

Determining Pesticides in Dietary Supplements with QuEChERS Extraction, Cartridge SPE, and GCxGC-TOFMS

Regulatory requirements are driving the development of new multiresidue pesticide methods for dietary supplements. Minimizing matrix interference is critical for data accuracy. The novel approach employed here combines QuEChERS extraction, cartridge SPE cleanup, and GCxGC-TOFMS analysis, and results in good recoveries across a range of compounds found in these complex matrices.

Introduction

Dietary supplement manufacturers must now comply with the current Good Manufacturing Practice (cGMP) regulations that also guide the manufacture of pharmaceuticals. cGMPs require testing that ensures product safety, and, since many dietary supplements are botanically based, pesticide residue methods are among the new analyses being developed. Methods that minimize matrix interference are especially important, as plant-based dietary supplements are extremely complex and data integrity can depend on removing or reducing matrix contributions.

Existing procedures for agricultural commodities are a good starting point for multiresidue pesticide methods. For example, the QuEChERS approach to sample extraction and cleanup was first developed as a fast, easy way to prepare fruit and vegetable samples for pesticide analysis, but it can also be applied to other areas. In recent work [1], we used a QuEChERS extraction method [2] with cartridge solid phase extraction (cSPE) cleanup to prepare dietary supplement samples for pesticide residue analysis by GC/MS. For dandelion root samples, matrix interferences were substantially reduced by using the higher capacity cSPE cleanup, and recoveries for a wide range of pesticides reported in dietary supplements [3] were very good. However, in more complex samples, quantification bias appeared for some pesticides, leading us to consider a relatively new technique, comprehensive two-dimensional gas chromatography (GCxGC) with time-of-flight MS.

GCxGC offers greater potential for accurate pesticide determinations than single dimension GC, because resolution is enhanced by applying two independent separations to a sample in one analysis. GCxGC involves a serial column configuration (differing phases) separated by a thermal modulator. A separation is performed on the first column, and then effluent from the first column is continually (and quickly) focused and injected onto the second column. By keeping the second column short, a series of high speed chromatograms are generated, and the first column separation can be maintained. Separation results are plotted as a retention plane (column 1 time x column 2 time). Use of orthogonal stationary phases optimizes peak resolution.

This work shows the application of QuEChERS, cSPE, and GCxGC-TOFMS with an Rxi®-5Sil MS x Rtx®-200 column combination to quantify pesticides in dietary supplements. The approach used here reduces matrix interferences and improves accuracy relative to one dimensional GC-TOFMS.



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Tubes & Standards

**for multi-residue pesticide analysis
from food and other matrices**



Experimental

Sample Wetting and Fortification

Samples of powdered dandelion root, sage, and finished product (a combination of botanicals) were obtained from a dietary supplement manufacturer and used for this work. Since the QuEChERS method was originally developed for high aqueous content fruits and vegetables, modification is necessary when testing dry samples. For powders, such as those used here, using a reduced amount of sample and then adding water increases extraction efficiency. Therefore, 1 g of powder was wetted with 9 mL organic-free water for each sample. After shaking to mix well, wetted powders were fortified as described below and then allowed to soak for 1 hour prior to QuEChERS extraction.

- *Unspiked Dietary Supplement*

Each control sample was fortified with 100 μ L of QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) containing PCBs 18, 28, and 52 (50 μ g/mL each), triphenylphosphate (20 μ g/mL), tris-(1,3-dichloroisopropyl)phosphate (50 μ g/mL), and triphenylmethane (10 μ g/mL).

- *400 ng/g Spiked Dietary Supplement*

Each spike was fortified with 200 μ L of a 2 ng/ μ L standard that contained 46 pesticides, representing different chemical classes, previously reported in dietary supplements [3]. 100 μ L of QuEChERS Internal Standard Mix for GC-MS Analysis was also added.

QuEChERS Extraction

The EN 15662 QuEChERS method was used for sample extraction [2]. 10 mL of acetonitrile was added to each wet sample. After a 1 minute shake, Q-sep™ Q110 buffering extraction salts (4 g MgSO₄, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate; cat.# 26235) were added. Following another 1 minute shake, the sample was centrifuged for 5 minutes at 3,000 g with a Q-sep™ 3000 centrifuge (cat.# 26230).

Extract Cleanup

Dispersive SPE (dSPE) cleanup is typically associated with the QuEChERS approach, but previous work indicated sorbent capacity with the EN dSPE PSA tubes was inadequate [1]; therefore, several different cleanup procedures were compared, including various dSPE cleanups and a cartridge SPE (cSPE) cleanup.

For dSPE, 1 mL portions of QuEChERS extracts were added to Q210 tubes (cat. # 26215) containing 150 mg MgSO₄ and 25 mg primary secondary amine (PSA). The tubes were shaken for 2 minutes and then centrifuged for 5 minutes in the Q-sep™ 3000 centrifuge. Supernatant extract was removed by Pasteur pipette for analysis. This procedure was also followed for other samples using tubes containing different sorbent materials, such as graphitized carbon black (GCB). Sorbents tested were Q211 (150 mg MgSO₄, 25 mg PSA, 25 mg C18; cat.# 26216), Q213 (150 mg MgSO₄, 25 mg PSA, 7.5 mg GCB; cat.# 26218), and Q252 (150 mg MgSO₄, 50 mg PSA, 50 mg C18, 50 mg GCB; cat.# 26219).

For cSPE, a 6 mL Resprep® Combo SPE Cartridge (cat.# 26194) containing 500 mg CarboPrep® 90 and 500 mg PSA for pesticide residue cleanup was used. Anhydrous MgSO₄ was added on top to a level approximately one-quarter height of the total bed followed by a cartridge rinse with 20 mL 3:1 acetonitrile:toluene, which was discarded. 1 mL of QuEChERS dietary supplement extract was then loaded onto the cartridge and eluted with 50 mL 3:1 acetonitrile:toluene. The eluent was evaporated and solvent exchanged using dry nitrogen gas and a 35-40 °C water bath. Evaporation proceeded until approximately 0.5-1 mL extract was left, at which point about 3 mL of toluene was added. The extract was evaporated to just under 0.5 mL and the evaporation vessel was rinsed with toluene to bring the sample to a final volume of 0.5 mL.

The resulting final extracts for all matrices, with cleanup by either a dSPE procedure or cSPE, were analyzed by both GC-TOFMS and GCxGC-TOFMS.

GC-TOFMS

A LECO Pegasus® 4D GCxGC-TOFMS instrument was used and all data were processed with LECO ChromaTOF® software. One-dimensional gas chromatography was performed using a 30 m x 0.25 mm x 0.25 μ m Rxi®-5Sil MS column (cat.# 13623) with a constant flow of helium at 1.5 mL/min. 1 μ L fast autosampler splitless injections were made into a 5 mm single gooseneck liner with wool (cat.# 22405) at 250 °C. The purge valve time was 90 seconds. The GC oven program was 90 °C (1.5 min.), 8 °C/min. to 340 °C. Electron ionization at 70 eV was used with a source temperature of 225 °C. Data acquisition was from 45 to 550 u at a rate of 5 spectra/sec.

GCxGC-TOFMS

The LECO Pegasus® 4D GCxGC-TOFMS was operated in comprehensive two-dimensional gas chromatography mode with a 30 m x 0.25 mm x 0.25 μ m Rxi®-5Sil MS column (cat.# 13623) connected to a 1.5 m x 0.18 mm x 0.20 μ m Rtx®-200 column (cut from a 10 m column, cat.# 45001) with a deactivated Universal Press-Tight® Connector (cat.# 20429). These orthogonal phases were chosen to maximize peak separation. Instrument conditions are shown in Figure 1.

Calibration and Quantification with Matrix-Matched Standards

Matrix-matched standards for each matrix were prepared at 80 pg/ μ L, representing 100% recovery of pesticides in a final extract, by adding standard solution to the final extract from an unspiked sample. Actual recoveries were calculated after quantification from one-point calibration in ChromaTOF®. The internal standard method of quantification was employed using PCB 52.

Results

We previously demonstrated that the dispersive SPE cleanup approach of QuEChERS, specifically 25 mg PSA per mL extract, was too weak to remove matrix interferences for complex dietary supplement extracts [1]. We saw similar results here for all matrices, even though we employed higher amounts of PSA and additional sorbents, including GCB, which is typically excellent for removing pigments and other compounds. In contrast, cartridge SPE has much higher capacity for removing matrix interferences and resulted in acceptable quantification for the dandelion root samples. However, even with cSPE cleanup, the sage and finished product extracts still showed quantification bias for some pesticides when using one-dimensional GC/MS, due to the overwhelming complexity of the matrix (Table I).

Table I GC-TOFMS and GCxGC-TOFMS recovery comparison for QuEChERS extracts and cartridge SPE cleanups of dietary supplements.

Compound	Dandelion			Sage		Finished Product	
	Quant Mass	GC Rec %	GCxGC Rec %	GC Rec %	GCxGC Rec %	GC Rec %	GCxGC Rec %
1,2,3,5-Tetrachlorobenzene	216	46	56	65	61	52	58
Pentachlorobenzene	250	51	57	75	68	55	60
Tetrachloronitrobenzene	261	72	64	93	85	57	64
2,3,5,6-Tetrachloroaniline	229	64	69	92	83	63	66
alpha-HCH	219	69	70	88	84	69	68
Hexachlorobenzene	284	56	61	74	67	62	61
Pentachloroanisole	265	62	73	77	78	62	64
beta-HCH	219	88	102	95	90	80	81
Pentachloronitrobenzene	237	62	70	97	87	65	68
Pentachlorobenzonitrile	275	70	74	81	81	71	72
gamma-HCH	219	85	76	100	87	83	72
Diazinon	179	71	72	98	103	70	64
delta-HCH	219	85	95	97	91	86	82
Pentachloroaniline	265	75	84	95	85	73	74
Pentachlorothioanisole	246	66	76	82	76	68	68
PCB 52	292	ISTD	ISTD	ISTD	ISTD	ISTD	ISTD
Chlorpyrifos	314	92	86	106	98	75	80
Dacthal	301	83	95	101	94	79	78
Parathion	291	91	94	89	91	90	80
Heptachlor epoxide	353	93	84	109	90	69	76
Procymidone	283	104	107	102	99	97	85
Endosulfan I	195	70	90	84	86	92	89
4,4'-DDE	318	90	100	84	88	102	106
Dieldrin	263	91	99	94	87	89	80
Myclobutanil	179	103	109	102	97	93	92
Endosulfan II	195	109	103	86	91	159	163
Oxadixyl	132	101	109	Int	97	86	91
4,4'-DDD	235	98	101	105	105	89	95
2,4'-DDT	235	94	102	88	90	86	82
Carfentrazone ethyl	312	112	106	102	100	88	93
Endosulfan sulfate	387	105	117	119	94	111	92
Fenhexamid	177	94	75	Int	85	110	86
4,4'-DDT	235	96	110	106	100	102	89
Piperonyl butoxide	176	93	106	123	93	73	91
Iprodione	187	112	125	Int	87	58	83
Cypermethrin	163	98	107	Int	88	Int	72
Pyraclostrobin	132	92	109	90	74	85	88
Fluvalinate	250	99	112	95	88	85	88
Difenoconazole	265	90	102	98	78	85	83
Azoxystrobin	344	93	105	118	80	52	86

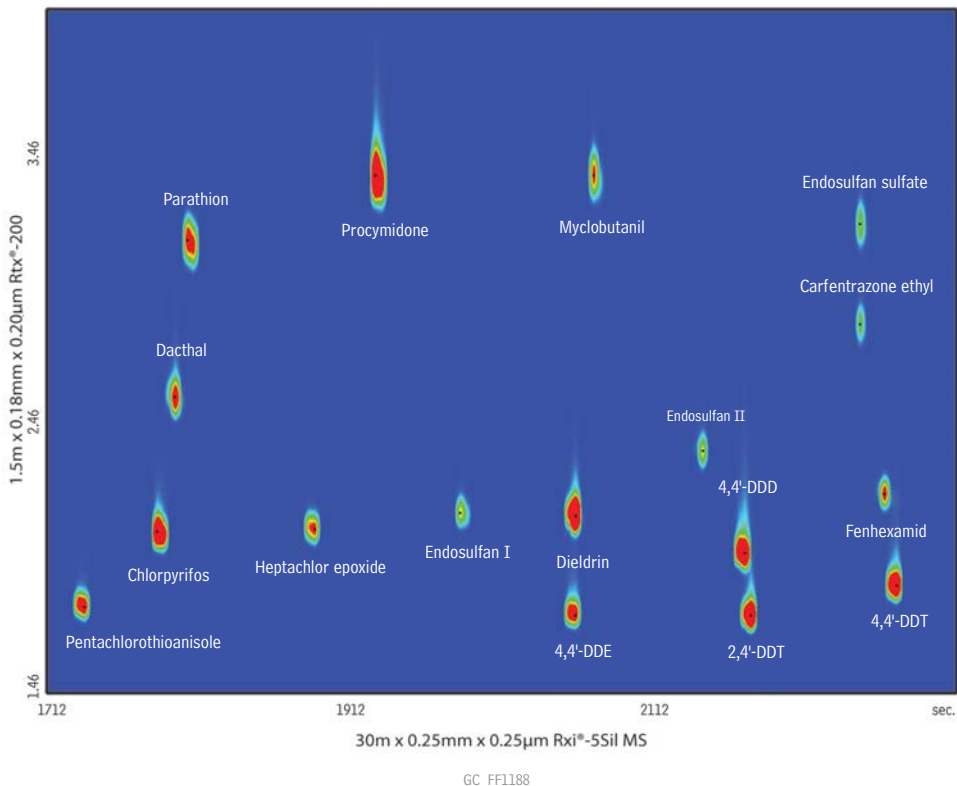
PCB 52 is the Internal Standard.

Cypermethrin, Fluvalinate, and Difenoconazole represent values from summed isomers.

Int = interference that prevented quantification.

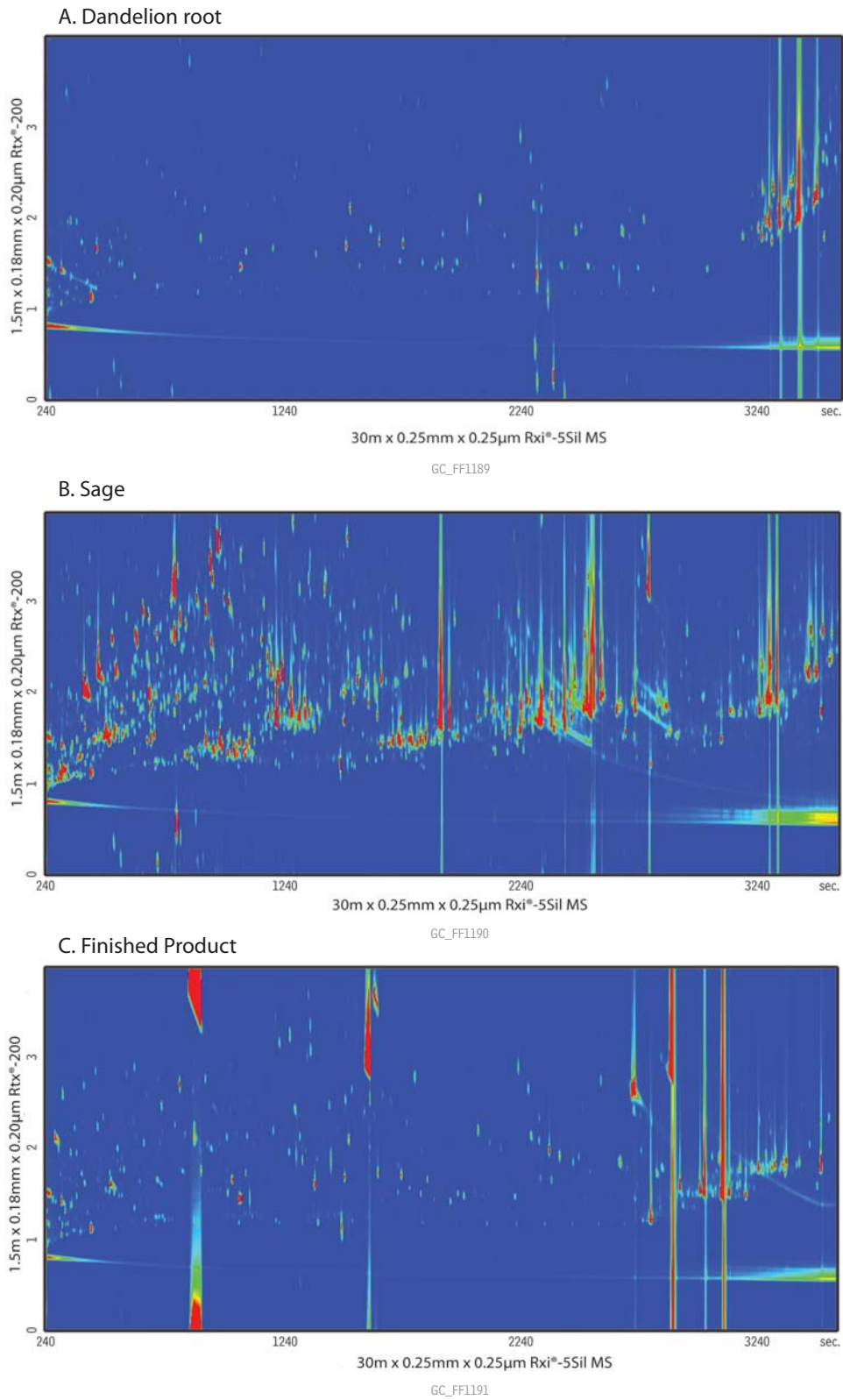
GCxGC allows two independent separations in one analytical run, which not only increases resolution among pesticides (Figure 1), but also spreads out all peaks, increasing the qualitative and quantitative accuracy of trace residue determinations in complex samples (Figure 2). Its specific value in the case of sage and finished product extracts was to allow the unbiased quantification of Oxadixyl, Fenhexamid, Iprodione, and Cypermethrin (Table I). As shown in Figure 3, Fenhexamid in sage was separated just enough when using GCxGC to not only get an accurate recovery value (Table I), but also to yield a mass spectrum that matches well with the reference spectrum (Figure 4).

Figure 1 GCxGC-TOFMS separation of a dietary supplement pesticide standard. GCxGC-TOFMS allows the separation 2,4'-DDT and 4,4'-DDD along the second dimension (Rtx®-200 column). These compounds coelute in the first dimension (Rxi®-5Sil MS column) and have very similar mass spectra.



Column: Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623); Rtx®-200 1.5 m, 0.18 mm ID, 0.20 µm (cat.# 45001); **Sample:** Mixed pesticide standard; Diluent: toluene; Conc.: 2 ng/µL; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min.), Liner: Gooseneck Splitless (4mm) w/Wool (cat.# 22405); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min.; **Oven:** Oven Temp: Rxi®-5Sil MS: 80 °C (hold 1 min.) to 310 °C at 4 °C/min. (hold 1.5 min.), Rtx®-200: 90 °C (hold 1 min.) to 320 °C at 4 °C/min. (hold 1.5 min.); Carrier Gas: He, constant flow; Flow Rate: 1.8 mL/min.; **Modulation:** Modulator Temp. Offset: 25 °C; Second Dimension Separation Time: 4 sec.; Hot Pulse Time: 1.2 sec.; Cool Time between Stages: 0.8 sec.; **Detector:** TOFMS; Transfer Line Temp.: 290 °C; Analyzer Type: TOF; Source Temp.: 225 °C; Electron Energy: 70 eV; Mass Defect: -20 mu/100 u; Solvent Delay Time: 4 min.; Ionization Mode: EI; Acquisition Range: 45 to 550 amu; Spectral Acquisition Rate: 100 spectra/sec; **Instrument:** LECO Pegasus 4D GCxGC-TOFMS

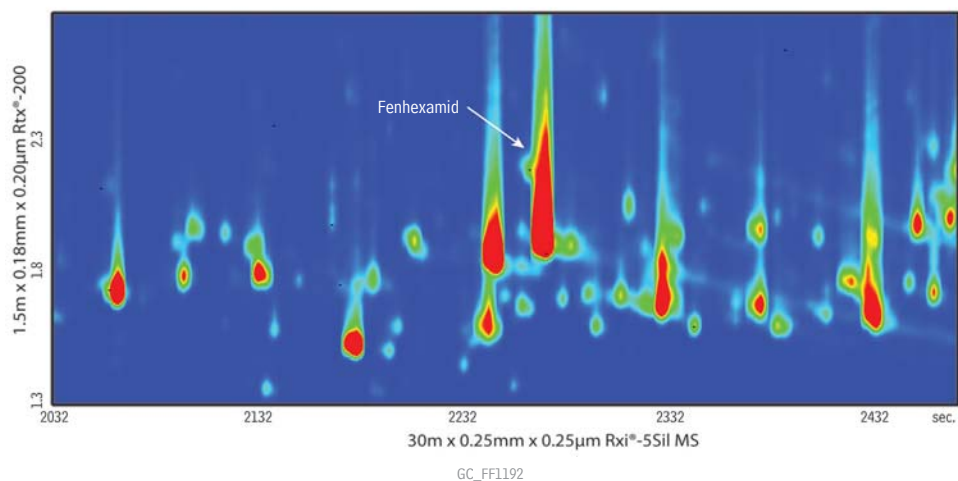
Figure 2 GCxGC-TOFMS can be used to separate compounds that coelute in complex dietary supplement matrices when analyzed by single dimension GC-TOFMS.



See Figure 1 for conditions.

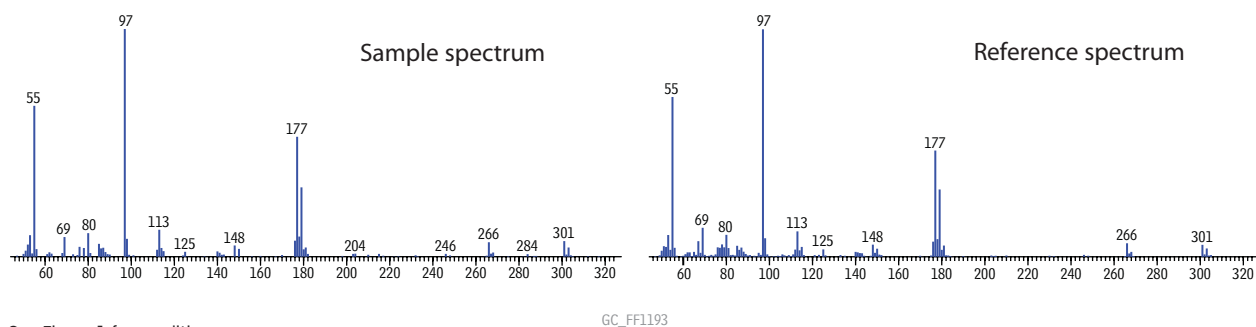
Figure 3 Fenhexamid coelutes with a major interference in one-dimensional GC-TOFMS, but the slight separation achieved with GCxGC allows quantification.

GCxGC-TOFMS extracted ion contour plot for the quantification mass (177 m/z) of Fenhexamid in sage extract.



See Figure 1 for conditions.

Figure 4 The excellent match between sample and library Fenhexamid spectra is achieved with the GCxGC separation using Rxi®-5Sil MS and Rtx®-200 columns.



See Figure 1 for conditions.

A more subtle correction on recovery for gamma-hexachlorocyclohexane (Lindane) in sage was achieved when using GCxGC-TOFMS by separating an isobaric interference that coeluted with Lindane in one-dimensional GC-TOFMS. This GCxGC separation is shown in Figure 5 as the peak immediately above gamma-hexachlorocyclohexane. A 100% recovery value was reported in Table I for GC-TOFMS, but a plot of the chlorine isotope m/z ions associated with the 219 ion used for Lindane quantification, indicates a high bias on the 219 ion versus a standard (Figure 6). In addition, the peak apexes do not line up properly for the Lindane in sage, another indication of coelution for one-dimensional GC. The 87% recovery value from GCxGC, although lower, is more accurate.

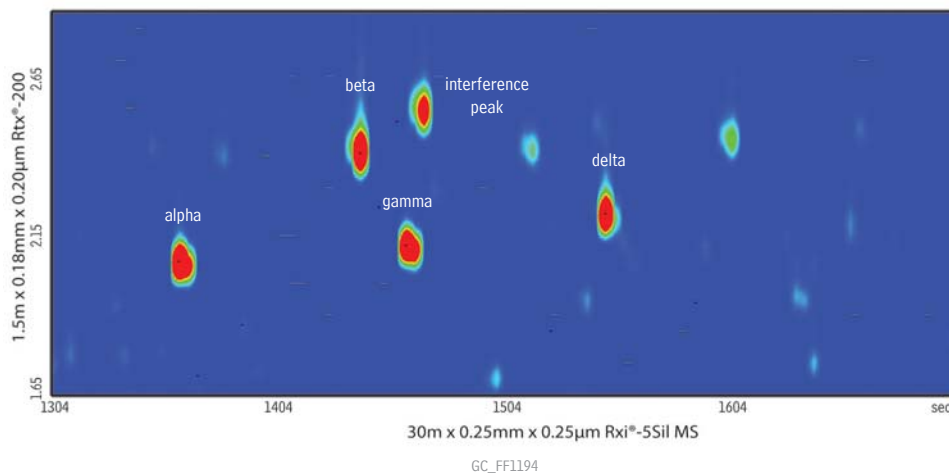
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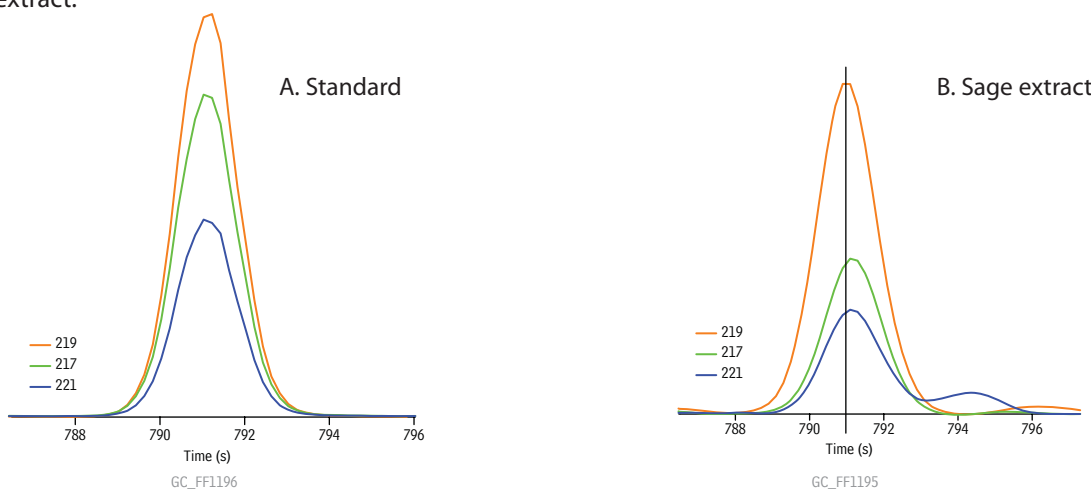
Figure 5 The interference just above the gamma-HCH peak at m/z 219 causes high quantification bias in one-dimensional GC-TOFMS, but the peaks are fully resolved and can be accurately quantified by GCxGC-TOFMS.

GCxGC-TOFMS extracted ion contour plot for the quantification mass (219 m/z) of the hexachlorocyclohexanes in sage extract.



See Figure 1 for conditions.

Figure 6 The correct chlorine isotope pattern for HCH can be seen in the standard, but is inaccurate for the sage extract due to a coeluting compound. In addition, the peak apexes for the ions do not align for the HCH in the sage extract.



Column: Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623); **Sample:** Diluent: Toluene; **Injection:** Inj. Vol.: 1 µL splitless (hold 1.5 min.), Liner: Gooseneck Splitless (4mm) w/Wool (cat.# 22405); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min.; **Oven:** Oven Temp: 90 °C (hold 1.5 min.) to 340 °C at 8 °C/min., Carrier Gas: He, constant flow, Flow Rate: 1.5 mL/min.; **Detector:** TOFMS; Transfer Line Temp.: 300 °C; Analyzer Type: TOF; Source Temp.: 225 °C; Electron Energy: 70 eV; Mass Defect: -20 mu/100 u; Solvent Delay Time: 4 min.; Ionization Mode: EI; Acquisition Range: 45 to 550 amu; Spectral Acquisition Rate: 5 spectra/sec; **Instrument:** LECO Pegasus 4D GCxGC-TOFMS
Notes: See application note PHAN1251 for extraction and cleanup details.

Conclusions

QuEChERS is a fast, solvent-saving approach originally developed for fruits and vegetables that can be extended to other matrices. As shown here, QuEChERS extraction with cartridge SPE cleanup of dietary supplement samples resulted in good recoveries for many pesticides, but a more powerful instrumental method such as GCxGC-TOFMS is sometimes necessary to minimize the impact of matrix interference in these complex samples. The benefits of GCxGC-TOFMS are maximized by using orthogonal stationary phases, such as Rxi®-5Sil MS and the Rtx®-200 columns, which allow optimized GCxGC separations.

References

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2. 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).
3. J.W. Wong, M.S. Wirtz, M.K. Hennessy, F.J. Schenck, A.J. Krynitsky, S.G. Capar, Acta Hort. (ISHS) 720 (2006) 113.

Product Listing

Q-sep™ QuEChERS Sample Prep Packets & Tubes

- Ready-to-use tubes, no glassware required.
- Preweighed, ultra-pure sorbents.
- Convenient, method-specific standards.

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Q110 kit	4g MgSO ₄ , 1g NaCl, 1g TSCD, 0.5g DHS with 50mL Centrifuge Tube	European EN 15662	50 packets & 50 tubes	26235
Q110 packets	4g MgSO ₄ , 1g NaCl, 1g TSCD, 0.5g DHS	European EN 15662	50 packets	26236
Empty 50mL Centrifuge Tube			50-pk.	26239
2mL Micro-Centrifuge Tubes for dSPE (clean-up of 1mL extract)				
Q210	150mg MgSO ₄ , 25mg PSA	European EN 15662	100-pk.	26215
Q211	150mg MgSO ₄ , 25mg PSA, 25mg C18		100-pk.	26216
Q213	150mg MgSO ₄ , 25mg PSA, 7.5mg GCB	European EN 15662	100-pk.	26218
Q252	150mg MgSO ₄ , 50mg PSA, 50mg C18, 50mg GCB	AOAC 2007.01	100-pk.	26219

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(midpolarity phase; Crossbond® trifluoropropylmethyl polysiloxane)

ID	df (µm)	temp. limits*	15-Meter
0.18mm	0.20µm	-20 to 310/330°C	45001

Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	30-Meter
0.25mm	0.25µm	-60 to 330/350°C	13623

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5.0mm ID Straight Inlet Liner w/ Wool

ID* x OD & Length	qty.	cat.#
Straight, Intermediate Polarity (IP), Semivolatiles Wool, 5.0mm x 6.5mm x 78.5mm	ea.	22975-231.1
5.0mm x 6.5mm x 78.5mm	5-pk.	22976-231.5

4.0mm ID Single Gooseneck Inlet Liner w/ Wool

ID* x OD & Length	qty.	cat.#
Single Gooseneck, Intermediate Polarity (IP), Deact. Wool, 4.0mm x 6.5mm x 78.5mm	ea.	22405
4.0mm x 6.5mm x 78.5mm	5-pk.	22406
4.0mm x 6.5mm x 78.5mm	25-pk.	22407
Single Gooseneck, Intermediate Polarity (IP), Semivolatiles Wool, 4.0mm x 6.5mm x 78.5mm	ea.	20798-231.1
4.0mm x 6.5mm x 78.5mm	5-pk.	20799-231.5
4.0mm x 6.5mm x 78.5mm	25-pk.	20800-231.25

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- Made from inert fused silica.

Description	5-pk.	25-pk.	100-pk.
Universal Press-Tight Connectors	20400	20401	20402
Universal Press-Tight Connectors, Deactivated	20429	20430	
Universal Press-Tight Connectors, Siltek Treated	20480	20449	

QuEChERS Quality Control Standards for GC/MS Analysis

Cat.# 33268: PCB 138 PCB 153	Cat.# 33264: anthracene
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50µg/mL each in acetonitrile, 5mL/ampul
cat. # 33268 (ea.)

100µg/mL in acetonitrile, 5mL/ampul
cat. # 33264 (ea.)

QuEChERS Internal Standard Mix for GC/MS Analysis

(6 components)

PCB 18	50µg/mL	tris-(1,3-	
PCB 28	50	dichloroisopropyl)phosphate	50
PCB 52	50		50
triphenyl phosphate	20	triphenylmethane	10

In acetonitrile, 5mL/ampul
cat. # 33267 (ea.)



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