Application Note: Analysis of Pesticides in Food Samples with High Fat Content Using DPX Cleanup Tips
(updated: 4/04/09)

Product: DPX-WAX (1 mL, TA)

INTRODUCTION

The conventional analysis of pesticides in food products that are high in fat content is labor intensive and time consuming. The high concentration of fatty acids from extracts of these samples greatly interferes with chromatographic analysis.

The QuEChERS[1] (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction was developed as a rapid method of analysis for pesticides in fruit and vegetables. This method uses the strategy of removing fatty acids and water from acetonitrile extracts of these samples. The sorbent used for the QuEChERS method is primary secondary amine, (PSA). Recently, a QuEChERS product (DPX-Q) has been developed using the DPX (disposable pipette extraction) tip format, referred to as DPX-Q. The main advantages of DPX-Q are that the extractions can be automated, additional centrifugation steps are unnecessary, and the sorbent is filtered to ensure solutions are free from particulate material.

Although the PSA used in QuEChERS and DPX-Q products are effective for fatty acid removal from fruit and vegetable extracts, this sorbent lacks capacity to remove fatty acids from samples high in fat content, such as grains and beans. In a previous study[2], we found that a polyamino sorbent has approximately 5 times the capacity of PSA resulting in more effective removal of fatty acids during extraction of high fat content samples. In this study, we examined the use of different sorbents in the DPX tip format to remove fatty acids from acetonitrile extracts of fatty foods. We present data showing that a new "Cleanup Tip" that uses a weak anion exchange (WAX) adsorbent effectively removed fatty acids from extracts of samples with as high as 50% fat content in as little as 60 seconds using a completely automated system.

EXPERIMENTAL

Sample preparation:

50 g of sample (corn muffin mix or cocoa beans), was blended with 100 mL of 85% acetonitrile in deionized water. The solutions were subsequently filtered under vacuum.

For the blended corn muffin mix sample, 1mL of the resulting acetonitrile solution was transferred into a clean test tube and spiked with the OC and OP pesticides. 0.5mL of saturated NaCl was then added and the solution was vortex mixed. Following centrifugation, the upper organic layer was transferred to a clean test tube prior to DPX extraction.

For the blended cocoa beans sample, 0.2mL of the resulting acetonitrile solution was transferred to a clean test tube and spiked with the OC and OP pesticides. An additional 0.1mL of acetonitrile was added to the solution, and 0.5mL of saturated NaCl was then added and the solution was vortex mixed. Following centrifugation, the upper organic layer was transferred to a clean test tube prior to DPX extraction.

DPX Extraction:

The DPX extraction was performed by aspirating the sample solution into and out of the DPX tip two times, aspirating a low volume of air to mix the solution with the sorbent. The final elution was dispensed directly into a GC vial. An additional 0.2 mL of acetonitrile was added to the top of the DPX tip and eluted into the same GC vial to remove residual analytes and obtain the highest recoveries. The eluate was then injected into the GC/MS for analysis. This procedure was performed automatically using a dual rail MPS-2 (see instrumentation below).

Instrumentation:

Fast GC chromatograms were recorded with an Agilent Technologies 6890 GC equipped with a MACH system (GERSTEL, equipped with a RTX-5 at 5 m with 0.18 mm ID and 0.2 um film) and a FID detector.

Analyses were performed on a 6890 GC with 5975 MSD (Agilent Technologies) and a PTV inlet (CIS 4, GERSTEL Inc). A dual rail MPS 2 Prepstation (GERSTEL Inc.) with a 2.5 mL syringe and 100 uL syringe was used for automated DPX extractions and injections.

1mL DPX tips with transport adaptors were obtained from DPX Labs, LLC (Columbia, SC). DPX tips containing three different sorbents were tested during this study: styrene divinyl benzene for reversed phase (DPX-RP), polyamino (DPX-PA), and a weak anion exchange sorbent (DPX-WAX).

Analysis Conditions

PTV:	Solvent Vent, 150 mL/min 50° C ; 12° C/sec**,** 280° C (3 min)
Column:	30 m RTX-5ms (Restek) di = 0.25 mm, df = 0.2 um
Pneumatics:	He; Pi = 7.47 psi constant flow = 1.0 mL/min
Oven:	60° C (2 min), 20° C/min, 280° C (5 min)

RESULTS AND DISCUSSION

We tested three adsorbent types in the DPX tip format for removal of fatty acids from acetonitrile extracts of fatty foods: a styrene divinyl benzene (sdvb, or DPX-RP for reverse phase), a polyamino sorbent (DPX-PA) and a high capacity weak anion exchange sorbent (DPX-WAX).

Figure 1 shows the results of the removal of fatty acids using the Cleanup Tips. Fatty acid removal was determined by measuring the intensity of the fatty acid peaks before and after use of the Cleanup Tips. Anion exchange sorbents were more effective than the RP sorbent, and the DPX-WAX was superior to the DPX-PA. All subsequent studies were therefore performed using DPX-WAX as the Cleanup Tips. Figure 2 compares fast GC chromatograms of corn muffin extract treated without and with DPX-WAX. The fatty acid peaks are completely removed following DPX-WAX cleanup. Figure 3 shows a GC/MS chromatogram of corn muffin mix extracted and "cleaned up" using DPX-WAX. Little interference is noted in the chromatogram, with the most intense peaks identified as esters of fatty acids.

Figures 4 and 5 show the GC/MS extracted ion chromatograms of OC and OP pesticides spiked at 0.5 ppm extracted from corn muffin matrix and cleaned up using the DPX-WAX Cleanup Tips. The recoveries and % RSDs are shown in Tables 1 and 2, with recoveries greater than 70% and good reproducibility. Figures 6 and 7 show the GC/MS chromatograms (selected ion monitoring, SIM) of OC and OP pesticides spiked at 50 ppb extracted from corn muffin mix and cleaned up using the DPX-WAX Cleanup Tips.

The same method was also applied to cocoa beans, but the amount of the sample solution was reduced (as indicated in the experimental section) because of the much greater amount of fatty acids. Figure 8 shows the GC/MS chromatogram (full scan) of cocoa beans after processing with the DPX-WAX Cleanup Tip. The high background observed is not due to fatty acids, but is due to the high concentration of caffeine and theobromine. The fatty acids were almost completely removed, and results for OP pesticides are shown in Table 3.

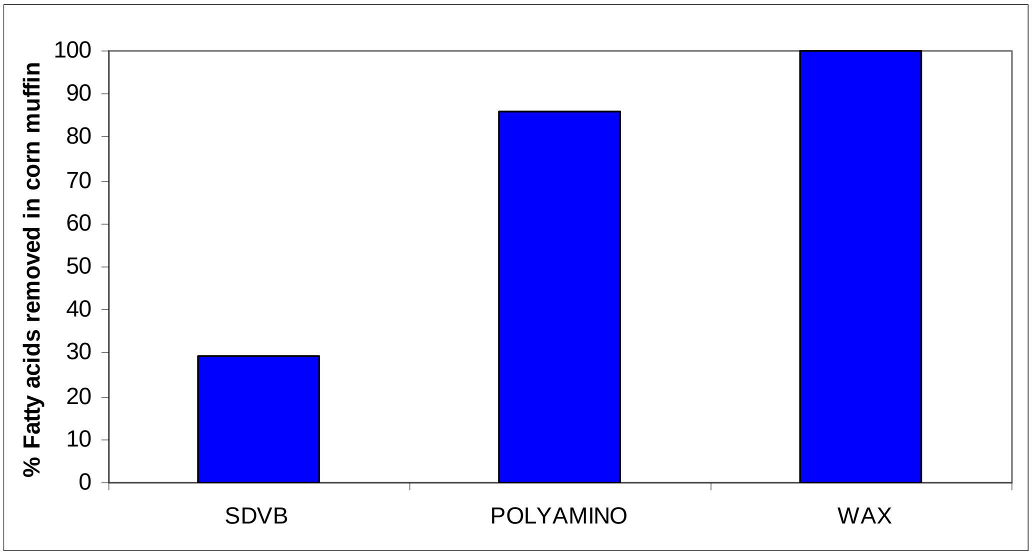

Figure 1. Graph showing the efficiency for removing fatty acids from the sample acetonitrile extract using various different sorbents. The weak anion exchange (WAX) sorbent performed the best at removing fatty acids.

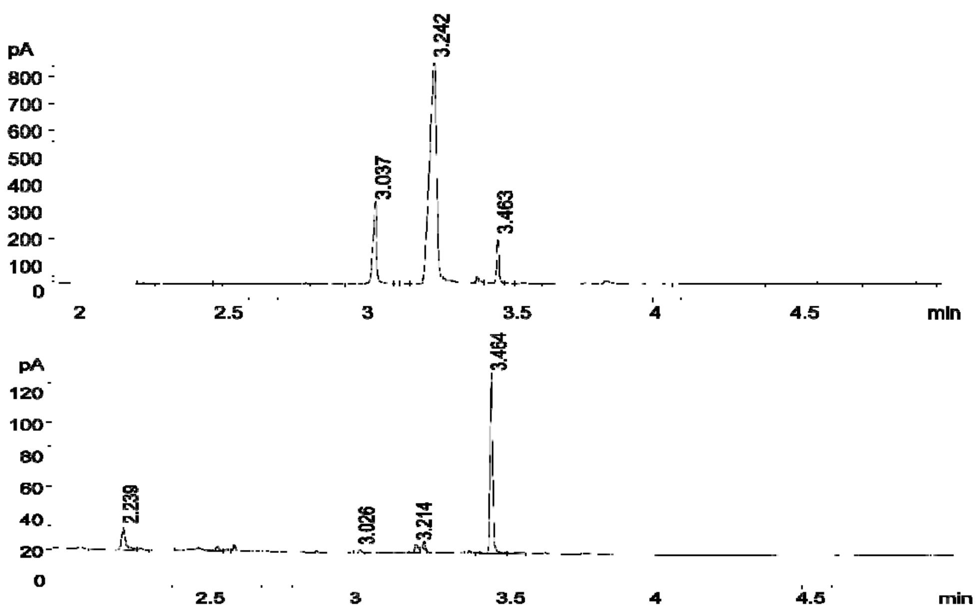

Figure 2. Fast GC chromatograms (recorded with MACH GC-FID) of corn muffin extract without (top) and with DPX-WAX Cleanup (bottom). Note the fatty acid peaks at 3.242 and 3.037 are completely removed in the bottom chromatogram. Only a relatively small peak at 3.46 minutes is observed, presumably from a methyl ester.

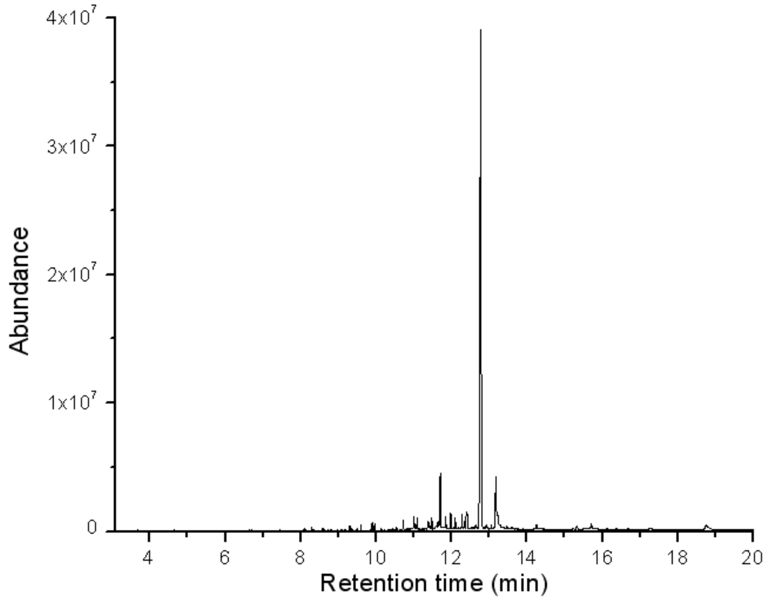

Figure 3. GC/MS chromatogram (full scan) of 0.5 ppm OC pesticide spiked corn muffin mix extract, after DPX-WAX Cleanup. The peaks at approximately 11.9, 12.9 and 13.2 minutes are fatty acid methyl esters, and no fatty acids are present in the chromatogram.

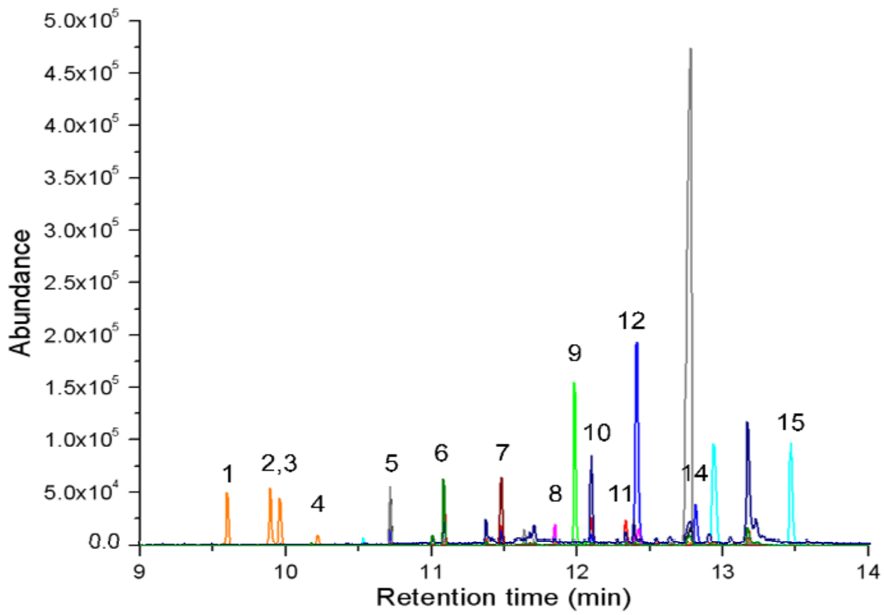

Figure 4. Extracted ion chromatogram of OC pesticides spiked at 0.5 ppm in corn muffin mix extract, after DPX-WAX Cleanup. (Compound number 13 is not shown because its extracted ion has a high background that obscures the display of several other compounds in this figure.)

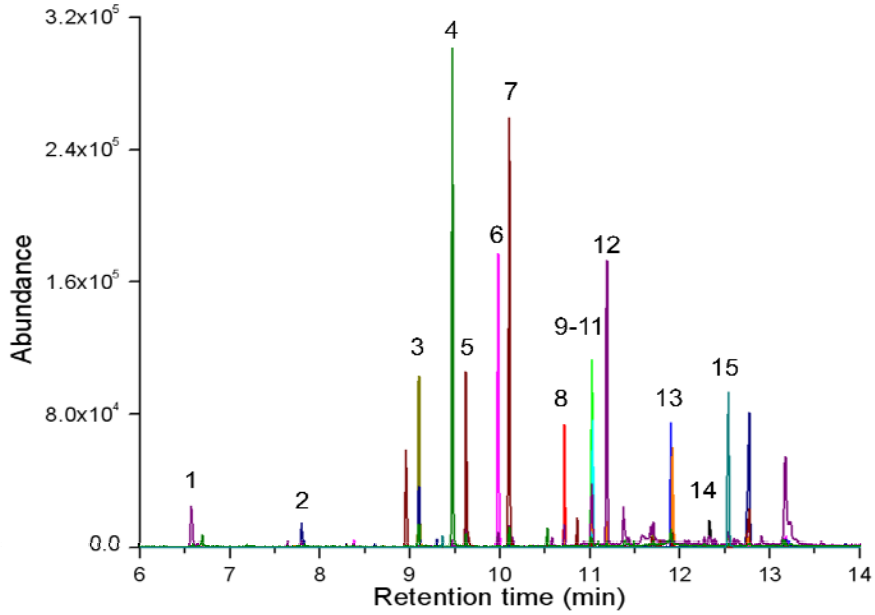

Figure 5. Extracted ion chromatogram of OP pesticides spiked at 0.5 ppm in corn muffin mix extract, after DPX-WAX Cleanup.

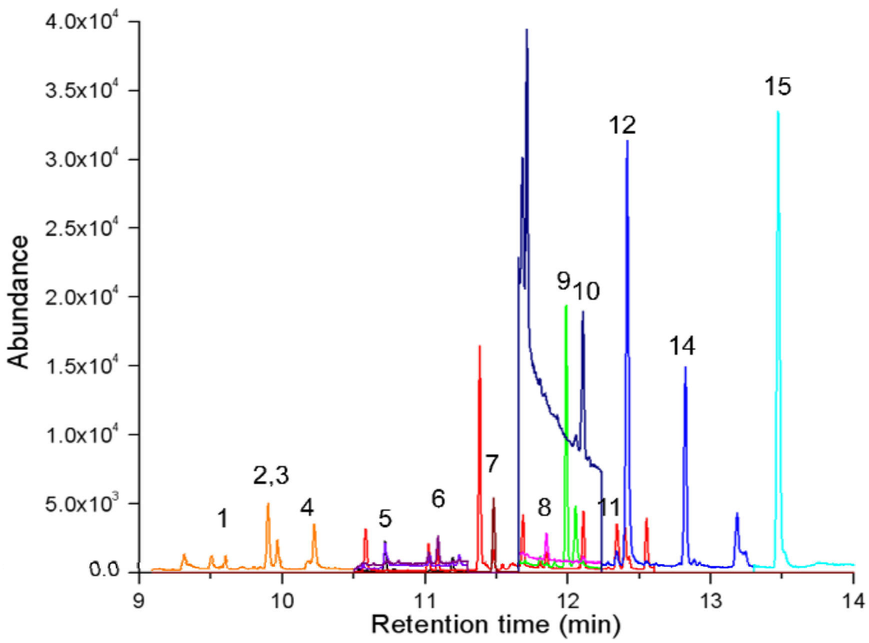

Figure 6. SIM chromatogram of OC pesticides spiked at 50 ppb in corn muffin mix extract, after DPX-WAX Cleanup. (Compound number 13 is not shown because its extracted ion has a high background that obscures the display of several other compounds in this figure.)

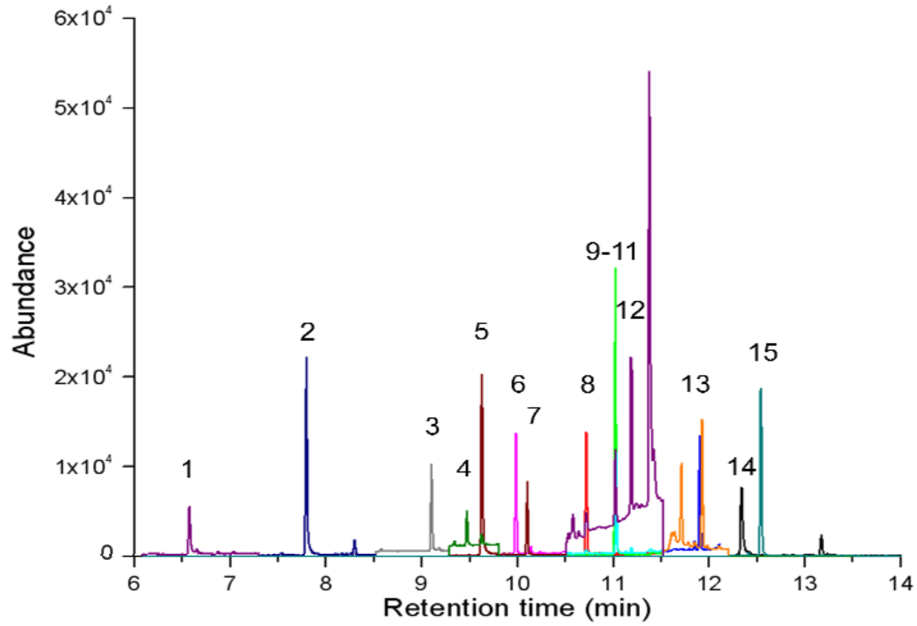

Figure 7. SIM chromatogram of OP pesticides spiked at 50 ppb in corn muffin mix extract, after DPX-WAX Cleanup.

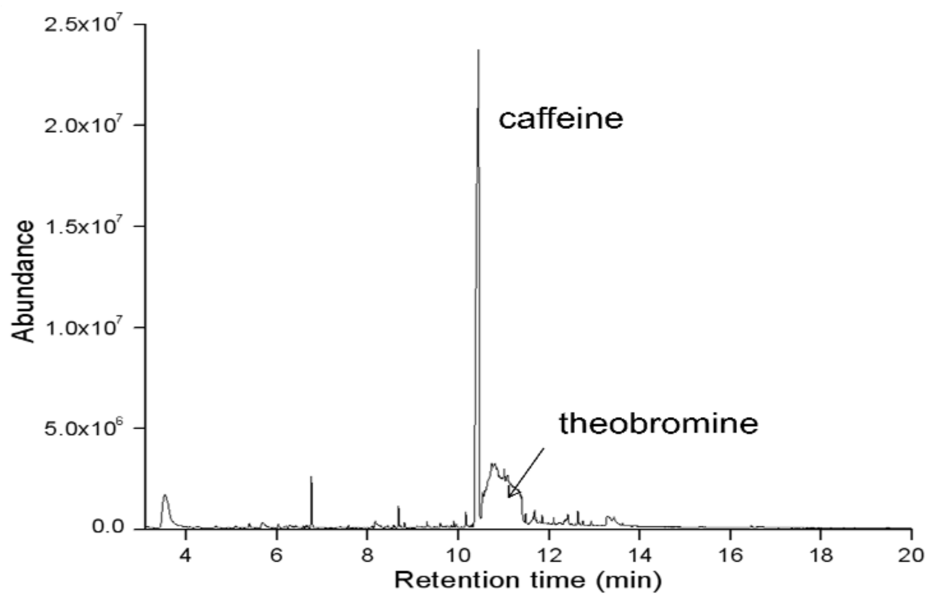

Figure 8. GC/MS chromatogram (full scan) of cocoa bean extract after DPX-WAX Cleanup.

Table 1. Data of DPX-WAX extractions of OC pesticides from corn muffin mix.

	OC Pesticide	Ret. Time	Ion	% Recov.	% RSD
1	α-BHC	6.574	181	82.46	5.01
2	β-BHC	7.801	181	88.37	4.91
3	γ-BHC	9.102	181	85.44	1.22
4	δ-BHC	9.473	181	98.54	14.05
5	Heptachlor	9.626	100	82.67	5.02
6	Aldrin	9.985	66	87.69	9.82
7	Heptachlor epoxide	10.105	353	88.50	4.85
8	p,p'-DDE	10.721	246	87.74	4.17
9	Endosulfan I	11.023	195	91.84	5.89
10	Diedrin	11.023	79	92.83	4.08
11	Endrin	11.034	263	93.01	6.70
12	p,p'-DDD	11.192	235	88.10	2.86
13	Endrin aldehyde	11.91	67	104.24	4.91
14	p,p'-DDT	12.33	235	76.08	5.78
15	Methoxychlor	12.54	227	84.08	6.38

Table 2. Data of DPX-WAX extractions of OP pesticides from corn muffin mix.

	OP Pesticide	Ret. Time	Ion	% Recov.	% RSD
1	Dichlorphos	6.574	109	app 120	NA
2	Mevinphos	7.801	127	app 120	NA
3	Ethoprophos	9.102	158	97.45	10.04
4	Phorate	9.473	75	78.42	13.88
5	Demeton-S	9.626	88	86.61	7.22
6	Diazinone	9.985	179	89.48	10.65
7	Disulfoton	10.105	88	84.43	12.00
9	Ronnel	10.721	285	116.64	9.08
8	Parathion-methyl	11.023	109	92.22	9.28
10	Fenthion	11.023	278	82.47	5.37
11	Chlorpyrifos	11.034	197	91.61	5.63
12	Trichloronat	11.192	109	83.93	8.09
13	Tokuthion	11.91	267	83.70	6.35
14	Merphos	11.92	169	63.40	5.57
15	Fensulfothion	12.33	293	96.51	19.20
16	Bolstar	12.54	322	82.20	5.41

Table 3. Data of DPX-WAX extractions of OP pesticides from cocoa beans.

	OP Pesticide	Ret. Time	Ion	% Recov.	% RSD
1	Dichlorphos	6.574	185	75.77	14.66
2	Mevinphos	7.801	127	71.33	11.67
3	Ethoprophos	9.102	158	106.24	16.75
4	Phorate	9.473	75	115.45	7.01
5	Demeton-S	9.626	88	108.51	4.78
6	Diazinone	9.985	152	117.55	7.33
7	Disulfoton	10.105	88	114.97	7.07
9	Ronnel	10.721	285	97.92	8.57
8	Parathion-methyl	11.023	125	109.93	5.08
10	Fenthion	11.023	278	99.12	5.08
11	Chlorpyrifos	11.034	197	108.05	2.11
12	Trichloronat	11.192	269	108.28	5.99
13	Tokuthion	11.91	267	105.91	4.44
14	Merphos	11.92	169	106.75	4.30
15	Fensulfothion	12.33	293	72.43	17.43
16	Bolstar	12.54	322	93.64	23.29

CONCLUSION

The automated clean up extractions using DPX-WAX provides a rapid and sensitive method for sample cleanup prior to determination of pesticides from samples high in fat content. This method did not require any solvent evaporation and used minimal solvent volumes.

References:

1. M. Anastassiades, S. J. Lehotay, D. Stajnbaher, and F. J. Schenck, J. AOAC Int. (2003) 86, 412.

2. W. E. Brewer, H. Guan, S. L. Morgan, S. T. Garris, and C. Craft, “Multi-residue pesticide analysis on incurred produce samples using variations of disposable pipette extraction”, Florida Pesticide Workshop, July 2008, St. Pete Beach, FL.

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