



Foods, Flavors & Fragrances Applications

Comparing Pesticide Residues in Amish and Commercially Grown Strawberries and Spinach Using QuEChERS, Various dSPE Sorbents, and GC-TOFMS

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Abstract

QuEChERS extraction, dSPE cleanup, and GC-TOFMS analysis were used to assess pesticide levels in strawberries and spinach from both commercial and Amish growers. Various dSPE cleanup products were compared to determine which were most effective. Good recoveries were obtained for most pesticides; however, low recoveries were observed for some base-sensitive or planar compounds. Incurred pesticides were generally low and varied by both matrix and source.

Introduction

Most produce available in grocery stores is grown using conventional agricultural practices that include the use of pesticides. However, movements toward locally obtained food have increased interest in roadside fruit and vegetable stands. These stands are common in areas with Amish communities, where the agricultural trades are an economic mainstay. The Amish are a group of Christian religious denominations that are characterized by simple living and the rejection of many basic conveniences, such as power line electricity, telephones, and cars. Since conservative principles limit the use of many modern technologies, there is a perception among the non-Amish that pesticides may not be used in Amish agricultural practices. In the United States the Amish are not governed by all of the same regulations as other citizens, which means that their farms are not always subjected to the same scrutiny as commercial farms. Here we tested fresh strawberries and spinach purchased from both an Amish farm and a local grocery store to determine if pesticide residues were present.

We used a QuEChERS-based sample preparation method and gas chromatography-time of flight mass spectrometry (GC-TOFMS) for analyzing pesticides. Several different dispersive solid phase extraction (dSPE) formulations in ready-to-use tubes were tested to determine which provided an optimum balance of sample cleanup along with adequate recoveries. QuEChERS is an approach developed by Anastassiades et al. [1] as a simple, rapid, effective, yet inexpensive, way to extract pesticide residues from fruits and vegetables, followed by a novel dSPE cleanup of the extract. We chose QuEChERS as an alternative to Pesticide Analytical Manual (PAM) [2] based methods because of its speed, simplicity, and low solvent use, as well as its ability to produce good extraction efficiencies for relatively polar pesticides [1,3]. QuEChERS extracts were analyzed by GC-TOFMS. TOFMS offers powerful data processing, due to fast acquisition rates and unbiased mass spectra, as well as picogram level sensitivity in full mass range mode.

Experimental

Pesticide Standard

We chose to test a group of pesticides that varied in volatility, polarity, and pH sensitivity. A 200 ng/μL (ppm) mixed pesticide stock solution was prepared and then diluted with acetonitrile to make 10 and 1 ng/μL fortification standards. The multi-component pesticide mix was a custom standard produced by Restek's Reference Standards group.

Sample Preparation

Strawberry and spinach samples were fortified to determine pesticide recoveries when compared to matrix-matched standards. Typically, QuEChERS methods use 10-15 grams of material per extraction and are ideal for commodities with high water content (>80%). In this work, 10 g samples of each commodity were used. Unfortified samples were also prepared to determine incurred pesticides and to produce matrix-matched standards.

Commodities were first homogenized, then 10 g sample aliquots were weighed into separate 50 mL centrifuge tubes (cat.# 26239). Fortified samples were prepared at 100 ng/g (ppb) by adding 100 μL of the 10 ng/μL pesticide spiking solution to 10 g of sample. Similarly, samples were prepared at 10 ng/g (ppb) by adding 100 μL of the 1 ng/μL pesticide spiking solution to 10 g of sample. Also, 100 μL of QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) was added to each sample. This internal standard mix requires no dilution ("snap-and-shoot") and contains six compounds specified in the QuEChERS method EN 15662 [4].

QuEChERS Extraction

The EN 15662 QuEChERS method was used for sample extraction [4]. 10 mL of acetonitrile was added to the 10 g homogenized sample. After a 1 minute manual shake, Q-sep™ Q110 buffering extraction salts (cat.# 26235), containing 4 g MgSO₄, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate, were added. Following another 1 minute shake, the sample was centrifuged for 5 minutes at 3,000 U/min. with a Q-sep™ 3000 centrifuge (cat.# 26230). The top layer (acetonitrile) was removed to a clean vial. Lastly, 5 μL of an anthracene quality control standard (cat.# 33264) was added per 1 mL of extract prior to cleanup. Anthracene was used to monitor potential losses of planar pesticides to graphitized carbon black (GCB) during QuEChERS dispersive cleanup.

QuEChERS Dispersive Solid Phase Extraction (dSPE) Cleanup

Ready-to-use tubes of different dSPE sorbent formulations, listed below, were tested to determine which sorbents provided the most sample cleanup in combination with high pesticide recovery values. Primary secondary amine (PSA) was used to remove matrix compounds like sugars and fatty acids. C18 sorbent was used to remove nonpolar matrix components, and graphitized carbon black (GCB) was used to remove pigments and sterols. GCB removes planar molecules so there is a risk of losing planar pesticides when GCB is part of the dSPE sorbent formulation. Magnesium sulfate was used to remove trace amounts of water from the acetonitrile extract. For dSPE, 1 mL of extract was added to each dSPE tube. Each tube was manually shaken for 30 seconds or 2 minutes, if containing GCB, and then centrifuged for 5 minutes. The resulting final extract was then analyzed by GC-TOFMS.

QuEChERS dSPE tubes:

- Restek Q-sep™ Q210 (cat.# 26215), 25 mg PSA, 150 mg MgSO₄
- Restek Q-sep™ Q212 (cat.# 26217), 25 mg PSA, 2.5 mg GCB, 150 mg MgSO₄
- Restek Q-sep™ Q213 (cat.# 26218), 25 mg PSA, 7.5 mg GCB, 150 mg MgSO₄
- Restek Q-sep™ Q252 (cat.# 26219), 50 mg PSA, 50 mg C18, 50 mg GCB, 150 mg MgSO₄

Matrix-Matched Standards

Matrix-matched standards were prepared at 100 ng/mL and 10 ng/mL (ppb), as these were the expected final concentrations (assuming 100% recovery values) in the 100 ng/g and 10 ng/g fortified samples. Matrix-matched standards were prepared by adding pesticide standard solution to a final (post-cleanup) extract of a non-fortified sample. Actual recoveries were calculated by comparing peak areas of fortified samples that were extracted and cleaned up to areas of a matrix-matched standard, using the internal standard quantification method with PCB 52 from the QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267), which was added prior to extraction.

GC-TOFMS Analysis

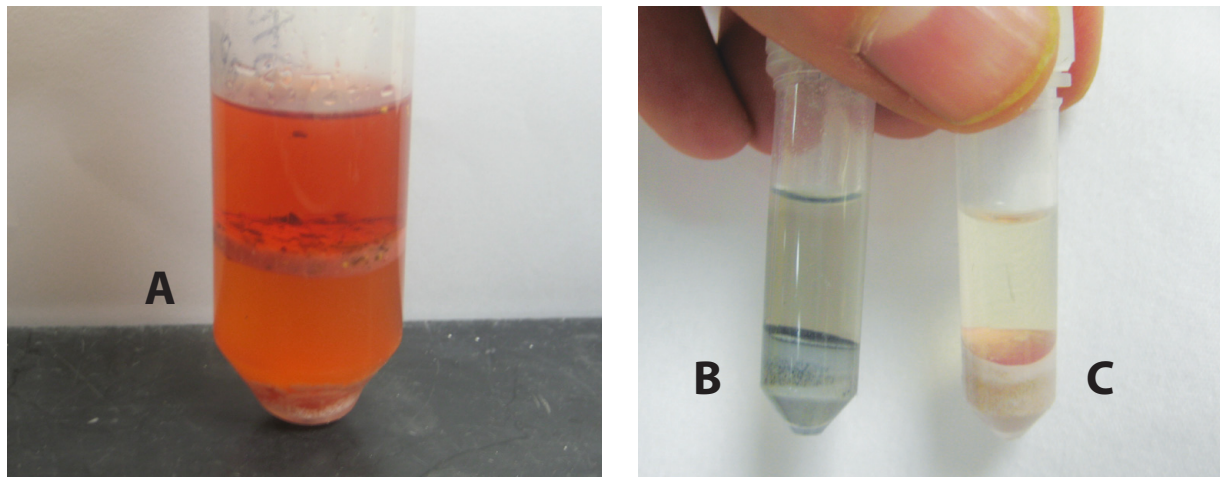
A LECO® Pegasus® 4D GCxGC-TOFMS was used for GC-TOFMS analysis and all data were processed with ChromaTOF® software. Gas chromatography was performed using a 30 m x 0.25 mm x 0.25 μm Rxi®-5Sil MS column (cat.# 13623) with a constant flow of helium at 2 mL/min. and a fast, autosampler, splitless injection of 1 μL, purge valve time of 1.5 minutes, into a 5 mm single goose-neck liner with wool (cat.# 22973-200.1). The inlet temperature was 250 °C and the GC oven program was 90 °C (hold 1.5 min.) to 340 °C at 10 °C/min. resulting in a 26.5 minute analysis time. Electron ionization at 70 eV was used with a source temperature of 225 °C. Data acquisition was from 45 to 550 u at an acquisition rate of 5 spectra/sec.

Results and Discussion

Strawberries

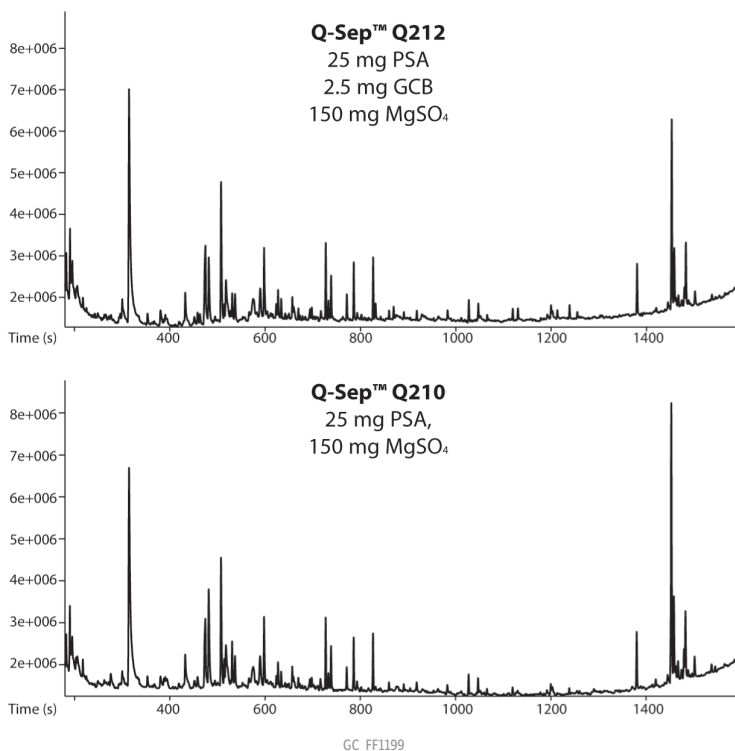
Strawberries produced a mid-intensity colored extract (Figure 1, A). The subsequent dSPE cleanup of the acetonitrile layer decreased levels of matrix co-extractives and removed some of the less volatile components, such as pigments. This reduces contamination of the GC inlet and front of the GC column by removing nonvolatile matrix co-extractives which can deposit in the front of the column. Two different QuEChERS dSPE cleanup products, Q-sep™ Q212 and Q-sep™ Q210 tubes, were tested and both removed most of the red color from the extract (Figure 1, B and C).

Figure 1 QuEChERS sample preparation for strawberry. Analytes were extracted in acetonitrile (A, top layer), then 2 dSPE cleanup formulations were compared: (B) Q212 tube, 25 mg PSA, 2.5 mg GCB, 150 mg MgSO₄, and (C) Q210 tube, 25 mg PSA, 150 mg MgSO₄.



In addition to pigments, many other matrix compounds can be extracted with target pesticides. Comparing chromatograms from strawberry samples processed with the 2 different dSPE tubes helps determine which sorbent formulation removes more matrix compounds overall. Total ion chromatograms (TICs) plotted on the same scale in Figure 2 show there is little obvious difference between the two cleanup formulations. For strawberry, both color intensity and TIC comparisons indicate that both cleanup formulations provide a similar degree of sample cleanup.

Figure 2 Reduction of matrix components in strawberry samples was similar for both Q-sep™ Q212 and Q-sep™ Q210 dSPE cleanup tubes.



GC_FF1199

Column Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)
Sample Strawberries processed with QuEChERS sample preparation
 QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267)
 anthracene for quality control (cat.# 33264)
 acetonitrile

Diluent:
Injection
 Inj. Vol.: 1 µL splitless (hold 1.5 min.)
 Liner: 5mm Splitless with wool (cat.# 22973-200.1)
 Inj. Temp.: 250 °C

Oven
 Oven Temp: 90 °C (hold 1.5 min.) to 340 °C at 10 °C/min.

Carrier Gas He, constant flow
Flow Rate: 2 mL/min.

Detector MS
 Mode:

Analyzer Type: TOF
Source Temp.: 225 °C
Electron Energy: 70 eV
Ionization Mode: EI
Acquisition Range: 45-550 amu
 Spectral

Acquisition Rate: 5 spectra/sec
Instrument LECO Pegasus 4D GCxGC-TOFMS

Notes
Sample Preparation:
 Sample: 10 g of homogenized strawberries, fortified with pesticides and QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267).
Extraction: 10 mL acetonitrile added, 1 minute shake, then addition of Q-sep™ Q110 (cat.# 26235), 1 minute shake, centrifuge with Q-sep™ 3000 Centrifuge (cat.# 26230).
dSPE: cleanup procedure according to EN 15662 method, add control standard anthracene (cat.# 33264) to 1 mL extract, add this to one of two different Q-sep™ dSPE tubes (either cat.# 26215 or cat.# 26217), shake, centrifuge.

Next, the recoveries of target pesticides were compared to determine if cleanup procedures were viable, and also to further evaluate which dSPE sorbent formulation was best. With few exceptions, both the Q-sep™ Q210 and Q-sep™ Q212 cleanup tubes resulted in strong recovery values near 100±20% for the 100 ppb fortification level (Table I). Chlorothalonil and dichlofluanid showed low recovery values, but this is not unexpected as these compounds are base-sensitive and known to be problematic [5]. However, since strawberries are acidic and the QuEChERS extraction buffers the solution below neutral pH, the low recovery values observed might be due to degradation in acetonitrile [6,7]. Commercially grown strawberries showed a relatively large amount of captan in the unfortified sample. Because of this, a recovery value was not determined.

The cleanup formulations tested differ in that Q-sep™ Q212 tubes include 2.5 mg of GCB, while Q-sep™ Q210 tubes do not contain any. Since GCB removes pigments and other planar molecules, anthracene was used as a quality control standard to track large losses of planar compounds. Near 100% recoveries of anthracene were obtained for both cleanup formulations, indicating that QuEChERS extraction and either cleanup formulation can provide satisfactory results for strawberries (Table I). Although both products provided good recoveries at 100 ppb, the Q-sep™ Q210 dSPE cleanup may be preferred to avoid the possibility of GCB-related loss of planar pesticides at lower levels.

Table I Good recoveries for a variety of pesticides were obtained for QuEChERS extracts of strawberries fortified at 100 ppb. Both dSPE cleanup formulations provided similar results.

Compound	RT (min.)	% Recovery Q210 (25 mg PSA)	% Recovery Q212 w (25 mg PSA, 2.5 mg GCB)	Class
Methamidophos	5.35	91	88	Organonitrogen
Dichlorvos	5.58	110	120	Organophosphorus
Mevinphos	7.95	110	110	Organophosphorus
Acephate	7.98	85	81	Organophosphorus
<i>o</i> -Phenylphenol	9.09	110	110	Other
Omethoate	9.91	75	53	Organonitrogen
Dimethoate	11.44	100	96	Organophosphorus
Diazinon	12.16	110	110	Organophosphorus
Chlorothalonil	12.20	37	31	Organohalogen
Anthracene	12.31	97	95	Quality control
Vinclozolin	13.16	110	120	Organonitrogen
Carbaryl	13.31	99	91	Organonitrogen
Metalaxyl	13.38	110	120	Organophosphorus
PCB 52	13.78	–	–	Internal standard
Dichlofluanid	13.84	61	53	Organonitrogen
Malathion	13.89	100	110	Organophosphorus
Thiabendazole	14.96	98	73	Organonitrogen
Captan	14.99	NQ	NQ	Organonitrogen
Folpet	15.10	94	89	Organonitrogen
Imazalil	15.76	110	110	Organonitrogen
Myclobutanil	16.01	110	120	Organophosphorus
Endrin	16.44	98	110	Organohalogen
Fenhexamid	17.42	100	95	Organonitrogen
4,4'-DDT	17.44	110	100	Organohalogen
Propargite	17.75	99	98	Other
Iprodione	18.15	110	100	Organonitrogen
Bifenthrin	18.33	110	110	Organohalogen
Fenpropathrin	18.50	110	110	Organonitrogen
<i>cis</i> -Permethrin	20.08	100	93	Organohalogen
<i>trans</i> -Permethrin	20.21	100	110	Organohalogen
Deltamethrin	22.53	120	88	Organohalogen

NQ = not quantifiable, due to high level of incurred pesticide.

Recovery was evaluated for the 10 ppb fortification level using the Q-sep™ Q210 dSPE tubes to avoid the loss of planar compounds (Table II). Six pesticides were not detected in the fortified sample. Since acephate and omethoate also were not found in the matrix-matched standard, these compounds were either lost during the analysis or fell below the detection limit of the GC-TOFMS method used for this work. The remaining four compounds that were not detected, methamidophos, chlorothalonil, *cis*-permethrin and deltamethrin, were observed in the 10 ppb matrix-matched standard, but not in the spiked sample. This indicates that these compounds can be detected at 10 ppb, but are lost or partially lost during sample preparation. Besides these six compounds, most of the recovery values were acceptable.

Table II Q-sep™ Q210 dSPE cleanup of QuEChERS extracts resulted in good recoveries for many pesticides fortified at 10 ppb in strawberries. However, several pesticides were not detected at this level.

Compound	RT (min.)	% Recovery Q210 (25 mg PSA)	Class
Methamidophos	ND	ND	Organonitrogen
Dichlorvos	5.59	70	Organophosphorus
Mevinphos	7.95	90	Organophosphorus
Acephate	ND	ND	Organophosphorus
<i>o</i> -Phenylphenol	9.09	90	Other
Omethoate	ND	ND	Organonitrogen
Dimethoate	11.44	100	Organophosphorus
Diazinon	12.16	70	Organophosphorus
Chlorothalonil	ND	ND	Organohalogen
Anthracene	12.31	100	Quality control
Vinclozolin	13.17	130	Organonitrogen
Carbaryl	13.31	100	Organonitrogen
Metalaxyl	13.38	130	Organophosphorus
PCB 52	13.78	–	Internal standard
Dichlofluanid	13.83	70	Organonitrogen
Malathion	13.89	110	Organophosphorus
Thiabendazole	14.96	100	Organonitrogen
Captan	14.99	NQ	Organonitrogen
Folpet	15.10	110	Organonitrogen
Imazalil	15.77	160	Organonitrogen
Myclobutanil	16.01	120	Organophosphorus
Endrin	16.44	90	Organohalogen
Fenhexamid	17.42	90	Organonitrogen
4,4'-DDT	17.44	80	Organohalogen
Propargite	17.70	70	Other
Iprodione	18.16	130	Organonitrogen
Bifenthrin	18.33	85	Organohalogen
Fenpropathrin	18.50	80	Organonitrogen
<i>cis</i> -Permethrin	ND	ND	Organohalogen
<i>trans</i> -Permethrin	20.21	100	Organohalogen
Deltamethrin	ND	ND	Organohalogen

ND = not detected; NQ = not quantifiable, due to high level of incurred pesticide.

Incurred Pesticides in Amish and Commercially Grown Strawberries

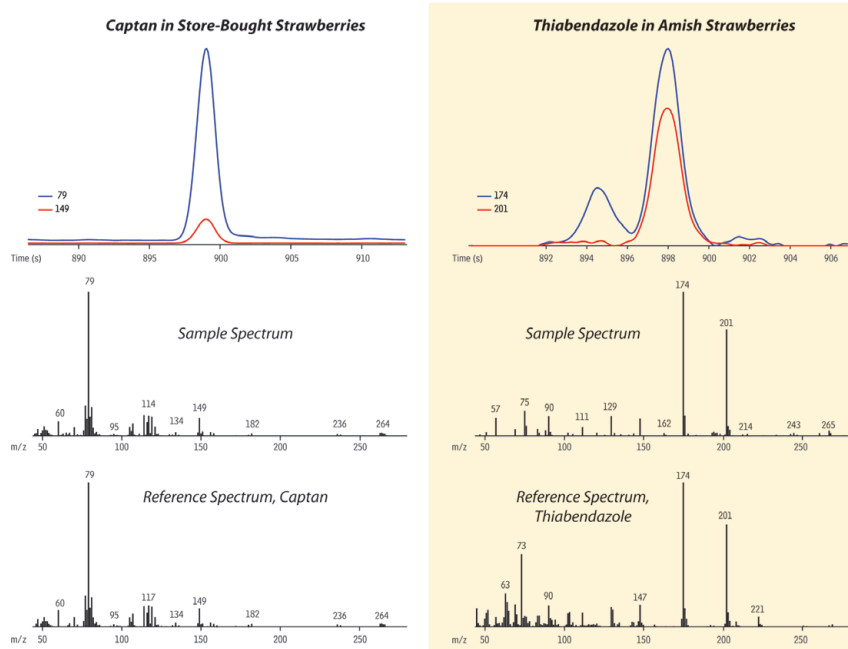
The QuEChERS dSPE sorbents discussed above both provided effective sample cleanup and good recovery values for most of the target pesticides. However, since GCB was not necessary for sample cleanup, Q-sep™ Q210 tubes were used to evaluate incurred samples in order to prevent the possible loss of low levels of planar pesticides. As shown in Table III, Amish strawberries contained small amounts of 6 pesticides: thiabendazole, fenhexamid, captan, 4,4'-DDT, *o*-phenylphenol and imazalil. Commercially grown strawberry samples contained fewer pesticides from the target list but had a much higher level of captan, estimated at 1.4 ppm. Because of this, the Amish (low captan) matrix-matched standard was used to estimate this value. Amounts of incurred pesticides in Amish strawberries, except for captan, were determined using the matrix-matched standard for store-bought strawberries because this sample did not contain the other five incurred pesticides found in the Amish sample.

Table III Levels of incurred pesticides found in strawberries from both Amish and commercial vendors.

Amish Vendor		Commercial Vendor	
Pesticide	ppb	Pesticide	ppb
Captan	<1	Captan	1,400
Thiabendazole	11		
Fenhexamid	8		
DDT	1		
<i>o</i> -Phenylphenol	3		
Imazalil	11		

Definitive compound identification of the incurred pesticides was made through retention time evaluation and comparison of experimental and reference spectra. For example, Figure 3 shows the overlay of extracted ion chromatograms (XICs) for ions characteristic of captan, m/z 79 and 149, as well as reference and sample spectra. The retention time of the incurred captan peak is 14.98 minutes which matches very closely to the retention time of 14.99 minutes for captan in the fortified samples (Tables I and II). Figure 3 also shows similar data for thiabendazole found in Amish strawberries. The retention time for thiabendazole in the incurred sample is 14.96 minutes, which is the same as in the spiked samples (Tables I and II). The spectrum from incurred thiabendazole also matches well with the reference spectrum. In addition to retention time matching, alignment of the peak apexes in the XICs, as well as the close match between empirical and full mass range reference spectra, make identification of these incurred pesticides straightforward.

Figure 3 Pesticide identification in incurred samples was based on retention time comparisons to fortified samples, as well as on good matches between deconvoluted sample and reference spectra.



GC_FF1201

Column Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)
Sample Strawberries processed with QuEChERS sample preparation QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) anthracene for quality control (cat.# 33264)
Diluent: acetonitrile
Conc.: captan incurred in store bought strawberries at 1,400 ppb and thiabendazole incurred in Amish strawberries at about 10 ppb
Injection
Inj. Vol.: 1 µL splitless (hold 1.5 min.)
Liner: 5mm Splitless with wool (cat.# 22975-200.1)
Inj. Temp.: 250 °C
Oven
Oven Temp: 90 °C (hold 1.5 min.) to 340 °C at 10 °C/min.
Carrier Gas He, constant flow
Flow Rate: 2 mL/min.
Detector MS
Mode:
Analyzer Type: TOF
Source Temp.: 225 °C
Electron Energy: 70 eV
Ionization Mode: EI
Acquisition Range: 45-550
Spectral
Acquisition Rate: 5 spectra/sec
Instrument LECO Pegasus 4D GCxGC-TOFMS
Notes Sample Preparation:
Sample: 10 g of homogenized strawberries, either Amish or store bought, and QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267).

Extraction: 10 mL acetonitrile added, 1 minute shake, then addition of Q-sep™ Q110 (cat.# 26235), 1 minute shake, centrifuge with Q-sep™ 3000 Centrifuge (cat.# 26230).

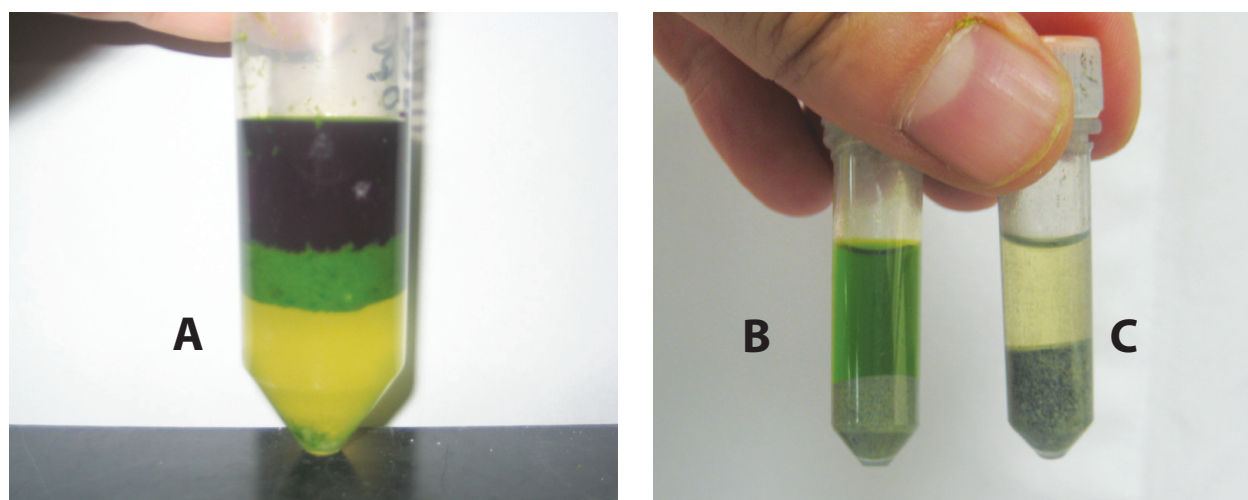
dSPE: cleanup procedure according to EN 15662 method, add control standard anthracene (cat.# 33264) to 1 mL extract, add this to Q-sep™ Q210 dSPE tubes (cat.# 26215), shake, centrifuge.

Analysis: Extracted ion chromatograms of m/z 79 and 149 for captan and m/z 174 and 201 for thiabendazole.

Spinach

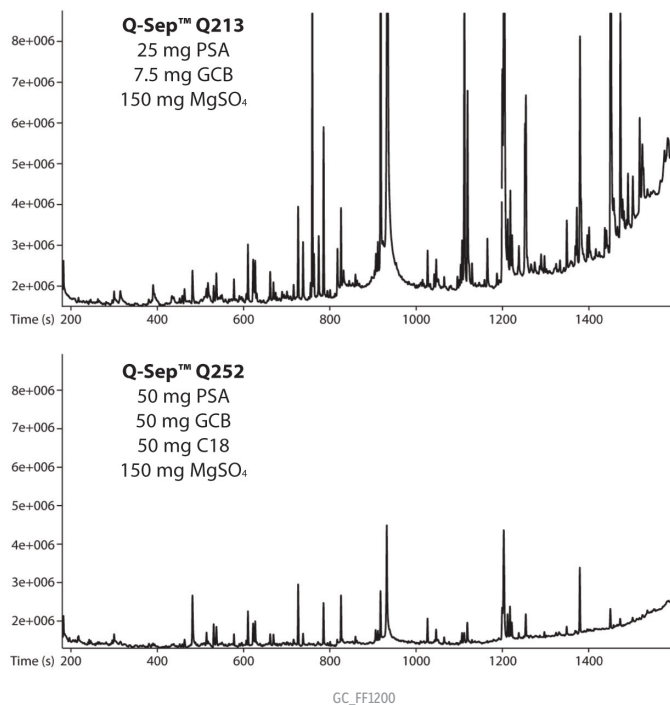
Spinach produced a very intensely colored extract (Figure 4, A). The concentrated color indicates that a large amount of pigment, chlorophyll, was extracted in acetonitrile along with the target analytes. Chlorophyll is not volatile enough to chromatograph, so it can deposit in the liner and column and cause performance issues. GCB was used during sample cleanup to help remove pigment and minimize system contamination. GCB can also remove planar pesticides causing low recovery values so caution should be used. Two ready-to-use dSPE cleanup products were tested, Q-sep™ Q213 tubes containing 25 mg PSA, 7.5 mg GCB, 150 mg MgSO₄, and Q-sep™ Q252 tubes containing 50 mg PSA, 50 mg C18, 50 mg GCB, 150 mg MgSO₄. Both formulations removed pigment from the extract, but the higher amount of GCB in the Q-sep™ Q252 tube provided more capacity to remove pigments and produced a less intensely colored sample (Figure 1, B and C). Although the Q-sep™ Q252 cleanup did not remove all the pigment, it significantly reduced the amount of nonvolatile material in the final extract, which can decrease contamination and increase the number of samples that can be analyzed before instrument maintenance is required.

Figure 4 QuEChERS sample preparation for spinach. Analytes were extracted in acetonitrile (A, top layer), then 2 dSPE cleanup formulations were compared: (B) Q213 tube, 25 mg PSA, 7.5 mg GCB, 150 mg MgSO₄, and (C) Q252 tube, 50 mg PSA, 50 mg C18, 50 mg GCB, 150 mg MgSO₄.



Cleanup treatments were also compared by examining the coextracted volatile component profiles on the total ion chromatograms (Figure 5). Results demonstrate that, as expected, the Q-sep™ Q252 tube, which contained C18 and more GCB, removed more matrix compounds. This dSPE formulation is preferred for spinach, because less matrix compounds in the final cleaned extract resulted in decreased background which can improve sensitivity and reduce matrix signal enhancement [8,9].

Figure 5 Q-sep™ Q252 tubes provided more effective cleanup of QuEChERS spinach extracts than Q-sep™ Q213 tubes, due to the presence of C18 and more GCB.



Column Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)
Sample Spinach processed with QuEChERS sample preparation
 QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267)
 anthracene for quality control (cat.# 33264)
 acetonitrile

Diluent:
Injection
 Inj. Vol.: 1 µL splitless (hold 1.5 min.)
 Liner: Gooseneck Splitless (5mm) (cat.# 22973-200.1)
 Inj. Temp.: 250 °C

Oven
 Oven Temp: 90 °C (hold 1.5 min.) to 340 °C at 10 °C/min.

Carrier Gas He, constant flow
 Flow Rate: 2 mL/min.

Detector MS

Mode:
 Analyzer Type: TOF
 Source Temp.: 225 °C
 Electron Energy: 70 eV
 Ionization Mode: EI
 Acquisition Range: 45-550 amu

Spectral
 Acquisition Rate: 5 spectra/sec
Instrument LECO Pegasus 4D GCxGC-TOFMS

Notes
 Sample Preparation:
 Sample: 10 g of homogenized spinach, fortified with pesticides and QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267).

Extraction: 10 mL acetonitrile added, 1 minute shake, then addition of Q-sep™ Q110 (cat.# 26235), 1 minute shake, centrifuge with Q-sep™ 3000 Centrifuge (cat.# 26230).

dSPE: Cleanup procedure according to EN 15662 method, add control standard anthracene (cat.# 33264) to 1 mL extract, add this to one of two different Q-sep™ dSPE tubes (either cat.# 26218 or cat.# 26219), shake, centrifuge.

Based on the post-cleanup color change of the extract and TIC results demonstrating the removal of nonvolatile compounds, the Q-sep™ Q252 cleanup formulation appeared to outperformed the Q-sep™ Q213 formulation for spinach. Recoveries were also evaluated to determine if adequate values, particularly of planar compounds, could still be obtained from the cleaner sample (Table IV). Most recoveries were in the 100±20% range, but there were some exceptions. Q-sep™ Q213 cleanup does not suffer from low recoveries, but elevated recovery values of 150% for carbaryl and iprodione were obtained. These elevated values may be a result of matrix signal enhancement because the values decrease with the higher capacity Q-sep™ Q252 cleanup. Low recoveries for base-sensitive pesticides chlorothalonil, dichlofluanid, captan, and folpet were observed with both cleanup products, but this was expected as spinach is a basic commodity and these compounds degrade in basic pH conditions [5]. Lower recoveries were also observed for thiabendazole and fenhexamid in the Q-sep™ Q252 sample, which likely was due to the higher GCB content in the formulation since these are planar compounds.

Table IV Recovery results for the 100 ppb fortification level demonstrate the importance of dSPE sorbent choice for spinach samples. Elevated recoveries were obtained for some compounds when using Q-sep™ Q213 tubes, due to matrix enhancement. The additional GCB in Q-sep™ Q252 tubes reduced this matrix effect, but lower recoveries of planar pesticides were observed.

Compound	RT (min.)	% Recovery Q213 (25 mg PSA, 7.5 mg GCB)	% Recovery Q252 (50 mg PSA, 50 mg GCB, 50 mg C18)	Class
Methamidophos	5.35	100	70	Organonitrogen
Dichlorvos	5.58	110	95	Organophosphorus
Mevinphos	7.94	110	100	Organophosphorus
Acephate	7.95	99	92	Organophosphorus
<i>o</i> -Phenylphenol	9.09	110	92	Other
Omethoate	9.89	120	120	Organonitrogen
Dimethoate	11.43	120	110	Organophosphorus
Diazinon	12.15	110	92	Organophosphorus
Chlorothalonil	12.20	64	21	Organohalogen
Anthracene	12.30	100	99	Quality control
Vinclozolin	13.16	110	110	Organonitrogen
Carbaryl	13.30	150	80	Organonitrogen
Metalaxyl	13.37	110	110	Organophosphorus
PCB 52	13.77	–	–	Internal standard
Dichlofluanid	13.82	11	6	Organonitrogen
Malathion	13.88	140*	110	Organophosphorus
Thiabendazole	14.96	92	42	Organonitrogen
Captan	ND	ND	ND	Organonitrogen
Folpet	15.09	11	17	Organonitrogen
Imazalil	15.77	86	69	Organonitrogen
Myclobutanil	16.01	110	100	Organophosphorus
Endrin	16.43	110	94	Organohalogen
Fenhexamid	17.41	86	66	Organonitrogen
4,4'-DDT	17.43	77	88	Organohalogen
Propargite	17.71	120	100	Other
Iprodione	18.14	150	120	Organonitrogen
Bifenthrin	18.32	110	91	Organohalogen
Fenpropathrin	18.49	93	96	Organonitrogen
<i>cis</i> -Permethrin	20.07	NQ	NQ	Organohalogen
<i>trans</i> -Permethrin	20.20	NQ	NQ	Organohalogen
Deltamethrin	22.52	NQ	NQ	Organohalogen

ND = not detected; NQ = not quantifiable, due to high level of incurred pesticide.

* Reprocessed with different quant ion due to matrix interference (m/z 125 to m/z 173).

Since matrix effects were observed for some pesticides at the 100 ppb level with Q-sep™ Q213 cleanup, samples from the Q-sep™ Q252 cleanup were used for evaluating recoveries at the 10 ppb level (Table V). Six compounds were not detected in the 10 ppb fortification samples. Of these, acephate, omethoate and folpet, also were not detected in the matrix-matched standard. Acephate and omethoate are not likely to be detected in either the fortified sample or the matrix-matched standard due to the presence of matrix interferences and also because at 10 ppb they are at the border of their detection limits. Folpet is a known base-sensitive pesticide that likely degraded quickly under the alkaline conditions [7]. The remaining compounds that were not detected in the fortified sample, methamidophos, chlorothalonil and captan, were observed in the matrix-matched standard. This indicates that these compounds can be detected at 10 ppb but were lost or partially lost during sample preparation. Chlorothalonil, captan, and dichlofluanid are base-sensitive pesticides and their loss or low recovery was probably due to degradation under basic conditions during sample preparation [7]. Compound degradation can be slowed by acidifying the sample and performing the extraction at low temperature [10]. Thiabendazole, a planar compound, had a low recovery value likely due to loss to GCB. Besides these compounds, most of the recovery values were acceptable.

Table V Low recoveries were observed for some base-sensitive or planar compounds in 10 ppb fortifications of spinach. Recoveries of most other pesticides from QuEChERS extracts cleaned up with Q-sep™ Q252 sorbents were acceptable.

Compound	RT (min.)	% Recovery Q252		Class
		(50 mg PSA, 50 mg GCB, 50 mg C18)		
Methamidophos	ND	ND		Organonitrogen
Dichlorvos	5.58	110		Organophosphorus
Mevinphos	7.94	80		Organophosphorus
Acephate	ND	ND		Organophosphorus
<i>o</i> -Phenylphenol	9.09	100		Other
Omethoate	ND	ND		Organonitrogen
Dimethoate	11.43	70		Organophosphorus
Diazinon	12.15	60		Organophosphorus
Chlorothalonil	ND	ND		Organohalogen
Anthracene	12.30	100		Quality control
Vinclozolin	13.16	100		Organonitrogen
Carbaryl	13.30	70		Organonitrogen
Metalaxyl	13.38	140		Organophosphorus
PCB 52	13.77	–		Internal standard
Dichlofluanid	13.83	20		Organonitrogen
Malathion	13.89	90		Organophosphorus
Thiabendazole	14.96	20		Organonitrogen
Captan	ND	ND		Organonitrogen
Folpet	ND	ND		Organonitrogen
Imazalil	15.76	60		Organonitrogen
Myclobutanil	16.01	90		Organophosphorus
Endrin	16.44	70		Organohalogen
Fenhexamid	17.42	80		Organonitrogen
4,4'-DDT	17.44	70		Organohalogen
Propargite	17.72	120		Other
Iprodione	18.15	90		Organonitrogen
Bifenthrin	18.33	80		Organohalogen
Fenprothrin	18.51	100		Organonitrogen
<i>cis</i> -Permethrin	20.07	100		Organohalogen
<i>trans</i> -Permethrin	20.20	100		Organohalogen
Deltamethrin	22.53	70		Organohalogen

ND = not detected; NQ = not quantifiable, due to high level of incurred pesticide.

Incurred Pesticides in Amish and Commercially Grown Spinach

Estimated values of incurred pesticides in both Amish and commercial spinach are presented in Table VI. Spinach samples from both sources contained a small amount of deltamethrin. In addition, commercially grown spinach contained *cis*- and *trans*-permethrin. Amounts of *cis*- and *trans*-permethrin in store-bought spinach were determined using the matrix-matched standard of Amish spinach, because it did not contain these pesticides. Deltamethrin for both store-bought and Amish spinach was estimated using the 100 ppb Amish matrix-matched spinach standard because all the spinach samples contained some incurred deltamethrin.

Table VI Levels of incurred pesticides found in spinach from both Amish and commercial vendors.

Amish Vendor		Commercial Vendor	
Pesticide	ppb	Pesticide	ppb
Deltamethrin	10	Deltamethrin	7
		<i>cis</i> -Permethrin	1,900
		<i>trans</i> -Permethrin	800

Conclusions

Incurred residues were found, generally at low levels, in strawberries and spinach samples from both Amish and commercial vendors. Overall, the QuEChERS extraction and dSPE cleanup, along with GC-TOFMS analysis, provided an effective approach for pesticide residue testing of these matrices. Good recoveries were obtained for most pesticides; however, low recoveries were observed for some base-sensitive or planar compounds, illustrating the importance of considering commodity pH and monitoring the loss of planar pesticides when using GCB.

The basic methodology presented here is a simple, effective strategy that can be applied to other pesticides and commodities. Analytical benefits to this approach include reduced interferences and good recoveries for most pesticides. Other benefits include an overall savings of both materials and sample preparation time compared to traditional methods, and better expected reproducibility due to the straightforward procedure with fewer manual preparations.

References

- [1] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC International 86 (2003) 412.
- [2] US Food and Drug Administration, Pesticide Analytical Manual, Volume I: Multiresidue Methods, 3rd ed. (1994).
- [3] S.J. Lehotay, K. Ae Son, H. Kwon, U. Koesukwiwat, W. Fu, K. Mastovska, E. Hoh, N. Leepipatpiboon, J. Chromatogr. A 1217 (2010) 2548.
- [4] Foods of Plant Origin—Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE (QuEChERS-method). (EN 15662 Version 2008.)
- [5] S.J. Lehotay, K. Mastovska, A.R. Lightfield, J. AOAC International 88 (2005) 615.
- [6] K. Mastovska, S.J. Lehotay, M. Anastassiades, Anal. Chem. 77 (2005) 8129.
- [7] K. Mastovska, S.J. Lehotay, J. Chromatogr. A 1040 (2004) 259.
- [8] C.F. Poole, J. Chromatogr. A 1158 (2007) 241.
- [9] T. Cajka, K. Mařtovská, S.J. Lehotay, J. Hajřlová, J. Sep. Sci. 28 (2005) 1048.
- [10] R.H. Savant, K. Banerjee, S.C. Utture, S.H. Patil, S. Dasgupta, M.S. Ghaste, P.G. Adsule, J. Agric. Food Chem. 58 (2010) 1447.

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