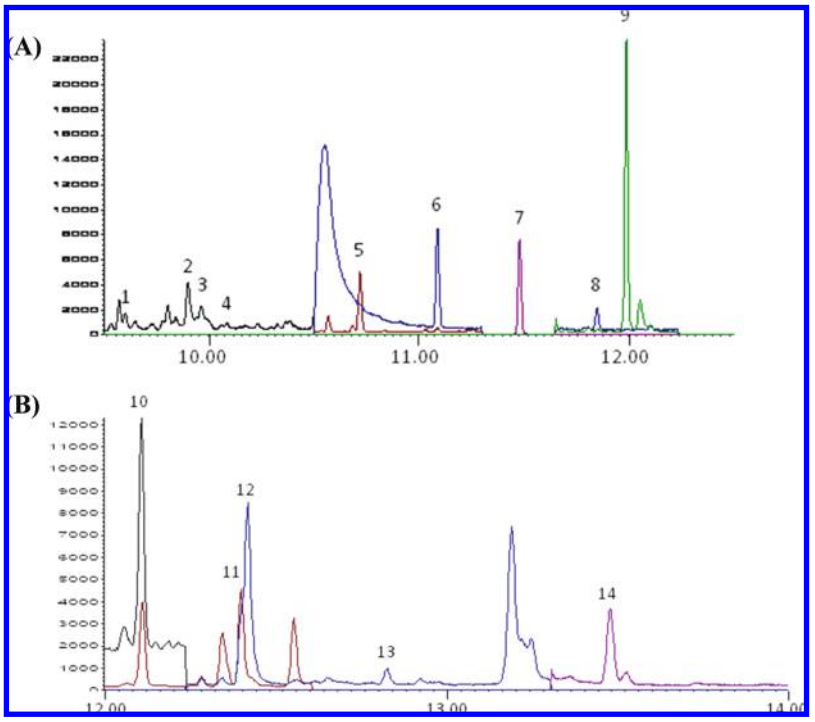

Figure 4. Extracted ion chromatograms of a SIM analysis of an acetonitrile extract of corn muffin mix spiked with 50 ppb OCs extracted ("cleaned up") using DPX-WAX. Peaks: 1, α-BHC; 2, β-BHC; 3, γ-BHC; 4, δ-BHC; 5, heptachlor; 6, aldrin; 7, heptachlor epoxide; 8, endosulfan I; 9, *p,p′*-DDE; 10, diedrin; 11, endrin; 12, *p,p′*-DDD; 13, *p,p′*-DDT; 14, methoxychlor.

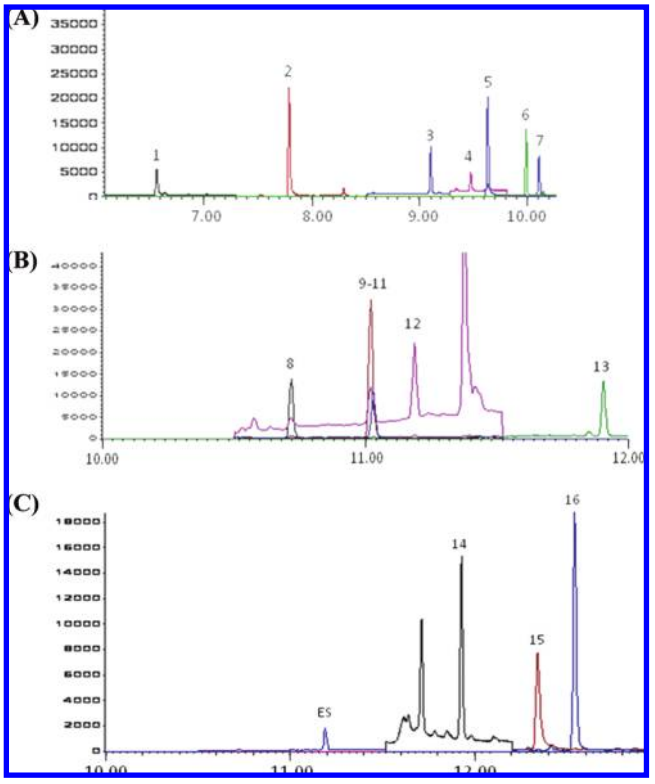

Figure 5. Extracted ion chromatograms of a SIM analysis of an acetonitrile extract of corn muffin mix spiked with 50 ppb OPs extracted ("cleaned up") using DPX-WAX. Peaks: 1, dichlorphos; 2, mevinphos; 3, ethoprophos; 4, phorate; 5, demeton-S; 6, diazinone; 7, disulfoton; 8, parathion-methyl; 9, ronnel; 10, fenthion; 11, chlorpyrifos; 12, trichloronat; 13, tokuthion; 14, merphos; 15, fensulfothion; 16, bolstar.

Table 3. Percent Recoveries and Relative Standard Deviations Based on Five Replicate Experiments Using DPX-WAX for Analysis of Organochlorine Pesticides in Corn Muffin Mix

	% recovery[a]	
pesticide	100 ppb	1000 ppb
aldrin	109.82 ± 5.25 (4.78)	101.97 ± 3.75 (3.68)
α-BHC	106.67 ± 2.37 (2.23)	106.57 ± 7.32 (6.87)
β-BHC	104.26 ± 4.88 (4.68)	100.84 ± 2.82 (2.80)
δ-BHC	98.88 ± 2.59 (2.62)	94.54 ± 3.82 (4.04)
γ-BHC	102.11 ± 1.01(0.99)	99.30 ± 4.12 (4.15)
diedrin	102.41 ± 7.27 (7.10)	100.89 ± 1.90 (1.88)
p,p′-DDD	105.48 ± 9.38 (8.89)	98.39 ± 0.84 (0.86)
p,p′-DDE	105.87 ± 9.03 (8.53)	100.50 ± 1.18 (1.18)
p,p′-DDT	87.48 ± 11.69 (13.37)	92.81 ± 8.70 (9.38)
endrin	98.11 ± 10.13 (10.32)	100.33 ± 1.84 (1.83)
endosulfan I	109.09 ± 2.24 (2.05)	101.09 ± 1.04 (1.03)
heptachlor	101.17 ± 1.89 (1.87)	101.62 ± 3.04 (2.99)
heptachlor epoxide	105.91 ± 5.06 (4.78)	101.42 ± 2.70 (2.66)
methoxychlor	94.69 ± 9.41 (9.94)	93.80 ± 5.58 (5.95)

[a] Mean ± standard deviation (% RSD) based on five replicate experiments.

GC-MS Analysis. GC-MS analyses were performed on a model 6890 GC coupled to a model 5975 mass selective detector (MSD) (Agilent Technologies). The instrument was equipped with a program temperature vaporization (PTV) inlet using the CIS-4 Cooled Injection System (Gerstel). Analysis of pesticides by GC-MS was carried out on an RTX-5 ms column (30 m × 0.25 mm i.d., 0.25 μm of film thickness) from Restek. The carrier gas was ultrapure helium at constant flow of 1.0 mL/min. For large volume injection (LVI) of 5 μL, a Gerstel MPS-2 was used for injection with the PTV using solvent vent mode. The PTV temperature was programmed to start at 50 °C for 1 min, ramped at 12 °C/s to 280 °C, and held for 3 min at this final temperature, with the split vent being closed at 1.01 min and reopened at 2 min. For full scan analysis, the GC oven was programmed to hold for 2 min at 60 °C, ramp at 20 °C/min to a final temperature of 280 °C, and then hold at 280 °C for 5 min. For selected ion monitoring (SIM), the GC oven was programmed to hold for 1 min at 60 °C, ramp at 40 °C/min to 200 °C, then ramp at 20 °C/min to a final temperature 300 °C, and hold for 11 min.

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. The mass spectrometer scanned the range of m/z 50−500. The identifications of pesticide peaks were confirmed by matching retention times of standards (within ±0.02 min) and by the presence of major ions. MS information for the OC and OP pesticides is summarized in **Tables 1** and **2**, respectively.

Method Validation. The following study was performed to determine the recovery of pesticides through DPX-WAX cleanup. Recovery studies for corn muffin mix were conducted by spiking pesticides at two different levels (100 and 1000 ppb) in 0.5 mL of acetonitrile solution following the initial extraction as described above. For cocoa beans, 0.2 mL of sample solution was used, and pesticides were spiked at 500 ppb. External standard (10 μL) was also added to each sample before extraction. To reduce or eliminate matrix effects for the recovery data, a matrix-matched sample was obtained by spiking the same amount of pesticides and external standard to a blank extract following the DPX procedures. A 5 μL aliquot of each sample was injected for GC-MS/SIM analysis. Calculations of recoveries in this study were based on peak area ratios of each pesticide to the external standard (D_{10}-parathion-diethyl) using matrix spiked solutions.

OC and OP pesticide working standards were spiked into acetonitrile extracts of corn muffin mix at five levels ranging from 20 to 1000 ppb. Calibration data were generated from 5 replicate samples at 20 ppb, 3 replicate samples at 50 ppb, 5 individual samples at 100 ppb, 3 replicate samples at 500 ppb, and 5 replicate samples at 1000 ppb. A 5 μL volume of final extract was injected for GC-MS/SIM analysis. Limits of detection (LOD) and limits of quantitation (LOQ) were calculated as (*28*)

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

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

Table 4. Percent Recoveries and Relative Standard Deviations Based on Five Replicate Experiments Using DPX-WAX for Analysis of Organophosphate Pesticides in Corn Muffin Mix

	% recovery[a]	
pesticide	100 ppb	1000 ppb
bolstar	98.09 ± 8.84 (9.02)	101.45 ± 2.85 (2.81)
chlorpyrifos	107.22 ± 4.62 (4.31)	99.38 ± 2.09 (2.10)
demeton-S	116.80 ± 4.35 (3.72)	102.79 ± 3.27 (3.18)
diazinon	119.28 ± 10.82 (9.07)	98.24 ± 4.66 (4.75)
dichlorphos	124.18 ± 3.56 (2.86)	100.74 ± 7.11 (7.06)
disulfoton	125.87 ± 13.06 (10.38)	97.32 ± 5.41 (5.56)
ethoprophos	129.89 ± 14.79 (11.38)	100.02 ± 5.99 (5.99)
fensulfothion	96.48 ± 15.85 (16.43)	109.77 ± 6.81 (6.20)
fenthion	111.22 ± 1.49 (1.34)	104.09 ± 0.41 (0.39)
merphos	101.77 ± 5.85 (5.75)	104.63 ± 1.48 (1.41)
mevinphos	135.73 ± 11.58 (8.53)	122.59 ± 4.57 (3.73)
parathion-methyl	114.49 ± 8.78 (7.67)	116.72 ± 3.28 (2.81)
phorate	124.45 ± 18.40 (14.79)	89.10 ± 7.71 (8.66)
ronnel	116.75 ± 3.63 (3.11)	108.04 ± 2.54 (2.35)
tokuthion	100.88 ± 3.55 (3.52)	100.51 ± 1.50 (1.49)
trichloronat	111.16 ± 4.96 (4.46)	103.24 ± 1.58 (1.53)

[a] Mean ± standard deviation (% RSD) based on five replicate experiments.

Table 5. Calibration and Statistics for Organochlorine Pesticides Using DPX-WAX for Cleanup of Corn Muffin Mix

pesticide	r^{2a}	LOD (ppb)	LOQ (ppb)
aldrin	0.9973	10.80	32.73
α-BHC	0.9927	5.43	16.44
β-BHC	0.9992	9.31	28.20
δ-BHC	0.9960	2.91	8.83
γ-BHC	0.9971	9.35	28.32
p,p′-DDD	0.9997	6.79	20.57
p,p′-DDE	0.9998	4.95	14.99
p,p′-DDT	0.9968	6.37	19.30
diedrin	0.9994	4.99	15.12
endosulfan I	0.9995	9.08	27.51
endrin	0.9993	8.08	24.47
heptachlor	0.9982	12.48	37.83
heptachlor epoxide	0.9991	6.56	19.89
methoxychlor	0.9924	5.39	16.34

[a] Coefficient of determination.

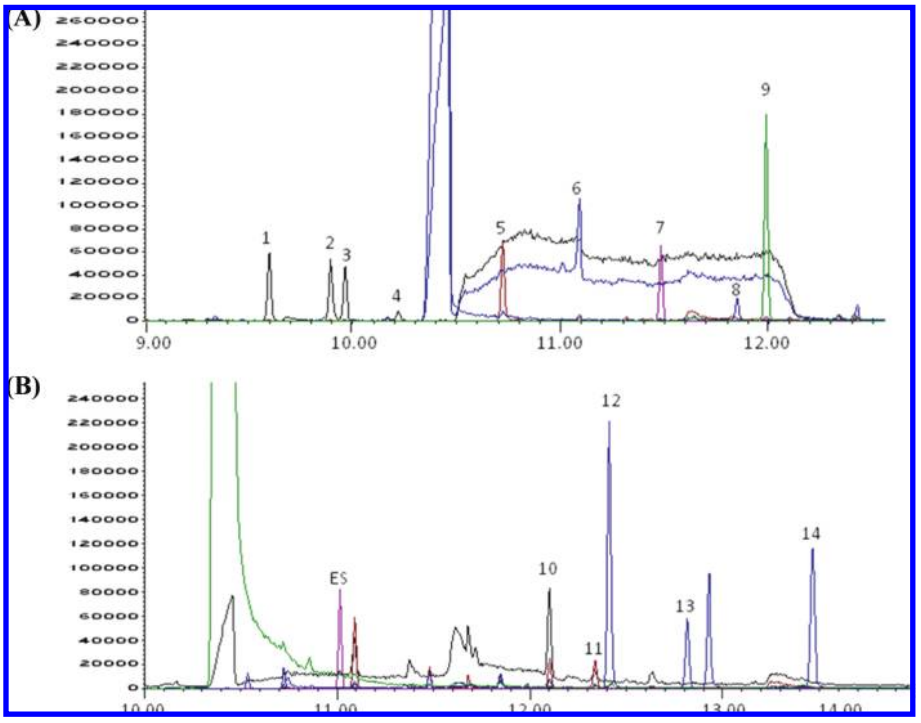

Figure 6. Extracted ion chromatograms of a full scan of an acetonitrile extract of cocoa beans spiked with 0.5 ppm OCs extracted ("cleaned up") using DPX-WAX. Peaks: 1, α-BHC; 2, β-BHC; 3, γ-BHC; 4, δ-BHC; 5, heptachlor; 6, aldrin; 7, heptachlor epoxide; 8, endosulfan I; 9, *p,p′*-DDE; 10, diedrin; 11, endrin; 12, *p,p′*-DDD; 13, *p,p′*-DDT; 14, methoxychlor.

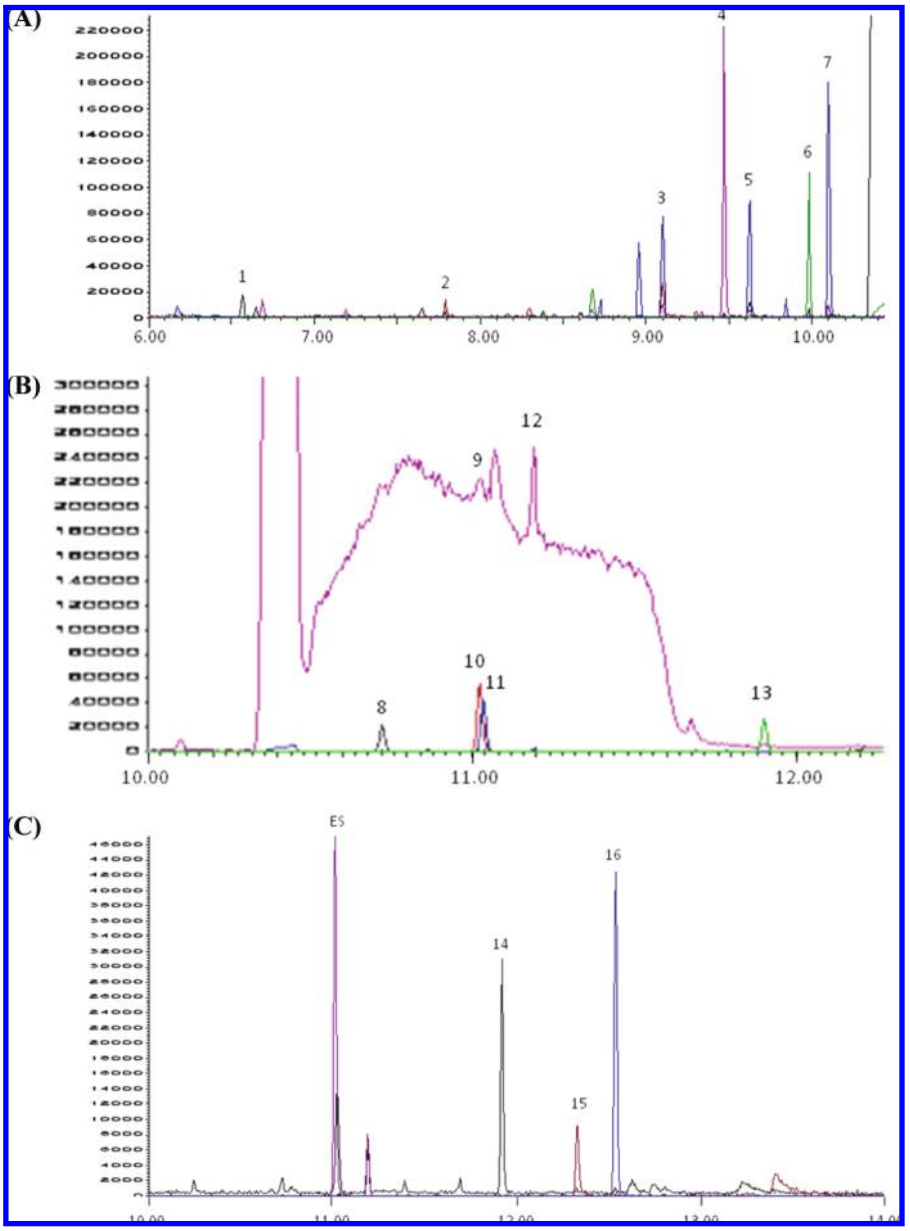

Figure 7. Extracted ion chromatograms of a full scan of an acetonitrile extract of cocoa beans spiked with 0.5 ppm OPs extracted ("cleaned up") using DPX-WAX. Peaks: 1, dichlorphos; 2, mevinphos; 3, ethoprophos; 4, phorate; 5, demeton-S; 6, diazinone; 7, disulfoton; 8, parathion-methyl; 9, ronnel; 10, fenthion; 11, chlorpyrifos; 12, trichloronat; 13, tokuthion; 14, merphos; 15, fensulfothion; 16, bolstar.

where m is the slope of the linear calibration plot 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 (20 ppb).

RESULTS AND DISCUSSION

DPX Cleanup. The DPX cleanup method is depicted in **Figure 1**. The sample solution is aspirated into the DPX tip, mixed with the sorbent, and then dispensed into a GC vial for chromatographic analysis. For highest recoveries, additional acetonitrile is added to the top of the DPX sorbent, eluted through the sorbent, and collected into the corresponding GC vial for analysis. These extra steps were used throughout this study. It should be noted that the final volume of the solution is approximately 0.5 mL because some of the acetonitrile is absorbed and retained in the sorbent, and the use of the external standard corrects for any volume flucutations without the need to do further measuring or solvent evaporation.

The total DPX cleanup method takes approximately 2 min. This extraction method focuses on extracting the matrix rather than extracting the analyte, which means that separate wash and elution steps are not required. This type of sample preparation is ideal for comprehensive analysis when analytes of interest differ in chemical structures and characteristics. This method can be particularly useful if the sample matrix interferences can be selectively removed with a sorbent without interaction with the analytes of interest.

Comparing DPX Sorbents for Fatty Acid Cleanup Using Fast GC-FID. Three sorbent types for DPX were investigated for removal of fatty acids from acetonitrile extracts of fatty foods: revered phase (styrene−divinylbenzene, DPX-RP), weak anion exchange using polyamino sorbent (DPX-PA), and high-capacity weak anion exchange sorbent (DPX-WAX).

The relative cleanup achieved with the various sorbents was evaluated using fast GC-FID and GC-MS. **Figure 2** compares fast GC-FID chromatograms of corn muffin extract before and after

Table 6. Calibration and Statistics for Organophosphate Pesticides Using DPX-WAX for Cleanup of Corn Muffin Mix

pesticide	r^{2a}	LOD (ppb)	LOQ (ppb)
bolstar	0.9990	3.35	10.16
chlorpyrifos	0.9994	6.65	20.15
demeton-S	0.9988	12.08	36.59
diazinon	0.9971	5.53	16.75
dichlorphos	0.9939	9.22	27.95
disulfoton	0.9960	8.16	24.73
ethoprophos	0.9954	7.97	24.15
fensulfothion	0.9957	6.57	19.91
fenthion	1.0000	0.90	2.73
merphos	0.9996	11.62	35.21
mevinphos	0.9980	3.26	9.89
parathion-methyl	0.9987	5.37	16.27
phorate	0.9940	8.43	25.54
ronnel	0.9994	2.73	8.27
trichloronat	0.9997	4.07	12.32
tokuthion	0.9997	2.11	6.40

[a] Coefficient of determination.

cleanup with different sorbents, and elimination of fatty acids was determined by comparing the intensity of the fatty acid peaks before and after cleanup using the same amount of different sorbents. As can be seen, reversed phase sorbent SDVB had little effect on reducing matrix fatty acids in the current study (**Figure 2B**), and it removed only 29% of fatty acids in corn muffin extract. This is consistent with the previously reported study that reversed phase sorbent using octadecylsilyl (C-18) did little to eliminate fatty acids (*5*). Weak anion exchange sorbents (DPX-PA and DPX-WAX) were more effective than SDVB (**Figures 2C2D**, respectively). Moreover, the high-capacity weak anion exchange sorbent (DPX-WAX, **Figure 2D**) was superior to polyamino (**Figure 2C**, eliminating 86% of fatty acids), and the fatty acid peaks in corn muffin extract are completely eliminated following DPX-WAX cleanup. All subsequent studies were therefore performed using DPX-WAX for cleanup.

GC-MS Analysis of Pesticides in Samples with High Fat Content. The fast GC-FID analysis indicated that high-capacity WAX sorbent was effective in removal of fatty acids in corn muffin mix. The following GC-MS analysis confirmed that WAX was able to eliminate fatty acid interferences in samples of high fat content, such as corn muffin mix and cocoa beans. **Figure 3** shows a full scan GC-MS chromatogram of a corn muffin sample spiked with 0.5 ppm OCs and extracted by DPX-WAX. There are no fatty acid interferences in the chromatogram. Only one major peak is found in the chromatogram, which appears to be an ester [tentatively identified as hexanedioic acid, bis(2-ethylhexyl) ester] that would probably not bind by anion exchange. Most importantly, all of the OCs are readily detected in the total ion chromatogram at this concentration. By using SIM, increased sensitivity for the analysis is realized.

Pesticide quantitation was performed using extracted ion chromatographic peak areas. **Figures 4** and **5** show the GC-MS extracted ion chromatograms (EIC) of OC and OP pesticides spiked at 50 ppb in corn muffin extract after DPX-WAX cleanup. As can be seen from these chromatograms, matrix components do not interfere with the analysis. By combining LVI with GC-MS/SIM, good sensitivity is achieved without any additional concentration steps incorporating solvent evaporation of the extract, as noted below in the calibration data. It is noted that these samples utilized only 5 μL for injection, so better sensitivity can be readily obtained by injecting larger volumes. However, increased volumes will necessitate more frequent changing of the injection port liner to maintain reproducible results. In this study, we chose to have reproducible results and less maintenance for this analysis.

Recoveries of Pesticides in Corn Muffin Mix. To evaluate potential losses of pesticides after DPX-WAX cleanup, we analyzed a series of samples spiked with 100 and 1000 ppb OC and OP pesticides in acetonitrile extracts of corn muffin mix and cocoa beans. **Table 3** shows recoveries of OCs in acetonitrile extracts of corn muffin mix. Of the studied OCs, recoveries ranged between 76.08 and 104.24%, with % RSDs (replicate of five samples) below 10% for all pesticides except δ-BHC, which has % RSD of 14.05%. **Table 4** shows recoveries of OPs in corn muffin mix. Recoveries for all targeted OPs approach 100%.

Analysis of Cocoa Beans. We found that cocoa beans, which contain approximately 50% fat, could likewise be extracted using DPX-WAX. However, the volume of the acetonitrile solution had to be decreased due to the capacity of the WAX sorbent. As shown in **Figures 6** and **7**, OC and OP pesticides can be detected with little interference from the sample matrix. The recoveries were found to be very high, as for the corn muffin extracts, but background matrix components include high intensities of caffeine and theobromine. These background peaks had no effect on most of the pesticides analyzed. It should be noted that this method used just 0.2 mL of sample solution, with a final volume of approximately 0.5 mL after cleanup, which results in a dilution factor. Sensitive detection of pesticides in cocoa beans using this method will require additional steps such as solvent evaporation or use of more sensitive instrumentation such as GC-MS/MS or LC-MS/MS.

Calibration, LODs, and LOQs of Target Pesticides in Corn Muffin Mix. Linear calibration of target pesticides in corn muffin sample using the current DPX-WAX cleanup method was performed from 20 to 1000 ppb. As shown in **Tables 5** and **6**, coefficients of determination (r^2) were > 0.99 for all studied OCs and OPs, with LODs and LOQs below 10 and 30 ppb for most of the target pesticides, respectively.

It should be noted that the LVIs utilized only 5 μL volumes. Although much larger volumes of solvent could be injected to improve sensitivity, this could lead to deleterious effects by contaminating the injection port linear. We found we can perform over 20 injections of these extracts without having to change the injection port liner.

The limitation of this study is that a minimal volume of sample solution is used, so the concentration factor is low, especially for cocoa beans that are actually diluted. However, it should be mentioned that this sample preparation method can be used with GC-MS/MS and LC-MS/MS methods that provide much higher sensitivity than GC-MS. Samples are often diluted for analysis with these types of instruments, and therefore this rapid 1−2 min cleanup method should be ideal for these methods of analysis.

This is the first reported method demonstrating a simple and rapid three-step process for the rapid and efficient cleanup of food extracts with high levels of fatty acids. The proposed DPX-WAX method offers an efficient method for removal of fatty acid interferences from samples of high fat content for multiresidue pesticide analysis. This method does not require any solvent evaporation and uses minimal solvent volumes. This method can be completely automated, providing a means for high-throughput analysis of pesticides in food with high fat content. Future work is focused on complete automation using DPX-WAX with GC-MS/MS and LC-MS/MS for both rapid and sensitive analysis of pesticides in high fat content samples.

ABBREVIATIONS USED

DPX, disposable pipette extraction; LVI, large volume injection; LOD, limit of detection; LOQ, limit of quantitation; OC,

organochlorine; OP, organophosphate; C-18, octadecylsilyl; 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 extraction; WAX, weak anion exchange.

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