Foods, Flavors & Fragrances Applications

A Comprehensive Approach to Pesticide Residue Testing, Including Non-Target Analysis, for Fruits, Vegetables, and Nuts, Using QuEChERS, LC-MS/MS, and GCxGC-TOFMS

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Abstract

Food commodities were fortified with pesticides and processed using the QuEChERS sample preparation technique. Samples were analyzed by both GCxGC-TOFMS and LC-MS/MS. The foods chosen varied in water, fat, and pigment content, so the ruggedness of QuEChERS as well as the performance of GCxGC-TOFMS and LC-MS/MS could be assessed. Commodities tested were red bell pepper, cucumber, black seedless grape, spinach, lemon, raisin, and hazelnut. Recovery values were determined by matrix-matched standards for the GC method and by solvent standards for the LC method. Evaluation of GCxGC-TOFMS and LC-MS/MS, along with the QuEChERS approach itself, was made by comparison of recovery values and incurred pesticide concentrations.

Good recoveries were obtained for most pesticides in most commodities as determined by GCxGC-TOFMS and LC-MS/MS. Sometimes GCxGC-TOFMS did not have the selectivity necessary for determining certain pesticides in the most complex samples. In this regard, dispersive SPE (dSPE) cleanup was ineffective at removing significant matrix interferences in lemon, raisin, and hazelnut extracts for some target pesticides. Corrupted LC-MS/MS quantification for some pesticides was observed, especially in lemon and hazelnut extracts, and likely resulted from ion suppression or was due to quantification by solvent-only standards. Incurred pesticide quantifications were comparable for GCxGC-TOFMS and LC-MS/MS. GCxGC-TOFMS was able to identify non-target pesticides.

Introduction

Pesticide residue testing of food has traditionally been performed using gas chromatography (GC), but there is increasing use of liquid chromatography (LC) with tandem mass spectrometry (MS/MS). LC is favored for polar, less thermally-stable, less volatile, compounds. GC-MS is preferred for volatile, thermally-stable species, and pesticides that do not ionize well in electrospray or atmospheric pressure chemical ionization LC sources. With MS, complete chromatographic resolution of compounds is not always essential, as selected ions or selected reaction monitoring (SRM) transitions are used for pesticide identification and quantification. However, data quality can be improved through better retention and separation of components, especially for structurally similar pesticides and high-level matrix coextractives. In GC, this better separation can come from comprehensive two-dimensional GC (GCxGC), an approach involving two separations on an orthogonal column set in a single analytical run. A fast time-of-flight (TOF) MS records data from the ~100 ms wide peaks produced by the GCxGC separation. TOFMS records full mass spectral data

to accomplish simultaneous target and non-target compound analysis. In LC, multiresidue pesticide methods based on standard C18 columns suffer from poor retention of small polar analytes. In addition, coelutions can be problematic if the analytes share MRM transitions. These difficulties can be improved by using a column that is both selective for small, polar compounds and that has balanced retention for a large number of compounds that vary in physiochemical properties. More balanced retention reduces the number of MRM transitions being monitored at any point in time, and improves data quality by allowing more time to be spent on a smaller number of MRM transitions.

QuEChERS (Quick–Easy–Cheap–Effective–Rugged–Safe) is a sample preparation 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 dispersive solid phase extraction (dSPE) cleanup of the extract. It is well established that QuEChERS can result in good recovery values not only for a large number of pesticides, but also for a wide variety of commodities [2,3,4]. In this work, the QuEChERS extraction approach was used for red bell pepper, cucumber, lemon, raisin, spinach, hazelnut, and black grape with subsequent pesticide determinations by LC-MS/MS and GCxGC-TOFMS. Benefits and weaknesses of the sample preparation and analysis approaches are reported.

Experimental

Chemicals and Materials

QuEChERS extraction and dSPE tubes, as well as QuEChERS internal and quality control standards, were from Restek Corporation (Bellefonte, Pennsylvania). A standard consisting of approximately 200 pesticides prepared in acetonitrile, was provided by the U.S. Food and Drug Administration/Center for Food Safety and Applied Nutrition. Food commodities were purchased at a local grocery store; the foods and their countries of origin are as follows: English cucumber (Canada), lemon (U.S.), black seedless grape (U.S.), red bell pepper (Mexico), spinach (U.S.), raisin (U.S.), and shelled hazelnut (U.S.)

Sample Wetting

Dry commodities, such as raisin and hazelnut, must be wetted prior to QuEChERS extraction. Wetting ratio recommendations from the EN 15622 QuEChERS method were used [5]. For raisin, 5 grams of homogenized raisin and 8.5 mL of deionized water were combined in a 50 mL centrifuge tube. For hazelnut, 10 mL water was added to 5 grams of homogenized hazelnut in a 50 mL centrifuge tube. These mixes of raisin and water and hazelnut and water are considered as "10 g homogenized sample" in the following sections.

Commodity Fortification

Commodities were first homogenized. For cucumber, lemon (the rind was not removed prior to homogenization), grape, red bell pepper, and spinach, a 10 gram sample of the commodity was weighed into a 50 mL centrifuge tube and fortified at 10 ng/g (ppb) by adding 100 µL of a 1 ng/µL pesticide spiking solution. Raisin and hazelnut samples were fortified at 10 ng/g (ppb) by adding 50 µL of a 1 ng/µL pesticide spiking solution because only 5 grams of material was used. 100 µL of QuEChERS internal standard mix for GC-MS analysis (cat.# 33267) and 100 µL of QuEChERS internal standard mix for LC-MS/MS analysis (cat.# 33261) were added to each sample. These internal standard mixes require no dilutions ("snap-and-shoot") and contain compounds specified in the EN 15662 QuEChERS method [5].

Unfortified samples were also prepared to determine incurred and non-target pesticides, and were also used to produce matrix-matched standards for GCxGC-TOFMS.

QuEChERS Extraction

The EN 15662 QuEChERS method was used for sample extraction [5]. First, 10 mL of acetonitrile were added to each homogenized sample. After a 1 minute manual shake, Q-sep™ QuEChERS extraction salts (cat.# 26235) containing 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate were added. At this point, lemon samples were pH adjusted by adding 600 µL of a 5 N (equivalent to a 5 M [molar, mol/L]) sodium hydroxide solution to the extraction tube. Following another 1 minute shake, samples were centrifuged for 5 minutes at 3,000 g with a Q-sep™ 3000 centrifuge (cat.# 26230). The top acetonitrile layer (extract) was transferred to a clean vial.

QuEChERS Dispersive Solid Phase Extraction (dSPE) Cleanup

Restek Q-sep™ QuEChERS dSPE tubes (cat.# 26216), containing 25 mg primary secondary amine (PSA), 25 mg octadecyl (C18), and 150 mg magnesium sulfate ($MgSO_4$), were used for 1 mL sample cleanup. Each tube was manually shaken for 30 seconds and then centrifuged for 5 minutes at 3,000 g. The resulting final extract was then analyzed directly by GCxGC-TOFMS. For LC-MS/MS analysis, the extract was diluted 10X with deionized water.

Matrix-Matched Standards and Solvent Standards for Calibration and Quantification

Calibration standards were prepared at 10 ng/mL (pg/µL), as these were the expected final concentrations in 10 ng/g (ppb) fortified samples, assuming 100% compound recovery. Matrix-matched standards for GCxGC-TOFMS were prepared by adding pesticide standard solution to a final (post-cleanup) extract of an unfortified sample. For GCxGC-TOFMS analysis, actual recoveries were calculated by comparing response factors for compounds in fortified samples that were extracted and cleaned up, to response factors for compounds in 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) added prior to extraction. For LC-MS/MS analysis, standards in solvent were used for recovery calculations.

GCxGC-TOFMS Analysis

A LECO Pegasus 4D GCxGC-TOFMS was used and all data were processed with ChromaTOF® software (Saint Joseph, Michigan). Gas chromatography was performed using a 30 m x 0.25 mm x 0.25 µm Rxi®-5Sil MS column (cat.# 13623) for the first dimension and a 1.5 m x 0.18 mm x 0.20 µm Rtx®-200 column (piece cut from cat.# 45001) for the second dimension. The carrier gas was a corrected constant flow of helium at 1.8 mL/min. A 1 µL sample was introduced with a fast autosampler splitless injection. The inlet was set to 250 °C and was outfitted with a 5 mm single taper liner with wool (cat.# 22973-200.1). The purge valve time was 1.0 minutes. The primary GC oven program was 90 °C (1 min), 4 °C/min to 310 °C and hold 2 minutes, and the secondary oven temperature program was 100 °C (1 min), 4 °C/min to 320 °C with a 2 minute hold. The modulation time was 4 seconds. Electron ionization at 70 eV was used with a source temperature of 225 °C and a transfer line temperature of 290 °C. Data acquisition was from 45 to 550 amu at a rate of 100 spectra/sec.

LC-MS/MS Analysis

A Shimadzu UFLCXR LC (Columbia, Maryland) and AB SCIEX 4000 QTRAP® LC-MS/MS system with Turbo V source (Foster City, California) were used for LC-MS/MS analysis. Analysis was performed using a 100 mm x 2.1 mm, 3 µm Ultra Aqueous C18 column (cat.# 9178312) with a 10 µL injection. Extracts were diluted by a factor of 10 with deionized water before analysis, resulting in an injection concentration of 1 ppb for each pesticide. A mobile phase gradient of water with 10 mM ammonium acetate and methanol with 10 mM ammonium formate and flow rate of 0.5 mL/min were used. Compounds were ionized with either positive or negative electrospray ionization. Two transitions were monitored in Scheduled MRM (sMRM) mode for each analyte as listed in Table I.

Table I: Pesticides and corresponding classes chosen for data analysis are listed here. GCxGC-TOFMS first and second dimension retention times, as well as the quantification ions, are shown. The LC-MS/MS retention time and two MRM transitions are shown for each pesticide.

		GCxGC-TOFMS			LC-MS/MS		
Pesticide	**Class**	**tR1 (sec)**	**tR2 (sec)**	**Q mass**	**tR (min)**	**MRM Transition 1 (Q1→Q3)**	**MRM Transition 2 (Q1→Q3)**
Propoxur	N-Methyl carbamate	372	1.60	110	5.46	210.1 → 111	210.1 → 168.1
Methamidophos	Organophosphorus	444	2.70	141	1.14	142 → 94	142 → 125
Acephate	Organophosphorus	772	3.63	136	1.35	184.1 → 143	184.1 → 125
Propham	Other carbamate	824	1.90	179	6.21	180 → 138	180 → 120
1-Naphthol	Breakdown product	908	1.73	144	NA	NA → NA	NA → NA
o-Phenylphenol	Phenol	916	1.59	170	NA	NA → NA	NA → NA
Tebuthiuron	Urea	924	2.52	156	6.18	229.2 → 172.4	229.2 → 116.1
Omethoate	Organophosphorus	1032	3.88	156	1.83	214 → 124.9	214 → 182.8
Dimethoate	Organophosphorus	1252	3.03	125	3.91	230 → 125	230 → 199.1
Prometon	Triazine	1292	1.78	168	7.27	226.1 → 142.1	226.1 → 86
Terbacil	Uracil	1388	2.63	161	NA	NA → NA	NA → NA
Pirimicarb	N-Methyl carbamate	1436	2.13	166	6.74	239.2 → 72.1	239.2 → 182.2
Metribuzin	Triazinone	1492	1.78	198	5.56	215.1 → 187.2	215.1 → 84.1
Fuberidazole	Benzimidazole	1512	2.22	184	5.95	185 → 157	185 → 65
Carbaryl	N-Methyl carbamate	1520	2.62	144	6.11	202.1 → 145	202.1 → 127
Metalaxyl	Xylylalanine	1540	2.39	160	6.58	280.2 → 220.2	280.2 → 192.3
Terbutryn	Triazine	1584	1.77	226	7.93	242.2 → 186.1	242.2 → 68.1
Ethofumesate	Unclassified	1596	2.71	161	6.97	304 → 121	304 → 161
Benthiocarb	Thiocarbamate	1628	1.74	257	NA	NA → NA	NA → NA
Cyprodinil	Pyrimidine	1724	1.62	224	8.51	226 → 93	226 → 77
Thiabendazole	Benzimidazole	1756	1.95	174	6.17	202.1 → 175.1	202.1 → 131.2
Furalaxyl	Xylylalanine	1776	2.21	242	7.04	302.1 → 95.1	302.1 → 242.1
Triadimenol	Triazole	1780	2.18	168	7.47	296.1 → 70.1	296.1 → 227.2
Siduron	Urea	1876	2.35	93	6.96	233.3 → 137.2	233.3 → 94
Imazalil	Imidazole	1884	2.43	173	8.42	297.1 → 159.2	297.1 → 161.2
Fludioxonil	Pyrrole	1888	2.73	248	NA	NA → NA	NA → NA
Myclobutanil	Triazole	1924	2.84	179	7.60	289 → 70	289 → 125
Buprofezin	Unclassified	1936	1.77	172	8.84	306.2 → 201.1	306.2 → 116.2
Oxadixyl	Anilide	2016	3.67	163	5.31	279.2 → 219.2	279.2 → 132.1
Mepronil	Anilide	2068	2.02	119	7.26	270.1 → 119.1	270.1 → 228
Carfentrazone ethyl	Unclassified	2100	2.43	312	7.90	412 → 346	412 → 366
Fenhexamid	Anilide	2116	1.94	177	7.48	302 → 97	302 → 55
Propargite	Organosulfur	2188	1.74	173	9.08	368 → 231	368 → 175
Piperonyl butoxide	Unclassified	2200	1.46	176	8.90	356.2 → 177.2	356.2 → 119
Pyriproxyfen	Juvenile hormone mimic	2380	1.47	136	9.01	322 → 96	322 → 185
Fenarimol	Pyrimidine	2416	2.02	219	7.72	331 → 268	331 → 81
Bitertanol	Triazole	2508	1.95	170	8.34	338 → 70	338 → 269
Prochloraz	Iimidazole	2544	2.69	180	8.62	376.1 → 308	376.1 → 70.1
Pyraclostrobin	Strobin	2784	1.92	132	8.30	388 → 194	388 → 163
Azoxystrobin	Strobin	2904	2.26	344	7.20	404.1 → 372.1	404.1 → 344.1
Dimethomorph	Morpholine	2920	2.31	301	7.63	388.2 → 301.1	388.2 → 165.2

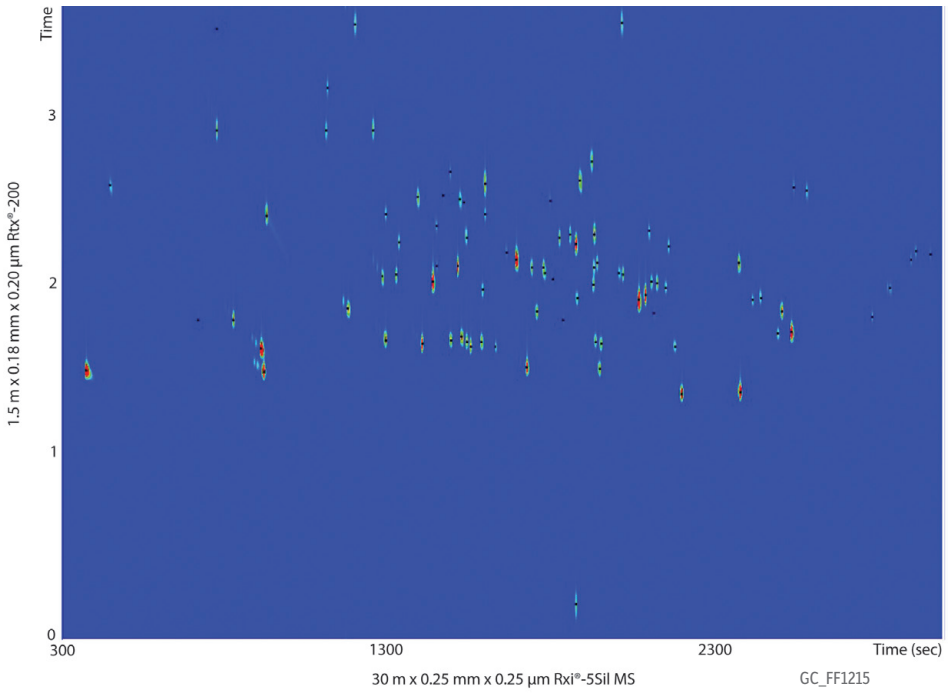
Results and Discussion

GCxGC Separation

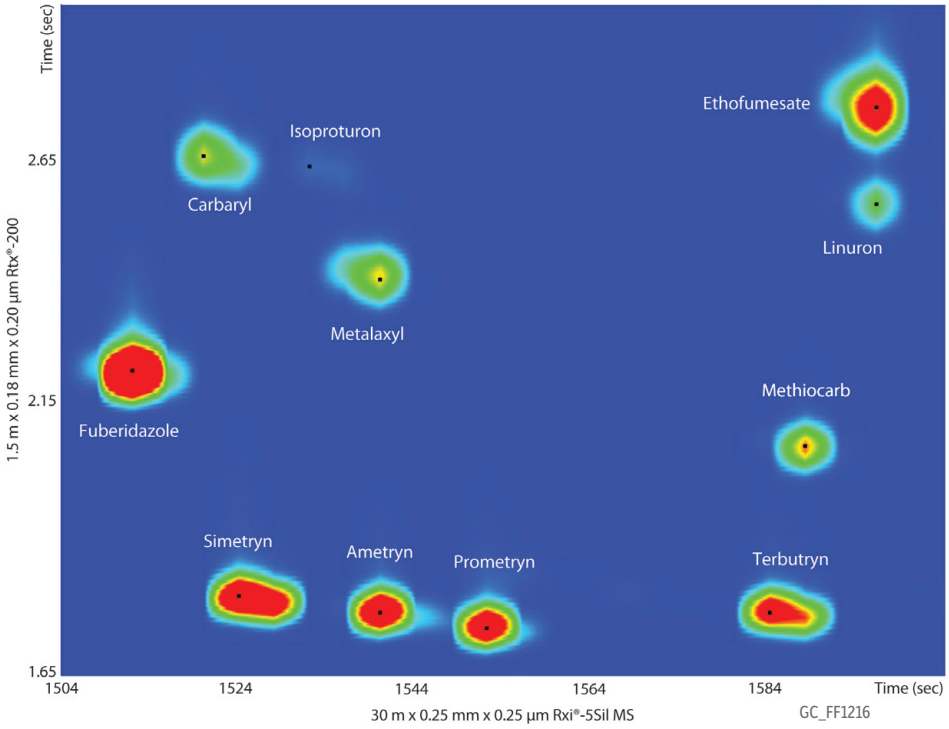
The GCxGC method was optimized to provide maximum separation of pesticides in two dimensions. Figure 1A shows a contour plot of the pesticide standard produced by GCxGC-TOFMS. In this plot, the x-axis is the retention time axis for the first dimension Rxi®-5Sil MS column. The y-axis corresponds to the retention time scale of the Rtx®-200 secondary column, and intensity data is depicted by color with red being the most intense and blue representing baseline. Figure 1B (magnification) demonstrates the increased resolving power of comprehensive two-dimensional gas chromatography. With one-dimensional GC, the following pairs of pesticides would coelute, but are separated in the second dimension: carbaryl and simetryn, metalaxyl and ametryn, and linuron and ethofumesate. This increased separation power is important for multiresidue pesticide methods consisting of a large number of compounds, and for separating large matrix interferences from trace-level analytes.

Figure 1: Contour plot of the pesticide standard produced by GCxGC-TOFMS. Both full scale and magnified images show good separation of pesticides using an Rxi®-5Sil MS column in the first dimension and an Rtx®-200 column in the second dimension.

A. Full scale



B. Enlarged area



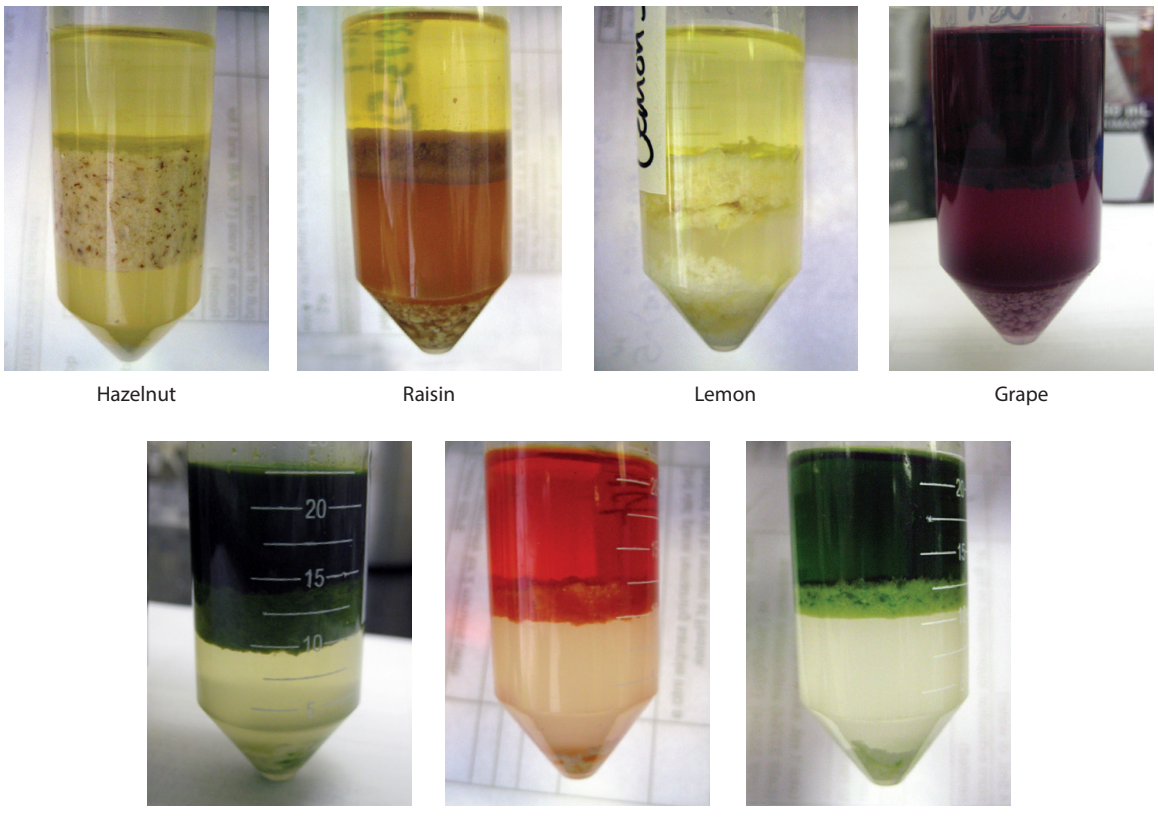
Column: Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623), Rtx®-200 1.5 m, 0.18 mm ID, 0.20 µm (cat.# 45001); **Sample:** Pesticide standard; Diluent: Acetonitrile; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: Gooseneck splitless (5 mm) w/deactivated wool (cat.# 22973-200.1); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min; **Oven:** Oven Temp: Rxi®-5Sil MS: 90 °C (hold 1 min) to 310 °C at 4 °C/min (hold 2 min); Rtx®-200: 100 °C (hold 1 min) to 320 °C at 4 °C/min (hold 2 min); **Carrier Gas:** He, corrected constant flow (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: 5 min; Tune Type: PFTBA; Ionization Mode: EI; Acquisition Range: 45-550 amu; Spectral Acquisition Rate: 100 spectra/sec; **Instrument:** LECO Pegasus 4D GCxGC-TOFMS; **Notes:** Rtx®-200 (cat.# 45001) is a 10 m column. A 1.5 m section was cut off and used as the second dimension column.

Commodity Type Characterizations

The commodities used for this pesticide residue analysis of food study represent different foods that vary in water content, fat content, pigment intensity, and acidity/basicity, and were expected to present different levels of difficulty in both extraction of pesticides and instrumental analysis. Lemon (including rind), cucumber, red bell pepper, grape, and spinach all have high water content, which is characteristic of the type of sample used to develop the original QuEChERS approach. Hazelnut has high fat content and is dry like raisin, which makes application of a QuEChERS procedure more difficult. As noted in the Experimental section, water must be added to dry samples to increase extraction efficiency. Higher fat content can lead to suppressed extraction efficiencies for hydrophobic pesticides, especially given that hydrophilic acetonitrile is used as the QuEChERS solvent. Lemon is acidic and spinach is basic. Some pesticides undergo degradation at pH extremes, so buffering is used to minimize this problem.

The QuEChERS extraction of the commodities in this work showed a wide spectrum of pigment intensities (Figure 2). Hazelnut, raisin, and lemon resulted in light colored extracts. Grape and spinach produced dark, pigment-rich extracts, while red bell pepper and cucumber produced mid-intensity extracts. Appreciably colored extracts contain nonvolatile pigments, like chlorophyll, that cannot be chromatographed by GC. If left in the sample these compounds rapidly contaminate the GC inlet liner, the inlet bottom seal, and the front of the GC column, resulting in performance issues and increased instrument maintenance. One strategy for the removal of chlorophyll and other pigments is using graphitized carbon black (GCB) during dSPE. Unfortunately, GCB can lead to serious losses of planar pesticides, so we avoided its use in favor of PSA and C18 dSPE. Given that most of the pesticides in this work are determined better by liquid chromatography where chlorophyll in the sample is a less significant issue, it was more important to try and maximize recoveries of all pesticides rather than produce a completely pigment-free extract.

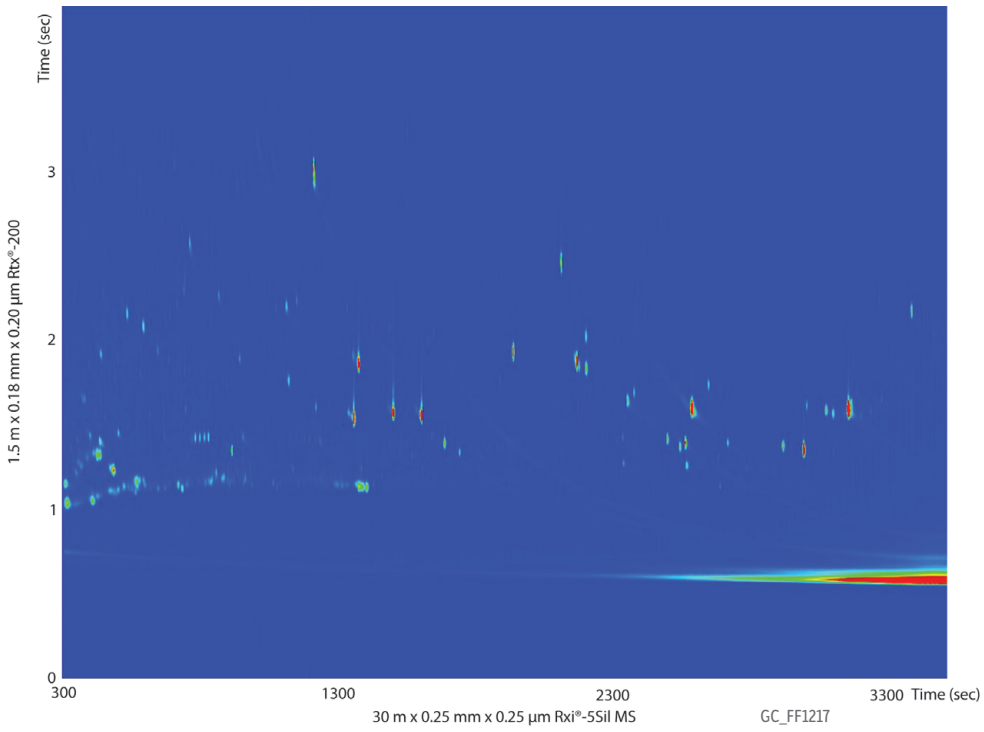
Figure 2: Photographs of QuEChERS extracts of studied commodities before dSPE cleanups were performed.



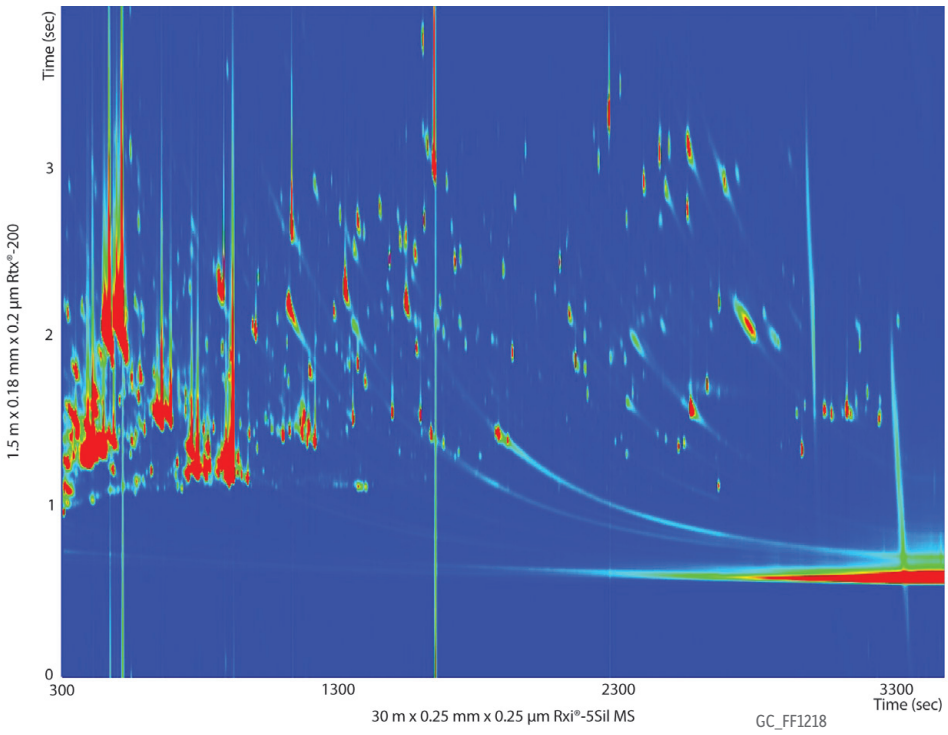
We assessed the complexity of different commodities by examining the total ion chromatogram (TIC) contour plots generated by GCxGC-TOFMS. Figure 3 shows TIC plots for two commodities, grape and lemon, which represent the range from least complex to most complex, as determined by a GC approach. It is clear that the lemon sample contained many more coextractives than the grape sample as demonstrated by the large number of intense (red) signals. While it should be possible to successfully analyze QuEChERS grape extracts for pesticides by one-dimensional GC, multidimensional techniques (e.g. GCxGC-MS or GC-MS/MS or LC-MS/MS) are necessary for determining pesticides in lemon.

Figure 3: GCxGC-TOFMS contour plots for grape and lemon QuEChERS extracts. The lemon extract is much more complex than the grape extract and could not be analyzed by one-dimensional GC.

A. Black seedless grape



B. Lemon



See Figure 1 for GCxGC-TOFMS conditions.

Pesticide Determinations

Of the more than 200 pesticides in the standard, over 150 were analyzed by LC-MS/MS. Many of the pesticides were not amenable to GC analysis due to their lack of volatility, high polarity, or poor thermal stability, so only 65 were determined using GCxGC-TOFMS. For brevity, 41 pesticides representing each chemical class (Table I) will be discussed here. Of these 41 representative pesticides, ten were analyzed by GCxGC-TOFMS only (acephate, 1-naphthol, *o*-phenylphenol, terbacil, pirimicard, benthiocarb, triadimenol, fludioxonil, fenarimol, and bitertanol). Imazalil showed calibration problems in every matrix except for lemon with GCxGC. Spinach extracts were not analyzed with LC-MS/MS, but GCxGC-TOFMS data are reported.

The QuEChERS sample preparation approach combined with GCxGC-TOFMS and LC-MS/MS showed successful pesticide detections and quantitative analysis for pepper, cucumber, grape, and spinach samples (Table II). Matrix compounds interfered with the determination of a few pesticides in raisin and hazelnut when analyzed by GCxGC-TOFMS, including propoxur (raisin, hazelnut), siduron (raisin), and buprofezin (hazelnut). Propoxur and siduron have relatively low m/z quantification ions (110, 93) in electron ionization MS, which fall in the range of many of the ions produced by coextractives in complex food extracts. Even GCxGC did not have the selectivity to chromatographically move the coextractive interferences away in these few cases. Interestingly, GC-MS/MS would likely not yield better results since a low m/z ion precursor ion would yield very low m/z product ions, a situation where coextractives could again produce high-biased quantification. LC-MS/MS has the advantage in this case with soft electrospray ionization, which yields higher m/z ions that, when subjected to MS/MS, show greater selectivity and less bias. This can be seen in Table II for propoxur, siduron, and buprofezin in raisin and hazelnut extracts, where LC-MS/MS produced reasonable recovery values.

Lemon proved to be a difficult matrix demonstrated by the fact that 11 pesticides were not detected by LC-MS/MS and two pesticides had interfering compounds using the GCxGC-TOFMS method. The pesticides not detected in lemon by LC were propham, fuberidazole, cyprodinil, thiabendazole, mepronil, fenhexamid, propargite, piperonyl butoxide, pyriproxyfen, prochloraz, and pyraclostrobin. Given lemon's complexity, ion suppression from coelution with coextractives is the likely culprit for the non-detects. There were coextractives interfering with propoxur and terbacil that prevented their determination using the GC method. These interference cases demonstrate that GCxGC-TOFMS did not always have the selectivity necessary for determining certain pesticides in the most complex samples. In this regard, dispersive SPE cleanup was ineffective at removing certain matrix interferences for lemon, raisin, and hazelnut extracts. Complex matrices like these might benefit from a more exhaustive sample cleanup. For example, we have used a cartridge SPE method to remove more matrix coextractives from QuEChERS extracts of dietary supplements, which resulted in good pesticide recovery values [6].

Pesticide Recovery Values

GCxGC-TOFMS and LC-MS/MS percent recovery values for the 41 representative pesticides in each commodity are listed in Table II. Percent recovery values were reasonable, most above 80%, for both GC and LC techniques, which demonstrates QuEChERS extraction efficiency for a large range of pesticide types. A summary examination of method performance was revealed by distilling data from Table II to an average recovery value for each commodity/analysis method combination (Figure 4).

Table II: Percent recovery values from QuEChERS sample preparation for the selected pesticides as determined by GCxGC-TOFMS and LC-MS/MS for each commodity. (IP = incurred pesticides, NA = not analyzed, ND = not detected, and INT = affected by interferences).

	Red Bell Pepper		Cucumber		Black Grapes		Lemon		Raisin		Hazelnut		Spinach
Pesticide	**GCxGC**	**LC**	**GCxGC**	**LC**	**GCxGC**	**LC**	**GCxGC**	**LC**	**GCxGC**	**LC**	**GCxGC**	**LC**	**GCxGC**
Propoxur	72	99	100	99	92	110	INT	75	INT	120	INT	100	120
Methamidophos	IP	IP	130	76	170	73	79	66	73	48	78	73	93
Acephate	IP	NA	48	NA	73	NA	88	NA	82	NA	78	NA	64
Propham	110	88	110	77	100	50	130	ND	94	66	78	80	100
1-Naphthol	IP	NA	86	NA	95	NA	110	NA	97	NA	87	NA	120
o-Phenylphenol	86	NA	70	NA	91	NA	100	NA	96	NA	81	NA	99
Tebuthiuron	140	100	110	88	92	90	110	42	110	110	100	100	86
Omethoate	IP	IP	56	98	68	98	100	89	66	96	87	65	83
Dimethoate	IP	IP	92	94	93	91	100	79	98	94	94	98	77
Prometon	79	89	110	76	96	73	110	47	100	96	82	87	93
Terbacil	100	NA	100	NA	110	NA	INT	NA	91	NA	83	NA	83
Pirimicarb	110	NA	96	NA	98	NA	100	NA	100	NA	90	NA	100
Metribuzin	100	110	98	80	110	76	110	58	87	26	110	41	98
Fuberidazole	50	89	77	46	96	85	98	ND	86	88	94	82	120
Carbaryl	IP	IP	88	170	120	150	72	14	100	190	86	160	77
Metalaxyl	IP	IP	120	81	93	81	95	52	89	76	86	78	93
Terbutryn	92	93	100	79	100	79	99	4	97	84	64	51	91
Ethofumesate	110	80	100	85	110	120	81	19	86	77	100	77	82
Benthiocarb	110	NA	86	NA	85	NA	110	NA	95	NA	56	NA	94
Cyprodinil	87	63	IP	IP	99	86	91	ND	80	55	57	6.4	84
Thiabendazole	IP	76	110	19	110	70	83	ND	65	72	68	57	100
Furalaxyl	90	88	100	89	130	85	110	37	95	86	85	87	97
Triadimenol	68	NA	93	NA	110	NA	100	NA	110	NA	120	NA	80
Siduron	98	110	96	88	98	96	120	35	INT	100	89	79	130
Imazalil	NA	IP	NA	87	NA	70	IP	IP	NA	130	NA	58	NA
Fludioxonil	84	NA	IP	NA	120	NA	96	NA	100	NA	89	NA	100
Myclobutanil	IP	IP	120	73	130	110	100	13	76	100	91	87	90
Buprofezin	110	70	100	90	IP	IP	94	24	80	110	INT	68	85
Oxadixyl	110	90	110	83	120	90	97	40	100	99	130	98	82
Mepronil	99	110	88	84	120	91	100	ND	91	97	88	ND	97
Carfentrazone ethyl	110	150	81	170	110	150	110	74	81	220	100	180	80
Fenhexamid	IP	38	89	82	120	51	87	ND	67	75	75	49	99
Propargite	110	73	85	100	110	130	100	ND	79	110	75	110	79
Piperonyl butoxide	140	IP	120	93	110	95	110	ND	92	110	80	98	110
Pyriproxyfen	99	64	77	86	96	100	99	ND	100	90	63	62	91
Fenarimol	67	NA	58	NA	89	NA	100	NA	81	NA	99	NA	91
Bitertanol	150	NA	85	NA	92	NA	110	NA	60	NA	110	NA	100
Prochloraz	53	73	48	55	78	80	100	ND	83	70	83	17	87
Pyraclostrobin	150	84	59	61	110	92	61	ND	55	130	53	94	53
Azoxystrobin	100	100	64	94	98	86	110	30	91	94	88	120	88
Dimethomorph	220	52	82	91	90	98	97	25	80	69	110	54	84

These average recovery values were produced using data from pesticides that could be quantified, excluding pesticides that were not analyzed, not detected, incurred, or suffered from interferences. As with Table II data, Figure 4 shows the QuEChERS approach worked well, as demonstrated by the average recovery values between 80 to 110% for most commodities and for both analysis methods. A notable exception was for lemon as determined by LC-MS/MS where average percent recovery for pesticides was just above 40%. The good GCxGC-TOFMS recovery values for lemon indicate that the QuEChERS sample preparation approach was not the cause of the low LC-MS/MS low values. In fact, low recovery values and non-detected pesticides are not unexpected, as other researchers have demonstrated extreme ion suppression for citrus fruits when using LC-MS/MS [7,8,9]. Results may be improved by adding a fat freezing step after the QuEChERS extraction to remove waxes, using a cleanup with higher sorbent capacity like cartridge SPE, or by increasing the sample dilution factor to minimize LC-MS/MS matrix effects.

Incurred Target Pesticides

Incurred target pesticides were detected in four of the seven commodities tested, including red bell pepper, lemon, grape, and cucumber. Concentrations for incurred pesticides as determined using QuEChERS with GCxGC-TOFMS and LC-MS/MS are shown in Table III. In general, there was good agreement between incurred pesticide concentrations for GCxGC-TOFMS and LC-MS/MS, with the exception of methamidophos and carbaryl in red bell pepper.

The number of incurred pesticides detected by GCxGC-TOFMS and LC-MS/MS is also comparable; however, GCxGC-TOFMS detected two additional incurred pesticides in red bell pepper, thiabendazole and fenhexamid, and one additional incurred pesticide in cucumber, fludioxonil. LC-MS/MS detected incurred pesticides in red bell pepper that either could not be analyzed or were not found using the GC method, including thiamethoxam, clothianidin, imidacloprid, propamocarb, diphenylamine, spinosyn A, and spinosyn D.

Figure 4: Average percent recovery values shown for each commodity determined by both GCxGC-TOFMS and LC-MS/MS. Ion suppression led to apparent low pesticide recovery values for LC-MS/MS analysis of lemon extract.

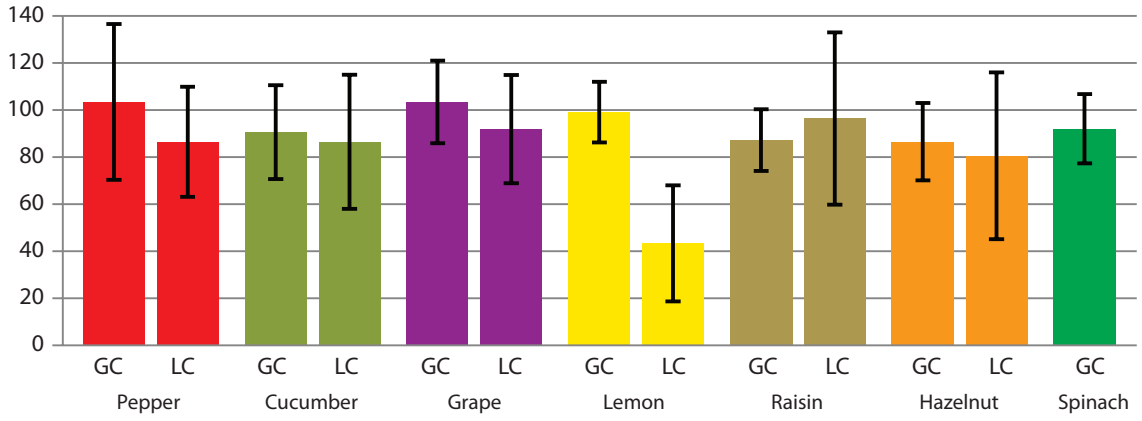


Figure 5 shows GC and LC chromatograms of red pepper extract from which incurred pesticides were determined. The GCxGC-TOFMS chromatogram demonstrates the power of that technique, especially for metalaxyl, which was accurately identified and quantified because the second dimension separated the peak from a more intense matrix component (below the metalaxyl peak on the contour plot). The LC-MS/MS chromatogram shows adequate retention and good peak shape for early eluting polar compounds (e.g. methamidophos and omethoate) by using the polar modified/functionally bonded aqueous C18 column. As noted above, LC-MS/MS detected incurred pesticides that either could not be analyzed or were not found using the GC method.

Non-Target Pesticide Analysis with GCxGC-TOFMS

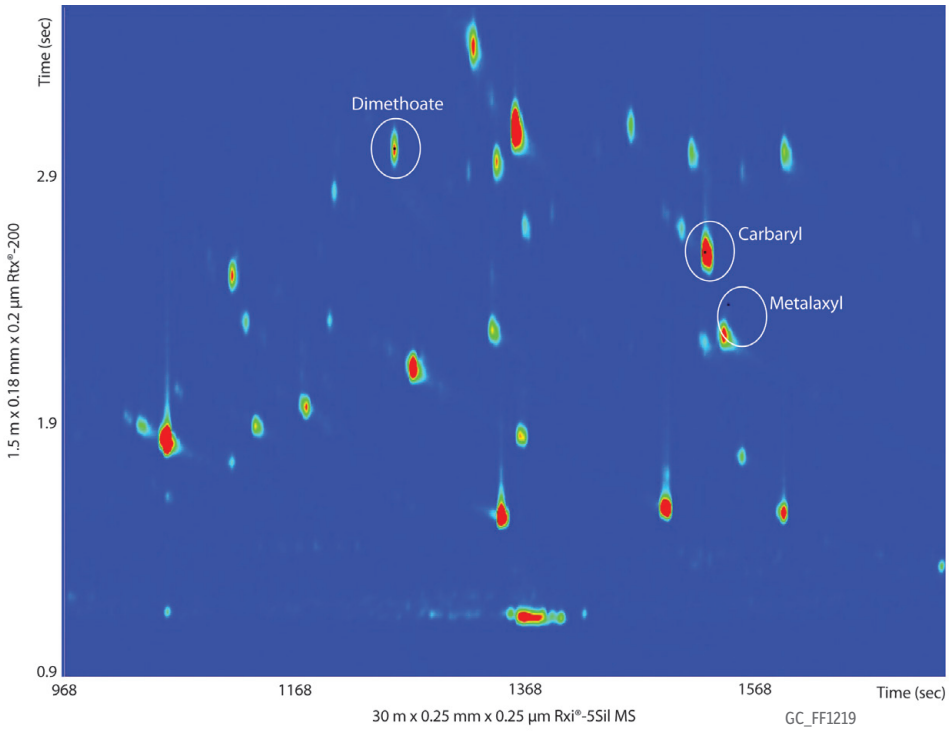
GCxGC-TOFMS can perform non-targeted and targeted analysis simultaneously because full mass spectral information is recorded during the entire analysis time. Automatic peak finding, spectral deconvolution, and library searching allowed full mass spectral data to be mined for pesticides not on the original GCxGC-TOFMS target compound list, e.g. imazalil in lemon. Other examples include the detection of endosulfans I and II, and endosulfan sulfate in red bell pepper extract. Figure 6 shows the contour plot for the elution region of the endosulfans and endosulfan sulfate, as well as the mass spectrum of endosulfan sulfate from the red bell pepper sample and the NIST library spectrum.

Table III: Incurred target pesticides and calculated ppb concentration determined by QuEChERS extraction with GCxGC-TOFMS and/or LC-MS/MS. (NA = not analyzed, ND = not detected)

	Concentration (ppb)	
Pesticide	**GCxGC**	**LC**
Red Bell Pepper		
Methamidophos	370	130
Acephate	560	NA
1-Naphthol	98	NA
o-Phenylphenol	0.62	NA
Omethoate	37	43
Dimethoate	58	57
Carbaryl	300	520
Metalaxyl	5.5	5.3
Thiabendazole	12	ND
Imazalil	NA	2.5
Myclobutanil	4.9	3.2
Fenhexamid	4.7	ND
Piperonyl butoxide	0.99	2.2
Bitertanol	0.40	NA
Lemon		
Imazalil	460	540
Black Seedless Grape		
Buprofezin	2.3	3.7
Cucumber		
Cyprodinil	100	95
Fludioxonil	30	NA

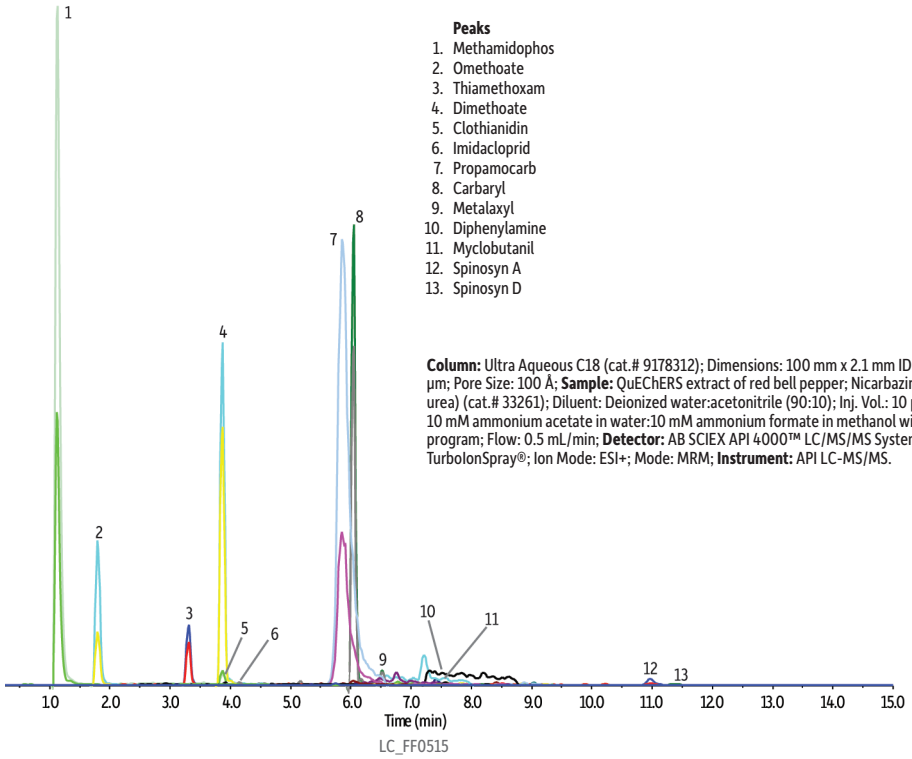
Figure 5: GCxGC-TOFMS contour plot showing incurred dimethoate, carbaryl, and metalaxyl pesticides in a QuEChERS extract of red bell pepper (A). The LC-MS/MS chromatogram (B) of incurred pesticides found in red bell pepper extract includes compounds that either could not be analyzed, or were not found, using GC.

A. GCxGC-TOFMS



See Figure 1 for GCxGC-TOFMS conditions.

B. LC-MS/MS

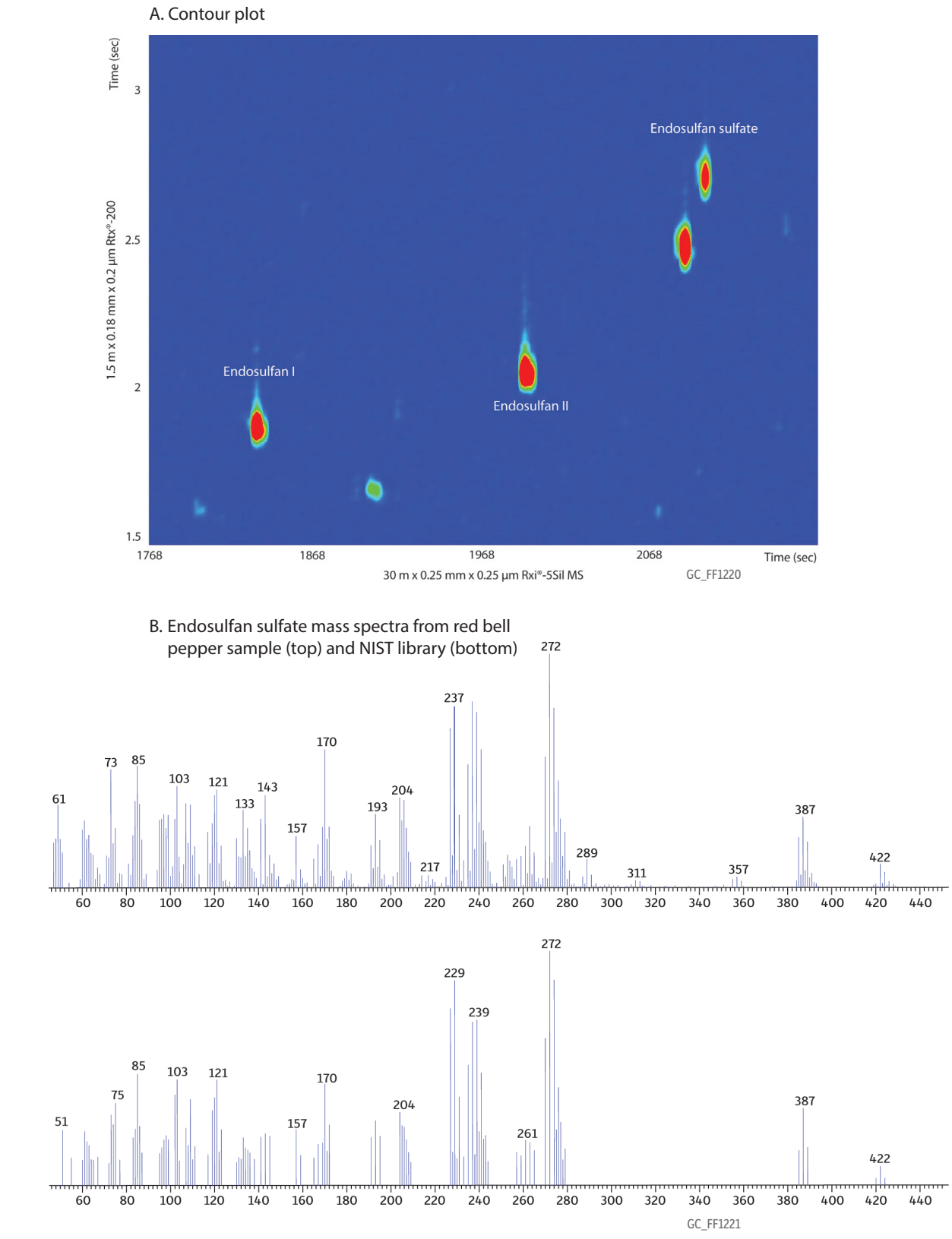


Peaks

1. Methamidophos
2. Omethoate
3. Thiamethoxam
4. Dimethoate
5. Clothianidin
6. Imidacloprid
7. Propamocarb
8. Carbaryl
9. Metalaxyl
10. Diphenylamine
11. Myclobutanil
12. Spinosyn A
13. Spinosyn D

Column: Ultra Aqueous C18 (cat.# 9178312); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 3 µm; Pore Size: 100 Å; **Sample:** QuEChERS extract of red bell pepper; Nicarbazin (bis-nitrophenol urea) (cat.# 33261); Diluent: Deionized water:acetonitrile (90:10); Inj. Vol.: 10 µL; **Mobile Phase:** 10 mM ammonium acetate in water:10 mM ammonium formate in methanol with gradient program; Flow: 0.5 mL/min; **Detector:** AB SCIEX API 4000™ LC/MS/MS System; Ion Source: TurboIonSpray®; Ion Mode: ESI+; Mode: MRM; **Instrument:** API LC-MS/MS.

Figure 6: Contour plot region for elution area of endosulfans and endosulfan sulfate in a QuEChERS extract of red bell pepper, as well as spectral comparison of endosulfan sulfate from the red bell pepper sample and the NIST library.



See Figure 1 for GCxGC-TOFMS conditions.

Conclusions

The pesticide residue analysis of food work presented here demonstrates that the QuEChERS sample preparation approach worked well for a variety of pesticides and commodities. In general, good pesticide recoveries were achieved for the QuEChERS approach as determined by both GCxGC-TOFMS and LC-MS/MS. However, more difficult matrices like lemon, raisin, and hazelnut proved to be a challenge. Sometimes GCxGC-TOFMS did not have the selectivity necessary for determining certain pesticides in the most complex samples, indicating dispersive SPE cleanup was unsuccessful at removing high-concentration, coeluting matrix interferences in lemon, raisin, and hazelnut extracts. Ion suppression and/or solvent standard calibration (versus matrix-matched standard calibration) adversely affected LC-MS/MS quantification for some pesticides, especially in lemon and hazelnut extracts. Generally, incurred pesticide quantifications were comparable for GCxGC-TOFMS and LC-MS/MS. Advantages and disadvantages of each methodology, QuEChERS, GCxGC-TOFMS and LC-MS/MS, presented themselves during this work, which highlighted the utility of QuEChERS and the desire for comprehensive and complementary instrumental determinations.

Acknowledgements

U.S. Food and Drug Administration/Center for Food Safety and Applied Nutrition; LECO Corporation

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