Rapid, Automated Extraction of Pesticides in Food Samples with High Fat Content Using Dispersive Pipette Extraction

HIGHLIGHTS: Highly reproducible results with reduced sample prep. time

WAX - XTR

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.

This study explores the use of dispersive pipette extraction technology for removing fatty acids and water from acetonitrile extracts of food samples. The sorbent used for the QuEChERS method is primary secondary amine, (PSA). XTR tips are compatible with automated liquid handlers making extractions easy to automate, and additional centrifugation steps are unnecessary. XTR tips contain loose or "dispersive sorbent that is filtered to ensure solutions are free from particulate material.

Although the PSA used in QuEChERS 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, 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. We examined the use of three different sorbents in the XTR tip to remove fatty acids from acetonitrile extracts of fatty foods. XTR tips containing weak anion exchange (WAX) sorbent showed the most effective removal of fatty acids from extracts of samples with as high as 50% fat content in as little as 60 seconds.

MATERIALS AND METHODS

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, 1 mL of the resulting acetonitrile solution was transferred into a clean test tube and spiked with the OC and OP pesticides. 0.5 mL of saturated NaCl was then

Dual Rail MPS System from GERSTEL

added and the solution was vortex mixed. Following centrifugation, the upper organic layer was transferred to a clean test tube prior to extraction.

For the blended cocoa beans sample, 0.2 mL of the resulting acetonitrile solution was transferred to a clean test tube and spiked with the OC and OP pesticides. An additional 0.1 mL of acetonitrile was 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 test tube prior to extraction.

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 μm film) and a FID detector.

A Dual Rail MPS 2 System (GERSTEL Inc.) with a 2.5 mL syringe and 100 μL syringe was used for automated dispersive pipette extraction and injections following the method in table 1. Analyses were performed on a 6890 GC with 5975 MSD (Agilent Technologies) and a PTV inlet (CIS 4, GERSTEL Inc). 1mL XTR tips with transport adaptors were obtained from DPX Technologies, Columbia, SC. XTR tips containing three different sorbents were tested during this study: reversed phase (RP), polyamino (PA), and weak anion exchange (WAX).

Table 1. Automated dispersive pipette extration steps

1	Aspirate and dispense sample solutions 2x with 1 mL XTR tips
2	Dispense solution into a GC vial
3	Add 0.2 mL of acetonitrile into the top of tip and dispense into the same GC vial. (This removes residual analytes to obtain high recoveries.)
4	Inject onto GC/MS

Analysis Conditions:
- PTV: Solvent Vent, 150 mL/minute
- 50°C ; 12°C/second, 280°C (3 minutes)
- 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/minute
- Oven: 60°C (2 minutes), 20°C/minute, 280°C (5 minutes)

RESULTS

We tested three sorbent types for removal of fatty acids from acetonitrile extracts of fatty foods. Figure 1 shows the results of the removal of fatty acids using XTR Tips with varying sorbents. Fatty acid removal was determined by measuring the intensity of the fatty acid peaks before and after use of tips for cleanup. WAX was more effective than the RP sorbent, and PA. All subsequent studies were therefore performed using XTR tips with WAX for cleanup.

Figure 2 compares Fast GC chromatograms of corn muffin extract treated with and without WAX-XTR cleanup. The fatty acid peaks are completely removed following WAX-XTR cleanup. Figure 3 shows a GC/MS chromatogram of corn muffin mix extracted using WAX-XTR tips. 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 WAX-XTR tips. The recoveries and % RSDs are shown in Tables 2 and 3, 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 WAX-XTR 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 WAX-XTR tips. The high background observed is not due to fatty acids, but is due to the high concentration of caffeine and the obromine. The fatty acids were almost completely removed, and results for OP pesticides are shown in Table 4.
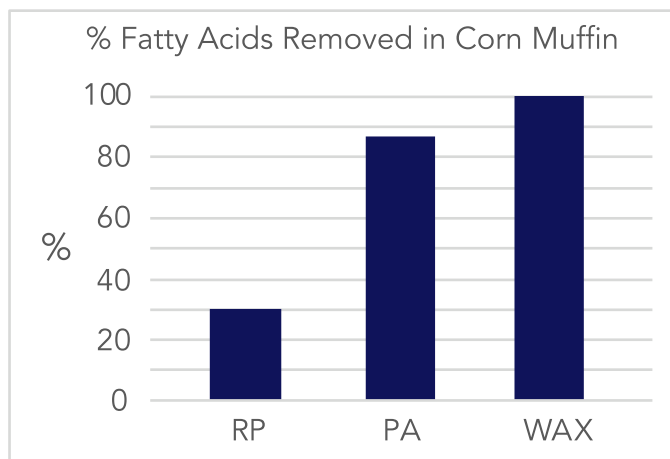

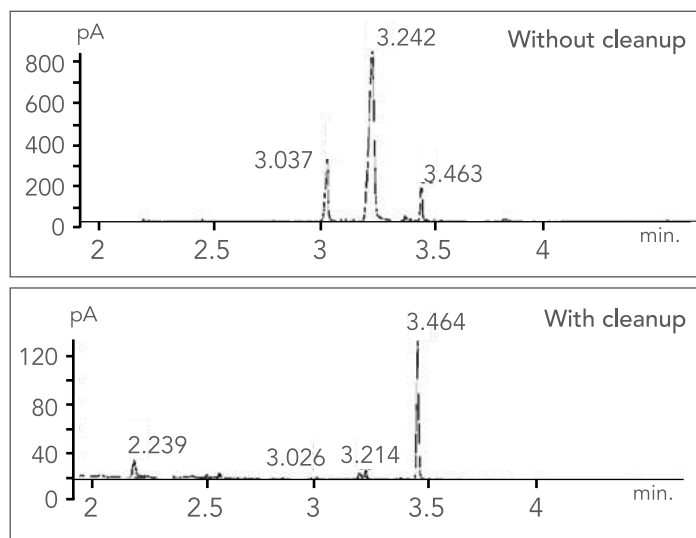Figure 1. WAX sorbent performed the best at removing fatty acids.

Figure 2. 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.
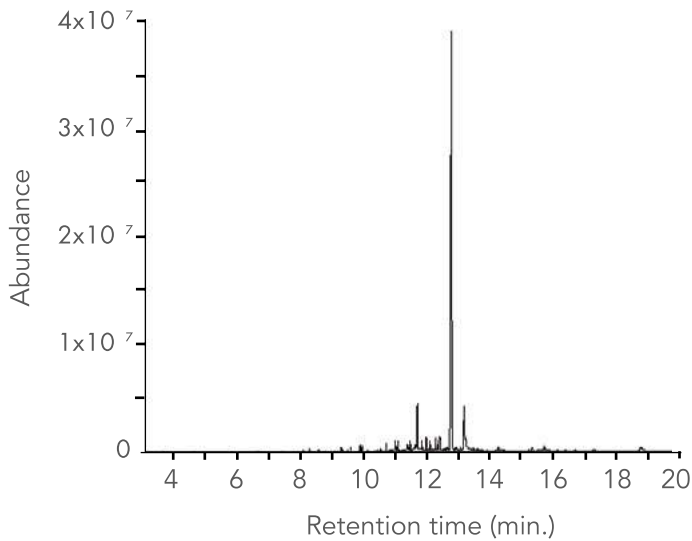
Figure 3. GC/MS chromatogram (full scan) of 0.5 ppm OC pesticide spiked corn muffin mix extract, after WAX-XTR cleanup. The peaks at ~ 11.9, 12.9 and 13.2 minutes are fatty acid methyl esters, and no fatty acids are present in the chromatogram.
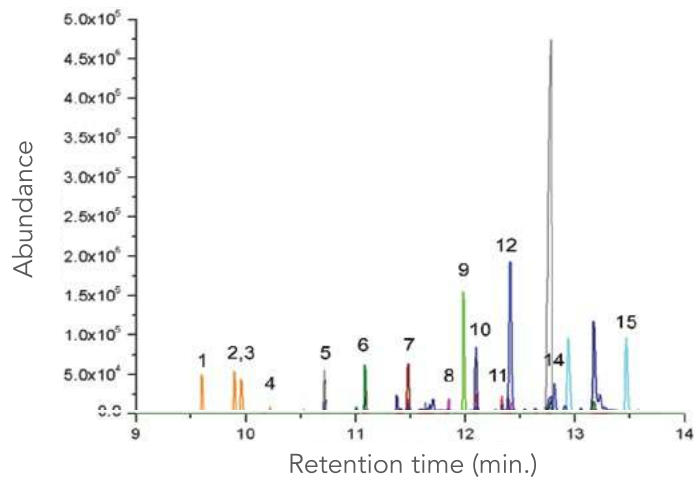

Figure 4. Extracted ion chromatogram of OC pesticides spiked at 0.5 ppm in corn muffin mix extract, after WAX-XTR cleanup. (Compound number 13 is not shown because its extracted ion has a high background that obscures the display of several other compounds in this figure.)
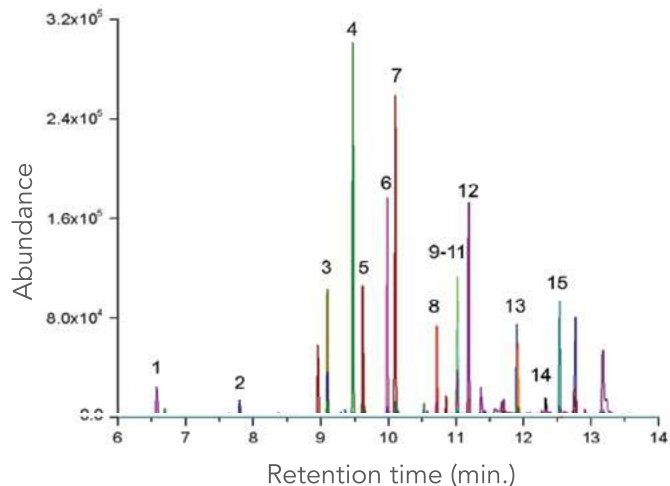

Figure 5. Extracted ion chromatogram of OP pesticides spiked at 0.5 ppm in corn muffin mix extract, after WAX-XTR cleanup.
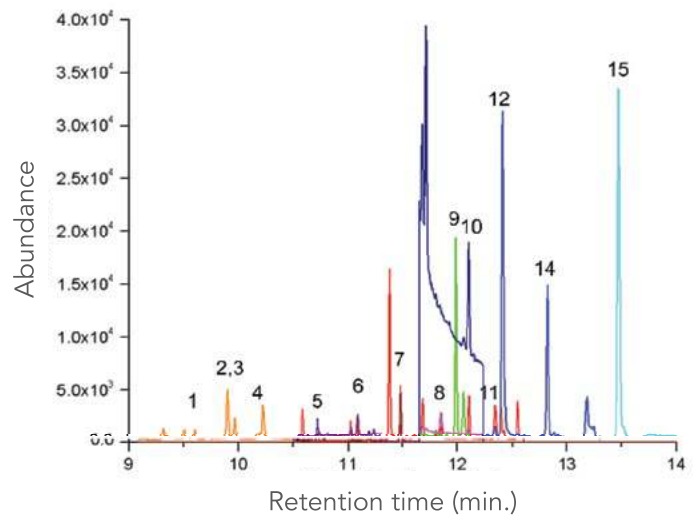

Figure 6. SIM chromatogram of OP pesticides spiked at 50 ppb in corn muffin mix extract, after WAX-XTR cleanup.
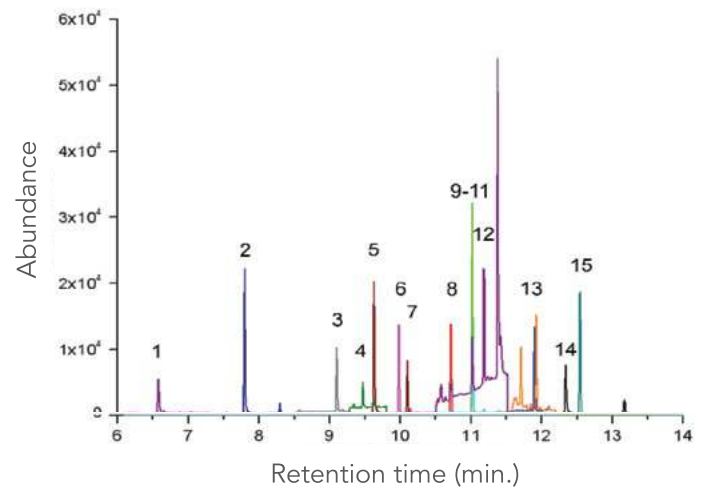

Figure 7. SIM chromatogram of OP pesticides spiked at 50 ppb in corn muffin mix extract, after WAX-XTR cleanup.
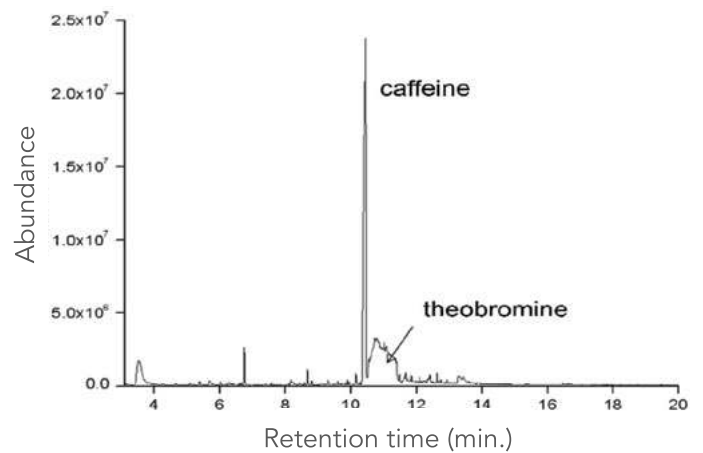

Figure 8. GC/MS chromatogram (full scan) of cocoa bean extract after WAX-XTR cleanup.

Table 2. Corn muffin mix extraction with WAX-XTR tips

OC Pesticides	Ret. Time	Ion	% Recov.	%RSD
α-BHC	6.574	181	82.46	5.01
β-BHC,	1.807	181	88.37	4.91
γ-BHC	9.102	181	85.44	1.22
δ-BHC	9.473	181	98.54	14.05
Heptachlor	9.626	100	82.67	5.02
Aldrin	9.985	66	87.69	9.82
Heptachlor epoxide	10.105	353	88.50	4.85
p,p'-DDE	10.721	246	87.74	4.17
Endosulfan I	11.023	195	91.84	5.89
Diedrin	11.023	79	92.83	4.08
Endrin	11.034	263	93.01	6.70
p,p'-DDD	11.192	235	88.10	2.86
Endrin aldehyde	11.91	67	104.24	4.91
p,p'=DDT	12.33	365	76.08	5.78
Methoxchlor	12.54	227	84.08	6.38

Table 4. Cocoa bean extraction with WAX-XTR tips

OP Pesticides	Ret. Time	Ion	% Recov.	%RSD
Dichlorphos	6.574	185	75.77	14.66
Mevinphos	1.807	127	71.33	11.67
Ethoprophos	9.102	158	106.24	16.75
Phorate	9.473	75	115.45	7.01
Dementon-S	9.626	88	108.51	4.78
Diazinone	9.985	152	117.55	7.33
Disulfoton	10.105	88	114.97	7.07
Ronnel	10.721	285	97.92	8.57
Parathion-methyl	11.023	125	109.93	5.08
Fenthion	11.023	278	99.12	5.08
Chlorpyrifos	11.034	197	108.05	2.11
Trichloronat	11.192	269	108.28	5.99
Tokuthion	11.91	267	105.91	4.44
Merphos	11.92	169	106.75	4.30
Fensulfothion	12.33	293	72.43	17.43
Bolstar	12.54	322	93.64	23.29

Table 3. Corn muffin mix extraction with WAX-XTR tips

OP Pesticides	Ret. Time	Ion	% Recov.	%RSD
Dichlorphos	6.574	109	~ 120	NA
Mevinphos	1.807	127	~ 120	NA
Ethoprophos	9.102	158	97.45	10.04
Phorate	9.473	75	78.42	13.88
Dementon-S	9.626	88	86.61	7.22
Diazinone	9.985	179	89.48	10.65
Disulfoton	10.105	88	84.43	12.00
Ronnel	10.721	285	116.64	9.08
Parathion-methyl	11.023	109	92.22	9.28
Fenthion	11.023	278	82.47	5.37
Chlorpyrifos	11.034	197	91.61	5.63
Trichloronat	11.192	109	83.93	8.09
Tokuthion	11.91	267	83.70	6.35
Merphos	11.92	169	63.40	5.57
Fensulfothion	12.33	293	96.51	19.20
Bolstar	12.54	322	82.20	5.41

CONCLUSIONS

The automated clean up extractions using WAX-XTR 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 dispersive pipette extraction", Florida Pesticide Workshop, July 2008, St. Pete Beach, FL.

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