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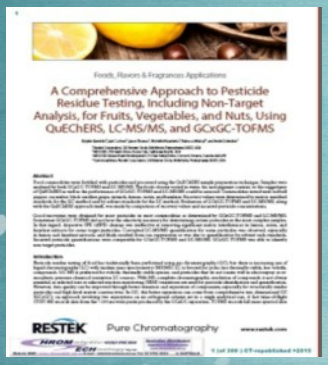

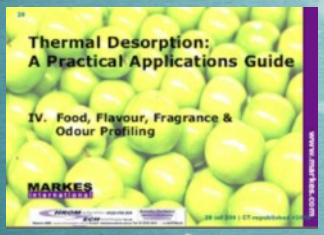

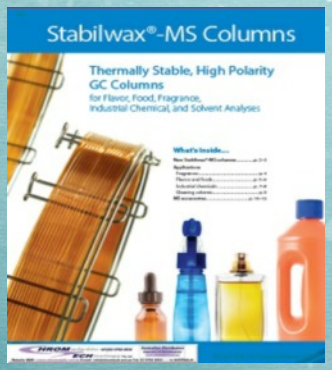

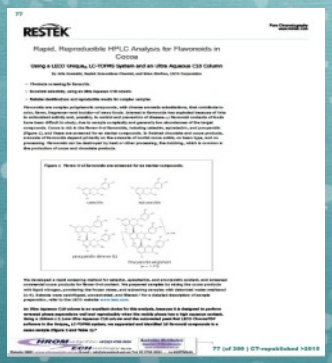

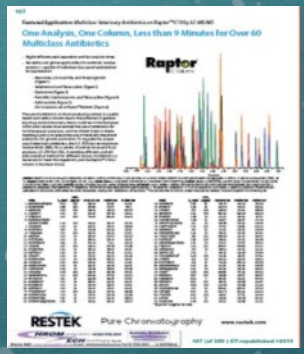

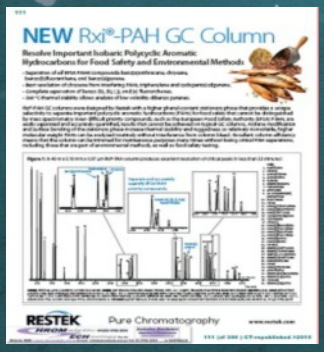

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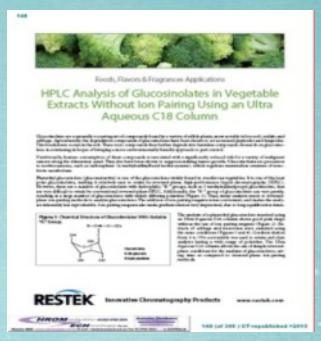

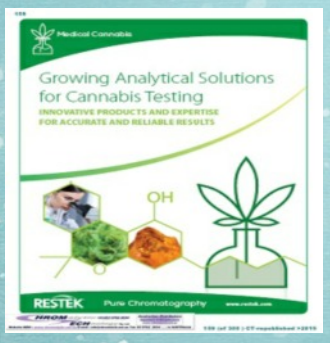

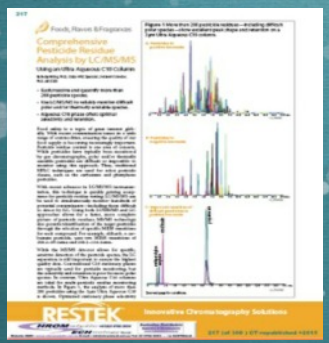

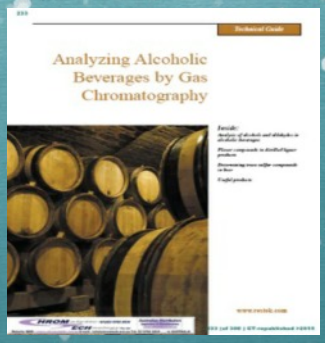

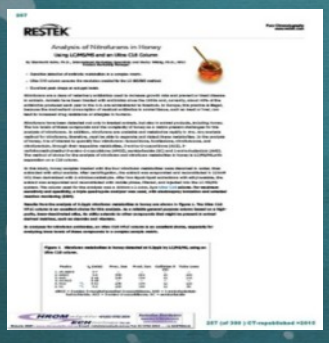

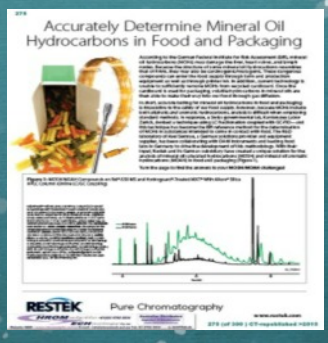

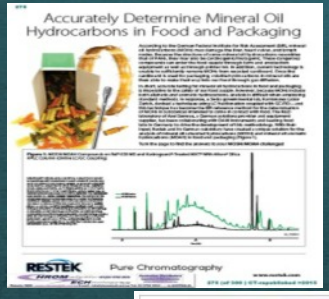

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Foods, Flavors & Fragrances Applications

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

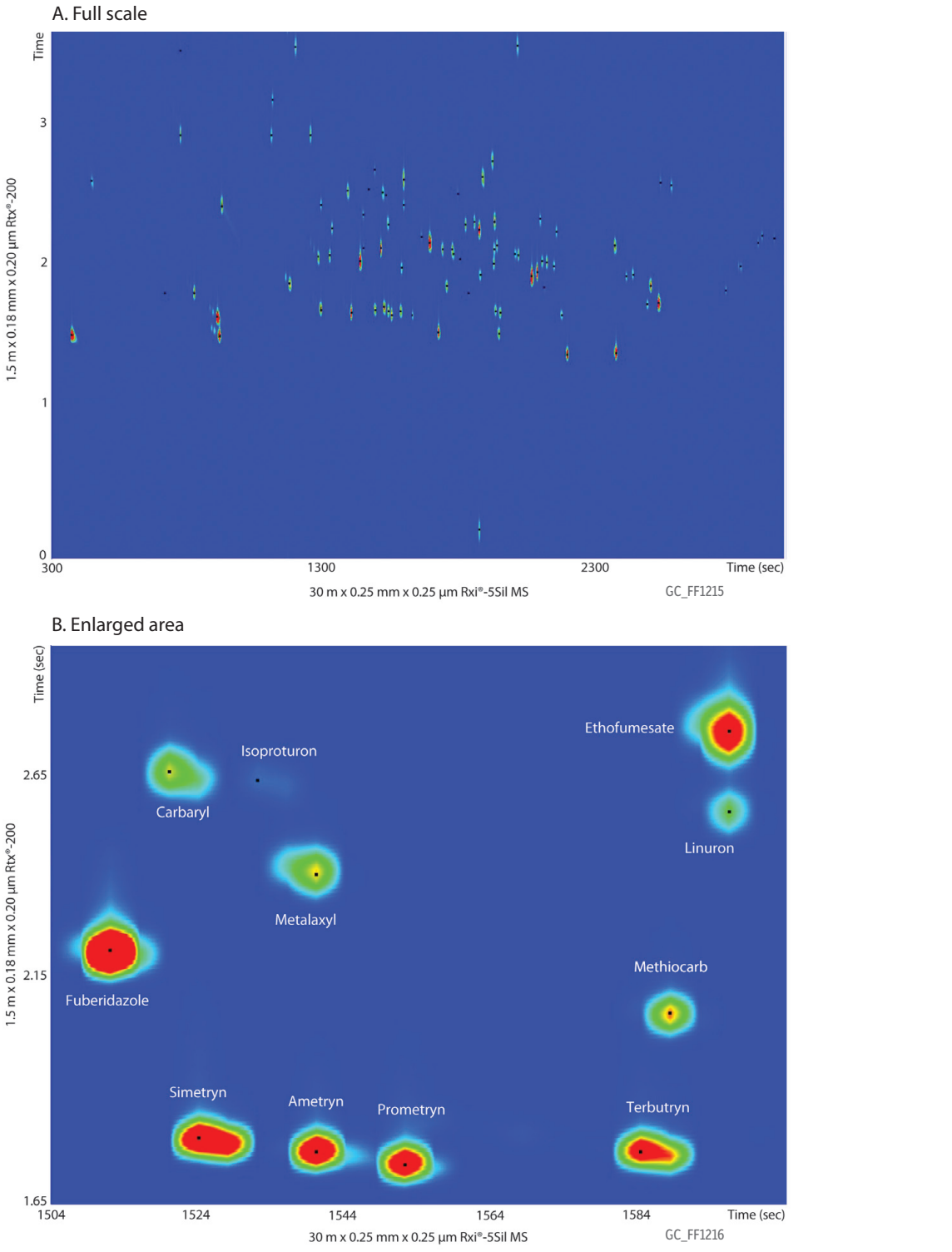
Results and Discussion

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.

RESTEK

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.



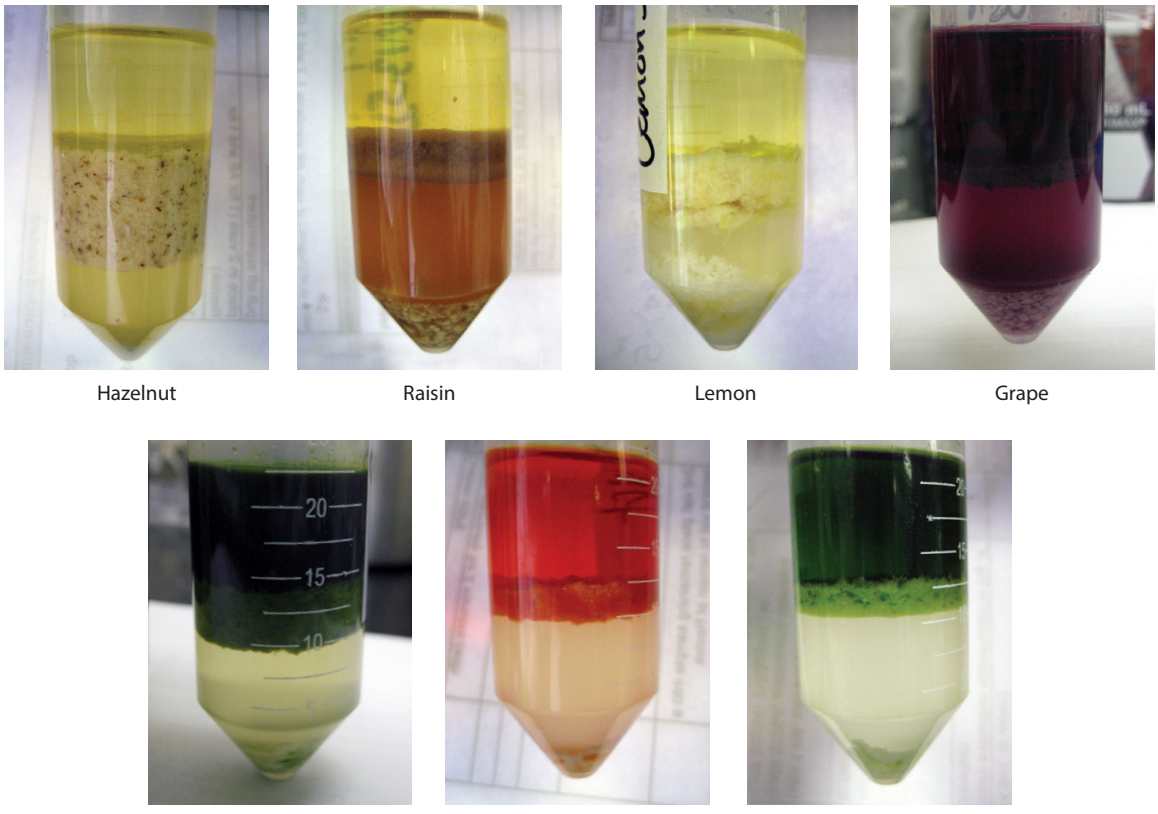
Column: Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623), Rtx®-200 1.5 m, 0.18 mm ID, 0.20 µm (cat.# 45001); **Sample:** Pesticide standard; Diluent: Acetonitrile; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: Gooseneck splitless (5 mm) w/deactivated wool (cat.# 22973-200.1); Inj. 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.

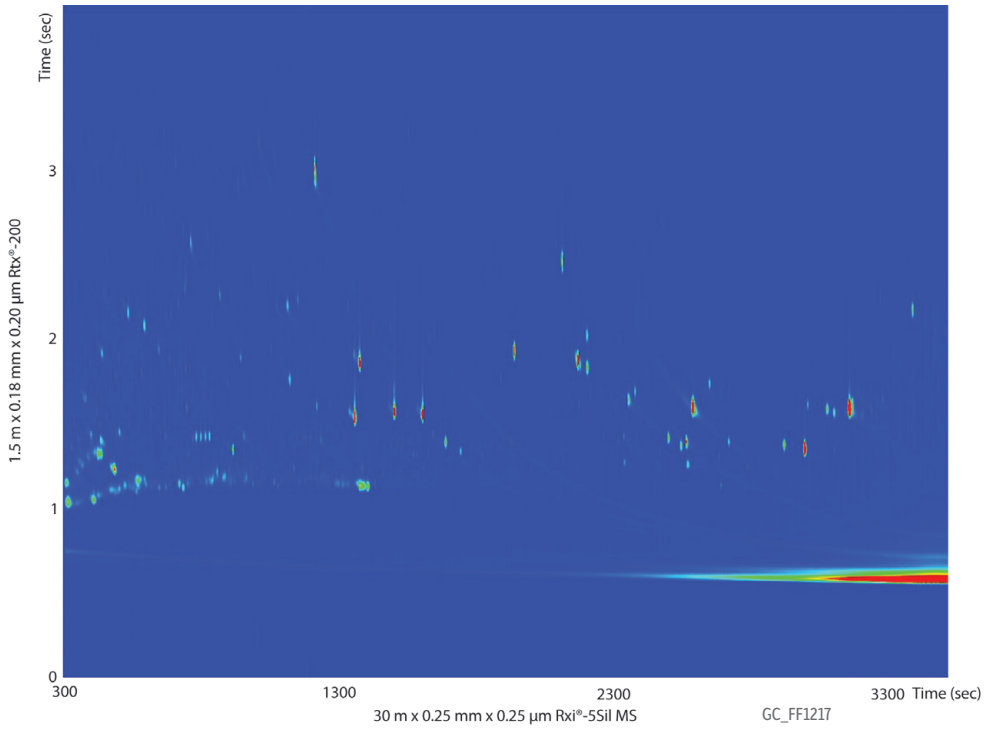
Figure 2: Photographs of QuEChERS extracts of studied commodities before dSPE cleanups were performed.



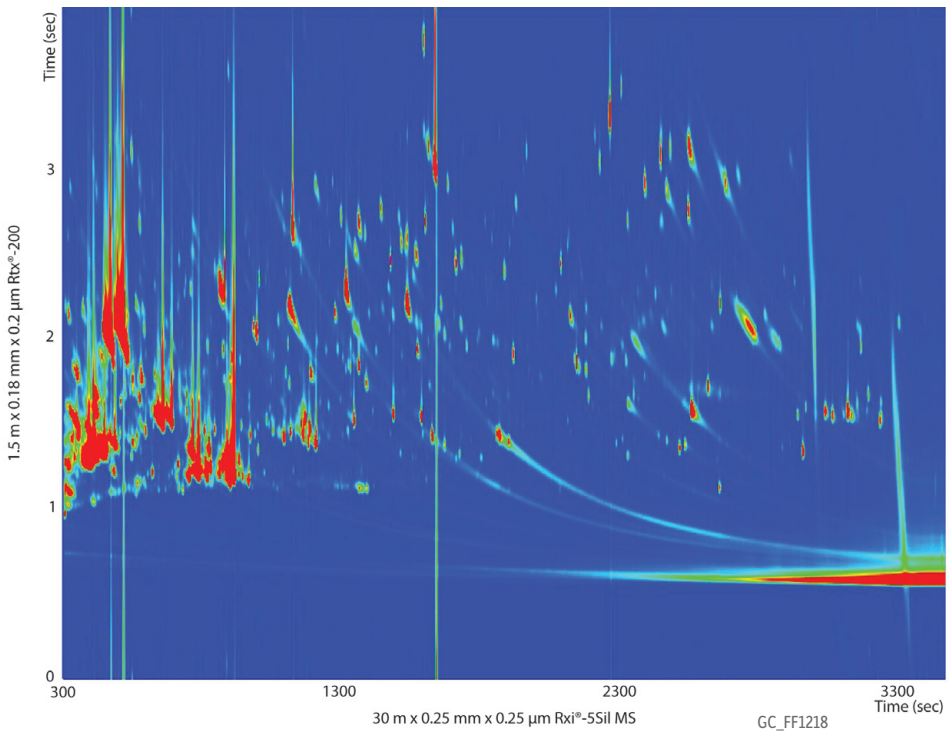
We assessed the complexity of different commodities by examining the total ion chromatogram (TIC) contour plots generated by GCxGC-TOFMS. Figure 3 shows TIC plots for two commodities, grape and lemon, which represent the range from least complex to most complex, as determined by a GC approach. It is 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.

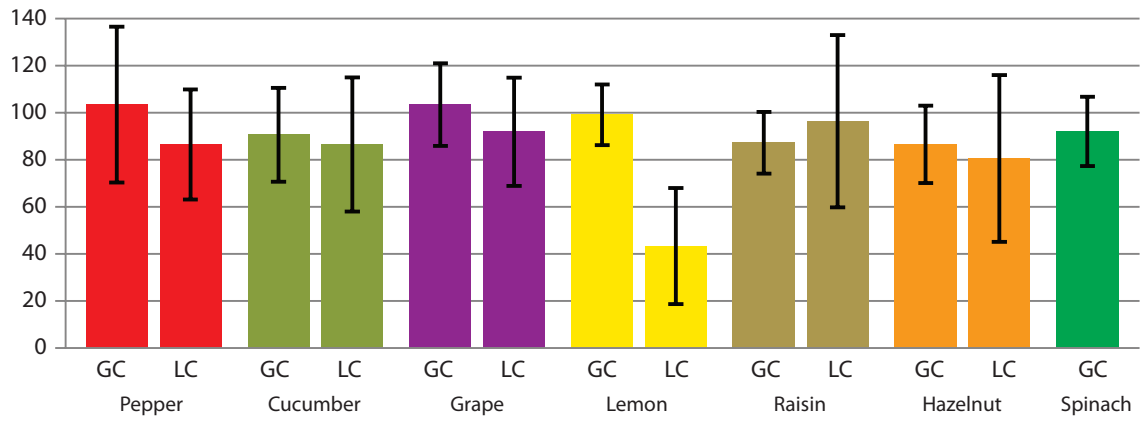


Figure 5 shows GC and LC chromatograms of red pepper extract from which incurred pesticides were determined. The GCxGC-TOFMS chromatogram demonstrates the power of that technique, especially for metalaxyl, which was accurately identified and quantified because the second dimension separated the peak 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

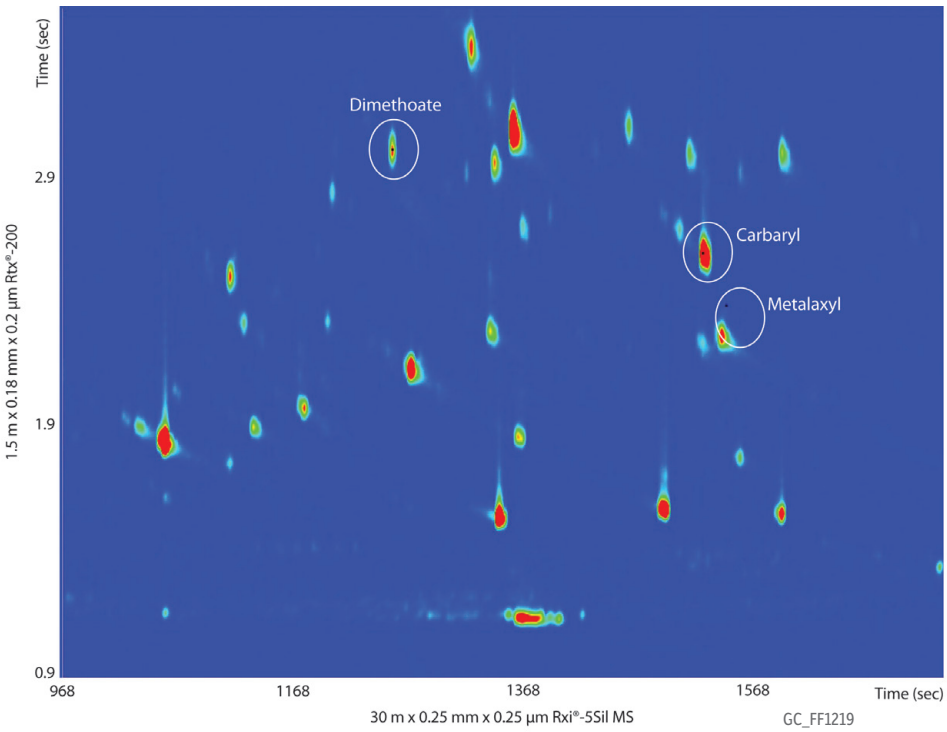
GCxGC-TOFMS can perform non-targeted and targeted analysis simultaneously because full mass spectral information is recorded during the entire analysis time. Automatic peak finding, spectral deconvolution, and library searching allowed full mass spectral data to be mined for pesticides not on the original 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

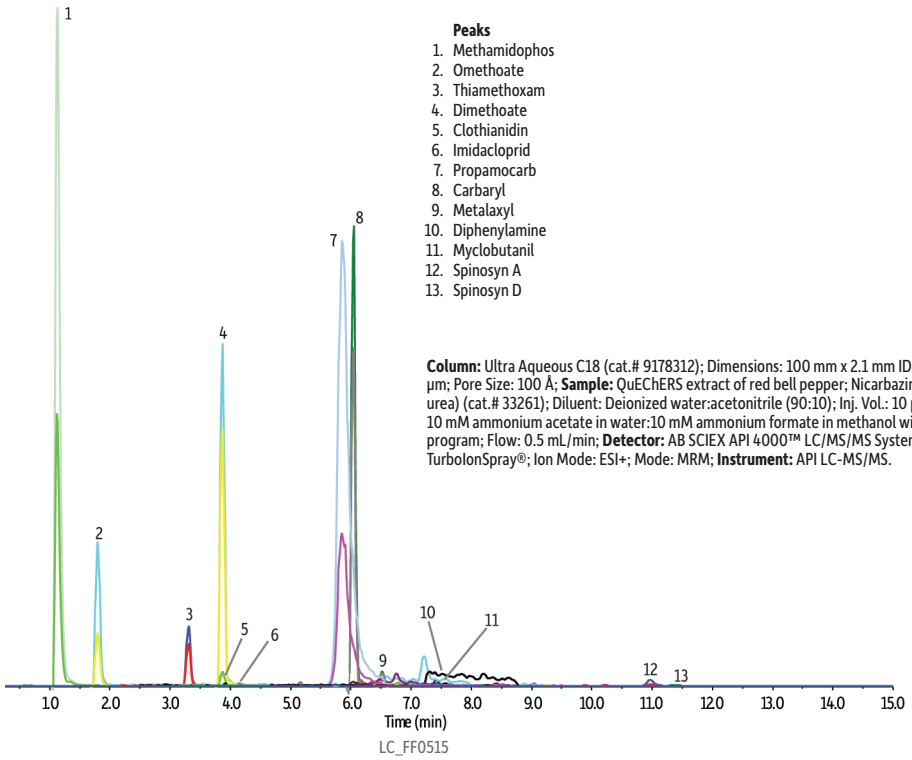
Figure 5: GCxGC-TOFMS contour plot showing incurred dimethoate, carbaryl, and metalaxyl pesticides in a QuEChERS extract of red bell pepper (A). The LC-MS/MS chromatogram (B) of incurred pesticides found in red bell pepper extract includes compounds that either could not be analyzed, or were not found,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

A. GCxGC-TOFMS



See Figure 1 for GCxGC-TOFMS conditions.

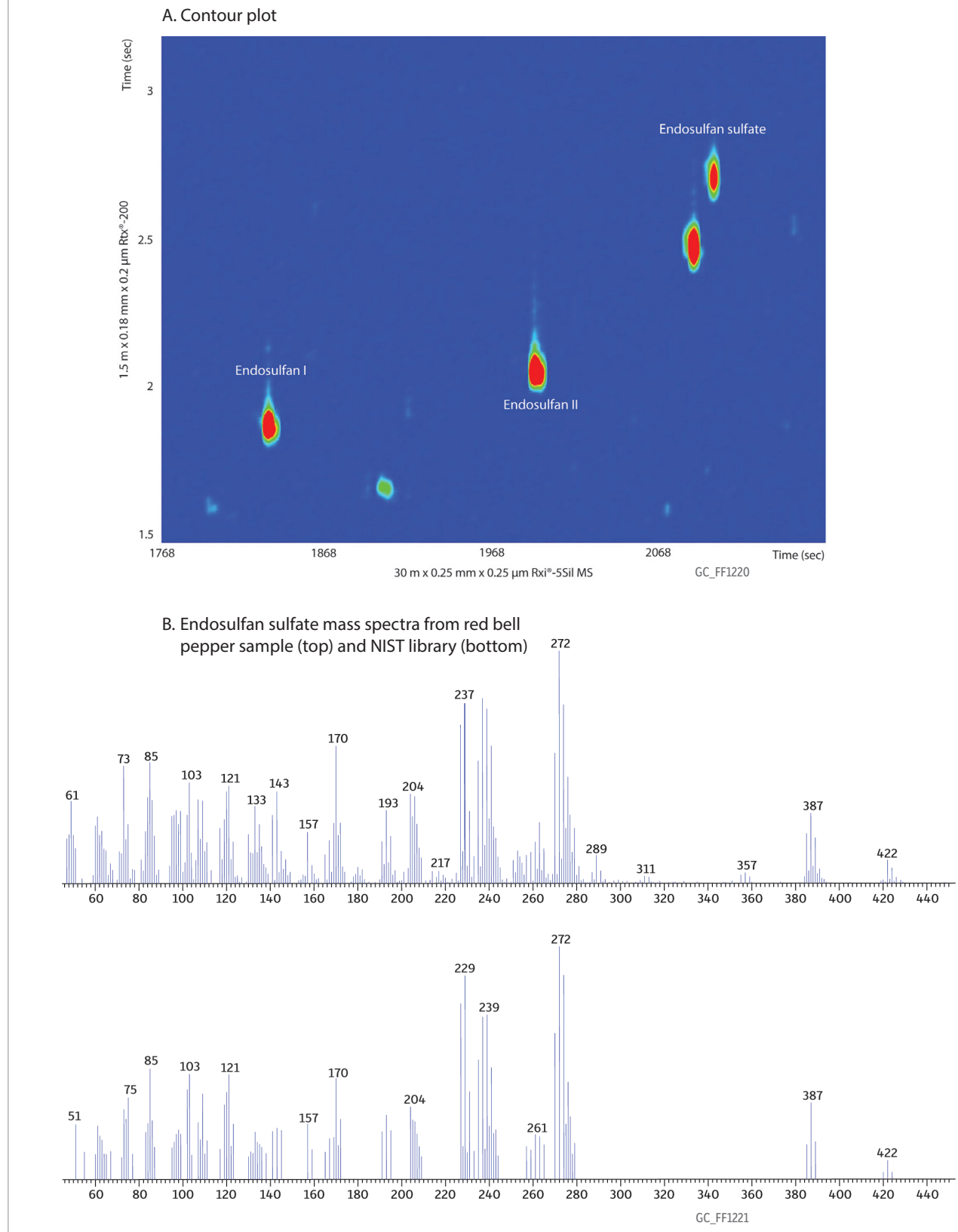
B. LC-MS/MS



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.

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General Interest

USLC™ Columns Put the Right Tools in Your LC Method Development Toolbox

Understanding Selectivity in Reversed Phase Separations – A Simplified Approach to HPLC and UHPLC Column Selection

By Rick Lake and Ty Kahler

The most significant influence on chromatographic peak separation, or resolution, is column selectivity. Unfortunately, column selectivity is also the least understood and most underutilized parameter. To improve selectivity, method developers often concentrate on manually altering mobile phases, operational parameters, and instrumentation. But because stationary phases offer more significant selectivity differences, you can drastically speed up HPLC and UHPLC method development by instead focusing on column choice. In this article, we discuss column selection for reversed phase separations and, using the hydrophobic-subtraction model (H-S model), identify a set of just 4 stationary phases—Restek's USLC™ column set—that encompasses the widest selectivity range available on the market.

The Role of Selectivity in Liquid Separations

When performing a liquid separation, we generally focus on choosing the right instrumentation—especially since the recent advent of UHPLC—and end up choosing columns rather hastily, either by proximity (using the column that is already on the instrument or in the closest drawer) or by habit (using a column that has offered problem-free service in the past). While never optimal, this practice should be particularly concerning for a method developer because improper column choice can lead to needlessly labor- and time-intensive method development. If we consider the impact of column selectivity on peak separation, or resolution, we can see why choosing the right column can be so advantageous.

Resolution is the result of 3 cumulative terms: efficiency (N), retention capacity (k), and selectivity (α). How well we resolve our analytes, and how quickly we do so, depends upon our ability to control these 3 factors. Of the 3, the selectivity term mathematically affects resolution to the greatest degree (Equation 1). Put another way, resolution is largely a function of selectivity.

Equation 1: Selectivity is the driving parameter of resolution, as it affects peak separation to the greatest degree.

$$R = \tfrac{1}{4}\sqrt{N} \times (k/(k+1)) \times (\alpha-1)$$

Efficiency *Retention Factor* *Selectivity*

Changing Columns to Create Significant Changes in Selectivity

Since resolution is largely a function of selectivity, any discussion about improving resolution should focus primarily on altering selectivity. It has often been taught in HPLC method development that one can effectively alter selectivity by adjusting mobile phases to reach a desired separation. This, of course, is true. However, mobile phase adjustment can be laborious—often involving many preparation adjustments and column equilibration times—and typically creates only marginal selectivity differences. In addition, some elution profiles are not practical with certain mobile phases and detection modes, including mass spectrometry (MS) and refractive index (RI).

On the other hand, changing stationary phases (i.e., columns) can be much easier and can also result in more significant selectivity differences because stationary phases can offer alternate and even orthogonal separations. These alternate separations can also be scouted very quickly using precise scouting gradients.

With the number of columns commercially available today, choosing the right one can be difficult, even overwhelming. By quantifying stationary phase selectivity, we can create new guidelines for effectively and easily choosing columns to help reduce method development time and increase method ruggedness.

Quantifying Column Selectivity Using the Hydrophobic-Subtraction Model (H-S Model)

Many models exist for choosing solvents and mobile phase additives, but not until recently has stationary phase characterization received much attention. Column selectivity has been largely overlooked due, in part, to its complexity, particularly for liquid separations. But now that Snyder et al. have proposed their popular hydrophobic-subtraction model (H-S model) [1], we can begin to compare and quantify stationary phase selectivity in reversed phase separations and determine (often through orthogonal separations) which stationary phases produce the greatest degree and range of selectivity differences. Only then can we identify a small set of columns that will form the contents of an efficient and effective method development toolbox.

The H-S model is a novel treatment that empirically defines reversed phase selectivity by analyzing a varied collection of solute test probes and then utilizing 5 established selectivity parameters—hydrophobicity (H), steric hindrance (S*), hydrogen bond acidity (A), hydrogen bond basicity (B), and cation exchange activity (C)—to identify the contributions of silica sorbents and stationary phases on selectivity. This model has been used by many organizations, including United States Pharmacopeia (USP), to find column equivalency.

The selectivity value (Fs) of the H-S model is normally used to find the similarity between columns, but it can conversely be used to find column ***dis***similarity, even orthogonality, to highlight selectivity differences and simplify column selection. Table I compares a variety of stationary phases and reveals which phases offer increased selectivity. (Because the H-S model evaluates the contributions of both stationary phase and silica support on selectivity, we intentionally kept the silica support constant throughout our experiments to isolate the effect of stationary phases on selectivity.) Each value was calculated relative to a C18 benchmark. The columns showing high Fs values—like the 4 Restek USLC™ phases shown in **blue**—exhibit the greatest dissimilarity in selectivity relative to the C18, so they are excellent choices when a C18 does not provide the selectivity needed.

Table I: The Fs term of the hydrophobic-subtraction model (H-S model) can numerically determine which stationary phases are most dissimilar to a C18, illustrating the phases needed to extend the selectivity range in reversed phase chromatography. The 4 Restek USLC™ phases are shown in **blue**.

Terms Calculated from the Hydrophobic-Subtraction Model (H-S Model)							
Stationary Phase Type	Hydrophobicity	Steric Hindrance	Hydrogen Bond Acidity	Hydrogen Bond Basicity	Cation Exchange Activity	Selectivity Function	Rank Dissimilarity
	H	S*	A	B	C	Fs	
Ultra C18 (control)	1.051	0.033	-0.032	-0.023	0.057	0.0	—
Ultra C8	0.0871	0.013	-0.0199	0.019	-0.032	11.2	8
Ultra C4	0.0738	-0.010	-0.276	0.019	0.032	11.3	7
Ultra C1	0.613	-0.054	-0.408	0.016	-0.032	17.9	6
Ultra Aqueous C18	0.808	-0.128	0.378	0.013	0.0229	25.4	5
Ultra Biphenyl	0.661	-0.189	-0.283	0.042	0.204	28.4	4
Ultra Cyano†	0.409	-0.041	-0.801	-0.011	-0.110	29.1	3
Ultra PFP Propyl	0.671	-0.092	-0.213	-0.007	0.658	52.0	2
Ultra IBD	0.672	-0.035	-0.052	0.233	-0.564	63.7	1

All columns were tested using the same silica support.
† NOTE: The cyano phase also ranks high in terms of dissimilarity, but the more rugged PFP Propyl phase was ultimately chosen for the USLC™ column set because it better withstands the low pH levels required for mass spectrometry while offering equally heightened retention of basic compounds.

Characterizing Selectivity at the Molecular Level

Often during method development, after we have made our initial column choice, we still find ourselves struggling to resolve compounds as we try to find a "better" column. This difficulty is often due to an inability to find a column with ***alternate*** selectivity. Quantifying stationary phase selectivity (Table I) is a very important step in identifying a small and effective column set for method development, but we must further define selectivity at a molecular level to ensure that the columns in our method development toolbox exhibit not just ***high*** selectivity, but also ***alternate*** selectivity based on potential analyte types.

Selectivity (α) is practically determined from the difference in retention factors (k) of 2 peaks. Therefore, to produce alternate selectivity, we must alter the retention of one peak relative to the other. (Increasing the retention of both peaks equally results in higher retention capacity, but no change in selectivity because the difference between the 2 peaks does not change.) If we focus column selection on intermolecular interactions, we can see how specific phases create selectivity by altering the retention profile of specific solutes in relation to others—true selectivity.

So before we can confirm alternate selectivity, we first need to characterize the types of intermolecular interactions commonly encountered in reversed phase chromatography (RPC). In our experiments, we measured 4 major types of interactions—dispersion, polarizability, hydrogen bonding, and cation exchange. To further simplify things and more easily define a guideline, we can relate these measured interactions to chemical properties as noted below:

- ***Dispersion*** is the term for the van der Waals interactions that exist to some extent in all organic molecules, including polar molecules. It is the major driver for RPC and is a major retention mechanism for alkyl phases (i.e., C1 through C18). Since the retention is proportionate to the hydrophobicity of the molecule, we can call these interactions ***hydrophobic retention***.
- ***Polarizability*** is the ability of a stationary phase to change its electron distribution in the presence of an analyte and induce a dipole interaction. It is commonly seen in phenyl-based columns and is the main reason we often switch from a C18 to a phenyl to find alternate selectivity. The Restek Biphenyl column has 2 phenyl rings to enhance polarizability. These interactions are most commonly seen in dipolar, unsaturated, or conjugated compounds and fused-ring compounds with electron withdrawing groups (like nitro groups). For our purposes, we will define these interactions simply as ***dipolar retention***.
- ***Hydrogen bonding*** is used in RPC when a solute and a stationary phase form a chemical bond in which a hydrogen atom of one molecule is attracted to an electronegative atom, especially a nitrogen, oxygen, or fluorine, of another molecule. Although hydrogen bonding results in retention of other solute types, we will focus on its ability to increase retention for acidic compounds and will call it ***acidic retention***.
- ***Cation exchange*** is an electrostatic interaction between a cationic solute and an anion within the stationary phase. Cation exchange, or electrostatic interaction, is most commonly employed in RPC for the retention of protonated bases. Therefore, for simplicity, we will call it ***basic retention***.

Table II outlines the common solute retention profiles for the specific interactions we measured in our experimentation. With these intermolecular interactions defined, we can now use their retention profiles to determine which highly selective columns produce alternate selectivity for specific compound types, thereby radically simplifying column selection.

Table II: Common retention profiles measured for modern reversed phase columns as they relate to molecular interactions.

Solute Interaction	Type of Solute Retained	Common Phase Category	H-S Model Term	Probes Measured
Dispersion	Hydrophobic	C18	H	Toluene, Ethylbenzene
Polarizability	Dipolar	Biphenyl	n/a*	Anisole, Benzonitrile
Hydrogen Bonding	Acidic	Polar Embedded	B	4-Butylbenzoic Acid, Mefenamic Acid
Cation Exchange	Basic	Fluorinated Phenyl	C	Berberine, Amitriptyline, Nortriptyline

* Because polarizability is not measured by the H-S model, Restek used anisole and benzonitrile probes to mathematically determine the degree of polarizability of each stationary phase.

Extending the H-S Model to Simplify Column Choice

To determine a simplified guideline for column selection, Restek has extended the H-S model by analyzing empirical selectivity data of our stationary phases (Table I) against the RPC molecular interactions described in Table II. Through matching stationary phases to specific solute types based on these measured intermolecular attractions, we can aid method development in 2 significant ways: First, we can find a small set of columns with a wide range of alternate selectivity for use in method development. Second, we can define a process for selecting columns based on the chemical properties of our analytes when scouting column selectivity.

Extrapolating the retention data for the solute probes in the H-S model allows us to correlate the retention characteristics of specific solutes to stationary phase types. Ultimately, this correlation has enabled us to match column type to the selective retention of our analytes' chemical properties, making column selection truly definable by the chemical composition of our analytes.

Figure 1 illustrates the retention profile of a C18 compared with the profiles of the 4 Restek Ultra Selective Liquid Chromatography™ (USLC™) columns. We can see changes in selectivity across these columns as illustrated by the circled areas showing heightened retention for particular solute types. (Selectivity is the retention of one solute relative to another.) The 4 USLC™ columns exhibit varied retention profiles based upon solute type and, therefore, will exhibit alternate selectivity relative to one another. Because we have a small, quantified column set—4 Restek USLC™ phases—that is highly selective ***and*** exhibits significantly different retention profiles based on specific solute chemical properties, we can now match columns to specific analytes and, thus, simplify method development.

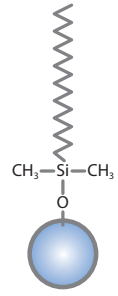
Figure 1: Stationary phase selectivity can be determined by looking for column types with varying retention profiles. When compared to a C18, the 4 Restek USLC™ phases offer diverse retention profiles—that is, a true range in selectivity.

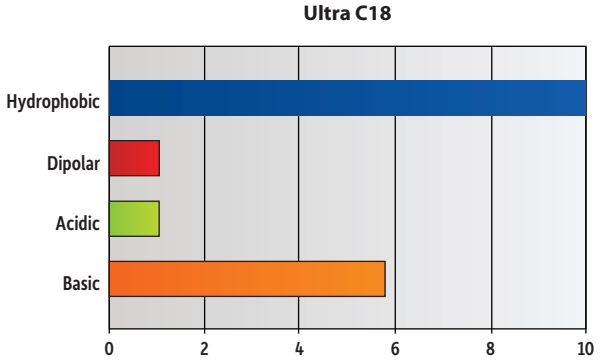
Restek Phase:
C18

Stationary Phase Category:
C18 (L1)

Ligand Type:
Densely bonded and fully end-capped octadecyl silane

Properties:
- General purpose.
- Strong hydrophobic retention.





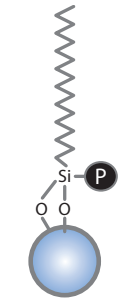
C18 Benchmark

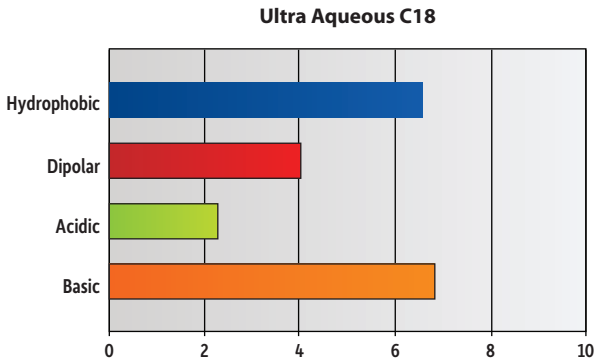
Restek USLC™ Phase:
Aqueous C18

Stationary Phase Category:
Modified C18 (L1)

Ligand Type:
Proprietary polar modified and functionally bonded C18

Properties:
- General purpose with a well-balanced retention profile.
- Compatible with 100% aqueous mobile phases.
- Ideal for multi-component LC-MS analyses.





Well-balanced retention profile.

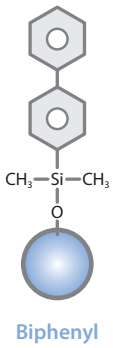
Figure 1, *continued*

Restek USLC™ Phase:
Biphenyl

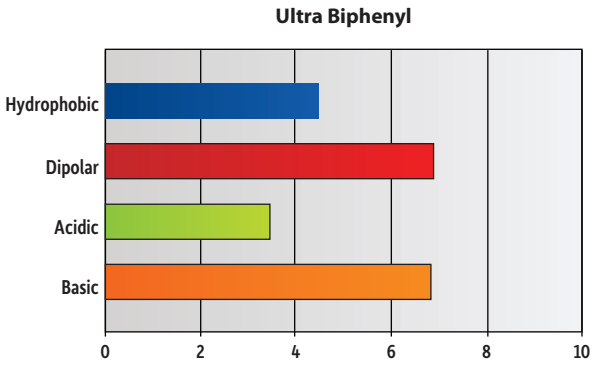
Stationary Phase Category:
Phenyl (L11)

Ligand Type:
Unique Biphenyl

Properties:
- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.



Biphenyl



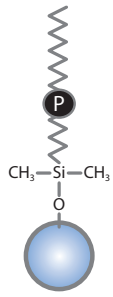
Heightened retention for dipolar compounds.

Restek USLC™ Phase:
IBD

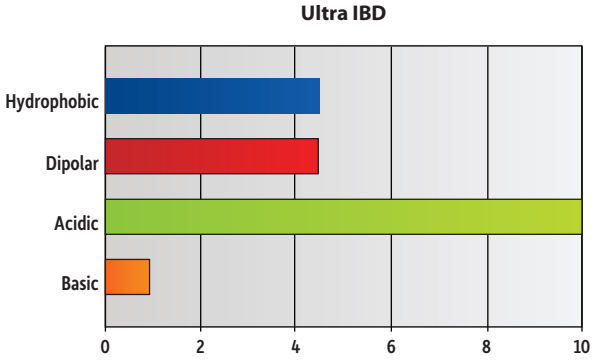
Stationary Phase Category:
Polar Embedded Alkyl (L68)

Ligand Type:
Proprietary polar functional embedded alkyl

Properties:
- Increased retention for acids and water-soluble compounds.
- Compatible with 100% aqueous mobile phases.
- Capable of reversed phase and HILIC separations.



IBD



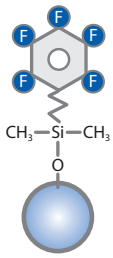
Heightened retention for acidic compounds.

Restek USLC™ Phase:
PFP Propyl

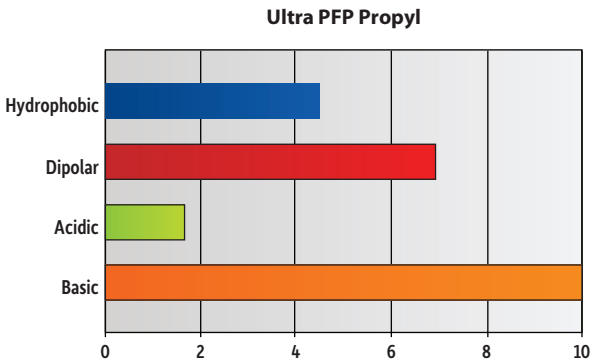
Stationary Phase Category:
Proprietary end-capped pentafluorophenyl propyl (L43)

Ligand Type:
Fluorophenyl

Properties:
- Increased retention for charged bases and electronegative compounds.
- Capable of reversed phase and HILIC separations.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.



PFP Propyl



Heightened retention for basic compounds.

All columns were tested using the same silica support.

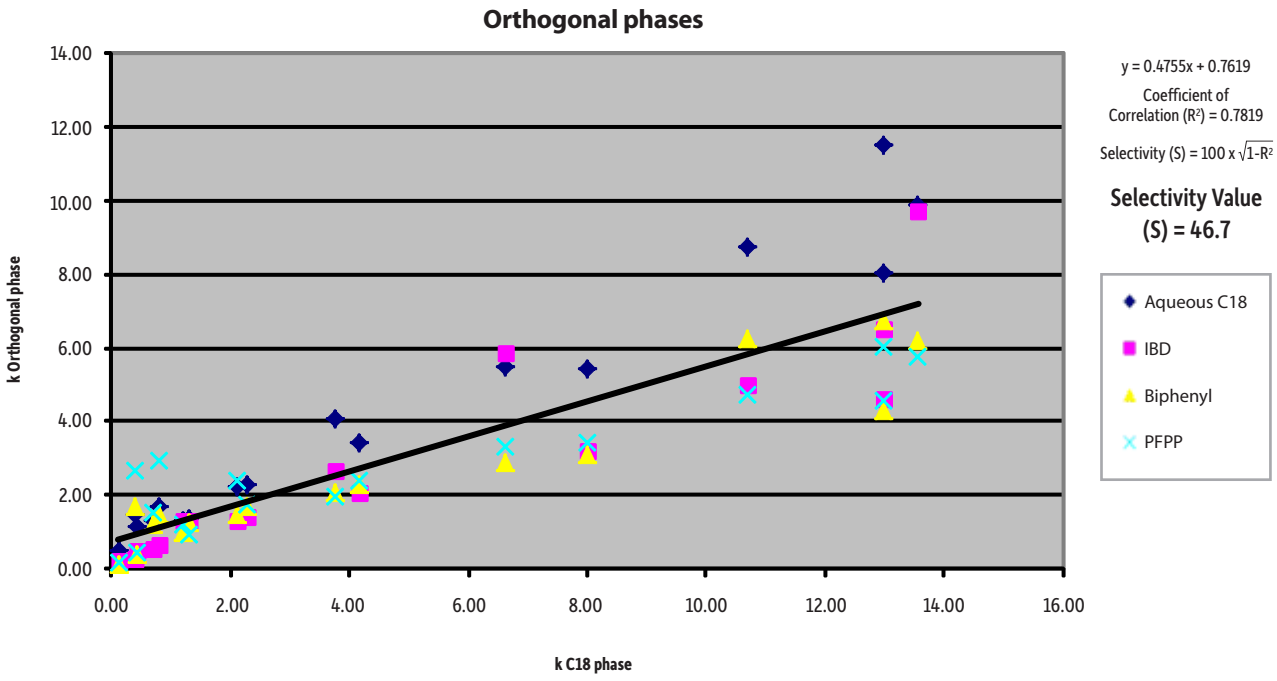
Confirming the Alternate Selectivity of the USLC™ Column Set

To further confirm that each USLC™ column provides alternate selectivity—not only when compared to the C18 benchmark, but also when compared to the other columns in the set—we quantified the column set's range of selectivity (S) as described by Neue et al. [2]. Looking at the retention characteristics of the H-S model solute probes, we can define selectivity as the degree of scatter along the regression line when comparing stationary phases to the conventional C18 benchmark (Figure 2).

Two very similar stationary phases will produce similar retention for the solute probes and, when graphed, will show high linearity and high correlation. Two very ***dis***similar, or alternately selective, stationary phases that differ in the retention of the solute probes will show a high degree of scatter around the regression line. More scatter reveals that columns are more different, or orthogonal, from one another because it shows larger differences in selectivity. To measure this difference and use it as a means of comparing stationary phases, we can calculate a selectivity (S) value for the columns in the USLC™ column set. Note that because silica and mobile phase contributions could also alter the retention of the test probes, it is important to use identical silica supports and mobile phase compositions as to not bias the results and to allow focus only on the stationary phase contributions to selectivity.

With a selectivity value (S) of 46.7, Restek USLC™ phases produce an incredible range of alternate selectivity —using only 4 columns.

Figure 2: Restek has extended the selectivity range for commercially available columns and defined a column set—the 4 USLC™ phases—that is ideal for simplified column selection and ease of method development.



All columns were tested using the same silica support.

Conclusion: The Right Tools for Maximum Selectivity

The H-S model offers the chromatographic method developer a practical approach to column selection. With a simplified model described above, we can now easily create predictable and alternate selectivity, effectively influencing the most significant factor contributing to resolution. Now that we have identified the small USLC™ column set with a wide range of quantified selectivity, we can quickly determine the best column for nearly any instrument platform and reversed phase or HILIC application by referencing predefined retention profiles. This column set can also be used to get the most out of column switching by providing a functional column set.

The Restek USLC™ column set, consisting of a balanced Aqueous C18, a Biphenyl, a fluorinated PFP Propyl, and a polar embedded IBD, has a profile that encompasses the widest range of reversed phase selectivity available today. Putting the right tools—like the USLC™ column set—in your method development toolbox means maximum alternate selectivity and peak separation with minimal effort.

Acknowledgements

The authors gratefully acknowledge the contributions of Dr. Lloyd Snyder from LC Resources and Dr. Frank Dorman from The Pennsylvania State University. The authors also wish to thank the contributing team of researchers Randy Romesberg, Bruce Albright, Mike Wittrig, Brian Jones, and Vernon Bartlett.

References

[1] L.R. Snyder, J.W. Dolan, P.W. Carr, The Hydrophobic-Subtraction Model of Reversed-Phase Column Selectivity, J. Chromatogr. A 1060 (2004) 77.

[2] U.D. Neue, J.E. O'Gara, A. Mendez, Selectivity in Reversed-Phase Separations Influence of the Stationary Phase, J. Chromatogr. A 1127 (2006) 161.

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Using Thermal Desorption to Enhance Aroma Profiling by GC/MS

Lower Detection Limits with Latest Technology

By Irene DeGraff, Product Marketing Manager, Lara Kelly, Markes International, and Liz Woolfenden, Markes International

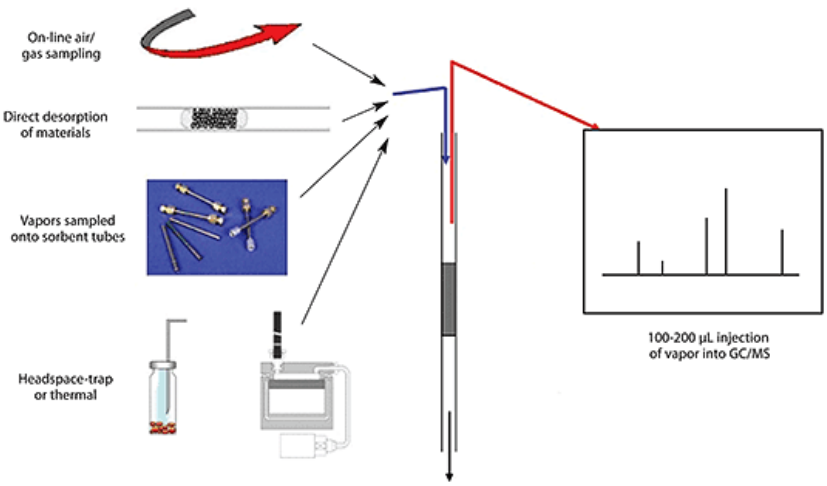
- Accommodates a wide range of sampling methods.
- Allows sample re-collection, for repeat analysis and result verification.
- Eliminates extraction solvents, purges volatile interferences, and concentrates sample vapors, for enhanced low-level detection.

Flavor and fragrance profiling by GC/MS presents significant analytical challenges, as profiles typically comprise hundreds of volatile organic compounds (VOCs), often with the lowest concentration analytes having the most profound effects on perceived aroma. Conventional sample preparation methods (solvent extraction, steam distillation, etc.) do not meet sensitivity requirements and often distort the vapor profile so that it is not representative of what the consumer experiences. Recently, thermal desorption (TD) has emerged as a useful complement to GC/MS, enabling more aroma profiling applications to be carried out using quantitative, automatic instrumentation. TD combines automated sample preparation with selective analyte enrichment, allowing VOCs to be injected into the GC/MS as a narrow concentrated band, free of most or all sample matrix effects.

Many Sampling Options, No Extraction Interferences

One of the strengths of thermal desorption for food, flavor, and fragrance profiling is that it offers a versatile range of sampling methodologies including sorbent tubes/traps, on-line sampling, direct desorption, and off-line thermal extraction (dynamic headspace) sampling. Whichever of these approaches is used, the compounds of interest are separated from the sample matrix and focused on a small, electrically-cooled sorbent trap (Figure 1). This focusing trap is subsequently desorbed by heating it rapidly in a reverse flow of carrier gas causing the VOCs to be injected into the GC/MS system as a narrow band of vapor. Since samples are extracted directly into the GC carrier gas stream, no manual sample preparation is required and the problems associated with solvents—masking of peaks of interest, loss of volatiles, and variable extraction efficiency—are eliminated.

Figure 1: Thermal desorption is compatible with a wide range of sampling strategies.

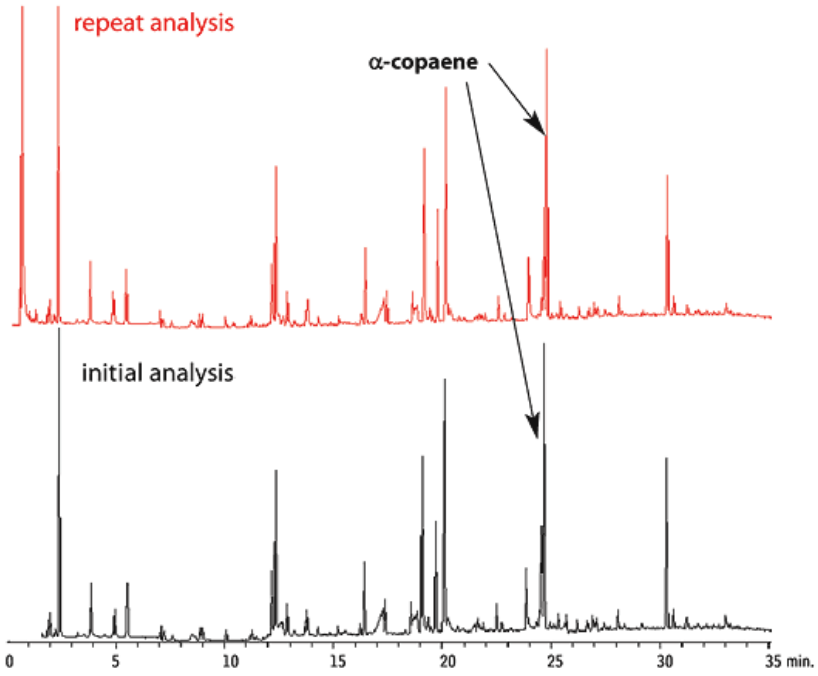


Lower Detection Limits and Repeat Analysis

The latest TD systems use thin-walled quartz traps capable of heating at rates over 100°C/sec.,

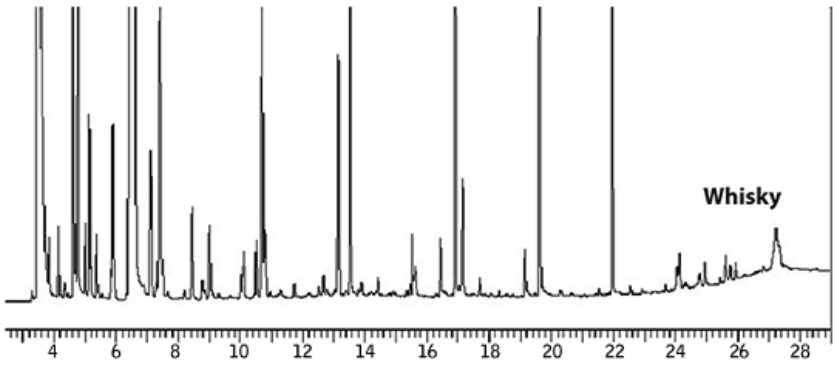
maximizing desorption efficiency and lowering detection limits. They also incorporate split re-collection for repeat analysis and simple validation of recovery (Figure 2) through the analytical system. Newer thermal desorption systems are also capable of transferring the vapor profile constituents into the GC capillary column in volumes of carrier gas as low as 100 µL. This means that significant concentration enhancement factors can be achieved—typically from 103 to 106—depending on the number of concentration/desorption steps. TD also allows volatile interferences such as water and ethanol to be purged to vent prior to analysis, making it easier to discriminate between samples according to the key olfactory components (Figure 3).

Figure 2: Verify initial results by analyzing re-collected samples.



Analysis of headspace collected above boiling genetically-modified potatoes. Repeat analysis of the recollected sample demonstrated excellent recovery of reactive monoterpenes, such as α-copaene.

Figure 3: Thermal desorption allows selective elimination of water and >99% of ethanol vapor, enhancing the determination of key olfactory components.



Analysis of whisky headspace by GC/FID.

Summary

Thermal desorption offers an automatic, high-sensitivity alternative to conventional liquid extraction methods for aroma profiling by GC/MS. It allows vapor profile constituents to be cleanly separated from the sample matrix and facilitates selective purging of volatile interferences in many cases. This helps to ensure that the vapor profile analyzed is most representative of the aroma perceived by consumers and

that key olfactory compounds can be identified and measured at the lowest levels possible.

RELATED SEARCHES

thermal desorption, *TD*, *aroma profiling*, *vapor profile*, *VOCs*, *monoterpenes*, *α-copaene*, *headspace*

RESTEK Pure Chromatography
Restek Corporation, U.S., 110 Benner Circle, Bellefonte, PA 16823

trans Fat: Resolving *cis* and *trans* FAME Isomers by GC

By Julie Kowalski, Innovations Chemist

- Highly polar Rt-2560 column resolves individual *cis* and *trans* FAME isomers.
- Analytical reference mixes for quantifying FAMEs in foods and dietary supplements.
- Use column and reference mixes to meet new *trans* fat labeling regulations.

Concern over the detrimental effects of diets high in *trans* fats has prompted the US Food and Drug Administration (FDA) to require *trans* fat content to be reported separately on food labels after January 2006. The FDA estimates that by 2009 this rule will save $900 million to $1.8 billion per year in medical costs and lost productivity. The monetary savings will far more than offset the FDA-estimated $140-250 million in one-time costs of determining amounts of *trans* fats, revising Nutrition Facts panels, and voluntarily reducing amounts of *trans* fats(1) that the food industry will incur to comply with the rule.

The highly polar Rt-2560 biscyanopropyl stationary phase has the selectivity needed for resolving *cis* and *trans* FAME isomers to comply with the FDA guidelines. Individual *cis* and *trans* isomers are resolved on a 100-meter Rt-2560 GC column (cat.# 13199), making this the column of choice for analyzing partially hydrogenated fats. The *trans* isomers elute before the *cis* isomers (Figure 1), a reverse of the elution order on Carbowax®-based phases such as FAMEWAX™ or Rtx®-Wax. AOAC method 996.06 (2) specifies the determination of total fat content based on the fatty acid content after conversion of the fatty acids to the methyl esters, and is the accepted analytical method for determining total fat content for nutritional labeling. A 100-meter Rt-2560 column meets the requirements of this procedure, and also allows quantification of the total *trans* fat content.

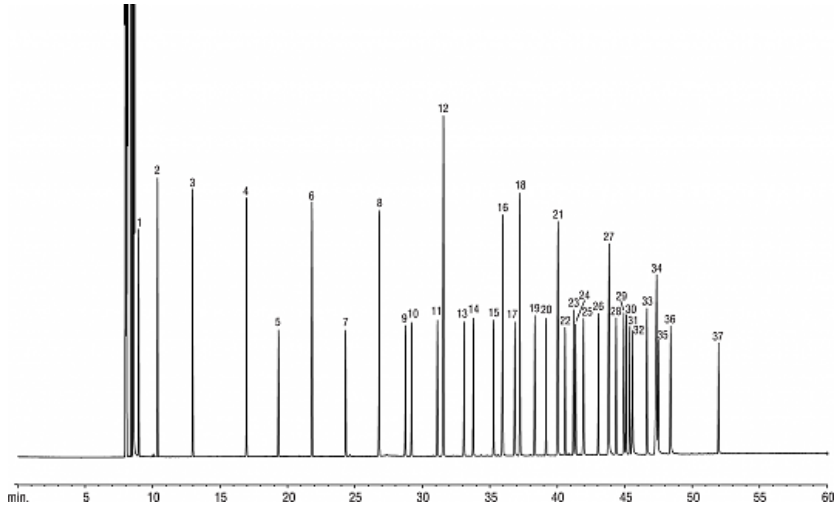
To calibrate the GC system for these assays, we recommend a carefully formulated FAME mixture, such as our 37-component Food Industry FAME Mix (cat.# 35077, Figure 1) or our 28-component NLEA FAME Mix (cat.# 35078). Each of these mixes includes a gravimetric certificate of analysis to help ensure accurate quantification. To ensure correct identifications of individual C18:1 *cis* or *trans* isomers, use our *cis/trans* FAME Mix (cat.# 35079), as shown in Figure 2.

An Rt-2560 column is the column of choice when determining *trans* fat content and total fat content in food products. Whatever your fatty acid analysis requirements, Restek can provide the consistent-performance analytical columns and reference materials that will help you to accurately characterize your materials.

Figure 1 Food Industry FAME Mix (cat.# 35077) resolved on an Rt-2560 column.

Peaks	% in Mix
1. C4:0 Methyl butyrate	4.0
2. C6:0 Methyl hexanoate	4.0
3. C8:0 Methyl octanoate	4.0
4. C10:0 Methyl decanoate	4.0
5. C11:0 Methyl undecanoate	2.0
6. C12:0 Methyl laurate	4.0
7. C13:0 Methyl tridecanoate	2.0
8. C14:0 Methyl myristate	4.0
9. C14:1 Methyl myristoleate (*cis*-9)	2.0
10. C15:0 Methyl pentadecanoate	2.0
11. C15:1 Methyl pentadecanoate (*cis*-10)	2.0
12. C16:0 Methyl palmitate	6.0
13. C16:1 Methyl palmitoleate (*cis*-9)	2.0
14. C17:0 Methyl heptadecanoate	2.0
15. C17:1 Methyl heptadecenoate (*cis*-10)	2.0
16. C18:0 Methyl stearate	4.0
17. C18:1 Methyl elaidate (*trans*-9)	2.0
18. C18:1 Methyl oleate (*cis*-9)	4.0
19. C18:2 Methyl linoleaidate (*trans*-9,12)	2.0
20. C18:2 Methyl linoleate (*cis*-9,12)	2.0
21. C20:0 Methyl arachidate	4.0
22. C18:3 Methyl γ-linolenate (*cis*-6,9,12)	2.0
23. C20:1 Methyl eicosenoate (*cis*-11)	2.0
24. C18:3 Methyl linolenate (*cis*-9,12,15)	2.0
25. C21:0 Methyl heneicosanoate	2.0
26. C20:2 Methyl eicosadienoate (*cis*-11,14)	2.0
27. C22:0 Methyl behenate	4.0
28. C20:3 Methyl eicosatrienoate (*cis*-8,11,14)	2.0
29. C22:1 Methyl erucate (*cis*-13)	2.0
30. C20:3 Methyl eicosatrienoate (*cis*-11,14,17)	2.0
31. C20:4 Methyl arachidonate (*cis*-5,8,11,14)	2.0
32. C23:0 Methyl tricosanoate	2.0
33. C22:2 Methyl docosadienoate (*cis*-13,16)	2.0
34. C24:0 Methyl lignocerate	4.0
35. C20:5 Methyl eicosapentaenoate (*cis*-5,8,11,14,17)	2.0
36. C24:1 Methyl nervonate (*cis*-15)	2.0

37.	C22:6 Methyl docosahexaenoate (*cis*-4,7,10,13,16,19)	2.0

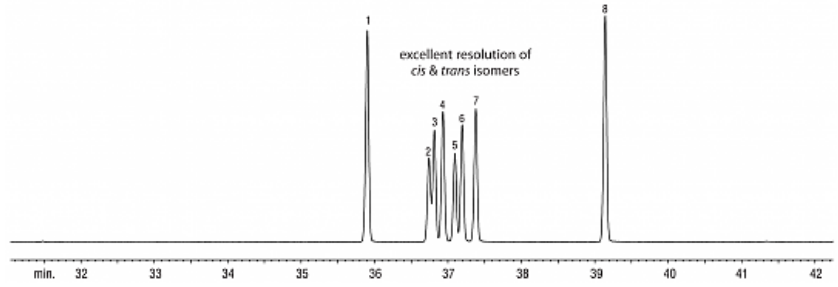


GC_FF00649

Column	Rt®-2560, 100 m, 0.25 mm ID, 0.20 µm (cat.# 13199)
Sample	Food industry FAME mix (cat.# 35077)
Diluent:	Methylene chloride
Conc.:	30 mg/mL total FAMEs
Injection	
Inj. Vol.:	2.0 µL split (split ratio 200:1)
Liner:	Splitless (4 mm ID) (cat.# 20814)
Inj. Temp.:	225 °C
Oven	
Oven Temp.:	100 °C (hold 4 min) to 240 °C at 3 °C/min (hold 10 min)
Carrier Gas	H_2, constant flow
Flow Rate:	1.2 mL/min
Detector	FID @ 250 °C

Figure 2 An Rt-2560 column resolves *cis* C18 FAME isomers from *trans* C18 isomers.

Peaks	Conc. (wt.%)	Peaks	Conc. (wt.%)
1. C18:0 methyl stearate	20.0	5. C18:1 methyl petroselinate (*cis*-6)	8.0
2. C18:1 methyl petroselaidate (*trans*-6)	8.0	6. C18:1 methyl oleate (*cis*-9)	10.0
3. C18:1 methyl elaidate (*trans*-9)	10.0	7. C18:1 methyl vaccenate (*cis*-11)	12.0
4. C18:1 methyl transvaccenate (*trans*-11)	12.0	8. C18:2 methyl linoleate (*cis*-9,12)	20.0



GC_FF00652

Column	Rt®-2560, 100 m, 0.25 mm ID, 0.20 µm (cat.# 13199)
Sample	cis/trans FAME mix (cat.# 35079)
Diluent:	Methylene chloride
Conc.:	10 mg/mL total FAMEs
Injection	
Inj. Vol.:	1 µL split (split ratio 20:1)
Liner:	Splitless (4 mm ID) (cat.# 20814)
Inj. Temp.:	225 °C
Oven	
Oven Temp.:	100 °C (hold 4 min) to 240 °C at 3 °C/min (hold 10 min)
Carrier Gas	H_2, constant flow
Flow Rate:	1.2 mL/min
Detector	FID @ 250 °C

References

1. www.cfsan.fda.gov/~dms/qatrans2.html
2. *Official Methods of Analysis, 17th ed.*, AOAC International, 2000.

RELATED SEARCHES

Rt-2560, *FAMEs*, *trans fat*, *biscyanopropyl*, *FDA*, *Carbowax*, *AOAC method 996.06*, *AOAC 996.06*, *dietary supplements*

Thermal Desorption: A Practical Applications Guide

IV. Food, Flavour, Fragrance & Odour Profiling

MARKES international

Introduction to Markes International Ltd.

Formed in 1997, Markes International Ltd. is one of the world's leading suppliers of thermal desorption (TD) equipment for monitoring trace toxic and odorous chemicals in air, gas and materials. Serving fast growing markets from environmental health and safety to materials testing and from food / flavour / fragrance to defence / forensic, Markes' global customer base includes major industry, government agencies, academia and the service laboratory sector.

Markes has introduced several highly successful brands of TD instruments to the market including: **UNITY™** – a universal TD platform for single tubes, the 100-tube **ULTRA™** TD autosampler, the **Air Server™** interface for canisters and on-line sampling, the **µ-CTE™** Micro-Chamber / Thermal Extractor for materials testing, the **TT24-7™** for continuous on-line monitoring and the **TC-20™** multi-tube conditioner.

Markes International also supplies a wide range of sampling accessories and consumables for all TD application areas.

What is TD?

Since the early 1980s, thermal desorption has provided the ultimate versatile sample introduction technology for GC / GC-MS. It combines selective concentration enhancement with direct extraction into the carrier gas and efficient transfer / injection all in one fully automated and labour-saving package.

Markes International Ltd., UK headquarters

Overview

Thermal desorption is now recognised as the technique of choice for environmental air monitoring and occupational health & safety. Relevant standard methods include: ISO/EN 16017, EN 14662 (parts 1 & 4), ASTM D6196, US EPA TO-17 and NIOSH 2549. Related applications include monitoring chemical warfare agents (CWA) in demilitarisation / destruction facilities & civilian locations (counter-terrorism).

TD is also routinely used for monitoring volatile and semi-volatile organic compounds (SVOC) in products and materials. Examples include residual solvents in packaging & pharmaceuticals, materials emissions testing and food / flavour / fragrance profiling.

This publication presents several of the real world applications of TD for measuring (semi) volatiles in food, flavour, fragrance and odours. Accompanying publications cover the applications areas of:

- Residual volatiles and materials emissions testing
- Defence & forensic
- Environmental monitoring and occupational health & safety

Applications

- Fragrance profiling of ingredients in toiletries and consumer products
- Identification of key olfactory components
- Characterisation / sourcing of natural products
- Odour profiling for potable spirits
- Quantitation of volatile components in dried foodstuffs
- Off-odour / taint analysis
- Biology / crop research
- Flavour profiling of GM foods

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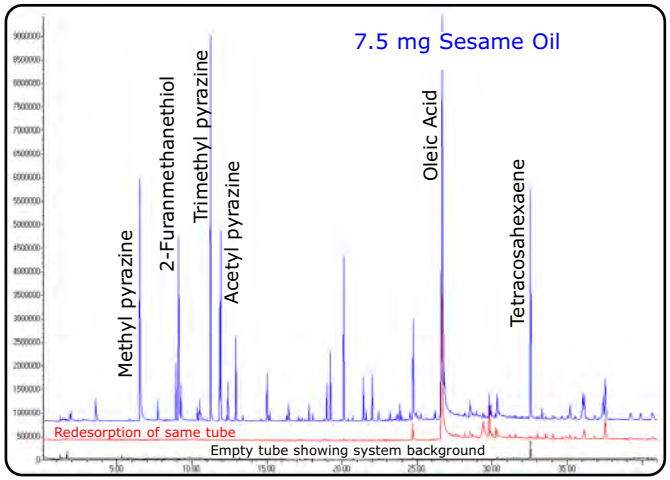
E: enquiries@markes.com **W:** www.markes.com

MARKES international

Profiling natural oils



Direct desorption of sesame oil sample, followed by a second desorption of the tube to illustrate efficient recovery of the components of interest in a single run

Background:

Detailed analysis of natural oils (*e.g.* sesame oil) may be required for several reasons; to identify key olfactory components, to characterise and source the material, and to identify oxidation products or other potential causes of taint. Traditionally this application has been carried out using multi-step liquid extraction or steam distillation with GC-MS analysis, but such procedures are lengthy, manual and inefficient. Direct thermal desorption/extraction of the oil using either the TD tube itself or a Markes Micro-Chamber / Thermal Extractor (µ-CTE) device, followed by TD-GC-MS analysis is a more efficient alternative.

TD conditions:

Sampling: Either incubate the oil sample in a µ-CTE chamber at 80 - 100ºC with vapour collection on Tenax TA™ tubes (gas flow ~100 ml/min) or load a few mg of oil onto a glass wool plug behind a 1 cm bed of Tenax in a standard TD tube.

Desorption: 10 mins at 300ºC

Cold trap: Tenax

Split ratio: >100:1

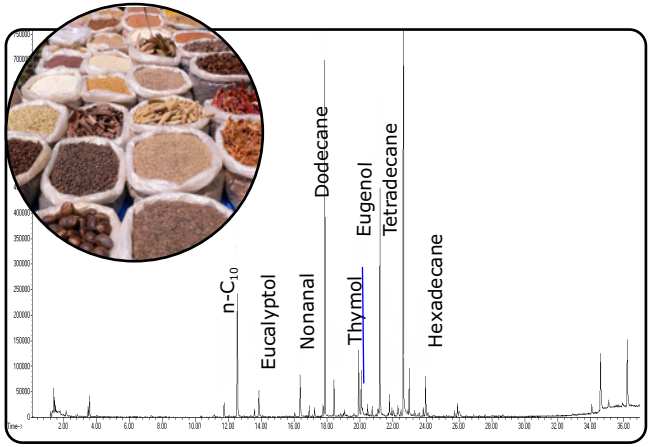
Analysis: GC-MS (SCAN)

Typical analytes:

Methyl pyrazines, fatty acids and ethyl vanillin

Concentration: Low to high ppm

Direct desorption of volatiles from dried foodstuffs



Direct desorption of dried animal-feed pellets weighed into an empty glass tube

Typical analytes:
Carvacrol, cineole, thymol, eugenol and hydrocarbons

Concentration: Sub to low ppm

Background:

Direct desorption of homogeneous dried foods provides a high sensitivity and labour saving alternative to solvent extraction and allows analysis of a wider volatility range of components than equilibrium headspace. Foodstuffs compatible with this approach include:

- Ground spices
- Freeze-dried products such as ground or instant coffee
- Animal feed pellets

Typical TD-GC analytical conditions:

Sampling: 100–200 mg weighed into empty glass tube or PTFE liner

TD system: ULTRA-UNITY

Desorption: 10 mins at 80°C

Trap: Quartz wool / Tenax

Split: ~25:1 split during trap desorption only

Analysis: GC-MS (SCAN)

Reference: TDTS23 Utilising the UNITY method development mode to analyse dried foodstuffs

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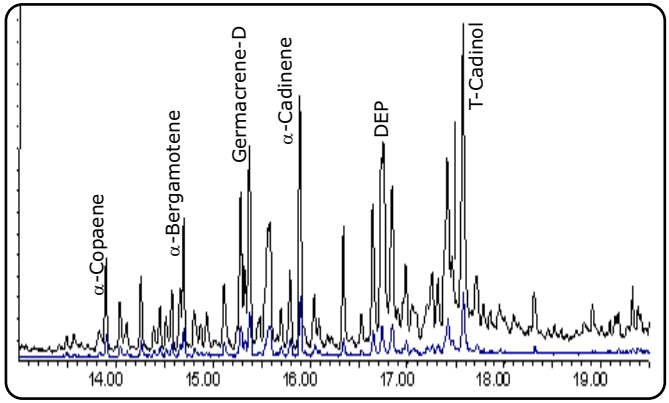
Natural products: Fragrance profiling



Vapours extracted from a leaf sample. Direct desorption (blue trace) followed by repeat analysis of re-collected sample (black trace) run with lower split ratio to enhance sensitivity

Typical analytes:

Range of terpenoid compounds, including:
α-Cedrene, α-Cadinene and T-Cadinol

Concentration: Sub to low ppm

Background:

Markes TD systems have an inert flow path that can be set at low temperatures, which makes them ideal for the direct desorption of labile volatiles such as terpenes and sulphur compounds. SecureTD-Q™ (*i.e.* quantitative re-collection of all split flow) facilitates repeat analysis of a sample under the same or different conditions (*e.g.* at a lower split setting, as shown) to demonstrate quantitative recovery through the system and to allow detailed analysis of minor components.

Typical TD-GC analytical conditions:

Sampling: ~100 mg of leaf sample weighed into an empty glass tube or PTFE liner secured with quartz wool

Re-collection on Tenax / UniCarb™ Silcosteel™ tubes

TD system: UltrA-UNITY

Desorption: 10 mins at 80°C

Trap: Sulphur trap

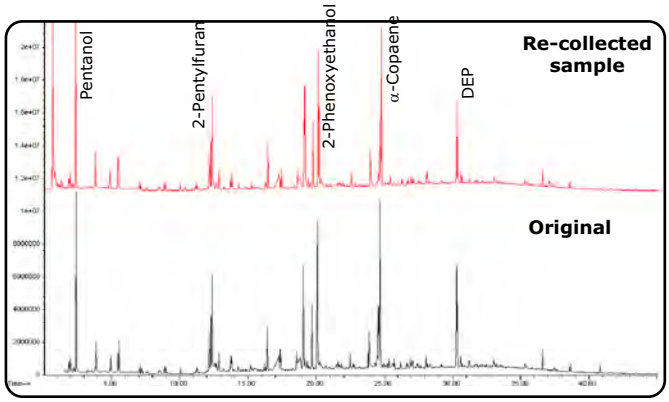
Flow path: 80°C to 150°C depending on target compounds

Split: ~25:1 & repeat analysis at 5:1 split.

Analysis: GC-MS (SCAN)

Reference: Markes brochure on TD validation featuring SecureTD-Q

Flavour profiling new crop varieties



TD-GC-MS analysis of volatiles from boiling potatoes using SecureTD-Q: Original sample and re-collected sample. Identical chromatographic profiles show recovery of labile analytes (*e.g.* terpenoids)

Analytes:

Diethyl phthalate, n-butyl butyrate, α-copaene, 2-(2-butoxyethoxy)-ethanol, 2-phenoxyethanol, decanal, octanoic acid, 2-ethylhexanoic acid, nonanal, 2-pentylfuran, pentanol, and hexanal

Concentration: Sub to low ppm

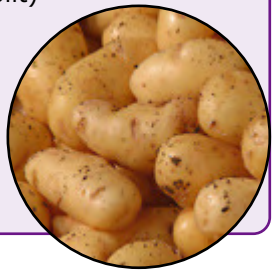
Background:

Development of new food crop species (*e.g.* genetic modification to aid pest resistance or to boost growth in arid areas) requires tests of the odour profile to make sure that the flavour is enhanced, or at least remains acceptable, in the new variety.

In the case of bulk, inhomogeneous materials like fresh fruit / vegetables, flavour profiles are best obtained by purging headspace volatiles from large (~ 1 kg) samples, cooked or raw, and collecting the vapours on tubes packed with Tenax sorbent. Tenax is completely hydrophobic so most water passes straight through during the vapour sampling process.

Typical TD-GC analytical conditions:

Sampling: 50 ml/min for 20 mins
Prepurge: 3 mins (to trap & split)
Desorption: 15 mins at 200°C
Trap: Tenax TA
Split flow: 20 ml/min
Analysis: GC-MS



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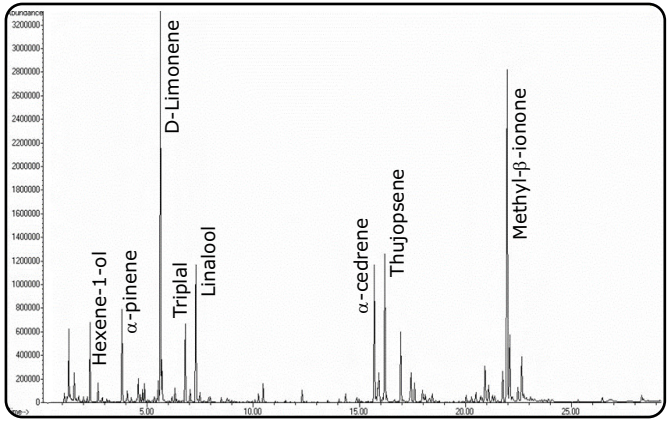
Fragrance profiling in consumer products



Headspace from sample of fabric conditioner

Analytes:

Isopropyl alcohol	α-Pinene	β-Pinene
α-Longipinene	Hexene-1-ol	Camphor
Methyl-β-ionone	γ-Terpinene	Myrcene
D-limonene	Triplal 1	Linalool
Hydroxy citronellal	α-Cedrene	γ-Muurolene
Thujopsene	β-Ionone	α-Chamigrene
Cyclamen aldehyde	α-Cedrol	α-Longipinene

Concentrations: ppm in headspace

Background:

Fragrance plays a major part in market acceptance and consumer satisfaction for products such as soaps & other toiletries, air fresheners and domestic cleaning materials.

TD provides a versatile, labour-saving and automated tool for GC-MS analysis of the fragrance profile of consumer products offering numerous sample handling options:

- Direct, in-tube desorption
- Dynamic purging of headspace vapours with on-line analysis
- Dynamic purging of headspace vapours onto sorbent tubes with off-line analysis

TD allows selective elimination of potential interferences such as water and some solvents thus simplifying fragrance analysis.

Typical TD-GC analytical conditions:

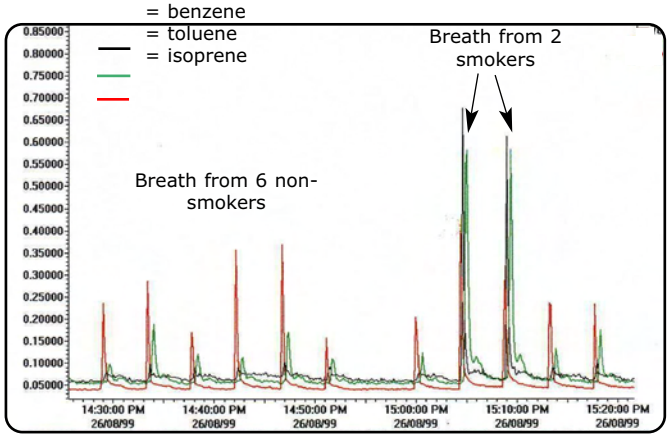
Sampling: ~200 ml headpace sampled onto Tenax tubes

Trap: U-T2GPH

Split flow: 30 ml/min during tube and trap desorption

Analysis: GC-MS

Halitosis – bad breath



Rapid TD-MS analysis of benzene and other hydrocarbons in the breath of smokers & non smokers

Typical analytes:
Hydrocarbons, oxygenates (esters, ketones alcohols, *etc.*) and other odorous VOCs

Concentrations: Low ppb

Background:

We are what we eat – and sometimes it comes back to haunt us in the shape of bad breath! Halitosis can also be caused by bacterial infections of the mouth / throat, some disease states and smoking.

The Bio-VOC™ Breath sampler from Markes International collects breath samples from the mouth & bronchial passages or from the alveoli (end-tidal air) and transfers them to sorbent tubes for subsequent analysis by TD-GC-MS. Applications of the Bio-VOC include biological monitoring of environmental / workplace exposure and disease diagnosis as well as breath odours.

Typical analytical conditions:

Sampling: Breath exhaled into Bio-VOC sampler and transferred to Tenax tube or Tenax / Carbograph 1TD focusing trap

Desorption: 280ºC for 10 mins

Trap: U-T2GPH

Trap conditions: +30ºC to 320ºC for 3 mins

Analysis: TD-GC-MS or TD with process-MS

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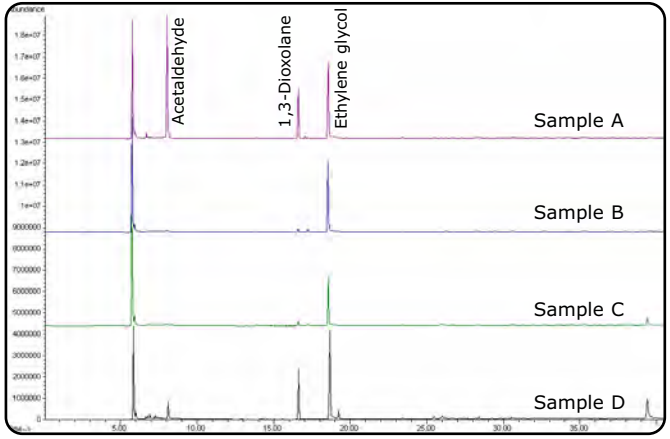
Taint and off-odour



Direct desorption of ground polyethylene terephthalate (PET) polymer to identify trace level VOCs contributing to taint. Comparison of PET polymers used in manfacture of soft drinks bottles

Typical analytes:
Residual monomers (*e.g.* acetaldehyde), pyrazoles and solvents

Concentrations: Sub to low ppm

Background:

Product taint can be introduced *via*:

- Odorous base materials
- Issues with the fragrance itself
- Packaging – everything from printed film to wood pallets
- Warehousing

Gentle direct desorption of the tainted product, *via* TD tubes, micro-chambers or bulk sample vessels and comparison with equivalent data from a control sample allows the taint components to be identified. The source can then be tracked *via* direct desorption of packaging, pallet fragments, base materials, additives *etc.* and analysis of vapour profiles and warehouse air collected on sorbent tubes.

Typical analytical conditions for PET:

Sampling: 200 mg of ground polymer in an empty tube

Desorption: 160ºC for 10 mins

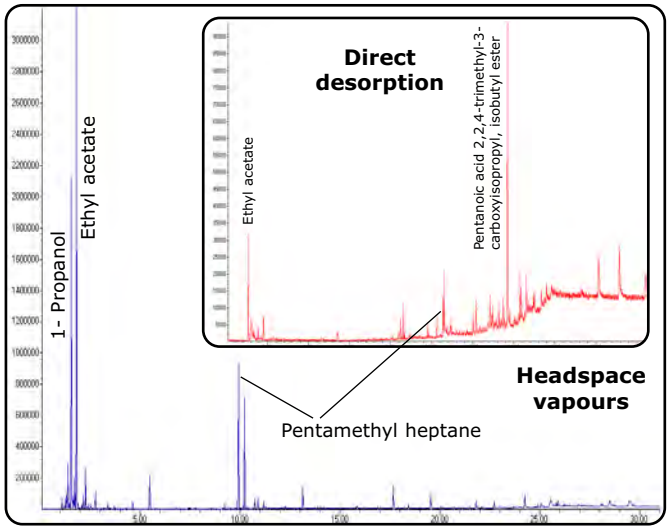
Trap: U-T6SUL

Trap conditions: -10ºC to 300ºC

Split: 40 ml/min during trap desorption

Analysis: GC-MS

Direct desorption of packaging



Direct desorption of residual solvents and semi-volatile additives from printed packaging film and sorbent tube sampling of headspace vapours from same

Typical analytes:

Alcohols, esters, ketones, alkanes and other odorous solvents

Background:

VOCs in food packaging can cause taint. In this example, TD was used to analyse printed biscuit wrappers in two ways:

- Direct desorption of the wrapper
- Desorption of Tenax TA tubes used to collect HS vapours from the sample

Note that headspace vapours from the packaging sample show high levels of volatile solvents which can migrate into fatty food stuffs, adversely affecting the taste.

Typical TD-GC conditions:

Sample: 10 x 5 cm area of film, rolled & inserted into an empty glass tube for direct desorption & 250 ml headspace sample drawn into a Tenax sorbent tube

TD system: UltrA-UNITY

Desorption: 10 mins at 60°C (direct TD) and 10 mins at 300°C (HS sample on Tenax tube)

Trap: Quartz wool, Tenax TA, Carbopack X™

Split: 30:1 during trap desorption

Analysis: GC-MS

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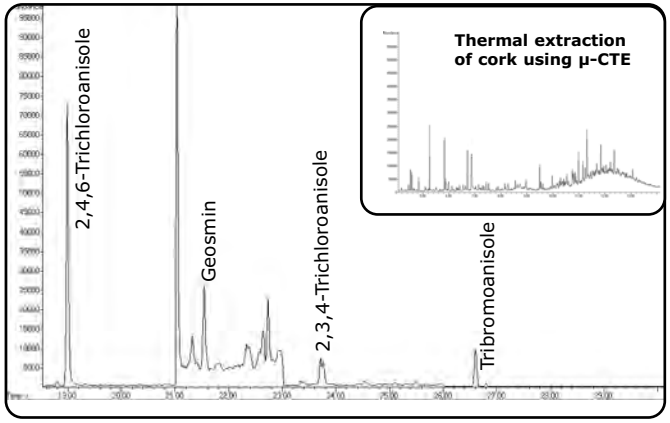
Trichloroanisoles in wine



TD analysis of 0.2 ng/L TCA and other odour compounds in the headspace of 1 L aqueous sample. Inset: Vapour profile from whole cork using µ-CTE

Analytes:

- 2,4,6-Trichloroanisole
- 2,3,4,6-Tetrachloroanisole
- 2,4,6-Tribromoanisole

Concentrations: Sub to low-ppb

Background:

A recent high-profile example of taint was linked to 2,4,6-trichloroanisole (TCA) in wine. TCA is produced from trichlorophenol by a microorganism that thrives in the production process of corks for wine bottles. This and other chemically similar analytes give the wine a mushroomy 'corked' aroma even at low concentrations (<5 ng/L).

The inert flow path of Markes TD systems facilitates TCA measurement in the headspace of aqueous samples at sub ng/L levels.

The Markes µ-CTE also facilitates direct thermal extraction of whole corks.

Typical TD-GC analytical conditions:

Sampling: On- or off-line sampling of headspace from 1 L aqueous samples at 60ºC onto Tenax trap. Whole cork incubated at 60ºC using µ-CTE with 70 ml/min flow of helium for 10 mins

Desorption: 280ºC for 10 mins

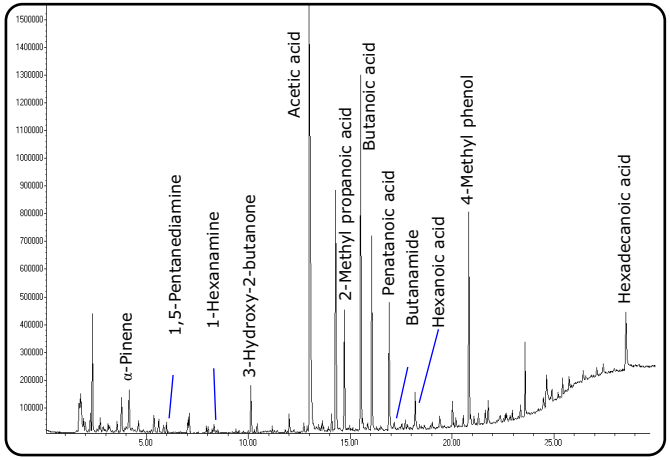
Trap: U-T2GPH

Trap conditions: -5ºC to 300ºC

Split flow: 30 ml/min

Analysis: GC-MS or GC-olfactometry

Odours from meat-processing



Chromatogram of odours from a swine facility

Background:

Thermal desorption is used extensively to monitor odours associated with meat processing. Applications include environmental / ambient-odour monitoring, product quality / flavour assessment, testing of animal odours (healthy and diseased) and at-line monitoring of production processes.

Typical TD-GC analytical conditions:

Sampling: 0.5-2 L vapour sampled onto Tenax / Carbograph 1TD tubes

Desorption: 300ºC for 5 mins then 320ºC for 5 mins

Trap: U-T2GPH

Trap conditions: +20ºC to 300ºC

Split: Low split during trap desorption only

Analysis: GC-MS

Chromatogram reproduced with the kind permission of APS Adamsen, of LugTek, Denmark - experts in odours from livestock production

Typical analytes:

Carboxylic acids, monoterpenes, phenolic compounds, amines and amides

Concentrations: ppb

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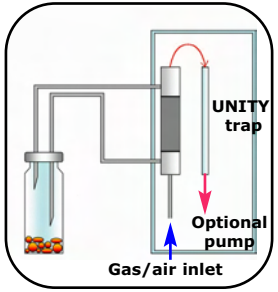
Multi-purpose Direct Inlet Accessory – For direct sampling / concentration of headspace vapours



The multi-purpose Direct Inlet Accessory (U-INLET) may be added to any manual UNITY thermal desorption platform to provide a simple and convenient mechanism for concentration of headspace vapours from a wide range of bulk sample containers.

This on-line approach allows vapours to be either pumped or swept through an inert, heated sampling line directly into the electrically-cooled focusing trap of UNITY without first being collected on a sorbent tube.

The UNITY-Direct Inlet system significantly improves the sensitivity of conventional headspace methods by allowing multi-stage extraction and concentration before analysis. The dynamic headspace approach also eliminates the need for equilibrium to be reached, thus reducing the time required for analysis.

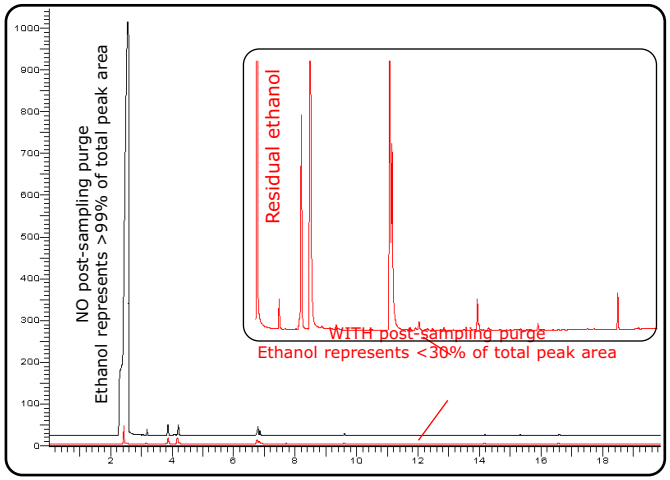
Sample vessel

The UNITY-Direct Inlet system is compatible with a wide range of sample vessels. It may be used for purging headspace vapours from smaller, sealed containers (such as reaction vessels or headspace vials) or for pumping air from open or compressible containers such as bell jars or Tedlar™ bags.

Key application areas include:

- Characterisation of VOC profiles from natural products and manufactured goods - food, flavour, fragrance analysis
- Monitoring emissions from living organisms - plants, microbes, fungi, insects, *etc.* as they change with time
- Monitoring malodours generated from food packaging (*e.g.* drink bottles)
- Off-odour / shelf-life testing
- Sampling from drinks / spirits, with the option of selectively purging the ethanol

Whisky: Aroma profiling



Purging of water and ethanol from whisky HS vapours allows selective concentration of key olfactory compounds – ketones, esters, *etc.*

Typical Analytes:

Ketones, aldehydes, esters
essential oils (*e.g.* juniper and coriander in gin)

Concentrations: Sub to low ppm

Background:

Thermal desorption facilitates detailed analysis of the flavour profile of potable spirit by allowing selective elimination of water and ethanol while key olfactory components – ketones, esters, essential oils, *etc.* – are quantitatively retained.

Headspace vapours are pumped / purged onto Tenax tubes under conditions which concentrate the target analytes while allowing most of the water, ethanol and other very volatile polar components to breakthrough. An example of whisky analysis, with and without selective elimination of water and ethanol, is shown opposite. Selective concentration of key olfactory components simplifies meaningful odour profiling.

Typical TD-GC analytical conditions:

Sampling: Sample placed in headspace vial at ~40°C. Connected to UNITY *via* Direct Heated Inlet Accessory

Sampling mode: Pulsed mode, 6 extractions of headspace

Trap: Tenax TA

Analysis: GC(-MS)

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Micro Chamber / Thermal Extractor (µ-CTE)

The µ-CTE offers a convenient approach for sampling volatiles from bulk samples at low to moderate temperatures.

The µ-CTE contains six (6) 28 mm deep x 45 mm diameter chambers into which samples are placed. A controlled flow of air or carrier gas is purged through all of the chambers simultaneously, sweeping the volatiles onto sorbent tubes attached to each chamber lid.

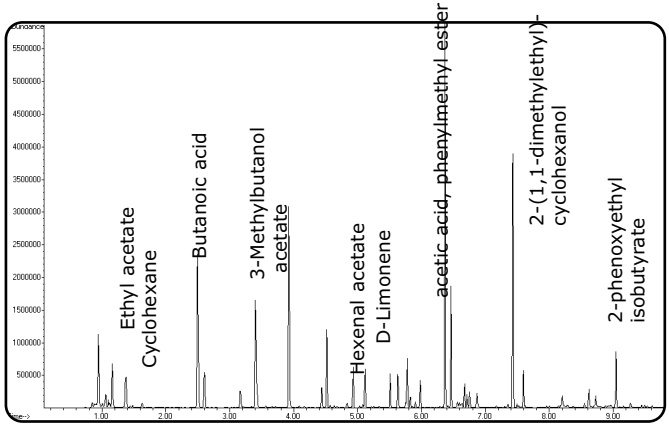
The µ-CTE can be heated from ambient to 120°C and is available with stainless steel or Silcosteel chambers. It is convenient for samples which are too inhomogeneous for direct desorption in empty tubes.

µ-CTE accessories are available to facilitate surface emissions testing and permeation studies (*e.g.* of packaging) as well as volatile analysis in bulk samples.

Key Applications include:

- Bulk sampling of volatiles from fruits, vegetables and other inhomogeneous foodstuffs
- Fragrance profiling of tobacco blends / substitutes
- Permeation testing of packaging
- Fragrance profiles from consumer products

Fragrance profiling of toiletries using the µ-CTE



Fragrance profile from shampoo obtained using the µ-CTE

Typical Analytes:
Esters, fatty acids, terpenes and solvents

Concentrations: ppm

Background:

The Markes µ-CTE is ideal for fragrance profiling of aqueous solutions and emulsions such as shampoo. Six replicate or different shampoo samples can be measured into individual micro-chambers, incubated at low temperatures (*e.g.* 30-40ºC) and the fragrance components purged onto attached sorbent tubes. Use of hydrophobic sorbents in the tubes allows quantitative retention of organic compounds of interest while water is purged to vent.

The µ-CTE is compatible with air or inert carrier gas to allow analysis of product fragrance under inert or oxygenating conditions.

Typical TD-GC analytical conditions:

Sampling: 5 ml shampoo incubated in the µ-CTE at 30ºC. Vapours swept onto Tenax tubes in a 70 ml/min flow of helium for 5 mins

Desorption: 280ºC for 10 mins

Trap: U-T2GPH

Trap conditions: 30ºC to 300ºC for 3 mins

Split flow: 30 ml/min during trap desorption

Analysis: GC-MS

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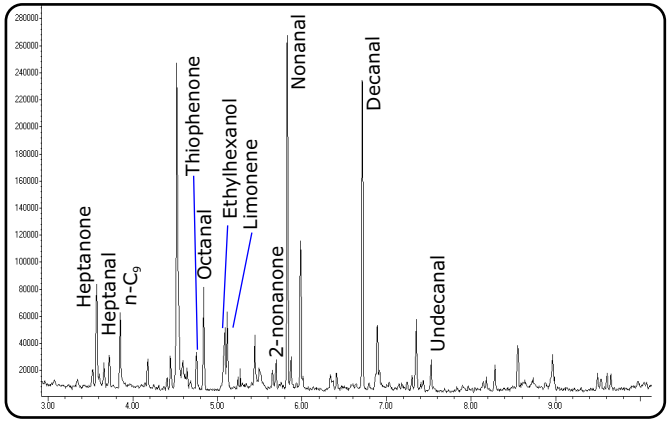
Flavour profiling of dairy products



Flavour profile obtained from natural greek yoghurt

Typical analytes:

Lactones from octalactone to tetradecalactone, fatty acids from C_6 to C_{16}, C_5 to C_{15} ketones, C_6 to C_{14} aldehydes, esters from ethyl butanoate to ethyl decanoate and C_{12} to C_{20} hydrocarbons

Concentrations: Sub to low-ppm in headspace

Background:

Milk and related dairy products have a complex aroma profile comprising fatty acids, lactones, ketones, aldehydes, esters, and hydrocarbons.

Several millilitres of milk or yoghurt can be conveniently measured into stainless or Silcosteeled micro-chambers and incubated at temperatures between ambient and 80ºC under a flow of pure air or inert carrier gas. Emitted vapours are collected on Tenax tubes connected to the exhaust of each micro-chamber. Water is selectively eliminated.

Typical TD-GC analytical conditions:

Sampling: 10 ml (g) yoghurt incubated at 70ºC in the µ-CTE, swept onto Tenax tubes in a 70 ml/min flow of helium for 10 mins

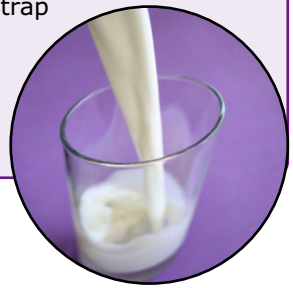
Desorption: 280ºC for 10 mins

Trap: U-T2GPH

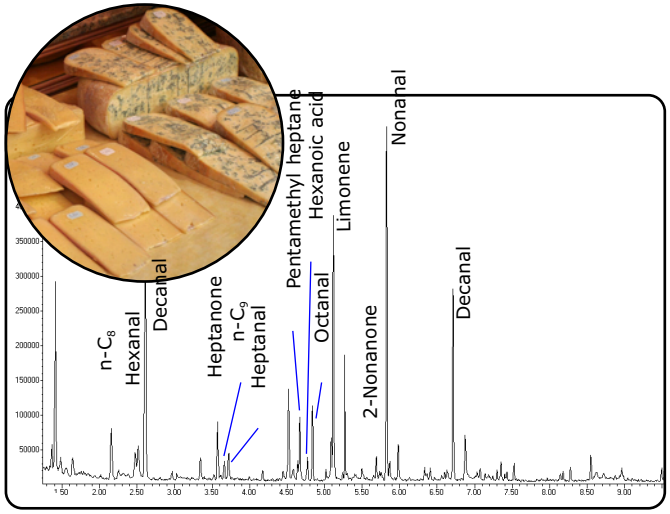
Trap conditions: 30ºC to 300ºC for 3 mins

Split flow: 30 ml/min during trap desorption

Analysis: GC-MS



Flavour profiling of cheese



Aroma / flavour profiling of cheese using the µ-CTE

Typical analytes:
Ketones, lactones, pyrazines, sulphurous compounds, esters, aldehydes, free fatty acids and alcohols

Concentrations: Sub to low-ppm in headspace

Background:

There is extensive research into the complex aroma profiles of different types of cheese. For example, over fifty aroma-active compounds have been detected in cheddar cheeses.

Thermal desorption / dynamic headspace offers an automated and versatile alternative to multi-step liquid extraction and vacuum distillation. Small cubes of cheese or grated cheese slurries mixed with distilled water can be incubated (*e.g.* using the Markes µ-CTE) purged with inert gas or pure air and the vapours collected using on- or off-line sorbent traps. Subsequent analysis is by TD-GC-MS or TD-GC with olfactometry.

Typical TD-GC analytical conditions:

Sampling: 2 g grated cheese mixed with 5 ml warm water and incubated in the µ-CTE. Vapours swept onto Tenax tubes in a 70 ml/min flow of helium for 10 mins

Desorption: 280ºC for 10 mins

Trap: U-T2GPH

Trap conditions: 30ºC to 300ºC for 3 mins

Split flow: 30 ml/min during trap desorption

Analysis: GC-MS

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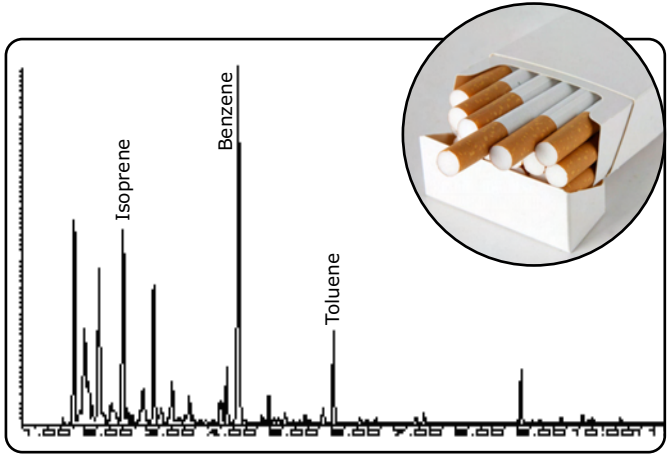
Applications for thermal desorption in the tobacco industry



VOC profile of tobacco smoke drawn through a cigarette filter, using a smoking machine

Analytes:
Benzene, toluene and isoprene

Concentrations: Sub- to low ppb levels in air. Higher levels in headspace vapours

Background:

In addition to sampling / analysis of environmental tobacco smoke (ETS), thermal desorption has extensive uses in the tobacco industry. Key applications include:

- Aroma profiling of tobacco / tobacco substitutes
- Monitoring filter efficiency by collecting vapours from smoking machines (see opposite)
- Tracking the cause of taint in batches of tobacco products

These applications are carried out using sorbent tube sampling, direct thermal desorption and sampling accessories such as the µ-CTE.

Typical analytical conditions:

Sampling: Multiple "puff" volumes taken into bag, then transferred onto sorbent tube or vapours sampled directly into tube

Desorption: 280ºC for 10 mins

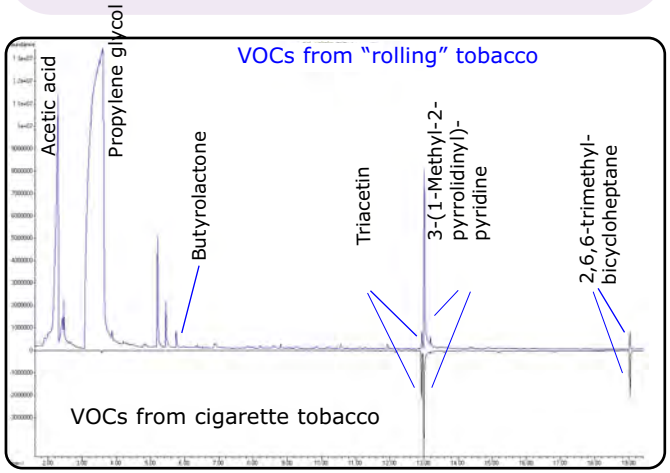
Trap: U-T2GPH, 30ºC to 300ºC for 3 mins

Split flow: 30 ml/min during trap desorption

Analysis: GC-MS

Reference: TDTS76 Applications of thermal desorption in the tobacco industry

Monitoring the aroma / flavour profile of tobacco using the µ-CTE



Vapour profiles from "rolling" tobacco (top) and cigarette tobacco (bottom) collected using a stainless steel micro-chamber at 50ºC

Typical analytes:
Glycols, triacetin, acetic acid, pyridines, hydrocarbons

Concentrations: Various

Background:

The Markes µ-CTE provides an ideal sampling accessory for inhomogeneous materials such as tobacco. Crumbled tobacco samples can be placed in Silcosteel micro-chambers, incubated at temperatures up to 120ºC and purged with air or inert carrier gas to sweep volatiles onto inert sorbent tubes. Subsequent analysis is *via* TD-GC-MS. The chromatograms opposite show comparative odour profiles from two types of tobacco.

The µ-CTE is similarly convenient for sampling whole cigarette filters (before or after smoking), cigarette paper and cigarette packaging materials.

Typical analytical conditions:

Sampling: 1 g of tobacco incubated in the µ-CTE at 50ºC. Vapours swept onto Silcosteel Tenax tubes in a 100 ml/min flow of helium for 10 mins
Desorption: 300ºC for 10 minutes
Trap: U-T2GPH
Trap conditions: -10ºC to 300ºC
Split: Double split, 300:1
Analysis: GC-MS (SCAN)

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Twin trap TT24-7 for continuous monitoring

Air Server for semi-continuous monitoring

Markes' continuous or semi-continuous TD systems for on-line monitoring of changing odour profiles

Background:

Versatile TD sampling accessories like the Markes Micro-Chamber / Thermal Extractor allow up to 6 product samples to be simultaneously incubated in a stream of air or inert gas for accelerated shelf-life tests and odour studies. Vapours are collected on attached sorbent tubes and analysed off-line using ULTRA-UNITY.

Custom made sample containers for bulk fresh or prepared foods – pizza, canned meat, *etc.* – can also be linked to off-line sorbent tubes or **monitored continuously** using either the TT24-7 or UNITY-Air Server / Direct Inlet. These on-line TD systems allow near real-time assessment of odour profiles as they change with time.

Typical on-line monitoring parameters:

TD system: TT24-7, UNITY-Direct Inlet or UNITY-Air Server

Sampling: 10-50 ml/min for 20 mins

Trap: Tenax or U-T2GPH at +30ºC

Trap desorption: 320ºC for 5 mins

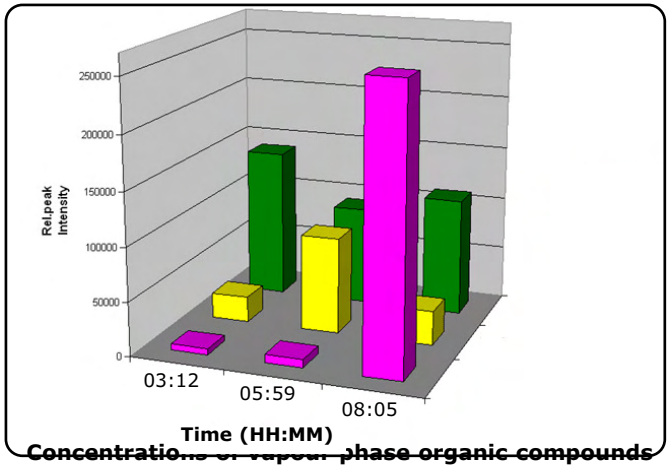
Analysis: GC-MS or GC-olfactometry

Typical Analytes:

Aldehydes, ketones, esters, sulphur and nitrogen containing compounds, fatty acids, *etc.*

Concentrations: ppb to ppm

On-line monitoring of fragrance profiles as they change with time



Concentrations of vapour phase organic compounds changing with time

Background:

Most natural (*e.g.* floral) fragrances and many of the fragrance profiles of consumer products, such as air fresheners, change with time and ambient conditions. Markes continuous and semi-continuous on-line monitoring vapour systems allow round-the-clock profiling of fragrance allowing changes to be tracked as a function of time and ambient conditions – temperature, humidity, sunlight intensity, *etc.*

Typical on-line monitoring parameters:

TD system: TT24-7, UNITY-Air Server, UNITY-Direct Inlet

Sampling: 10-50 ml/min for 20 mins

Trap: U-T2GPH

Trap conditions: -10ºC to 320ºC for 3 mins

Analysis: GC-MS

Typical Analytes:

Aldehydes, ketones, esters, sulphur and nitrogen containing compounds, fatty acids, *etc.*

Concentrations: ppb to ppm

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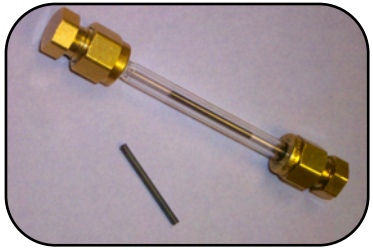
SPE-tD™ cartridges

Markes' SPE-tD cartridges offer a simple, convenient method for sampling less volatile impurities in aqueous samples – applications which would otherwise require manually-intensive extraction or distillation techniques before GC(-MS) analysis.



SPE-tD cartridges comprise a hollow tube, coated inside and out with polydimethyl siloxane (PDMS) for optimum capacity. The cartridge is placed into the aqueous sample and agitated. Volatile and semi-volatile organics in the sample, partition between the aqueous matrix and PDMS, reaching an equilibrium state over time. This allows semi-quantitative analysis of less volatile organics and direct comparison of organic impurity levels in two similar samples.

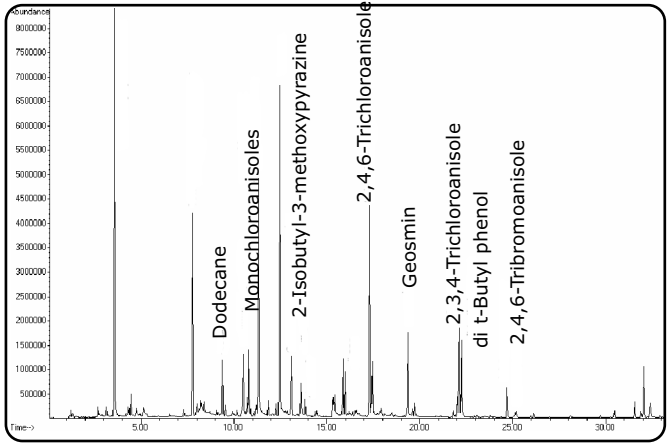
After equilibration, the SPE-tD cartridge is removed from the sample, rinsed in pure water to remove solid residues (if necessary) and placed into an empty TD tube. The cartridge is then dry purged with pure carrier gas, on- or off-line, prior to analysis by direct TD-GC-MS.

Solid phase extraction / TD methods provide a complementary sample preparation tool to automated headspace (HS) and purge-and-trap (P&T) techniques, which favour volatiles. Use of SPE-tD cartridges in combination with HS or P&T allows full characterisation of aqueous samples.

Key Applications include:

- Off-odours / taints in drinking water
- Semi-volatiles in processed fruit juices
- Profiling of hydrosols (aqueous fraction from steam distillation of natural oils)

SPE-tD extraction of organics from drinking water



Profile of sub-ppb level organics extracted from drinking water using the SPE-tD cartridge

Typical analytes:

Geosmin, methyl isoborneol, phenols and trichloroanisoles

Concentrations: Sub to low ppb

Background:

High capacity solid phase extraction using Markes SPE-tD cartridges provides a convenient approach to monitoring semi-volatile off-odour components in drinking water. SPE-tD cartridges used in combination with subsequent high sensitivity TD-GC-MS analysis offer trace detection limits (sub-ppb) and complement purge-and-trap / equilibrium headspace methods for volatiles.

The example opposite shows sub-ppb impurities absorbed by a SPE-tD cartridge from a 1 L sample of drinking water.

Typical TD-GC analytical conditions:

Sampling: SPE-tD cartridge placed into 1 L water sample and agitated for 2 hours

Desorption: 60ºC for 10 mins

Trap: U-T2GPH

Trap Conditions: 30ºC to 300ºC

Split flow: 10 ml/min during trap desorption

Analysis: GC-MS

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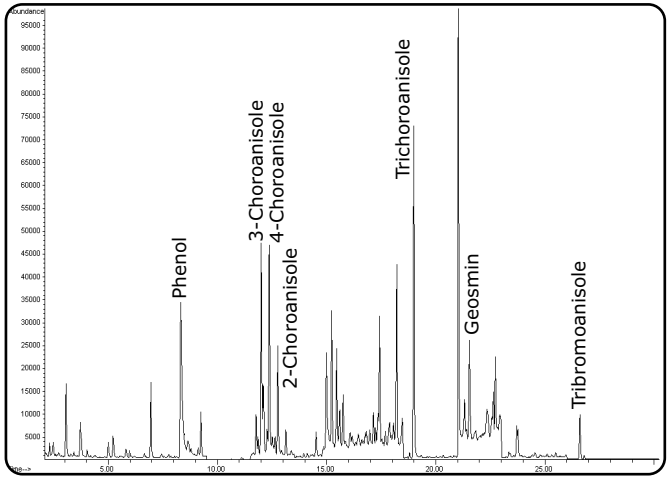
Drinking water: Pre-empting odour complaints



On-line monitoring of key odour components in the headspace of river water

Typical analytes:
Methyl i-borneol, chloroanisoles and geosmin

Concentration: 200 ppt in example shown

Background:

Much drinking water is sourced from natural rivers and streams. Continuous on-line monitoring of the headspace of river water, for unusually high levels of key odour components such as geosmin, can be used to prevent tainted water entering the drinking water supply and causing public concern.

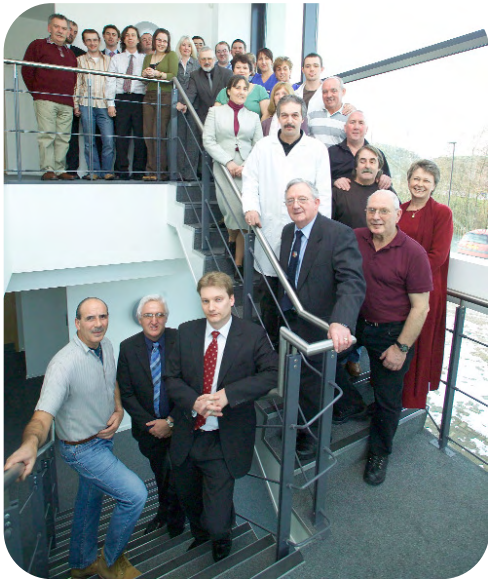
Analytical options include either UNITY-Air Server or the TT24-7 on-line TD systems operating continuously with GC-MS in unattended monitoring stations at strategically important points along the river system. Hourly data from multiple remote field monitoring stations can be sent to a central network hub by telemetry.

TD conditions:

Trap: Tenax TA / Carbograph 1TD
Heated inlet temp: ~70°C
Sampling flow rate / time: 50 ml/min; 15-20 mins
Post sampling purge time: 15 mins at 50 ml/min
Trapping temperature: 40°C
Desorption: 300°C for 5 mins
Flow path temperature: 200°C
Analysis: GC-MS

The Markes International advantage

- Markes is the market leader in TD
- Unparalleled reputation for product quality and reliability
- Excellence in technical and applications support
- For further information on Markes comprehensive range of instruments, sampling accessories and consumables please use one of the contact numbers / email address below or browse the web site



The Markes International team

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Stabilwax®-MS Columns

Thermally Stable, High Polarity GC Columns

for Flavor, Food, Fragrance,
Industrial Chemical, and Solvent Analyses

What's Inside...

NEW! Stabilwax®-MS Columns

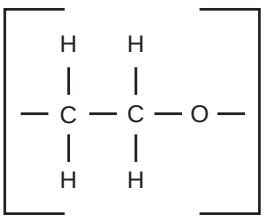
Stabilwax®-MS Features	Your Benefit
High thermal stability polyethylene glycol (PEG) stationary phase	Able to couple to MS detector Temperature range: 40 °C to 260 °C
Lower bleed than VF-WAXms	Lower detection limits
Ultra-clean, Restek-manufactured phase and bonding chemistries	Extraordinary inertness and stability against chemicals and high temperatures
Withstand repeated water injections with no phase loss or degradation	Longer column lifetime and solvent rinseable
Equivalent to USP G14, G15, G16, G20, and G39 phases	Ideal for polar analytes in foods, flavors, fragrances, industrial chemicals, and solvents

Challenges of Polar Compound Analysis by GC

One of the most widely used columns in gas chromatography is a polyethylene glycol (PEG) or "wax" phase. This unique column is highly polar compared to nonpolar methyl phases like Rxi®-1 columns (100% dimethyl polysiloxane) or Rxi®-5 columns (5% diphenyl/95% dimethyl polysiloxane) due to the presence of a polyethylene glycol backbone (Figure 1). The incorporation of the oxygen group in the backbone creates a phase with high selectivity for polar analytes such as alcohols, glycols, esters, and ethers. These compound classes are commonly found in pharmaceutical raw materials, alcoholic beverages, industrial chemicals, flavors and fragrances. A wax phase is capable of providing resolution of these compound classes that will not be achievable on nonpolar and intermediate polarity columns.

Due to phase structure, wax columns typically have lower maximum operating temperatures (240-250 °C) than nonpolar columns (e.g., max temp of an Rxi®-5ms column is 360 °C) and exhibit higher column bleed levels than silicone phases. Because of this, retention time shifting can occur on some wax columns due to the loss of stationary phase (column bleed) that occurs during GC oven temperature cycling. Wax phases are also susceptible to oxygen contamination and can degrade quickly if exposed to oxygen from a leak in the GC at high temperatures. Oxygen contamination is chromatographically seen as a high column baseline that cannot be decreased by column conditioning or maintenance. A Restek® electronic leak detector is the best way to ensure a leak-free system and long column lifetimes. See page 12 for details on this product and view the leak checking demo on our website for how to properly use an electronic leak detector.

Figure 1: The highly polar nature of the Stabilwax®-MS column makes it ideal for separating polar compounds found in food, flavors, fragrances, pharmaceutical raw materials, and industrial chemicals.



RESTEK

Over the past several years, benchtop mass spectrometer (MS) detectors have become the GC detector of choice since they provide high sensitivity, quantitative retention time data, and compound identification. GC-MS users have long wanted thermally stable polar phases to use with their MS systems to take advantage of their unique selectivity, without the worry of the column bleed seen on most wax columns.

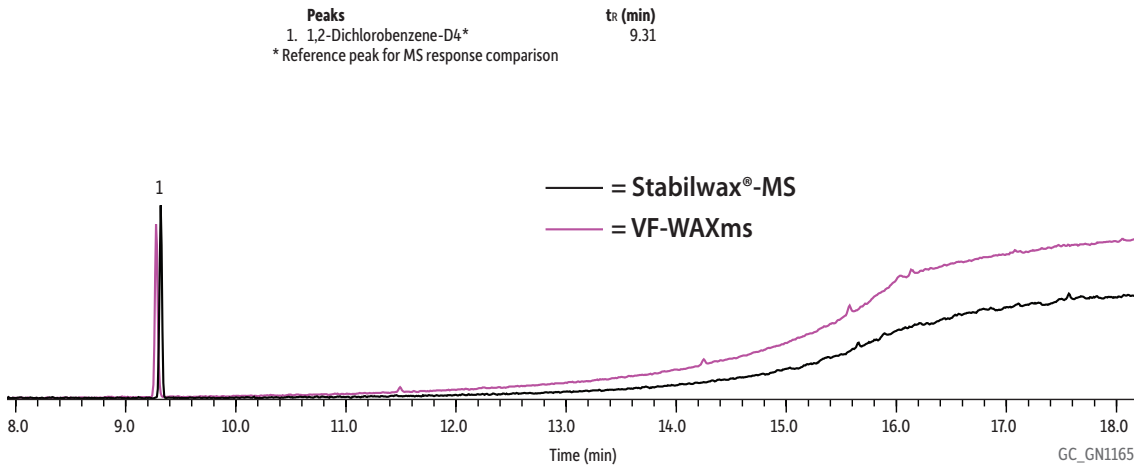
Stabilwax®-MS Columns: A Wax Phase Suitable for GC-MS

The new Stabilwax®-MS column from Restek meets a GC-MS user's challenges. The polar deactivated surface tightly binds the polyethylene glycol polymer to the fused silica tubing, resulting in a high maximum operating temperature (260 °C). This allows for faster elution of higher molecular weight compounds since the column can be taken to high temperatures. In addition, low bleed levels are ensured by strict quality testing that specifies maximum allowable bleed levels of 4.0 pA for 0.25 mm ID columns and 5.0 pA for 0.32 mm ID columns. When comparing actual bleed levels on a mass spectrometer, Stabilwax®-MS columns outperform the VF-WAXms column (Figure 2). When tested at the 250 °C temperature limit of the VF-WAXms column, less bleed is seen on the Stabilwax®-MS column.

When methods require trace analysis of polar compounds, the new **Stabilwax®-MS** column produces excellent sensitivity and low bleed levels.

The low bleed level of the Stabilwax®-MS column makes it suitable for GC-MS analysis of a wide range of polar compounds and matrices including: FAMEs, flavor compounds, essential oils, solvents, aromatics (including xylene isomers), acrolein/ acrylonitrile, and oxygenated compounds. The Stabilwax®-MS column is also useful for purity testing of chemicals and analyzing impurities in water and alcoholic beverages. When methods require trace analysis, the highly polar, low-bleed Stabilwax®-MS column produces excellent results compared to conventional wax columns. Review the applications in this brochure and try a low-bleed Stabilwax®-MS column for yourself!

Figure 2: Stabilwax®-MS columns exhibit lower bleed levels than VF-WAXms columns and are stable up to 260 °C.



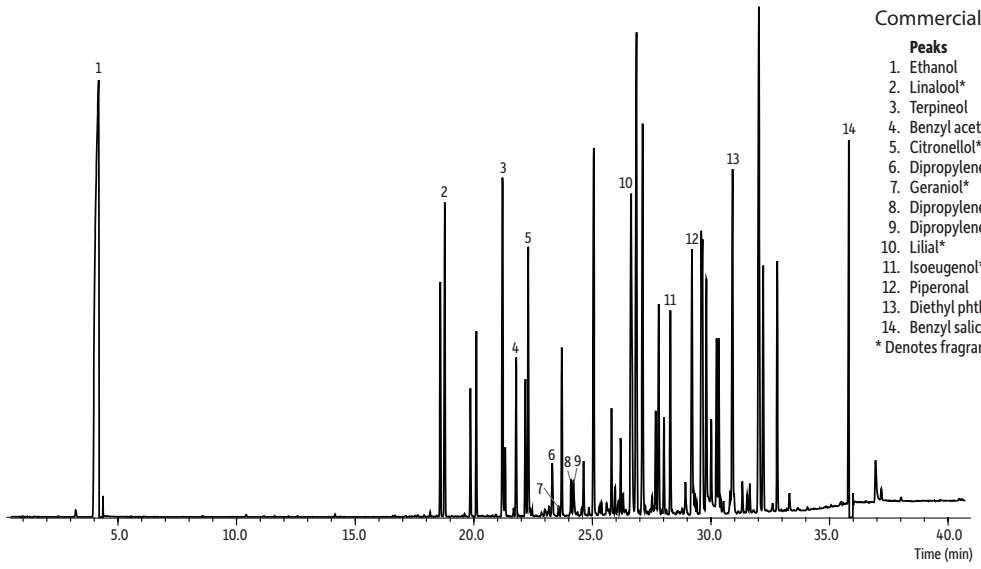
Columns: Stabilwax®-MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 10673), VF-WAXms, 30 m, 0.25 mm ID, 0.25 µm; **Sample**: 2 µg/mL 1,2-Dichlorobenzene-d4 in acetonitrile (reference peak 1 for MS response comparison), (cat.# 30049); **Injection**: 1 µL splitless (hold 0.50 min); Liner: Sky® 3.5 mm single taper w/wool (cat.# 23322), Inj. Temp.: 250 °C; Oven: 40 °C (hold 2 min) to 250 at 15 °C/min (hold 5 min); Carrier Gas: He, constant flow; Flow Rate: 1.0 mL/min; Linear Velocity: 36.1 cm/sec @ 40 °C; **Detector**: MS, scan mode; Scan Range (amu): 45-550; Scan Rate (scans/sec): 2; Transfer Line Temp: 260 °C; Analyzer Type: Quadrupole; Source Temp.: 250 °C; Ionization Mode: EI; **Instrument**: Shimadzu 2010 GC & QP2010+ MS.

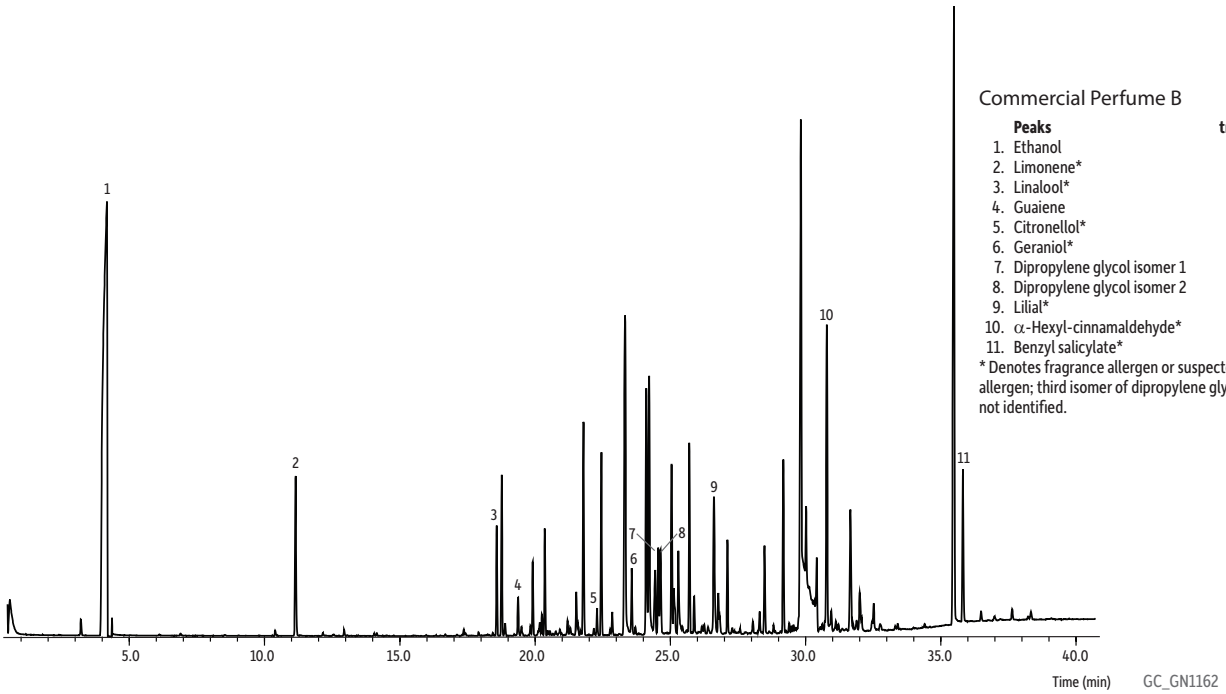
Fragrance Analysis

Commercial Perfumes

Materials containing fragrances, such as personal care products and perfumes, can be challenging to analyze by GC-MS due to their complex nature. Manufacturers analyze these difficult mixtures for quality control and stability purposes, as well as during formulation. Because these mixtures contain a diverse range of compounds at varying concentrations, a stationary phase that offers good selectivity and excellent resolution for a wide range of analytes, high inertness, and low bleed for low-level analysis is necessary. The Stabilwax®-MS column provides excellent separation of the alcohols, glycols, and terpenes in a commercial perfume sample analyzed by GC-MS in Figure 3.

Figure 3: Fragrance allergens and common compounds in commercial perfume separated on a Stabilwax®-MS column.





Columns: Stabilwax®-MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 10673); **Sample**: commercial perfumes, neat; **Injection**: 1 µL split (split ratio 200:1); Liner: Sky® 3.5 mm single taper w/wool (cat.# 23322), Inj. Temp.: 250 °C; **Oven:** 35 °C (hold 5 min) to 250 °C at 7 °C/min (hold 5 min); **Carrier Gas:** He, constant linear velocity; Linear Velocity: 36 cm/sec; **Detector**: MS, scan mode; Scan Range (amu): 40-550; Scan Rate (scans/sec): 2; Transfer Line Temp: 260 °C; Analyzer Type: Quadrupole; Source Temp.: 250 °C; Ionization Mode: EI; **Instrument**: Shimadzu 2010 GC & QP2010+ MS.

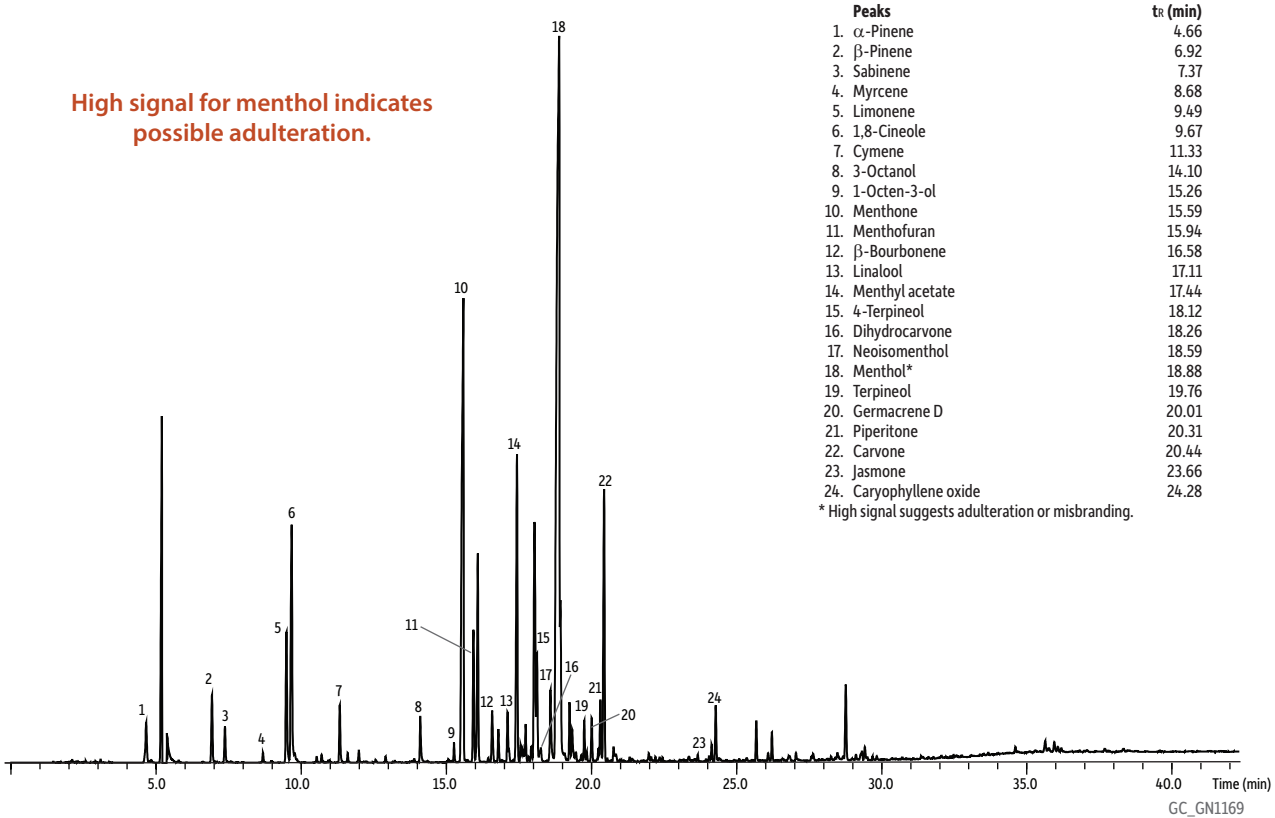
Flavor and Food Analysis

Spearmint Oil

Flavor and food samples contain numerous aromatic compounds; some naturally present in the raw materials and some forming during processing. GC-MS is extensively used for the analysis of these compounds, which include esters, fatty acids, alcohols, aldehydes, and terpenes. It is also used to detect and measure contaminants from spoilage or adulteration that may be harmful to humans and, therefore, are often controlled by governmental agencies.

Spearmint oil is used in a variety of commercially available products, including food and personal care items. Companies manufacturing materials containing spearmint oil generally control quality by testing for carvone, the main active component that gives spearmint oil its minty flavor. Menthol is also often a target compound as it should be a minor component in spearmint oil, but is commonly added as an adulterant. The large menthol peak shown in the spearmint oil sample in Figure 4 indicates that this sample is likely either spearmint oil with menthol added or a different type of oil (e.g., misbranded peppermint oil). The Stabilwax®-MS column provides the required selectivity to give excellent separation of this complex natural sample, while exhibiting minimal column bleed at 250 °C by GC-MS.

Figure 4: Commercial spearmint oil analyzed on a Stabilwax®-MS column.



	Peaks	t_R (min)
1.	α-Pinene	4.66
2.	β-Pinene	6.92
3.	Sabinene	7.37
4.	Myrcene	8.68
5.	Limonene	9.49
6.	1,8-Cineole	9.67
7.	Cymene	11.33
8.	3-Octanol	14.10
9.	1-Octen-3-ol	15.26
10.	Menthone	15.59
11.	Menthofuran	15.94
12.	β-Bourbonene	16.58
13.	Linalool	17.11
14.	Menthyl acetate	17.44
15.	4-Terpineol	18.12
16.	Dihydrocarvone	18.26
17.	Neoisomenthol	18.59
18.	Menthol*	18.88
19.	Terpineol	19.76
20.	Germacrene D	20.01
21.	Piperitone	20.31
22.	Carvone	20.44
23.	Jasmone	23.66
24.	Caryophyllene oxide	24.28

* High signal suggests adulteration or misbranding.

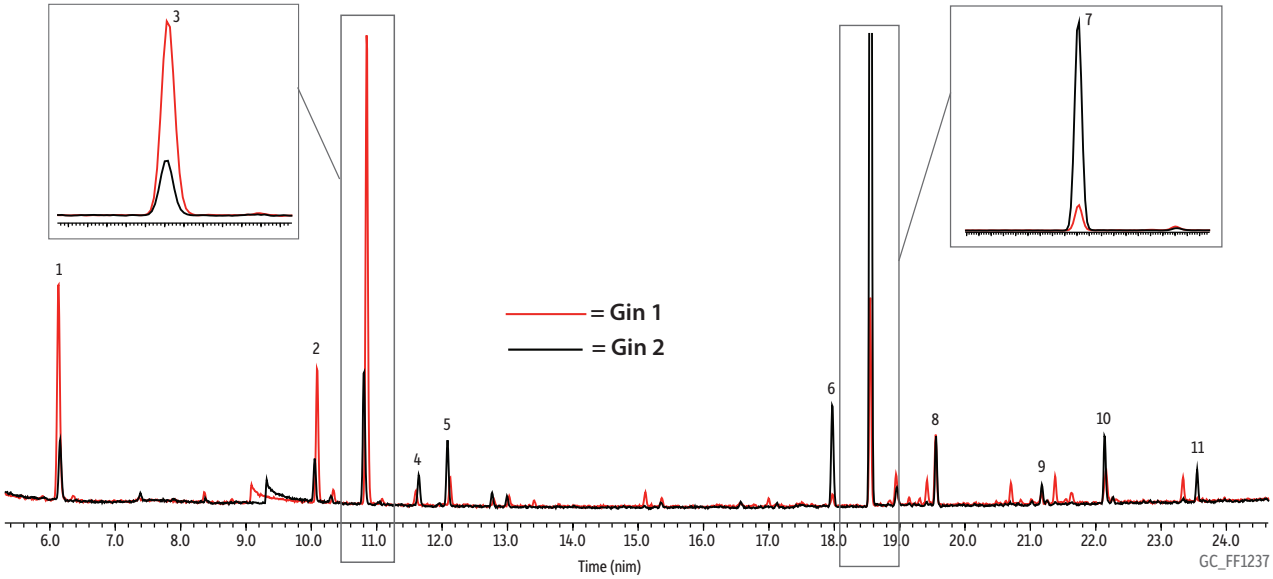
Columns: Stabilwax®-MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 10673); **Sample**: commercial spearmint oil, neat; **Injection**: 1 µL split (split ratio 150:1); Liner: Sky® 3.5 mm single taper w/wool (cat.# 23322), Inj. Temp.: 250 °C; **Oven:** 45 °C (hold 5 min) to 250 °C at 7 °C/min (hold 10 min); **Carrier Gas:** He, constant linear velocity; Linear Velocity: 36 cm/sec; **Detector**: MS, scan mode; Scan Range (amu): 40-550; Scan Rate (scans/sec): 3.3 ; Transfer Line Temp: 260 °C; Analyzer Type: Quadrupole; Source Temp.: 250 °C; Ionization Mode: EI; **Instrument**: Shimadzu 2010 GC & QP2010+ MS.

Flavor and Food Analysis *(cont.)*

Alcoholic Beverages

Alcoholic beverages contain a wide range of volatile compounds, including alcohols and short-chain aldehydes, which manufacturers analyze for quality control, authenticity, and brand identification purposes. Gas chromatography can be used to determine these compounds since capillary columns offer efficient separations. Capillary GC is especially useful in the analysis of structurally similar compounds, such as fusel alcohols (i.e., isoamyl alcohol, 4-terpeniol, linalool, geraniol, etc.). The unique polarity of the Stabilwax®-MS stationary phase ensures excellent resolution of a range of alcohols and fusel alcohols (also known as fusel oils) as shown in the analysis of a gin sample in Figure 5. The low bleed level obtained with a Stabilwax®-MS column permits excellent response and quantitation of the gin volatiles to aid in accurate brand identification.

Figure 5: Different brands of gin are compared using a Stabilwax®-MS column.



Column	Stabilwax®-MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 10673)
Sample	Two different brands of gin
Conc.:	Neat
Injection	
Inj. Vol.:	1 µL split (split ratio 20:1)
Liner:	Sky® 3.5 mm Precision® liner w/wool (cat.# 23320.1)
Inj. Temp.:	250 °C
Oven	
Oven Temp.:	35 °C (hold 5 min) to 250 °C at 7 °C/min (hold 5 min)
Carrier Gas	He, constant linear velocity
Linear Velocity:	36 cm/sec

Detector	MS
Mode:	Scan
Scan Program:	

Group	Start Time (min)	Scan Range (amu)	Scan Rate (scans/sec)
1	0.5	40-550	2

Transfer Line Temp.:	260 °C
Analyzer Type:	Quadrupole
Source Temp.:	250 °C
Solvent Delay Time:	0.5 min
Ionization Mode:	EI
Instrument	Shimadzu 2010 GC & QP2010+ MS

	Peaks	t_R (min)
1.	α-Pinene	6.16
2.	Beta-myrcene	10.05
3.	D-Limonene	10.81
4.	Isoamyl alcohol*	11.64
5.	γ-Terpinene	12.08
6.	Camphor*	17.97
7.	Linalool	18.56
8.	4-Terpineol	19.56
9.	α-Terpineol*	21.18
10.	Nerol acetate*	22.14
11.	Geraniol*	23.55

* Not found in gin represented by red trace.

RESTEK

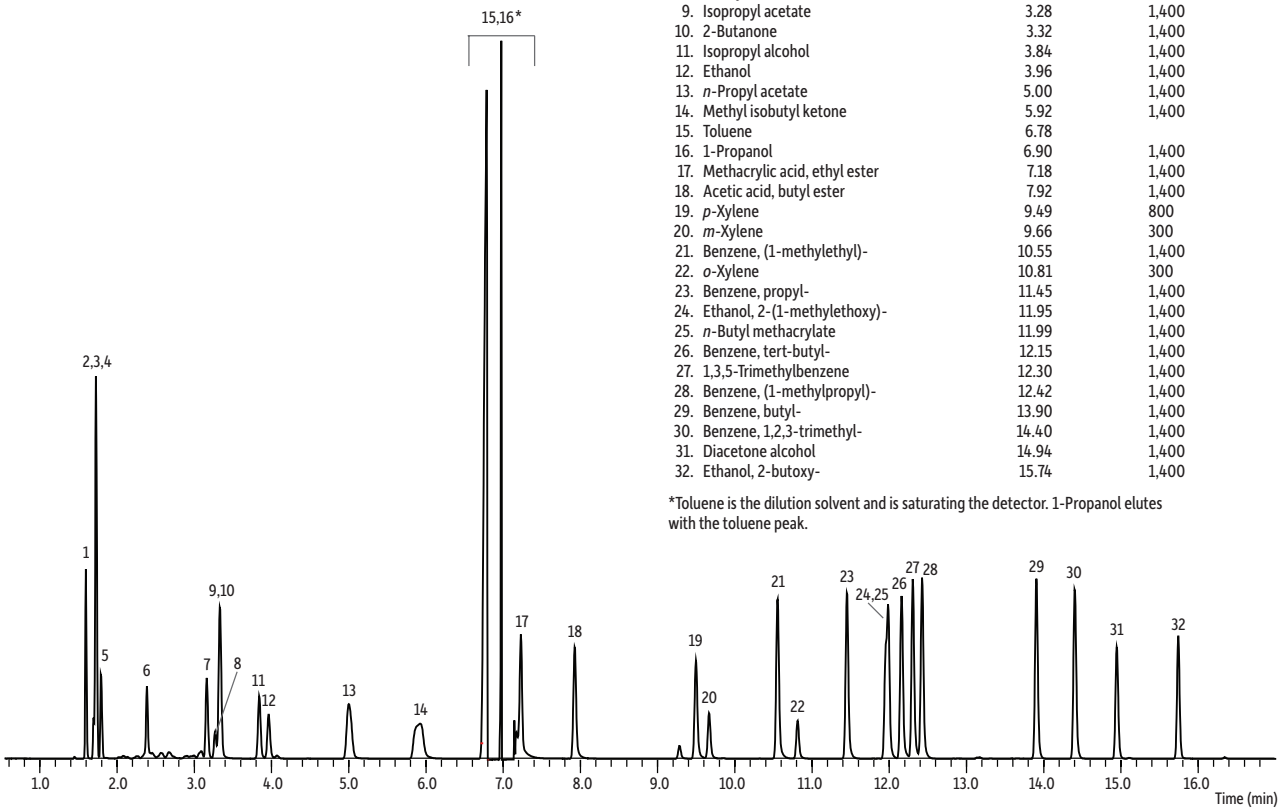
Industrial Chemicals Analysis

Industrial chemicals and solvents are used in dry cleaning agents, paint thinners, spot removers, perfumes, inks, adhesives, and hundreds of other materials. Many also are used to manufacture polymers, fine chemicals, celluloid cements, and lacquers, such as wood stains and printing applications, as well as in the manufacture of coatings, pharmaceuticals, paints, and packaging material. Analysis of these chemicals and solvents is performed to monitor incoming purity, process control, and disposal (drum waste). Many of the compounds analyzed in Figure 6 are found in packaging samples and industrial hygiene samples. Figure 7 shows excellent separation of chemicals and solvents commonly identified in process control and purity samples. The thermal stability of the Stabilwax®-MS column permits fast analysis times for a wide range of compounds in a temperature programmed run and results in low column bleed at 250 °C by GC-MS.

Figure 6: Excellent resolution and inertness of alcohols and acetates on a Stabilwax®-MS column.

	Peaks	t_R (min)	Conc. (µg/mL)
1.	*n*-Hexane	1.59	1,400
2.	Hexane, 2-methyl-	1.72	200
3.	Pentane, 2,3-dimethyl-	1.70	200
4.	Hexane, 3-methyl-	1.72	500
5.	Heptane	1.79	500
6.	Acetone	2.38	1,400
7.	Ethyl acetate	3.16	1,400
8.	Methyl alcohol	3.26	1,400
9.	Isopropyl acetate	3.28	1,400
10.	2-Butanone	3.32	1,400
11.	Isopropyl alcohol	3.84	1,400
12.	Ethanol	3.96	1,400
13.	*n*-Propyl acetate	5.00	1,400
14.	Methyl isobutyl ketone	5.92	1,400
15.	Toluene	6.78	
16.	1-Propanol	6.90	1,400
17.	Methacrylic acid, ethyl ester	7.18	1,400
18.	Acetic acid, butyl ester	7.92	1,400
19.	*p*-Xylene	9.49	800
20.	*m*-Xylene	9.66	300
21.	Benzene, (1-methylethyl)-	10.55	1,400
22.	*o*-Xylene	10.81	300
23.	Benzene, propyl-	11.45	1,400
24.	Ethanol, 2-(1-methylethoxy)-	11.95	1,400
25.	*n*-Butyl methacrylate	11.99	1,400
26.	Benzene, tert-butyl-	12.15	1,400
27.	1,3,5-Trimethylbenzene	12.30	1,400
28.	Benzene, (1-methylpropyl)-	12.42	1,400
29.	Benzene, butyl-	13.90	1,400
30.	Benzene, 1,2,3-trimethyl-	14.40	1,400
31.	Diacetone alcohol	14.94	1,400
32.	Ethanol, 2-butoxy-	15.74	1,400

*Toluene is the dilution solvent and is saturating the detector. 1-Propanol elutes with the toluene peak.

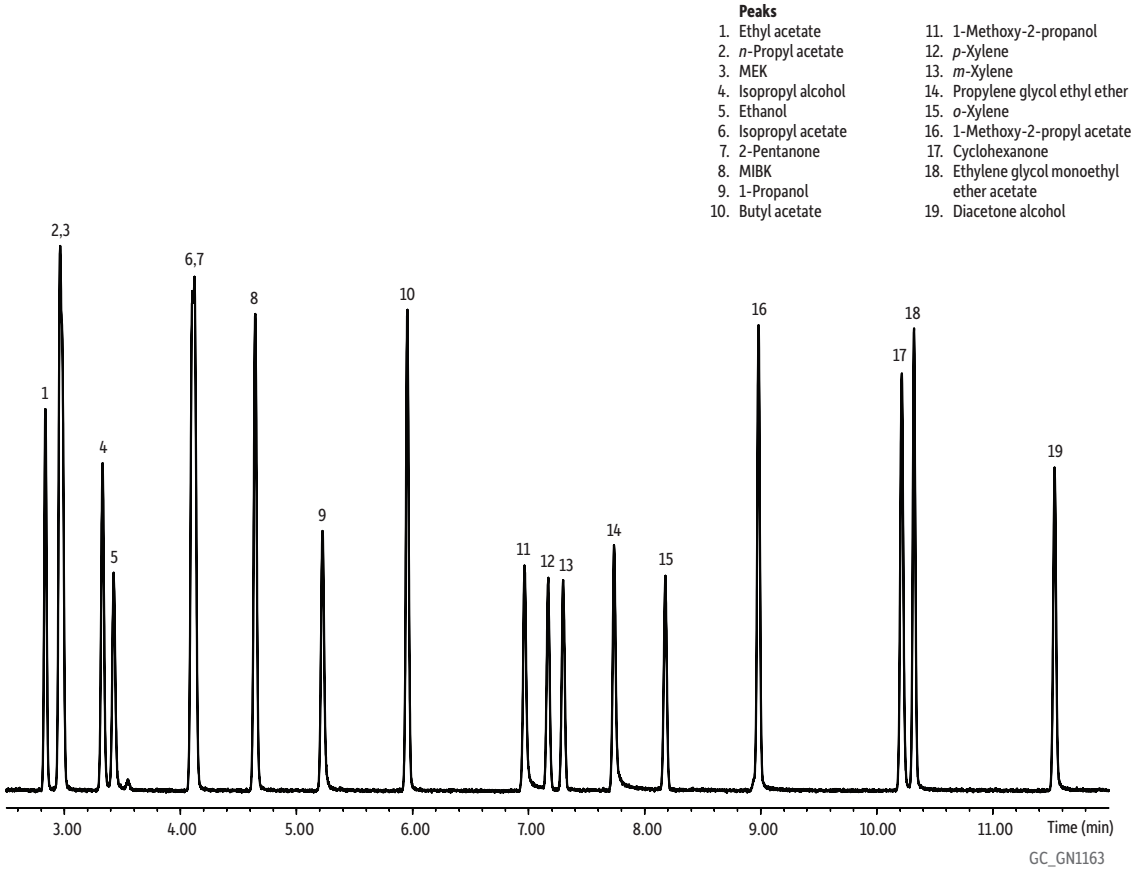


GC_FF1224

Columns: Stabilwax®-MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 10673); **Sample**: custom standard prepared in toluene; **Injection**: 1 µL split (split ratio 200:1); Liner: Sky® 3.5 mm single taper w/wool (cat.# 23322), Inj. Temp.: 250 °C; **Oven:** 35 °C (hold 5 min) to 250 °C at 7 °C/min (hold 5 min); **Carrier Gas:** He, constant flow; Flow Rate: 1 mL/min; Linear Velocity: 36.1 cm/sec @ 35 °C; **Detector**: MS, scan mode; Scan Range (amu): 30-400; Transfer Line Temp: 260 °C; Analyzer Type: Quadrupole; Source Temp.: 250 °C; Ionization Mode: EI; **Instrument**: Shimadzu 2010 GC & QP2010+ MS.

Industrial Chemicals Analysis *(cont.)*

Figure 7: Analysis of common industrial chemicals and solvents on a Stabilwax®-MS column by GC-MS in less than 12 min.



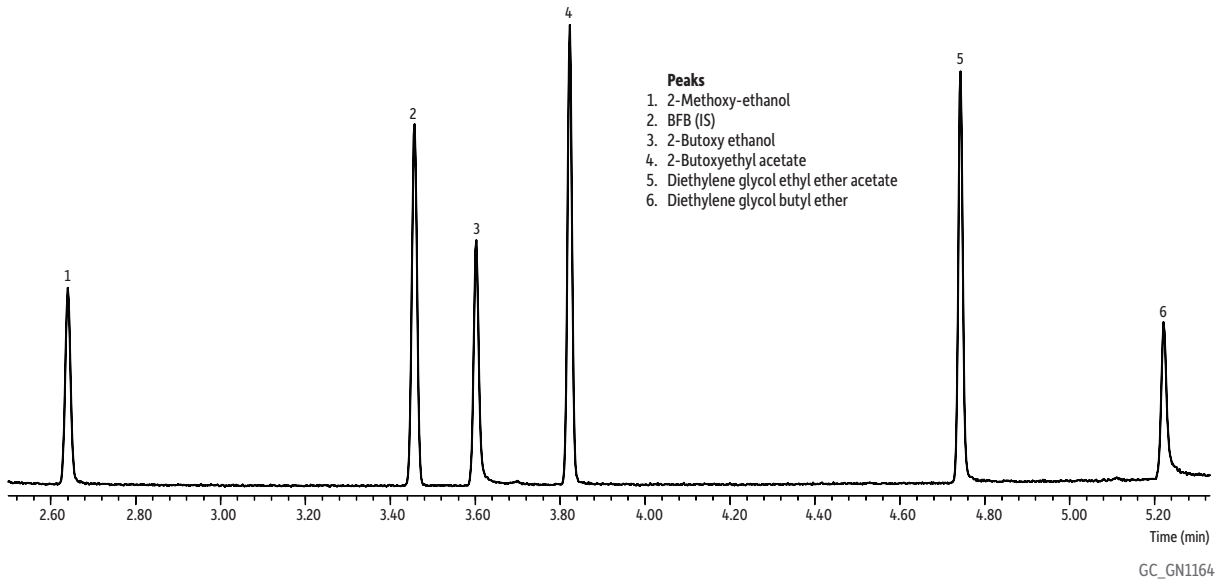
Columns: Stabilwax®-MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 10673); **Sample**: 400 ppm custom solvent standard prepared in cyclohexane; **Injection:** 0.5 µL split (split ratio 200:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305), Inj. Temp.: 200 °C; **Oven:** 40 °C (hold 3 min) to 130 °C at 8 °C/min; **Carrier Gas:** He, constant flow; Flow Rate: 1 mL/min; **Detector**: MS, scan mode; Scan Program: 30-150 amu; Transfer Line Temp: 200 °C; Analyzer Type: Quadrupole; Source Temp.: 200 °C; Quad Temp: 150 °C; Ionization Mode: EI; **Instrument**: Agilent 7890A GC & 5975C MSD.

RESTEK®

Cleaning Solvent Analysis

Cleaning solvents are used primarily to dissolve organic material. They clean without leaving residue, making them very useful in products such as glass cleaners. The main criterion for cleaning solvents is water miscibility, as the solvent must form a solution with the other water-soluble components. Thus, alcohols and glycols are popular choices. Glycol ethers are made from ethylene and propylene, and they prove to be excellent degreasers, cleaners, and intermediates. There are more than 30 different commonly used glycol ethers with varying technical properties and toxicity profiles. For example, diethylene glycol ethyl ether acetate (EGEEA) has been identified by the European Union as a reproductive toxin and is not manufactured or used in France. In fact, the use of glycol ethers is controlled or has been eliminated in many European countries. The Stabilwax®-MS column has excellent selectivity and inertness for alcohols and glycol ethers found in cleaning solvents, with the add advantage of good thermal stability. Figure 8 shows baseline resolution is achieved with a fast 5-minute, temperature programmed run to 220 °C. The thermal stability of the Stabilwax®-MS column would allow a bake out ramp to 250 °C to remove any high molecular weight contaminants in the cleaning solvents, which would prolong column lifetime and reduce column maintenance.

Figure 8: Cleaning solvents analyzed on a Stabilwax®-MS column.



Columns: Stabilwax®-MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 10673); **Sample**: 200 ppm custom standard prepared in methanol; **Injection**: 1.0 µL split (split ratio 300:1); Liner: Sky® 4 mm Precision® liner w/ wool (cat.# 23305), Inj. Temp.: 200 °C; **Oven:** 60 °C to 220 °C at 30 °C/min; **Carrier Gas:** He, constant flow; Flow Rate: 1 mL/min; **Detector**: MS, scan mode; Scan Range: 30-200 amu; Transfer Line Temp: 200 °C; Analyzer Type: Quadrupole; Source Temp.: 200 °C; Quad Temp: 150 °C; **Instrument**: Agilent 7890A GC & 5975C MSD.

Recommended Products

Thermally stable, high polarity

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columns are ideal for flavor, food, fragrance, industrial chemical, and solvent analyses.

Stabilwax-MS Columns

Cat.#	Length	ID	df
10673	30 m	0.25 mm	0.25 µm
10674	30 m	0.32 mm	0.25 µm

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9 mm	27133	27134
9.5 mm (3/8")	27136	27137
10 mm	27139	27140
11 mm (7/16")	27142	27143
11.5 mm	27145	27146
12.7 mm (1/2")	27148	27149
17 mm	27151	27152
Shimadzu Plug	27154	27155

Note: Due to differences in inlet design, the actual septum temperature for a given inlet setpoint can vary by manufacturer. Restek recommends using only BTO® septa in Thermo TRACE and Focus GCs.
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21314

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- Improved design reduces chance of leaks.

Description	qty.	cat.#
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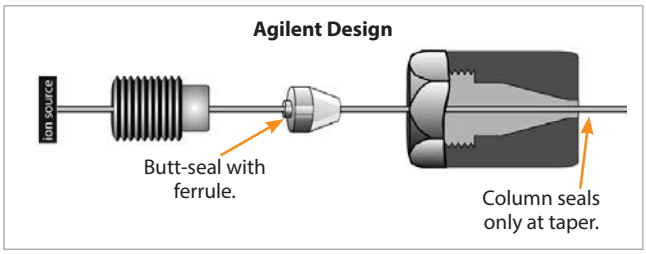
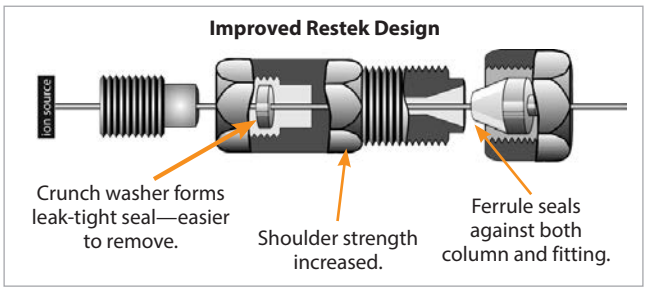
Replacement Ring Seal for MSD Conversion Fitting	2-pk.	21313





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Poor sensitivity, loss of sensitivity at high masses, or high multiplier gain during an auto tune are all indicators that your mass spectrometer source may need to be cleaned. Restek has assembled all of the necessary components for cleaning and polishing your ion source.

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Mass Spec Cleaning Kit with Dremel Tool	kit	27194
Mass Spec Cleaning Kit without Dremel Tool	kit	27195
Mass Spec Cleaning Kit Replacement Parts Kit Includes: cloths, micro mesh sheets, small and large gloves	kit	27196

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Use this aluminum oxide powder to clean surfaces that contact the sample or ion beam when you encounter poor sensitivity and inadequate abundances at high masses.

Description	Similar to Agilent part #	qty.	cat.#
Ion Source Cleaning Powder	8660-0791	1 kg	22685

Recommended Products

Dynamic Duo (Restek Leak Detector and ProFLOW 6000 Flowmeter)

Protect your instrument and improve data quality with this powerful pair from Restek. Checking for leaks and verifying flows before you start helps you avoid costly problems later.

Description	qty.	cat.#
Dynamic Duo Combo Pack (Restek Leak Detector and ProFLOW 6000 Flowmeter)	kit	22654
Related Products and Accessories		
Leak Detector With Hard-Sided Carrying Case and Universal Charger Set (U.S., UK, European, Australian)	ea.	22655
Small Probe Adaptor for Leak Detector	ea.	22658
Restek ProFLOW 6000 Electronic Flowmeter With Hard-Sided Carrying Case	ea.	22656
Soft-Sided Storage Case for Leak Detector or ProFLOW 6000 Flowmeter	ea.	22657

Restek's New Leak Detector

Redesigned and better than ever, our new leak detector is an essential tool for trouble-shooting and routine maintenance of your gas chromatograph. Don't risk damaging your system or losing sensitivity; check for leaks often and protect your GC column and instrument with a Restek leak detector!

Leak Detector Specifications

Detectable Gases:	Helium, nitrogen, argon, carbon dioxide, hydrogen
Battery:	Rechargeable lithium ion internal battery pack (12 hours normal operation)
Operating Temp. Range:	32–120 °F (0–48 °C)
Humidity Range:	0–97%
Warranty:	One year
Certifications:	CE, Ex, Japan
Compliance:	WEEE, RoHS

Limits of Detection

These gases can be detected with the Restek electronic leak detector at the following leak rates:

Minimum Detectable Gas Limits and Indicating LED Color:
Helium, 1.0 x 10^{-5}, red LED
Hydrogen*, 1.0 x 10^{-5}, red LED
Nitrogen, 1.4 x 10^{-3}, yellow LED
Argon, 1.0 x 10^{-4}, yellow LED
Carbon dioxide, 1.0 x 10^{-4}, yellow LED

Gas detection limits measured in atm cc/sec.

ProFLOW 6000 Flowmeter

With its wide range of capabilities, the ProFLOW 6000 flowmeter simplifies gas flow measurement in the lab. Real-time measurements can be made for various types of flow paths, including continually changing gas types.

Flowmeter Specifications:

Type of Flowmeter:	Volumetric
Battery:	2-AA
Operating Temp. Range:	32–120 °F (0–48 °C)
Warranty:	One year
Certifications:	CE, Ex
Compliance:	WEEE, RoHS

Patented.

Optional Accessories

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Soft-Side Carry/Storage Case
Ideal for storing your leak detector or flowmeter in smaller spaces such as a tool box.

22658

Small Probe Adaptor for Leak Detector
Verify hard-to-reach leaks using the small probe adaptor.

Lit. Cat.# GNBR1818-UNV

Simple, Reliable HPLC Analyses of Organic Acids

Using Water-Compatible Allure® or Ultra C18 Columns

by Julie Kowalski, Ph.D., Innovations Chemist, and Becky Wittrig, Ph.D., HPLC Product Marketing Manager

- Use 100% aqueous mobile phases without losing retention.
- Simple, isocratic method.
- Complete resolution of critical organic acids, including quinic and tartaric.

Organic acids are common components in foods and beverages, and play a critical role in product characteristics like taste and aroma. They can be tested for in many food products including fruits, cheeses, and various beverages such as juices and wines. Organic acids can originate in the foods themselves (e.g. cranberries) or can be produced by food processing (e.g. alcoholic fermentation). A method that allows resolution of organic acids, as well as their quantification, can help determine product quality and authenticity.

Reversed phase HPLC coupled with UV-Vis detection is a popular technique for organic acid analysis because it is easy to use. One common method, AOAC method 986.13, stipulates reversed phase HPLC using two C18 stationary phase columns in series. Because organic acids are low in molecular weight, and have polar functionalities, 100% aqueous buffer is the mobile phase of choice. A low pH buffer is used to ensure that all acidic groups are protonated (otherwise, the organic acids are neutral), thus allowing the best interaction between the organic acids and the C18 stationary phase. However, using a 100% aqueous mobile phase can cause the C18 chain in conventional C18 columns to collapse. Phase collapse results in loss of retention, and the column must be flushed with organic mobile phase, a time consuming step, to restore chain structure and column performance.

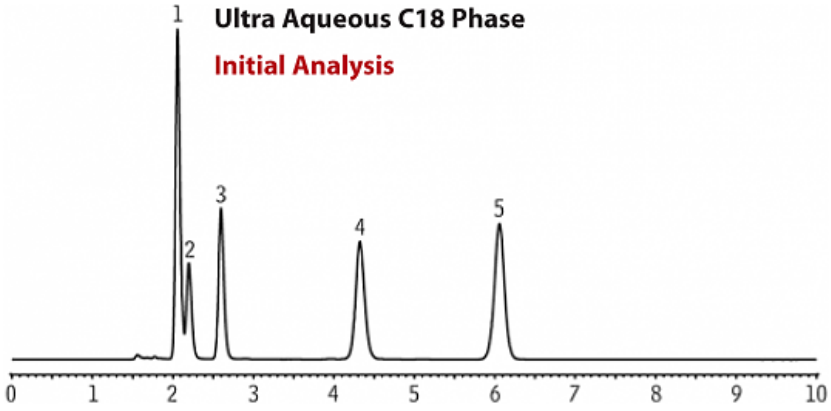
Three Restek columns—the Ultra Aqueous C18 column, the Allure® Aqueous C18 column, and the Allure® Organic Acids column—were designed using aqueous-compatible C18 phases that combat phase collapse. The advantage of using these columns is demonstrated in **Figure 1** by the fast analysis of organic acids on a Shimadzu Prominence 20A system. Here, we compared the ability of the Ultra Aqueous C18 phase and a conventional C18 phase to withstand phase collapse. The Ultra Aqueous C18 phase resolves organic acids in a 100% aqueous mobile phase without loss of retention. In comparison, the conventional C18 phase suffers a complete loss of retention following phase collapse when used under the same conditions. Thus, in an analysis that requires, or is improved by, a mobile phase with a high aqueous content, an Ultra Aqueous C18 column is the superior choice.

In analyses of organic acids, specifically, under high aqueous mobile phase conditions, the Allure® Organic Acids column is the column of choice. We have developed a method using a 300mm Allure® Organic Acids column to separate critical organic acids: tartaric, quinic, malic, citric and fumaric acids. This method uses 100% aqueous mobile phase as recommended by AOAC method 986.13. In addition to allowing repeated injections in 100% aqueous mobile phase without the severe lose of retention observed with conventional C18 column technology, the Allure® Organic Acids column is prepared and tested specifically for separating organic acids. **Figure 2** shows that tartaric and quinic acids are resolved to baseline; **Figure 3** shows typical analyses under the conditions we recommend.

Figure 1 Restek's water-compatible C18 phase does not collapse in a 100% aqueous mobile phase, compared to a conventional C18 column which shows a complete loss of retention.

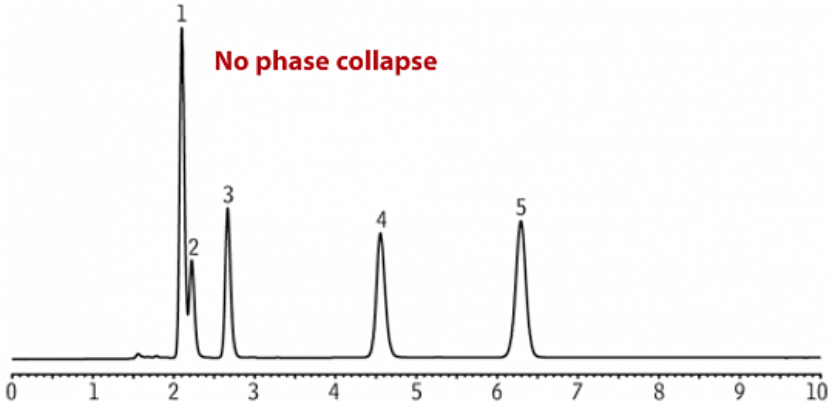
Peaks	Conc. (µg/mL)

1. Tartaric acid	2,000
2. Quinic acid	2,000
3. Malic acid	2,000
4. Citric acid	2,000
5. Fumaric acid	10



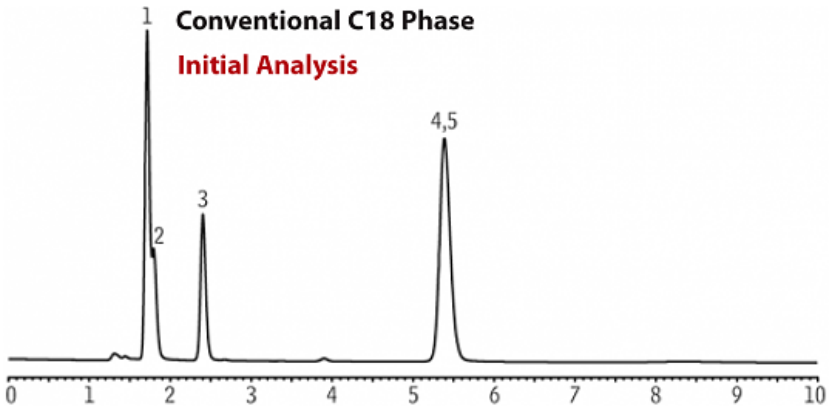
LC_FF0433

Peaks	Conc. (µg/mL)
1. Tartaric acid	2,000
2. Quinic acid	2,000
3. Malic acid	2,000
4. Citric acid	2,000
5. Fumaric acid	10



LC_FF0434

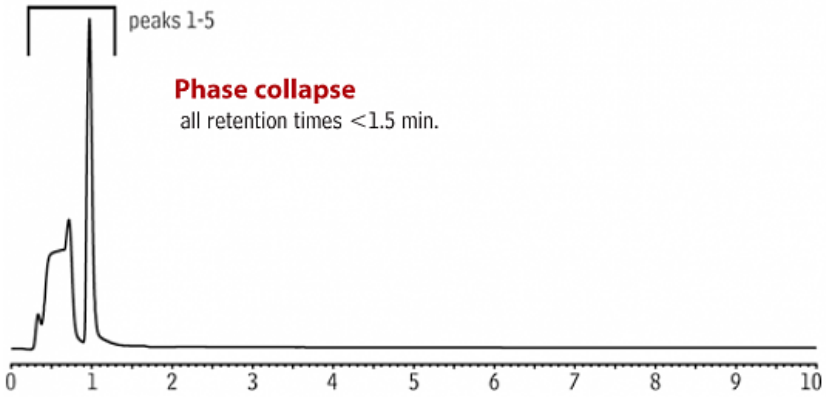
Peaks	Conc. (µg/mL)
1. Tartaric acid	2,000
2. Quinic acid	2,000
3. Malic acid	2,000
4. Citric acid	2,000
5. Fumaric acid	10



LC_FF00431

Peaks	Conc. (µg/mL)
1. Tartaric acid	2,000
2. Quinic acid	2,000
3. Malic acid	2,000
4. Citric acid	2,000

5. Fumaric acid		10

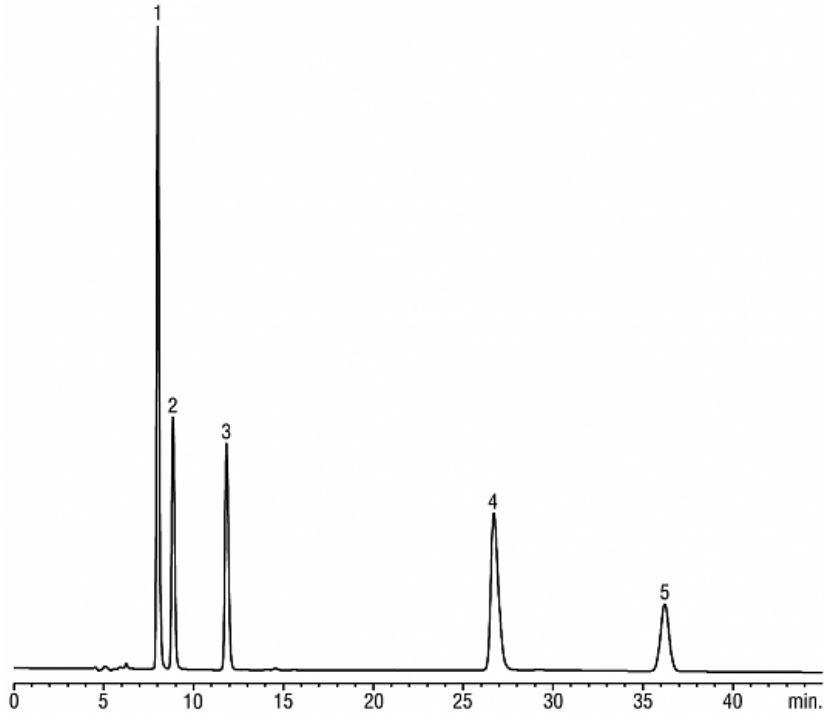


LC_FF0432

Column	Conventional Ultra C18 (cat.# 9174565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	30 °C
Sample	Fruit Juice Organic Acid Standard (cat.# 35080)
Diluent:	deionized water
Inj. Vol.:	10 µL
Mobile Phase	20 mM potassium phosphate (pH 2.5)
Flow:	1.0 mL/min
Detector	UV/Vis @ 226 nm
Instrument	Shimadzu Prominence

Figure 2 Excellent resolution of organic acids, including tartaric and quinic acids, on an Allure® Organic Acids column.

Peaks		Conc. (mg/mL)
1. Tartaric acid		1.0
2. Quinic acid		1.0
3. Malic acid		1.0
4. Citric acid		1.0
5. Fumaric acid		0.005

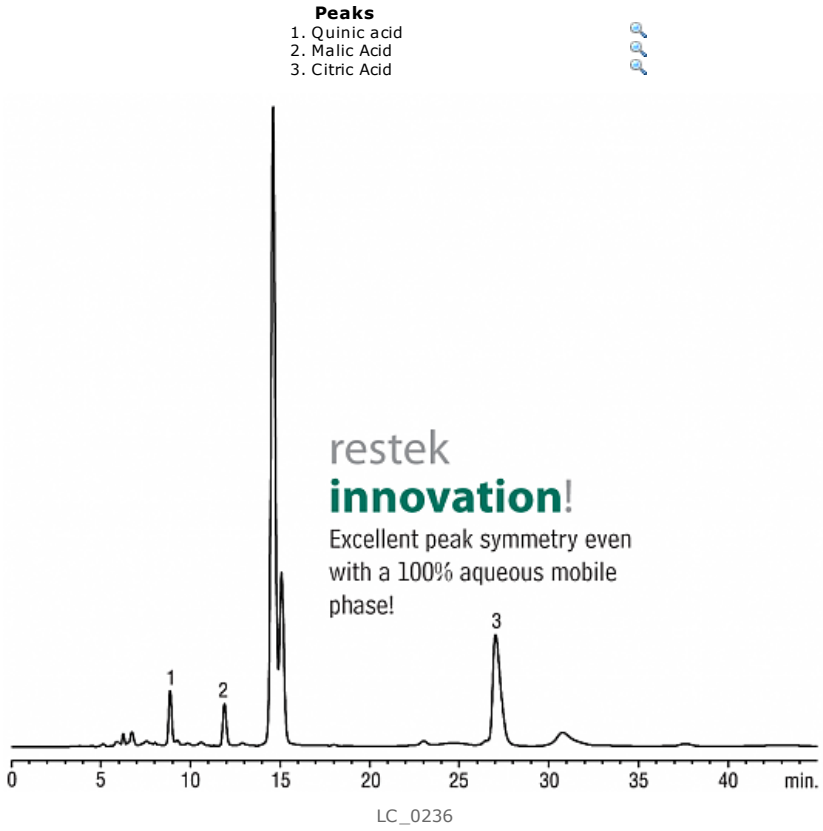


LC_0238

Column	Allure® Organic Acids (cat.# 9165585)
Dimensions:	300 mm x 4.6 mm ID

Pore Size:	60 Å
Temp.:	ambient
Sample	standard solution
Diluent:	water
Inj. Vol.:	10.0 µL
Mobile Phase	100 mM phosphate buffer, pH 2.5
Flow:	0.5 mL/min
Detector	UV/Vis @ 226 nm

Figure 3 Sharp, easily differentiated organic acid profiles for cranberry juice cocktail on an Allure® Organic Acids column.



LC_0236

Column	Allure® Organic Acids (cat.# 9165585)
Dimensions:	300 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	60 Å
Temp.:	ambient
Sample	cranberry juice:water (50:50, v/v)
Diluent:	water
Inj. Vol.:	10.0 µL
Mobile Phase	100mM phosphate buffer, pH 2.5
Flow:	0.5 mL/min
Detector	UV/Vis @ 226 nm

References

1. Verify Fruit Juice Quality from Organic Acid Profiles from *The Restek Advantage, 2003*, vol. 3
2. Official Methods of Analysis (2000). AOAC International, 17th edition, method # 986.13
3. Manolaki, P. et al., Food Chemistry, 98 (2006), page 658-663
4. Kafkas, E. et al., Food Chemistry, 97 (2006), page 732-736

RELATED SEARCHES

AOAC method 986.13, *Allure Organic Acids*, *cranberry juice*, *fruit juice*, *Ultra Aqueous C18*

Simple HPLC Analysis for Sudan Dyes

Monitor Sudan I, II, III, and IV in a Single, Isocratic Analysis

By Julie Kowalski, Innovations Chemist

- Ultra Aqueous C18 HPLC column separates the four Sudan dyes in 20 minutes.
- Simple methanol and water mobile phase.
- Two wavelengths detect all four dyes.

Sudan dyes are synthetic industrial azo-dyes traditionally used in waxes, plastics, oils, and polishes. Although recognized as carcinogens, Sudan dyes recently have been found in food products in some European countries. They are added to foods such as chili powders to mimic, intensify, and prolong the appearance of natural red hues. In the UK, more than six hundred products containing Sudan dyes have been recalled, the largest food recall in British history.(1)

Sudan dyes are categorized as Class 3 carcinogens by the International Agency for Research on Cancer (IARC) and, therefore, are illegal as food additives according to both the FDA and the EU. The European Commission requires products to have documentation confirming the absence of Sudan dyes.(2,3) Since 2003, European nations have required random product testing and testing of suspected adulterated products. Items found to contain Sudan dyes must be disposed of as hazardous waste.(4)

Laboratories performing analyses for Sudan dyes are not required to follow defined methods. The EU has set detection limits at 0.5-1 mg/kg, and any food material containing more than the limit should be withdrawn from the market.(1) Here, we describe a simple reversed phase HPLC separation of Sudan I, Sudan II, Sudan III, and Sudan IV (Scarlet Red).

We prepared 1mg/mL stock solutions of Sudan I or Sudan II in HPLC grade methanol, and equivalent solutions of Sudan III or Sudan IV in ethyl acetate. To avoid reductive cleavage, we stored the stock solutions at 4°C in foil-wrapped containers. We prepared sample solutions by combining the four stock solutions and diluting with methanol to 20µg/mL each dye. We used a 150 x 4.6mm Ultra Aqueous C18 HPLC column (cat.# 9178565) for the analysis.

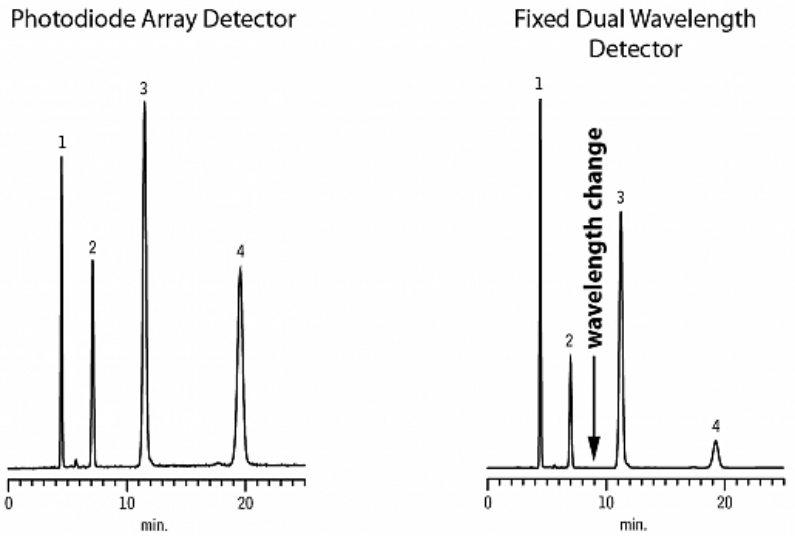
Results

Figure 1 shows the Ultra Aqueous C18 column separates the four dyes in approximately 20 minutes. Sudan I can be detected at 476nm or 418nm, Sudan II at 493nm or 604nm, Sudan III at 508nm to 512nm, and Sudan IV at 357nm or 520nm. For each dye except Sudan III, we observed the higher response at the first listed wavelength; for Sudan III there was little difference. The dyes can be detected by monitoring at 488nm for Sudan I and II and at 520nm for Sudan III and IV, allowing all four dyes to be detected with a fixed dual wavelength instrument.

This method is simple, yet efficient, requiring only a simple mobile phase, isocratic elution, and detection at two wavelengths. The Ultra Aqueous C18 column provides the selectivity needed to assure the separation.

Figure 1 Monitor Sudan I, II, III and Sudan IV in a single, isocratic analysis.

Peaks
1. Sudan I
2. Sudan II
3. Sudan III
4. Sudan IV



LC_FF0326

Column	Ultra Aqueous C18 (cat.# 9178565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	ambient
Sample	mixture of Sudan I, Sudan II, Sudan III, Sudan IV
Diluent:	methanol
Conc.:	20 µg/mL each
Inj. Vol.:	20 µL
Mobile Phase	water:methanol (3:97)
Flow:	1.0 mL/min
Detector	UV/Vis
Notes	Detector: 476/493/512/357 nm (photodiode array), 488/520 nm (fixed dual wavelength)

References

1. www.ift.org/news_bin/news/newsBody.shtml
2. Commission Decision of 20 June 2003 on emergency measures regarding hot chili and hot chili products, notified under document number C(2003) 1970, (2003/460/EC), OJ L. 154/114, 21.6.2003.
3. Implementation of Commission Decision 2003/460/EC of 21 January 2004.
4. www.food.gov.uk/foodindustry/guidancenotes/foodguid/sudanguidance

RELATED SEARCHES

Ultra Aqueous C18, *Sudan I*, *Sudan II*, *Sudan III*, *Sudan IV*, *Scarlet Red*, *azo-dyes*, *food contaminants*, *RPLC*, *reversed phase*

Robust 9-Minute GC Analysis of Cholesterol

Excellent Sample Throughput with an Rxi®-5ms Column

By Julie Kowalski and Lydia Nolan, Innovations Chemists, and Aaron Muscarella, Innovations Intern

- Highly inert Rxi™-5ms column enables analysis of underivatized or derivatized sterol.
- Isothermal approach greatly increases throughput.
- Highly reproducible retention and quantification.

Cholesterol is a vital component in human and animal blood and cell membranes, but cholesterol receives attention primarily because of its association with impaired blood circulation and heart disease. Because of this involvement with cardiovascular disease, many countries require the cholesterol content of food products to be a part of nutritional labels. Much effort is spent on promoting low cholesterol foods and diets and much money is spent on cholesterol-reducing medications.[1]

Capillary GC affords qualitative and quantitative analysis of cholesterol and is incorporated into AOAC International methods 970.51E and 976.26.2; According to these methods, cholesterol and other sterols are extracted from the unsaponified fraction of an ether extract of a sample material. The residue is dissolved in chloroform and evaporated (see AOAC methods 933.08 and 970.51A for details[1]). Other methods, including AOAC method 976.26 and AOCS method Ce 3-74[2,3], describe derivatization of sterols prior to chromatographic analysis.

A highly inert Rxi™-5ms capillary column allows qualitative and quantitative analysis of either underivatized or derivatized cholesterol. Figure 1A shows the separation of underivatized cholesterol and internal standard 5-α-cholestane in less than 9 minutes. The temperature program maximizes separation of the analytes and any early-eluting contaminants or extracted matrix interferences encountered when evaluating complex samples. Figure 1B illustrates a rapid, isothermal method that maximizes throughput when interferences are not a concern.

The same analytical conditions can be applied when analyzing derivatized cholesterol, using the standard extraction and derivatization methods referred to above. Figure 2A and Figure 2B show the chromatographic results. In a series of 9 replicates, using the temperature program, the Rxi™-5ms column showed both exceptional inertness and excellent reproducibility for either cholesterol or derivatized cholesterol. Table 1 summarizes the results.

If cholesterol analyses are part of your work regimen, we highly recommend using Rxi™-5ms columns to ensure reliable results and increase sample throughput.

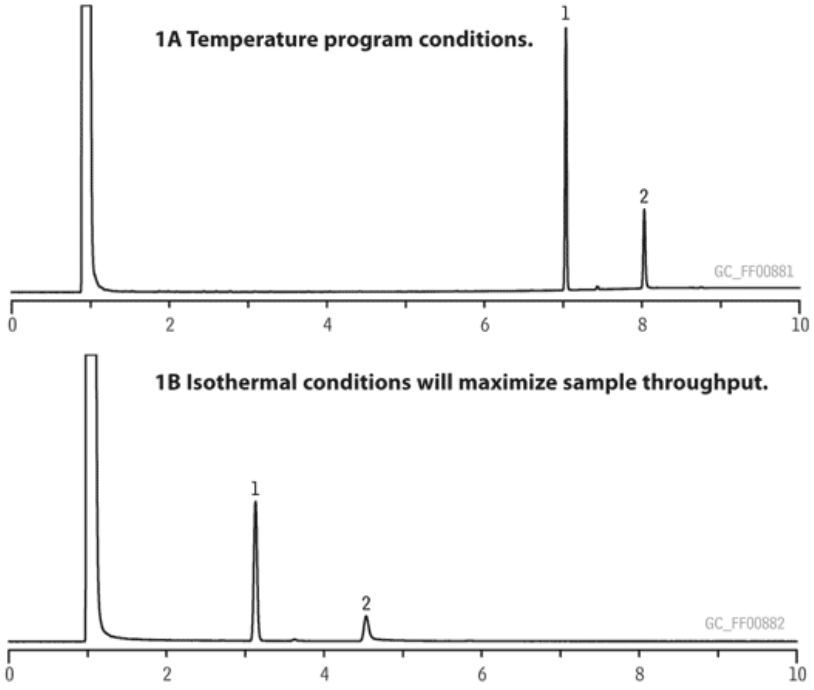
Table 1 Reproducible results for cholesterol, using an Rxi™-5ms column (n=9).

Analyte		%RSD
Quantitative Data, Area Count		
cholesterol*	32.5	1.78
cholesterol, derivatized**	17.3	1.08
Retention Time		
cholesterol*	8.030	0.008
cholesterol, derivatized**	8.111	0.044

*25µg/mL in dimethylformamide.
**50µg/mL in hexane.

Figure 1 Analyze underivatized cholesterol in less than 9 minutes, using an Rxi™-5ms column.

1. 5-α-cholestane (IS)
2. cholesterol

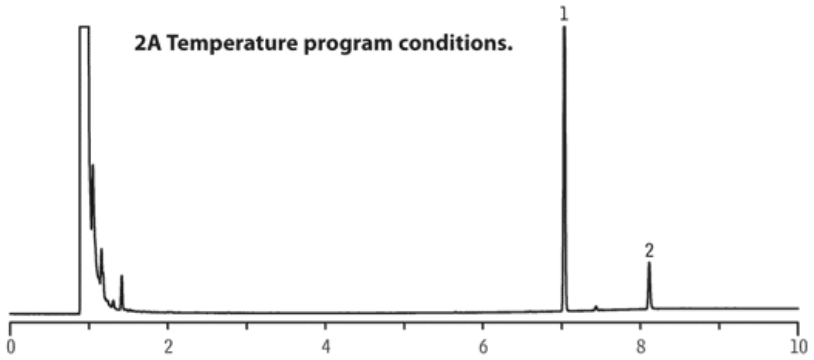


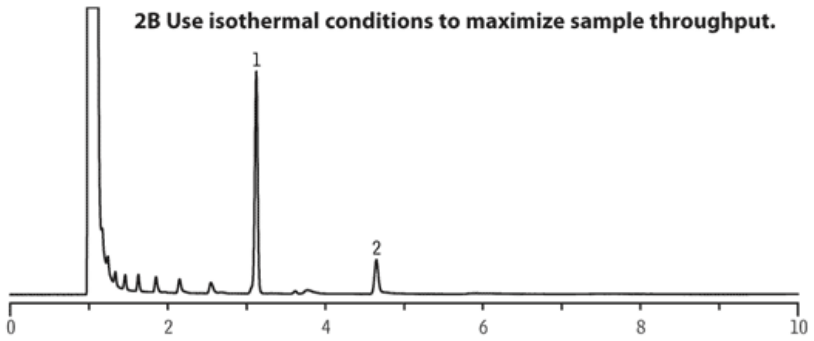
GC_FF00881, GC_FF00882

Column:	Rxi™-5ms, 15m, 0.25mm ID, 0.25µm (cat.# 13420)
	1000µg/mL cholesterol in DMF, 1000µg/mL 5-α-cholestane in hexane; 25ng cholesterol,
Sample:	150ng 5-α-cholestane on column
Inj.:	1.0µL, split (20:1), single gooseneck inlet liner w/ wool (cat.# 22405)
Inj. temp.:	250°C
Carrier gas:	helium, constant pressure (9.7psi @ 200°C)
Linear velocity:	24cm/sec.
Oven temp.:	Figure 1A: 200°C (hold 1 min.) to 330°C @ 20°C/min. (hold 7.5 min.) Figure 1B: 300°C (hold 10 min.)
Det.:	FID
Det. temp.:	340°C

Figure 2 Results for derivatized cholesterol are equal to those for underivatized cholesterol.

1. 5-α-cholestane (IS)
2. cholesterol





GC_FF00883, GC_FF00884

Column:	Rxi™-5ms, 15m, 0.25mm ID, 0.25µm (cat.# 13420)
	1000µg/mL cholesterol in hexane, 1000µg/mL 5-α-cholestane in hexane; 50ng derivatized cholesterol,
Sample:	150ng 5-α-cholestane on column
Inj.:	1.0µL, split (20:1), single gooseneck inlet liner w/ wool (cat.# 22405)
Inj. temp.:	250°C
Carrier gas:	helium, constant pressure (9.7psi @ 200°C)
Linear velocity:	24cm/sec.
	Figure 2A: 200°C (hold 1 min.) to 330°C @ 20°C/min. (hold 7.5 min.) Figure 2B:
Oven temp.:	300°C (hold 10 min.)
Det.:	FID
Det. temp.:	340°C

References

1. www.cholesterol lowdown.org /what_is_cholesterol/index.html
2. AOAC Official Methods, 15th ed., pp 976-977, 1103-1105 (1990).
3. AOCS Official Methods and Recommended Practices, 4th ed. (1994).

RELATED SEARCHES

derivatized cholesterol, *underivatized cholesterol*, *AOAC method 970.51E*, *AOAC method 976.26.2*, *AOAC 970.51E*, *AOAC 976.26.2*, *Rxi-5ms*

Rapid, Reproducible HPLC Analysis for Flavonoids in Cocoa

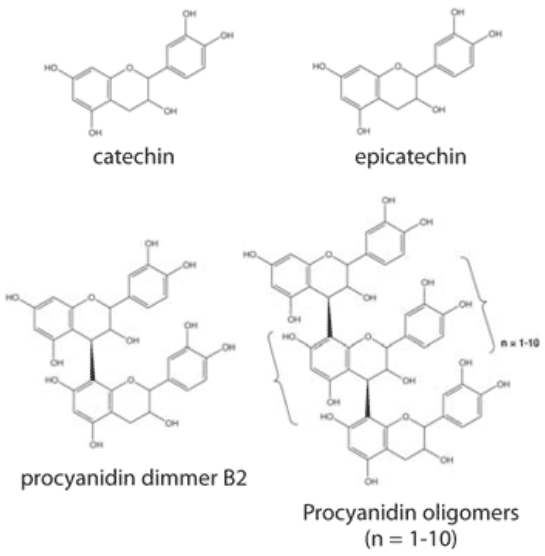
Using a LECO Unique® LC-TOFMS System and an Ultra Aqueous C18 Column

By Julie Kowalski, Restek Innovations Chemist, and Brian Shofran, LECO Corporation

- 15-minute screening for flavonoids.
- Excellent selectivity, using an Ultra Aqueous C18 column.
- Reliable identifications and reproducible results for complex samples.

Flavonoids are complex polyphenolic compounds, with diverse aromatic substitutions, that contribute to color, flavor, fragrance—and toxicity—of many foods. Interest in flavonoids has exploded because of links to antioxidant activity and, possibly, to control and prevention of disease.[1,2] Flavonoid contents of foods have been difficult to study, due to sample complexity and generally low abundances of the target compounds. Cocoa is rich in the flavan-3-ol flavonoids, including catechin, epicatechin, and procyanidin (Figure 1), and these are screened for as marker compounds. In finished chocolate and cocoa products, amounts of flavonoids depend primarily on the amounts of nonfat cocoa solids, on bean type, and on processing. Flavonoids can be destroyed by heat or other processing, like dutching, which is common in the production of cocoa and chocolate products.

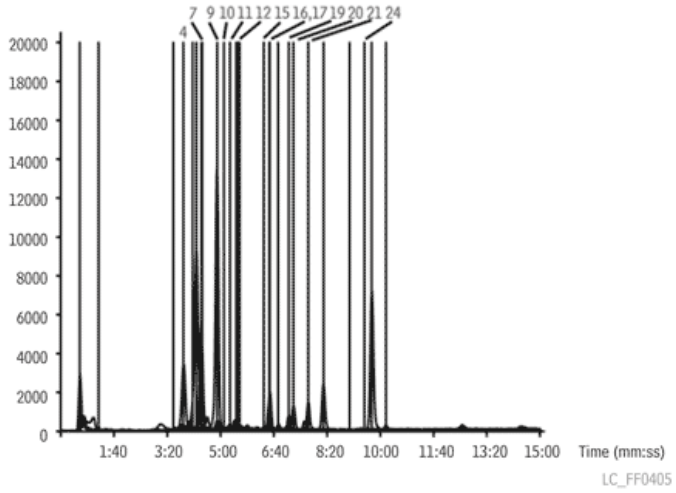
Figure 1 Flavan-3-ol flavonoids are screened for as marker compounds.



We developed a rapid screening method for catechin, epicatechin, and procyanidin content, and screened commercial cocoa products for flavan-3-ol content. We prepared samples by mixing the cocoa products with liquid nitrogen, powdering the frozen mixes, and extracting samples with deionized water:methanol (1:4). Extracts were centrifuged, concentrated, and filtered.[3] For a detailed description of sample preparation, refer to the LECO website www.leco.com.

An Ultra Aqueous C18 column is an excellent choice for this analysis, because it is designed to perform reversed phase separations well and reproducibly when the mobile phase has a high aqueous content. Using a 100mm x 2.1mm Ultra Aqueous C18 column and the automated peak find LECO ChromaTOF software in the Unique® LC-TOFMS system, we separated and identified 26 flavonoid compounds in a cacao sample (Figure 2 and Table 1).*

Figure 2 Extracted ion chromatogram of a cacao sample.



Sample:

Inj.:	5µL
Conc.:	500 mg sample extract
Sample diluent:	70% water/methanol
Autosampler temp:	10°C
Column:	**Ultra Aqueous C18**
Cat.# :	9178312
Dimensions:	100 x 2.1 mm
Particle size:	3µm
Pore size:	100Å

Conditions:

Mobile phase: A: 0.1% formic acid in water; B: acetonitrile:methanol, 50:50 (v/v)

Time (min.)	%B
0	10
10	60
15	60

Flow:	400µL/min.
Temp.:	30°C
Det.:	UV @ 210nm

Mass Spectrometry

Instrument:	Leco Unique® LC-TOFMS High Flow ESI Source
ESI voltage:	(-) 3500 V
Desolv. temp.:	300°C
Nebulizer pres.:	375kPa
Desolv. gas:	nitrogen, 7L/min.
Interface temp.:	100°C
Nozzle:	(-) 160V
Data acq. rate:	4 spectra/sec.

Numbered peaks are listed in Table 1

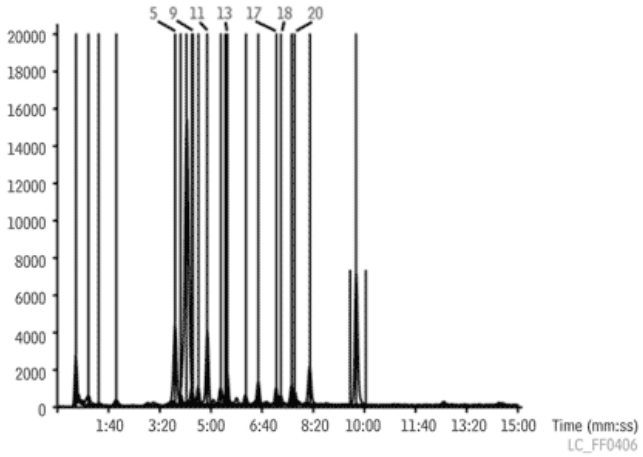
Table 1 Components in the cacao sample.

Peak	RT (min:sec)	Unique Mass	Area	Area %
1. unknown	00:35.5	273.0613	16924	3.9
2. unknown	01:11.0	383.3092	1821	0.4
3. unknown	03:31.2	294.181	5757	1.3
4. catechin (monomer)	03:50.4	289.1818	28618	6.7
5. unknown	04:07.7	369.1762	4530	1.1
6. unknown	04:14.4	305.1884	85897	20.0
7. procyanidin B2	04:24.0	577.3722	34559	8.0
8. unknown	04:25.9	278.1767	4378	1.0
9. epicatechin	04:53.8	289.1841	93682	21.8
10. procyanidin C1	05:06.2	865.5671	10221	2.4
11. procyanidin (tetramer)	05:17.8	1153.8179	1585	0.4
12. clovamide	05:29.3	358.2409	3528	0.8
13. unknown	05:33.1	275.2085	6160	1.4
14. unknown	05:36.0	353.177	1586	0.4
15. procyanidin II-g	06:21.1	737.4785	5246	1.2

16. procyanidin B5	06:31.7	577.3745	10339	2.4
17. procyanidin II-a	06:32.6	707.4643	4043	0.9
18. unknown	06:48.0	393.3242	2778	0.6
19. dideoxyclovamide	07:08.2	326.2384	4839	1.1
20. quercetin-galactoside	07:16.8	463.279	9471	2.2
21. quercetin-arabinoside	07:44.6	433.2524	9797	2.3
22. unknown	08:13.4	497.536	17417	4.1
23. unknown	09:02.4	201.191	3097	0.7
24. quercetin	09:30.2	301.1595	2179	0.5
25. unknown	09:43.7	723.8071	52646	12.3
26. unknown	10:10.6	391.2756	8550	2.0

Next, using the automated peak find software in ChromaTOF, we identified flavonoids in cocoa powder (Figure 3 and Table 2). Processing of cacao reduces the amount of catechins and procyanidins in cocoa components. If an alkalizing step is present in the process, this also leads to a remarkable decrease in the content of catechins and procyanidins. For peaks identified in the cocao and cocoa powder samples, retention time did not differ by more than 0.01 seconds (Tables 1 and 2). The analysis was completed and conditions returned to the initial mobile phase composition in 15 minutes.

Figure 3 The flavonoid composition of cocoa powder is readily distinguished from that of cacao, using our column and detection system.



Sample:
Inj.: 5µL
Conc.: 500 mg sample extract
Sample diluent: 70% water/methanol
Autosampler temp: 10°C
Column: **Ultra Aqueous C18**
Cat.# : 9178312
Dimensions: 100 x 2.1 mm
Particle size: 3µm
Pore size: 100Å
Conditions:
Mobile phase: A: 0.1% formic acid in water; B: acetonitrile:methanol, 50:50 (v/v)

Time (min.)	%B
0	10
10	60
15	60

Flow: 400µL/min.
Temp.: 30°C
Det.: UV @ 210nm
Mass Spectrometry
Instrument: Leco Unique® LC-TOFMS High Flow ESI Source
ESI voltage: (-) 3500 V
Desolv. temp.: 300°C
Nebulizer pres.: 375kPa
Desolv. gas: nitrogen, 7L/min.
Interface temp.: 100°C
Nozzle: (-) 160V

Data acq. rate: 4 spectra/sec.

Numbered peaks are listed in Table 2

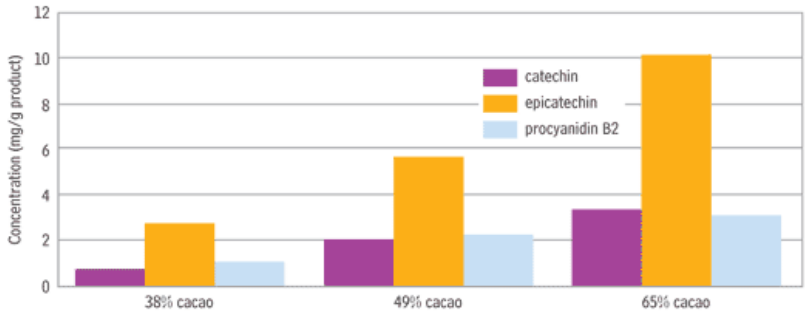
Table 2 Flavonoid components in cocoa powder exhibit virtually the same retention times as in cacao.

Peak	RT (min:sec)	Unique Mass	Area	Area %
1. Unknown	00:36.5	273.0620	16827	4.2
2. Unknown	01:00.5	405.1844	3064	0.8
3. Unknown	01:20.6	283.1889	2835	0.7
4. Unknown	01:55.2	299.1908	3542	0.9
5. catechin	03:50.4	289.1806	35151	8.7
6. Unknown	04:01.0	431.3734	4779	1.2
7. Unknown	04:12.5	305.1866	156954	38.9
8. Unknown	04:23.0	381.3214	6868	1.7
9. procyanidin B2	04:25.0	577.3661	3928	1.0
10. Unknown	04:35.5	381.3273	6601	1.6
11. epicatechin	04:52.8	289.1802	28030	6.9
12. Unknown	05:19.7	333.1894	9199	2.3
13. clovamide	05:28.3	358.2432	3287	0.8
14. Unknown	05:32.2	275.2074	12865	3.2
15. Unknown	06:08.6	333.1899	5070	1.3
16. Unknown	06:32.6	393.3275	9841	2.4
17. dideoxyclovamide	07:08.2	326.2279	7088	1.8
18. quercetin-galactoside	07:16.8	463.2485	6002	1.5
19. Unknown	07:37.9	516.4572	8285	2.1
20. quercetin-arabinoside	07:43.7	433.2532	6047	1.5
21. Unknown	08:13.4	497.5329	15347	3.8
22. Unknown	09:43.7	723.8036	52001	12.9

Subsequently, we analyzed three samples from Venezuela, containing differing amounts of cacao. Quantitative results were determined through ChromaTOF. Analytical results for these samples are shown in Figure 4. As expected, based on data in Table 1, epicatechin was substantially higher than catechin in each sample. Also as expected, catechin, epicatechin, and procyanidin B2 content increased with increasing amounts of cacao.

A LECO Unique® LC-TOFMS system and an Ultra Aqueous C18 column assure rapid, excellent resolution, reliable identification and quantification, and highly reproducible retention times for flavonoid compounds — even in very complex mixtures.

Figure 4 Concentrations of flavonoids in Venezuelan cacao, determined using an Ultra Aqueous C18 column and a LECO Unique® LC-TOFMS system.



References

1. Prior, R.L., et al., *Procyanidin and catechin content and antioxidant capacity of cocoa and chocolate products,*
J. Agric. Food Chem. 54: 4057-4061 (2006).
2. Hurst, W.J., *et al., Antioxidant activity and polyphenols and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States, J. Agric. Food Chem.* 54: 4062-4068 (2006).
3. Andreas-Lacueva, *et al., An LC method for the analysis of cocoa phenolics, LC*GC Eur.* 902-905 (2000).

Footnotes

*Cacao is the sum of the products derived from the cacao bean — chocolate liquor, cocoa, and cocoa butter(2).

LECO Corporation, 14950 Technology Court, Fort Myers, FL, 33912 54: 4062-4068 (2006).

RELATED SEARCHES

flavonoids, *cocoa*, *catechin*, *epicatechin*, *procyanidin*, *LECO Unique LC-TOFMS*, *ChromaTOF*, *cacao*, *polyphenols*, *Ultra Aqueous C18*

Rapid Screening Method for Carbamates in Orange Oil

Using an Ultra Carbamate HPLC Column

By Julie Kowalski, PhD., Innovations Chemist

- Fast analysis times, for increased sample throughput.
- Simple methodology saves time—no sample preparation.
- Accurate mass identification, for definitive results.

Concern over the presence of pesticides in food products, particularly citrus, is growing, resulting in an increasing number of countries regulating insecticides such as carbamates. EPA Method 531.1 describes a method for the analysis of carbamates in water, but not in other commodities. Matrices like citrus oil contain numerous interferences and often require time-consuming sample preparation. However, the method described here requires no sample preparation and provides fast analysis times, significantly increasing sample throughput.

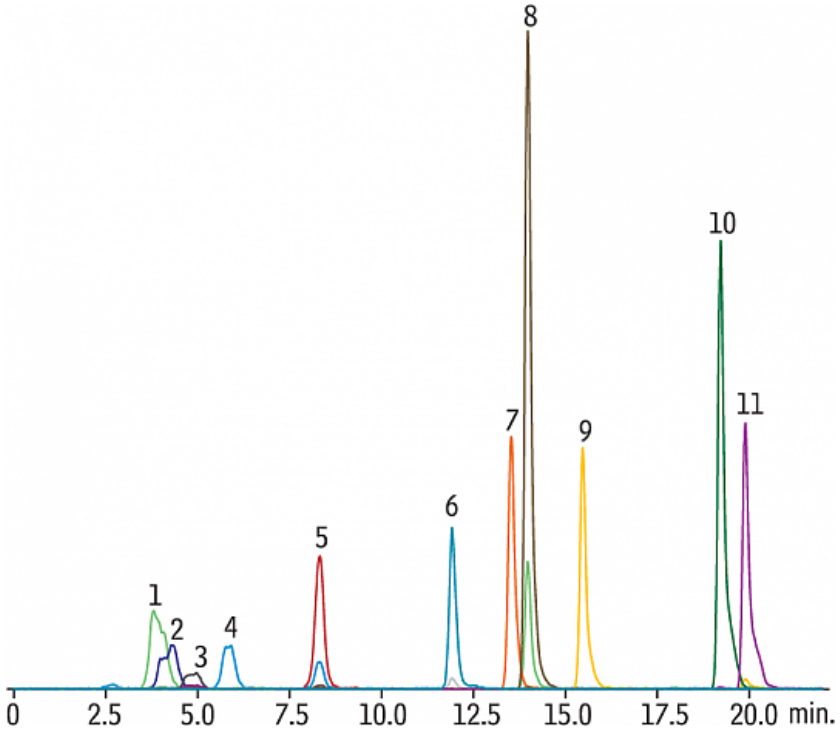
Fast analysis times and no sample preparation allow faster sample throughput.

Carbamates are most easily determined via HPLC analysis because derivatization is required for GC analysis. The rapid screening method shown here uses the Ultra Carbamate HPLC column, which is designed specifically for analyzing carbamates and is compatible with both traditional detectors and mass spectrometry. This column works well with mass spectrometry amenable buffers and allows an initial mobile phase composition of 20% organic, which promotes complete ionization at the electrospray source.

Orange oil was spiked at 10ppm with a carbamate mix and analyzed (Figures 1-2). The monoisotopic masses and retention times were compared to an injected standard and found to match closely (Table I). The high mass accuracy of the Leco Unique TOF- MS allowed positive analyte identification, even in a complex mixture containing compounds with the same nominal mass (within 1 amu) as the target carbamate. By using the Ultra Carbamate column in conjunction with the Leco Unique TOF- MS, we were able to develop a quick, easy, and accurate screening method for carbamates in a complex matrix such as orange oil.

Figure 1 Reference standard carbamates resolve quickly on an Ultra Carbamate HPLC column. (extracted ion chromatograms)

Peaks	Peaks
1. Aldicarb sulfone	6. Aldicarb
2. Aldicarb sulfoxide	7. Propoxur
3. Oxamyl	8. Carbofurane
4. Methomyl	9. Carbaryl
5. 3-Hydroxycarbofuran	10. Methiocarb
	11. BDMC (IS)



LC_FF0473

Column	Ultra Carbamate (cat.# 9177352)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	3 µm
Pore Size:	100 Å
Temp.:	ambient
Sample	531.1 Carbamate Pesticide Calibration Mixture (cat.# 32273) 4-bromo-3,5-dimethylphenyl-N-methylcarbamate (BDMC) (cat.# 32274)
Diluent:	methanol
Conc.:	50 µg/mL
Inj. Vol.:	1 µL
Mobile Phase	
A:	2 mM ammonium acetate:methanol (v/v, 90/10)
B:	2 mM ammonium acetate:methanol (v/v, 10/90)

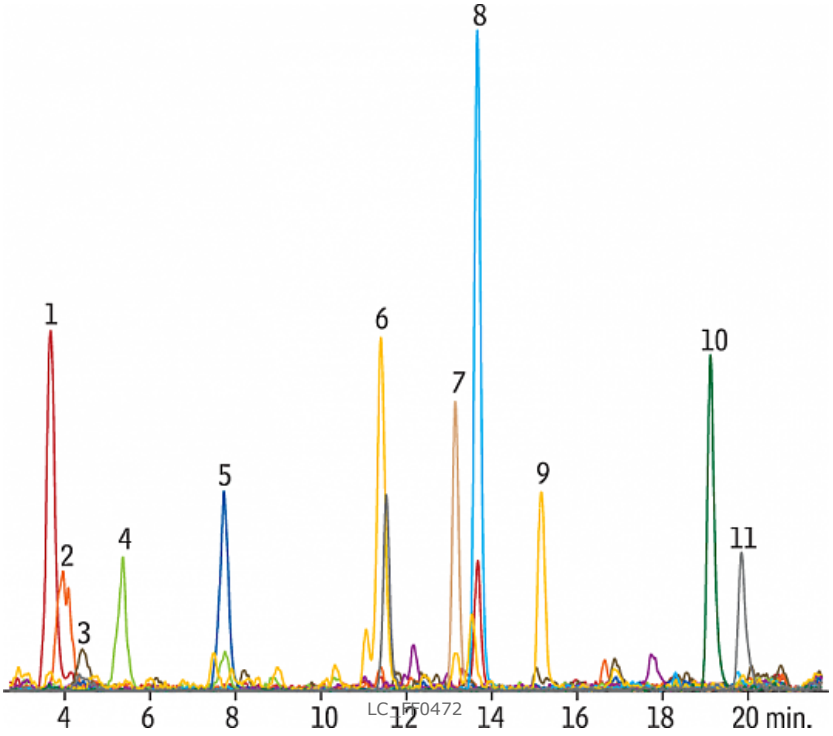
Time (min)	%A	%B
0.00	80	20
20	0	100
25	0	100

Flow:	0.2 mL/min
Detector	LECO Unique® TOFMS
Run Length:	25 min
Ionization Source Type:	high flow ESI
Ion Mode:	positive
Desolvation Temp.:	130 °C
Nebulizing Pressure:	100 kPa
Desolvation Gas (N2):	4 L/min
Interface Temp.:	120 °C
Nozzle Voltage:	62 V
Capillary Voltage:	2.75 kV
Instrument	Agilent 1100
Acknowledgement	LECO Corporation

Figure 2 Positive identification of carbamates in orange oil injected with no sample preparation. (extracted ion chromatograms)

Peaks

1. Aldicarb sulfone
2. Aldicarb sulfoxide
3. Oxamyl
4. Methomyl
5. 3-Hydroxycarbofuran
6. Aldicarb
7. Propoxur
8. Carbofuran
9. Carbaryl
10. Methiocarb
11. BDMC (IS)



Column
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 3 µm
Pore Size: 100 Å
Temp.: ambient

Sample
531.1 Carbamate Pesticide Calibration Mixture (cat.# 32273)
4-bromo-3,5-dimethylphenyl-N-methylcarbamate (BDMC) (cat.# 32274)
Diluent: methanol
Conc.: 10 ppm spiked into unprocessed orange oil
Inj. Vol.: 3 µL

Mobile Phase
A: 2 mM ammonium acetate:methanol, 90:10
B: 2 mM ammonium acetate:methanol, 10:90

Time (min)	%B
0.00	20
20	100
25	100

Flow: 0.2 mL/min

Detector LECO Unique® TOFMS
Ionization Source Type: high flow ESI
Ion Mode: positive
Nebulizing Pressure: 100 kPa
Desolvation Gas (N2): 4 L/min
Interface Temp.: 120 °C
Nozzle Voltage: 62 V
Capillary Voltage: 2.75 kV

Table I Carbamates were positively identified in matrix using both retention time and mass.

		Calculated ion monoisotopic mass	Standard ion monoisotopic mass	Standard Retention Time (min.)	Orange oil ion monoisotopic mass	Orange oil Retention Time (min.)
aldicarb sulfone	[M+H]+	223.075	223.099	3.81	223.142	3.67
aldicarb sulfoxide	[M+H]+	207.080	207.103	4.31	207.122	4.09
oxamyl	[M+NH4]+	237.102	237.085	4.97	237.110	4.41
methomyl	[M+H]+	163.054	163.074	5.84	163.086	5.36
3-hydroxycarbofuran	[M+H]+	238.108	238.121	8.32	238.128	7.73
aldicarb	[M+H]+	191.085	191.0728 116.0751*	11.92	116.052*	11.53
propoxur	[M+H]+	210.113	210.152	13.53	210.153	13.14
carbofuran	[M+H]+	222.113	222.140	13.98	222.120	13.66
carbaryl	[M+H]+	202.087	202.084	15.48	202.101	15.17
methiocarb	[M+H]+	226.090	226.097	19.22	226.060	19.12
BDMC	[M+H]+	258.013	258.042	19.89	258.005	19.84

** m/z 116.052 is a fragment ion with higher intensity than the [M+H]+ ion and was used for identification in*

orange oil

RELATED SEARCHES

Carbamates, *ultra carbamate*, *pesticide residue*, *epa 531.1*, *epa method 531.1*, *orange*, *orange oil*, *citrus oil*

Rapid Characterization of Garlic Volatiles—No Sample Prep Required!

Using Headspace GC/MS and an Rxi®-5ms Capillary Column

By Julie Kowalski, Innovations Chemist; Michelle Long, Innovations Chemist; Jason Thomas, Innovations Chemist; and William Goodman†, GC/MS Applications Specialist

- No sample preparation! Eliminate complicated steps required by other methods.
- Rapid screening of garlic-specific flavor and odor compounds.
- Speedy determination of volatiles profile.

Introduction:

Garlic, Allium sativum (L)., is a member of the onion family and is related to onions, shallots, and leek. It has a rich history in cooking and is characterized by a strong hot flavor which sweetens as it is cooked. Garlic has also been used for medicinal purposes for thousands of years and, at times, has been claimed to help prevent everything from high cholesterol to cancer. Recently, garlic supplements have gained popularity for boosting immune and cardiovascular health. Determining garlic flavor and odor components is important to the food industry since the quality of garlic and garlic powder affects overall food quality. Similarly, chromatographic methods for garlic are used by the dietary supplements industry to detect garlic volatiles that may affect the acceptability of supplements to the consumer.

Garlic odor and flavor components are produce enzymatically from precursors when the plant is cut or crushed. Allicin is the key compound of interest produced in this manner but it degrades quickly to other sulfide compounds. These sulfide degradents help produce garlicky odor and taste and are more stable than allicin and retain health benefits. Many of these components are volatile and thus are well-suited for headspace analysis.

This work describes the determination of garlic flavor and odor components by headspace gas chromatography mass spectrometry (HS GC/MS). The method shown here requires no sample preparation making the bench work simple and fast. Other methods of analysis involve steam distillation, solid phase trapping solvent exchange, headspace solid phase microextraction, and simultaneous distillation and solvent extraction which can be difficult and time-consuming.

Method:

This comparative analysis of fresh garlic and garlic powder was done on a PerkinElmer TurboMatrix 40 Trap Headspace Sampler. Several headspace conditions were adjusted to optimize the comparison. The column and injection pressures used were 15psi. Column flow is dictated by the headspace unit pressure when the headspace unit is directly coupled to the GC column. The vial was pressurized for 1 min., with a thermostat time of 15min. The oven, needle, and transfer temperatures were 80°C, 90°C and 110°C respectively. A 0.32mm ID IP deactivated guard column was used as a transfer line from the headspace unit to the GC oven. The transfer line was inserted into the injector of the Clarus 500 in a manner which allowed the flow from the HS-trap to be split (10:1). The analytical column used was a 30m x 0.25mm ID x 1.0µm Rxi®-5ms column. The GC oven temperature program started with an initial temperature of 35°C (1 min.) increasing at 15°C/min. to 20°C then increasing at 45°C/min. to the final temperature of 300°C. The mass spectrometer was used as the detector with a scan range of 35-350amu.

Results:

Several sulfur components were identified including allyl methylsulfide, 3,3'-thiobis-1-propene, allyl mercaptan and diallyl disulfide. Diallyl disulfide appeared to be the dominant component for both garlic preparations. The fingerprint, or relative ratios, of the other components were distinct for fresh garlic and powdered garlic. Figure 1 shows the total ion chromatograms and for both fresh garlic and garlic powder.

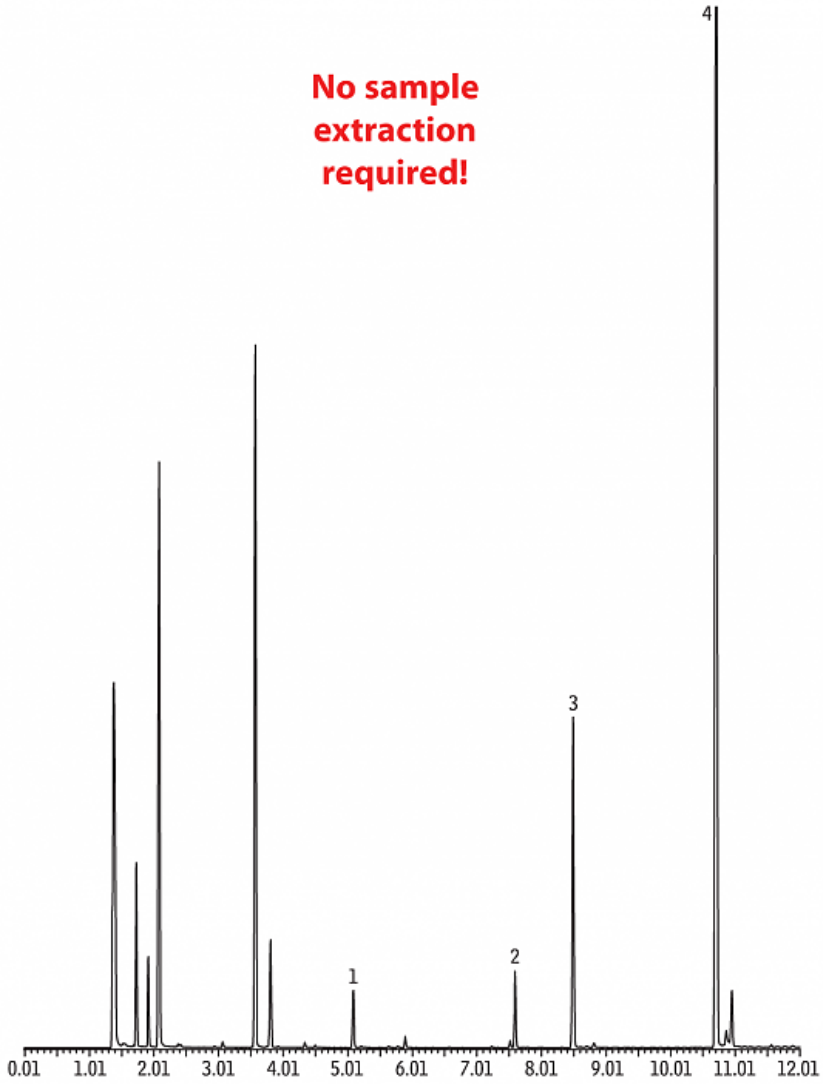
Conclusions:

This work demonstrates the simplicity of using headspace GC/MS for rapid characterization of garlic and garlic powder samples. The experimental set-up is ideal for both screening analysis and low-level trace analysis. This method will allow for rapid determination of garlic quality and could be used for the determination of low levels of sulfur containing compounds from odorless supplements.

Figure 1 Rapid screening of garlic volatiles—analyze samples in less than 11 minutes!

Peaks
1. Allyl methylsulfide
2. 3,3'-Thiobis-1-propene

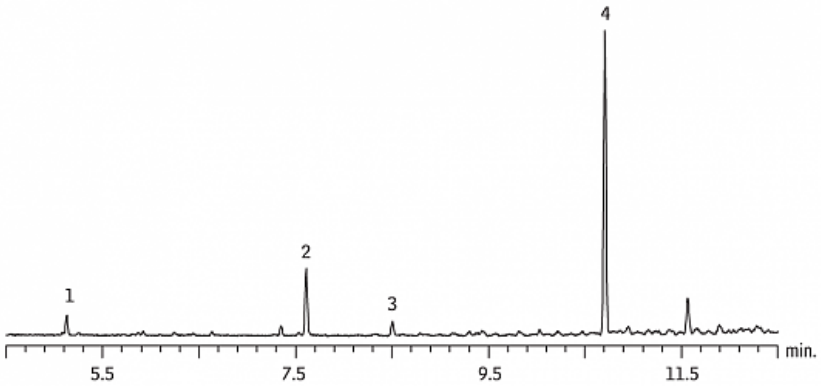
Peaks
3. Allyl mercaptan
4. Diallyl disulphide



GC_FF00958

Column	Rxi® -5ms, 30 m, 0.25 mm ID, 1.00 µm (cat.# 13453) using IP Deactivated Guard Column 5 m, 0.32 mm ID (cat.# 10044)
Sample	Fresh garlic
Injection	headspace-trap
Headspace-Trap	
Instrument:	PerkinElmer Turbomatrix 40 Trap Headspace Sampler
Column Pressure:	15 psi
Inj. Time:	0.02 min
Inj. Temp.:	220 °C
Inj. Pressure:	30 psi
Withdraw Time:	0.2 min
Transfer Line Temp.:	110 °C
Oven Temp.:	80 °C
Needle Temp.:	90 °C
Vial Pressurize Time:	1 min
Thermostat Time:	15 min
Oven	
Oven Temp.:	35 °C (hold 1 min) to 220 °C at 15 °C/min to 300 °C at 45 °C/min
Carrier Gas	He, constant flow
Flow Rate:	1.5 mL/min
Detector	MS
Mode:	Scan
Ionization Mode:	EI
Scan Range:	35-350 amu
Notes	Injection: split (10:1)

2. 3,3'-Thiobis-1-propene 4. Diallyl disulphide



GC_FF00959

Column	Rxi® -5ms, 30 m, 0.25 mm ID, 1.00 µm (cat.# 13453) using IP Deactivated Guard Column 5 m, 0.32 mm ID (cat.# 10044)
Sample	Garlic Powder
Injection	headspace-trap
Headspace-Trap	
Instrument:	PerkinElmer Turbomatrix 40 Trap Headspace Sampler
Column Pressure:	15 psi
Inj. Time:	0.02 min
Inj. Temp.:	220 °C
Inj. Pressure:	30 psi
Withdraw Time:	0.2 min
Transfer Line Temp.:	110 °C
Oven Temp.:	80 °C
Needle Temp.:	90 °C
Vial Pressurize Time:	1 min
Thermostat Time:	15 min
Oven	
Oven Temp.:	35 °C (hold 1 min) to 220 °C at 15 °C/min to 300 °C at 45 °C/min
Carrier Gas	He, constant flow
Flow Rate:	1.5 mL/min
Detector	MS
Mode:	Scan
Ionization Mode:	EI
Scan Range:	35-350 amu
Notes	Injection: split (10:1)

RELATED SEARCHES

Garlic, *fresh garlic*, *rxi-5ms*, *sulfides*, *garlic powder*, *headspace trap*, *headspace*, *Rxi-5ms*

Q-sep™ New Product Supplement

Restek Q-sep™ QuEChERS Products

Fast, Simple Sample Prep for Multi-Residue Pesticide Analysis

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

QuEChERS methods are fast, easy, and cost-effective, and Restek Q-sep™ products make QuEChERS procedures even simpler. All sorbents and sample tubes are included—no specialized equipment or glassware is required. Prepare samples more efficiently with a complete line of QuEChERS supplies from Restek.

MORE!

Visit **www.restek.com/quechers** for new products & detailed technical information.

Table I Prepare samples more quickly, easily, and cost-effectively with QuEChERS.

	Mini-Luke or Modified Luke Method	QuEChERS	Savings with QuEChERS
Estimated time to process 6 samples (min.)	120	30	4x faster
Solvent used (mL)	60-90	10	6-9x less solvent
Chlorinated waste (mL)	20-30	0	Safer, cheaper, greener
Glassware/specialized equipment	capacity for 200mL, quartz wool, funnel, water bath or evaporator	none	Ready-to-use

Q-sep™ QuEChERS Sample Prep Packets & Tubes

Description	Material	Methods	qty.	cat#
Extraction Salt Packets and 50mL Centrifuge Tubes				
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
Q150 kit	6g $MgSO_4$, 1.5g NaOAc, with 50mL Centrifuge Tube	AOAC 2007.01	50 packets & 50 tubes	26237
Q150 packets	6g $MgSO_4$, 1.5g NaOAc	AOAC 2007.01	50 packets	26238
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
Q212	150mg $MgSO_4$, 25mg PSA, 2.5mg GCB	European EN 15662	100-pk.	26217
Q213	150mg $MgSO_4$, 25mg PSA, 7.5mg GCB	European EN 15662	100-pk.	26218
Q250	150mg $MgSO_4$, 50mg PSA	AOAC 2007.01	100-pk.	26124
Q251	150mg $MgSO_4$, 50mg PSA, 50mg C18	AOAC 2007.01	100-pk.	26125
Q253	150mg $MgSO_4$, 50mg PSA, 50mg GCB		100-pk.	26123
Q252	150mg $MgSO_4$, 50mg PSA, 50mg C18, 50mg GCB	AOAC 2007.01	100-pk.	26219
15mL Centrifuge Tubes for dSPE (clean-up of 6mL extract)				
Q350	1200mg $MgSO_4$, 400mg PSA	AOAC 2007.01	50-pk.	26220
Q351	1200mg $MgSO_4$, 400mg PSA, 400mg C18	AOAC 2007.01	50-pk.	26221
Q352	1200mg $MgSO_4$, 400mg PSA, 400mg C18, 400mg GCB	AOAC 2007.01	50-pk.	26222
Q370	900mg $MgSO_4$, 150mg PSA	European EN 15662	50-pk.	26223
Q371	900mg $MgSO_4$, 150mg PSA, 15mg GCB	European EN 15662	50-pk.	26224
Q372	900mg $MgSO_4$, 150mg PSA, 45mg GCB	European EN 15662	50-pk.	26225
Q373	900mg $MgSO_4$, 150mg PSA, 150mg C18		50-pk.	26226
Q374	900mg $MgSO_4$, 300mg PSA, 150mg GCB		50-pk.	26126

TSCD = trisodium citrate dihydrate
DHS = disodium hydrogen citrate sesquihydrate
NaOAc = sodium acetate

Sorbent Guide

Sorbent	Removes
PSA*	sugars, fatty acids, organic acids, anthocyanine pigments
C18	lipids, nonpolar interferences
GCB**	pigments, sterols, nonpolar interferences

*PSA—primary and secondary amine exchange material
**GCB—graphitized carbon black

Q-sep™ 3000 Centrifuge

- Meets requirements of AOAC and European QuEChERS methodology.
- Supports 50mL, 15mL, and 2mL centrifuge tubes.
- Small footprint requires less bench space.
- Safe and reliable—UL, CSA, and CE approved, 1-year warranty.

Centrifuge includes 50mL tube carriers (6), 50mL conical tube inserts (6), 4-place 15mL tube carriers (6), and 2mL tube adaptors (24).

Description	qty.	cat.#
Q-sep 3000 Centrifuge, 110V	ea.	26230
Q-sep 3000 Centrifuge, 220V	ea.	26231
Replacement Accessories		
50mL Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26232
50mL Conical Tube Insert for Q-sep 3000 Centrifuge	6-pk.	26249
4-Place Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26233
2mL Tube Adaptors for Q-sep 3000 Centrifuge	4-pk.	26234

GC and HPLC Columns

Rxi®-5Sil MS

(low polarity Crossbond® silarylene phase; selectivity same as DB-5MS)

- Engineered to be a low bleed fused silica GC/MS column.
- Excellent inertness for active compounds.
- Temperature range: -60°C to 350°C.

ID	df (µm)	temp. limits	30-Meter
0.25mm	0.25	-60 to 330/350°C	13623
	0.50	-60 to 330/350°C	13638
ID	**df (µm)**	**temp. limits**	**20-Meter**
0.18mm	0.18	-60 to 330/350°C	43602
	0.36	-60 to 330/350°C	43604

Ultra Aqueous C18 Columns (USP L1)

Physical Characteristics:

particle size: 3µm or 5µm, spherical; pore size: 100Å; carbon load: 15%; endcap: no; pH range: 2.5 to 7.5; temperature limit: 80°C

Chromatographic Properties:

Highly retentive and selective for reversed phase separations of polar analytes. Highly base-deactivated. Compatible with highly aqueous (up to 100%) mobile phases.

	1.0mm ID	2.1mm ID	3.2mm ID	4.6mm ID
Length	cat.#	cat.#	cat.#	cat.#
3µm Columns				
30mm	9178331	9178332	9178333	9178335
50mm	9178351	9178352	9178353	9178355
100mm	9178311	9178312	9178313	9178315
5µm Columns				
30mm	9178531	9178532	9178533	9178535
50mm	9178551	9178552	9178553	9178555
100mm	9178511	9178512	9178513	9178515
150mm	9178561	9178562	9178563	9178565
200mm	9178521	9178522	9178523	9178525
250mm	9178571	9178572	9178573	9178575

QuEChERS Standards

- Ready to use for QuEChERS extractions—no dilutions necessary.
- Support for GC and HPLC with MS, MS/MS, and selective detectors.

QuEChERS Internal Standard Mix for GC/ECD Analysis

PCB 18
PCB 28
PCB 52
tris-(1,3-dichloroisopropyl) phosphate

50µg/mL each in acetonitrile, 5mL/ampul

cat. # 33265 (ea.)

QuEChERS Internal Standard Mix for GC/MS Analysis

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

In acetonitrile, 5mL/ampul

cat. # 33267 (ea.)

QuEChERS Internal Standard Mix for GC/NPD and LC/MS/MS Analysis

triphenyl phosphate 20µg/mL
tris-(1,3-dichloroisopropyl)phosphate 50µg/mL

In acetonitrile, 5mL/ampul

cat. # 33266 (ea.)

QuEChERS Single-Component Reference Standards

Concentration is µg/mL. ACN=acetonitrile

Compound	Solvent	Conc.	cat.# (ea.)
PCB 18 (5mL)	ACN	50	33255
PCB 28 (5mL)	ACN	50	33256
PCB 52 (5mL)	ACN	50	33257
PCB 138 (5mL)	ACN	50	33262
PCB 153 (5mL)	ACN	50	33263
triphenylmethane (5mL)	ACN	10	33260
triphenylphosphate (5mL)	ACN	20	33258
tris(1,3-dichloroisopropyl) phosphate (5mL)	ACN	50	33259

QuEChERS Internal Standard Mix for LC/MS/MS Analysis

nicarbazin

10µg/mL in acetonitrile, 5mL/ampul

cat. # 33261 (ea.)

QuEChERS Quality Control Standards for GC/MS Analysis

PCB 138 PCB 153

50µg/mL each in acetonitrile, 5mL/ampul

cat. # 33268 (ea.)

anthracene

100µg/mL in acetonitrile, 5mL/ampul

cat. # 33264 (ea.)

Lit. Cat.# FFTS1199A

Save Time and Money with QuEChERS

- Ready-to-use extraction and dSPE tubes, no glassware required.
- Preweighed adsorbents for dSPE cleanup.
- Convenient, method-specific internal and QC standards.

Quick, Easy, Cheap, Effective, Rugged, and Safe, the QuEChERS ("catchers") method is a fast, simple, and effective alternative to conventional sample prep for multiresidue pesticide analysis. QuEChERS is based on work done by the U.S. Department of Agriculture Eastern Regional Research Center in Wyndmoor, PA.[1] Researchers there were looking for a simple, effective, and inexpensive way to extract and clean pesticide residues from the many varied sample matrices that they worked with routinely. They had been using the modified Luke extraction method, which is highly effective and rugged, but is solvent, labor, and glassware intensive, leading to a relatively high cost per sample. In contrast, QuEChERS employs a very short shake-extraction step, making it faster and less labor intensive. Solid phase extraction cleanup of extracts from other methods also had been effective, but the complex matrices the investigators were dealing with required multiple individual cartridges to remove the many classes of interferences, which added significant cost and complexity to the process. To reduce costs and speed up sample preparation, the[illegible] tion (dSPE) technique, which effectively removes sugars, lipids, organic acids, sterols, proteins, pigments and excess water, but is far simpler and less expensive than conventional methods (Table I).

Using QuEChERS, samples are prepared in three simple steps. As shown on the following page, samples are first homogenized, then extracted and partitioned with an organic solvent and salt solution, with the extracts finally cleaned using the dSPE technique. Using the dSPE approach, the quantity and type of sorbents can easily be optimized for different matrix interferences and difficult analytes. Results from this approach have been verified and modified at several USDA and Food and Drug Administration labs, and the method now is widely accepted for many types of pesticide residue samples. Validation and proficiency data for the QuEChERS method are available for a wide variety of pesticides in several common food matrices at **www.quechers.com**

Restek Q-sep™ products make QuEChERS even simpler. All extraction salts, adsorbents, and sample tubes are included—no specialized equipment or glassware is required. The dSPE centrifuge tube format, available in 2 mL and 15 mL sizes, contains magnesium sulfate (to partition water from organic solvent) and PSA adsorbent (to remove sugars and fatty acids), with or without graphitized carbon (to remove pigments and sterols) or C18 (to remove nonpolar interferences). Custom products are available by request. If you are frustrated with the time and expense of your current pesticide sample cleanup procedure, we suggest you try this simple, economical new method.

Table I: Prepare samples more quickly, easily, and cost-effectively with QuEChERS.

	Mini-Luke or Modified Luke Method	QuEChERS	Savings with QuEChERS
Estimated time to process 6 samples (min)	120	30	4x faster
Solvent used (mL)	60-90	10	6-9x less solvent
Chlorinated waste (mL)	20-30	0	Safer, cheaper, greener
Glassware/specialized equipment	capacity for 200 mL, quartz wool, funnel, water bath, or evaporator	none	Ready-to-use

Try QuEChERS risk-free today!

Call 1-814-353-1300 or 1-800-356-1688 to request a free sample pack of Q-sep™ QuEChERS tubes.

Quick and Easy...

Prepare Samples for LC or GC Analysis in 3 Simple Steps

1. Blend

Homogenize the sample.

See QuEChERS in Action!

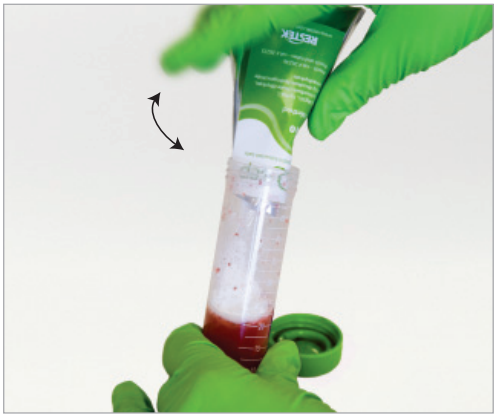
Restek Learning Network

visit **www.restek.com/webinars**

2. Extract and Dry

Add acetonitrile and internal standard, then shake vigorously for 1 minute.

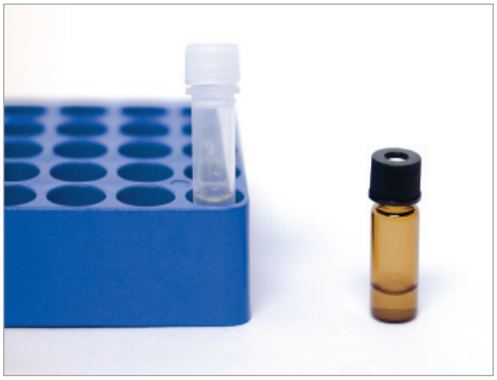
Add buffering salts and shake, then centrifuge for 5 minutes to separate the phases.



3. Clean Up

Transfer supernatant to dSPE tube.

Shake, centrifuge, and transfer to an autosampler vial for analysis by GC or LC.

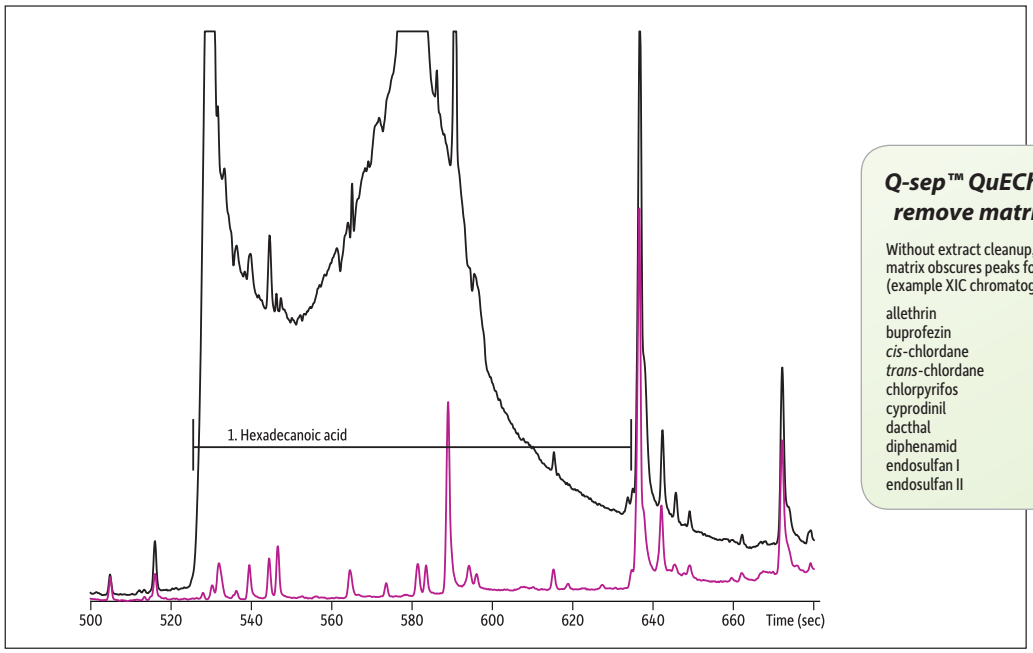


Effective...

QuEChERS dSPE Cleanup Assures Optimal Results for Pesticide Analysis

- Improves integration and mass spectral matches.
- Removes matrix interferences that obscure target analytes or cause ion suppression.
- Protects GC inlet, and LC and GC columns from contamination.

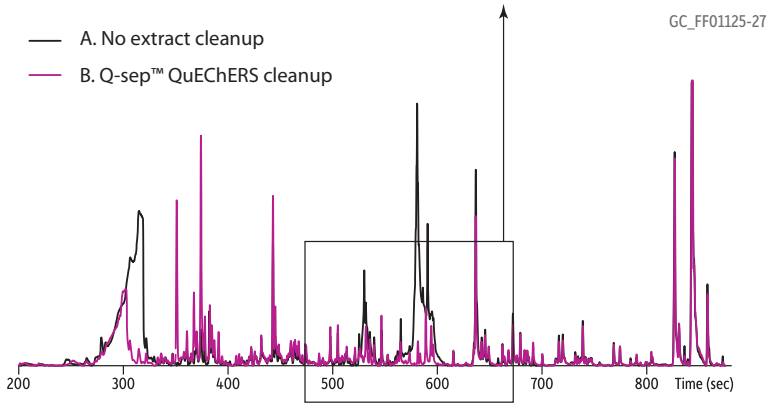
Figure 1: QuEChERS dSPE cleanup removes interferences that obscure target pesticides.



Q-sep™ QuEChERS tubes easily remove matrix interferences.

Without extract cleanup, hexadecanoic acid from the matrix obscures peaks for all the following pesticides. (example XIC chromatogram = endosulfan I)

allethrin
buprofezin
cis-chlordane
trans-chlordane
chlorpyrifos
cyprodinil
dacthal
diphenamid
endosulfan I
endosulfan II
fenthion
metolachlor
myclobutanil
oxyfluorfen
pendimethalin
pentachlorothioanisole
pirimiphos methyl
triadimefon
triadimenol

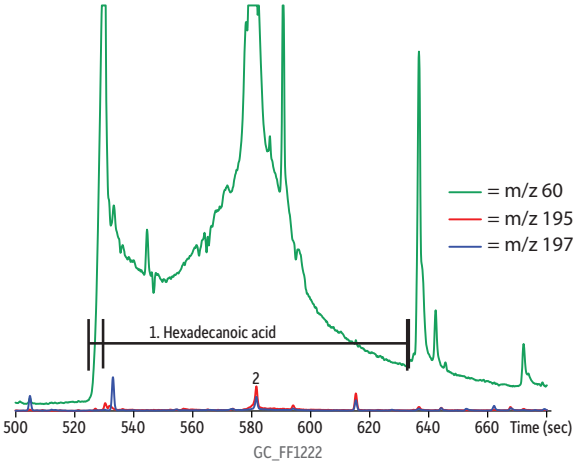


Column	Rxi®-5Sil MS, 20 m, 0.18 mm ID, 0.18 µm (cat.# 43602)
Sample	Sweet potato spiked with pesticide mix and extracted with acetonitrile and Q-sep™ QuEChERS EN Method 15662 extraction salts
Injection	
Inj. Vol.:	1.0 µL splitless (hold 1 min)
Liner:	Gooseneck splitless (4 mm) w/deact. wool (cat.# 22405)
Inj. Temp.:	250 °C
Oven	
Oven Temp:	72.5 °C (hold 1 min) to 350 °C at 20 °C/min
Carrier Gas	He, constant flow
Flow Rate:	1.2 mL/min

Detector	MS
Mode:	
Transfer Line Temp.:	300 °C
Analyzer Type:	TOF
Ionization Mode:	EI
Acquisition Range:	45-550 amu
Instrument	Agilent/HP6890 GC
Notes	A. Extract (without cleanup step) acidified with formic acid to pH 5 B. Extract with cleanup using Q-sep™ QuEChERS dSPE cleanup tube (cat.# 26124) acidified with formic acid to pH 5. Scan range: m/z 60, 73, 87, 129, 256 plotted

Figure 2: QuEChERS dSPE cleanup significantly improves quantification and identification.

Without cleanup, matrix masks Endosulfan I.

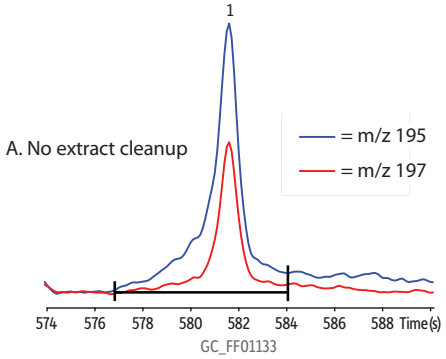


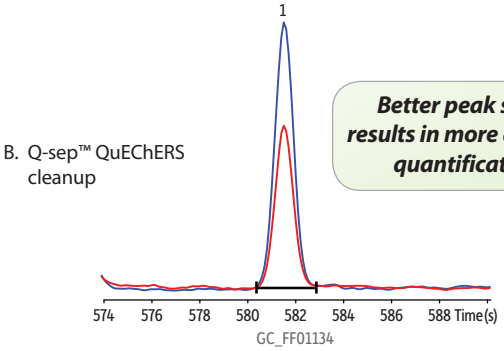
Peak List
1. Hexadecanoic acid
2. Endosulfan I

Column:	Rxi®-5Sil MS, 20 m, 0.18 mm ID, 0.18 µm (cat.# 43602)
Sample:	sweet potato spiked with pesticide mix, extracted with acetonitrile and Q-sep™ QuEChERS EN Method 15662 extraction salts, then acidified with formic acid to pH 5
Inj.:	1.0 µL splitless (hold 1 min.), 4 mm single gooseneck liner with w/wool (cat.# 22405)
Inj. temp.:	250°C
Carrier gas:	helium, constant flow
Flow rate:	1.2 mL/min.
Oven temp.:	72.5°C (hold 1 min.) to 350°C @ 20°C/min.
Det:	TOFMS
Transfer line temp.:	225°C
Scan range:	45-550 amu, m/z 60, 195, 197 plotted
Ionization:	EI
Instrument:	Agilent 6890, LECO Pegasus III

QuEChERS dSPE cleanup improves quantification and identification.

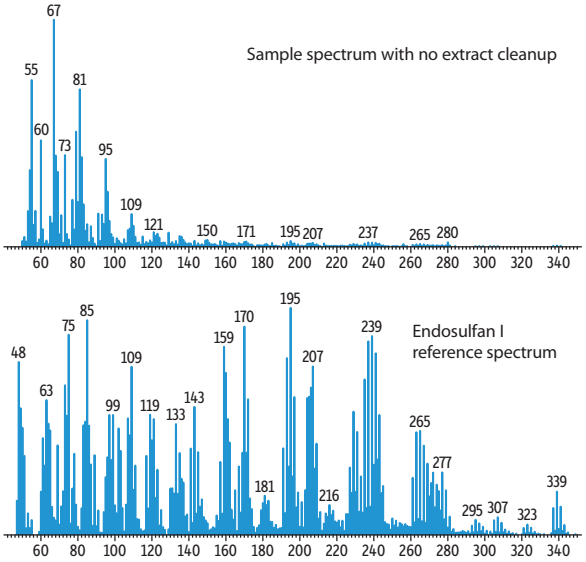
Peak Integration (extracted ion chromatograms)

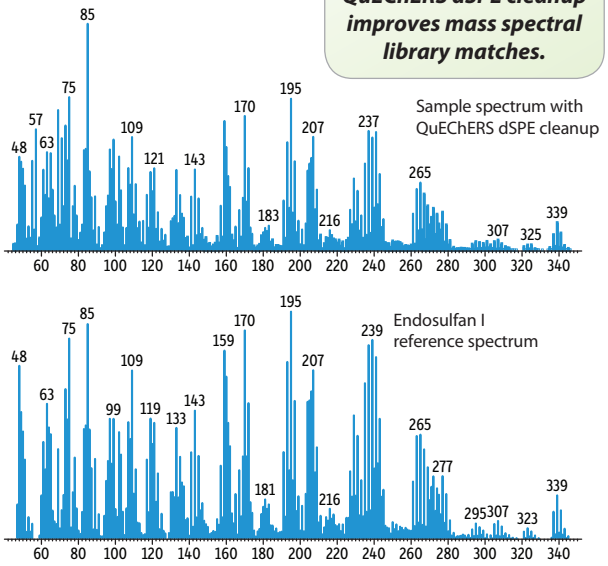




Better peak shape results in more accurate quantification.

Spectral Identification





QuEChERS dSPE cleanup improves mass spectral library matches.

Optimize Analysis with Sorbent Choice

Choosing a QuEChERS dSPE Sorbent

Primary and secondary amine exchange material (PSA) is the base sorbent used for QuEChERS dSPE cleanup of fruit and vegetable extracts because it removes many organic acids and sugars that might act as instrumental interferences. In addition, C18 or graphitized carbon black (GCB) may be used to remove lipids or pigments, respectively. Choice of sorbent should be based on matrix composition and target analyte chemistry. Most methods make specific recommendations for acidic, basic, and planar pesticides, which may require additional considerations.

As seen in Table II, GCB can have a negative effect on the recoveries of certain pesticides that can assume planar shapes (e.g. chlorothalonil and thiabendazole). The work shown here was done with 50 mg GCB per mL extract, which emphasizes this effect. The EN 15662 QuEChERS method recommends less GCB, which improves recoveries of planar pesticides, but still assures the removal of pigments that can degrade GC-MS performance. To simplify and speed up sample prep, Restek QuEChERS tubes are available in the sorbent combinations and amounts specified by EN 15662 and AOAC methods.

Table II: Select sorbents based on matrix and target analyte chemistry. (Percent recovery using C18 or GCB, relative to PSA alone).

Rt (min)	pesticide	CAS Number	action/use	classification	C18*	GCB**
9.50	dichlorvos	62-73-7	insecticide	organophosphorus	111	116
9.67	methamidophos	10265-92-6	insecticide	organophosphorus	105	107
11.75	mevinphos	7786-34-7	insecticide	organophosphorus	112	130
12.02	*o*-phenylphenol	90-43-7	fungicide	unclassified	106	97
12.14	acephate	30560-19-1	insecticide	organophosphorus	128	147
13.89	omethoate	1113-02-6	insecticide	organophosphorus	120	119
14.74	diazinon	333-41-5	insecticide	organophosphorus	108	127
14.98	dimethoate	60-51-5	insecticide	organophosphorus	124	151
15.69	chlorothalonil	1897-45-6	fungicide	organochlorine	125	13
15.86	vinclozolin	50471-44-8	fungicide	organochlorine	102	98
16.21	metalaxyl	57837-19-1	fungicide	organonitrogen	105	117
16.28	carbaryl	63-25-2	insecticide	carbamate	114	111
16.60	malathion	121-75-5	insecticide	organophosphorus	124	160
16.67	dichlofluanid	1085-98-9	fungicide	organohalogen	122	103
17.51	thiabendazole	148-79-8	fungicide	organonitrogen	88	14
17.70	captan	133-06-2	fungicide	organochlorine	88	91
17.76	folpet	133-07-3	fungicide	organochlorine	108	63
18.23	imazalil	35554-44-0	fungicide	organonitrogen	115	95
18.39	endrin	72-20-8	insecticide	organochlorine	104	101
18.62	myclobutanil	88671-89-0	fungicide	organonitrogen	119	114
19.07	4,4-DDT	50-29-3	insecticide	organochlorine	102	95
19.22	fenhexamid	126833-17-8	fungicide	organochlorine	118	77
19.40	propargite 1	2312-35-8	acaricide	organosulfur	110	95
19.43	propargite 2	2312-35-8	acaricide	organosulfur	121	114
19.75	bifenthrin	82657-04-3	insecticide	pyrethroid	106	81
20.04	dicofol	115-32-2	acaricide	organochlorine	98	54
20.05	iprodione	36734-19-7	fungicide	organonitrogen	118	90
20.21	fenpropathrin	39515-41-8	insecticide	pyrethroid	113	96
21.32	*cis*-permethrin	52645-53-1	insecticide	pyrethroid	106	65
21.47	*trans*-permethrin	51877-74-8	insecticide	pyrethroid	109	71
23.74	deltamethrin	52918-63-5	insecticide	pyrethroid	97	52

*50 mg PSA, 50 mg C18, **50 mg PSA, 50 mg GCB

$$\% \text{ recovery} = \frac{\text{RRF C18 or GCB}}{\text{RRF PSA}} \times 100$$

Strawberry extracts were spiked at 200 ng/mL with pesticides and subjected to dSPE with PSA only. Results were used to generate single point calibration curves. Spiked extracts were then subjected to additional dSPE sorbents (either C18 or GCB). Results are shown as percent recoveries relative to PSA alone.

Sorbent Guide

Sorbent	Removes
PSA*	sugars, fatty acids, organic acids, anthocyanine pigments
C18	lipids, nonpolar interferences
GCB**	pigments, sterols, nonpolar interferences

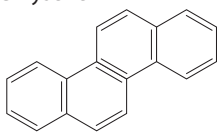
*PSA—primary and secondary amine exchange material
**GCB—graphitized carbon black

Example dSPE Cleanup: PAHs in Infant Formula

Analyzing polycyclic aromatic hydrocarbons (PAHs) in infant formula can be difficult as both the target analytes and certain matrix elements are lipophilic in nature and difficult to separate. Proper sorbent choice is critical to removing matrix interferences, while assuring good PAH recoveries. When choosing a sorbent, target analyte and matrix component chemistry must be considered. PAHs are relatively nonpolar, planar compounds with no pH-dependent functional groups. Infant formula typically contains significant amount of sugars and can be fortified with fatty acids.

Here, PSA was chosen for dSPE cleanup since both sugars and fatty acids can be removed through hydrogen bonding. Using PSA to remove these matrix compounds is optimal, because it will not bind to the relatively nonpolar PAHs, thus ensuring they remain available for analysis. GCB is not recommended here because it also can bind planar PAHs. (Note: GCB is not needed since infant formula does not contain pigments.) Based on the chemical structure of the analytes of interest, as well as the most dominant matrix compounds, PSA is the best choice when analyzing PAHs in infant formula.

Chrysene



Phenanthrene

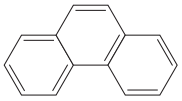
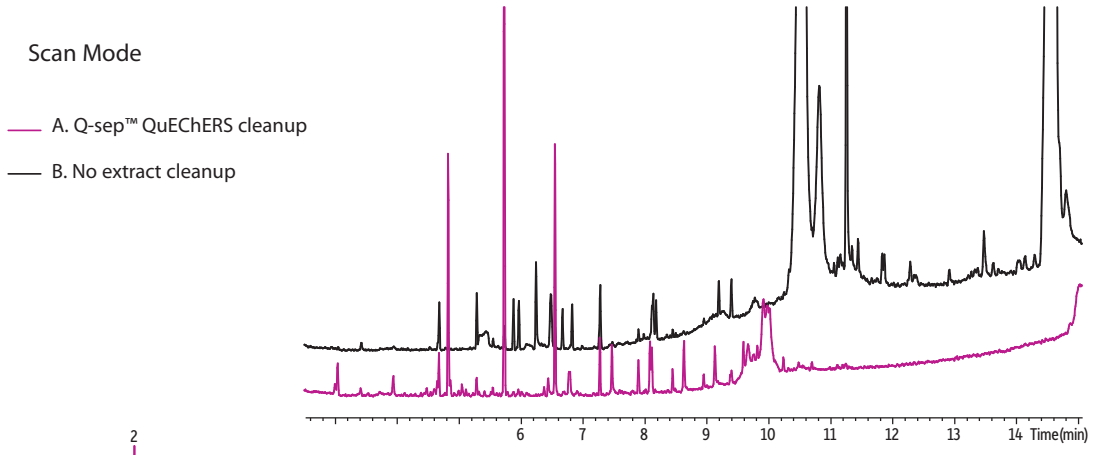
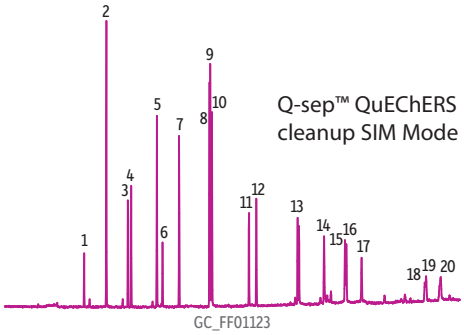


Figure 3: PSA is ideal for removing matrix sugars and fatty acids, while leaving PAHs behind for analysis.





Compound list and ions monitored (SIM mode)			
Compound	m/z		
1. decafluorobiphenyl (SS)	265	10. Anthracene	178
2. Naphthalene	128	11. Fluoranthene	202
3. 2-Methylnaphthalene	142	12. Pyrene	202
4. 1-Methylnaphthalene	142	13. Benzo[a]anthracene	228
5. Acenaphthylene	152	14. Chrysene	228
6. Acenaphthene	152	15. Benzo[b]fluoranthene	252
7. Fluorene	166	16. Benzo[k]fluoranthene	252
8. Phenanthrene-d10 (IS)	188	17. Benzo[a]pyrene	252
9. Phenanthrene	178	18. Indeno[1,2,3-cd]pyrene	276
		19. Dibenz[a,h]anthracene	278
		20. Benzo[ghi]perylene	276

Column Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)
Sample See notes
Injection
Inj. Vol.: 1 µL splitless (hold 0.15 min)
Liner: 3.5 mm Gooseneck Splitless w/Wool (cat.# 22286-200.1)
Inj. Temp.: 300 °C
Oven
Oven Temp: 50 °C (hold 0.5 min) to 290 °C at 25 °C/min to 320 °C at 5 °C/min (hold 5 min)
Carrier Gas He, constant flow
Flow Rate: 1.4 mL/min

Detector MS
Mode: Scan
Transfer Line Temp.: 290 °C
Analyzer Type: Quadrupole
Ionization Mode: EI
Scan Range: 100-350 amu
Instrument Shimadzu 2010 GC & QP2010+ MS
Notes Sample Details:
Liquid infant formula spiked at 1 µg/mL with decafluorobiphenyl (cat.# 31842) and EPA Method 8310 PAH mixture (cat.# 31841) and at 0.5 µg/mL with internal standard phenanthrene-d10 (cat.# 31045), then extracted with acetonitrile and Q-sep™ QuEChERS extraction salts.
A. Extract with cleanup using Q-sep™ QuEChERS dSPE cleanup tube (cat.# 26215)
B. Extract without dSPE cleanup

QuEChERS Methods for Complex and Varied Matrices

QuEChERS has been successfully applied to many different types of matrices. When developing procedures for your lab, start with these selected references—or visit **www.restek.com/quechers** for an expanded version that includes hyperlinks. (Note: references not available from Restek.)

General/Original

1. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce. (M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC International 86 (2003) 412.)
2. QuEChERS—A Mini-Multiresidue Method for the Analysis of Pesticide Residues in Low-Fat Products. (http://www.quechers.com (accessed July 15, 2008).)
3. Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. (AOAC Official Method 2007.01.)
4. Foods of Plant Origin—Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Cleanup by Dispersive SPE (QuEChERS-method). (EN 15662 Version 2008.)

General Fruits and Vegetables

5. Validation of a Fast and Easy Method for the Determination of Residues from 229 Pesticides in Fruits and Vegetables Using Gas and Liquid Chromatography and Mass Spectrometric Detection. (S.J. Lehotay, A. de Kok, M. Hiemstra, P. Van Bodegraven, J. AOAC Int. 88 (2005) 595.)
6. Multiresidue Analysis of 102 Organophosphorus Pesticides in Produce at Parts-Per-Billion Levels Using a Modified QuEChERS Method and Gas Chromatography with Pulsed Flame Photometric Detection. (F. Schenck, J. Wong, C. Lu, J. Li, J.R. Holcomb, L.M. Mitchell, J. AOAC Int. 92 (2009) 561.)

Dairy and Fatty Matrices

7. Evaluation of the QuEChERS Sample Preparation Approach for the Analysis of Pesticide Residues in Olives. (S.C. Cunha, S.J. Lehotay, K. Mastovska, J.O. Fernandes, M. Beatriz, P.P. Oliveira, J. Sep. Sci. 30 (2007) 620.)
8. Dispersive Solid-Phase Extraction Followed by Liquid Chromatography-Tandem Mass Spectrometry for the Multi-Residue Analysis of Pesticides in Raw Bovine Milk. (T. Dagnac, M. Garcia-Chao, P. Pulleiro, C. Garcia-Jares, M. Llompart, J. Chromatogr. A 1216 (2009) 3702.)

Grains, Nuts, and Seeds

9. A Multi-Residue Method for the Determination of 203 Pesticides in Rice Paddies Using Gas Chromatography/Mass Spectrometry. (T.D. Nguyen, E.M. Han, M.S. Seo, S.R. Kim, M.Y. Yun, D.M. Lee, G.H Lee, Anal. Chim. Acta 619 (2008) 67.)
10. Development of a Multi-Residue Method for the Determination of Pesticides in Cereals and Dry Animal Feed Using Gas Chromatography-Tandem Quadrupole Mass Spectrometry II. Improvement and Extension to New Analytes. (S. Walorczyk, J. Chromatogr. A 1208 (2008) 202.)

Oils

11. Simplified Pesticide Multiresidue Analysis of Soybean Oil by Low-Temperature Cleanup and Dispersive Solid-Phase Extraction Coupled with Gas Chromatography/Mass Spectrometry. (L. Li, Y. Xu, C. Pan, Z. Zhou, S. Jianc, F. Liu, J. AOAC Int. 90 (2007) 1387.)

Baby Food

12. Determination of 142 Pesticides in Fruit- and Vegetable-Based Infant Foods by Liquid Chromatography/Electrospray Ionization-Tandem Mass Spectrometry and Estimation of Measurement Uncertainty. (J. Wang, D. Leung, J. AOAC Int. 92 (2009) 279.)
13. Method for Routine Screening of Pesticides and Metabolites in Meat Based Baby-Food Using Extraction and Gas Chromatography-Mass Spectrometry. (C. Przybylski, C. Segard, J. Sep. Sci. 32 (2009) 1858.)

Non-Food Matrices

14. Multiresidue Analytical Method Using Dispersive Solid-Phase Extraction and Gas Chromatography/Ion Trap Mass Spectrometry to Determine Pharmaceuticals in Whole Blood. (F. Plössl, M. Giera, F. Bracher, J. Chromatogr. A 1135 (2006) 19.)
15. Comparison of Four Extraction Methods for the Analysis of 24 Pesticides in Soil Samples with Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Ion Trap-Mass Spectrometry. (C. Lesueur, M. Gartner, A. Mentler, M. Fuerhacker, Talanta 75 (2008) 284.)

Muscle and Tissues

16. The Development and Validation of a Multiclass Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Procedure for the Determination of Veterinary Drug Residues in Animal Tissue Using a QuEChERS (QUick, Easy, CHeap, Effective, Rugged and Safe) Approach. (G. Stubbings, T. Bigwood, Anal. Chim. Acta 637 (2009) 68.)

Q-sep™ QuEChERS Products

Fast, Simple Sample Prep for Multiresidue Pesticide Analysis

- Ready-to-use tubes, no glassware required.
- Preweighed, ultra-pure sorbents.
- Support original unbuffered, AOAC (2007.01) and European (EN 15662) QuEChERS methods.

QuEChERS methods are fast, easy, and cost-effective, and Restek Q-sep™ products make QuEChERS procedures even simpler. All extraction salts, sorbents and sample tubes are included—no specialized equipment or glassware is required. Prepare samples more efficiently with a complete line of QuEChERS supplies from Restek.

MORE!

Visit **www.restek.com/quechers** for new products & detailed technical information.

Q-sep™ QuEChERS Sample Prep Packets & Tubes

Q-sep™ QuEChERS Extraction Salts

- Salt packets eliminate the need for a second empty tube to transfer salts.
- Go green by using packets with reusable tubes.
- Convenient and easy to use.

26235

Description	Material	Methods	qty.	cat#
Q-sep Kit	4 g $MgSO_4$, 1 g NaCl with 50 mL Centrifuge Tube	Original Unbuffered	50 packets & 50 tubes	23991
Q-sep Packets	4 g $MgSO_4$, 1 g NaCl	Original Unbuffered	50 packets	23992
Q-sep Kit	4 g $MgSO_4$, 1 g NaCl, 1 g TSCD, 0.5 g DHS with 50 mL Centrifuge Tube	European EN 15662	50 packets & 50 tubes	26235
Q-sep Packets	4 g $MgSO_4$, 1 g NaCl, 1 g TSCD, 0.5 g DHS	European EN 15662	50 packets	26236
Q-sep Kit	6 g $MgSO_4$, 1.5 g NaOAc with 50 mL Centrifuge Tube	AOAC 2007.01	50 packets & 50 tubes	26237
Q-sep Packets	6 g $MgSO_4$, 1.5 g NaOAc	AOAC 2007.01	50 packets	26238
Empty 50 mL Centrifuge Tube, Polypropylene			50-pk.	26239
Empty 50 mL Centrifuge Tube, FEP			2-pk.	23997

TSCD—trisodium citrate dihydrate; DHS—disodium hydrogen citrate sesquihydrate; NaOAc—sodium acetate

Q-sep™ QuEChERS dSPE Tubes for Extract Cleanup

- Packaged in mylar subpacks for enhanced protection and storage stability.
- Individually labeled tubes for easy sorbent identification.

26126

26123

Description	Methods	qty.	cat#
2 mL Micro-Centrifuge Tubes for dSPE (cleanup of 1 mL extract)			
150 mg $MgSO_4$, 25 mg PSA	Original Unbuffered, Mini-Multiresidue, European EN 15662	100-pk.	26215
150 mg $MgSO_4$, 25 mg PSA, 25 mg C18	Mini-Multiresidue	100-pk.	26216
150 mg $MgSO_4$, 25 mg PSA, 2.5 mg GCB	Mini-Multiresidue, European EN 15662	100-pk.	26217
150 mg $MgSO_4$, 25 mg PSA, 7.5 mg GCB	Mini-Multiresidue, European EN 15662	100-pk.	26218
150 mg $MgSO_4$, 50 mg PSA	AOAC 2007.01	100-pk.	26124
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18	AOAC 2007.01	100-pk.	26125
150 mg $MgSO_4$, 50 mg PSA, 50 mg GCB	AOAC 2007.01	100-pk.	26123
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, 50 mg GCB	AOAC 2007.01	100-pk.	26219
150 mg $MgSO_4$, 50 mg C18	NA	100-pk.	26242
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, 7.5 mg GCB	Universal	100-pk.	26243
15 mL Centrifuge Tubes for dSPE (cleanup of 6 mL and 8 mL extract)			
1200 mg $MgSO_4$, 400 mg PSA	AOAC 2007.01	50-pk.	26220
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18	AOAC 2007.01	50-pk.	26221
1200 mg $MgSO_4$, 400 mg PSA, 400 mg C18, 400 mg GCB	AOAC 2007.01	50-pk.	26222
1200 mg $MgSO_4$, 400 mg C18	similar to AOAC 2007.01	50-pk.	26244
900 mg $MgSO_4$, 150 mg PSA	Original Unbuffered, European EN 15662	50-pk.	26223
900 mg $MgSO_4$, 150 mg PSA, 15 mg GCB	European EN 15662	50-pk.	26224
900 mg $MgSO_4$, 150 mg PSA, 45 mg GCB	European EN 15662	50-pk.	26225
900 mg $MgSO_4$, 150 mg PSA, 150 mg C18	similar to European EN 15662	50-pk.	26226
900 mg $MgSO_4$, 300 mg PSA, 300 mg C18, 45 mg GCB	similar to European EN 15662	50-pk.	26245
900 mg $MgSO_4$, 300 mg PSA, 150 mg GCB	NA	50-pk.	26126

PSA—primary and secondary amine exchange material; GCB—graphitized carbon black

Sorbent Guide

Sorbent	Removes
PSA	sugars, fatty acids, organic acids, anthocyanine pigments
C18	lipids, nonpolar interferences
GCB	pigments, sterols, nonpolar interferences

Q-sep™ Accessories

Q-sep™ Bottle Top Solvent Dispenser

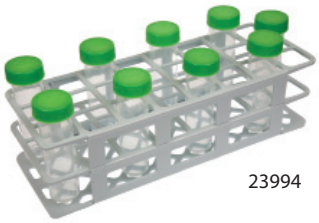
- Adjustment knob offers 56 output volume settings from 2.5 mL to 30 mL per stroke (0.5 mL increments)—ideal for QuEChERS methods!
- Base features 30 mm threads and includes four adaptors (25 mm, 28 mm, 38 mm, and 45 mm).
- Individually calibrated in accordance with ISO 8655 standards (certificate included) and can also be recalibrated by the user.
- PTFE, glass, and polypropylene construction for excellent chemical compatibility and 100% autoclavability.
- Integral safety discharge reduces risk of accidental dispensing and nozzle cap prevents dripping.
- Easy to disassemble for cleaning and servicing.

Accurately and precisely dispense liquids for QuEChERS extractions with this versatile pump. A quick, simple adjustment lets you set the output volume anywhere from 2.5 mL to 30 mL per stroke, and the included adaptors will accommodate most reagent bottles.

Description	qty.	cat.#
Q-sep Bottle Top Solvent Dispenser, 2.5 mL - 30 mL	ea.	23990

Q-sep™ Tube Racks

- Available for 2 mL, 15 mL, and 50 mL tubes.
- Alphanumerical grid reference on top tier for easy identification of samples.
- Easy to assemble, simply fold and snap together securely.



23994

23995

Description	Size	Material	qty.	cat.#
Q-sep Tube Rack for 2 mL Centrifuge Tube	Holds 100	Polypropylene, White	ea.	23995
Q-sep Tube Rack for 15 mL Centrifuge Tube	Holds 60	Polypropylene, White	ea.	23993
Q-sep Tube Rack for 50 mL Centrifuge Tube	Holds 24	Polypropylene, White	ea.	23994

Q-sep™ 3000 Centrifuge

- Meets or exceeds requirements of original unbuffered, AOAC, and European QuEChERS methodology.
- Supports 50 mL, 15 mL, and 2 mL centrifuge tubes.
- Small footprint requires less bench space.
- Safe and reliable—UL, CSA, and CE approved, 1-year warranty.

Centrifuge includes 50 mL tube carriers (6), 50 mL conical tube inserts (6), 4-place 15 mL tube carriers (6), and 2 mL tube adaptors (24).

Dimensions:
9"h x 14.5"w x 17"d
(22.9 cm x 36.8 cm
x 43.2 cm)

Description	qty.	cat.#
Q-sep 3000 Centrifuge, 110V	ea.	26230
Q-sep 3000 Centrifuge, 220V	ea.	26231
Replacement Accessories		
50 mL Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26232
50 mL Conical Tube Insert for Q-sep 3000 Centrifuge	6-pk.	26249
4-Place Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26233
2 mL Tube Adaptors for Q-sep 3000 Centrifuge	4-pk.	26234

GC and HPLC Columns

Rxi®-5Sil MS Columns (fused silica)

(low polarity Crossbond® silarylene phase; similar to 5% phenyl/95% dimethyl polysiloxane)

- Engineered to be a low bleed GC-MS column.
- Excellent inertness for active compounds.
- Temperature range: -60 °C to 350 °C.

Description	temp. limits	cat.#
20 m, 0.18 mm ID, 0.18 µm	-60 to 330/350 °C	43602
20 m, 0.18 mm ID, 0.36 µm	-60 to 330/350 °C	43604
30 m, 0.25 mm ID, 0.25 µm	-60 to 330/350 °C	13623
30 m, 0.25 mm ID, 0.50 µm	-60 to 330/350 °C	13638

Ultra Aqueous C18 Columns (USP L1)

particle size: 3µm or 5µm, spherical
pore size: 100Å
carbon load: 15%
endcap: no
pH range: 2.5 to 8
temperature limit: 80 °C

Highly retentive and selective for reversed phase separations of polar analytes. Highly base-deactivated. Compatible with highly aqueous (up to 100%) mobile phases.

Length		1.0 mm ID cat.#	2.1 mm ID cat.#	3.2 mm ID cat.#	4.6 mm ID cat.#
3µm Columns	30 mm	9178331	9178332	9178333	9178335
	50 mm	9178351	9178352	9178353	9178355
	100 mm	9178311	9178312	9178313	9178315
	150 mm	9178361	9178362	9178363	9178365
5µm Columns	30 mm	9178531	9178532	9178533	9178535
	50 mm	9178551	9178552	9178553	9178555
	100 mm	9178511	9178512	9178513	9178515
	150 mm	9178561	9178562	9178563	9178565
	200 mm	9178521	9178522	9178523	9178525
	250 mm	9178571	9178572	9178573	9178575

Inlet Liners

23303.1

For Agilent GCs equipped with split/splitless inlets

Sky™ 2.0 mm ID Inlet Liners for Agilent MMI

2.0 mm ID x 6.4 mm x 78.5 mm Length	ea.	5-pk.
Single Taper w/Dimple, Sky Technology, Borosilicate Glass	23334.1	23334.5

Sky™ 4.0 mm ID Single Taper Inlet Liner w/ Wool

4.0 mm ID x 6.5 mm OD x 78.5 mm Length	ea.	5-pk.	25-pk.
Single Taper, Sky Technology, Wool	23303.1	23303.5	23303.25

Sky™ 4.0 mm ID Single Taper Inlet Liner

4.0 mm ID x 6.5 mm OD x 78.5 mm Length	ea.	5-pk.	25-pk.
Single Taper, Sky Technology	23302.1	23302.5	23302.25

Sky™ 4.0 mm ID Cyclo Double Taper Inlet Liner

4.0 mm ID x 6.5 mm OD x 78.5 mm Length	ea.	5-pk.	25-pk.
Cyclo Double Taper, Sky Technology	23310.1	23310.5	23310.25

Sky™ 4.0 mm ID Double Taper Inlet Liner

4.0 mm ID x 6.5 mm OD x 78.5 mm Length	ea.	5-pk.	25-pk.
Double Taper, Sky Technology	23308.1	23308.5	23308.25

5.0 mm ID Single Taper Inlet Liner

5.0 mm ID x 6.5 mm OD x 78.5 mm Length	ea.	5-pk.
Single Taper, Intermediate Polarity (IP), Borosilicate Glass	22973	22974

5.0 mm ID Single Taper Inlet Liner w/ Wool

5.0 mm ID x 6.5 mm OD x 78.5 mm Length	ea.	5-pk.
Single Taper, Intermediate Polarity (IP), Deact. Wool, Borosilicate Glass	22973-200.1	22974-200.5

QuEChERS Standards

- Ready to use for QuEChERS extractions—no dilutions necessary.
- Support for GC and HPLC with MS, MS/MS, and selective detectors.

QuEChERS Internal Standard Mix for GC-ECD Analysis (4 components)

PCB 18 PCB 52
PCB 28 tris-(1,3-dichloroisopropyl)phosphate

50 µg/mL each in acetonitrile, 5 mL/ampul cat.# 33265 (ea.)

QuEChERS Internal Standard Mix for GC-MS

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

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

QuEChERS Internal Standard Mix for GC-NPD and LC-MS/MS Analysis

triphenyl phosphate 20µg/mL
tris-(1,3-dichloroisopropyl)phosphate 50µg/mL

In acetonitrile, 5 mL/ampul cat.# 33266 (ea.)

QuEChERS Single-Component Reference Standards

Concentration is µg/mL.

Compound	CAS #	Solvent	Conc.	cat.#
PCB 18 (5 mL)	37680-65-2	ACN	50	33255
PCB 28 (5 mL)	7012-37-5	ACN	50	33256
PCB 52 (5 mL)	35693-99-3	ACN	50	33257
PCB 138 (5 mL)	35065-28-2	ACN	50	33262
PCB 153 (5 mL)	35065-27-1	ACN	50	33263
triphenylmethane (5 mL)	519-73-3	ACN	10	33260
triphenylphosphate (5 mL)	115-86-6	ACN	20	33258
tris(1,3-dichloroisopropyl) phosphate (5 mL)	13674-87-8	ACN	50	33259

ACN = acetonitrile

QuEChERS Internal Standard Mix for LC-MS/MS Analysis

nicarbazin

10 µg/mL in acetonitrile, 5 mL/ampul cat.# 33261 (ea.)

QuEChERS Quality Control Standards for GC-MS Analysis

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

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

AOAC QuEChERS QC Spike Mix (27 components)

40 µg/mL each in acetonitrile:acetic acid (99.9:0.1), 5 mL/ampul cat.# 31999 (ea.)

AOAC QuEChERS Triphenylphosphate Solution

triphenylphosphate

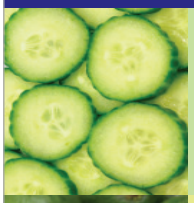
2 µg/mL in acetonitrile:acetic acid (99:1), 5 mL/ampul cat.# 31964 (ea.)

AOAC QuEChERS IS Solution

α-BHC-d6 (α-HCH-d6) parathion-d10

40 µg/mL each in acetonitrile, 5 mL/ampul cat.# 31963 (ea.)

Selection Guide for Q-sep™ dSPE Tubes

Commodity types and examples	AOAC 2007.01	EN 15662	Mini-multiresidue	Additional products
 **Low fat & low pigment fruits & vegetables** • Celery • Cucumber • Head lettuce • Melon	**2 mL, 100-pk.** (cat.# 26124) **15 mL, 50-pk.** (cat.# 26220)	**2 mL, 100-pk.** (cat.# 26215) **15 mL, 50-pk.** (cat.# 26223)	**2 mL, 100-pk.** (cat.# 26215)	
 **Fatty or waxy fruits & vegetables** • Cereals • Avocado • Nuts & seeds • Dairy	**2 mL, 100-pk.** (cat.# 26125) **15 mL, 50-pk.** (cat.# 26221)		**2 mL, 100-pk.** (cat.# 26216)	**15 mL, 50-pk.** (cat.# 26226) **2 mL, 100-pk.** (cat.# 26242) **15 mL, 50-pk.** (cat.# 26244)
 **Pigmented fruits & vegetables** • Strawberries • Sweet potatoes • Tomatoes	**15 mL, 50-pk.** (cat.# 26222)	**2 mL, 100-pk.** (cat.# 26217) **15 mL, 50-pk.** (cat.# 26224)	**2 mL, 100-pk.** (cat.# 26217)	**2 mL, 100-pk.** (cat.# 26123)
 **Highly pigmented fruits & vegetables** • Red peppers • Spinach • Blueberries	**2 mL, 100-pk.** (cat.# 26219)	**2 mL, 100-pk.** (cat.# 26218) **15 mL, 50-pk.** (cat.# 26225)	**2 mL, 100-pk.** (cat.# 26218)	**15 mL, 50-pk.** (cat.# 26126)
 **Universal use** Wide range of commodities, including fatty & pigmented fruits & vegetables.				**2 mL, 100-pk.** (cat.# 26243) **15 mL, 50-pk.** (cat.# 26245)
Download free instructions at **www.restek.com/quechers**	Instruction sheet# 805-01-002	Instruction sheet# 805-01-001	Instruction sheet# 805-01-001	Generic dSPE 805-01-003

RESTEK
Lit. Cat.# FFBR1183D-UNV

U.S.

Technical Article

Prepare Olive Oil Samples for Pesticide Residue Analysis in Half the Time With a Fraction of the Solvent Using dSPE

By Michelle Misselwitz[1], Julie Kowalski[1], Mark Crawford[2], Michael Halvorson[2], and Joan M. Stevens[2]
1. Restek Corporation, 2. Gilson, Inc.

*Simplify and speed up sample preparation with **Resprep® dSPE tubes**! Here we show the extraction and cleanup of pesticide residues from olive oil samples—twice as fast as GPC, with only a fraction of the solvent required for conventional SPE.*

Increase sample throughput with a quick, easy sample preparation method, while protecting your column from matrix contamination.

Olive oil is considered a healthy fat source and is a staple in many recommended diets. However, concerns about potentially negative health effects associated with pesticide residues have increased consumer interest in testing. While organophosphorus pesticides are currently used in olive orchards to control pests, organochlorine pesticides are still tested, even though they are no longer in commercial use, because they are persistent organic pollutants. There are several existing methods for measuring pesticide residues in olive oil, all of which involve sample extraction and cleanup.[1] The common goal of these methods is to remove lipids that are harmful to the analytical system.[2] Efficient sample cleanup procedures are critical to maximizing sample throughput and minimizing labor and material costs. Here we demonstrate the efficiency of a dSPE cleanup procedure, as well as the capabilities of a method-specific analytical column.

Simple Procedure Uses Half the Time and Minimal Solvent

Sample extraction and cleanup can be accomplished with gel permeation chromatography (GPC), solid phase extraction (SPE), or dispersive solid phase extraction (dSPE) methods. However the dSPE method shown here is much less expensive than GPC (which requires specialized equipment) and uses substantially less solvent than comparable GPC or SPE methods (Table I).[3] The method is simple to use and allows sample extraction and cleanup to be accomplished in half the time of other techniques (Table II).

Extraction and dSPE Cleanup for Pesticide Residues in Olive Oil

Test sample: A 1.5 mL sample of commercially obtained virgin olive oil was spiked with a standard organochlorine pesticide mix. The spiked sample was processed as follows:

1. Dilute with 1.5 mL hexane.
2. Add 6 mL of acetonitrile (ACN).
3. Mix for 30 minutes on a shaker
4. Allow layers to separate (approximately 20 minutes), then collect the top (ACN) layer.
5. Repeat the liquid-liquid extraction (steps 2–4) and combine both ACN extract layers.
6. Place 1 mL of the combined ACN extract in a 1.5 mL tube containing 150 mg magnesium sulfate and 50 mg PSA.
7. Shake the tube for 2 minutes.
8. Centrifuge at 3,000 U/min for approximately 5 minutes.
9. Remove the top layer and inject directly into the gas chromatograph system.

Extracts were analyzed using the Rtx®-CLPesticides2 column (Figure 1). The Rtx®-CLPesticides2 column is a method-specific column that resolves all chlorinated pesticide residues tested. Recoveries of 70%–80% were obtained, levels comparable to conventional SPE—without the necessity of vacuum manifolds or high-pressure systems. The GPC method attained recoveries of > 95%. However this method requires large amounts of solvent and takes over twice as long as other methods.

The dSPE method shown here is an efficient, cost-effective way to clean up chlorinated pesticide residues in olive oil. With good recoveries and minimal matrix interference, it is an easy way to reduce solvent usage, compared to conventional SPE, and is more cost-effective than GPC.

Table I: The Resprep® dSPE method uses 42% and 89% less solvent than SPE and GPC methods respectively.

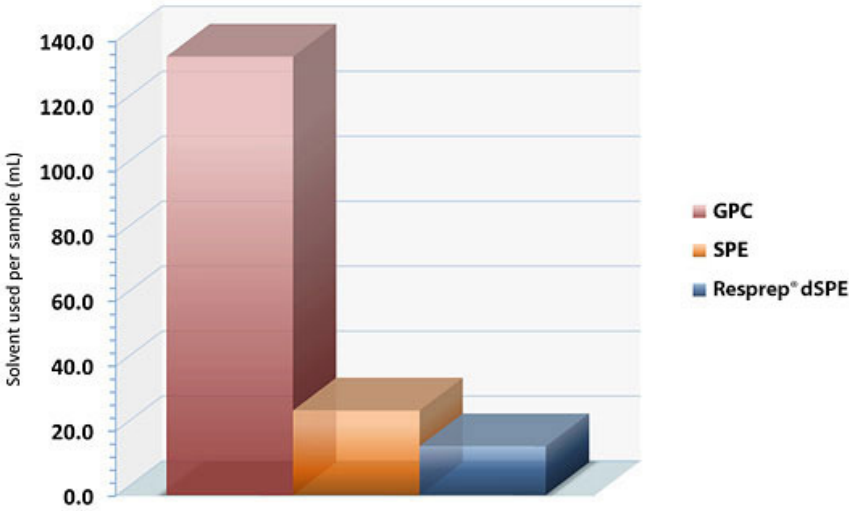


Table II: Cut extraction/cleanup time by 50% using a Resprep® dSPE method.

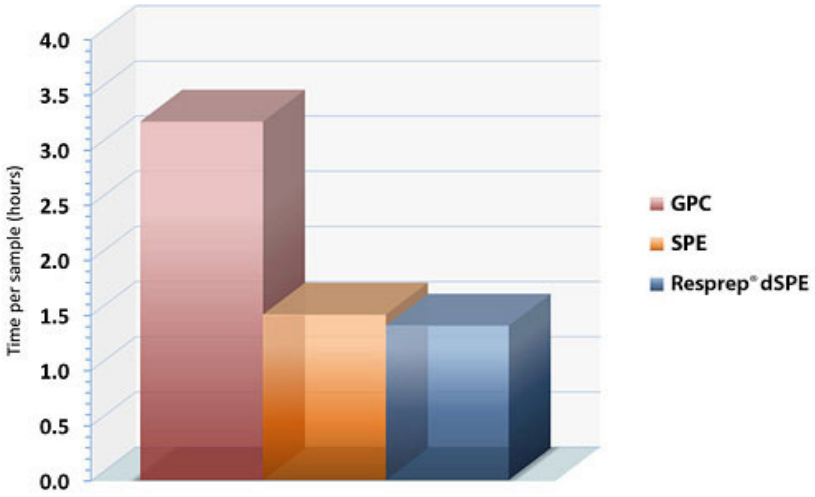
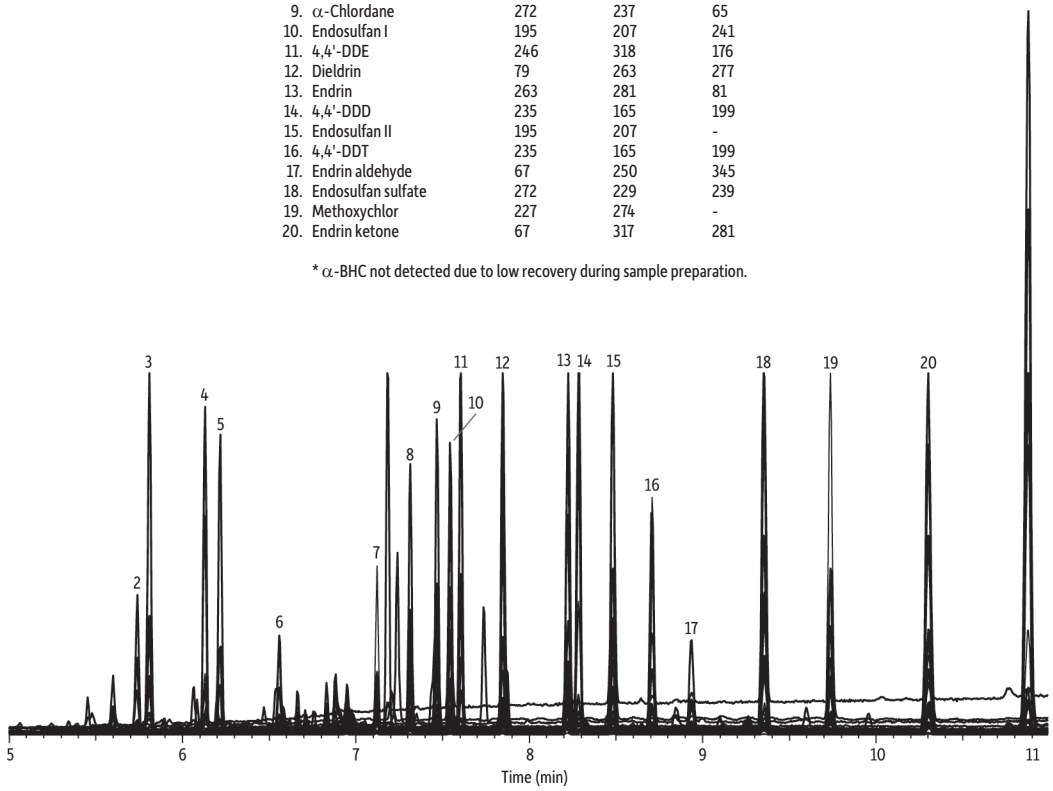


Figure 1: Chlorinated pesticide residues in olive oil are easily separated on the Rtx®-CLPesticides2 column.

Peaks	Quant. ion	Qual. ion 1	Qual. ion 2
1. α-BHC*	219	181	109
2. γ-BHC	219	181	109
3. β-BHC	219	181	109
4. δ-BHC	219	181	109
5. Heptachlor	272	237	100
6. Aldrin	263	293	220
7. Heptachlor epoxide	263	237	81
8. δ-Chlordane	272	237	65
9. α-Chlordane	272	237	65
10. Endosulfan I	195	207	241
11. 4,4'-DDE	246	318	176
12. Dieldrin	79	263	277
13. Endrin	263	281	81
14. 4,4'-DDD	235	165	199
15. Endosulfan II	195	207	-
16. 4,4'-DDT	235	165	199
17. Endrin aldehyde	67	250	345
18. Endosulfan sulfate	272	229	239
19. Methoxychlor	227	274	-
20. Endrin ketone	67	317	281

* α-BHC not detected due to low recovery during sample preparation.



GC_FF01043

Column Rtx®-CLPesticides2 30 m, 0.25 mm ID, 0.20 µm (cat.# 11323)
Sample Olive oil spiked with Organochlorine Pesticide Mix AB # 3 (cat.# 32415)
Conc.: 10 µg/mL
Injection
Inj. Vol.: 1 µL splitless (hold 0.5 min)
Liner: Single Taper w/Wool (cat.# 22286-200.1)
Inj. Temp.: 225 °C
Oven
Oven Temp.: 140 °C (hold 0.5 min) to 268 °C at 20 °C/min to 290 °C at 3 °C/min to 330 °C at 20 °C/min (hold 5 min)
Carrier Gas He, constant flow
Flow Rate: 1 mL/min
Detector MS
Mode: SIM
Transfer Line Temp.: 320 °C
Ionization Mode: EI
Notes Extraction and dSPE Cleanup for Pesticide Residues in Olive Oil

Test sample: A 1.5 mL sample of commercially obtained virgin olive oil was spiked with a standard organochlorine pesticide mix. The spiked sample was processed as follows:
1. Dilute with 1.5 mL hexane.
2. Add 6 mL of acetonitrile (ACN).
3. Mix for 30 minutes on a shaker.
4. Allow layers to separate (approximately 20 minutes), then collect the top (ACN) layer.
5. Repeat the liquid-liquid extraction (steps 2–4) and combine both ACN extract layers.
6. Place 1 mL of the combined ACN extract in a 1.5 mL tube containing 150 mg magnesium sulfate and 50 mg PSA.
7. Shake the tube for 2 minutes.
8. Centrifuge at 3,000 U/min for approximately 5 minutes.
9. Remove the top layer and inject directly into the gas chromatograph system.

References
[1] C. Lentza-Rizos, E.J. Avramides, Rev. Environ. Contam. Toxicol. 141 (1995) 111.
[2] S. Cunha, S. Lehotay, K. Mastovska, J. Sep. Sci. 30 (2007) 620.
[3] M. Crawford, M. Halvorson, J. Stevens, The Examination and Automation of GPC, SPE and QuEChERS Utilized in Extracting Pesticides from Olive Oil. HPLC 2008 poster presentation.

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Lit. Cat.# FFAR1924-UNV

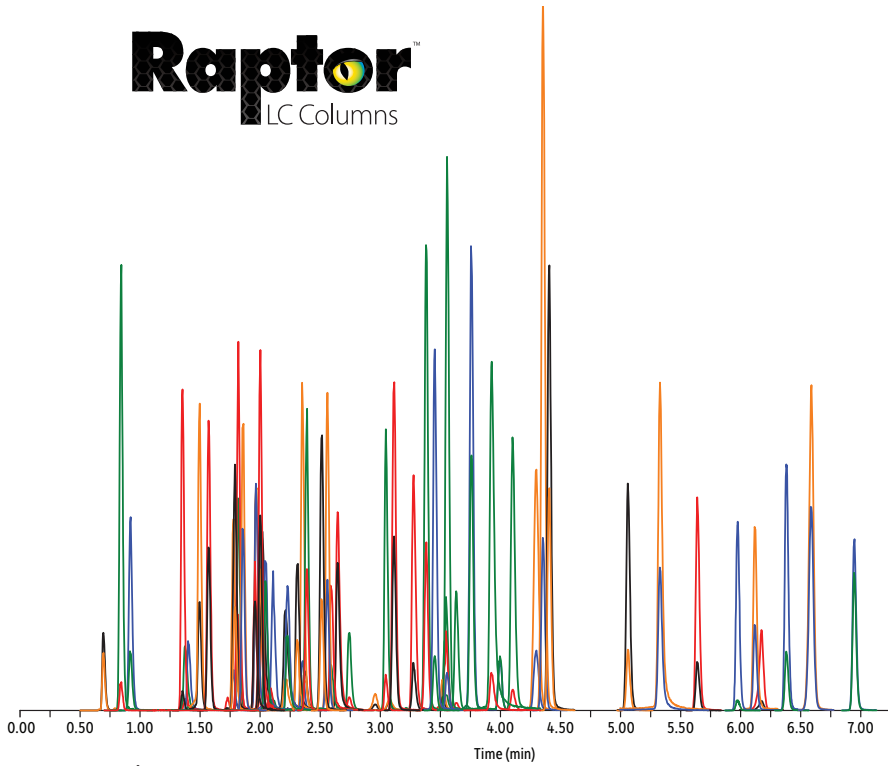
Featured Application: *Multiclass Veterinary Antibiotics on Raptor™ C18 by LC-MS/MS*

One Analysis, One Column, Less than 9 Minutes for Over 60 Multiclass Antibiotics

- Highly efficient peak separation and fast analysis times.
- Versatility and global applicability for antibiotic residue analysis—capable of individual class panel optimization for quantitation:
  - Macrolide, Lincosamide, and Streptogramin (Figure 1)
  - Amphenicol and Tetracycline (Figure 2)
  - Quinolone (Figure 3)
  - Penicillin, Cephalosporin, and Tetracycline (Figure 4)
  - Sulfonamide (Figure 5)

  (For Ionophore, use on Raptor™ Biphenyl. [Figure 6])

The use of antibiotics on food-producing animals is a public health and safety concern due to the potential of generating drug-resistant bacteria. Many countries in the European Union and Canada have banned the use of antibiotics for nontherapeutic purposes, and the United States is implementing a policy to reduce the use of medically important antibiotics for growth promotion. To regulate the proper use of veterinary antibiotics, the U.S. FDA has set maximum residue limits (MRL) for a variety of animal tissue and food products (21 CFR Part 556). A sensitive, efficient, and reliable analytical method for different classes of antibiotics is necessary to meet this regulation, and the Raptor™ C18 LC column is the ideal choice.



Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 35 °C; **Sample:** Diluent: Water; Conc.: 5–300 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0.00 min (10%), 4.50 min (35%), 7.00 min (55%), 7.01 min (10%); 9.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+/ESI-; Mode: Scheduled MRM; **Instrument:** UHPLC; **Notes:** 1. Positive and negative polarity data were collected simultaneously from a single injection. 2. Amphenicol compounds (chloramphenicol, thiamphenicol, and florfenicol) were detected with negative polarity. 3. The MRM was scheduled at +/- 20 to 30 seconds for each analyte. 4. Multiclass antibiotics include penicillin, cephalosporin, tetracycline, sulfonamide, macrolide, lincosamide, streptogramin, amphenicol, and quinolone. **The retention time for Tylosin is noted in the peak list; however, it was not included in the chromatogram.

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Desacetyl cephapirin	0.70	150	382.03	111.92	124.21
2. Sulfanilamide	0.85	200	172.98	93.07	75.23
3. Amoxicillin	0.92	100	366.24	349.10	208.07
4. Cephapirin	1.36	50	424.17	292.08	124.14
5. Tildipirosin	1.38	200	734.59	561.45	204.15
6. Desfuroyl ceftiofur cysteine disulfide	1.40	300	549.16	183.02	126.00
7. Lincomycin	1.50	50	407.32	359.23	389.28
8. Sulfadiazine	1.57	20	251.18	156.04	92.08
9. Cefquinome	1.73	200	529.19	134.10	125.12
10. Ampicillin	1.78	50	350.19	106.07	160.06
11. Sulfathiazole	1.79	10	256.16	156.03	92.08
12. Marbofloxacin	1.81	10	363.20	72.11	320.10
13. Cefalexin	1.82	100	348.10	158.05	174.05
14. Sulfapyridine	1.86	10	250.13	156.10	92.08
15. Norfloxacin	1.96	20	320.23	276.20	233.13
16. Ofloxacin	1.98	10	362.21	318.20	261.15
17. Sulfamerazine	2.00	20	265.08	156.03	92.08
18. Cefalonium	2.01	100	459.16	337.03	123.10
19. Oxytetracycline	2.02	25	461.27	426.15	443.32
20. Ciprofloxacin	2.04	20	332.18	288.22	245.15
21. Cefacetrile	2.09	300	362.07	258.08	178.01
22. Tulathromycin A	2.11	100	806.65	577.42	420.31
23. Tetracycline	2.21	25	445.28	154.07	427.32
24. Danofloxacin	2.23	20	358.22	340.16	314.21
25. Enrofloxacin	2.32	10	360.29	316.22	245.13
26. Orbifloxacin	2.35	10	396.22	352.17	226.12
27. Thiamphenicol*	2.38	200	354.16	290.04	184.98
28. Sulfamethazine	2.39	10	279.23	186.08	124.08
29. Sulfamethizole	2.52	10	271.17	156.02	108.02
30. Sulfamethoxypyridazine	2.56	10	281.14	156.03	126.07
31. Sarafloxacin	2.59	10	386.20	342.20	368.15
32. Difloxacin	2.65	10	400.23	356.17	299.13
33. Cefazolin	2.75	100	455.10	323.06	295.09
34. Spiramycin	2.96	200	843.64	540.36	699.48
35. Pirlimycin	3.05	20	411.32	363.18	327.21
36. Chlortetracycline	3.08	25	479.27	154.07	371.06
37. Sulfachlorpyridazine	3.12	20	285.05	156.03	108.09
38. Gamithromycin	3.28	100	777.63	619.52	601.45
39. Sulfadoxine	3.39	10	311.17	156.03	108.09
40. Sulfamethoxazole	3.46	20	254.18	155.98	147.06
41. Cefoperazone	3.52	100	646.26	143.07	148.02
42. Florfenicol*	3.55	200	356.10	336.02	184.98
43. Sulfaethoxypyridazine	3.56	20	295.17	267.07	156.03
44. Tilmicosin	3.64	100	869.72	696.50	522.42
45. Sulfisoxazole	3.76	20	268.14	156.03	113.10
46. Oxolinic acid	3.94	5	262.10	244.06	215.96
47. Chloramphenicol*	4.00	200	321.16	151.99	257.04
48. Ceftiofur	4.11	50	524.14	241.08	125.24
49. Erythromycin	4.31	25	734.64	576.40	558.38
50. Sulfadimethoxine	4.36	10	311.17	156.09	108.09
51. Sulfaquinoxaline	4.42	20	301.18	156.04	108.02
52. Tylosin**	4.67	100	916.62	772.49	598.36
53. Penicillin G	5.07	100	335.18	176.07	160.07
54. Flumequine	5.34	5	262.15	244.11	202.03
55. Penicillin V	5.56	100	351.10	160.06	114.07
56. Oxacillin	5.99	100	402.15	160.05	114.06
57. Virginiamycin M1	6.13	50	526.43	508.31	355.10
58. Tylvalosin	6.19	50	1042.71	814.46	640.39
59. Cloxacillin	6.39	100	436.15	277.06	160.05
60. Nafcillin	6.60	25	415.19	199.09	171.06
61. Dicloxacillin	6.96	100	470.11	160.05	311.02

*Acquired in negative ion mode

Figure 1: Macrolide, Lincosamide, and Streptogramin Antibiotics on Raptor™ C18 by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Tildipirosin	1.01	200	734.59	561.45	204.15
2. Lincomycin	1.11	50	407.32	359.23	389.28
3. Tulathromycin A	1.19	100	806.65	577.42	420.31
4. Spiramycin	1.41	200	843.64	540.36	699.48
5. Pirlymycin	1.49	20	411.32	363.18	327.21
6. Gamithromycin	1.50	100	777.63	619.52	601.45
7. Tilmicosin	1.58	100	869.72	696.50	522.42
8. Erythromycin	1.80	25	734.64	576.40	558.38
9. Tylosin	1.87	100	916.62	772.49	598.36
10. Tylvalosin	2.30	50	1042.71	814.46	640.39
11. Virginiamycin M1	2.45	50	526.43	508.31	355.10

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 20–200 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0.00 min (10%), 3.00 min (80%), 3.01 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC

LC_FS0502

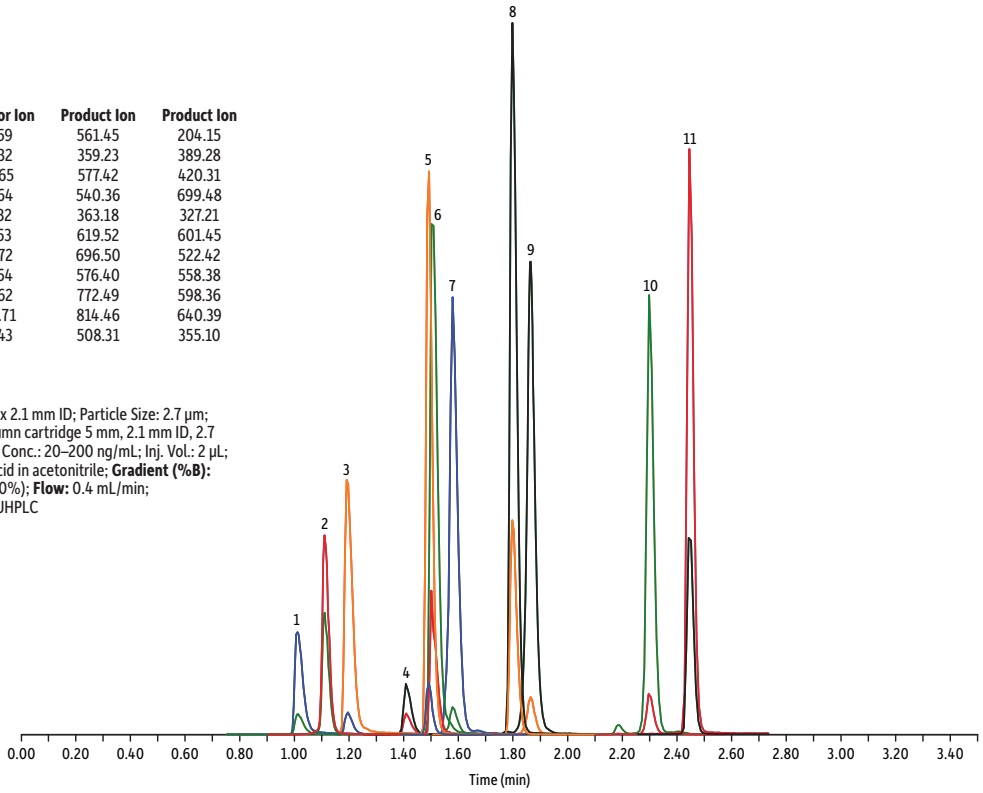


Figure 2: Amphenicol and Tetracycline Antibiotics on Raptor™ C18 by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Oxytetracycline	1.28	25	461.27	426.15	443.32
2. Tetracycline	1.34	25	445.28	154.07	427.32
3. Thiamphenicol*	1.48	200	354.16	290.04	184.98
4. Chlortetracycline	1.56	25	479.27	154.07	371.06
5. Florfenicol*	1.86	200	356.10	336.02	184.98
6. Chloramphenicol*	1.95	200	321.16	151.99	257.04

*Acquired in negative ion mode.

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 25–200 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B)** 0.00 min (10%), 3.00 min (80%), 3.01 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+/ESI-; Mode: MRM; **Instrument:** UHPLC; **Notes:** Tetracyclines and amphenicols were analyzed with ESI+ and ESI- mode, respectively.

LC_FS0504

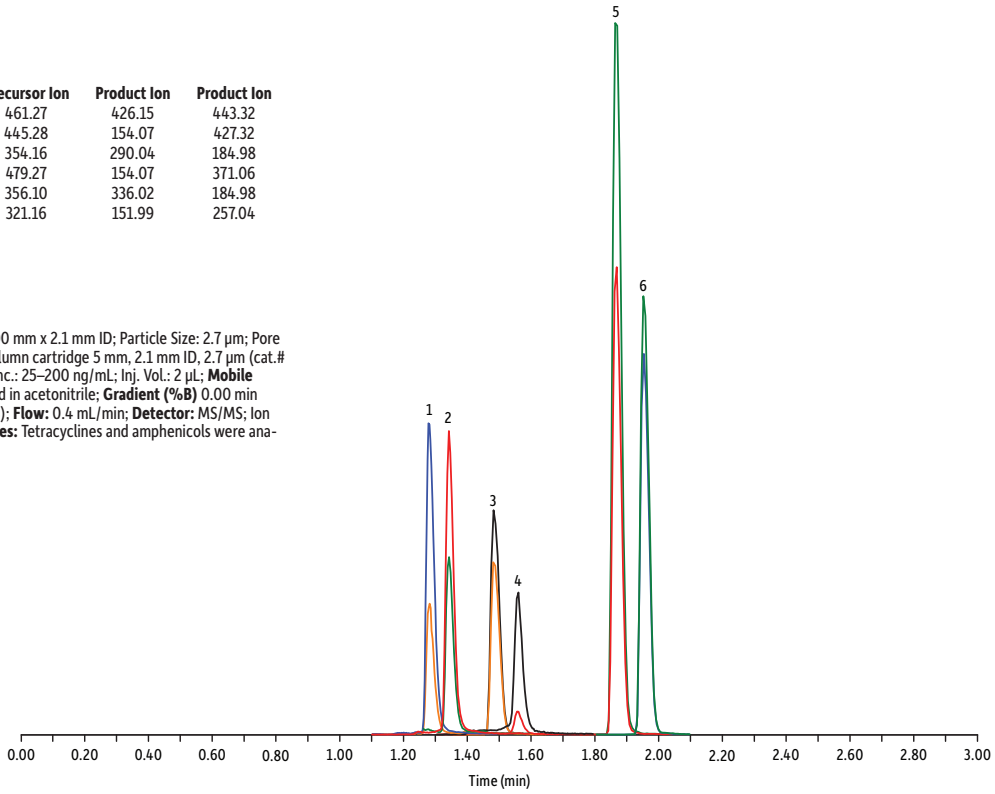


Figure 3: Quinolone Antibiotics on Raptor™ C18 by LC-MS/MS

	Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1.	Marbofloxacin	1.28	10	363.20	72.11	320.10
2.	Norfloxacin	1.32	20	320.23	276.20	233.13
3.	Ofloxacin	1.32	10	362.21	318.20	261.15
4.	Ciprofloxacin	1.35	20	332.18	288.22	245.15
5.	Danofloxacin	1.40	20	358.22	340.16	314.21
6.	Enrofloxacin	1.44	10	360.29	316.22	245.13
7.	Orbifloxacin	1.47	10	396.22	352.17	226.12
8.	Sarafloxacin	1.55	10	386.20	342.20	368.15
9.	Difloxacin	1.57	10	400.23	356.17	299.13
10.	Oxolinic acid	2.23	5	262.10	244.06	215.96
11.	Flumequine	2.78	5	262.15	244.11	202.03

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 5–20 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0.00 min (10%), 3.00 min (60%), 3.01 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC

LC_FS0505

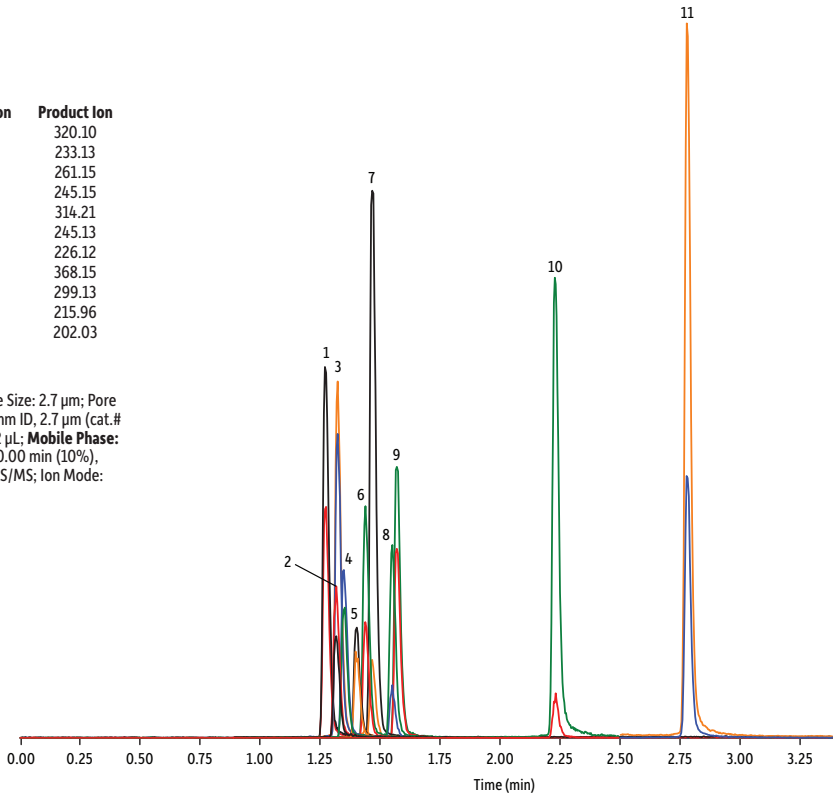


Figure 4: Penicillin, Cephalosporin, and Tetracycline Antibiotics on Raptor ™ C18 by LC-MS/MS

	Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1.	Desacetyl cephapirin	0.68	150	382.03	111.92	124.21
2.	Amoxicillin	0.89	100	366.24	349.10	208.07
3.	Cephapirin	1.05	50	424.17	292.08	124.14
4.	Desfuroyl ceftiofur cysteine disulfide	1.06	300	549.16	183.02	126.00
5.	Cefquinome	1.16	200	529.19	134.10	125.12
6.	Ampicillin	1.18	50	350.19	106.07	160.06
7.	Cefalexin	1.19	100	348.10	158.05	174.05
8.	Oxytetracycline	1.26	50	461.27	426.15	443.32
9.	Cefalonium	1.29	100	459.16	337.03	123.10
10.	Tetracycline	1.32	50	445.28	154.07	427.32
11.	Cefacetrile	1.37	300	362.07	258.08	178.01
12.	Cefazolin	1.50	100	455.10	323.06	295.09
13.	Chlortetracycline	1.54	50	479.27	154.07	371.06
14.	Cefoperazone	1.69	100	646.26	143.07	148.02
15.	Ceftiofur	1.85	50	524.14	241.08	125.24
16.	Penicillin G	2.18	100	335.18	176.07	160.07
17.	Penicillin V	2.33	100	351.10	160.06	114.07
18.	Oxacillin	2.44	100	402.15	160.05	114.06
19.	Cloxacillin	2.56	100	436.15	277.06	160.05
20.	Nafcillin	2.63	25	415.19	199.09	171.06
21.	Dicloxacillin	2.76	100	470.11	160.05	311.02

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 25–300 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0.00 min (10%), 3.00 min (80%), 3.01 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC.

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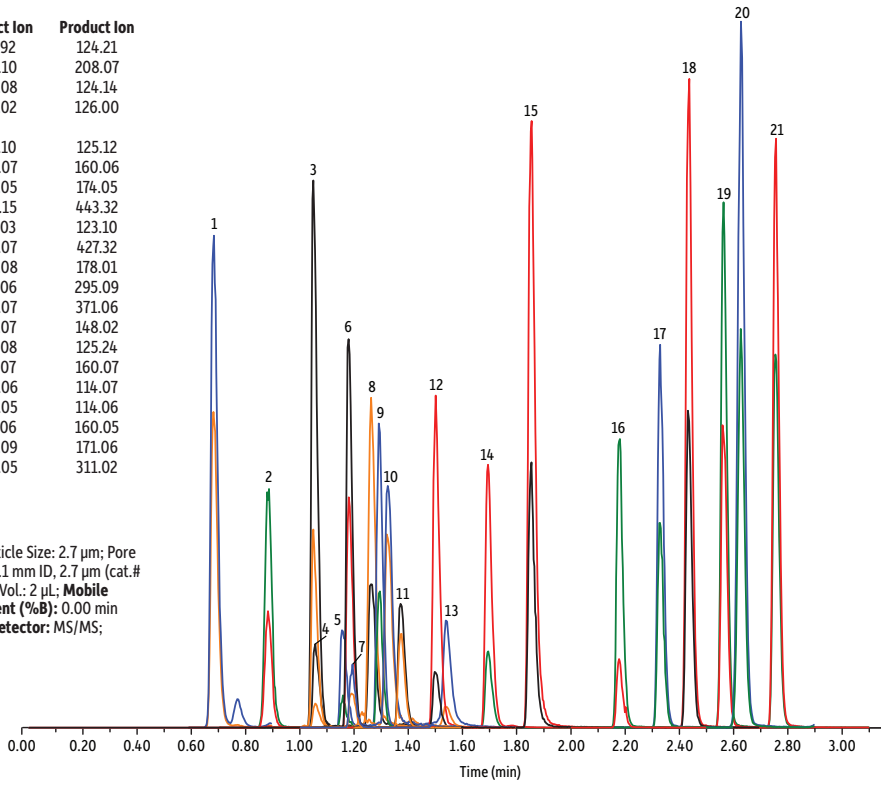


Figure 5: Sulfonamide Antibiotics on Raptor™ C18 by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Sulfanilamide	0.83	200	172.98	93.07	75.23
2. Sulfadiazine	1.45	20	251.18	156.04	92.08
3. Sulfathiazole	1.60	10	256.16	156.03	92.08
4. Sulfapyridine	1.67	10	250.13	156.10	92.08
5. Sulfamerazine	1.79	20	265.08	156.03	92.08
6. Sulfamethazine	2.07	10	279.23	186.08	124.08
7. Sulfamethizole	2.11	10	271.17	156.02	108.02
8. Sulfamethoxypyridazine	2.16	10	281.14	156.03	126.07
9. Sulfachlorpyridazine	2.55	20	285.05	156.03	108.09
10. Sulfadoxine	2.75	10	311.17	156.03	108.09
11. Sulfamethoxazole	2.78	20	254.18	155.98	147.06
12. Sulfaethoxypyridazine	2.84	20	295.17	267.07	156.03
13. Sulfisoxazole	2.98	20	268.14	156.03	113.10
14. Sulfadimethoxine	3.37	10	311.17	156.09	108.09
15. Sulfaquinoxaline	3.40	20	301.18	156.04	108.02

Column: Raptor™ C18 (cat.# 9304A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ C18 EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252); Temp.: 40 °C; **Sample:** Diluent: Water; Conc.: 10–200 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile; **Gradient (%B)** 0.00 min (10%), 3.50 min (40%), 3.51 min (10%), 5.00 min (10%); **Flow:** 0.4 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument:** UHPLC

LC_FS0501

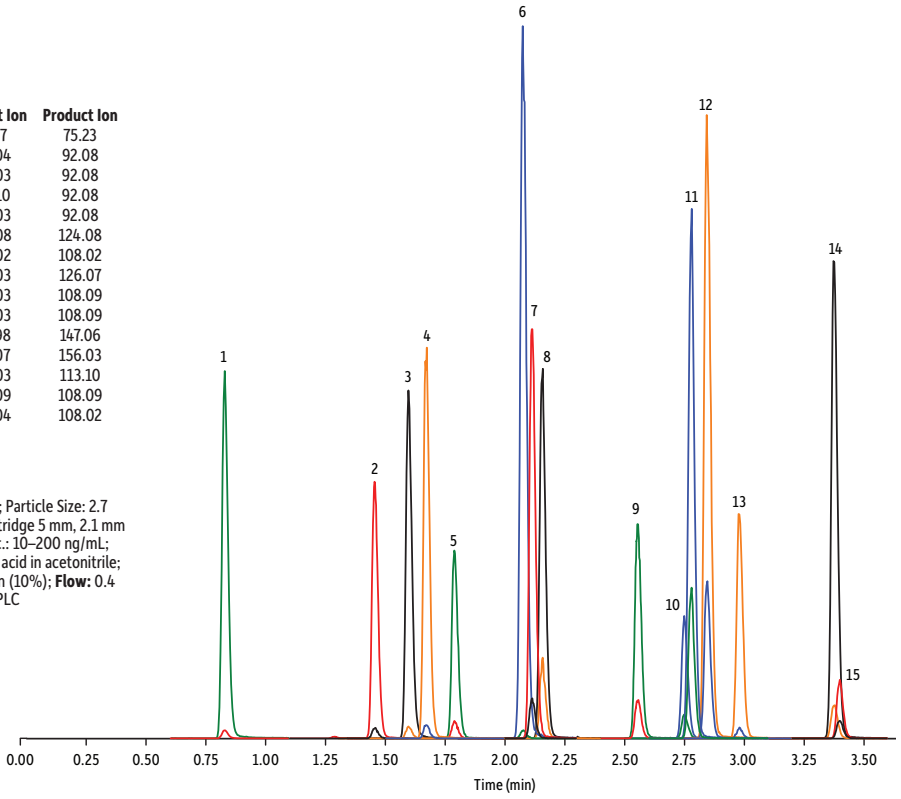
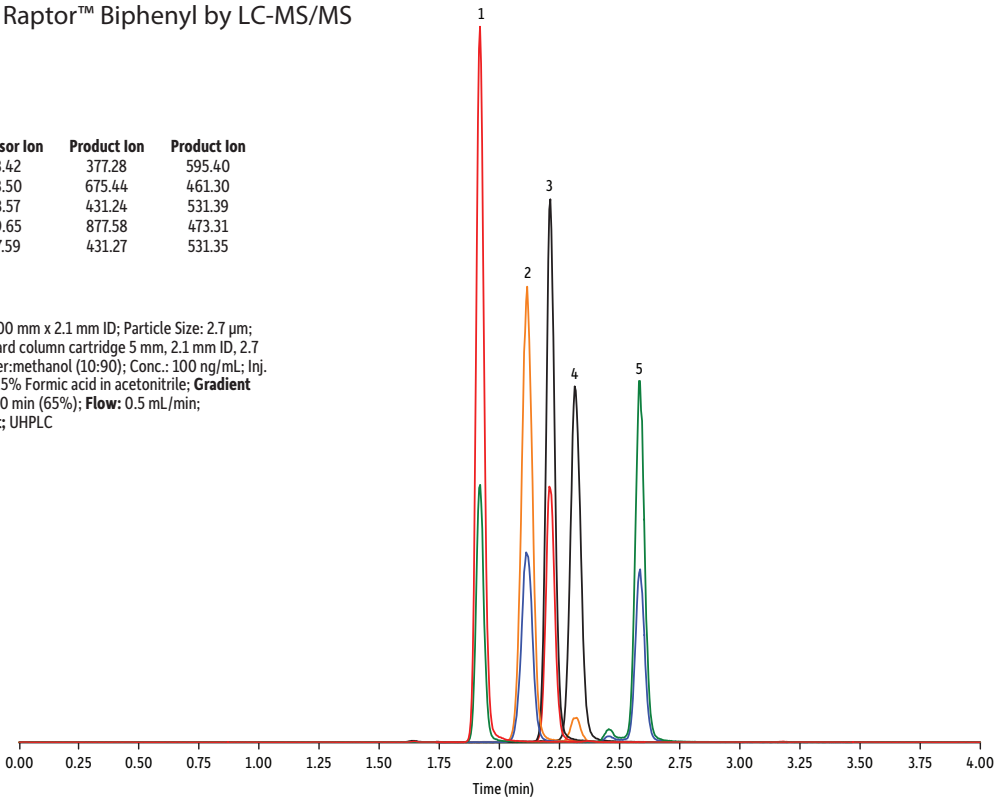


Figure 6: Ionophore Antibiotics on Raptor™ Biphenyl by LC-MS/MS

Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. Lasalocid A	1.92	100	613.42	377.28	595.40
2. Monensin	2.12	100	693.50	675.44	461.30
3. Salinomycin	2.19	100	773.57	431.24	531.39
4. Maduramicin	2.30	100	939.65	877.58	473.31
5. Narasin	2.58	100	787.59	431.27	531.35

Column: Raptor™ Biphenyl (cat.# 9309A12); Dimensions: 100 mm x 2.1 mm ID; Particle Size: 2.7 µm; Pore Size: 90 Å; Guard Column: Raptor™ Biphenyl EXP® guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252); Temp.: 40 °C; **Sample:** Diluent: Water:methanol (10:90); Conc.: 100 ng/mL; Inj. Vol.: 2 µL; **Mobile Phase:** A: 0.5% Formic acid in water; B: 0.5% Formic acid in acetonitrile; **Gradient (%B)** 0.00 min (65%), 3.00 min (75%), 3.01 min (65%), 5.00 min (65%); **Flow:** 0.5 mL/min; **Detector:** MS/MS; Ion Mode: ESI+; Mode: MRM; **Instrument;** UHPLC

LC_FS0503



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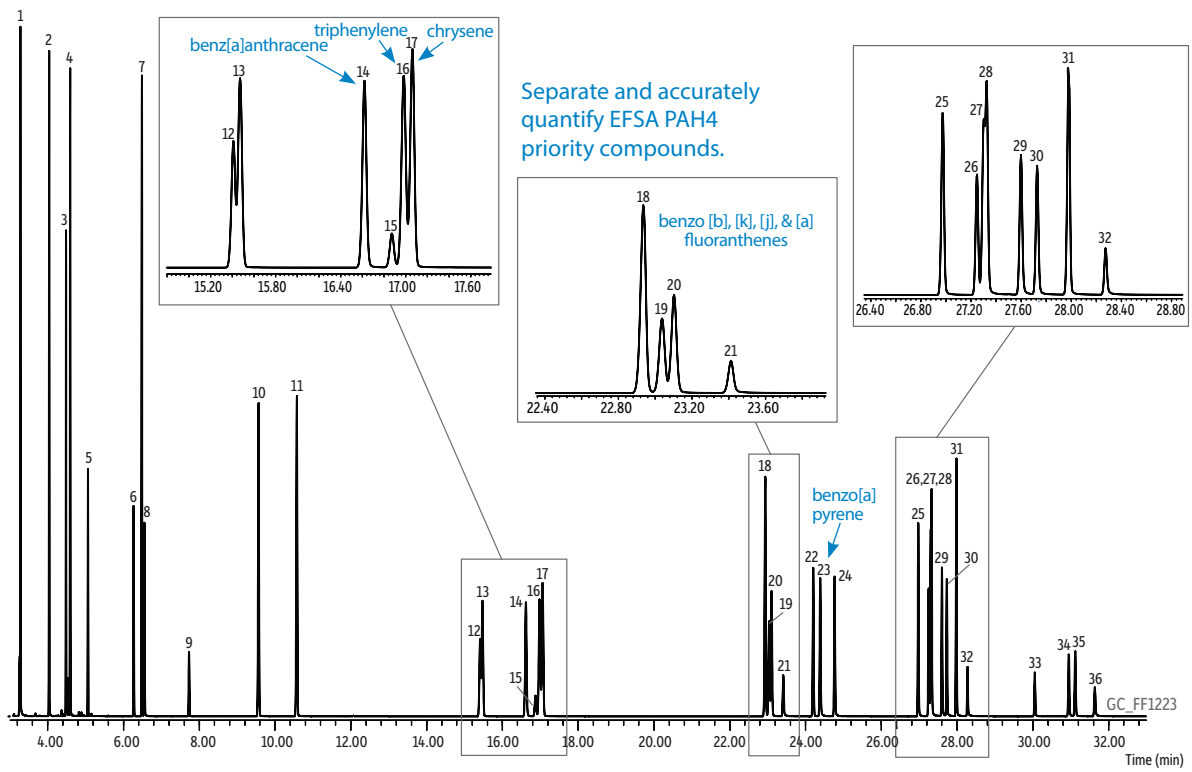
Resolve Important Isobaric Polycyclic Aromatic Hydrocarbons for Food Safety and Environmental Methods

- Separation of ***all*** EFSA PAH4 compounds: benz[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene.
- ***Best resolution*** of chrysene from interfering PAHs, triphenylene and cyclopenta[cd]pyrene.
- ***Complete separation*** of benzo [b], [k], [j], and [a] fluoranthenes.
- 360 °C thermal stability allows analysis of low volatility dibenzo pyrenes.

Rxi®-PAH GC columns were designed by Restek with a higher phenyl-content stationary phase that provides a unique selectivity to separate important polycyclic aromatic hydrocarbons (PAHs) for food safety that cannot be distinguished by mass spectrometry. Even difficult priority compounds, such as the European Food Safety Authority (EFSA) PAH4, are easily separated and accurately quantified, results that cannot be achieved on typical GC columns. Arylene modification and surface bonding of the stationary phase increase thermal stability and ruggedness so relatively nonvolatile, higher molecular weight PAHs can be analyzed routinely without interference from column bleed. Excellent column efficiency means that the column can be trimmed for maintenance purposes many times without losing critical PAH separations, including those that are part of environmental methods, as well as food safety testing.

Figure 1: A 40 m x 0.18 mm x 0.07 µm Rxi®-PAH column produces excellent resolution of critical peaks in less than 33 minutes!



Peaks
1. Naphthalene
2. Biphenyl
3. Acenaphthylene
4. Acenaphthene
5. Fluorene
6. Dibenzothiophene
7. Phenanthrene
8. Anthracene
9. 4H-Cyclopenta[def] phenanthrene
10. Fluoranthene
11. Pyrene
12. Benzo[ghi]fluoranthene
13. Benzo[c]phenanthrene
14. Benz[a]anthracene
15. Cyclopenta[cd]pyrene
16. Triphenylene
17. Chrysene
18. Benzo[b]fluoranthene
19. Benzo[k]fluoranthene
20. Benzo[j]fluoranthene
21. Benzo[a]fluoranthene
22. Benzo[e]pyrene
23. Benzo[a]pyrene
24. Perylene
25. Dibenz[a,j]anthracene
26. Dibenz[a,c]anthracene
27. Indeno[1,2,3-cd]pyrene
28. Dibenz[a,h]anthracene
29. Benzo[b]chrysene
30. Picene
31. Benzo[ghi]perylene
32. Anthanthrene
33. Dibenzo[b,k]fluoranthene
34. Dibenzo[a,e]pyrene
35. Coronene
36. Dibenzo[a,h]pyrene

Column: Rxi®-PAH, 40 m, 0.18 mm ID, 0.07 µm (cat.# 49316); **Sample**: NIST SRM 2260a PAH mix; Diluent: Toluene; Conc.: 0.2 - 2 µg/mL (SRM 2260a PAH mix was diluted 5x in toluene); **Injection:** 0.5 µL pulsed splitless (hold 0.58 min); Liner: Sky® 2 mm single taper w/wool (cat.# 23316.1); Inj. Temp.: 275 °C; Pulse Pressure: 80 psi (551.6kPa); Pulse Time: 0.6 min; Purge Flow: 40 mL/min; **Oven:** 110 °C (hold 1 min) to 210 °C at 37 °C/min to 260 °C at 3 °C/min to 350 °C at 11 °C/min (hold 4.5 min); **Carrier Gas:** He, constant flow; Flow Rate: 1.4 mL/min; **Detector:** MS; Mode: SIM; Transfer Line Temp.: 350 °C; Analyzer Type: Quadrupole; Source Temp.: 350 °C; Quad Temp.: 200 °C; Solvent Delay Time: 3.00 min; Tune Type: PFTBA; Ionization Mode: EI; **Instrument:** Agilent 7890A GC & 5975C MSD. For SIM program and quant ion information, visit visit www.restek.com and enter GC_FF1223 in the search.

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Cat.#	Length	ID	df	Description
49316	40 m	0.18 mm	0.07 µm	Narrow inside diameter, thinner film, faster analysis, excellent separation of important PAHs, less sample loading capacity
49317	60 m	0.25 mm	0.10 µm	0.25 mm inner diameter, better sample loading capacity, highest resolution of important PAHs, longer analysis than 0.18 mm column, thin film allows elution of dibenzo pyrenes
49318	30 m	0.25 mm	0.10 µm	0.25 mm inside diameter, better sample loading capacity, faster analysis time than 60 m column, adequate resolution of important PAHs, lower cost column

Recommended for PAH Analysis

23316

Sky® 2.0 mm ID Single Taper Inlet Liner

Suggested for 0.18 mm ID columns.

For Agilent GCs equipped with split/splitless inlets

ID x OD x Length	qty.	cat.#
Single Taper, Sky Technology, Borosilicate Glass		
2.0 mm x 6.5 mm x 78.5 mm	ea.	23315.1
2.0 mm x 6.5 mm x 78.5 mm	5-pk.	23315.5
2.0 mm x 6.5 mm x 78.5 mm	25-pk.	23315.25
Single Taper, Sky Technology, Wool, Borosilicate Glass		
2.0 mm x 6.5 mm x 78.5 mm	ea.	23316.1
2.0 mm x 6.5 mm x 78.5 mm	5-pk.	23316.5
2.0 mm x 6.5 mm x 78.5 mm	25-pk.	23316.25

Sky® 4.0 mm ID Single Taper Inlet Liner

Suggested for 0.25 mm ID columns.

For Agilent GCs equipped with split/splitless inlets

ID x OD x Length	qty.	cat.#
Single Taper, Sky Technology, Borosilicate Glass		
4.0 mm x 6.5 mm x 78.5 mm	ea.	23302.1
4.0 mm x 6.5 mm x 78.5 mm	5-pk.	23302.5
4.0 mm x 6.5 mm x 78.5 mm	25-pk.	23302.25
Single Taper, Sky Technology, Wool, Borosilicate Glass		
4.0 mm x 6.5 mm x 78.5 mm	ea.	23303.1
4.0 mm x 6.5 mm x 78.5 mm	5-pk.	23303.5
4.0 mm x 6.5 mm x 78.5 mm	25-pk.	23303.25

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Gold-Plated	21246	21247	
Siltek-Treated	21248	21249	
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Lit. Cat.# GNTS1718-UNV

Monitor Anitoxidants in Tea Extract

Using an Ultra Aqueous C18 HPLC Column and Unique® TOFMS

by Julie Kowalski, Ph.D., Innovations Chemist

- Use highly aqueous mobile phases without collapsing the stationary phase.
- Extract data for specific compounds and manually inspect spectra for other compounds.
- Simple sample preparation.

Much focus has been given to the health benefits of foods and beverages that contain antioxidant compounds. By reacting with free radical-forming compounds before they can cause cell damage, antioxidants protect the body against oxidative stress.[1] Some foods and beverages naturally contain antioxidants, but supplementing foodstuffs has been on the rise due to demands by health conscious consumers. Recently, green tea has been successfully promoted as a health drink because it contains antioxidant phenolic compounds.

Using LC/TOFMS, we show a straightforward method for determining the presence of antioxidant compounds in commercial tea formulations. Samples were prepared by adding approximately 15g of dry tea product to 200mL of methanol which was cooled to approximately 20°C. The mixture was stirred for 5 minutes and decanted. The tea product was rinsed with an additional 20mL of cooled methanol. The 200mL and 20mL solutions were combined, then filtered through a 0.45µm syringe filter to capture particles. The filtered solution was used directly for analysis.

We used a 150 x 2.1mm Ultra Aqueous C18 HPLC column for the analysis and, because a tea extract is a complex matrix, we used a gradient elution and mobile phases with a high water content. The Ultra Aqueous C18 stationary phase is ideal for such an application: the phase is specifically designed to prevent collapse of the C18 alkyl chains in highly aqueous mobile phases.[2]

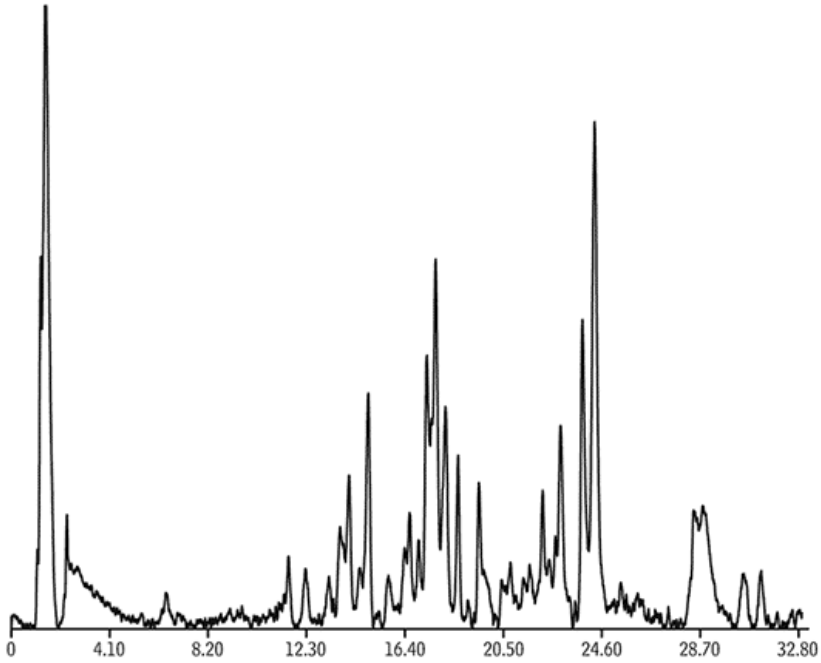
The Ultra Aqueous C18 phase proved ideal for resolving the complex tea matrix, as shown by the large number of peaks in Figure 1. The resolving power of this chromatographic system, in combination with the LECO Unique® TOF Mass Spectrometer, allow the analyst to both extract data for specific compounds of interest and manually inspect spectra for other compounds, including phenolic glycosides and esters of phenolic acid.

If you are analyzing antioxidants in tea, or other complex mixtures of compounds, an Ultra Aqueous C18 column gives you the reliable results you need, without restricting your ability to use the mobile phase composition that works best for your application.

Table I Phenolic compounds of interest.

Compound	[M-H]-
gallocatechin-3-gallate	457.206
catechin	289.154
epigallocatechin-3-methyl gallate	471.208
epicatechin di-gallate	609.318
epicatechin-3-gallate	441.208
catechin gallate	
Note: m/z 441.2 can be either epicatechin-3-gallate or catechin gallate.	

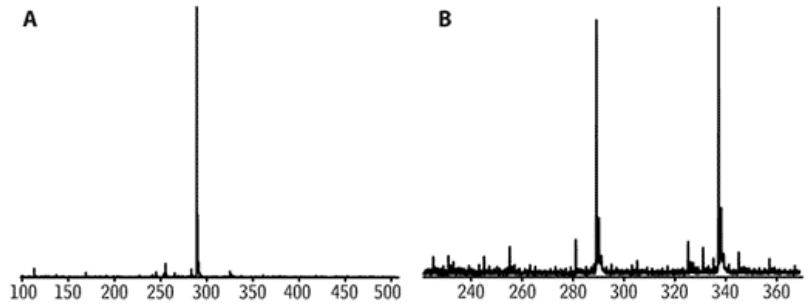
Figure 1 A complex mix of tea extract components is best separated on an Ultra Aqueous C18 column with a highly aqueous mobile phase (total ion chromatograms of Table 1 compounds).



LC_FF0425

For conditions see Figure 3.

Figure 2 (-)-Epicatechin produced by infusion of a standard (A) and spectrum of (-)-epicatechin created from an extracted ion chromatogram (B).

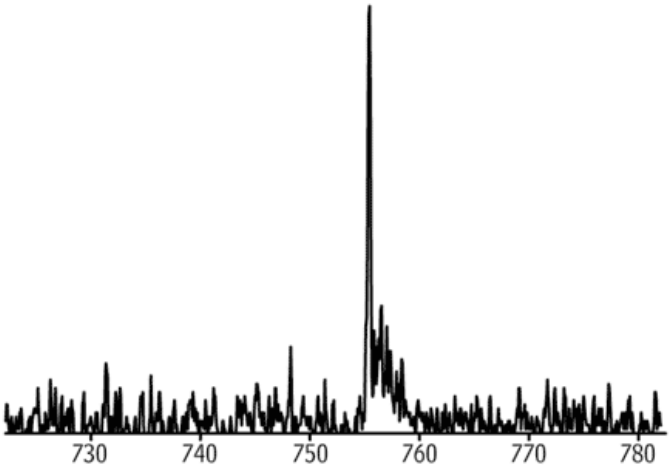


LC_FF0425A & LC_FF0425B

For conditions see Figure 3.

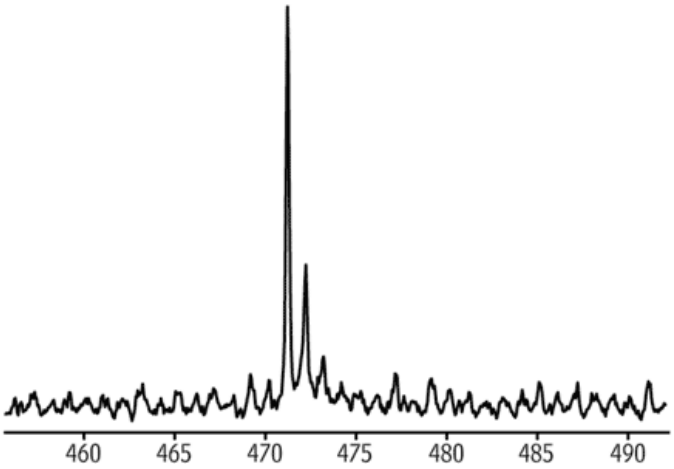
Figure 3 Spectra of phenolic compounds identified in the tea extract.

A) quercitin dicoumaryl glycoside



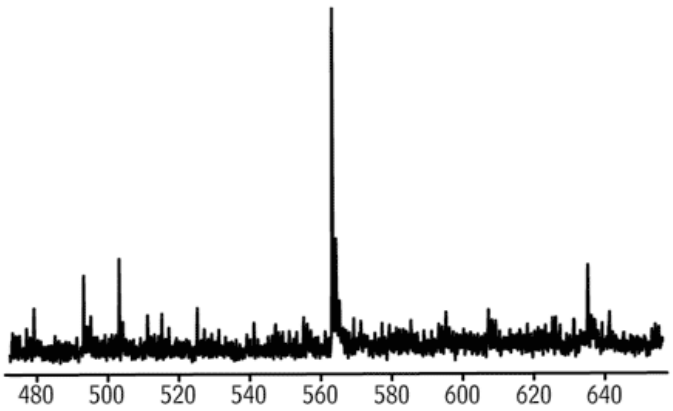
LC_FF0425G

B) epigallocatechin 3-methyl gallate



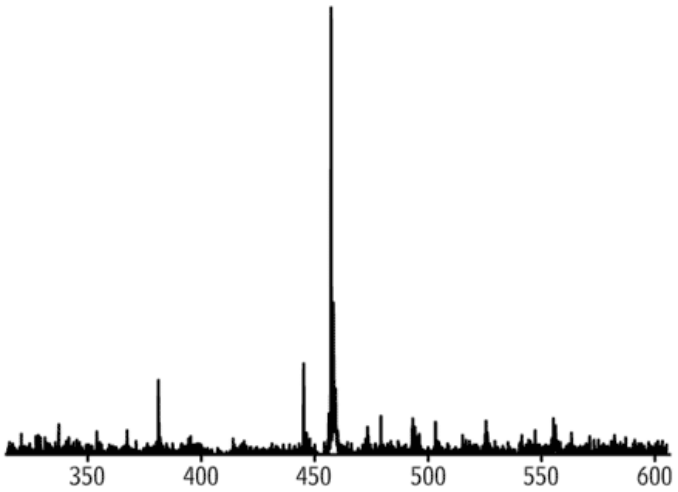
LC_FF0425F

C) caffeic acid ester



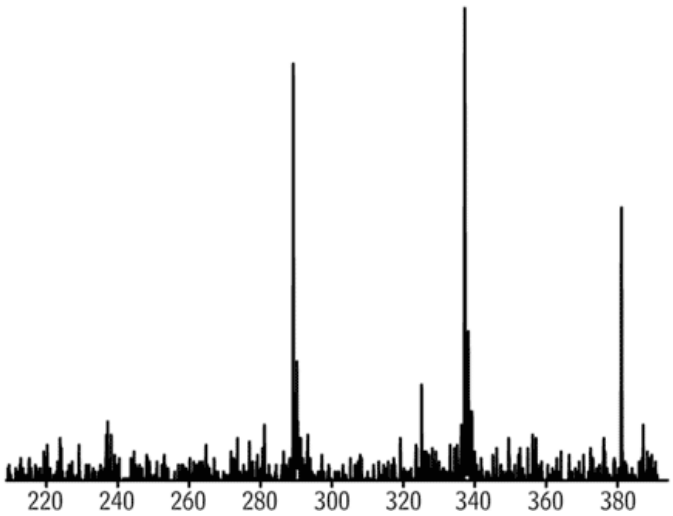
LC_FF0425E

D) gallocatechin-3-gallate



LC_FF0425C

E) catechin



LC_FF0425D

Sample:

Inj.:	10µL
Conc.:	15g tea extracted w/ 200mL + 20mL methanol
Sample diluent:	methanol
Column:	Ultra Aqueous C18
Cat.#:	9178562
Dimensions:	150 x 2.1 mm
Particle size:	5µm
Pore size:	100Å
Conditions:	
Mobile phase:	A: 0.15% formic acid in water; B: 0.15% formic acid in acetonitrile (v/v)

Time (min.)	**%B**
0	5
60	9.5

Flow:	0.3mL/min.
Temp.:	ambient
Det.:	Leco Unique® LC-TOFMS
ESI voltage:	-3500 V
Desolvation temp.:	300°C
Nebulizer pressure:	375 kPa
Desolvation gas:	7000 cc/min.
Interface temp.:	100°C
Nozzle:	-80 V
Data	

acquisition: 3.13 spectra/sec.

For information about the LECO Unique® TOFMS, please visit the LECO website: www.leco.com

References

1. Free radical damage is implemented in many disease models, including cancer, in many degenerative illnesses, and in the aging process.
2. When the long, hydrophobic alkyl chain of a conventional C18 stationary phase is exposed to a highly aqueous mobile phase it folds down on itself, causing loss of retention. A prolonged equilibration time in a high organic solution is needed to restore the phase. The Ultra Aqueous C18 stationary phase is not susceptible to phase collapse — not even in mobile phases with very highly aqueous content.

RELATED SEARCHES

phenolic glycosides, *esters of phenolic acid*, *LECO Unique TOF Mass Spectrometer*, *Ultra Aqueous C18*



Food Safety Applications

Mitigating Matrix Effects: Examination of Dilution, QuEChERS, and Calibration Strategies for LC-MS/MS Analysis of Pesticide Residues in Diverse Food Types

By Julie Kowalski, Sharon Lupo, and Jack Cochran

Abstract

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is popular for monitoring pesticide residues in food. The increased selectivity and sensitivity of LC-MS/MS have impacted how multiresidue methods are performed, sometimes decreasing the need for rigorous sample preparation. However, this approach suffers from matrix effects causing poor data quality and difficult quantification. Matrix effects can be mitigated by sample preparation that reduces the concentration of coextracted matrix material or by experimental strategies like matrix-matched calibration that compensate for matrix effects. We considered these two aspects of multiresidue methods, sample preparation and calibration strategies, in order to determine recommendations that balanced data quality as well as time and financial investments.

We performed matrix effects studies investigating two approaches for reducing matrix interferences, QuEChERS sample preparation and dilution, in combination with the compensation strategy of matrix-matched calibration compared to solvent calibration. There are compromises with each method regarding time and financial resources. A variety of food types were tested including high water (celery), high pigment (kale), high fat (avocado), citrus (lime), and dry (brown rice flour) foods, with subsequent pesticide residue analysis by LC-MS/MS. Samples were fortified at high and low ppb levels with over 100 pesticides representing multiple classes.

We determined that with the easiest commodities, the dilution method and solvent-only calibration gave acceptable recovery values. However, for other commodities either a matrix-matched calibration curve and/or cleanup were needed to obtain good recovery values. The high carbohydrate and citrus commodities proved to be too difficult with the specific methods we tested here. In almost every case, use of matrix-matched calibration provided improvement.

Introduction

There are many challenges for chemists performing trace analysis like pesticide residues. Some challenges are associated with the diverse commodities that need to be tested and the large number of analytes. In recent years, the trend has been for analytical methods to become faster and simpler, but at the same time detect lower levels and test many analytes in one analysis (multiresidue methods).

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is popular for this type of testing because it addresses some of these challenges and is amenable to many more pesticides than gas chromatographic techniques. Retention times, ion transitions, and transition ratios are used for pesticide identification and quantification. By monitoring ion transitions, tandem mass spectrometry increases selectivity by filtering specific ions. This selectivity removes noise, resulting in a large increase in the signal-to-noise ratio, thereby enhancing sensitivity.

Although tandem mass spectrometry is highly selective and sensitive, coeluting compounds can cause interferences at the LC-MS interface during electrospray ionization (ESI), which occurs before mass analysis and signal generation. Inconsistent ionization causes poor data quality and makes accurate quantification extremely difficult. We use the term "matrix effect" to discuss this ionization issue. The matrix effect of a compound is the change in ionization in solvent compared to ionization in matrix. Matrix coextractives can change the ionization efficiency of an analyte causing signal suppression or enhancement. Matrix effects must be considered to ensure acceptable quantitative results for any LC-MS/MS method.

Matrix effects can be compensated for by experimental design, for example, by employing labeled isotopic standards or matrix-matched calibration. Matrix-matched calibration is proven to be effective but adds time and cost to analysis. Also, it can be challenging to find commodities that do not contain pesticides that are target analytes. Efforts have been made to use one matrix-matched sample set for different commodities, but this has shown varying success [1,2,3].

Matrix compounds can be selectively removed by applying sample cleanup procedures. This lowers the concentrations of coextractives while maintaining the concentrations of target analytes, but it can be labor-intensive and costly. There is also the potential of losing analytes during sample processing. Common sample preparation techniques include liquid-liquid extraction, solid phase extraction, and the QuEChERS approach. QuEChERS has extraction and cleanup steps that remove matrix compounds, while maintaining analytes at initial concentrations. Another approach is to reduce matrix effects by directly diluting the sample. This decreases the matrix coextractives concentration, but it also decreases the concentration of target analytes. Higher dilutions reduce or eliminate matrix effects more successfully, but better and better sensitivity is required as dilution factors increase. Dilutions can range from 10 to 100-fold [4] and choosing the proper dilution factor depends on the concentrations of analytes and coextractives, as well as on chemical properties and instrument sensitivity. For example, 10 ppb is often used as a benchmark concentration because it is a common maximum residue limit for many pesticides in many foods. If we diluted the sample 50-fold, we need to detect and quantify pesticides at 0.2 ppb or 200 ppt (parts-per-trillion). The excellent sensitivity of tandem mass spectrometry can sometimes accommodate this inherent sensitivity challenge of dilution-based methods.

The dilution technique has been heavily promoted in recent years and sometimes endorsed as a universal method. It has been shown to be successful in many applications, including pesticides residue analysis. Dilution is attractive because it eliminates sample preparation and associated costs. However, pesticide residue testing involves a wide variety of food types as well as a large number of pesticides that vary in physiochemical properties and generally speaking a single method is not adequate to address this complexity. For that reason, it is important to evaluate the effectiveness of sample preparation techniques and define recommendations for applying different strategies.

We evaluated a variety of pesticides that are typical of multiresidue methods. We tested approximately 100 pesticides from different chemical classes including carbamate, organophosphorus, aniline, conazole, macrocyclic lactone, phenylurea, benzoylphenylurea, and strobilurin pesticides. We tested a variety of commodities representing different food types and ranging from easy to difficult. High water, low fat, low carbohydrate, and low pigment foods are considered the easiest commodities to analyze because their extracts do not contain the high amount of coextracted compounds that are more common with high fat, high carbohydrate, high pigment, and dry commodities.

Method performance was evaluated using matrix effect values, acceptable pesticide recovery values, and the number of pesticides detected. We fortified foods and processed samples according to QuEChERS and dilution methods. We then determined recovery values. Quantification was performed two ways, by using both a solvent calibration curve and a matrix-matched calibration curve. Calibration methods were evaluated by comparing recovery values. The experimental design yields four recovery value data sets for each commodity: dilution with solvent calibration, dilution with matrix-matched calibration, QuEChERS with solvent calibration, and QuEChERS with matrix-matched calibration. This allows assessment of sample preparation/calibration method combinations.

Experimental

Chemicals and Materials

All solvents were LC-MS grade and purchased from Fisher Scientific (Pittsburgh, PA). Formic acid was obtained from Sigma-Aldrich (Milwaukee, WI) and ammonium formate from Alfa Aesar (Ward Hill, MA). QuEChERS extraction and dispersive solid phase extraction (dSPE) tubes, as well as QuEChERS internal and quality control standards, were from Restek Corporation (Bellefonte, PA). The approximately 100 pesticide mix in acetonitrile was a custom standard that was combined with AOAC spike mix (cat.# 31999). Internal standards, atrazine-d5 and diazinon-d10, were purchased from Sigma-Aldrich (Milwaukee, WI). Food commodities were purchased at a local grocery store and included celery, kale, avocado, lime, and brown rice flour. Commodities were stored at -20 °C.

Sample Preparation

Commodity Selection

Celery is considered the easiest commodity with high water content, medium pigment, and low fat and carbohydrates. Kale represents a high water, high pigment commodity that produces an intensely colored extract. Lime is the representative citrus fruit. Although citrus fruits are known to be difficult for LC-MS/MS techniques, we included lime (with peel) to observe any differences among methods. Avocado represents the high fat category. Brown rice flour is a dry grain, which has very high carbohydrate content. Table I shows the nutritional composition of each food tested.

Table I: Nutritional content for each commodity as grams per 100 grams of edible material.[1]

	Water	Sugar	Lipids	Carbohydrates	Protein	Fiber
Celery	95	2	0	3	1	2
Kale	84	na	1	10	3	2
Lime	88	2	0	10	1	3
Avocado	73	1	15	8	2	7
Brown rice flour	12	1	3	77	7	5

[1] USDA National Nutrient Database for Standard Reference, http://ndb.nal.usda.gov/ (last modified: Dec 7, 2011).

Commodity Fortification

Frozen commodities were homogenized using a food processor, weighed, and fortified with pesticides at 10 and 500 µg/kg (ppb) levels. Internal standards atrazine-d5, diazinon-d10, and AOAC IS mix (cat.# 31963) were added at 250 ppb final extract concentration. Unfortified samples were prepared to determine incurred pesticides and produce matrix-matched standards.

AOAC QuEChERS Extraction

The AOAC QuEChERS extraction method was used for celery, kale, and lime. For these samples, 15 mL of acetonitrile with 1% acetic acid (v/v) was added to 15 g of fortified homogenized sample [5]. Q-sep® AOAC buffering extraction salts (cat.# 26237) containing 6 g $MgSO_4$, 1.5 g sodium acetate were added. At this point, lime samples were pH adjusted by adding 900 µL of a 5 N (or M, mol/L*) sodium hydroxide solution to the extraction tube. This pH adjustment was based on the EN method recommendation, but scaled for a 15 g sample [6]. Following 1 minute of manual shaking, samples were centrifuged for 5 minutes at 3,000 xg with a Q-sep® 3000 centrifuge (cat.# 26230). The top acetonitrile layer was removed to a clean vial.

For dilution samples, the extract was diluted 20-fold with initial mobile phase, 90:10 (v:v) ratio of water with 4 mM ammonium formate and 0.1% formic acid to methanol with 4 mM ammonium formate and 0.1% formic acid.

*A 5 N sodium hydroxide solution is equivalent to a 5 M (molar, mol/L) solution.

Unbuffered QuEChERS Extraction

The original unbuffered extraction method was used for avocado and brown rice flour with adjustments for sample wetting [7]. For brown rice flour, 5 g of brown rice flour, 15 mL of deionized water, and 10 mL of acetonitrile were combined in a 50 mL centrifuge tube. The sample was vortexed briefly and shaken for one hour using a shaker table. For avocado, 3 mL water, 10 g of partially thawed homogenized avocado, and 10 mL of acetonitrile were added to a 50 mL centrifuge tube and shaken for one minute. The samples were centrifuged for 5 minutes at 3,000 xg with a Q-sep® 3000 centrifuge (cat.# 26230). The top acetonitrile layer was removed to a clean vial.

For dilution LC-MS/MS analysis, the extract was diluted 20-fold with initial mobile phase, 90:10 (v:v) ratio of water with 4 mM ammonium formate and 0.1% formic acid to methanol with 4 mM ammonium formate and 0.1% formic acid.

QuEChERS Dispersive Solid Phase Extraction (dSPE) Cleanup

Restek Q-sep® dSPE tubes were used and sorbent formulations were chosen based on commodity nutrient composition and preliminary experiments examining pigment removal. Table II shows the dSPE formulation, amount of extract processed, manual shake time, and specific dSPE formulation method and catalog number. Cleanup tubes contained one or more of three sorbents: primary secondary amine (PSA), octadecyl (C18), and graphitized carbon black (GCB). In addition to the contents listed in Table II, each dSPE tube contained 150 mg magnesium sulfate per mL extract processed. After the acetonitrile extract was added to the dSPE tube, the tube was shaken for the period specified in Table II. The samples were then centrifuged for 5 minutes at 3,000 xg. For LC-MS/MS analysis, the extract was diluted 10-fold with initial mobile phase, 90:10 (v:v) ratio of water with 4 mM ammonium formate and 0.1% formic acid to methanol with 4 mM ammonium formate and 0.1% formic acid.

Table II: The dSPE sorbent formula and extract volume used during sample cleanup for each food are listed below. The shake time required, method associated with each formulation, and Restek catalog number are also presented.

	dSPE Contents						
	PSA (mg)	C18 (mg)	GCB (mg)	Extract (mL)	Shake (min)	Method	Cat.#
Celery	25	—	7.5	1	2	EN 15662	26218
Kale	300	—	150	6	2	NA	26126
Lime	25	—	2.5	1	2	EN 15662	26217
Avocado	50	50	—	1	0.5	AOAC 2007.01	26125
Brown rice flour	50	50	—	1	0.5	AOAC 2007.01	26125

Solvent and Matrix-Matched Calibration Standards

Two calibration strategies commonly used for LC-MS/MS pesticide residue analysis are a solvent-only calibration curve and a matrix-matched calibration curve. The solvent curve uses analytes in a common solvent. The solvent calibration curve generated contained pesticides at various levels in the initial mobile phase of 90% water with 4 mM ammonium formate and 0.1% formic acid to 10% methanol with 4 mM ammonium formate and 0.1% formic acid (v/v). Solvent curves for the dilution method were generated daily with calibration levels of 0.25, 0.5, 1, 2, 10, 25, and 50 ppb with internal standard compounds at 12.5 ppb. Because only 5 g of brown rice flour was initially processed, the expected injection concentration of the 10 ppb spike samples was 0.25 ppb. Therefore, a 0.1 ppb calibration level was added for the brown rice flour samples. Solvent calibration curves for the QuEChERS method were also prepared daily with concentrations of 0.5, 1, 2, 10, 25, 50, and 75 ppb and internal standard compounds at 25 ppb. All solvent standards were made in 90% water with 4 mM ammonium formate and 0.1% formic acid to 10% methanol with 4 mM ammonium formate and 0.1% formic acid (v/v).

Dilution method matrix-matched calibration curves were produced by combining 50 µL matrix solution (pre-cleanup) with the pesticide stock standard solution and water with 4 mM ammonium formate and 0.1% formic acid totaling 1 mL and resulting in a 20-fold dilution of the matrix. This was done for calibration standards at 0.25, 0.5, 1, 2, 10, 25, and 50 ppb with internal standard compounds at 12.5 ppb. Again, a 0.1 ppb calibration standard was added for brown rice flour samples. The QuEChERS method matrix-matched curves combined 100 µL matrix solution (post cleanup), with pesticide stock solution and water with 4 mM ammonium formate and 0.1% formic acid totaling 1 mL and resulting in a 10-fold dilution of the matrix. Calibration standards included pesticides at 0.5, 1, 2, 10, 25, 50, and 75 ppb and internal standard compounds at 25 ppb.

LC-MS/MS Analysis

A Shimadzu UFLCXR LC (Columbia, MD) and Applied Biosystems/MDS SCIEX AB SCIEX API 4000™ LC-MS/MS system with Turbo V™ source (Foster City, CA) were used for LC-MS/MS pesticide residue analysis. Testing was performed using a 100 mm x 2.1 mm, 3 µm Ultra Aqueous C18 column (Restek, cat.# 9178312) and 20 µL injections. The column was held at 50 °C. A mobile phase gradient of solvent A, water with 4 mM ammonium formate and 0.1% formic acid, and solvent B, methanol with 4 mM ammonium formate and 0.1% formic acid, and 0.5 mL/min flow rate were used. The mobile phase gradient is shown in Table III and includes a 3 minute re-equilibration step. Compounds were ionized by positive electrospray ionization. The interface parameters are as follows: interface temperature at 450 °C, ion spray voltage of 5.5 kV, curtain gas at 30 psi (206.8 kPa), ion source gas 1 at 40 psi (275.8 kPa), and ion source gas 2 at 45 psi (310.3 kPa). Two transitions were optimized for each compound and monitored in Scheduled MRM™ (sMRM) mode. The MRM window was 45 seconds and target scan time was 0.33 seconds. The optimized MRM transitions and retention times for each analyte are listed in Table IV and the optimized voltages can be found in Table V. Pesticide identification was based on retention time matching and MRM transition ratios.

Table III: LC-MS/MS mobile phase gradient program.

Time (min)	%A	%B
0	90	10
1.5	90	10
6	30	70
9	30	70
10	0	100
12	0	100
12.01	90	10
15	90	10

Table IV: LC-MS/MS MRM transitions and retention times for each analyte.

Component Name	Q1/Q3 Transition 1, m/z	Q1/Q3 Transition 2, m/z	t_R (min)
Methamidophos	142.1 / 94.1	142.1 / 112.2	1.27
Acephate	184.1 / 125.1	184.1 / 95.1	1.78
Propamocarb	189.2 / 102.0	189.2 / 144.2	2.65
Omethoate	214.1 / 125.2	214.1 / 155.1	2.94
Aldicarb sulfone	223.2 / 148.0	223.2 / 76.2	3.45
Aldicarb sulfoxide	207.2 / 132.1	207.2 / 89.1	3.71
Pymetrozine	218.1 / 105.0	218.1 / 78.2	3.82
Oxamyl	237.1 / 71.9	237.1 / 90.1	3.9
Methomyl	163.1 / 88.1	163.1 / 106.2	4.02
Monocrotophos	224.1 / 127.1	224.1 / 98.1	4.38
Dimethoate	230.1 / 125.2	230.1 / 171.2	4.81
Mevinphos E	225.2 / 193.3	225.2 / 127.2	4.99
Thiabendazole	202.2 / 175.0	202.2 / 131.2	4.99
Imidacloprid	256.3 / 209.1	256.3 / 175.2	5.02
Mevinphos Z	225.1 / 193.2	225.1 / 127.1	5.43
Aldicarb	208.2 / 116.2	208.2 / 89.1	5.55
Carbetamide	237.1 / 192.0	237.1 / 118.1	5.73
Imazethapyr	290.1 / 245.2	290.1 / 177.3	5.9
Thidiazuron	221.2 / 102.0	221.2 / 128.1	5.93
Thiophanate methyl	343.2 / 151.1	343.2 / 93.1	5.96
Propoxur	210.2 / 168.1	210.2 / 111.2	5.99
Bendiocarb	224.1 / 109.2	224.1 / 167.2	6.00
Dichlorvos	220.9 / 109.2	220.9 / 95.0	6.01
Carbofuran	222.3 / 165.2	222.3 / 123.1	6.09
Pirimicarb	239.2 / 72.2	239.2 / 182.2	6.15
Carbaryl	202.3 / 127.1	202.3 / 117.2	6.32
Imazalil	297.1 / 159.0	297.1 / 173.1	6.40
Isoprocarb	194.3 / 95.2	194.3 / 137.3	6.53
Metalaxyl	280.4 / 192.3	280.4 / 160.2	6.72
Metalaxyl-M	280.4 / 220.3	280.4 / 192.1	6.72
Atrazine	216.2 / 174.3	216.2 / 132.1	6.75
Isoproturon	207.2 / 72.3	207.2 / 134.3	6.86
Azinphos methyl	318.2 / 160.0	318.2 / 132.1	6.96
Diuron	233.1 / 72.1	233.1 / 160.0	6.97
Phosmet	318.1 / 160.2	318.1 / 133.0	6.97
Demeton-O	259.0 / 89.1	259.0 / 61.2	6.98
Demeton-S	259.2 / 88.9	259.2 / 61.1	6.98
Nuarimol	315.0 / 252.1	315.0 / 81.0	7.08
Propanil	218.2 / 162.0	218.2 / 127.0	7.11
Azoxystrobin	404.3 / 372.3	404.3 / 344.0	7.12
Malathion	331.1 / 127.1	331.1 / 99.1	7.13
Methiocarb	226.1 / 169.1	226.1 / 121.1	7.14
Chlorpropham	214.1 / 154.0	214.1 / 126.1	7.20
Linuron	249.2 / 160.1	249.2 / 182.1	7.20
Crotoxyphos	332.2 / 211.2	332.2 / 167.2	7.21
Promecarb	208.8 / 109.2	208.8 / 151.3	7.21
Propetamphos	282.1 / 138.0	282.1 / 110.2	7.22
Boscalid	343.2 / 307.2	343.2 / 140.0	7.25
Triadimefon	294.3 / 197.1	294.3 / 69.2	7.26
Triadimenol	296.3 / 70.2	296.3 / 227.1	7.35
Fenhexamid	302.1 / 97.1	302.1 / 55.0	7.40

Component Name	Q1/Q3 Transition 1, m/z	Q1/Q3 Transition 2, m/z	t_R (min)
Myclobutanil	289.3 / 70.2	289.3 / 124.9	7.43
Dichlofluanid	332.9 / 224.0	332.9 / 123.1	7.44
Triazophos	314.1 / 162.0	314.1 / 119.2	7.48
Alachlor	270.2 / 238.1	270.2 / 162.2	7.60
Fenarimol	331.0 / 268.0	331.0 / 81.0	7.61
Iprodione	330.3 / 245.2	332.3 / 247.0	7.66
Ethoprop	243.1 / 131.0	243.1 / 173.0	7.70
Parathion	292.1 / 236.0	292.1 / 140.1	7.77
Fenamiphos	304.4 / 217.2	304.4 / 202.0	7.83
Diflubenzuron	311.1 / 158.2	311.1 / 141.1	7.85
Fenoxycarb	302.1 / 88.0	302.1 / 116.1	7.87
Etaconazole-1	328.2 / 159.1	328.2 / 123.0	7.89
Fenbuconazole	337.3 / 125.3	337.3 / 70.3	7.89
Kresoxim methyl	314.2 / 115.9	314.2 / 131.0	7.9
Tolyfluanid	364.0 / 238.0	364.0 / 137.1	8.00
Etaconazole-2	328.3 / 159.2	328.3 / 123.1	8.01
Fenthion	279.1 / 169.1	279.1 / 105.1	8.06
Quinalphos	299.3 / 243.1	299.3 / 163.2	8.07
Cyprodinil	226.1 / 93.3	226.1 / 77.1	8.16
Tebuconazole	308.3 / 70.1	308.3 / 125.1	8.24
Chlorfenvinphos	359.2 / 155.1	359.2 / 99.2	8.32
Diazinon	305.2 / 169.3	305.2 / 153.1	8.34
Pirimiphos methyl	306.1 / 164.3	306.1 / 108.1	8.35
Phosalone	368.1 / 182.1	368.1 / 138.0	8.38
Coumaphos	363.1 / 227.2	363.1 / 211.1	8.46
Propiconazole-1	342.3 / 159.0	342.3 / 69.3	8.60
Pyraclostrobin	388.0 / 164.2	388.0 / 194.3	8.61
Chlorpyrifos methyl	323.9 / 125.0	323.9 / 291.8	8.72
Propiconazole-2	342.4 / 159.1	342.4 / 69.4	8.77
Dialifos	394.3 / 208.0	394.3 / 187.0	8.81
Prochloraz	376.1 / 308.1	376.1 / 266.0	9.01
Indoxacarb	528.6 / 218.0	528.6 / 150.2	9.04
Trifloxystrobin	409.4 / 186.0	409.4 / 145.1	9.18
Spinosyn A	733.1 / 142.4	733.1 / 98.4	9.58
Difenoconazole-1	406.3 / 251.1	408.2 / 253.1	9.60
Triflumizole	346.2 / 278.2	346.2 / 73.1	9.79
Difenoconazole-2	406.4 / 251.2	408.3 / 253.2	9.85
Ethion	385.3 / 199.0	385.3 / 171.0	10.20
Spinosyn D	746.8 / 142.4	746.8 / 98.3	10.38
Chlorpyrifos	350.0 / 198.0	350.0 / 97.0	10.40
Pendimethalin	282.3 / 212.2	282.3 / 194.3	10.47
Emamectin B1a Benzoate	887.2 / 158.3	887.2 / 126.3	10.48
Propargite	368.4 / 175.1	368.4 / 231.2	10.61
Fenpropathrin	350.3 / 125.0	350.3 / 97.4	10.67
Flufenoxuron	489.5 / 158.2	489.5 / 141.1	10.72
Lambda-cyhalothrin	467.4 / 225.1	467.4 / 181.0	10.72
Deltamethrin	523.3 / 280.9	523.3 / 181.0	10.78
trans-Permethrin	408.4 / 183.3	408.4 / 153.2	10.90
Leptophos	413.2 / 171.0	413.2 / 77.1	10.92
cis-Permethrin	408.5 / 183.1	408.5 / 153.2	10.95
Bifenthrin	440.3 / 181.2	440.3 / 166.2	10.98

Table V: LC-MS/MS optimized voltages for MRM mode. Voltages for first and second transitions are designated by 1 and 2, respectively.

Component Name	Declustering Potential (DP), V	Entrance Potential (EP), V	Collision Energy (CE), V	Collision Cell Exit Potential (CXP), V
Methamidophos 1	57	11	20	6
Methamidophos 2	57	11	17	8
Acephate 1	53	5	25	7
Acephate 2	53	5	32	6
Propamocarb 1	61	10	25	8
Propamocarb 2	48	11	19	11
Omethoate 1	55	5	31	7
Omethoate 2	55	5	22	10
Aldicarb sulfone 1	71	5	14	11
Aldicarb sulfone 2	71	5	12	14
Aldicarb sulfoxide 1	58	3	10	10
Aldicarb sulfoxide 2	58	3	20	5
Pymetrozine 1	66	10	29	6
Pymetrozine 2	66	10	53	4
Oxamyl 1	34	5	20	6
Oxamyl 2	34	5	12	6
Methomyl 1	33	5	13	6
Methomyl 2	33	5	15	7
Monocrotophos 1	60	5	21	6
Monocrotophos 2	60	5	18	6
Dimethoate 1	56	11	29	6
Dimethoate 2	56	11	21	11
Mevinphos E 1	59	5	20	8
Mevinphos E 2	59	5	10	6
Imidacloprid 1	62	11	21	6
Imidacloprid 2	62	11	25	10
Thiabendazole 1	91	9	36	11
Thiabendazole 2	91	9	47	9
Mevinphos Z 1	59	5	10	6
Mevinphos Z 2	59	5	20	8
Aldicarb 1	30	5	11	9
Aldicarb 2	30	5	22	5
Carbetamide 1	56	10	13	12
Carbetamide 2	56	10	19	10
Atrazine d5 (IS) 1	64	10	26	11
Atrazine d5 (IS) 2	64	10	36	5
Imazethapyr 1	77	5	30	15
Imazethapyr 2	77	5	38	10
Thidiazuron 1	52	11	22	6
Thidiazuron 2	52	11	22	7
Thiophanate methyl 1	69	11	28	11
Thiophanate methyl 2	69	11	68	6
Propoxur 1	56	5	21	8
Propoxur 2	56	5	12	11
Bendiocarb 1	60	11	25	34
Bendiocarb 2	60	11	110	15
Dichlorvos 1	74	5	27	7
Dichlorvos 2	74	5	46	7
Carbofuran 1	64	10	17	11
Carbofuran 2	64	10	30	8
Pirimicarb 1	48	10	32	5
Pirimicarb 2	48	10	23	10
Carbaryl 1	57	15	37	11
Carbaryl 2	57	15	34	9
Imazalil 1	79	11	30	11
Imazalil 2	79	11	35	11
Isoprocarb 1	62	10	22	6
Isoprocarb 2	62	10	14	11
Metalaxyl-M 1	60	11	20	6
Metalaxyl-M 2	60	11	26	14
Metalaxyl 1	54	5	41	5
Metalaxyl 2	54	5	63	15

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Component Name	Declustering Potential (DP), V	Entrance Potential (EP), V	Collision Energy (CE), V	Collision Cell Exit Potential (CXP), V
Atrazine 1	72	9	25	10
Atrazine 2	72	9	32	8
Isoproturon 1	68	5	31	5
Isoproturon 2	68	5	31	7
Phosmet 1	64	4	17	12
Phosmet 2	64	4	50	10
Demeton-S 1	50	5	16	6
Demeton-S 2	50	5	48	7
Demeton-O 1	40	5	14	6
Demeton-O 2	40	5	47	6
Diuron 1	74	10	44	5
Diuron 2	74	10	36	12
Azinphos methyl 1	57	5	11	11
Azinphos methyl 2	57	5	21	10
Nuarimol 1	81	10	31	16
Nuarimol 2	81	10	45	14
Azoxystrobin 1	58	10	20	9
Azoxystrobin 2	58	10	34	9
Propanil 1	66	15	23	11
Propanil 2	66	15	36	9
Malathion 1	63	15	19	7
Malathion 2	63	15	33	7
Methiocarb 1	61	5	15	10
Methiocarb 2	61	5	27	8
Promecarb 1	63	5	22	9
Promecarb 2	63	5	14	11
Crotoxyphos 1	34	4	13	6
Crotoxyphos 2	34	4	21	11
Chlorpropham 1	54	5	25	11
Chlorpropham 2	54	5	35	8
Linuron 1	69	10	25	11
Linuron 2	69	10	23	10
Propetamphos 1	60	6	23	11
Propetamphos 2	60	6	41	8
Boscalid 1	98	10	28	11
Boscalid 2	98	10	27	11
Triadimefon 1	59	11	22	6
Triadimefon 2	59	11	32	6
Triadimenol 1	42	4	26	5
Triadimenol 2	42	4	14	6
Fenhexamid 1	81	10	35	6
Fenhexamid 2	81	10	71	10
Myclobutanil 1	65	11	36	5
Myclobutanil 2	65	11	39	9
Dichlofluanid 1	70	4	16	15
Dichlofluanid 2	70	4	44	15
Triazophos 1	76	9	26	11
Triazophos 2	76	9	50	9
Alachlor 1	46	5	12	15
Alachlor 2	46	5	28	11
Fenarimol 1	61	10	31	4
Fenarimol 2	61	10	49	15
Diazinon d10 (IS) 1	74	10	31	11
Diazinon d10 (IS) 2	74	10	32	11
Iprodione 1	82	4	21	6
Iprodione 2	82	4	31	7
Ethoprop 1	64	11	21	11
Ethoprop 2	64	11	29	9
Parathion 1	70	4	22	15
Parathion 2	70	4	33	10
Parathion d10 1	74	4	49	28
Parathion d10 2	74	4	49	28
Fenamiphos 1	82	5	31	6
Fenamiphos 2	82	5	47	6

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Component Name	Declustering Potential (DP), V	Entrance Potential (EP), V	Collision Energy (CE), V	Collision Cell Exit Potential (CXP), V
Diflubenzuron 1	70	4	22	10
Diflubenzuron 2	70	4	46	8
Fenoxycarb 1	66	10	31	6
Fenoxycarb 2	66	10	17	8
Fenbuconazole 1	78	10	38	6
Fenbuconazole 2	78	10	43	5
Kresoxim methyl 1	66	10	33	8
Kresoxim methyl 2	66	10	19	6
Etaconazole (isomer 1) 1	93	10	35	10
Etaconazole (isomer 1) 2	93	10	75	9
Etaconazole (isomer 2) 1	93	10	35	10
Etaconazole (isomer 2) 2	93	10	75	9
Tolyfluanid 1	46	10	39	8
Tolyfluanid 2	46	10	19	14
Fenthion 1	71	10	25	11
Fenthion 2	71	10	34	7
Quinalphos 1	56	11	24	15
Quinalphos 2	56	11	34	11
Cyprodinil 1	92	11	49	5
Cyprodinil 2	92	11	61	14
Tebuconazole 1	75	11	46	6
Tebuconazole 2	75	11	48	7
Chlorfenvinphos 1	78	5	19	11
Chlorfenvinphos 2	78	5	44	7
Diazinon 1	58	10	31	11
Diazinon 2	58	10	28	12
Phosalone 1	78	9	20	9
Phosalone 2	78	9	41	11
Pirimiphos methyl 1	75	11	31	11
Pirimiphos methyl 2	75	11	43	9
Coumaphos 1	92	11	35	6
Coumaphos 2	130	10	52	12
Pyraclostrobin 1	46	10	18	6
Pyraclostrobin 2	46	10	26	11
Propiconazole (isomer 1) 1	81	5	37	11
Propiconazole (isomer 1) 2	81	5	37	5
Propiconazole (isomer 2) 1	81	5	37	11
Propiconazole (isomer 2) 2	81	5	37	5
Chlorpyrifos methyl 1	74	5	28	9
Chlorpyrifos methyl 2	74	5	22	18
Dialifos 1	71	5	12	7
Dialifos 2	71	5	20	6
Indoxacarb 1	90	11	33	14
Indoxacarb 2	90	11	34	11
Prochloraz 1	51	11	17	11
Prochloraz 2	51	11	24	18
Trifloxystrobin 1	56	6	22	13
Trifloxystrobin 2	56	6	61	11
Difenoconazole (isomer 1) 1	86	11	34	6
Difenoconazole (isomer 1) 2	76	10	33	4
Spinosyn A 1	111	11	44	11
Spinosyn A 2	111	11	93	7
Triflumizole 1	53	5	15	12
Triflumizole 2	53	5	26	5
Difenoconazole (isomer 2) 1	86	11	34	6
Difenoconazole (isomer 2) 2	76	10	33	4
Ethion 1	71	5	15	6
Ethion 2	71	5	22	11
Spinosyn D 1	112	11	41	11
Spinosyn D 2	112	11	93	7
Chlorpyrifos 1	71	10	45	6
Chlorpyrifos 2	71	10	23	12

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Component Name	Declustering Potential (DP), V	Entrance Potential (EP), V	Collision Energy (CE), V	Collision Cell Exit Potential (CXP), V
Pendimethalin 1	45	4	16	6
Pendimethalin 2	45	4	26	7
Emamectin B1a Benzoate 1	127	5	50	11
Emamectin B1a Benzoate 2	127	5	62	6
Propargite 1	61	5	15	7
Propargite 2	61	5	23	11
Fenpropathrin 1	103	10	17	11
Fenpropathrin 2	103	10	44	5
Lambda-cyhalothrin 1	59	5	22	6
Lambda-cyhalothrin 2	59	5	49	15
Flufenoxuron 1	91	5	27	11
Flufenoxuron 2	91	5	65	12
Deltamethrin 1	56	4	22	19
Deltamethrin 2	130	10	52	12
trans-Permethrin 1	50	10	22	6
trans-Permethrin 2	50	10	61	11
Leptophos 1	85	11	32	13
Leptophos 2	85	11	77	6
cis-Permethrin 1	49	10	27	13
cis-Permethrin 2	49	10	61	11
Bifenthrin 1	53	5	18	9
Bifenthrin 2	53	5	59	11

Determination of Matrix Effects

Matrix effect values were determined for each pesticide in each commodity. Data were generated from the solvent and matrix-matched calibration curves discussed previously. Matrix effect values were calculated by comparing the ratio of the solvent curve slope to the matrix-matched curve slope. Equation 1 shows the calculation used to determine matrix effect. Values above 100% indicate enhanced ionization in matrix and values below 100% define ion signal suppression due to matrix.

$$\text{Equation 1: } \%ME = \left(\frac{\text{slope MM curve}}{\text{slope sol curve}}\right) * 100$$

Pesticide Percent Recovery Determination

Fortified samples at two levels, 10 and 500 µg/kg (ppb), were prepared for both the QuEChERS method and the dilution method. Atrazine-d5 was used as an internal standard. Quantification was performed two ways: one way used a solvent calibration curve and the other way used a matrix-matched calibration curve. Calculated concentrations were compared to the expected concentration, assuming 100% recovery. For each pesticide there is a set of four recovery values per fortification level: two recovery values for the QuEChERS method and two values for the dilution method. These values are based on pesticide quantification using a solvent curve and a matrix-matched curve. The four percent recovery categories will be abbreviated as shown in Table VI.

Table VI: Abbreviations for the sample preparation and calibration method combinations.

Abbreviation	Sample Preparation Method	Calibration Method
Q/Sol	QuEChERS	solvent calibration curve
Q/MM	QuEChERS	matrix-matched calibration curve
D/Sol	dilution	solvent calibration curve
D/MM	dilution	matrix-matched calibration curve

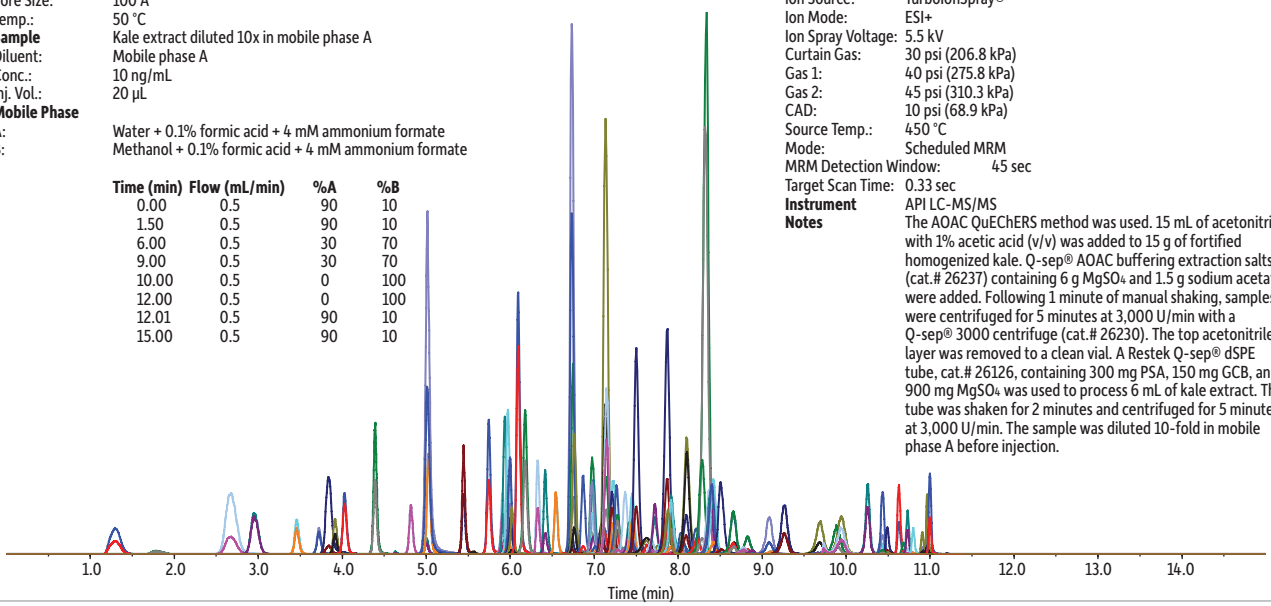
Figure 1: Chromatogram of QuEChERS extract of kale fortified with pesticides.

	Peaks	t_R (min)	MRM 1	MRM 2
1.	Methamidophos	1.27	142.1 / 94.1	142.1 / 112.2
2.	Acephate	1.78	184.1 / 125.1	184.1 / 95.1
3.	Propamocarb	2.65	189.2 / 102.0	189.2 / 144.2
4.	Omethoate	2.94	214.1 / 125.2	214.1 / 155.1
5.	Aldicarb sulfone	3.45	223.2 / 148.0	223.2 / 76.2
6.	Aldicarb sulfoxide	3.71	207.2 / 132.1	207.2 / 89.1
7.	Pymetrozine	3.82	218.1 / 105.0	218.1 / 78.2
8.	Oxamyl	3.90	237.1 / 71.9	237.1 / 90.1
9.	Methomyl	4.02	163.1 / 88.1	163.1 / 106.2
10.	Monocrotophos	4.38	224.1 / 127.1	224.1 / 98.1
11.	Dimethoate	4.81	230.1 / 125.2	230.1 / 171.2
12.	Mevinphos E	4.99	225.2 / 193.3	225.2 / 127.2
13.	Thiabendazole	4.99	202.2 / 175.0	202.2 / 131.2
14.	Imidacloprid	5.02	256.3 / 209.1	256.3 / 175.2
15.	Mevinphos Z	5.43	225.1 / 193.2	225.1 / 127.1
16.	Aldicarb	5.55	208.2 / 116.2	208.2 / 89.1
17.	Carbetamide	5.73	237.1 / 192.0	237.1 / 118.1
18.	Imazethapyr	5.90	290.1 / 245.2	290.1 / 177.3
19.	Thidiazuron	5.93	221.2 / 102.0	221.2 / 128.1
20.	Thiophanate methyl	5.96	343.2 / 151.1	343.2 / 93.1
21.	Propoxur	5.99	210.2 / 168.1	210.2 / 111.2
22.	Bendiocarb	6.00	224.1 / 109.2	224.1 / 167.2
23.	Dichlorvos	6.01	220.9 / 109.2	220.9 / 95.0
24.	Carbofuran	6.09	222.3 / 165.2	222.3 / 123.1
25.	Pirimicarb	6.15	239.2 / 72.2	239.2 / 182.2
26.	Carbaryl	6.32	202.3 / 127.1	202.3 / 117.2
27.	Imazalil	6.40	297.1 / 159.0	297.1 / 173.1
28.	Isoprocarb	6.53	194.3 / 95.2	194.3 / 137.3
29.	Metalaxyl	6.72	280.4 / 192.3	280.4 / 160.2
30.	Metalaxyl-m	6.72	280.4 / 220.3	280.4 / 192.1
31.	Atrazine	6.75	216.2 / 174.3	216.2 / 132.1
32.	Atrazine-d5 (IS)	6.77	221.1 / 179.0	221.1 / 101.2
33.	Isoproturon	6.86	207.2 / 72.3	207.2 / 134.3
34.	Azinphos-methyl	6.96	318.2 / 160.0	318.2 / 132.1
35.	Diuron	6.97	233.1 / 72.1	233.1 / 160.0
36.	Phosmet	6.97	318.1 / 160.2	318.1 / 133.0
37.	Demeton-O	6.98	259.0 / 89.1	259.0 / 61.2
38.	Demeton-S	6.98	259.2 / 88.9	259.2 / 61.1
39.	Nuarimol	7.08	315.0 / 252.1	315.0 / 81.0
40.	Propanil	7.11	218.2 / 162.0	218.2 / 127.0
41.	Azoxystrobin	7.12	404.3 / 372.3	404.3 / 344.0
42.	Malathion	7.13	331.1 / 127.1	331.1 / 99.1
43.	Methiocarb	7.14	226.1 / 169.1	226.1 / 121.1
44.	Chlorpropham	7.20	214.1 / 154.0	214.1 / 126.1
45.	Linuron	7.20	249.2 / 160.1	249.2 / 182.1
46.	Crotoxyphos	7.21	332.2 / 211.2	332.2 / 167.2
47.	Promecarb	7.21	208.8 / 109.2	208.8 / 151.3
48.	Propetamphos	7.22	282.1 / 138.0	282.1 / 110.2
49.	Boscalid	7.25	343.2 / 307.2	343.2 / 140.0
50.	Triadimefon	7.26	294.3 / 197.1	294.3 / 69.2
51.	Triadimenol	7.35	296.3 / 70.2	296.3 / 227.1
52.	Fenhexamid	7.40	302.1 / 97.1	302.1 / 55.0
53.	Myclobutanil	7.43	289.3 / 70.2	289.3 / 124.9
54.	Dichlofluanid	7.44	332.9 / 224.0	332.9 / 123.1
55.	Triazophos	7.48	314.1 / 162.0	314.1 / 119.2
56.	Alachlor	7.60	270.2 / 238.1	270.2 / 162.2
57.	Fenarimol	7.61	331.0 / 268.0	331.0 / 81.0
58.	Iprodione	7.66	330.3 / 245.2	332.3 / 247.0
59.	Ethoprop	7.70	243.1 / 131.0	243.1 / 173.0
60.	Parathion	7.77	292.1 / 236.0	292.1 / 140.1
61.	Fenamiphos	7.83	304.4 / 217.2	304.4 / 202.0
62.	Diflubenzuron	7.85	311.1 / 158.2	311.1 / 141.1
63.	Fenoxycarb	7.87	302.1 / 88.0	302.1 / 116.1
64.	Etaconazole isomer 1	7.89	328.2 / 159.1	328.2 / 123.0
65.	Fenbuconazole	7.89	337.3 / 125.3	337.3 / 70.3
66.	Kresoxim-methyl	7.90	314.2 / 115.9	314.2 / 131.0
67.	Tolyfluanid	8.00	364.0 / 238.0	364.0 / 137.1
68.	Etaconazole isomer 2	8.01	328.3 / 159.2	328.3 / 123.1
69.	Fenthion	8.06	279.1 / 169.1	279.1 / 105.1
70.	Quinalphos	8.07	299.3 / 243.1	299.3 / 163.2
71.	Cyprodinil	8.16	226.1 / 93.3	226.1 / 77.1
72.	Tebuconazole	8.24	308.3 / 70.1	308.3 / 125.1
73.	Chlorfenvinphos	8.32	359.2 / 155.1	359.2 / 99.2
74.	Diazinon	8.34	305.2 / 169.3	305.2 / 153.1
75.	Pirimiphos methyl	8.35	306.1 / 164.3	306.1 / 108.1
76.	Phosalone	8.38	368.1 / 182.1	368.1 / 138.0
77.	Diazinon-d10 (IS)	8.39	315.3 / 170.1	315.3 / 154.1
78.	Coumaphos	8.46	363.1 / 227.2	363.1 / 211.1
79.	Propiconazole isomer 1	8.60	342.3 / 159.0	342.3 / 69.3
80.	Pyraclostrobin	8.61	388.0 / 164.2	388.0 / 194.3
81.	Chlorpyrifos methyl	8.72	323.9 / 125.0	323.9 / 291.8
82.	Propiconazole isomer 2	8.77	342.4 / 159.1	342.4 / 69.4
83.	Dialifos	8.81	394.3 / 208.0	394.3 / 187.0
84.	Prochloraz	9.01	376.1 / 308.1	376.1 / 266.0
85.	Indoxacarb	9.04	528.6 / 218.0	528.6 / 150.2
86.	Trifloxystrobin	9.18	409.4 / 186.0	409.4 / 145.1
87.	Spinosyn A	9.58	733.1 / 142.4	733.1 / 98.4
88.	Difenoconazole isomer 1	9.60	406.3 / 251.1	408.2 / 253.1
89.	Triflumizole	9.79	346.2 / 278.2	346.2 / 73.1
90.	Difenoconazole isomer 2	9.85	406.4 / 251.2	408.3 / 253.2
91.	Ethion	10.20	385.3 / 199.0	385.3 / 171.0
92.	Spinosyn D	10.38	746.8 / 142.4	746.8 / 98.3
93.	Chlorpyrifos	10.40	350.0 / 198.0	350.0 / 97.0
94.	Pendimethalin	10.47	282.3 / 212.2	282.3 / 194.3
95.	Emamectin B1a benzoate	10.48	887.2 / 158.3	887.2 / 126.3
96.	Propargite	10.61	368.4 / 175.1	368.4 / 231.2
97.	Fenpropathrin	10.67	350.3 / 125.0	350.3 / 97.4
98.	Flufenoxuron	10.72	489.5 / 158.2	489.5 / 141.1
99.	Lambda cyhalothrin	10.72	467.4 / 225.1	467.4 / 181.0
100.	Deltamethrin	10.78	523.3 / 280.9	523.3 / 181.0
101.	*trans*-Permethrin	10.90	408.4 / 183.3	408.4 / 153.2
102.	Leptophos	10.92	413.2 / 171.0	413.2 / 77.1
103.	*cis*-Permethrin	10.95	408.5 / 183.1	408.5 / 153.2
104.	Bifenthrin	10.98	440.3 / 181.2	440.3 / 166.2

Isomers were designated 1 or 2 by elution order

Column Ultra Aqueous C18 (cat.# 9178312)
Dimensions: 100 mm x 2.1 mm ID
Particle Size: 3 µm
Pore Size: 100 Å
Temp.: 50 °C
Sample Kale extract diluted 10x in mobile phase A
Diluent: Mobile phase A
Conc.: 10 ng/mL
Inj. Vol.: 20 µL
Mobile Phase
A: Water + 0.1% formic acid + 4 mM ammonium formate
B: Methanol + 0.1% formic acid + 4 mM ammonium formate

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	90	10
1.50	0.5	90	10
6.00	0.5	30	70
9.00	0.5	30	70
10.00	0.5	0	100
12.00	0.5	0	100
12.01	0.5	90	10
15.00	0.5	90	10



Detector ABSCIEX MS/MS
Model #: API 4000
Ion Source: TurboIonSpray®
Ion Mode: ESI+
Ion Spray Voltage: 5.5 kV
Curtain Gas: 30 psi (206.8 kPa)
Gas 1: 40 psi (275.8 kPa)
Gas 2: 45 psi (310.3 kPa)
CAD: 10 psi (68.9 kPa)
Source Temp.: 450 °C
Mode: Scheduled MRM
MRM Detection Window: 45 sec
Target Scan Time: 0.33 sec
Instrument API LC-MS/MS
Notes The AOAC QuEChERS method was used. 15 mL of acetonitrile with 1% acetic acid (v/v) was added to 15 g of fortified homogenized kale. Q-sep® AOAC buffering extraction salts (cat.# 26237) containing 6 g $MgSO_4$ and 1.5 g sodium acetate were added. Following 1 minute of manual shaking, samples were centrifuged for 5 minutes at 3,000 U/min with a Q-sep® 3000 centrifuge (cat.# 26230). The top acetonitrile layer was removed to a clean vial. A Restek Q-sep® dSPE tube, cat.# 26126, containing 300 mg PSA, 150 mg GCB, and 900 mg $MgSO_4$ was used to process 6 mL of kale extract. The tube was shaken for 2 minutes and centrifuged for 5 minutes at 3,000 U/min. The sample was diluted 10-fold in mobile phase A before injection.

RESTEK

LC_FF0516

Results and Discussion

LC-MS/MS Analysis

Figure 1 shows a chromatogram of kale extract processed with the QuEChERS method. This is representative of the chromatograms produced in this work. Analytes eluted in 11 minutes followed by a short high-organic rinse and an equilibration step. The high-organic rinse was important in helping to maintain the column and also in avoiding matrix carryover into the next sample. Elution of remaining matrix material during a subsequent analysis can cause unexpected matrix effects. There is evidence that late eluting matrix compounds in a reverse phase HPLC analysis can cause significant ion suppression [2].

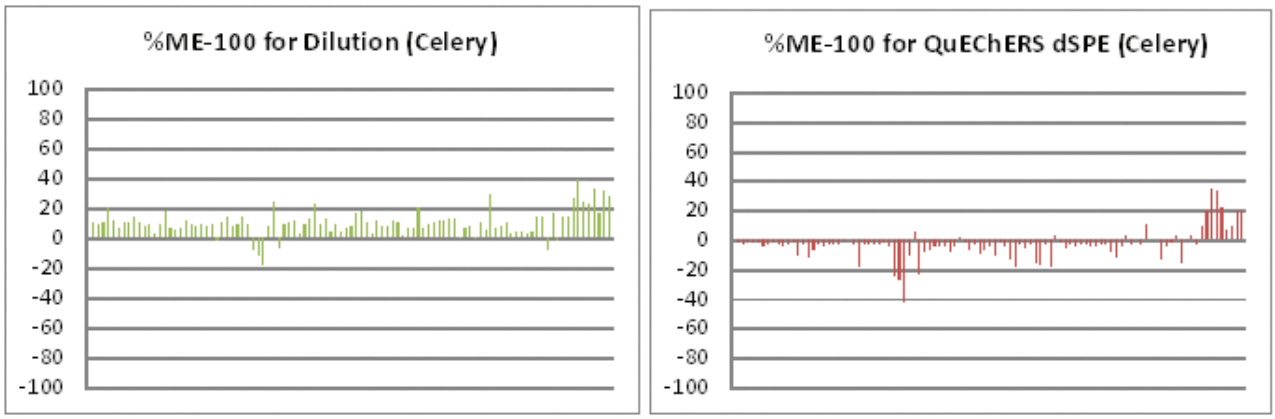
Retention times were an integral part of pesticide identifications and were used to produce the scheduled MRM program. For these reasons, retention time reproducibility is extremely important. We tested approximately 380 samples; each sample tracked 204 MRM transitions resulting in 77,520 MRM transitions. Only four transitions, or 0.005%, shifted and all of these were isomers that could be manually integrated and reported as one peak. Early eluting polar pesticides typically are difficult to analyze on C18 columns and usually are characterized by little or no retention and poor peak shape. However, we obtained excellent results using an Ultra Aqueous C18 column. We tested polar pesticides methamidophos, acephate, propamocarb, and omethoate using an Ultra Aqueous C18 column (100 mm x 2.1 mm, 3 µm). This column is a polar modified bonded C18 stationary phase, meaning it can interact with polar and nonpolar compounds. This resulted in significant retention and good peak shapes for these early-eluting polar pesticides. Also, the remaining analytes were distributed across the elution window. This can help ensure a proper scan rate for scheduled MRM methods with many analytes.

Matrix Effects Interpretation

The matrix effect for each compound was calculated as described by Equation 1 above. If we assume that the slopes of the solvent calibration curve and the matrix-matched curve are equal, then no matrix effect is present and the slope ratio is 1, meaning the signal of a compound in matrix and in solvent is exactly the same. A matrix effect value above 100% indicates signal enhancement when the analyte is tested in matrix. When the value is below 100% this means that the signal of an analyte in matrix is lower or suppressed compared to analyte in solvent.

We illustrate total matrix effects by plotting the calculated matrix effect minus 100 for each compound ordered by increasing retention time. These plots help identify matrix effect trends, like overall suppression or enhancement of a specific commodity or an association between retention time and matrix effects. These matrix effect plots were generated for samples processed by QuEChERS and dilution methods and can be used to compare trends. For example, the plots for celery in Figure 2 show that the dilution method tended to produce signal enhancement, while celery processed with QuEChERS tended to show signal suppression. However, both methods produce samples that show signal enhancement at the end of the chromatogram.

Figure 2: Matrix effect for celery plotted in retention time order. The dilution method tended to produce signal enhancement, whereas the QuEChERS method usually resulted in signal suppression.



More drastic matrix effects at specific points in the chromatogram can indicate what type of coextracted compounds might be in the sample. For example, strong matrix effects at the end of a reverse phase chromatographic analysis indicate that hydrophobic compounds, like lipids, might be causing ionization problems. If we look at the plots for high fat avocado in Figure 3, we see ion suppression at the end of the elution window for samples produced by both sample treatments. It is reasonable to conclude that lipid content remains in both samples.

Figure 3: Matrix effect for avocado plotted in retention time order. Signal suppression is evident for late eluting compounds for both dilution and QuEChERS samples.

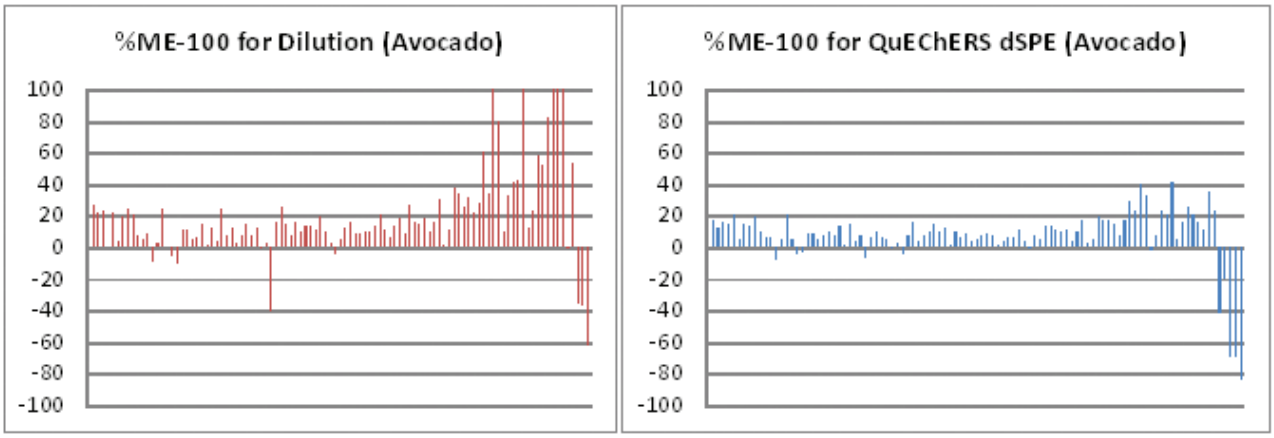
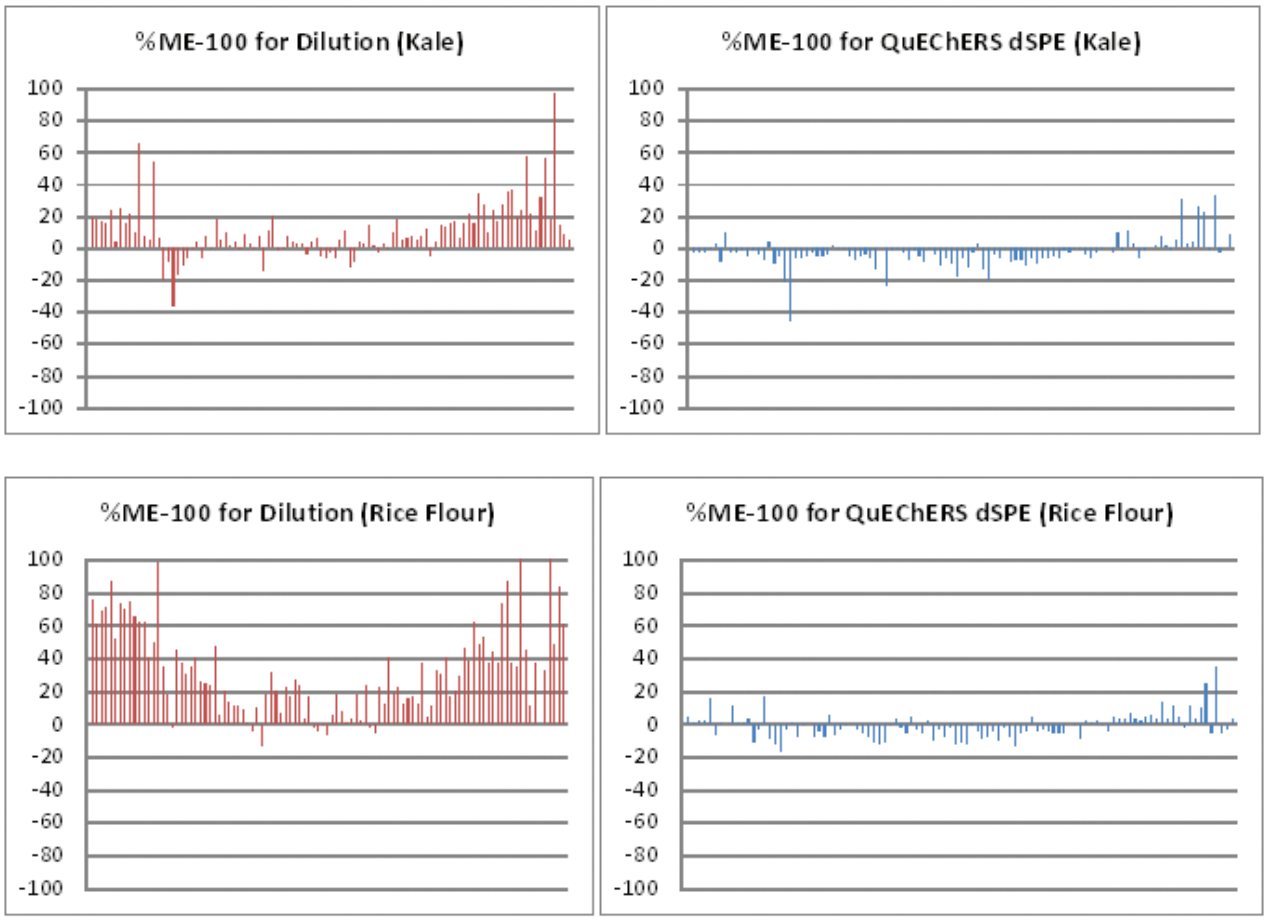


Figure 4: Matrix effects plots for kale (top) and brown rice flour (bottom). For many pesticides, the QuEChERS method reduced matrix effects.

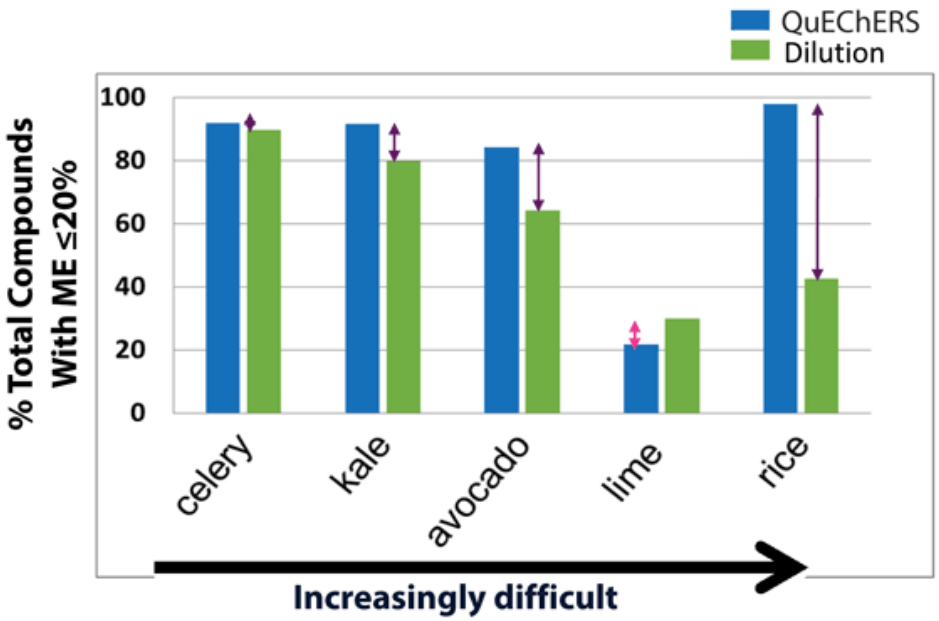


Corresponding plots for kale and rice show the QuEChERS method was able to mitigate matrix effects for some portions of the chromatogram. Signal enhancement at the beginning and end of kale chromatograms is decreased when the sample is treated with the QuEChERS method (Figure 4). The brown rice plots, also in Figure 4, demonstrate a clear example of matrix effect reduction with the QuEChERS sample for many pesticides, especially for early and late eluting pesticides as shown by the U-shaped plot of the dilution method. Lime plots did not show any clear trends.

Matrix Effects Evaluation

Matrix effect values from 80-120% are considered suitable values indicating minor matrix effects. The range is considered acceptable by many people testing for pesticide residues. Often, this 100±20% range is used as a cutoff value to justify using solvent calibration as opposed to matrix-matched standards. We evaluated the different testing strategies by comparing the percent of compounds tested that fell within ± 20% of the solvent curve values (Figure 5). The total number of compounds is 102, but it is adjusted here for incurred pesticides. Incurred pesticides determined by either QuEChERS or dilution methods were removed from both data sets. This ranged from three incurred pesticides for brown rice flour, to fifteen incurred pesticides in celery. Commodities are listed in order of increasing difficulty from left to right in Figure 5.

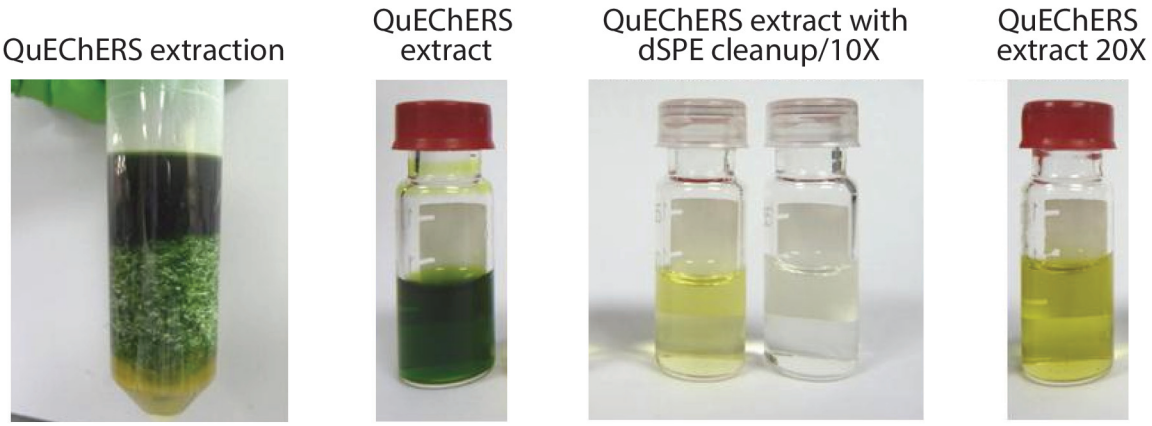
Figure 5: Comparison of the percent of analytes that have matrix effects within 80-120% by commodity and sample preparation method.



Celery has high water, intermediate color, and is low in fat. The performances of QuEChERS and dilution methods are almost identical, 92% and 90% matrix effects, respectively. Both strategies for decreasing the concentration of coextracted material are successful. For celery, the dilution method saved time and eliminated the potential loss of analytes by sample cleanup.

As shown in Figure 6, the initial QuEChERS extraction step was used for both the QuEChERS and the dilution methods. The dark top layer is the acetonitrile extract that was prepared two ways for analysis. For the dilution sample, this extract was diluted 20-fold and analyzed; whereas for the QuEChERS method, this extract was further processed by dispersive solid phase extraction and then diluted 10-fold and analyzed. Much of the pigment was removed by GCB during dSPE cleanup; however, even at this level of dilution significant pigment remains. This impacts the cleanliness of the LC-MS/MS interface requiring more frequent cleaning to maintain the same level of data quality. Based on Figure 5, the QuEChERS and dilution methods performed similarly well for kale with respect to analyte matrix effects. The QuEChERS method resulted in 91% of analytes having low matrix effects compared to the dilution method that achieved low matrix effects for 80% of analytes. That is a relatively small difference of about ten compounds. The QuEChERS method provides a slight advantage in minimizing matrix effects, which in general can help increase the time between interface cleanings. The dilution method performs well and requires less sample preparation time and expense. We estimate that the QuEChERS method added about one and a half hours to total processing time.

Figure 6: Preparation of kale samples for analysis. From left to right, sample layers separated in extraction tube, extract (solvent layer) removed to a clean vial, extract treated with dSPE cleanup, post-cleanup extract diluted 10x (QuEChERS sample for analysis), and pre-cleanup extract diluted 20x (dilution sample for analysis).



Avocado is more challenging chiefly because of its high fat content and lower water content. The QuEChERS cleanup used 50 mg of C18 sorbent per one milliliter of extract to help remove coextracted fat compounds. Figure 5 shows that both the QuEChERS and dilution methods were less effective at minimizing matrix effects in avocado than they were in easier commodities like celery and kale. The QuEChERS method produced low matrix effects for 84% of analytes, which can still be considered tolerable performance. In contrast, the dilution method produced low matrix effects for just 64% of analytes, indicating that it was not able to mitigate matrix effects as well at the QuEChERS method for this matrix. This difference in performance is equivalent to about 20 analytes or one fifth of all target analytes. Figure 3 shows that the removal of matrix lipid material by dSPE cleanup improved matrix effects of analytes in the latter part of the analysis where coelution with lipid type compounds is expected.

The poor performance of both methods for lime is not surprising as citrus fruits are known to be difficult to analyze by LC-MS/MS methods [2,3,4,8]. Despite the challenges, pesticide residue testing for citrus commodities is still performed by LC-MS/MS so we included lime in this work. The QuEChERS method produced low matrix effects for only 22% of analytes while the dilution method did so for 30% of analytes, which is a difference of about eight compounds. It is thought that compounds specific to citrus fruit peel interfere with ionization [7,8].

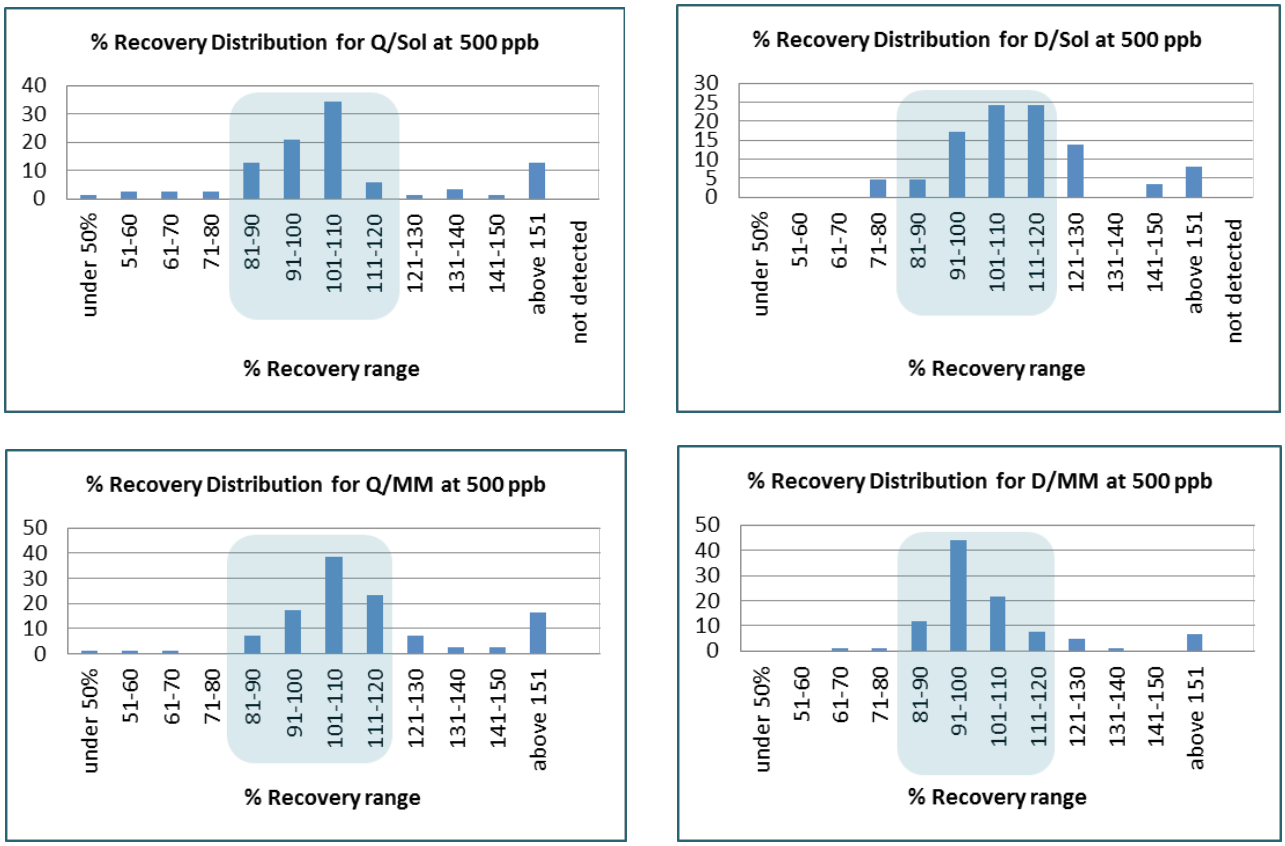
Grains also present challenges for sample preparation because they are dry and contain high levels of coextracted material. For these reasons, a modified QuEChERS method was used as described earlier. The high amount of coextractives can intensify matrix effects, making LC-MS/MS pesticide residue analysis difficult. For brown rice flour, the performance of the methods is significantly different (Figure 5). The modified QuEChERS method shows low matrix effects for 98% of analytes, while the dilution method did so for only 42%. This equates to about 55 analytes for which the QuEChERS method produces acceptably low matrix effects, but that fall outside the range of the dilution method. This indicates that the dilution factor of 20 was not able to reduce the coextractives concentration to the degree needed to produce workable matrix effects. The QuEChERS cleanup step was able to remove carbohydrates and fatty acids that are commonly found in high levels in grains. This was accomplished by the use of PSA sorbent and makes a significant difference with respect to matrix effects.

Both QuEChERS and dilution methods performed well for high water commodities. With these types of samples, the dilution approach offers time savings in both sample processing and also in standard preparation because solvent standards can be used for calibration. As commodities become more challenging with higher concentrations of coextractives, especially fat and carbohydrates, QuEChERS shows better performance by removing more coextracted material compared to the dilution method used in this work. This is demonstrated by the significant differences observed for avocado and brown rice flour. To mitigate matrix effects, QuEChERS provides a good option for pesticide residue testing because it works well for many foods and pesticides and the time and cost expense is small compared to other sample cleanup techniques.

Sample Preparation/Calibration Method Combinations Evaluation

We evaluated the performance of the two sample processing strategies combined with two different calibration methods to determine the best combinations for different commodity types. We know from the discussion above that a dilution method can save time during sample processing. However, if a dilution method requires matrix-matched calibration, this adds significant time to the overall experiment. In some cases, it may be beneficial to process the samples with a more time consuming sample preparation method that would allow the use of solvent calibration. When feasible, analyzing dilution-only samples with solvent calibration is faster and less expensive; however, sometimes acceptable results can only be achieved by using sample cleanup and matrix-matched calibration, which requires significantly more resources. Our evaluation of sample preparations paired with different calibration methods will help establish recommendations for choosing which strategies to apply.

Figure 7: Percent recovery distribution comparing sample preparation and calibration strategy combinations for celery samples. (See Table VI for abbreviation key.)

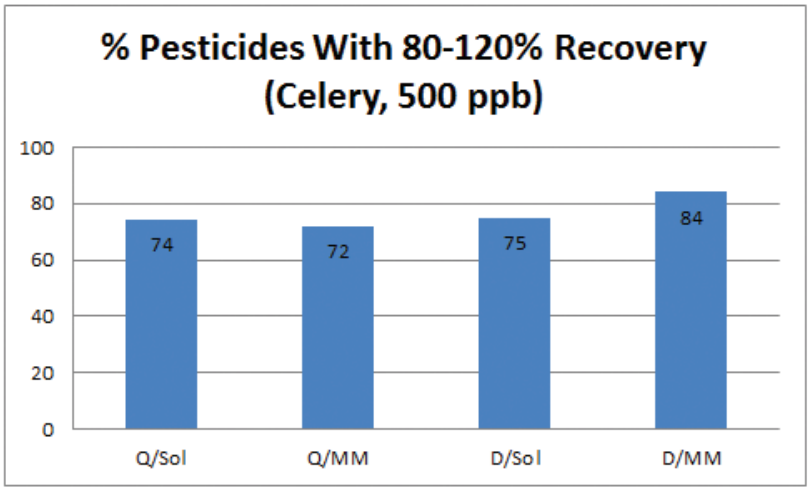


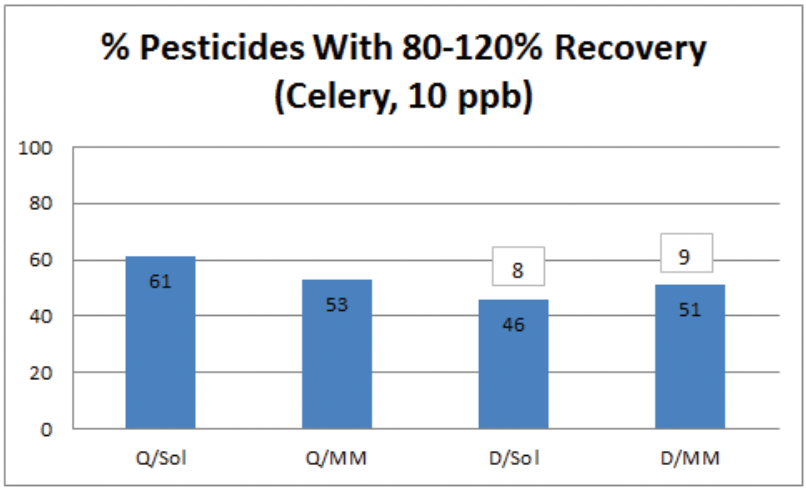
Percent recovery values for all analytes, excluding incurred pesticides, were calculated for each commodity and sample preparation approach using both solvent and matrix-matched calibrations. This yields percent recovery values for each pesticide for the four sample preparation/calibration combinations. This was done at two fortification levels, 500 ppb and 10 ppb. The 500 ppb level was selected to begin with because we thought based on experience that all or most of the pesticides at this level could be quantified even after 10x and 20x dilutions. Fortified samples were also prepared at 10 ppb because this is the default maximum residue limit (MRL) for pesticides in foods if a specific value is not set.

We sorted pesticides into recovery value ranges and plotted these to produce the graphs in Figure 7. The plot shows the percentage of total compounds that fell within the recovery range labeled on the x-axis for celery. The 80-120% recovery range is considered satisfactory for quantitative work. These plots can be used to compare biasing of recovery values between sample preparation/calibration strategies. When these plots were compared for each commodity, generally we saw the same biasing trend towards either high or low recovery values for all four strategies. There were a couple of exceptions where biasing was removed because of the use of matrix-matched calibration, not because of different sample preparation (e.g., lime using QuEChERS method at 500 ppb and avocado using the dilution method at 10 ppb [data not shown]). Biasing was only removed by the use of matrix-matched calibration.

We calculated the percent of compounds that fell within the acceptable recovery range (80 to 120%) and used this to compare sample preparation/calibration method combinations. This is done for each commodity at both the 500 and 10 ppb fortification levels (Figures 8-12). Graphs are used for easy visual comparison and the percent values are listed inside the top of each bar. During analysis, the signal response for some analytes fell below the quantification level or was not detected. In this case, the number of compounds that were not able to be detected or quantified is given above the bar. This is an important parameter for evaluating different testing strategies. For example, in Figure 8, all analytes could be quantified at the 500 ppb level as anticipated. At the 10 ppb level, the QuEChERS method with either solvent or matrix-matched calibration allowed quantification of all analytes. However, for the dilution method, eight analytes could not be quantified by solvent calibration and nine compounds could not be quantified using matrix-matched standards.

Figure 8: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in celery. (See Table VI for abbreviation key.)

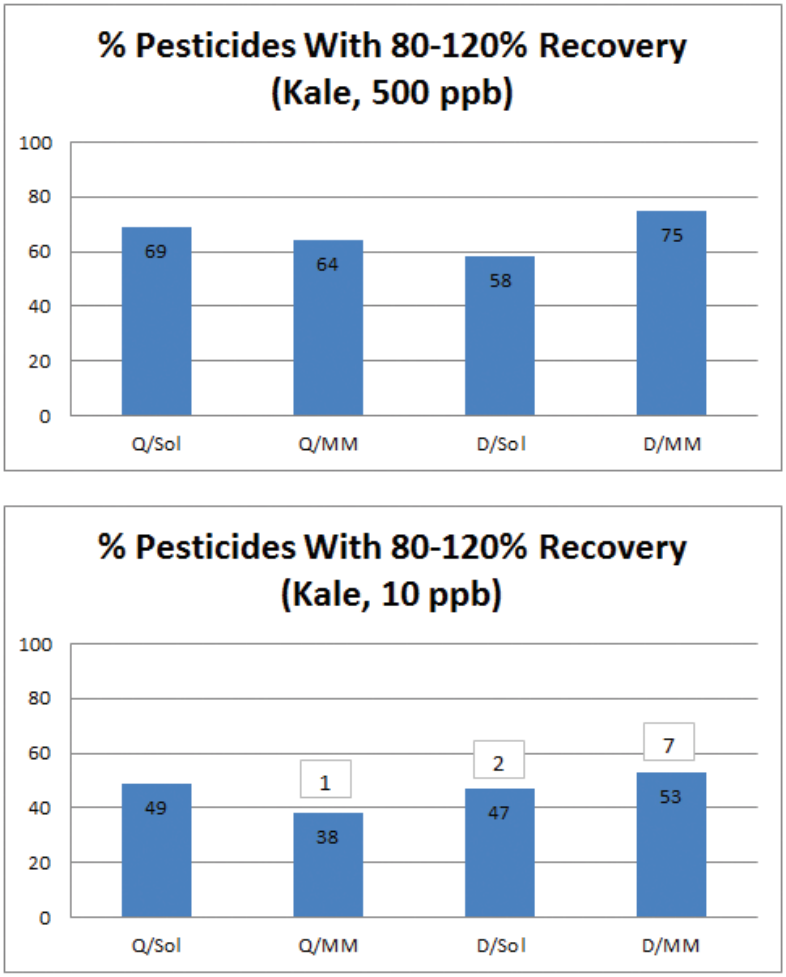




Of the commodities tested, celery is the easiest to analyze because it is mainly water. While the extract contained some pigmentation, color intensity was reduced significantly by both the dilution and QuEChERS methods. The 500 ppb data indicate that both the QuEChERS and dilution techniques work well, but there is some advantage using a matrix-matched calibration with the dilution method. This equates to about 10 compounds.

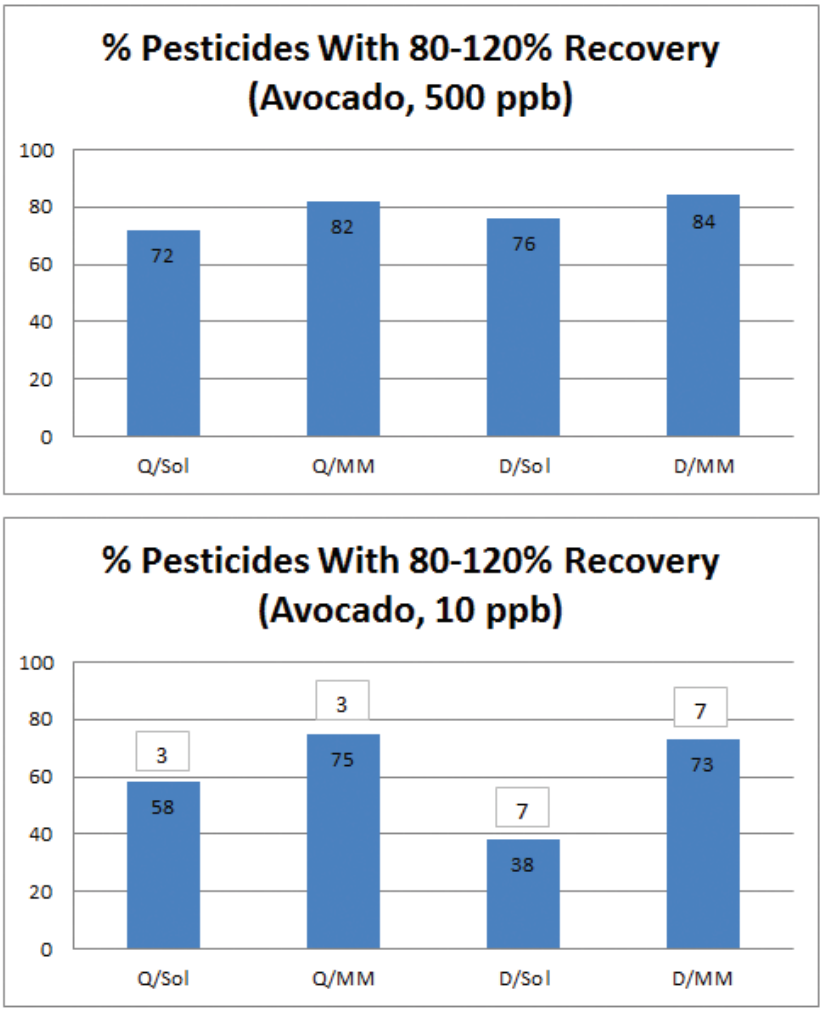
At the 10 ppb level results for the four strategies show similar results. However, the dilution method was not able to detect all of the pesticides regardless of calibration strategy. The injection concentration was 0.5 ppb, which is close the detection limit for some of the 102 pesticides tested. Because there is no advantage with respect to recovery values using the dilution method, choosing the QuEChERS method or at least decreasing the dilution factor are the best options for detectability reasons.

Figure 9: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in kale. (See Table VI for abbreviation key.)



Kale is considered slightly more difficult due to its higher pigment content, but it is still a relatively easy commodity because it has high water content. For the 500 ppb level shown in Figure 9, the performance is similar between the four sample preparation/calibration strategies. We see again a small increase for the dilution method when using a matrix-matched calibration curve. For the 10 ppb level, the methods performed similarly with the exception of the QuEChERS/matrix-matched calibration scheme. This is surprising as the trend is to see improvement with matrix-matched calibration. The dilution method performs as well as the QuEChERS method but the tradeoff at the lower concentration is failure to detect some pesticides.

Figure 10: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in avocado. (See Table VI for abbreviation key.)



Avocado is a high fat commodity with about 15% lipid content. It can be a difficult matrix to analyze because some lipids are coextracted in acetonitrile along with the pesticides. At the 500 ppb level, the four different strategies produced similar results, but improvement was seen when using matrix-matched calibration (Figure 10). For the lower fortification level, the benefit seen from using matrix-matched calibration is even stronger. When employing solvent calibration, the QuEChERS approach resulted in more compounds with acceptable recovery values than the dilution method, likely because the QuEChERS procedure used C18 sorbent to remove some of the coextracted lipid material. The dilution method was noticeably inferior, probably because dilution was not able to decrease ionization problems caused by coextracted lipids. This is supported by the matrix effects data showing that matrix effects were more pronounced at the end of the chromatographic analysis where lipids would be expected to elute (Figure 3).

Matrix effects are significant for the dilution/solvent calibration scheme, but using matrix-matched calibration compensated well and produced better recovery values. The QuEChERS/matrix-matched calibration data is almost identical to the dilution/matrix-matched calibration method, but with fewer compounds lost due to sensitivity at the 10 ppb level. In the cases of the compounds that are not detected, the three pesticides not detected using QuEChERS methods are the same and are included in the seven that were not detected for the dilution methods. This is common for undetected pesticides and indicates that the losses are due to sensitivity limitation and are not associated with loss due to QuEChERS sample cleanup. However, there are occasions when the commodity (rice) or the cleanup (GCB) causes low recovery and at 10 ppb these pesticides are difficult to detect.

It is well known that LC-MS based techniques struggle with citrus fruits but we still wanted to compare the sample preparation and calibration methods. At the 500 ppb level, recovery values are poor for all methods (Figure 11). There is a 20% increase, or about 20 compounds, for the QuEChERS method when matrix-matched calibration is used. This demonstrates the influence matrix-matched calibration can have on data quality. The dilution method shows similar results between solvent and matrix-matched calibration. At 10 ppb, it is apparent that this is a difficult matrix for trace analysis by the 21 and 39% values produced with solvent calibration. It is also apparent that the use of a matrix-matched curve can compensate for matrix effects for many pesticides. Using a matrix-matched curve for both the dilution and QuEChERS sample preparation methods significantly increases the number of pesticides that show good recovery, by about 20 and 40 compounds respectively. It is important to note that the dilution methods lost detectability for 8 compounds.

Brown rice flour has low water content and high carbohydrate composition. Dry commodities, like grains, present analytical challenges as they produce extracts with a high concentration of coextractives. The dilution/solvent calibration strategy suffers greatly and is not a viable method for this type of matrix (Figure 12). Matrix-matched calibration is extremely advantageous when used with the dilution method at both concentration levels. For the QuEChERS method, with both solvent and matrix-matched calibration, data show the benefit of removing some of the coextractives with dSPE cleanup. This cleanup step used here included PSA, which can remove fatty acids. This should be helpful for grains that are known to contain high amounts of fatty acids in the extract. Because of the low values at the 10 ppb level for all four schemes, it is likely that some combination of rigorous cleanup, high dilution, and matrix-matched calibration will be needed to yield acceptable results.

Several ideas result from the evaluation of different sample preparation/calibration approaches. All strategies will struggle to produce good recovery values as commodity types become more difficult and are susceptible to stronger matrix effects. This might require adjustment to experimental design to achieve acceptable results. For all foods tested in this work, there is a trad-eoff between spending time on sample cleanup and losing detectability of some analytes due to sensitivity limitations. This can be alleviated by higher dilution factors when instrumentation allows, but for some commodities actual removal of coextractives may be needed. Matrix-matched calibration is a powerful strategy to ensure data quality.

Figure 11: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in lime. (See Table VI for abbreviation key.)

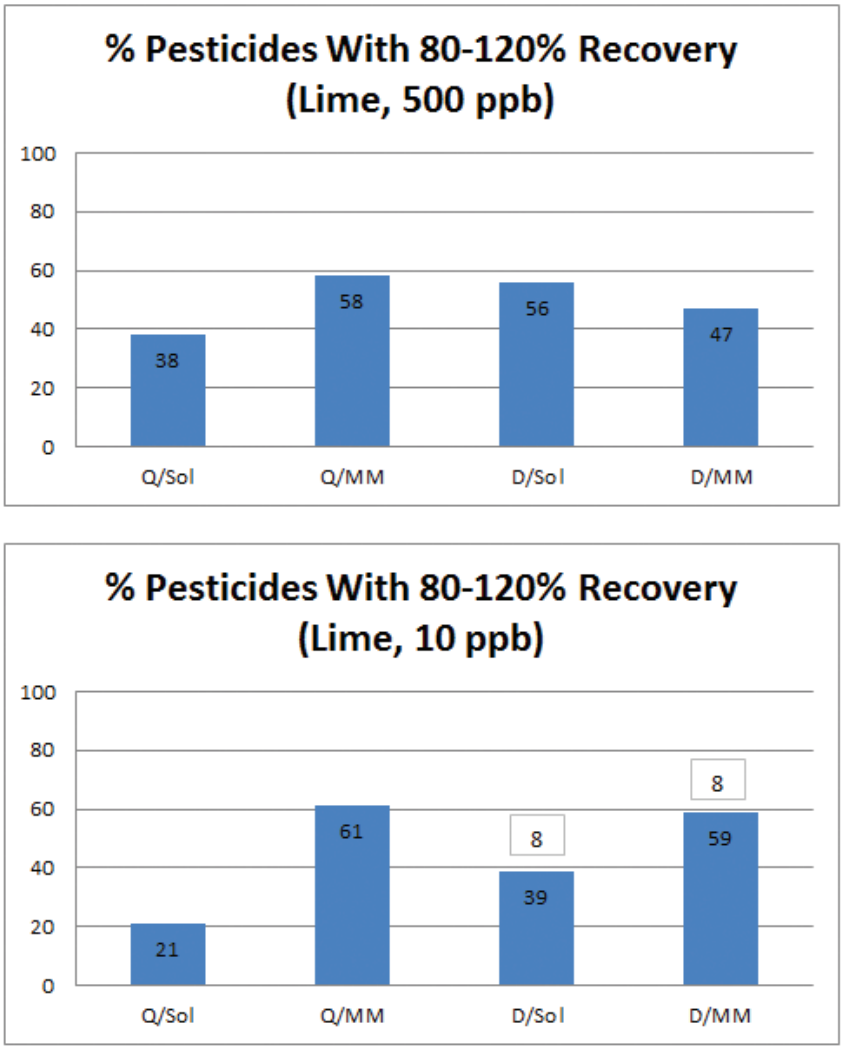
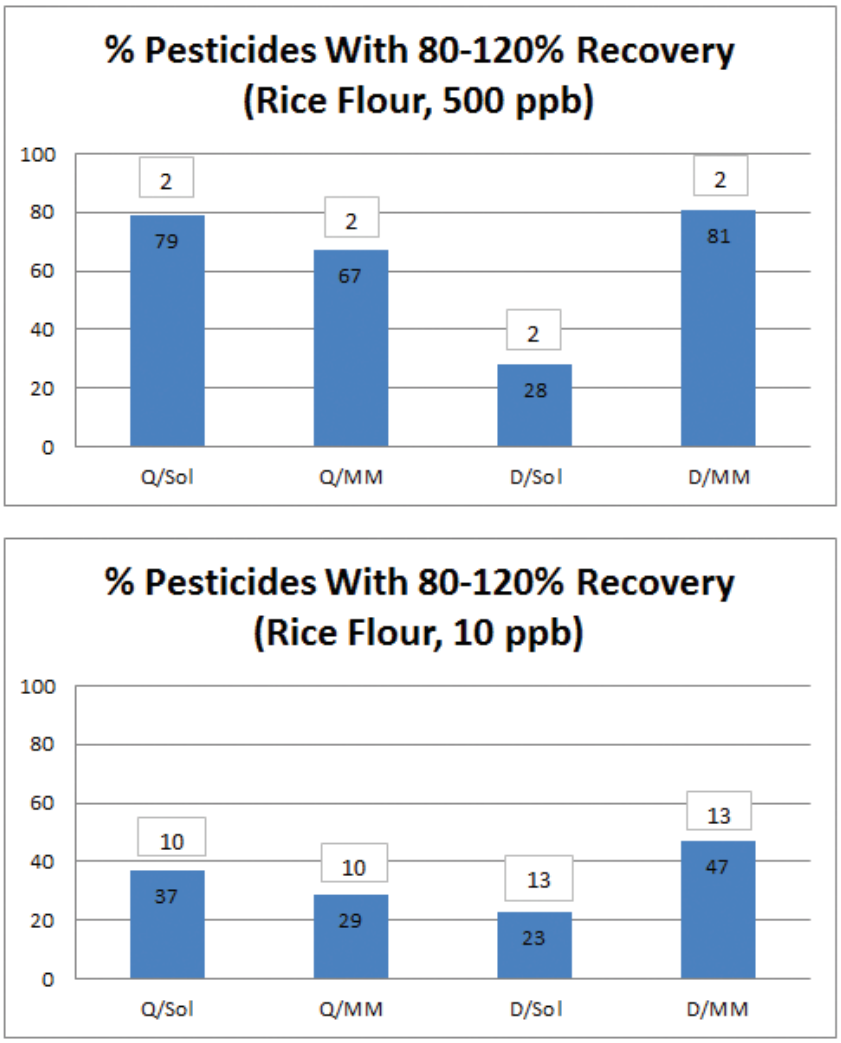


Figure 12: Comparison of percent of pesticides with acceptable recoveries (80-120%) by sample preparation/calibration strategy at 500 and 10 ppb fortification levels in brown rice flour. (See Table VI for abbreviation key.)



Conclusions

Matrix effects must be considered during method development of any pesticide residue method because of their drastic impact on data quality. Sample preparation strategies should be designed and tested to minimize matrix effects and ensure good data quality. Matrix effect studies can be useful for determining the regions of a chromatogram that are severely affected by matrix effects and, therefore, yield information about the nature of the interfering compounds. This information can steer changes to chromatographic conditions to minimize analytes eluting with interfering compounds or identify the best sample cleanup approach. In general, severe matrix effects require more sample preparation or higher dilution factors.

The choice of sample preparation and calibration methods is highly dependent on the commodity. Easy commodities with high water content are likely to be successful with either QuEChERS or dilution approaches, and the use of matrix-matched calibration may not be needed. Commodities that produce severe matrix effects, like grains, require some sample preparation or higher dilution factors, as well as matrix-matched calibration. Foods that have high fat content can be challenging and will require some sample cleanup, high dilutions and likely matrix-matched calibration. For the most difficult commodities, like dry grains, successful methods will likely involve substantial sample cleanup in combination with dilution and matrix-matched calibration. Matrix-matched calibration was the most effective way to improve results, regardless of sample preparation technique. Sample preparation is costly and time consuming and can sometimes result in the removal of analytes during processing, but it is sometimes needed for these types of commodities. Dilution methods do not require this time and financial investment during sample processing. However, high dilutions will be needed in many cases and this will cause some analytes to fall below limits of detection and may require the use of newer, more expensive instrumentation. These are some of the variables and tradeoffs that need to be considered when developing pesticide residue testing schemes.

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GC/MS Analysis of Melamine and Cyanuric Acid Below 1µg/g in Infant Formula

Abstract
The recent establishment of a 1µg/g safety threshold for melamine in infant foods has led to an immediate need for more sensitive methods. Here we established GC/MS conditions for highly reproducible analyses and evaluated the effectiveness of both solvent-based and matrix-matched standards. Using this method, melamine and cyanuric acid were reliably detected at and below 1µg/g in infant formula.

Introduction

Recent reports linking the presence of melamine in pet food and infant formula to illnesses and deaths have led to the recall of a wide variety of tainted food products and to calls for stricter product testing. Melamine is not considered toxic alone at low doses; however, the observed toxicity has been attributed to melamine exposure in the presence of cyanuric acid. In combination, these compounds form insoluble crystals in the kidneys, causing illness and eventual renal failure. Melamine is not a legal food additive; it is a nitrogen-rich industrial compound used for plastics, flame-resistant products, and some cleaning agents. However, melamine and related byproducts have been added illegally to food products in order to falsely represent the amount of protein present, since protein level in many products is determined using nonspecific assays for nitrogen content (Figure 1).

In response to the increasing need for more rigorous melamine and cyanuric acid testing, the US Food and Drug Administration (FDA) has set three different commodity-based minimum reporting levels (MRLs): 10µg/g for pet food, 2.5µg/g for human food, and 1µg/g for infant formula.[1,2] These limits place stringent demands on the analyst to demonstrate adequate instrument sensitivity. The following work was performed to establish conditions for melamine analysis down to 1µg/g in infant formula and is based on the FDA method, *GC-MS Screen for the Presence of Melamine, Ammeline, Ammelide, and Cyanuric Acid.*[1] Please refer to this FDA method for overall experimental design and semi-quantitative calculations.

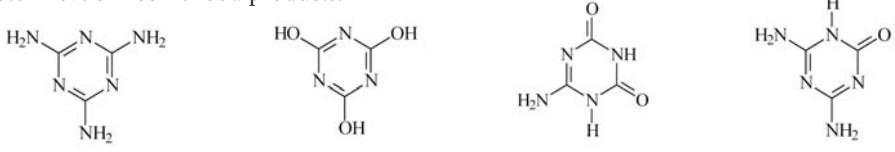
Procedure

Sample Preparation

All solutions were made using a 1,000µg/mL (each compound) mixed stock solution of melamine, cyanuric acid, ammeline, and ammelide (cat.# 33253). Working standard solutions were then prepared at 1µg/mL, 10µg/mL, and 100µg/mL in extraction solvent (10/40/50 diethylamine/water/acetonitrile). The 10µg/mL working solution was used to fortify control infant formula at 0.5µg/g, 1µg/g, and 5µg/g (dry formula was prepared according to label instructions prior to fortification). These matrix spike levels were chosen to demonstrate method performance at the MRL for infant formula (1µg/g) and the MRL for adult human food commodities (2.5µg/g). High and low standards were also prepared from the working solutions. Standards were prepared in solvent alone and also in extracted matrix at concentrations equivalent to sample fortification levels (0.0125µg/mL, 0.0249µg/mL, and 0.123µg/mL) in order to evaluate possible matrix effects and to determine which technique would yield the most reliable GC/MS data.

The extraction procedure was performed as follows. Multiple 0.5g matrix samples were prepared in 50mL centrifuge tubes (cat.# 26227) and fortified if necessary (matrix control blanks were not spiked). After fortification, 20mL of extraction solvent (10/40/50 diethylamine/water/acetonitrile) was added to each tube, and the samples were then sonicated for 30 minutes. Following sonication, the samples were centrifuged for 10 minutes to pellet particulate matter. The supernatant was then filtered through a 0.45µm nylon filter (cat.# 26147) to remove any remaining particles. 200µL aliquots of the resulting extracts were transferred to autosampler vials for derivatization.

Figure 1 Melamine and related compounds are rich in nitrogen and have been used to misrepresent protein levels in some food products.



Derivatization

Aliquots of standard (50µL solvent-based, 200µL matrix-based) and sample extracts (200µL) were evaporated to dryness at 70°C with nitrogen gas. Derivatization was performed as follows: 200µL pyridine, 200µL BSTFA with 1% TMCS (cat.# 35606), and 100µL of benzoguanamine (internal standard, cat.# 33251) were added to each vial. Vials were shaken or vortexed for 10 minutes and then incubated at 70°C for 45 minutes. Derivatization was also tested using 50µL of BSTFA with 1% TMCS in attempt to minimize instrument contamination from excess derivatization material.[3] Samples processed with the reduced amount of reagent were not completely derivatized; therefore, the FDA method for derivatization using 200µL of reagent was followed.[1]

Analysis

Analyses were performed on a Shimadzu QP 2010 Plus gas chromatograph mass spectrometer (GC/MS) equipped with an AOC 20i+s auto injector and sampler. An Rxi®-5Sil MS (30m x 0.25mm ID x 0.25µm) column with a 5m Integra-Guard™ integrated guard column (cat.# 13623-124) was used for the analysis. The integrated guard column was chosen since it protects the analytical column from matrix contamination, thus extending column lifetime, and prevents the possibility of leaks at a press-fit connection.

A splitless liner packed with wool (cat.# 22286-200.1) was used to help vaporize the compounds and also to further protect the column by trapping any nonvolatile compounds. GC conditions are shown in Figure 2; mass spectrometer conditions follow and are shown in Table I. The mass spectrometer was operated in SIM acquisition mode with selected ions for each analyte of interest. The transfer line temperature was set at 290°C and the ion source temperature at 190°C. The filament delay was set at 8.1 minutes and the dwell time for each ion was 0.15 seconds, giving a total run time of 18.67 minutes.

Calculations were performed using relative response factors as described in the FDA method.[1] Samples were quantified using both solvent-based and matrix-matched quantification standards, in order to assess matrix effect and determine which procedure was more effective for infant formula.

Results

This method successfully detected melamine and cyanuric acid to the MRLs required for routine analysis of infant formula and human foods. Highly reproducible chromatographic separation was achieved (Figure 2) and was critical for compound identification, since several quantitation ions were also found in other peaks. Matrix interference was a significant issue for the analysis of ammelide and ammeline at lower concentrations.

Quantitation Ions and Importance of Retention Data

The quantitation ions given in the method were used. However, several of these ions (including m/z 344 and 345) were shared among analytes and were also observed for many of the peaks in samples and derivatizing reagent blanks. This resulted in heavy reliance on chromatographic retention time data for peak identification (in conjunction with confirmation of the ion ratios listed in Table I). The FDA method requires retention times to be within 0.05 minutes for compound identification.[1] This was easily achieved using the Rxi®-5Sil MS column, which produced highly reproducible results, even after the approximately 150 injections made during testing (Table II).

Recovery of Target Compounds

Recovery data can be determined using solvent-based standards (no matrix) or matrix-matched standards (prepared in matrix extract and then derivatized). Since some matrices provide an analyte protecting effect that results in an increased response compared to an identical analysis with solvent-based standards, we evaluated both methods here.[4,5,6] Recovery values using solvent-based standards varied from 50 to 250%, compared to the 81 to 143% recoveries shown in Table III, which were determined using matrix-matched standards. Though this method is not intended for true quantitation, recoveries based on matrix-matched standards indicate that determining melamine and cyanuric acid at different minimum reporting levels is possible, even below the 1µg/g MRL required for infant formula.

Table I MS conditions (SIM mode).

Compound	t_R (min.)	Quant. ion	Qual. ion	Qual. ion	Qual. ion
cyanuric acid	10.23	345 (100)*	330 (36)	346 (30)	347 (15)
ammelide	11.07	344 (100)	329 (58)	345 (30)	330 (16)
ammeline	11.76	328 (100)	343 (79)	329 (29)	344 (24)
melamine	12.31	327 (100)	342 (53)	328 (30)	343 (17)
benzoguanamine	14.54	316 (100)	331 (68)	332 (20)	330 (9)

*Expected relative ion ratios from FDA method.

Table II Retention time is critical to accurate peak identification. Highly reproducible results were achieved using an Rxi®-5Sil MS column (n=3).

Compound	Retention time (min.)		
	0.5µg/g	1µg/g	5µg/g
cyanuric acid	10.26 ± 0.05	10.23 ± 0.0006	10.23 ± 0.001
ammelide	11.08 ± 0.003	11.07 ± 0.002	11.08 ± 0.003
ammeline	11.76 ± 0.001	11.76 ± 0.003	11.76 ± 0.002
melamine	12.31 ± 0.002	12.31 ± 0.000	12.31 ± 0.004
benzoguanamine	14.54 ± 0.002	14.54 ± 0.001	14.54 ± 0.002

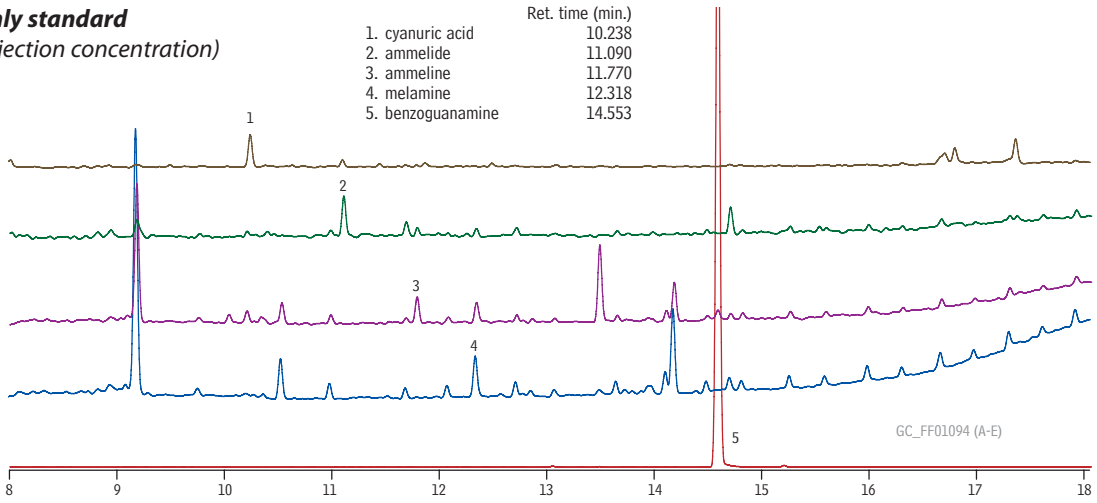
Table III Recovery of melamine and related compounds from spiked infant formula using matrix-matched standards (n = 3).

	µg/g Detected	% Recovery (mean±std.dev.)
0.5µg/g fortification		
cyanuric acid	0.4	81±29
ammelide	MI	MI
ammeline	MI	MI
melamine	0.42	85±35
1µg/g fortification		
cyanuric acid	1.3	131±17
ammelide	1.3	132±20
ammeline	MI	MI
melamine	1.3	132±57
5µg/g fortification		
cyanuric acid	7.2	143±46
ammelide	4.9	97±18
ammeline	5.3	106±20
melamine	4.3	86±34

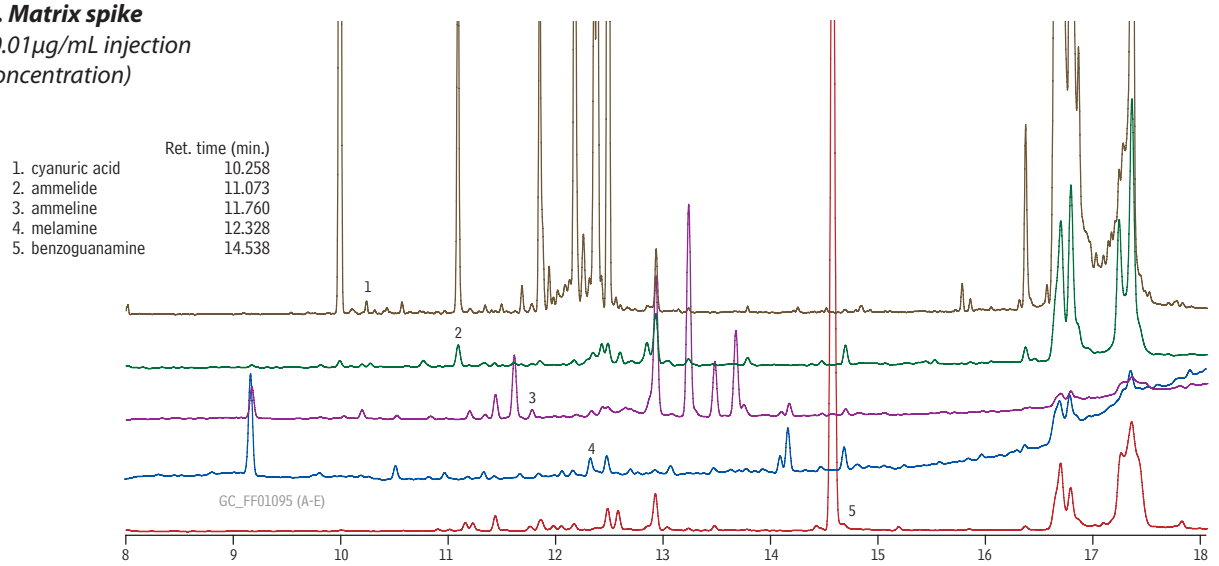
MI= matrix interference prevented accurate integration.

Figure 2 Analysis of melamine and related compounds in infant formula (1µg/g MRL spike level).

A. Solvent-only standard
(0.01µg/mL injection concentration)



B. Matrix spike
(0.01µg/mL injection concentration)



Column:	Rxi®-5Sil MS, 30m, 0.25mm ID, 0.25µm, w/ 5m Integra-Guard™ (cat.# 13623-124)
Instrument:	Shimadzu QP 2010 Plus
Sample:	A. Melamine and Related Analogs Stock Solution (cat.# 33253), Benzoguanamine (cat.# 33251) as tri-TMS derivatives, injection concentration: 0.01µg/mL B. infant formula fortified at 1µg/g with Melamine, Related Analogs Stock Solution (cat.# 33253), Benzoguanamine (cat.# 33251), analyzed as tri-TMS derivatives, injection concentration: 0.01µg/mL
Inj.:	1.0µL splitless (hold 1 min.), 3.5mm splitless inlet liner with wool (cat.# 22286-200.1)
Inj. temp.:	280°C
Carrier gas:	helium, constant linear velocity
Flow rate:	1mL/min.
Oven temp.:	75°C to 320°C @ 15°C/min. (hold 4 min.)
Det:	MS
Transfer line temp.:	290°C
Ionization:	EI
Mode:	SIM (all method ions in table, only quantification ions were plotted)

Compound	Quant. ion	Qual. ion	Qual. ion	Qual. ion
cyanuric acid	345	330	346	347
ammelide	344	329	345	330
ammeline	328	343	329	344
melamine	327	342	328	343
benzoguanamine	316	331	332	330

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Matrix Interference

As shown in Table III, ammelide and ammeline could not be integrated reliably below the MRL, due to partial coelution with a matrix compound that had isobaric interferences. The same effect was observed for ammeline at 1µg/g. Accurate integration was not possible since the ion ratios of the target analyte did not align with that expected based on known spectra or spectra of standards determined on the same system. While this method was optimized for melamine and cyanuric acid, the matrix interference seen for the other compounds illustrates the importance of evaluating and minimizing matrix carryover, especially when analyzing a new matrix. In this work, derivatizing reagent blanks were analyzed between each sample to ensure there was no carryover to subsequent injections.

Conclusion

Analysis of melamine and related compounds in infant formula is challenging since it has the lowest MRL of all commodities, and also because it is rich in sugars, which derivatize and chromatograph easily, thus increasing the potential for significant interferences. While this method successfully determined melamine and cyanuric acid to the MRLs required for routine analysis, coelutions complicated the analysis of ammelide and ammeline. Reliable retention time identification was critical for compound identification and the Rxi®-5Sil MS column used here detected target analytes reproducibly and thus is recommended for GC/MS analysis of melamine. In addition, this column includes an Integra-Guard™ integrated guard column, which extends analytical column lifetime without the risk of leaks that can occur with manually connected guards.

Product Listing

Description	cat.#
GC Column	
Rxi-5Sil MS w/5m Integra-Guard Column (30m, 0.25mm ID, 0.25µm)	13623-124
Standards	
Volume is 1mL/ampul. Concentration is 1,000µg/mL in diethylamine:water (20:80).	
Melamine Stock Standard	33247
Cyanuric Acid Stock Standard	33248
Ammelide Stock Standard	33249
Ammeline Stock Standard	33250
Benzoguanamine Internal Standard	33251
Melamine and Related Analogs Stock Standard (1mL ampul containing 1,000µg/mL each: ammelide, ammeline, cyanuric acid, and melamine)	33253
Derivatization Reagent	
25g vial BSTFA w/ 1% TMCS	35607
Accessories	
50mL empty centrifuge tube, 25-pk.	26227*
13mm, 0.45µm nylon syringe filter, 100-pk.	26147*
*Kit contains a 5-pk.	
Melamine Analysis Kit with Detailed Instructions	
Includes all items above (column, standards, derivatization reagents, and accessories) and easy-to-follow instructions with a procedural checklist to simplify documentation.	33254

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Malachite Green and Leucomalachite Green Analysis

By Julie Kowalski, Innovations Chemist

Introduction

Malachite green (MG) is an effective and inexpensive fungicide with a long history of use, mainly in Asian countries. There is concern about illegal use of MG because of potential harm to humans. MG entering water cycles is easily absorbed by fish tissue and is reduced to leucomalachite green (LMG) during metabolism. LMG accumulates in, and is stored in, fat tissue. Based on European Commission decision 2002/657/EC, a test method must be able to detect 2 micrograms of MG + LMG per kilogram of fish muscle.

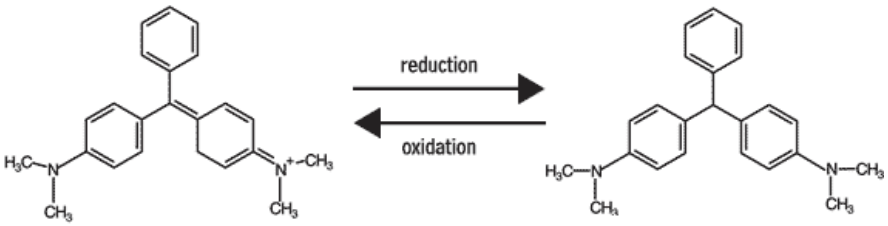
Law amendment to prohibit use of malachite green, *news.gov.hk, August 19, 2005*

General Chromatographic Methodology

Often reversed phase HPLC, typically on an octadecylsilane column with a guard column with the same stationary phase, is used to analyze for MG and LMG. Mobile phase composition is an organic and aqueous buffer mixture, often close to a 50:50 ratio, with acetonitrile being most common organic component. The aqueous buffer is adjusted to approximately pH 4.0 to 4.5.

The column typically is 100 mm or longer. The diameter is dictated by the detector: spectrometric instruments with large detector cells require larger diameter columns (4.6 mm) while spectrometric instruments with small detector cells, or with a mass spectrometer, a smaller diameter column is recommended, (2.1 mm or less).

Several detection methods can be employed, including UV-visible, fluorescence spectrophotometry, and mass spectrometry. MG is active at 620 nm and LMG at 265 nm, making it difficult to observe both in one spectrophotometric method. This can be circumvented by utilizing an oxidizing post column (e.g. lead oxide), in addition to the HPLC analytical column, to convert LMG to MG.



Malachite Green Oxalate Salt **MG** Leucomalachite Green **LMG**

LMG converted to MG can be distinguished from MG by the different retention times. Alternatively a fluorescence detector can be added to the eluent flow path, to detect LMG by excitation at 265 nm and emission at 360nm. Mass spectrometry allows detection of both compounds without oxidation or use of two detectors.

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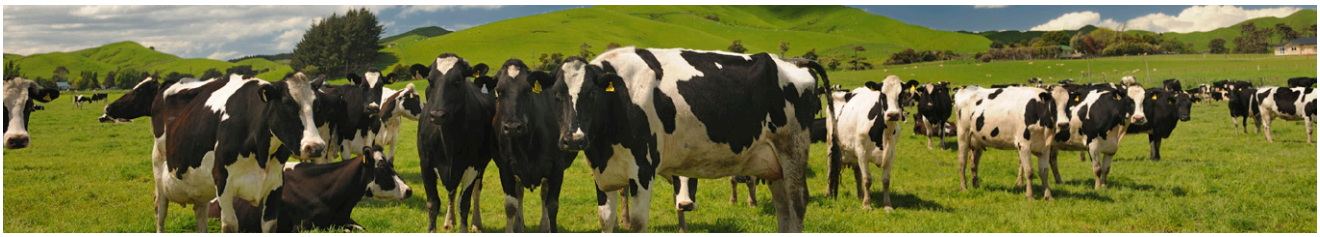
RELATED SEARCHES

Malachite Green, *Leucomalachite Green*, *MG*, *fungicide*, *MG metabolite*, *LMG*, *fish tissue*



Food Safety Applications

Increase Data Quality for Sulfonamide Residue Analysis by HPLC and UHPLC Using Unique Biphenyl Column Selectivity

By Rick Lake and Ty Kahler

- Improve reporting accuracy with better selectivity and retention.
- Biphenyl column and MS-friendly mobile phases allow easy transfer between UV and MS detection.
- Maximize sample throughput by combining USLC® selectivity with UHPLC speed.

The analysis of antibiotic residues in food-producing animals is important worldwide for evaluating food safety and maintaining compliance with export regulations. Sulfonamides are a specific concern, as drugs in this antibiotic class are commonly used in feed additives for livestock in order to fight infections and maintain desired growth levels. The analysis of sulfonamides usually involves a liquid chromatographic separation and detection by either UV or mass spectrometry. In both cases, the highly selective separation produced by a Biphenyl HPLC or UHPLC column can significantly improve data quality and reporting accuracy.

Increase Accuracy With Ultra Selective Biphenyl Columns

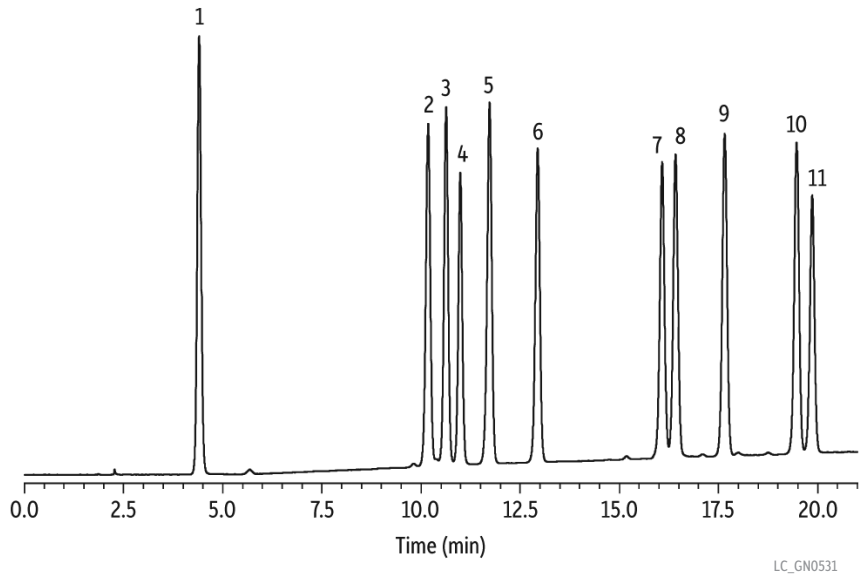
Since selectivity is the most important factor affecting peak resolution, we chose a Biphenyl column, part of our USLC® family of phases, for this work. Due to the column's unique selectivity and high retention, we were able to develop a very effective HPLC separation of 11 common sulfonamides with complete resolution (Figure 1). Use of the Biphenyl column produced much better chromatographic data compared to results obtained from a phenyl hexyl column used under identical conditions (Figure 2). The positive identifications and easy integrations obtained on the Biphenyl column allow more accurate reporting of sulfonamide residues.

In addition to providing improved separation of target analytes, focusing on stationary phase selectivity when choosing the analytical column allowed us to use simple, MS-friendly mobile phases. This approach provides several advantages for sulfonamide residue analysis. First, the separation can be easily transferred from UV to MS without further method development. Second, the use of simple mobile phases saves time and money, since they are quick to prepare and do not require complex additives.

Higher Retention Reduces Matrix Interferences in MS Detection

When developing a separation for UV detection, selectivity is critical for positive analyte identification. If MS detection is used, selectivity may not be required for analyte identification, but it still may be needed for adequate sensitivity and separation from matrix interferences. Matrix interferences can play a significant role in MS analyses by lowering sensitivity through suppressing ionization. Ion suppression in reversed phase mode often occurs with early eluting compounds, so it is good practice to retain them to a retention factor (k) of 2. In this example, we can see that the retention factor of sulfanilamide on the Biphenyl column is approximately twice as high as it is on the phenyl hexyl column (Figure 2). As a result, sulfanilamide is more susceptible to sample matrix interference if a phenyl hexyl column is used. The increased retention provided by the Biphenyl column, in combination with the MS-friendly mobile phases used here, ensure good sensitivity and allow easy method transfer between detectors.

Figure 1: Due to their unique selectivity, Biphenyl columns can provide the retention and separation needed for accurate sulfonamides analysis with simple, MS-friendly mobile phases.



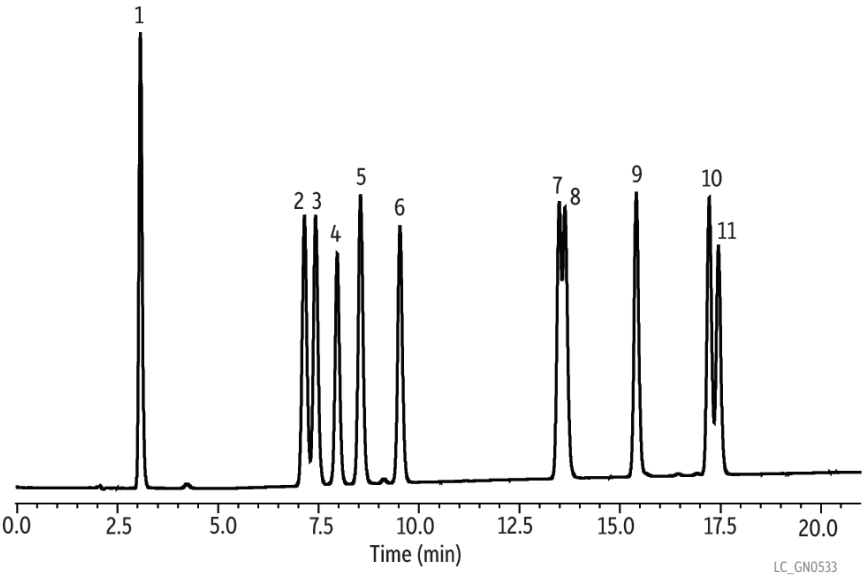
	Peaks	RT (min)
1.	Sulfanilamide	4.40
2.	Sulfadiazine	10.18
3.	Sulfapyridine	10.63
4.	Sulfathiazole	10.99
5.	Sulfamerazine	11.72
6.	Sulfamethazine	12.94
7.	Sulfachlorpyridazine	16.08
8.	Sulfadoxine	16.42
9.	Sulfisoxazole	17.65
10.	Sulfadimethoxine	19.47
11.	Sulfaquinoxaline	19.86

Column Ultra Biphenyl (cat.# 9109565)
Dimensions: 150 mm x 4.6 mm ID
Particle Size: 5 µm
Pore Size: 100 Å
Temp.: 25 °C
Sample
Diluent: 0.1% Formic acid in water
Conc.: 50 µg/mL
Inj. Vol.: 10 µL
Mobile Phase
A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

Time (min)	%A	%B
0	90	10
3.0	90	10
20.0	60	40
21.0	60	40

Flow: 1.0 mL/min
Detector UV/Vis @ 265
Instrument Shimadzu UFLCXR

Figure 2: A phenyl hexyl column, used under identical conditions, does not provide adequate retention or selectivity for sulfonamide residue analysis.



	Peaks	RT (min)
1.	Sulfanilamide	3.07
2.	Sulfadiazine	7.15
3.	Sulfapyridine	7.43
4.	Sulfathiazole	7.96
5.	Sulfamerazine	8.54
6.	Sulfamethazine	8.53
7.	Sulfachlorpyridazine	13.49
8.	Sulfadoxine	13.63
9.	Sulfisoxazole	15.41
10.	Sulfadimethoxine	17.22
11.	Sulfaquinoxaline	17.45

Column Waters XSELECT™ CSH Phenyl-Hexyl
Dimensions: 150 mm x 4.6 mm ID
Particle Size: 5 µm
Temp.: 25 °C
Sample
Diluent: 0.1% Formic acid in water
Conc.: 50 µg/mL
Inj. Vol.: 10 µL
Mobile Phase
A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

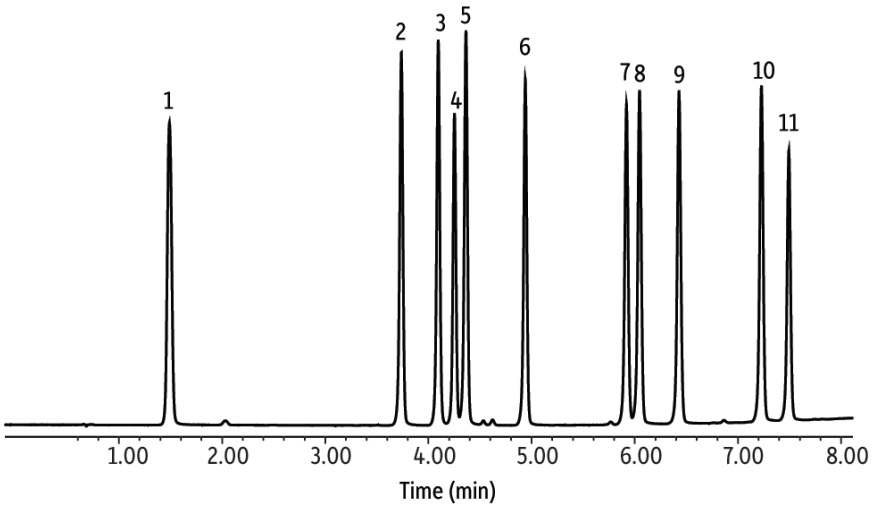
Time (min)	%A	%B
0	90	10
3.0	90	10
20.0	60	40
21.0	60	40

Flow: 1.0 mL/min
Detector UV/Vis @ 265
Instrument Shimadzu UFLCXR

Combining USLC® Selectivity and UHPLC Speed—The Most Powerful Approach

Selectivity has the greatest influence on resolution, but efficiency is the best tool for decreasing analysis time. While high efficiency separations can help increase sample throughput, efficiency is volume dependent and resolution can be affected by changes in instrumentation. By optimizing column selectivity first, we can then easily transfer a robust separation to UHPLC for faster analysis. Figure 3 illustrates the power of combining USLC® selectivity with UHPLC efficiency. By using a 1.9 µm Biphenyl UHPLC column we are able to fully separate all 11 sulfonamide peaks in a fast, 8-minute analysis.

Figure 3: Ultra selective analysis of sulfonamides on a unique Biphenyl column can be used in conjunction with UHPLC for higher sample throughput.



	Peaks	RT (min)
1.	Sulfanilamide	1.55
2.	Sulfadiazine	3.74
3.	Sulfapyridine	4.09
4.	Sulfathiazole	4.24
5.	Sulfamerazine	4.35
6.	Sulfamethazine	4.91
7.	Sulfachlorpyridazine	5.87
8.	Sulfadoxine	5.99
9.	Sulfisoxazole	6.37
10.	Sulfadimethoxine	7.14
11.	Sulfaquinoxaline	7.40

Column	Pinnacle® DB Biphenyl (cat.# 9409212)
Dimensions:	100 mm x 2.1 mm ID
Particle Size:	1.9 µm
Pore Size:	140 Å
Temp.:	25 °C
Sample	
Diluent:	0.1% Formic acid in water
Conc.:	50 µg/mL
Inj. Vol.:	2 µL
Mobile Phase	
A:	0.1% Formic acid in water
B:	0.1% Formic acid in acetonitrile

Time (min)	%A	%B
0	95	5
8	60	40

Flow:	0.4 mL/min
Detector	UV/Vis @ 265 nm
Instrument	Shimadzu UFLCXR

Conclusion

Focusing first on selectivity when choosing an analytical column for sulfonamide residue analysis is an easy way to improve data quality. The unique selectivity and high retention of Biphenyl columns produce complete separations and benefit both UV and MS detection. In addition, Biphenyl columns in a UHPLC format allow faster sample throughput, while maintaining good separation of target compounds.

RESTEK®

Lit. Cat.# FFAN1578-UNV

Foods, Flavors & Fragrances Applications

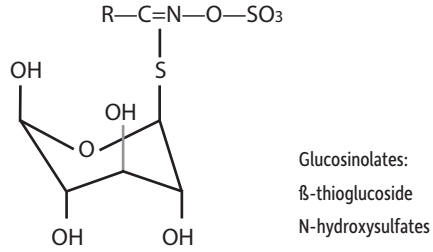
HPLC Analysis of Glucosinolates in Vegetable Extracts Without Ion Pairing Using an Ultra Aqueous C18 Column

Glucosinolates are a naturally occurring set of compounds found in a variety of edible plants, most notably in broccoli, radish, and cabbage. Agriculturally, the degradation compounds of glucosinolates have been shown to act as natural pesticides and fungicides. This breakdown occurs in the soil. These toxic compounds then further degrade into harmless compounds. Research on glucosinolates is continuing in hopes of bringing a more environmentally friendly approach to pest control.

Nutritionally, human consumption of these compounds is associated with a significantly reduced risk for a variety of malignant cancers along the alimentary canal. They also have been shown to suppress existing tumor growth. Glucosinolates are precursors to isothiocyanates, such as sulforaphane (4-methylsulfinylbutyl isothiocyanate), which regulates mammalian enzymes of xenobiotic metabolism.

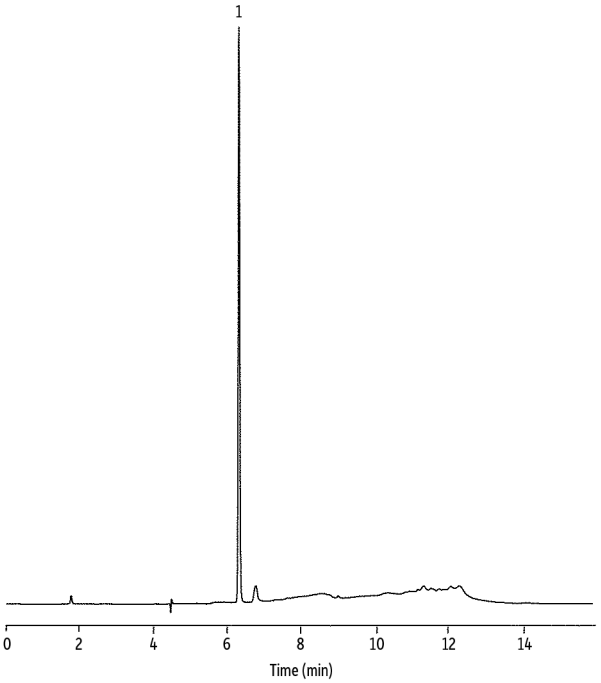
Phenethyl glucosinolate (gluconasturtiin) is one of the glucosinolates widely found in cruciferous vegetables. It is one of the least polar glucosinolates, making it relatively easy to retain by reversed-phase, high-performance liquid chromatography (HPLC). However, there are a number of glucosinolates with hydrophilic "R-" groups, such as 3-methylsulfinylpropyl glucosinolate, that are very difficult to retain by conventional reversed-phase HPLC. Additionally, the "R-" group of glucosinolates can vary greatly, resulting in a large number of glucosinolates with widely differing polarities (Figure 1). Thus, many analysts resort to reversed-phase ion-pairing methods to analyze glucosinolates. The addition of ion-pairing reagents is less convenient, and makes the analyses inherently less reproducible. Ion-pairing reagents also make gradient elution very impractical, due to long equilibration times.

Figure 1: Chemical Structure of Glucosinolates With Variable "R-" Group.



The analysis of a phenethyl glucosinolate standard using an Ultra Aqueous C18 column shows good peak shape without the use of ion-pairing reagents (Figure 2). Extracts of cabbage and watercress were analyzed using the same conditions (Figures 3 and 4). Gradient elution from 0 to 75% acetonitrile was used to retain and elute analytes having a wide range of polarities. The Ultra Aqueous C18 column allows the use of simple reversed-phase conditions for the analyses of glucosinolates, saving time as compared to reversed-phase ion-pairing methods.

Figure 2: Phenethyl Glucosinolate on Ultra Aqueous C18



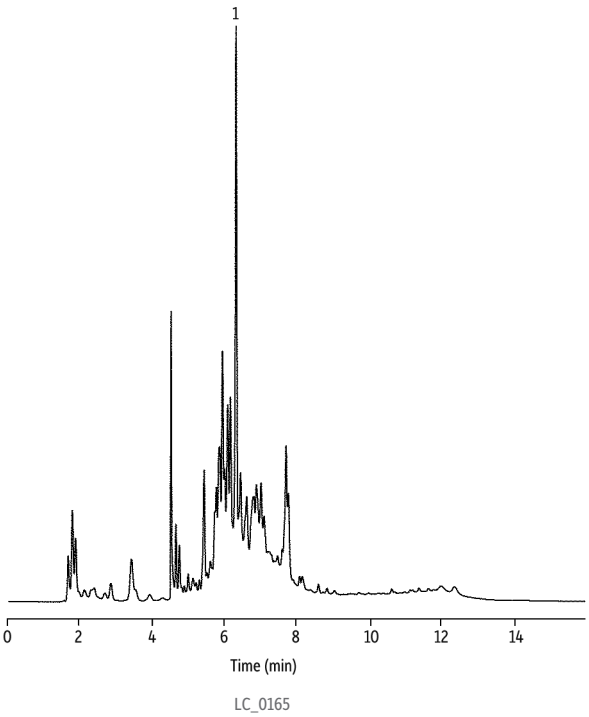
Peak
1. Phenethyl glucosinolate

Column	Ultra Aqueous C18 (cat.# 9178565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	ambient
Sample	
Diluent:	water
Conc.:	1,000 µg/mL
Inj. Vol.:	10 µL
Mobile Phase	
A:	50 mM potassium phosphate, pH 2.5
B:	acetonitrile

Time (min)	**%B**
0	0
10	75
11	0
16	0

Flow:	1.0 mL/min
Detector	UV/Vis @ 210 nm

Figure 3: Cabbage Extract on Ultra Aqueous C18



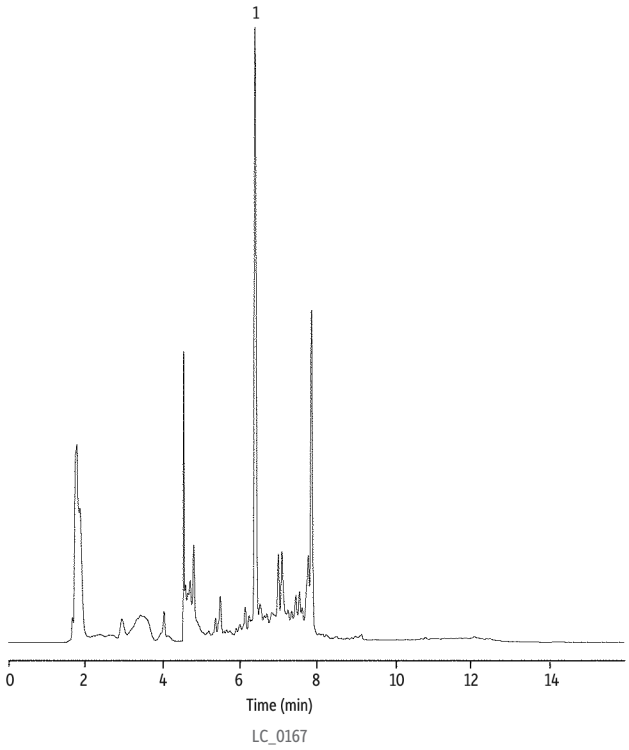
Peak
1. Phenethyl glucosinolate

Column	Ultra Aqueous C18 (cat.# 9178565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	Ambient
Sample	
Diluent:	Water
Inj. Vol.:	20 µL
Mobile Phase	
A:	50 mM Potassium phosphate, pH 2.5
B:	Acetonitrile

Time (min)	**%B**
0.00	0
10	75
11	0
16	0

Flow:	1.0 mL/min
Detector	UV/Vis @ 210 nm

Figure 4: Watercress Extract on Ultra Aqueous C18



Peak
1. Phenethyl glucosinolate

Column	Ultra Aqueous C18 (cat.# 9178565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	Ambient
Sample	Watercress extract
Diluent:	Water
Inj. Vol.:	100 µL
Mobile Phase	
A:	50 mM Potassium phosphate, pH 2.5
B:	Acetonitrile

Time (min)	**%B**
0.00	0
10	75
11	0
16	0

Flow:	1.0 mL/min
Detector	UV/Vis @ 210 nm

Acknowledgement: The phenyl glucosinolate standard and extracts of cabbage and watercress were generously provided by Dr. Gerard Engelen-Eigles, University of Minnesota, Horticulture Department.

Ultra Aqueous C18 Columns (USP L1)

Chromatographic Properties

The Restek® Aqueous C18 is a rugged, reversed-phase column with a well-balanced retention profile. It can effectively retain more types of solutes than a conventional C18 and is ideal for multi-component LC-MS analyses. The general-purpose Aqueous C18 boasts high reproducibility and compatibility with many mobile phase conditions—even 100% aqueous and acidic. And when used with a gradient, it eliminates the all-too-common issue of multiple compounds eluting near the column void time.

Column Characteristics:

particle size:	3 µm or 5 µm, spherical
pore size:	100 Å
carbon load:	15%
end-cap:	no
pH range:	2.5 to 8
temperature limit:	80 °C
USP phase code:	L1
phase category:	modified C18
ligand type:	proprietary polar modified and functionally bonded C18

Length	1.0 mm ID cat.#	2.1 mm ID cat.#	3.0 mm ID cat.#	4.6 mm ID cat.#
3 µm Columns				
30 mm	9178331	9178332	917833E	9178335
50 mm	9178351	9178352	917835E	9178355
100 mm	9178311	9178312	917831E	9178315
150 mm	9178361	9178362	917836E	9178365
5 µm Columns				
30 mm	9178531	9178532	917853E	9178535
50 mm	9178551	9178552	917855E	9178555
100 mm	9178511	9178512	917851E	9178515
150 mm	9178561	9178562	917856E	9178565
200 mm	9178521	9178522	917852E	9178525
250 mm	9178571	9178572	917857E	9178575

Ultra Aqueous C18 Guard Cartridges

Guard Cartridges	3-pk. (10 x 2.1 mm)	3-pk. (10 x 4.0 mm)
Ultra Aqueous C18 Guard Cartridge	917850212	917850210

RESTEK®

Lit. Cat.# FFAR1522-UNV

Technical Article

High-Quality Analysis of Pesticides in Cannabis

Using QuEChERS, Cartridge SPE Cleanup, and GCxGC-TOFMS

By Jack Cochran, Julie Kowalski, Sharon Lupo, Michelle Misselwitz, and Amanda Rigdon

- Quickly and effectively extract medical marijuana samples for pesticide analysis.
- Cartridge SPE cleanup of dirty extracts improves GC inlet and column lifetimes.
- Selective GC columns increase accuracy of pesticide determinations for complex samples.

Over 20 states in the U.S. have legalized the use of recreational or medical cannabis because of therapeutic benefits for ailments such as cancer, multiple sclerosis, and ALS. Dosing methods include smoking or vaporizing and baked goods. Unlike other prescribed medicines regulated by U.S. FDA, marijuana is a Schedule 1 drug and is illegal on the federal level. As a result, medical cannabis patients have no safety assurances for their medication, which could contain harmful levels of pesticide residues. Currently, medical marijuana pesticide residue analysis methods are poorly defined and challenging to develop due to matrix complexity and a long list of potential target analytes.

In order to address matrix complexity, we combined a simple QuEChERS extraction approach with cartridge SPE (cSPE) cleanup, followed by GCxGC-TOFMS. Acceptable recoveries were obtained for most pesticides, and incurred pesticide residues were detected in some of the illicit marijuana samples used for method development.

QuEChERS Extraction Saves Time and Reduces Hazardous Solvent Use

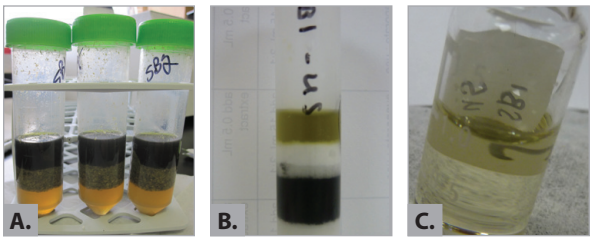
Trace residue extraction procedures from dry materials like medical cannabis typically involve large amounts of solvent, long extraction times, and tedious concentration steps similar to the Soxhlet procedure or multiresidue methods from the Pesticide Analytical Manual. QuEChERS, with its simple 10 mL acetonitrile shake extraction and extract partitioning with salts and centrifugation, offers time savings, glassware use reduction, and lower solvent consumption.

Water was added to finely ground, dry cannabis samples to increase QuEChERS extraction efficiency, especially for more polar pesticides. A vortex mixer was used to shake the solvent and sample for at least 30 minutes prior to extract partitioning. When finished, it was easy to transfer the supernatant from the QuEChERS extraction tube for subsequent cSPE cleanup prior to analysis with GC or LC (Figure 1).

Cartridge SPE Cleanup Improves GC Inlet Uptime

Injecting chlorophyll-laden extracts into a GC gives reduced recoveries for less volatile pesticides, and results in degradation of sensitive pesticides like DDT and Dicofol (Table I). SPE cleanup with a 500 mg graphitized carbon black/500 mg PSA cartridge removes chlorophyll and traps fatty acids that interfere with qualitative pesticide identification and bias quantification. cSPE has increased sorbent capacity over dispersive SPE for thorough cleanup of complex extracts.

Figure 1: A quick and easy QuEChERS extraction, combined with cSPE, effectively prepared extracts for pesticide residue analysis from highly complex marijuana samples.



A. Post-centrifugation QuEChERS extracts

B. QuEChERS extracts loaded on SPE cartridge

C. Final extract

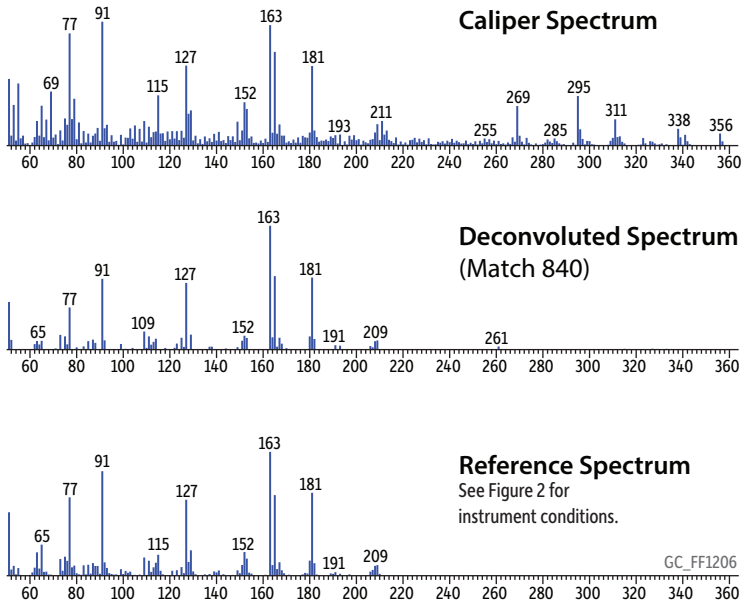
Orthogonal GC Columns Increase Separation Power for More Accurate Pesticide Results

GCxGC is a powerful multidimensional approach that gives two independent separations in one instrumental analysis. An Rxi®-5Sil MS and Rtx®-200 column combination distributes pesticides broadly in both dimensions, providing a highly orthogonal GCxGC system. More important though is separating pesticides from potential isobaric matrix interferences, as seen in the surface plot for the insecticide cypermethrin (Figure 2). Cypermethrin gas chromatographs as four isomers, and all would have experienced qualitative interference and quantitative bias from peaks in the foreground of the surface plot had only 1-dimensional GC been used. With GCxGC-TOFMS, cypermethrin was unequivocally identified in a marijuana sample at a low ppm level (Figure 3).

Summary

QuEChERS and cSPE produced usable extracts from highly complex cannabis samples for high-quality pesticide residue analysis. The multidimensional separation power of GCxGC-TOFMS was then used to correctly identify and quantify pesticides in these complex extracts.

Figure 3: Positive mass spectral identification of incurred cypermethrin in illicit marijuana.



Acknowledgment: Randy Hoffman, a Police Evidence Technician at The Pennsylvania State University (PSU), supplied the seized marijuana samples while overseeing their handling. Frank Dorman at PSU assisted with QuEChERS extractions.

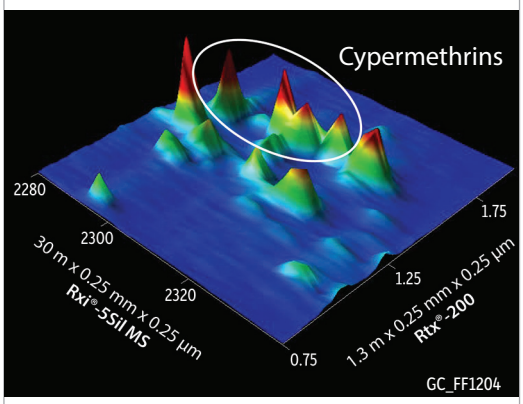
Initially published in *Restek® Advantage*.

Table I: Pesticide recoveries for a QuEChERS extract of cannabis give higher results when cSPE is used for cleanup. Dicofol and DDT are degraded in the inlet for the dirtier extract, yielding high DDD results.

Pesticide	Classification	With cSPE Cleanup (%)	Without cSPE Cleanup (%)
4,4'-DDD	Organochlorine	83	230
4,4'-DDT	Organochlorine	77	9
Bifenthrin	Pyrethroid	86	89
Dicofol	Organochlorine	84	ND
Azinphos methyl	Organophosphorus	79	53
trans-Permethrin	Organochlorine	68	17
Pyraclostrobin	Strobilurin	73	19
Fluvalinate	Pyrethroid	72	23
Difenoconazole	Triazole	67	21
Deltamethrin	Pyrethroid	68	20
Azoxystrobin	Strobilurin	72	27

ND = no peak detected

Figure 2: GCxGC-TOFMS and orthogonal Rxi®-5Sil MS and Rtx®-200 columns allow incurred cypermethrins in a marijuana extract to be separated from interferences (m/z 163 quantification ion).



Peaks	RT 1 (sec.)	RT 2 (sec.)
1. Cypermethrin 1	2292	1.50
2. Cypermethrin 2	2304	1.54
3. Cypermethrin 3	2310	1.53
4. Cypermethrin 4	2313	1.58

Column: Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623), Rtx®-200 1.3 m, 0.25 mm ID, 0.25 µm (cat.# 15124); **Sample:** Diluent: Toluene; **Injection:** Inj. Vol.: 1 µL splitless (hold 1 min); Liner: Sky® 4mm single taper w/wool (cat.# 23303.1); Inj. Temp.: 250 °C; Purge Flow: 40 mL/min; **Oven:** Oven Temp: Rxi®-5Sil MS: 80 °C (hold 1 min) to 310 °C at 5 °C/min, Rtx®-200: 85 °C (hold 1 min) to 315 °C at 5 °C/min; **Carrier Gas:** He, corrected constant flow (2 mL/min); **Modulation**: Modulator Temp. Offset: 20 °C; Second Dimension Separation Time: 3 sec.; Hot Pulse Time: 0.9 sec.; Cool Time between Stages: 0.6 sec.; **Instrument**: LECO Pegasus 4D GCxGC-TOFMS; For complete conditions, visit www.restek.com and enter GC_FF1204 in the search.

High Sensitivity Melamine GC/MS Analysis of Cat Food

Modified Conditions Save Costs and Reduce Maintenance

By Michelle Misselwitz, Innovations Chemist and Julie Kowalski, Ph.D., Food Flavor and Fragrance Innovations Chemist

- Excellent results in pet food matrix; lower pyridine background for better sensitivity.
- Easy sample preparation; reduced derivatization reagent volume lowers costs and keeps inlet and column clean. Modified conditions reduce maintenance and extend filament lifetime.

Abstract

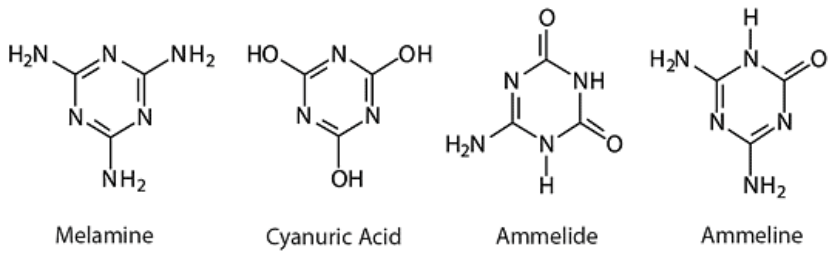
Gas chromatography/mass spectrometry (GC/MS) was used to analyze for melamine and related compounds cyanuric acid, ammelide, and ammeline. The method used was based on FDA method *GC-MS Method for Screening and Confirmation of Melamine and Related Analogs*, Version 2, May 7, 2007.[1] Several changes were made to this method to optimize performance. The analytes were easily identified by reliable retention time matching and mass spectra. Analyses of high and low spike levels in dry cat food were successful as matrix components did not coelute with compounds of interest.

Introduction

A large pet food recall occurred in 2007 when animals became ill after eating contaminated food, some animals eventually died. There was a suspected link between the sick animals and melamine contamination, although melamine was believed to have low or no toxicity.[2] Melamine is an industrial chemical used for the production of plastics, adhesives, flame retardants, fabrics and other materials.[3] Melamine is not a food ingredient but was found to be present in pet food and other protein-containing goods. It is believed that the toxicity is due to the combination of melamine and cyanuric acid forming insoluble crystals. The crystals can form in the kidneys, causing sickness and eventual renal failure.[4]

As the investigation continued, it was determined that melamine and melamine byproducts were intentionally added to vegetable protein products like wheat gluten and rice protein. This is done to artificially elevate the protein content values of products. The Kjeldahl method is used to determine protein content. This method works by testing for nitrogen content. When protein is digested, nitrogen is released and converted to ammonia. The amount of ammonia is determined by titration and is correlated to the amount of protein present. This method yields falsely high numbers when nonprotein nitrogen is in the sample. Melamine and melamine production byproducts were added to protein products to gain higher protein content. The structures of melamine and related compounds, pictured below, show very high nitrogen content, which make them ideal as nonprotein nitrogen sources. Unfortunately, the melamine production byproducts contain not only melamine, but also cyanuric acid, ammeline, and ammelide, which cause toxicity in pets. Due to the adverse effects of melamine contamination, imported products need to be tested for the presence of melamine, cyanuric acid and related compounds.

Figure 1 Melamine and related compounds are nitrogen rich and can artificially raise labeled protein content when used as an additive.



Procedure

The procedure for this experiment was adapted from the U.S Food and Drug Administration (FDA), *GC-MS Method for Screening and Confirmation of Melamine and Related Analogs*, Version 2, May 7, 2007. [1]

Sample prep: Melamine and its related analogs were purchased from TCI America. All solutions were prepared under the specified method conditions. This includes all stock solutions, mixed standard spiking solution, as well as high and low standards. The high standard consisted of the mixed standard solution (100 µg/mL) diluted to 10µg/mL and the low standard was diluted to 1µg/mL. Dilutions were made with 10:40:50 diethylamine:water:acetonitrile. The extraction procedure was performed with dry cat food purchased from a local grocery market. Three 0.5g matrix samples were prepared: one as the control, one with a high spike level and one with a low spike level. The mixed standard solution was also used to spike the matrix. The high level spike used 250µL of the mixed standard solution and the low spike level contained 50µL.

Derivitization: A total of eleven samples went through the entire derivitization procedure. These samples included solvent only, singles of each compound, the internal standard, high and low standards, and the blank, high and low matrix. The derivitization procedure closely followed the recommended method. Two modifications were made to the FDA method. The amount of derivitizing reagent was reduced from 200µL to 50µL of BSTFA with 1% TMCS (cat # 35606). This is still a molar excess of 50:1 for the derivitization reagent. Incubation time was subsequently increased from 45 min. to 120 min.

Instrument Parameters: Analyses were performed on a Shimadzu QP-2010 Plus gas chromatograph mass spectrometer (GC/MS) equipped with an AOC 20i+s auto injector and sampler. An Rtx®-5MS 30m x 0.25mm ID x 0.25µm column was used for the analysis. The injector was in splitless mode (1 min. hold) with 1µL injection volume at an inlet temperature of 280°C. Helium was used as the carrier gas set at constant flow 1mL/min. The oven program had an initial temperature of 75°C, then immediately ramped at 15°C/min. to 320°C (hold 4 min.). The transfer line temperature was set at 290°C and the ion source temperature at 190°C. The mass spectrometer data was acquired in SIM acquisition mode with selected ions for each analyte of interest (Table I). The filament delay was set at 8.1 min. to decrease the presence of pyridine, and the dwell time for each ion was 0.15 sec. This gave a total run time of 18.67 min.

Table I MS conditions (SIM mode)

Compound	Retention Time (min.)	Target Ions	Reference Ions	Reference Ions	Reference Ions
Cyanuric Acid	8.97	345 (100)*	330 (36)	346 (30)	347 (15)
Ammelide	9.79	344 (100)	329 (58)	345 (30)	330 (16)
Ammeline	10.44	328 (100)	343 (79)	329 (29)	344 (24)
Melamine	10.97	327 (100)	342 (53)	328 (30)	343 (17)
Benzoguanamine	13.18	316 (100)	331 (68)	332 (20)	330 (9)

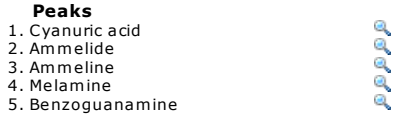
* *Relative ion ratio*

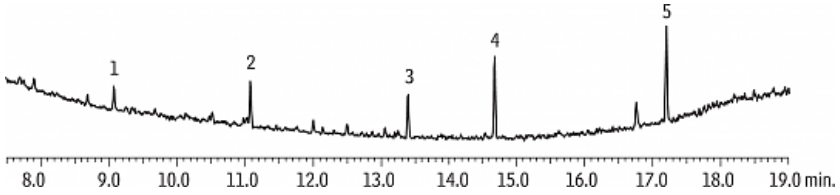
Data Processing: All data processing was done on the Shimadzu Lab Solutions software (GCMS solution version 2.5 SU1). The individual standards were used to create a user library in order to identify the compounds in the matrix samples. Chromatograms of standards and matrices were compared using the data comparison function of the software.

Results

Figure 2 shows a chromatogram of the high standard mixture using the method conditions in the FDA screening method. The chromatogram shows an elevated baseline at the beginning of data collection that slowly decreases.

Figure 2 Original method produces an elevated baseline, compromising integration and reducing sensitivity (10µg/mL standard).





GC_FF00979

Column	Rtx®-5MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 12623)
Sample	melamine, cyanuric acid, ammelide, ammeline, benzoguanamine
Conc.:	10 µg/mL prederivatized
Injection	
Inj. Vol.:	1 µL splitless (hold 1 min)
Liner:	3.5mm Gooseneck Splitless (cat.# 22286)
Inj. Temp.:	280 °C
Oven	
Oven Temp.:	75 °C (hold 1 min) to 320 °C at 15 °C/min (hold 4 min)
Carrier Gas	He, constant flow
Flow Rate:	1 mL/min
Detector	MS
Mode:	Scan
Transfer Line Temp.:	290 °C
Ionization Mode:	EI
Scan Range:	50-450 amu

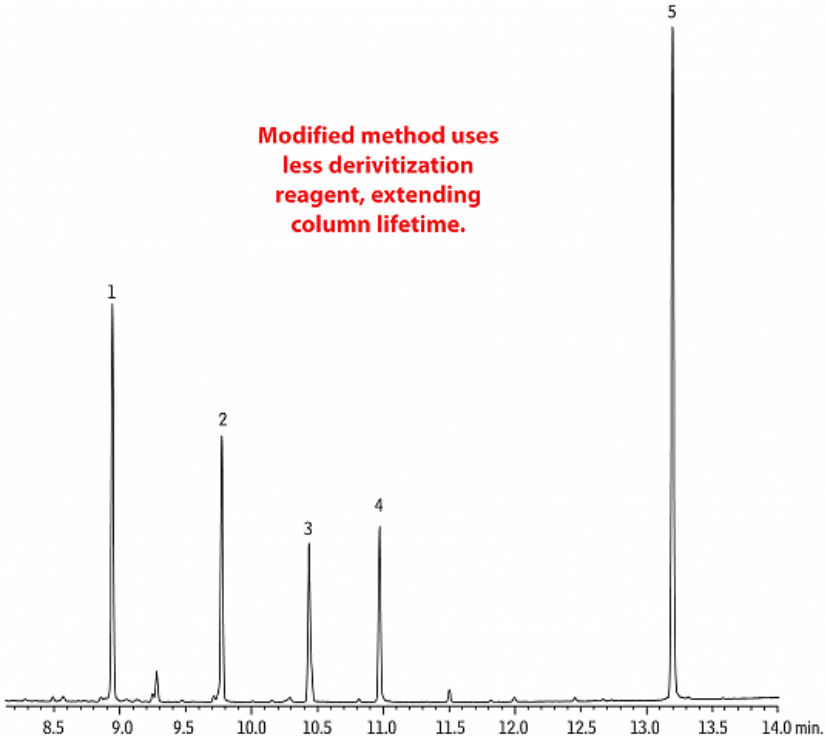
This is due to the presence of pyridine which is the sample solvent. Pyridine is necessary for the derivatization reaction performed during sample preparation and can increase ion signal background over a long period of time. To combat this, pyridine can sometimes be evaporated and the remaining analytes can be dissolved in a different more GC-amenable solvent. It is possible to lose some analytes during this process and it adds a lengthy step to sample preparation. Pyridine ion signal can be eliminated by simply changing the mass range used for the scanning mass spectrometer method. All of the analytes have characteristic ions of interest well above m/z 79, which is associated with pyridine. The scan method was modified to begin scanning at m/z 85.

A couple of modifications were made to help minimize stress to the instrument. The amount of derivatizing reagent was reduced to help maintain a clean inlet and column, as well as reduce the cost of analysis. The FDA method suggests a solvent delay time of 6 minutes. This was increased to approximately 8 minutes because of high pyridine levels, derivatization reagent, and matrix components. Adding this extra time helps to increase the filament lifetime but still ensures all the analytes will be detected.

Figure 3 shows a chromatogram of the high mix standard using the new method. This method provides excellent separation of melamine and cyanuric acid, the suspected toxic compounds, as well as ammelide and ammeline.

Figure 3 Excellent separation of melamine and related compounds using modified conditions (10µg/mL standard).

Peaks
1. Cyanuric acid
2. Ammelide
3. Ammeline
4. Melamine
5. Benzoguanamine



GC_FF00978

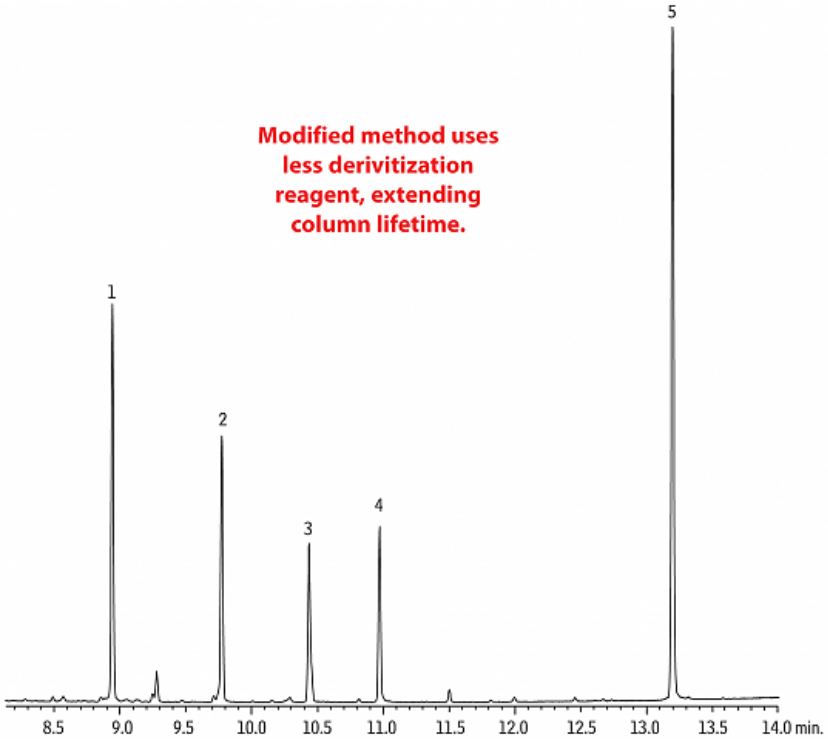
Column	Rtx®-5MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 12623)
Sample	melamine, cyanuric acid, ammelide, ammeline, benzoguanamine
Conc.:	10 µg/mL prederivatized
Injection	
Inj. Vol.:	1.0 µL splitless (hold 1 min)
Liner:	3.5mm Gooseneck Splitless (cat.# 22286)
Inj. Temp.:	280 °C
Oven	
Oven Temp.:	75 °C to 320 °C at 15 °C/min (hold 4 min)
Carrier Gas	He, constant flow
Flow Rate:	1 mL/min
Detector	MS
Mode:	SIM
Transfer Line Temp.:	290 °C
Ionization Mode:	EI

Figure 4 shows the analysis of dry cat food spiked with melamine, cyanuric acid, ammelide, ammeline, and the internal standard benzoguanamine. Reproducible and reliable retention times, along with SIM mass spectrometric detection, allow easy identification of analytes at both the high and low spike levels.

Figure 4 Melamine production analytes are easily identified in cat food using SIM analysis (50µg/g spike).

Peaks

1. Cyanuric acid
2. Ammelide
3. Ammeline
4. Melamine
5. Benzoguanamine



GC_FF00978

Column	Rtx®-5MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 12623)
Sample	melamine, cyanuric acid, ammelide, ammeline, benzoguanamine
Conc.:	10 µg/mL prederivatized
Injection	
Inj. Vol.:	1.0 µL splitless (hold 1 min)
Liner:	3.5mm Gooseneck Splitless (cat.# 22286)
Inj. Temp.:	280 °C
Oven	
Oven Temp.:	75 °C to 320 °C at 15 °C/min (hold 4 min)
Carrier Gas	He, constant flow
Flow Rate:	1 mL/min
Detector	MS
Mode:	SIM
Transfer Line Temp.:	290 °C
Ionization Mode:	EI

Conclusions

This work demonstrates that the FDA method is a valuable guideline for analysts screening melamine and related analogs. Using an Rtx®-5MS column and modifying the original method provides additional benefits: 1) decreasing the derivitization reagent volume results in longer column lifetime and less inlet maintenance, and 2) increasing the solvent delay decreases pyridine ion background, resulting in approximately 5-fold higher sensitivity for the analytes of interest.

References

1. *GC-MS Method for Screening and Confirmation of Melamine and Related Analogs*, Version 2, May 7, 2007, U.S Food and Drug Administration , http://www.fda.gov/cvm/GCMSscreen.htm.
2. FDA Recalls Animal Feed Tainted With Melamine From U.S. Supplier, Steven Reinberg, *HealthDay Reporter*, http://www.healthfinder.gov/news/printnewsstory.asp?docID=605070.
3. *Flame-retarding plastics and Elastomers with Melamine*, Weil ED and Choudhary V, *Journal of Fire Sciences* 13 (2): 104-126 Mar-Apr 1995.
4. Final report on the safety assessment of melamine/formaldehyde resin, Anderson FA, *Journal of the American College of Toxicology* 14 (5): 373-385 Oct 1995.

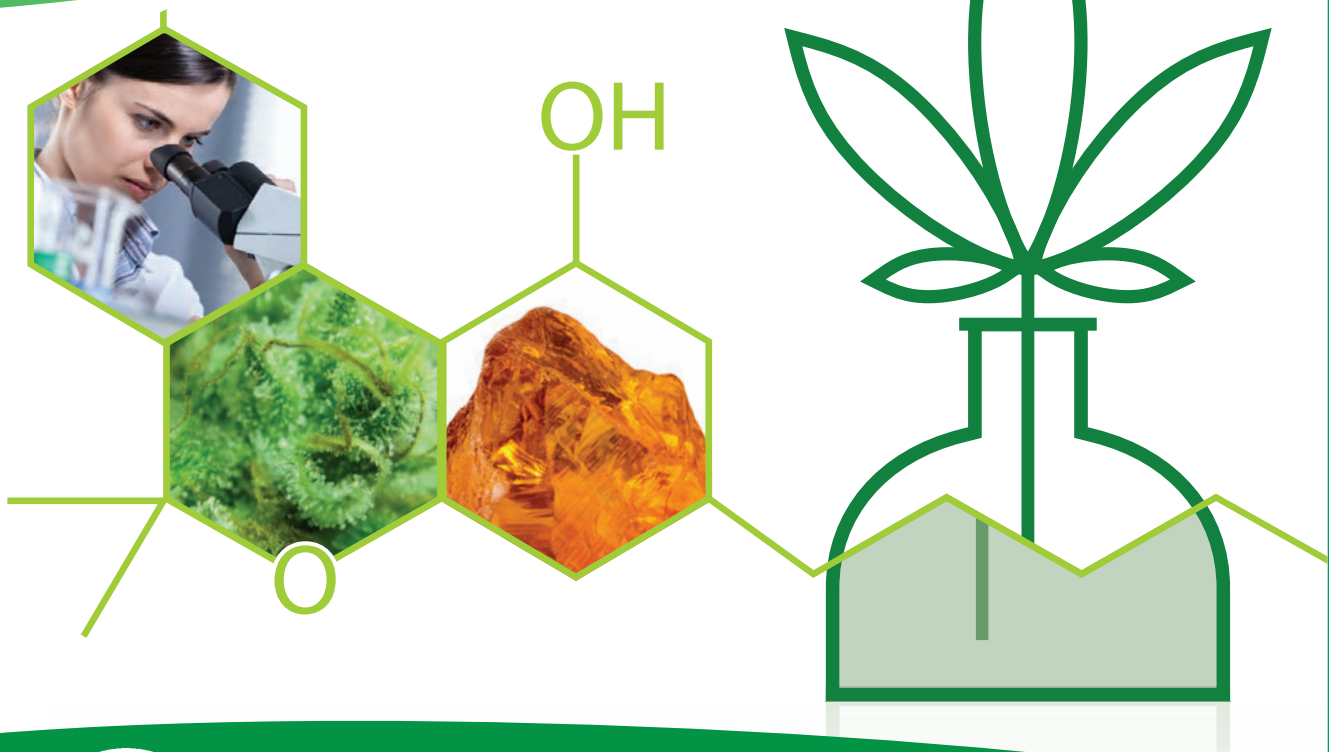
RELATED SEARCHES

Melamine, *cyanuric acid*, *ammelide*, *ammeline*, *pet food*, *cat food*, *dog food*, *food adulterants*, *melamine analogs*, *derivatized melamine*, *Rtx-5ms*, *Benzoguanamine*

Medical Cannabis

Growing Analytical Solutions for Cannabis Testing

INNOVATIVE PRODUCTS AND EXPERTISE FOR ACCURATE AND RELIABLE RESULTS



RESTEK® Pure Chromatography www.restek.com

Technical Expertise &

By Breaking Boundaries in Our Industry, We Help You Succeed in Yours

Restek has been helping cannabis labs establish innovative, cost-effective analytical solutions from the very beginning, and we will continue to help you manage your ever-changing analytical challenges every step of the way.

We get it. Your market is quickly changing and you need a chromatography partner that understands that. Whether you are part of a well-established safety and potency lab or starting a new lab, Restek has the products and expertise you need for successful cannabis analyses. Being an employee-owned and independent chromatography company, every employee at Restek has a vested interest in your success. We design the best solutions for your lab, regardless of the instrumentation and techniques used. In this brochure, you will find innovative LC and GC products and methodologies designed to fit your toughest analytical problems.

We've been in your shoes. That's why we understand your challenges and focus on solving them. Using our expertise to develop innovative products that help chromatographers has always been, and continues to be, Restek's top priority. We strive to develop industry-leading technologies that fit the needs of today's analysts. When setting up a laboratory for cannabis testing, we realize that you need dependable products that deliver high quality data without considerable capital investment. We know you need to work with a company that understands the challenges of your market and supports you with tailored solutions and superior customer service.

Rxi® GC COLUMNS

Lower Costs With Rugged, Long-Lasting Rxi® Columns

The chemists at Restek have combined their analytical expertise and wide range of polymer chemistries to provide a solution for straightforward analysis of terpenes and residual solvents on a single Rxi® column platform, streamlining workflows for busy labs. Rxi® columns deliver more accurate, reliable results than any other fused silica column on the market. To ensure the highest level of performance, all Rxi® capillary columns for the cannabis industry are manufactured and individually tested to meet stringent requirements for exceptional inertness, low bleed, and unsurpassed column-to-column reproducibility.

Sky® GC INLET LINERS

True Blue Performance—State-of-the-Art Deactivation With a 100% Satisfaction Guarantee

Whether you're determining cannabinoids, residual solvents, pesticides, or terpenes by GC, the inertness of your inlet is crucial for the success of your analyses. Sky® inlet liners from Restek use a comprehensive, state-of-the-art deactivation and are the only blue liners on the market—making them an easy-to-recognize solution to common inlet problems. The innovative deactivation used for Sky® liners results in exceptional inertness for a wide range of analyte chemistries. In addition to improved data quality, you'll benefit from fewer liner changes and less downtime for maintenance.

Product Innovation

Raptor™ LC COLUMNS

Maximize Analytical Performance and Minimize Your Capital Investment

Raptor™ LC columns combine the speed of a superficially porous particle (SPP or "core-shell") with the separation power of optimized USLC phase chemistry. These columns are ideal for cannabis testing because they quickly separate your target compounds, providing higher sample throughput. Raptor™ LC columns maximize your instrument performance so you won't need to buy expensive UHPLC equipment or extend your capital investment when the sample volume increases. Build a solid analytical foundation on any instrument with fast, rugged Raptor™ LC columns.

Q-sep® SAMPLE PREP SUPPLIES

Everything You Need for Fast, Simple Sample Prep

Cannabis products present a broad array of challenging matrices, from foods, to plant materials, to concentrates. For pesticides analysis, a fast, easy cleanup method is required to remove the matrix background for accurate, reliable results. Restek's versatile line of Q-sep™ QuEChERS extraction and cleanup salts allows for the development of quick, easy, and affordable sample preparation methods without capital investment in extraction equipment. The friendly experts at Restek are always willing to help with method development questions, too.

CERTIFIED REFERENCE MATERIALS (CRMs)

Get Results You can Trust With World-Class CRMs Produced in ISO-Accredited Labs

In order to achieve accurate results, samples must be quantified using certified reference materials. Restek has the widest offering of cannabinoid standards in the industry, and we are continually expanding our product line in order to meet the evolving needs of the cannabis industry. Restek's certified reference materials are manufactured and QC tested under our ISO Guide 34 and ISO/IEC 17025 accreditations, helping ensure confidence in results and compliance with changing regulations.

Restek® Certified Reference Materials

www.restek.com/cannabis

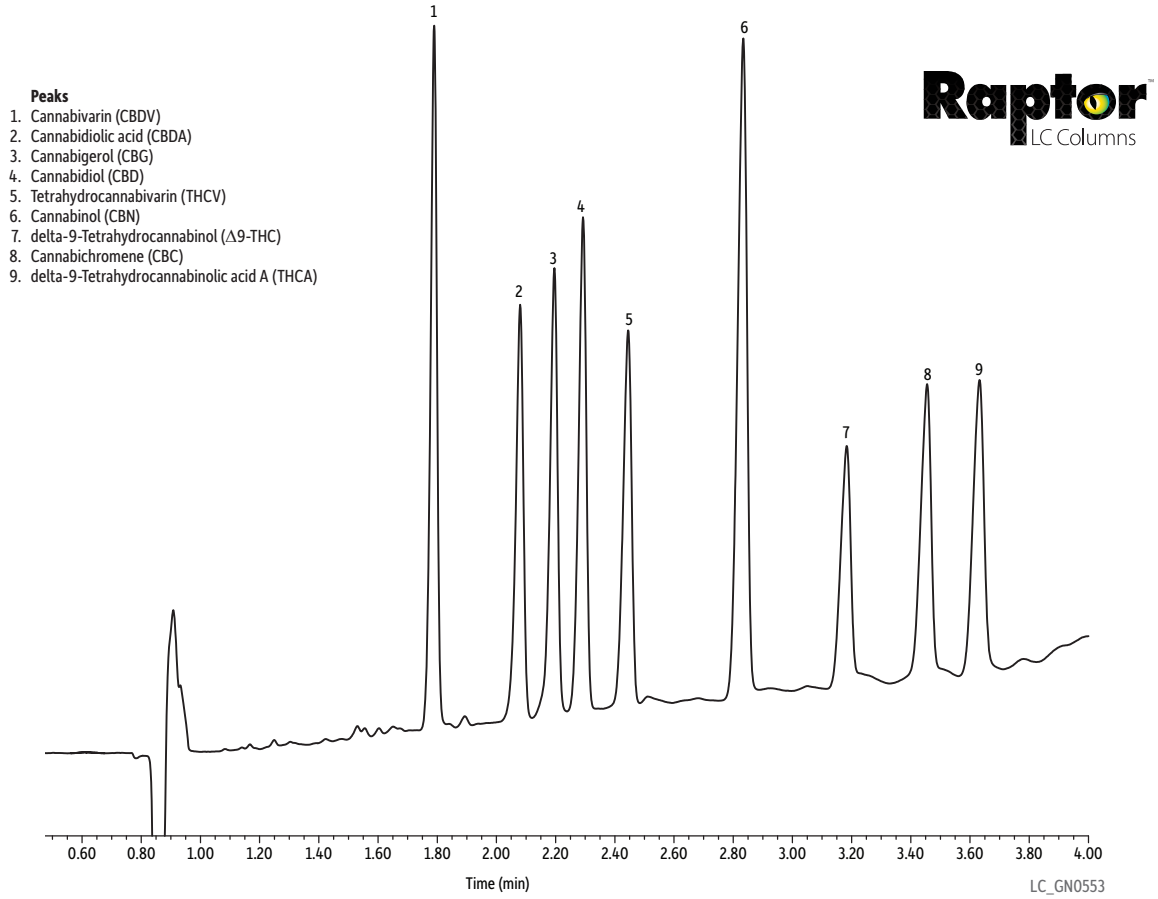
Applications

PRODUCT POTENCY TESTING

Our High-Throughput LC and GC Cannabinoids Methods Produce Results Quickly Without the Cost of New Equipment

When setting up a lab, often you just can't invest in the latest instrumentation, but you still need to get results fast. We understand that. That's why Restek has developed both LC and GC methods for cannabinoids that let you report potency results quickly. For LC, we created a fast analysis that can be performed on any LC instrument. By utilizing Raptor™ column technology, as shown in Figure 1, we developed a 3.7 minute analysis (7 minutes total cycle time) that is compatible with any HPLC instrument—so you get UHPLC speed on your existing equipment without the capital investment. Also, we specifically chose an easy-to-make mobile phase that can be directly transferred to LC-MS, if you ever need to move to MS due to regulation changes. For labs using GC equipment, you can analyze cannabinoids in just minutes using an Rxi®-35Sil MS column and the instrument conditions shown in Figure 2. We also offer a similar 35-type stationary phase on metal MXT® tubing for labs using SRI GC instruments. Why did we focus on fast cannabinoid analyses? Potency testing is the cornerstone of your lab. Building a fast method means your productivity increases and you can analyze more samples per day on the same instrument, delaying the need for expensive capital investments in new equipment.

Figure 1: Raptor™ LC columns give you fast analysis times for cannabinoids without the expense of UHPLC equipment.

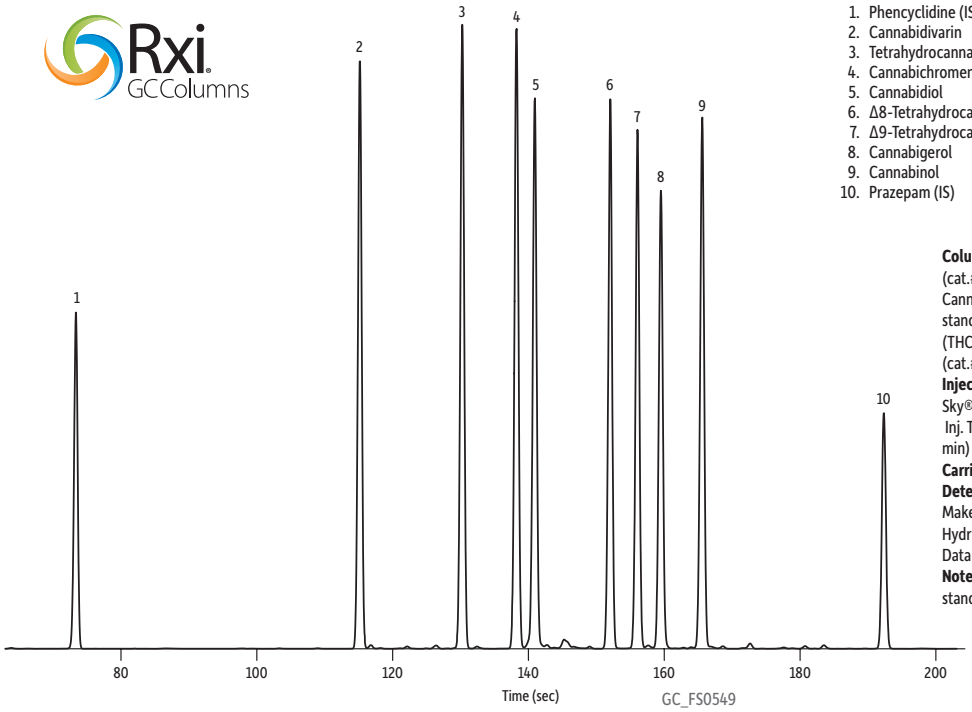


Column: Raptor™ ARC-18 (cat.# 9314A65), Dimensions: 150 mm x 4.6 mm ID, Particle Size: 2.7 µm, Temp.: 50 °C; **Sample:** Cannabidiolic acid (cat.# 34094), Cannabigerol (cat.# 34091), Cannabidiol (cat.# 34011), Cannabinol (cat.# 34010), delta-9-Tetrahydrocannabinol (THC) (cat.# 34067), Cannabichromene (cat.# 34092), delta-9-Tetrahydrocannabinolic acid A (THCA) (cat.# 34093), Diluent: 50:50 Methanol:water, Conc.: 50 µg/mL, Inj. Vol.: 5 µL; **Mobile Phase:** A: 0.1% Formic acid in water, B: 0.1% Formic acid in acetonitrile; **Gradient (%B):** 0.00 min (75%), 4.00 min (100%), 4.01 min (75%), 7.00 min (75%); **Flow:** 1.5 mL/min; **Detector:** UV/Vis @ 220 nm; **Instrument:** HPLC

TECH TIP

Using syringe filters is an economical way to remove particulate matter that could clog your column. Visit **www.restek.com/filters** to access our solvent/syringe filter compatibility guide and quickly find the best filter for your method.

Figure 2: Determine critical cannabinoids in minutes by GC using an Rxi®-35Sil MS column.



Peaks	Conc. (µg/mL)	tR (sec)
1. Phencyclidine (IS)	50	73.4
2. Cannabidivarin	100	115.2
3. Tetrahydrocannabivarin	100	130.3
4. Cannabichromene	100	138.3
5. Cannabidiol	100	150.0
6. Δ8-Tetrahydrocannabinol	100	152.1
7. Δ9-Tetrahydrocannabinol	100	156.1
8. Cannabigerol	100	159.5
9. Cannabinol	100	165.6
10. Prazepam (IS)	50	192.4

Column: Rxi®-35Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13820), **Sample:** Phencyclidine (cat.# 34027); Cannabichromene (CBC) (cat.# 34092); Cannabinoids standard (cat.# 34014); delta-8-Tetrahydrocannabinol (THC) (cat.# 34090); Cannabigerol (CBG) (cat.# 34091); Prazepam (cat.# 34055); **Injection:** Inj. Vol.: 1 µL split (split ratio 20:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 190 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.9 min); **Carrier Gas:** H_2, constant flow; Flow Rate: 2.5 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument:** Agilent/HP6890 GC; **Notes:** Cannabidivarin and tetrahydrocannabivarin standards were obtained from BOC Sciences.

POTENCY TESTING PRODUCTS

Raptor™ ARC-18 LC Columns (USP L1)

Properties:

- Well-balanced retention profile.
- Sterically protected and acid-resistant to resist harsh, low-pH mobile phases.
- Ideal for use with sensitive detectors like mass spec.

Description	cat.#
2.7 µm Columns 150 mm, 4.6 mm ID	9314A65

For guard cartridges, visit our website at **www.restek.com**

Rxi®-35Sil MS Columns (fused silica)

(midpolarity Crossbond® phase)

- Provides superior separation for cannabinoids.
- Very low-bleed phase for GC-MS analysis.
- Extended temperature range: 50 °C to 340/360 °C.

Description	temp. limits	qty.	cat.#
15 m, 0.25 mm ID, 0.25 µm	50 to 340/360 °C	ea.	13820

Sky® 4.0 mm ID Precision® Inlet Liner w/Wool

For Agilent GCs equipped with split/splitless inlets

ID x OD x L	qty.	cat.#
Precision, Sky Technology, Borosilicate Glass with Quartz Wool		
4.0 mm x 6.3 mm x 78.5 mm	ea.	23305.1
4.0 mm x 6.3 mm x 78.5 mm	5-pk.	23305.5
4.0 mm x 6.3 mm x 78.5 mm	25-pk.	23305.25

Patent pending

Medical Marijuana Singles

Concentration is µg/mL. Volume is 1 mL/ampul.

Compound	CAS #	Solvent	Conc.	cat.#
Cannabichromene (CBC)	20675-51-8	PTM	1,000	34092
Cannabidiol (CBD)	13956-29-1	PTM	1,000	34011
Cannabidiolic Acid (CBDA)	1244-58-2	ACN	1,000	34094
Cannabigerol	25654-31-3	PTM	1,000	34091
Cannabinol (CBN)	521-35-7	PTM	1,000	34010
delta-8-Tetrahydrocannabinol (THC)	5957-75-5	PTM	1,000	34090
delta-9-Tetrahydrocannabinol (THC)	1972-08-3	M	1,000	34067
delta-9-Tetrahydrocannabinolic acid A (THCA-A)	23978-85-0	PTM	1,000	34093
Tetrahydrofuran-d8	1693-74-9	PTM	2,000	30112
(±)11-nor-9-carboxy-Δ^9-THC	104874-50-2	M	100	34068

M = methanol; PTM = purge-and-trap grade methanol; ACN = acetonitrile

Cannabinoids Standard (3 components)

Cannabidiol (13956-29-1)
Cannabinol (521-35-7)
delta-9-Tetrahydrocannabinol (Δ^9-THC) (1972-08-3)

1,000 µg/mL each in P&T methanol, 1 mL/ampul
cat.# 34014 (ea.)

Quantity discounts not available.

Phencyclidine

Phencyclidine (956-90-1)

1,000 µg/mL in P&T methanol, 1 mL/ampul
cat.# 34027 (ea.)

Prazepam

Prazepam (2955-38-6)

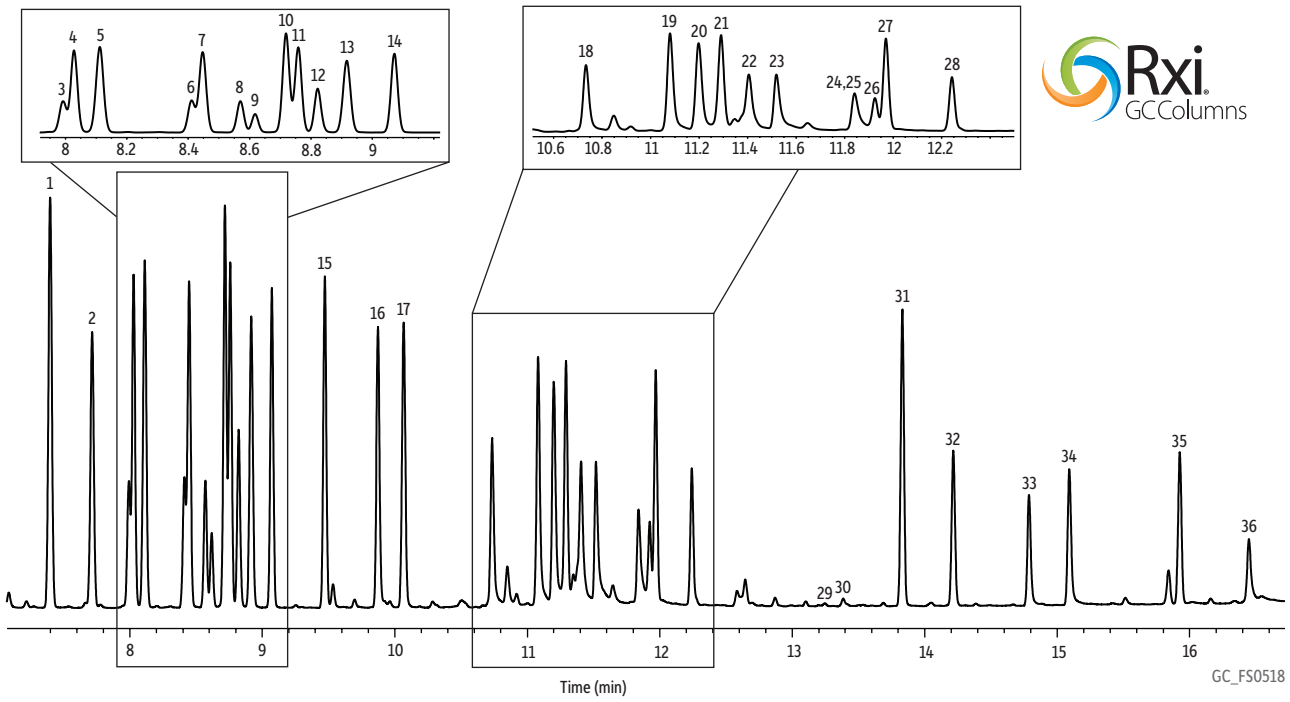
1,000 µg/mL in P&T methanol, 1 mL/ampul
cat.# 34055 (ea.)

TERPENE PROFILING

Reduce Capital Investments—Analyze Terpenes by GC on the Same Setup Used for Residual Solvents

Cannabis has a complex terpene profile, which is theorized to increase its therapeutic effects. Terpene profiling is used for both product quality testing and strain identification. These complex and sometimes problematic compounds are challenging to analyze, but the experts at Restek have developed GC methodology for terpene profiling that fits easily into required laboratory workflows. To keep things simple, the GC terpene profile analysis in Figure 3 can be performed on the same instrument and column that we recommend for residual solvent testing (see page 8).

Figure 3: Comprehensive terpene analysis by headspace GC-FID can be done on the same instrument and GC column as residual solvents analysis, which simplifies setup and improves lab productivity.



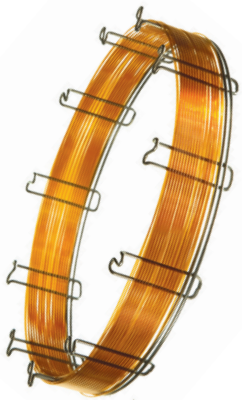
Peaks	tR (min)						
1. α-Pinene	7.39	10. Limonene	8.71	19. dl-Menthol	11.08	28. Citral 2	12.24
2. Camphene	7.71	11. *p*-Cymene	8.75	20. Borneol	11.19	29. Citral 3	13.19
3. β-Myrcene	7.98	12. β-Ocimene	8.82	21. α-Terpineol	11.29	30. Citral 4	13.43
4. Sabinene	8.02	13. Eucalyptol	8.91	22. Dihydrocarveol	11.40	31. β-caryophyllene	13.83
5. β-Pinene	8.11	14. γ-Terpinene	9.06	23. Citronellol	11.51	32. α-Humulene	14.21
6. α-Phellandrene	8.4	15. Terpinolene	9.47	24. Geraniol	11.82	33. Nerolidol 1	14.78
7. δ3-Carene	8.44	16. Linalool	9.87	25. 2-Piperidinone	11.88	34. Nerolidol 2	15.08
8. α-Terpinene	8.57	17. Fenchone	10.06	26. Citral 1	11.92	35. Caryophyllene oxide	15.92
9. Ocimene	8.61	18. Isopulegol	10.73	27. Pulegone	11.97	36. α-Bisabolol	16.43

Column: Rxi® -624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868); **Sample:** Terpenes mix; Diluent: Isopropyl alcohol; Conc.: 200 ng/µL (0.02% wt/vol). The sample was prepared by placing 10 µL into the headspace vial.; **Injection:** headspace-loop split (split ratio 10:1); Liner: Sky® 1.0 mm ID straight inlet liner (cat.# 23333.1); **Headspace-Loop**: Inj. Port Temp.: 250 °C; Instrument: Tekmar HT-3; Inj. Time: 1.0 min; Transfer Line Temp.: 160 °C; Valve Oven Temp.: 160 °C; Needle Temp.: 140 °C; Sample Temp.: 140 °C; Sample Equil. Time: 30.0 min; Vial Pressure: 20 psi; Loop Pressure: 15 psi; **Oven:** Oven Temp.: 60 °C (hold 0.10 min) to 300 °C at 12.50 °C/min (hold 3.0 min); **Carrier Gas:** He, constant flow; Linear Velocity: 33 cm/sec; **Detector:** FID @ 320 °C; Make-up Gas Flow Rate: 45 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument** Agilent/HP6890 GC

TECH TIP

For full method details on headspace GC analysis of terpenes, visit **www.restek.com/cannabis_terpenes**

TERPENE TESTING PRODUCTS



Rxi®-624Sil MS Columns (fused silica)
(midpolarity Crossbond® phase)

- Low-bleed, high-thermal stability column—maximum temperatures up to 320 °C.
- Inert—excellent peak shape for a wide range of compounds.
- Selective—G43 phase highly selective for volatile organics and residual solvents, great choice for USP<467>.
- Manufactured for column-to-column reproducibility—well-suited for validated methods.

Description	temp. limits	qty.	cat.#
30 m, 0.25 mm ID, 1.40 µm	-20 to 300/320 °C	ea.	13868

Sky® 1.0 mm ID Straight Inlet Liner
for Agilent GCs equipped with split/splitless inlets

ID x OD x L	qty.	cat.#
Straight, Sky Technology, Borosilicate Glass		
1.0 mm x 6.3 mm x 78.5 mm	ea.	23333.1
1.0 mm x 6.3 mm x 78.5 mm	5-pk.	23333.5
1.0 mm x 6.3 mm x 78.5 mm	25-pk.	23333.25

* 100% SATISFACTION GUARANTEE: If your Sky® inlet liner does not perform to your expectations for any reason, simply contact Restek® Technical Service or your local Restek® representative and provide a sample chromatogram showing the problem. If our GC experts are not able to quickly and completely resolve the issue to your satisfaction, you will be given an account credit or replacement product (same cat.#) along with instructions for returning any unopened product. (Do not return product prior to receiving authorization.) For additional details about Restek's return policy, visit www.restek.com/warranty

Headspace Crimp Vials (20 mm)

Description	Volume	Color	Dimensions	100-pk.	1,000-pk.
Headspace Vial, Flat Bottom	20 mL	Clear	23 x 75 mm	24685	24686

Vial-to-instrument compatibility are designated in instrument reference chart on the product web page.

Medical Cannabis Terpenes Standards

Medical Cannabis Terpenes Standard #1 (19 components)

(-)-alpha-Bisabolol (23089-26-1)
Camphene (79-92-5)
delta-3-Carene (13466-78-9)
beta-Caryophyllene (87-44-5)
Geraniol (106-24-1)
(-)-Guaiol (489-86-1)
alpha-Humulene (6753-98-6)
p-Isopropyltoluene (*p*-cymene) (99-87-6)
(-)-Isopulegol (89-79-2)
d-Limonene (5989-27-5)
Linalool (78-70-6)
beta-Myrcene (123-35-3)
Nerolidol (7212-44-4)
Ocimene (13877-91-3)
alpha-Pinene (80-56-8)
(-)-beta-Pinene (18172-67-3)
alpha-Terpinene (99-86-5)
gamma-Terpinene (99-85-4)
Terpinolene (586-62-9)

2,500 µg/mL each in isopropanol, 1 mL/ampul
cat.# 34095 (ea.)

Medical Cannabis Terpenes Standard #2 (2 components)

(-)-Caryophyllene oxide (1139-30-6)
1,8-Cineole (Eucalyptol) (470-82-6)

2,500 µg/mL each in isopropanol, 1 mL/ampul
cat.# 34096 (ea.)

Did you **know**?

You'll save money ordering from Restek because we understand the need to control costs and build efficient workflows. We develop as many analyses as possible using the same columns and consumables, so you can minimize the number of products you need to stock.

TECH TIP

Did you know that headspace analysis eliminates the possibility of column contamination from nonvolatile matrix components? This results in an extremely clean chromatogram, minimal instrument maintenance, and longer column lifetimes.

RESIDUAL SOLVENT ANALYSIS

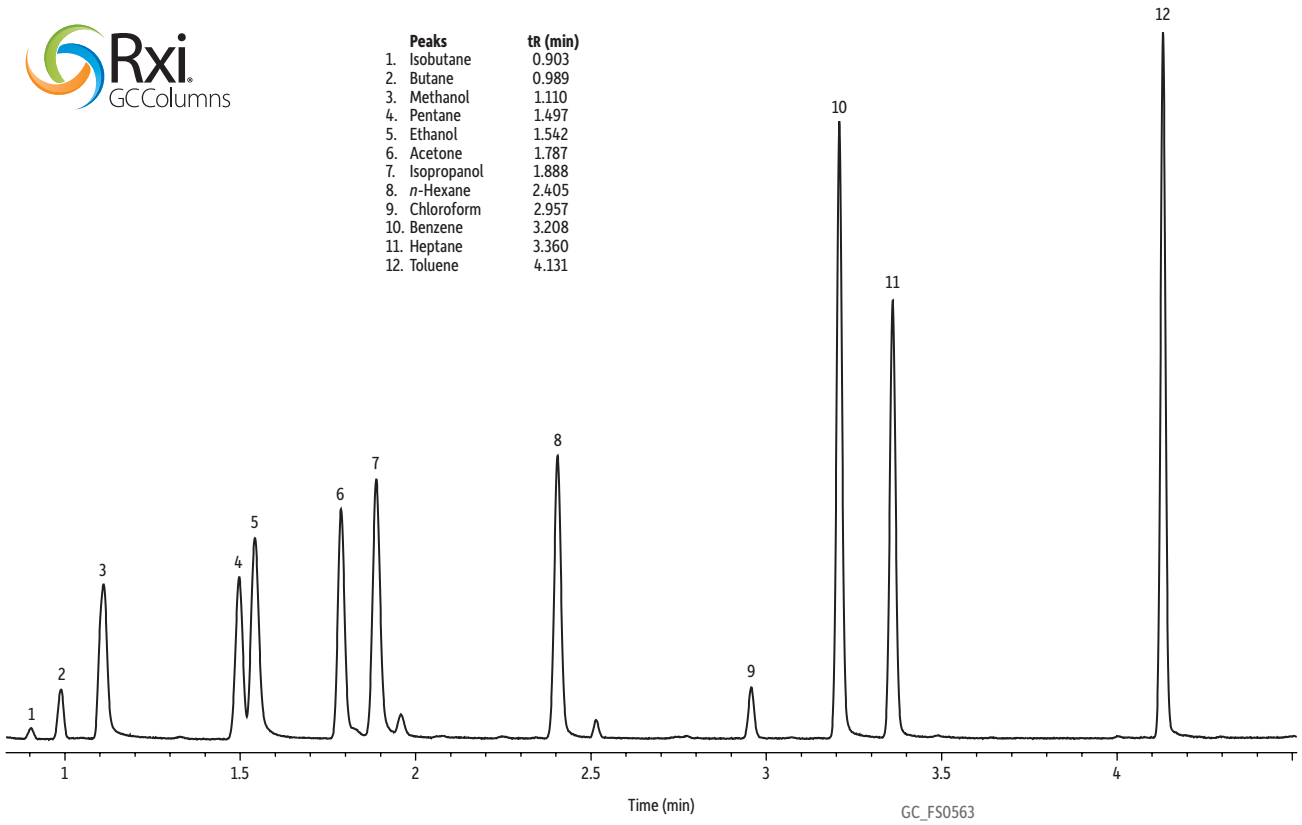
Improve Productivity—Keep Analyzing Samples Instead of Changing Columns Between Residual Solvent and Terpene Methods.

As the popularity of medical cannabis grows, so does concern over the safety of the drug products. Cannabis concentrates can contain residual solvents left over from manufacturing that can be harmful to human health. Because of this risk, many states will require residual solvent testing of cannabis concentrates. Due to their high volatility, residual solvents can only be analyzed using GC techniques. The chemists at Restek have developed a quick and easy method that allows for residual solvent analysis (Figure 4) and terpene profiling (Figure 3) on the same column and instrument platform with minimal sample preparation (see page 6 for terpene profiling).

TECH TIP

For full method details on headspace GC analysis of residual solvents, visit **www.restek.com/cannabis_solvents**

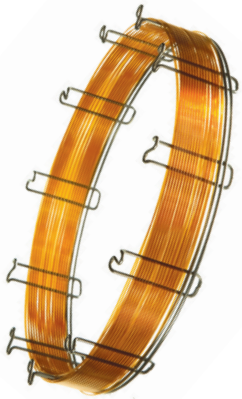
Figure 4: Improve productivity and reduce downtime for column changes—this sensitive headspace GC-FID analysis of residual solvents can be accomplished on the same instrument and Rxi®-624Sil MS column that is used in Restek's terpenes profiling method.



Column: Rxi®-624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868); **Sample**: Residual solvent mix; Diluent: Dimethyl sulfoxide (DMSO); Conc.: 25 ppm (For the HS-FET technique, 10 µL of a 50 µg/mL standard was placed into a 20 mL headspace vial to represent a 25 ppm sample concentration, assuming a 20 mg sample weight.); **Injection:** headspace-loop split (split ratio 10:1); Liner: Sky® 1.0 mm ID straight inlet liner (cat.# 23333.1); **Headspace-Loop:** Inj. Port Temp.: 250 °C; Instrument: Tekmar HT3; Inj. Time: 1.0 min; Transfer Line: Temp.: 160 °C; Valve Oven Temp.: 160 °C; Needle Temp.:140 °C; Sample Temp.: 140 °C; Platen temp equil. time: 1.0 min; Sample Equil. Time: 30.0 min; Vial Pressure: 20 psi; Pressurize Time: 5.0 min; Loop Pressure: 15 psi; Loop Fill Time: 2.0 min; Oven Temp.:35 °C (hold 1.5 min) to 300 °C at 30 °C/min (hold 2.0 min); **Carrier Gas**: He, constant flow; Linear Velocity: 80 cm/sec; **Detector:** FID @ 320 °C; Make-up Gas Flow Rate: 45 mL/min; Make-up Gas Type: N2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument**: Agilent/HP6890 GC; **Notes**: The butane used for standard preparation was a mixture of butane and isobutane in an unknown ratio. The concentrations should be considered approximate, but do not exceed 50 ppm for any component.

RESIDUAL SOLVENT TESTING PRODUCTS

Rxi®-624Sil MS Columns (fused silica)
(midpolarity Crossbond® phase)



- Low-bleed, high-thermal stability column—maximum temperatures up to 320 °C.
- Inert—excellent peak shape for a wide range of compounds.
- Selective—G43 phase highly selective for volatile organics and residual solvents, great choice for USP<467>.
- Manufactured for column-to-column reproducibility—well-suited for validated methods.

Description	temp. limits	qty.	cat.#
30 m, 0.25 mm ID, 1.40 µm	-20 to 300/320 °C	ea.	13868

Sky® 1.0 mm ID Straight Inlet Liner
for Agilent GCs equipped with split/splitless inlets

ID x OD x L	qty.	cat.#
Straight, Sky Technology, Borosilicate Glass		
1.0 mm x 6.3 mm x 78.5 mm	ea.	23333.1
1.0 mm x 6.3 mm x 78.5 mm	5-pk.	23333.5
1.0 mm x 6.3 mm x 78.5 mm	25-pk.	23333.25

* 100% SATISFACTION GUARANTEE: If your Sky® inlet liner does not perform to your expectations for any reason, simply contact Restek® Technical Service or your local Restek® representative and provide a sample chromatogram showing the problem. If our GC experts are not able to quickly and completely resolve the issue to your satisfaction, you will be given an account credit or replacement product (same cat.#) along with instructions for returning any unopened product. (Do not return product prior to receiving authorization.) For additional details about Restek's return policy, visit www.restek.com/warranty

Headspace Crimp Vials (20 mm)

Description	Volume	Color	Dimensions	100-pk.	1,000-pk.
Headspace Vial, Flat Bottom	20 mL	Clear	23 x 75 mm	24685	24686

Vial-to-instrument compatibility are designated in instrument reference chart on the product web page.

Did you **know**?

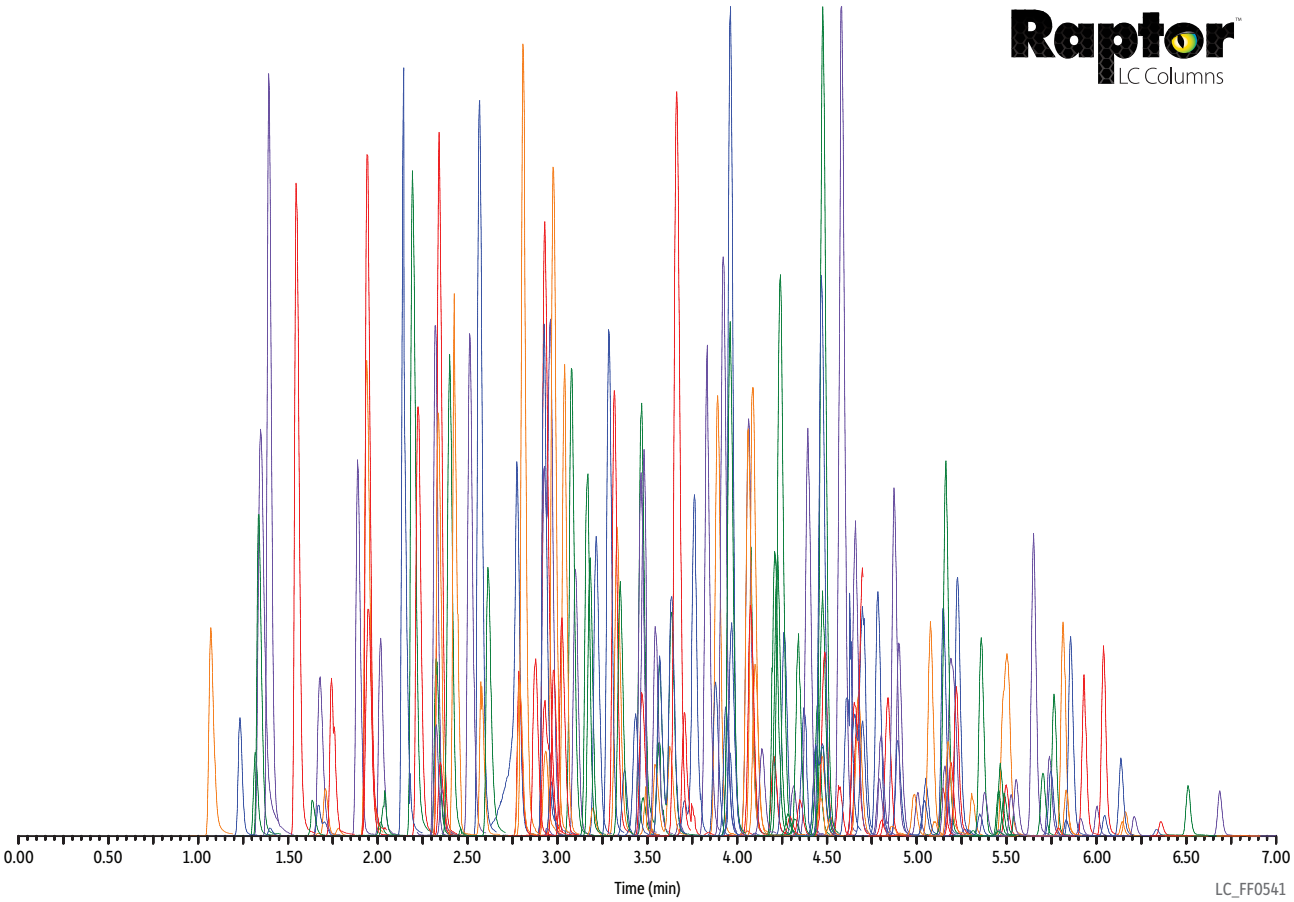
You'll save money ordering from Restek because we understand the need to control costs and build efficient workflows. We develop as many analyses as possible using the same columns and consumables, so you can minimize the number of products you need to stock.

PESTICIDE ANALYSIS

Ensure Product Safety With Fast, Selective Multiresidue Pesticide Analysis

In addition to residual solvents, cannabis products can contain residues of pesticides that were applied to cannabis plants during growth in order to control agricultural pests. These pesticides can be analyzed by LC-MS/MS, GC-MS/MS, and GC-MS. Regardless of the technique used, lists of target compounds can be extensive, so column selectivity is an important factor in achieving good separations. Both Raptor™ ARC-18 LC columns (Figure 5) and Rxi®-5ms GC columns (Figure 6) provide the selectivity needed for accurate and reliable multiresidue pesticides analysis. Removing matrix interferences while also recovering the analytes of interest is also crucial for a successful pesticide analysis using either LC or GC, and Restek's Q-sep® QuEChERS products allow for fast, easy, adaptable cleanup of a wide variety of matrices.

Figure 5: A high-throughput separation of 204 pesticides by LC-MS/MS can be achieved in only 7 minutes with the Raptor™ ARC-18 column.



Column: Raptor™ ARC-18 (cat.# 9314A12), Dimensions: 100 mm x 2.1 mm ID, Particle Size: 2.7 µm, Temp.: 50 °C; **Sample:** LC multiresidue pesticide kit (cat.# 31971), Diluent: Water, Conc.: 20 ng/mL, Inj. Vol.: 5 µL; **Mobile Phase:** A: Water + 2 mM ammonium formate + 0.2% formic acid, B: Methanol + 2 mM ammonium formate + 0.2% formic acid; **Gradient (%B):** 0.00 min (5%), 2.00 min (60%), 4.00 min (75%), 6.00 min (100%), 7.00 min (100%), 7.01 min (5%), 9.50 min (5%); **Flow:** 0.4 mL/min; Max Pressure: 525 bar; **Detector:** Waters Xevo TQ-S, Ion Source: Waters Zspray™ ESI, Ion Mode: ESI+, Mode: MRM, **Instrument:** Waters ACQUITY UPLC® I-Class; **Notes:** When combining a large number of compounds with different chemical functionalities, mix stability can be an issue. In formulating our LC multi-residue pesticide standard kit (cat.# 31971), we extensively studied the 204 compounds involved, then grouped them into as few mixes as possible while still ensuring maximum long-term stability and reliability. Several of these compounds are isomeric and separation of the isomers accounts for 216 peaks in the chromatogram compound list. For quantitative analysis, we recommend analyzing each mix separately to ensure accurate results for every compound.

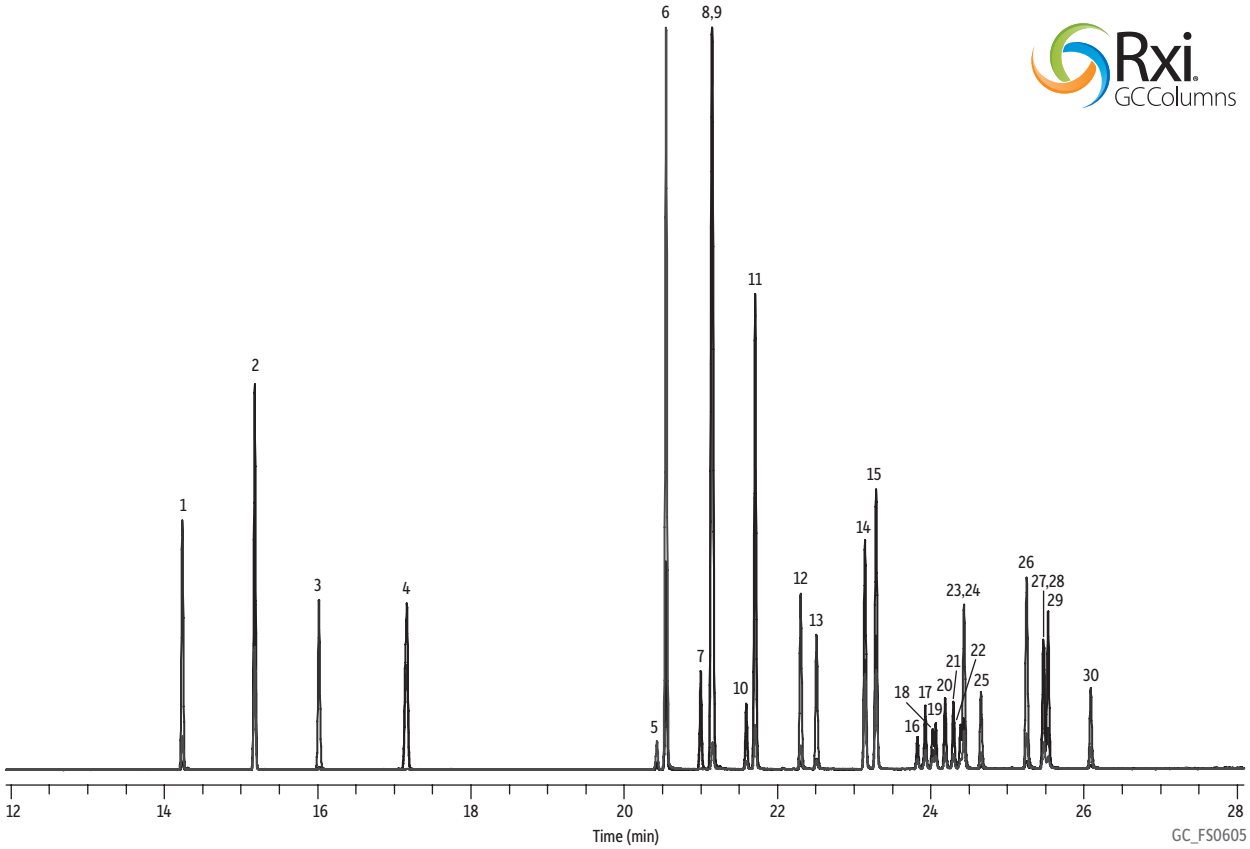
Figure 6: Peak List

	Peaks	t_R (min)	Precursor Ion	Product Ion 1	Product Ion 2
1.	Cyromazine	1.07	167.0	85.0	108.1
2.	Methamidophos	1.23	142.0	93.9	124.9
3.	Formetanate HCl	1.32	222.0	165.0	46.0
4.	Aminocarb	1.34	209.0	137.0	152.0
5.	Pymetrozine	1.35	218.0	105.0	79.0
6.	Acephate	1.40	184.1	143.0	125.1
7.	Propamocarb	1.40	189.1	102.0	144.0
8.	Omethoate	1.55	214.1	125.1	183.1
9.	Aldicarb sulfoxide	1.64	207.0	89.0	132.0
10.	Dinotefuran	1.64	203.0	129.0	157.0
11.	Butoxycarboxim	1.67	223.0	106.0	166.0
12.	Nitenpyram	1.68	271.1	125.9	224.9
13.	Aldicarb sulfone	1.71	240.0	148.0	86.0
14.	Carbendazim	1.74	192.1	160.1	132.1
15.	Oxamyl	1.78	237.0	72.0	90.0
16.	Flonicamid	1.89	230.0	203.1	174.1
17.	Methomyl	1.91	163.0	106.0	88.0
18.	Thiabendazole	1.94	202.0	175.0	131.0
19.	Thiamethoxam	1.94	292.0	211.0	181.0
20.	Mexacarbate	1.95	222.9	151.1	166.1
21.	Monocrotophos	2.02	224.1	127.1	98.1
22.	Fuberidazole	2.04	185.0	157.0	156.0
23.	Dicrotophos	2.14	238.0	112.0	193.0
24.	Imidacloprid	2.19	256.1	175.1	209.1
25.	Clothianidin	2.22	250.0	169.0	132.0
26.	Trichlorfon	2.32	257.0	109.0	79.0
27.	3-Hydroxycarbofuran	2.33	238.0	181.0	163.0
28.	Fenuron	2.33	165.0	71.9	45.9
29.	Dimethoate	2.34	230.1	125.0	199.0
30.	Vamidothion	2.34	288.0	146.0	118.0
31.	Dioxacarb	2.35	224.1	123.1	167.1
32.	Mevinphos isomer 1	2.36	225.1	127.1	193.1
33.	Acetamiprid	2.40	223.0	126.0	56.1
34.	Ethirimol	2.43	210.1	140.0	98.0
35.	Cymoxanil	2.46	199.0	128.0	111.0
36.	Pirimicarb	2.51	239.1	72.0	182.1
37.	Thiacloprid	2.56	253.0	126.0	90.1
38.	Mevinphos isomer 2	2.58	225.1	127.1	193.1
39.	Mesotrione	2.62	340.1	228.1	104.0
40.	Butocarboxim	2.68	213.0	156.0	116.0
41.	Aldicarb	2.71	213.1	89.1	116.1
42.	Oxadixyl	2.77	279.0	219.0	132.0
43.	Carbetamide	2.79	237.0	118.0	192.0
44.	Tricyclazole	2.79	190.0	163.0	136.0
45.	Simetryn	2.81	214.0	124.0	95.9
46.	Thiophanate-methyl	2.88	343.0	151.0	93.0
47.	Bendiocarb	2.93	224.1	109.0	167.0
48.	Prometon	2.93	226.0	184.3	86.3
49.	Secbumeton	2.93	226.2	100.2	170.2
50.	Thidiazuron	2.93	221.0	101.9	93.9
51.	Propoxur	2.95	210.0	111.0	168.0
52.	Metribuzin	2.96	215.0	131.0	89.0
53.	Terbumeton	2.96	226.1	114.1	170.1
54.	Carbofuran	2.98	222.1	123.0	165.1
55.	Imazalil	2.98	297.0	159.0	69.0
56.	Sulfentrazone	3.03	387.0	307.0	145.8
57.	Pyracarbolid	3.04	218.1	125.1	97.1
58.	Tebuthiuron	3.08	229.0	172.0	116.0
59.	Carbaryl	3.09	202.0	145.0	127.0
60.	Carboxin	3.10	236.0	143.0	87.0
61.	Monolinuron	3.17	215.0	126.0	99.0
62.	Fluometuron	3.18	233.2	72.2	46.4
63.	Ethiofencarb	3.20	226.1	107.0	164.0
64.	Ametryn	3.21	228.1	186.1	68.1
65.	Chlortoluron	3.29	213.0	72.0	46.0
66.	Metobromuron	3.32	259.1	170.0	148.1
67.	Methoprotryne	3.33	272.2	170.2	198.2
68.	Propham	3.33	180.0	138.0	120.1
69.	Flutriafol	3.35	302.1	123.1	70.2
70.	Isoprocarb	3.37	194.1	95.1	137.1
71.	Fenpropimorph	3.44	304.2	147.1	57.2
72.	Methabenzthiazuron	3.46	222.0	165.0	150.0
73.	Diuron	3.47	233.0	72.1	46.3
74.	Forchlorfenuron	3.47	248.1	129.0	93.0
75.	Isocarbophos	3.48	291.1	121.1	231.1
76.	Isoproturon	3.48	207.0	72.0	47.0
77.	Pyrimethanil	3.48	200.0	107.0	82.0
78.	Desmedipham	3.55	318.0	182.0	154.0
79.	Metalaxyl	3.56	280.1	220.1	192.1
80.	Spiroxamine isomer 1	3.57	298.0	144.0	100.0
81.	Phenmedipham	3.63	301.0	168.0	136.0
82.	Spiroxamine isomer 2	3.63	298.0	144.0	100.0
83.	Chlorantraniliprole	3.66	483.9	286.0	453.0
84.	Cycluron	3.68	199.0	89.1	69.2
85.	Prometryn	3.71	242.0	158.0	200.1
86.	Terbutryn	3.76	242.1	186.1	91.0
87.	Linuron	3.83	249.1	160.0	182.0
88.	Fenobucarb	3.84	208.0	94.9	152.0
89.	Diethofencarb	3.88	268.0	226.0	124.0
90.	Ethofumesate	3.89	287.1	121.1	259.1
91.	Azoxystrobin	3.92	404.1	372.0	329.0
92.	Ethiprole	3.94	396.9	350.9	255.2
93.	Fenamidone	3.96	312.1	236.1	92.0
94.	Methiocarb	3.96	226.0	121.0	169.0
95.	Siduron	3.96	233.0	93.8	137.0
96.	Fludioxonil	3.97	249.1	229.1	158.1
97.	Furalaxyl	3.97	302.1	270.1	242.2
98.	Halofenozide	3.99	331.1	104.9	275.0
99.	Acibenzolar-S-methyl	4.06	210.9	91.0	135.9
100.	Boscalid	4.06	342.9	307.0	139.9
101.	Dimethomorph isomer 1	4.06	388.1	300.9	165.0
102.	Nuarimol	4.08	315.0	252.0	81.1
103.	Mandipropamid	4.09	412.3	328.2	356.2
104.	Flutolanil	4.10	324.1	262.1	65.0
105.	Promecarb	4.10	208.1	151.0	109.0
106.	Paclobutrazol	4.14	294.1	125.1	70.2
107.	Thiofanox	4.19	219.1	172.9	129.0
108.	Cyproconazole isomer 1	4.21	292.2	125.1	70.2
109.	Mepronil	4.21	270.1	119.0	91.0
110.	Bupirimate	4.22	317.0	166.0	108.0
111.	Dimethomorph isomer 2	4.24	388.1	300.9	165.0
112.	Myclobutanil	4.26	289.1	70.2	125.1
113.	Clethodim isomer 1	4.28	360.0	164.0	268.1
114.	Methoxyfenozide	4.30	369.1	149.1	313.2
115.	Chloroxuron	4.31	291.1	164.1	111.0
116.	Cyprodinil	4.32	226.0	93.0	108.0
117.	Triadimefon	4.34	294.1	197.2	69.3
118.	Bifenazate	4.35	301.1	198.0	170.0
119.	Triadimenol	4.35	296.1	99.1	70.2
120.	Cyproconazole isomer 2	4.38	292.2	125.1	70.2
121.	Mefenacet	4.39	299.0	148.0	120.0
122.	Mepanipyrim	4.40	224.1	106.0	77.0
123.	Iprovalicarb isomer 1	4.44	321.1	119.1	203.1
124.	Fluquinconazole	4.45	376.0	348.8	306.9
125.	Fenhexamid	4.46	302.1	97.2	55.3
126.	Bromuconazole isomer 1	4.47	376.0	158.9	70.1
127.	Fluoxastrobin	4.47	459.0	427.0	188.0
128.	Iprovalicarb isomer 2	4.47	321.1	119.1	203.1
129.	Butafenacil	4.48	492.0	180.0	331.0
130.	Tetraconazole	4.48	372.0	159.0	70.1
131.	Flufenacet	4.49	364.0	152.1	194.1
132.	Triticonazole	4.52	318.1	70.1	124.9
133.	Cyazofamid	4.57	325.0	107.9	261.0
134.	Spirotetramat	4.58	374.2	330.3	302.2
135.	Diflubenzuron	4.63	311.1	141.0	158.1
136.	Epoxiconazole	4.66	330.0	121.0	101.0
137.	Etaconazole isomer 1	4.66	328.1	205.0	159.0
138.	Fenbuconazole	4.67	337.0	125.0	70.1
139.	Fenarimol	4.68	331.0	268.0	81.0
140.	Etaconazole isomer 2	4.70	328.1	205.0	159.0
141.	Fipronil	4.70	437.0	367.9	290.0
142.	Flusilazole	4.78	316.0	247.0	165.0
143.	Picoxystrobin	4.79	368.0	145.1	205.1
144.	Fenoxycarb	4.80	302.1	116.1	88.0
145.	Neburon	4.80	275.0	88.0	57.0
146.	Rotenone	4.84	395.0	213.1	192.1
147.	Tebufenozide	4.87	353.1	133.0	297.1
148.	Dimoxystrobin	4.88	327.1	116.1	205.2
149.	Bromuconazole isomer 2	4.89	376.0	158.9	70.1
150.	Flubendiamide	4.89	683.0	408.0	274.0
151.	Carfentrazone ethyl	4.90	412.0	346.0	266.0
152.	Diclobutrazol	4.91	328.0	70.0	59.1
153.	Kresoxim-methyl	4.92	314.1	206.0	116.0
154.	Tebuconazole	4.98	308.0	70.1	125.0
155.	Penconazole	5.00	284.0	70.1	159.0
156.	Spinosyn A	5.04	732.6	142.0	98.1
157.	Prothioconazole	5.05	344.0	326.0	189.0
158.	Alanycarb	5.06	400.0	238.2	254.1
159.	Zoxamide	5.08	336.0	187.1	159.0
160.	Famoxadone	5.10	392.2	331.1	238.0
161.	Prochloraz	5.15	376.0	308.0	70.1
162.	Triflumuron	5.15	359.0	156.1	139.1
163.	Benalaxyl	5.16	326.1	148.0	91.0
164.	Hexaconazole	5.16	314.0	70.1	159.0
165.	Hydramethylnon	5.17	495.1	323.2	151.1
166.	Metconazole	5.19	320.1	70.0	125.0
167.	Propiconazole isomer 1 & 2	5.19	342.0	159.0	69.0
168.	Clofentezine	5.22	303.0	138.0	102.0
169.	Pyraclostrobin	5.23	388.1	163.0	193.9
170.	Bitertanol	5.27	338.1	269.2	70.1
171.	Benzoximate	5.29	364.0	199.1	105.0
172.	Spinosyn D	5.31	746.5	142.0	98.1
173.	Thiobencarb	5.31	257.9	125.1	100.1
174.	Diniconazole	5.35	326.1	70.2	159.0
175.	Pencycuron	5.36	329.1	125.0	218.0
176.	Spinetoram	5.38	748.5	142.2	98.1
177.	Hexaflumuron	5.46	461.0	158.0	141.0
178.	Indoxacarb	5.46	528.0	203.0	218.0
179.	Ipconazole isomer 1	5.46	334.2	70.0	125.1
180.	Triflumizole	5.49	346.0	277.9	60.0
181.	Difenoconazole isomer 1 & 2	5.50	406.0	251.1	111.1
182.	Trifloxystrobin	5.50	409.0	186.0	145.0
183.	Novaluron	5.53	493.0	158.0	141.0
184.	Ipconazole isomer 2	5.56	334.2	70.0	125.1
185.	Emamectin benzoate B1b	5.57	872.4	158.2	126.1
186.	Clethodim isomer 2	5.65	360.0	164.0	268.1
187.	Buprofezin	5.70	306.1	201.0	57.4
188.	Teflubenzuron	5.74	380.9	158.0	140.9
189.	Emamectin benzoate B1a	5.75	886.5	158.1	126.1
190.	Benfuracarb	5.76	411.1	195.0	190.0
191.	Fluazinam	5.78	464.8	373.0	338.1
192.	Metaflumizone	5.79	507.0	287.2	267.1
193.	Furathiocarb	5.82	383.2	194.9	252.0
194.	Lufenuron	5.83	511.2	158.0	141.0
195.	Temephos	5.83	467.1	125.0	418.9
196.	Tebufenpyrad	5.86	334.0	117.0	145.0
197.	Pyriproxifen	5.91	322.1	96.0	227.1
198.	Piperonyl butoxide	5.93	356.3	176.9	119.0
199.	Hexythiazox	6.01	353.0	228.1	168.1
200.	Quinoxyfen	6.04	308.0	197.0	161.9
201.	Flufenoxuron	6.05	489.1	158.0	141.0
202.	Amitraz	6.14	294.0	163.0	122.0
203.	Propargite	6.14	368.2	175.0	231.1
204.	Etoxazole	6.16	360.2	304.2	177.2
205.	Spiromesifen	6.20	371.1	273.1	255.1
206.	Chlorfluazuron	6.21	539.8	382.9	158.0
207.	Spirodiclofen	6.33	411.1	313.0	71.2
208.	Fenpyroximate	6.36	422.2	366.1	138.1
209.	Abamectin B1b	6.48	876.6	553.4	291.0
210.	Pyridaben	6.51	365.1	147.1	309.1
211.	Eprinomectin	6.53	914.6	186.0	154.0
212.	Abamectin B1a	6.61	890.5	305.2	567.3
213.	Fenazaquin	6.69	307.2	161.0	57.2
214.	Doramectin	6.82	916.6	331.2	593.4
215.	Moxidectin	6.82	640.5	498.3	528.4
216.	Ivermectin	7.01	892.6	569.4	551.4

TECH TIP

Using syringe filters is an economical way to remove particulate matter that could clog your column. Visit **www.restek.com/filters** to access our solvent/syringe filter compatibility guide and quickly find the best filter for your method.

Figure 6: Rxi®-5ms GC columns reliably separate many commonly used pesticides.



	Peaks	t_R (min)					
1.	Tefluthrin	14.23	12.	lambda-Cyhalothrin	22.30	23.	Cypermethrin 4*	24.43
2.	Transfluthrin	15.18	13.	Acrinathrin	22.51	24.	Flucythrinate 1*	24.43
3.	Anthraquinone	16.02	14.	*cis*-Permethrin	23.14	25.	Flucythrinate 2*	24.66
4.	Bioallethrin	17.17	15.	*trans*-Permethrin	23.29	26.	Fenvalerate 1*	25.25
5.	Resmethrin 1*	20.43	16.	Cyfluthrin 1*	23.83	27.	tau-Fluvalinate 1*	25.47
6.	Resmethrin 2*	20.55	17.	Cyfluthrin 2*	23.93	28.	Fenvalerate 2*	25.48
7.	Tetramethrin 1*	21.00	18.	Cyfluthrin 3*	24.02	29.	tau-Fluvalinate 2*	25.53
8.	Tetramethrin 2*	21.14	19.	Cyfluthrin 4*	24.06	30.	Deltamethrin	26.09
9.	Bifenthrin	21.15	20.	Cypermethrin 1*	24.19	*Isomers numbered according to elution order.		
10.	Phenothrin 1*	21.59	21.	Cypermethrin 2*	24.30			
11.	Phenothrin 2*	21.71	22.	Cypermethrin 3*	24.39			

Column: Rxi®-5ms, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13423); **Sample:** GC multiresidue pesticide standard #6-SPP (cat.# 32568); Diluent: Toluene; Conc.: 100 µg/mL; **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); Liner: Sky® 4.0 mm ID Precision® inlet liner w/wool (cat.# 23305.1); Inj. Temp.: 250 °C; **Oven:** 90 °C (hold 1 min) to 330 °C at 8.5 °C/min (hold 5 min); **Carrier Gas:** He, constant flow; Flow Rate: 1.4 mL/min; **Detector:** MS; Mode: Scan; Start Time: 5 min; Scan Range: 55-550 amu; Scan Rate: 7 scans/sec; Transfer Line Temp.: 290 °C; Analyzer Type: Quadrupole; Source Temp.: 325 °C; Electron Energy: 70 eV; Solvent Delay Time: 5 min; Ionization Mode: EI; **Instrument:** Thermo Scientific TSQ 8000 Triple Quadrupole GC-MS; **Notes:** Bioallethrin isomers are only slightly resolved with this method, so they are treated as one peak. Chromatogram is reconstructed from select ions.

TECH TIP

Struggling with matrix interferences or high back pressures? Contact Restek's Technical Service team at **support@restek.com** for guard column recommendations.

PESTICIDE ANALYSIS PRODUCTS

Raptor™ ARC-18 LC Columns (USP L1)

Properties:

- Well-balanced retention profile.
- Sterically protected and acid-resistant to resist harsh, low-pH mobile phases.
- Ideal for use with sensitive detectors like mass spec.

Description	cat.#
2.7 µm Columns 100 mm, 2.1 mm ID	9314A12

For guard cartridges, visit our website at **www.restek.com**

26237

Q-sep® QuEChERS Extraction Salts

Fast, Simple Sample Prep for Multiresidue Pesticide Analysis

- Salt packets eliminate the need for a second empty tube to transfer salts.
- Go green by using packets with reusable tubes.
- Convenient and easy to use.

Description	Material	Methods	qty.	cat.#
Q-sep Kit	6 g $MgSO_4$, 1.5 g NaOAc with 50 mL Centrifuge Tube	AOAC 2007.01	50 packets & 50 tubes	26237

NaOAc—sodium acetate

For LC Analysis

Q-sep® QuEChERS dSPE Tubes for Extract Cleanup

Fast, Simple Sample Prep for Multiresidue Pesticide Analysis

Packaged in foil subpacks of 10 for enhanced protection and storage stability.

Multiple sorbents are used to extract different types of interferences.

- $MgSO_4$ removes excess water
- PSA removes sugars, fatty acids, organic acids, and anthocyanine pigments
- C18 removes nonpolar interferences

Description	Methods	qty.	cat.#
2 mL Micro-Centrifuge Tubes for dSPE (cleanup of 1 mL extract)			
150 mg $MgSO_4$, 50 mg PSA, 50 mg C18	AOAC 2007.01	100-pk.	26125

PSA—primary and secondary amine

Rxi®-5ms Columns (fused silica)

(low-polarity phase; Crossbond® diphenyl dimethyl polysiloxane)

- General-purpose columns for semivolatiles, phenols, amines, residual solvents, drugs of abuse, pesticides, PCB congeners (e.g., Aroclor mixes), solvent impurities.
- Most inert column on the market.
- Tested and guaranteed for ultra-low bleed; improved signal-to-noise ratio for better sensitivity and mass spectral integrity.
- Equivalent to USP G27 and G36 phases.

Description	temp. limits	qty.	cat.#
30 m, 0.25 mm ID, 0.25 µm	-60 to 330/350 °C	ea.	13423

QuEChERS Performance Standards Kit

- Kit contains organochlorine, organonitrogen, organophosphorus, and carbamate pesticides commonly used on fruits and vegetables.
- Ideal for initial method evaluations and ongoing method performance validations.
- Analytes are divided into three ampuls based on compatibility for maximum stability and shelf life.*
- Precise formulations improve data quality and operational efficiency; spend more time running samples and less time sourcing and preparing standards.

Contains 1 mL each of these mixtures.
31153: QuEChERS Performance Standard A
31154: QuEChERS Performance Standard B
31155: QuEChERS Performance Standard C

300 µg/mL each in acetonitrile/acetic acid (99.9:0.1), 1 mL/ampul.
Blend equal volumes of all three ampuls for a 100 µg/mL final solution.

cat.# 31152 (kit)

*When combining compounds with different functionalities, chemical stability can be an issue. The analytes in this kit are separated into three mixes to ensure maximum long-term storage stability. For analysis, a fresh working standard should be prepared by combining the three kit mixes in a 1:1:1 ratio to prepare a 100 µg/mL working standard solution. Once blended, Restek does not recommend storing working standards or subsequent dilutions for future use.

For GC Analysis

Pesticide Residue Cleanup SPE Cartridges

- Convenient, multiple adsorbent beds in a single cartridge.
- For use in multiresidue pesticide analysis to remove matrix interferences.
- Excellent for cleanup of dietary supplement extracts.

SPE Cartridge	qty.	cat.#
6 mL Combo SPE Cartridge Packed with 500 mg CarboPrep 90/500 mg PSA, Polyethylene Frits	30-pk.	26194

PSA–primary and secondary amine

26125

26194

PESTICIDE ANALYSIS PRODUCTS (CONT.)

LC Multiresidue Pesticide Kit

- Accurately detect and quantify pesticides of global food safety concern in a wide range of fruits, vegetables, and other commodities by LC-MS/MS.
- Full kit contains 204 compounds of interest, covering many LC-determined pesticides listed by government agencies; individual ampuls also sold separately.
- Formulated and grouped for maximum long-term stability* and well-balanced chromatographic performance, even for early eluting compounds.
- Quantitatively tested to confirm composition; detailed support documentation provided.
- Optimized multiresidue pesticide method is offered free of charge; downloadable XLS file includes conditions and transition tables.
- Certified reference material (CRM) manufactured and QC-tested in Restek's ISO-accredited labs satisfies your ISO requirements.

Restek® Certified Reference Materials

Cat.# 31972: LC Multiresidue Pesticide Standard #1 (13 components)
Organophosphorus Compounds
Acephate (30560-19-1)
Carbaryl (Sevin) (63-25-2)
Dicrotophos (141-66-2)
Dimethoate (60-51-5)
Dimethomorph (110488-70-5)
Isocarbophos (24353-61-5)
Methamidophos (10265-92-6)
Mevinphos (7786-34-7)
Monocrotophos (6923-22-4)
Omethoate (1113-02-6)
Temephos (Abate) (3383-96-8)
Trichlorfon (Dylox) (52-68-6)
Vamidothion (Vamidoate) (2275-23-2)

Cat.# 31973: LC Multiresidue Pesticide Standard #2 (16 components)
Carbamate/Uron Compounds
Alanycarb (83130-01-2)
Aldicarb (116-06-3)
Aldicarb sulfone (1646-88-4)
Aldicarb sulfoxide (1646-87-3)
Benfuracarb (82560-54-1)
Butocarboxim (34681-10-2)
Butoxycarboxim (34681-23-7)
Ethiofencarb (29973-13-5)
Furathiocarb (65907-30-4)
Methabenzthiazuron (18691-97-9)
Methiocarb (2032-65-7)
Methomyl (16752-77-5)
Oxamyl (23135-22-0)
Tebuthiuron (34014-18-1)
Thidiazuron (51707-55-2)
Thiophanate-methyl (23564-05-8)

Cat.# 31974: LC Multiresidue Pesticide Standard #3 (38 components)
Carbamate/Uron Compounds
Bendiocarb (22781-23-3)
Bifenazate (149877-41-8)
Carbofuran (1563-66-2)
Chlorfluazuron (71422-67-8)
Chloroxuron (1982-47-4)
Chlortoluron (15545-48-9)
Cycluron (2163-69-1)
Diethofencarb (87130-20-9)
Diflubenzuron (35367-38-5)
Dioxacarb (6988-21-2)
Diuron (330-54-1)
Fenobucarb (BPMC) (3766-81-2)
Fenoxycarb (79127-80-3)
Fenuron (101-42-8)
Flufenoxuron (101463-69-8)
Fluometuron (2164-17-2)
Forchlorfenuron (68157-60-8)
Hexaflumuron (86479-06-3)
3-Hydroxycarbofuran (16655-82-6)
Indoxacarb (173584-44-6)
Iprovalicarb (140923-17-7)
Isoprocarb (2631-40-5)
Isoproturon (34123-59-6)
Linuron (330-55-2)
Lufenuron (103055-07-8)
Metobromuron (3060-89-7)
Monolinuron (1746-81-2)
Neburon (555-37-3)
Novaluron (116714-46-6)
Pirimicarb (23103-98-2)
Promecarb (2631-37-0)
Propham (122-42-9)
Propoxur (Baygon) (114-26-1)
Pyraclostrobin (175013-18-0)
Siduron (1982-49-6)
Teflubenzuron (83121-18-0)
Thiobencarb (28249-77-6)
Triflumuron (64628-44-0)

Cat.# 31975: LC Multiresidue Pesticide Standard #4 (63 components)
Organonitrogen Compounds
Abamectin (71751-41-2)
Acetamiprid (135410-20-7)
Ametryn (834-12-8)
Amitraz (33089-61-1)
Azoxystrobin (131860-33-8)
Benalaxyl (71626-11-4)
Benzoximate (29104-30-1)
Boscalid (188425-85-6)
Butafenacil (134605-64-4)
Carbetamide (16118-49-3)
Carfentrazone-ethyl (128639-02-1)
Chlorantraniliprole (500008-45-7)
Clofentezine (74115-24-5)
Cymoxanil (57966-95-7)
Cyprodinil (121552-61-2)
Cyromazine (66215-27-8)
Dimoxystrobin (149961-52-4)
Dinotefuran (165252-70-0)
Doramectin (117704-25-3)
Eprinomectin (123997-26-2)
Famoxadon (131807-57-3)
Fenazaquin (120928-09-8)
Fenhexamid (126833-17-8)
Fenpyroximate (111812-58-9)
Flonicamid (158062-67-0)
Fluazinam** (79622-59-6)
Fludioxonil (131341-86-1)
Fluoxastrobin (361377-29-9)
Flutolanil (66332-96-5)
Furalaxyl (57646-30-7)
Halofenozide (112226-61-6)
Imazalil (35554-44-0)
Imidacloprid (138261-41-3)
Ivermectin (70288-86-7)
Kresoxim-methyl (143390-89-0)
Mandipropamid (374726-62-2)
Mepanipyrim (110235-47-7)
Mepronil (55814-41-0)
Metaflumizone (139968-49-3)
Metalaxyl (57837-19-1)
Methoxyfenozide (161050-58-4)
Moxidectin (113507-06-5)
Myclobutanil (88671-89-0)
Nitenpyram (120738-89-8)
Oxadixyl (77732-09-3)
Picoxystrobin (117428-22-5)
Piperonyl butoxide (51-03-6)
Prochloraz (67747-09-5)
Prometon (1610-18-0)
Pymetrozine (123312-89-0)
Pyracarbolid (24691-76-7)
Pyrimethanil (53112-28-0)
Pyriproxyfen (95737-68-1)
Quinoxyfen (124495-18-7)
Rotenone (83-79-4)
Secbumeton (26259-45-0)
Spiroxamine (118134-30-8)
Tebufenozide (112410-23-8)
Tebufenpyrad (119168-77-3)
Terbumeton (33693-04-8)
Triadimefon (43121-43-3)
Trifloxystrobin (141517-21-7)
Zoxamide (156052-68-5)

Cat.# 31976: LC Multiresidue Pesticide Standard #5 (30 components)
Organonitrogen Compounds
Acibenzolar-S-methyl (135158-54-2)
Bupirimate (41483-43-6)
Buprofezin (69327-76-0)
Carboxin (5234-68-4)
Clethodim (99129-21-2)
Clothianidin (210880-92-5)
Cyazofamid (120116-88-3)
Ethiprole (181587-01-9)
Ethofumesate (26225-79-6)
Fenamidone (161326-34-7)
Fipronil (120068-37-3)
Flubendimide (272451-65-7)
Flufenacet (Fluthiamide) (142459-58-3)
Hexythiazox (78587-05-0)
Mefenacet (73250-68-7)
Mesotrione (104206-82-8)
Methoprotryne (841-06-5)
Metribuzin (21087-64-9)
Prometryne (7287-19-6)
Propargite (2312-35-8)
Prothioconazole (178928-70-6)
Pyridaben (96489-71-3)
Simetryn (1014-70-6)
Sulfentrazone (122836-35-5)
Terbutryn (886-50-0)
Thiabendazole (148-79-8)
Thiacloprid (111988-49-9)
Thiamethoxam (153719-23-4)
Thiofanox (39196-18-4)
Tricyclazole (Beam) (41814-78-2)

Cat.# 31977: LC Multiresidue Pesticide Standard #6 (28 components)
Organonitrogen Compounds
Baycor (Bitertanol) (55179-31-2)
Bromuconazole (116255-48-2)
Cyproconazole (113096-99-4)
Diclobutrazol (75736-33-3)
Difenoconazole (119446-68-3)
Diniconazole (83657-24-3)
Epoxiconazole (133855-98-8)
Etaconazole (60207-93-4)
Ethirimol (23947-60-6)
Etoxazole (153233-91-1)
Fenarimol (60168-88-9)
Fenbuconazole (114369-43-6)
Fluquinconazole (136426-54-5)
Flusilazole (85509-19-9)
Flutriafol (76674-21-0)
Fuberidazole (3878-19-1)
Hexaconazole (79983-71-4)
Ipconazole (125225-28-7)
Metconazole (125116-23-6)
Nuarimol (63284-71-9)
Paclobutrazol (76738-62-0)
Penconazole (66246-88-6)
Propiconazole (Tilt) (60207-90-1)
Tebuconazole (107534-96-3)
Tetraconazole (112281-77-3)
Triadimenol (55219-65-3)
Triflumizole (68694-11-1)
Triticonazole (131983-72-7)

Cat.# 31978: LC Multiresidue Pesticide Standard #7 (7 components)
Organonitrogen Compounds
Emamectin-benzoate (155569-91-8)
Fenpropimorph (67564-91-4)
Spirodiclofen (148477-71-8)
Spinosad (168316-95-8)
Spirotetramat (203313-25-1)
Spinetoram (J&L) (187166-40-1)
Spiromesifen (283594-90-1)

Cat.# 31979: LC Multiresidue Pesticide Standard #8
Organonitrogen Compounds
Hydramethylnon (67485-29-4)

Cat.# 31980: LC Multiresidue Pesticide Standard #9 (7 components)
Carbamate/Uron Compounds
Aminocarb (2032-59-9)
Desmedipham (13684-56-5)
Formetanate HCL (23422-53-9)
Mexacarbate (Zectran) (315-18-4)
Monceren (Pencycuron) (66063-05-6)
Phenmedipham (13684-63-4)
Propamocarb free base (24579-73-5)

Cat.# 31981: LC Multiresidue Pesticide Standard #10
Carbamate/Uron Compounds
Carbendazim (10605-21-7)

Contains 1 mL each of these mixtures.
cat.# 31971 (kit)

Quantity discounts not available.

* NOTE: When combining a large number of compounds with different chemical functionalities, mix stability can be an issue. In formulating these standards, we extensively studied the 204 compounds involved, then grouped them into as few mixes as possible while still ensuring maximum long-term stability and reliability. For quantitative analysis, we recommend analyzing each mix separately to ensure accurate results for every compound.

** NOTE: In this standard, fluazinam should only be used for qualitative analysis. A single-component standard (cat.# 31982) is available for quantitative analysis.

GC Multiresidue Pesticide Kit

- Accurately identify and quantify pesticide residues by GC-MS/MS in fruits, vegetables, botanicals, and herbals like tea, ginseng, ginger, Echinacea, and dietary supplements.
- Comprehensive 203-compound kit covers food safety lists by the FDA, USDA, and other global governmental agencies; individual ampuls also sold separately.
- Formulated and grouped for maximum long-term stability* and well-balanced chromatographic performance, even for early eluting compounds.
- Quantitatively tested to confirm composition; detailed support documentation provided.
- Certified reference material (CRM) manufactured and QC-tested in Restek's ISO-accredited labs satisfies your ISO requirements.

Restek® Certified Reference Materials

Cat.# 32563: GC Multiresidue Pesticide Standard #1 (16 components)
Organophosphorus Compounds
Azinphos ethyl (2642-71-9)
Azinphos-methyl (86-50-0)
Chlorpyrifos (2921-88-2)
Chlorpyrifos methyl (5598-13-0)
Diazinon (333-41-5)
EPN (2104-64-5)
Fenitrothion (122-14-5)
Isazophos (42509-80-8)
Phosalone (2310-17-0)
Phosmet (732-11-6)
Pirimiphos ethyl (23505-41-1)
Pirimiphos methyl (29232-93-7)
Pyraclofos (77458-01-6)
Pyrazophos (13457-18-6)
Pyridaphenthion (119-12-0)
Quinalphos (13593-03-8)

Cat.# 32564: GC Multiresidue Pesticide Standard #2 (40 components)
Organochlorine Compounds
Aldrin (309-00-2)
alpha-BHC (319-84-6)
beta-BHC (319-85-7)
delta-BHC (319-86-8)
gamma-BHC (Lindane) (58-89-9)
Chlorbenside (103-17-3)
cis-Chlordane (5103-71-9)
trans-Chlordane (5103-74-2)
Chlorfenson (Ovex) (80-33-1)
Chloroneb (2675-77-6)
2,4'-DDD (53-19-0)
4,4'-DDD (72-54-8)
2,4'-DDE (3424-82-6)
4,4'-DDE (72-55-9)
2,4'-DDT (789-02-6)
4,4'-DDT (50-29-3)
4,4'-Dichlorobenzophenone (90-98-2)
Dieldrin (60-57-1)
Endosulfan I (959-98-8)
Endosulfan II (33213-65-9)
Endosulfan ether (3369-52-6)
Endosulfan sulfate (1031-07-8)
Endrin (72-20-8)
Endrin aldehyde (7421-93-4)
Endrin ketone (53494-70-5)
Ethylan (Perthane) (72-56-0)
Fenson (80-38-6)
Heptachlor (76-44-8)
Heptachlor epoxide (Isomer B) (1024-57-3)
Hexachlorobenzene (118-74-1)
Isodrin (465-73-6)
2,4'-Methoxychlor (30667-99-3)
4,4'-Methoxychlor olefin (2132-70-9)
Mirex (2385-85-5)
cis-Nonachlor (5103-73-1)
trans-Nonachlor (39765-80-5)
Pentachloroanisole (1825-21-4)
Pentachlorobenzene (608-93-5)
Pentachlorothioanisole (1825-19-0)
Tetradifon (116-29-0)

Cat.# 32565: GC Multiresidue Pesticide Standard #3 (25 components)
Organonitrogen Compounds
Benfluralin (1861-40-1)
Biphenyl (92-52-4)
Chlorothalonil (1897-45-6)
Dichlofluanid (1085-98-9)
Dichloran (99-30-9)
3,4-Dichloroaniline (95-76-1)
2,6-Dichlorobenzonitrile (Dichlobenil) (1194-65-6)
Diphenylamine (122-39-4)
Ethalfluralin (55283-68-6)
Fluchloralin (33245-39-5)
Isopropalin (33820-53-0)
Nitralin (4726-14-1)
Nitrofen (1836-75-5)
Oxyfluorfen (42874-03-3)
Pendimethalin (40487-42-1)
Pentachloroaniline (527-20-8)
Pentachlorobenzonitrile (20925-85-3)
Pentachloronitrobenzene (Quintozene) (82-68-8)
Prodiamine (29091-21-2)
Profluralin (26399-36-0)
2,3,5,6-Tetrachloroaniline (3481-20-7)
Tetrachloronitrobenzene (Tecnazene) (117-18-0)
THPI (Tetrahydrophthalimide) (1469-48-3)
Tolylfluanid (731-27-1)
Trifluralin (1582-09-8)

Cat.# 32566: GC Multiresidue Pesticide Standard #4 (28 components)
Organonitrogen Compounds
Acetochlor (34256-82-1)
Alachlor (15972-60-8)
Allidochlor (93-71-0)
Clomazone (Command) (81777-89-1)
Cycloate (1134-23-2)
Diallate (*cis* and *trans*) (2303-16-4)
Dimethachlor (50563-36-5)
Diphenamid (957-51-7)
Fenpropathrin (39515-41-8)
Fluquinconazole (136426-54-5)
Flutolanil (66332-96-5)
Linuron (330-55-2)
Metazachlor (67129-08-2)
Methoxychlor (72-43-5)
Metolachlor (51218-45-2)
N-(2,4-Dimethylphenyl) formamide (60397-77-5)
Norflurazon (27314-13-2)
Oxadiazon (19666-30-9)
Pebulate (1114-71-2)
Pretilachlor (51218-49-6)
Prochloraz (67747-09-5)
Propachlor (1918-16-7)
Propanil (709-98-8)
Propisochlor (86763-47-5)
Propyzamide (23950-58-5)
Pyridaben (96489-71-3)
Tebufenpyrad (119168-77-3)
Triallate (2303-17-5)

Cat.# 32567: GC Multiresidue Pesticide Standard #5 (34 components)
Organonitrogen Compounds
Atrazine (1912-24-9)
Bupirimate (41483-43-6)
Captafol (2425-06-1)
Captan (133-06-2)
Chlorfenapyr (122453-73-0)
Cyprodinil (121552-61-2)
Etofenprox (80844-07-1)
Etridiazole (2593-15-9)
Fenarimol (60168-88-9)
Fipronil (120068-37-3)
Fludioxonil (131341-86-1)
Fluridone (Sonar) (59756-60-4)
Flusilazole (85509-19-9)
Flutriafol (76674-21-0)
Folpet (133-07-3)
Hexazinone (Velpar) (51235-04-2)
Iprodione (36734-19-7)
Lenacil (2164-08-1)
MGK-264 (113-48-4)
Myclobutanil (88671-89-0)
Paclobutrazol (76738-62-0)
Penconazole (66246-88-6)
Procymidone (32809-16-8)
Propargite (2312-35-8)
Pyrimethanil (53112-28-0)
Pyriproxyfen (95737-68-1)
Tebuconazole (107534-96-3)
Terbacil (5902-51-2)
Terbuthylazine (5915-41-3)
Triadimefon (43121-43-3)
Triadimenol (55219-65-3)
Tricyclazole (Beam) (41814-78-2)
Triflumizole (68694-11-1)
Vinclozolin (50471-44-8)

Cat.# 32568: GC Multiresidue Pesticide Standard #6 (18 components)
Synthetic Pyrethroid Compounds
Acrinathrin (101007-06-1)
Anthraquinone (84-65-1)
Bifenthrin (82657-04-3)
Bioallethrin (584-79-2)
Cyfluthrin (68359-37-5)
lambda-Cyhalothrin (91465-08-6)
Cypermethrin (52315-07-8)
Deltamethrin (52918-63-5)
Fenvalerate (51630-58-1)
Flucythrinate (70124-77-5)
tau-Fluvalinate (102851-06-9)
cis-Permethrin (61949-76-6)
trans-Permethrin (61949-77-7)
Phenothrin (*cis* & *trans*) (26002-80-2)
Resmethrin (10453-86-8)
Tefluthrin (79538-32-2)
Tetramethrin (7696-12-0)
Transfluthrin (118712-89-3)

Cat.# 32569: GC Multiresidue Pesticide Standard #7 (10 components)
Herbicide Methyl Esters
Acequinocyl (57960-19-7)
Bromopropylate (18181-80-1)
Carfentrazone ethyl (128639-02-1)
Chlorobenzilate (510-15-6)
Chlorpropham (101-21-3)
Chlozolinate (84332-86-5)
DCPA methyl ester (Chlorthal-dimethyl) (1861-32-1)
Fluazifop-*p*-butyl (79241-46-6)
Metalaxyl (57837-19-1)
2-Phenylphenol (90-43-7)

Cat.# 32570: GC Multiresidue Pesticide Standard #8 (24 components)
Organophosphorus Compounds
Bromfenvinfos-methyl (13104-21-7)
Bromfenvinphos (33399-00-7)
Bromophos ethyl (4824-78-6)
Bromophos methyl (2104-96-3)
Carbophenothion (786-19-6)
Chlorfenvinphos (470-90-6)
Chlorthiophos (60238-56-4)
Coumaphos (56-72-4)
Edifenphos (17109-49-8)
Ethion (563-12-2)
Fenamiphos (22224-92-6)
Fenchlorphos (Ronnel) (299-84-3)
Fenthion (55-38-9)
Iodofenphos (18181-70-9)
Leptophos (21609-90-5)
Malathion (121-75-5)
Methacrifos (62610-77-9)
Profenofos (41198-08-7)
Prothiofos (34643-46-4)
Sulfotepp (3689-24-5)
Sulprofos (35400-43-2)
Terbufos (13071-79-9)
Tetrachlorvinfos (22248-79-9)
Tolclofos-methyl (57018-04-9)

Cat.# 32571: GC Multiresidue Pesticide Standard #9 (8 components)
Organophosphorus Compounds
Disulfoton (298-04-4)
Fonofos (944-22-9)
Methyl parathion (298-00-0)
Mevinphos (7786-34-7)
Parathion (Ethyl parathion) (56-38-2)
Phorate (298-02-2)
Piperonyl butoxide (51-03-6)
Triazophos (24017-47-8)

Contains 1 mL each of these mixtures.
cat.# 32562 (kit)

* NOTE: When combining a large number of compounds with different chemical functionalities, mix stability can be an issue. In formulating these standards, we extensively studied the 203 compounds involved, then grouped them into as few mixes as possible while still ensuring maximum long-term stability and reliability. For quantitative analysis, we recommend analyzing each mix separately to ensure accurate results for every compound.

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Sample Preparation Applications

Fast, Simple QuEChERS Extraction and Cleanup of Pesticide Residue Samples

- Achieve a four-fold increase in sample throughput.
- Significantly reduce material costs.
- Convenient, ready to use centrifuge tubes with ultra pure, pre-weighed adsorbent mixtures.

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innovative chromatography solutions

Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe, the QuEChERS ("catchers") method for extracting pesticides from food is based on research by the US Department of Agriculture.[1] In addition to using less solvent and materials versus conventional SPE methods, QuEChERS employs a novel and much quicker dispersive solid phase extraction cleanup (dSPE). QuEChERS methods, including an AOAC Official Method[2] and modifications to the methods, have been posted on the internet.[3] These methods have several basic steps in common:

Step 1: Sample preparation and extraction – Commodities are uniformly homogenized. Acetonitrile solvent is added for a shake extraction. Salts, acids, and buffers may be added to enhance extraction efficiency and protect sensitive analytes. Surrogate standards can be added to monitor extraction efficiencies.

Step 2: Extract cleanup – A subsample of solvent extract is cleaned up using dSPE, a key improvement incorporated in the QuEChERS technique. Small polypropylene centrifuge tubes are prefilled with precise weights of $MgSO_4$ and SPE adsorbents to remove excess water and unwanted contaminants from the extracted samples. After agitation and centrifugation, the cleaned extracts are ready for analysis by a variety of techniques.[4]

Step 3: Sample analysis – Samples may be pH adjusted to protect sensitive pesticides and/or solvent-exchanged to improve analysis by either GC/MS or LC/MS. Internal standards can be added.

QuEChERS methods are convenient, rugged methods that simplify extract cleanup, reduce material costs, and improve sample throughput. Here we demonstrate the effectiveness of QuEChERS sample cleanup using a multiresidue analysis of pesticides on strawberries.

Experimental

Strawberry extracts were prepared, spiked, and dSPE treated according to Table I. One microliter splitless injections of the extracts were performed by a Shimadzu AOC-20i autosampler using "mid" injection speed into a Shimadzu QP-2010 Plus GC-MS system operated under the conditions in Table II.

Results and Discussion

Primary and secondary amine exchange material (PSA) is the base sorbent used for dSPE cleanup of QuEChERS fruit and vegetable extracts because it removes many organic acids and sugars that might act as instrumental interferences.

A pesticide-spiked strawberry extract (200ng/mL) subjected to dSPE with PSA was used to generate one-point calibration curves. Spiked strawberry extracts subjected to additional dSPE sorbents were analyzed and the results versus PSA dSPE are shown as percent recoveries in Table III. C18 is suggested for use when samples might contain fats; not an issue for a strawberry extract, but it was important to verify that gross losses of more hydrophobic pesticides (e.g., endrin and DDT) would not occur. Graphitized carbon black (GCB) is used to remove pigments, and when treated, the pink/red strawberry extract became clear. However, GCB can also have a negative effect on certain pesticides, especially those that can assume a planar shape like chlorothalonil and thiabendazole.

Restek dSPE products, available in a variety of standard sizes, make QuEChERS even simpler. The centrifuge tube format contains magnesium sulfate (to partition water from organic solvent) and a choice of SPE sorbents, including PSA (to remove sugars and fatty acids), C18 (to remove nonpolar interferences such as fats), and GCB (to remove pigments and sterols). Custom products also are available by request. If you are frustrated by the time and cost involved with your current approach to pesticide sample cleanup, we suggest you try this simple and economical new method.

References

1. Michelangelo Anastassiades, Steven J. Lehotay, Darinka Štajnbaher, Frank J. Schenck. "Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and Dispersive Solid-Phase Extraction for the Determination of Pesticide Residues in Produce." *J. AOAC International*, 2003, vol. 86(22), pp.412-431.
2. AOAC Official Method 2007.01, "Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate."
3. http://www.quechers.com/
4. Schenck, F.J., *SPE Cleanup and Analysis of PPB Levels of Pesticides in Fruits and Vegetables*. Florida Pesticide Residue Workshop, 2002.

References not available from Restek

Table I QuEChERS extraction and cleanup procedure for pesticides from strawberries.

Sample preparation and extraction	
Sample:	10g of strawberries were homogenized and placed in a 50mL PTFE centrifuge tube (cat.# 26227)
Solvent:	10mL of acetonitrile were added to homogenate Shake for 1 minute, until uniform
Salts:	4.0g $MgSO_4$ (powder or granular) 1.0g NaCl 1.0g trisodium citrate dihydrate 0.5g disodium hydrogencitrate sesquihydrate (cat.# 26213) Salts were added and vigorously shaken for 1 minute. Sample was centrifuged and the supernatant removed for cleanup. Pesticides standards (200ng/mL) were spiked in at this point.
Sample extract cleanup	
QuEChERS tubes:	1mL of supernatant from the previous step was placed into several 2mL polypropylene centrifuge tubes, each containing one of the following adsorbent mixes: A. 50mg PSA + 150mg $MgSO_4$ (cat.# 26124) B. 50mg PSA + 150mg $MgSO_4$ + 50mg C18 (cat.# 26125) C. 50mg PSA + 150mg $MgSO_4$ + 50mg GCB (cat.# 26123)
Cleanup:	Samples were shaken with the adsorbents for 30 seconds (carbon for 2 minutes), then centrifuged to produce a clear supernatant for GC/MS analysis.
Internal standard:	Pentachloronitrobenzene in a formic acid solution, pH 5.

PSA—primary and secondary amine exchange material.
GCB—graphitized carbon black

Table II Instrument conditions.

Column:	Rtx®-CLPesticides2, 20m, 0.18mm ID, 0.14µm (cat.# 42302)
Sample:	custom pesticide mix, 200µg/mL each pesticide, internal standards: 8140-8141 ISTD, 1000µg/mL (cat.# 32279), 508.1 ISTD 100µg/mL (cat.# 32091), triphenylphosphate 1000µg/mL (cat.# 32281)
Inj.:	1.0µL splitless (hold 1 min.)
Inj. temp.:	250°C
Carrier gas:	helium
Flow rate:	constant linear velocity @ 40cm/sec
Oven temp.:	40°C (hold 1 min.) to 320°C @ 12°C/min.
Det:	Shimadzu GCMS-QP2010 Plus
Transfer line temp.:	300°C
Ionization:	Electron ionization
Mode:	Selected ion monitoring

did you **know**?

Multiple sorbents are used to extract different types of interferences.

$MgSO_4$	removes excess water
PSA*	removes sugars, fatty acids, organic acids, and anthocyanine pigments
C18	removes nonpolar interferences
GCB**	removes pigments, sterols, and nonpolar interferences

*PSA—primary and secondary amine exchange material.
**GCB—graphitized carbon black

Table III Pesticide percent recoveries in strawberry extracts treated with C18 or GCB dSPE, relative to PSA only.

Rt (min.)	pesticide	CAS Number	action/use	classification	C18*	GCB**
9.50	dichlorvos	62-73-7	insecticide	organophosphorus	111	116
9.67	methamidophos	10265-92-6	insecticide	organophosphorus	105	107
11.75	mevinphos	7786-34-7	insecticide	organophosphorus	112	130
12.02	*o*-phenylphenol	90-43-7	fungicide	unclassified	106	97
12.14	acephate	30560-19-1	insecticide	organophosphorus	128	147
13.89	omethoate	1113-02-6	insecticide	organophosphorus	120	119
14.74	diazinon	333-41-5	insecticide	organophosphorus	108	127
14.98	dimethoate	60-51-5	insecticide	organophosphorus	124	151
15.69	chlorothalonil	1897-45-6	fungicide	organochlorine	125	13
15.86	vinclozolin	50471-44-8	fungicide	organochlorine	102	98
16.21	metalaxyl	57837-19-1	fungicide	organonitrogen	105	117
16.28	carbaryl	63-25-2	insecticide	carbamate	114	111
16.60	malathion	121-75-5	insecticide	organophosphorus	124	160
16.67	dichlofluanid	1085-98-9	fungicide	organohalogen	122	103
17.51	thiabendazole	148-79-8	fungicide	organonitrogen	88	14
17.70	captan	133-06-2	fungicide	organochlorine	88	91
17.76	folpet	133-07-3	fungicide	organochlorine	108	63
18.23	imazalil	35554-44-0	fungicide	organonitrogen	115	95
18.39	endrin	72-20-8	insecticide	organochlorine	104	101
18.62	myclobutanil	88671-89-0	fungicide	organonitrogen	119	114
19.07	4,4-DDT	50-29-3	insecticide	organochlorine	102	95
19.22	fenhexamid	126833-17-8	fungicide	organochlorine	118	77
19.40	propargite 1	2312-35-8	acaricide	organosulfur	110	95
19.43	propargite 2	2312-35-8	acaricide	organosulfur	121	114
19.75	bifenthrin	82657-04-3	insecticide	pyrethroid	106	81
20.04	dicofol	115-32-2	acaricide	organochlorine	98	54
20.05	iprodione	36734-19-7	fungicide	organonitrogen	118	90
20.21	fenpropathrin	39515-41-8	insecticide	pyrethroid	113	96
21.32	*cis*-permethrin	52645-53-1	insecticide	pyrethroid	106	65
21.47	*trans*-permethrin	51877-74-8	insecticide	pyrethroid	109	71
23.74	deltamethrin	52918-63-5	insecticide	pyrethroid	97	52

*50mg PSA, 50mg C18, **50mg PSA, 50mg GCB

$$\% \text{ recovery} = \frac{\text{RRF C18 or GCB}}{\text{RRF PSA}} \times 100$$

Rtx®-CLPesticides2 (proprietary Crossbond® phase)

- Application-specific columns for organochlorine pesticides and herbicides.
- Low bleed—ideal for GC/ECD or GC/MS analyses.
- Baseline separations in less than 10 minutes.

ID	df (µm)	temp. limits	length	cat. #
0.18mm	0.14	-60 to 310/330°C	20-meter	42302

Q-sep™ 3000 Centrifuge for QuEChERS

- Meets requirements of AOAC and European QuEChERS methodology.
- Supports 50mL, 15mL, and 2mL centrifuge tubes.
- Small footprint requires less bench space.
- Safe and reliable—UL, CSA, and CE approved, 1-year warranty.

Description	qty.	cat.#
Q-sep 3000 Centrifuge, 110V	ea.	26230
Q-sep 3000 Centrifuge, 220V	ea.	26231
Replacement Accessories		
50mL Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26232
50mL Conical Tube Insert for Q-sep 3000 Centrifuge	6-pk.	26249
4-Place Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26233
2mL Tube Adaptors for Q-sep 3000 Centrifuge	4-pk.	26234

QuEChERS SPE Tubes

Quick, Easy, Cheap, Effective, Rugged, and Safe, the QuEChERS ("catchers") method is a fast, simple, and effective alternative to conventional sample prep for multiresidue pesticide analysis. Restek Q-sep™ products make QuEChERS even simpler. All extraction salts, adsorbents, and sample tubes are included—no specialized equipment or glassware is required.

Q-sep™ innovative chromatography solutions


cat. # 26123


cat. # 26124


cat. # 26227 cat. # 26214


cat. # 26125


cat. # 26126

Free Sample Packs Available!

To receive your free sample pack, add -248 to the item number. (One sample per customer.)

Description	Material	Methods	qty.	cat#
50mL Centrifuge Tubes for Sample Extraction				
Q110	4g $MgSO_4$, 1g NaCl, 1g trisodium citrate dihydrate, .5g disodium hydrogencitrate sesquihydrate	European EN 15662	50-pk.	26213
Q150	6g $MgSO_4$, 1.5g NaOAc	AOAC 2007.1	50-pk.	26214
Empty 50mL Centrifuge Tube	—	European EN 15662, AOAC 2007.1	25-pk.	26227
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
Q212	150mg $MgSO_4$, 25mg PSA, 2.5mg GCB	European EN 15662	100-pk.	26217
Q213	150mg $MgSO_4$, 25mg PSA, 7.5mg GCB	European EN 15662	100-pk.	26218
Q250	150mg $MgSO_4$, 50mg PSA	AOAC 2007.1	100-pk.	26124
Q251	150mg $MgSO_4$, 50mg PSA, 50mg C18	AOAC 2007.1	100-pk.	26125
Q253	150mg $MgSO_4$, 50mg PSA, 50mg GCB	—	100-pk.	26123
Q252	150mg $MgSO_4$, 50mg PSA, 50mg C18, 50mg GCB	AOAC 2007.1	100-pk.	26219
15mL Centrifuge Tubes for dSPE (clean-up of 6mL extract)				
Q350	1200mg $MgSO_4$, 400mg PSA	AOAC 2007.1	50-pk.	26220
Q351	1200mg $MgSO_4$, 400mg PSA, 400mg C18	AOAC 2007.1	50-pk.	26221
Q352	1200mg $MgSO_4$, 400mg PSA, 400mg C18, 400mg GCB	AOAC 2007.1	50-pk.	26222
Q370	900mg $MgSO_4$, 150mg PSA	European EN 15662	50-pk.	26223
Q371	900mg $MgSO_4$, 150mg PSA, 15mg GCB	European EN 15662	50-pk.	26224
Q372	900mg $MgSO_4$, 150mg PSA, 45mg GCB	European EN 15662	50-pk.	26225
Q373	900mg $MgSO_4$, 150mg PSA, 150mg C18	—	50-pk.	26226
Q374	900mg $MgSO_4$, 300mg PSA, 150mg GCB	—	50-pk.	26126

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	Mini-Luke or Modified Luke Method	QuEChERS	Savings with QuEChERS
Estimated time to process 6 samples (min.)	120	30	4x faster
Solvent used (mL)	60-90	10	6-9x less solvent
Chlorinated waste (mL)	20-30	0	Safer, cheaper, greener
Glassware/specialized equipment	capacity for 200mL, quartz wool, funnel, water bath or evaporator	none	Ready-to-use

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Fast, Simple Sample Cleanup

Using QuEChERS SPE Tubes

By Julie Kowalski, Innovations Chemist, Lydia Nolan, Innovations Chemist, Jack Cochran, Director of New Business and Technology, and Irene DeGraff, Product Marketing Manager.

- Achieve a four-fold increase in sample throughput.
- Significantly reduce material costs.
- Convenient, ready to use centrifuge tubes with ultra pure, pre-weighed adsorbent mixtures.

Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe, the QuEChERS ("catchers") method for extracting pesticides from food is based on research by the US Department of Agriculture.[1] In addition to using less solvent and materials versus conventional SPE methods, QuEChERS employs a novel and much quicker dispersive solid phase extraction cleanup (dSPE). QuEChERS methods, including an AOAC Official Method[2] and modifications to the methods, have been posted on the Internet.[3] These methods have several basic steps in common:

Step 1: Sample preparation and extraction – Commodities are uniformly comminuted. Acetonitrile solvent is added for a shake extraction. Salts, acids, and buffers may be added to enhance extraction efficiency and protect sensitive analytes. Surrogate standards can be added to monitor extraction efficiencies.

Step 2: Extract cleanup – A subsample of solvent extract is cleaned up using dSPE, a key improvement incorporated in the QuEChERS technique. Small polypropylene centrifuge tubes are prefilled with precise weights of $MgSO_4$ and SPE adsorbents to remove excess water and unwanted contaminants from the extracted samples. After agitation and centrifugation, the cleaned extracts are ready for analysis.

Step 3: Sample analysis – Samples may be pH adjusted to protect sensitive pesticides and/or solvent-exchanged to improve analysis by either GC/MS or LC/MS. Internal standards can be added.

QuEChERS methods are convenient, rugged methods that simplify extract cleanup, reduce material costs, and improve sample throughput. Here we demonstrate the effectiveness of QuEChERS sample cleanup using a multiresidue analysis of pesticides on strawberries.

Experimental

Strawberry extracts were prepared, spiked, and dSPE treated according to Table I. Analytical conditions are presented in Table II.

One microliter splitless injections of the extracts were performed by a Shimadzu AOC-20i autosampler using "mid" injection speed into a Shimadzu QP-2010 Plus GC/MS system operated under the conditions in Table II.

Table I Modified mini-multiresidue QuEChERS for pesticides from strawberries.

Sample preparation and extraction

Sample:	10g of strawberries were homogenized and placed in a 50mL PTFE centrifuge tube
Solvent:	10mL of acetonitrile were added to homogenate Shake for 1 minute, until uniform
Salts:	4.0g $MgSO_4$ (powder or granular) 1.0g NaCl 1.0g trisodium citrate dihydrate 0.5g disodium hydrogencitrate sesquihydrate Salts were added and vigorously shaken for 1 minute. Sample was centrifuged and the supernatant removed for cleanup. Pesticides standards (200ng/mL) were spiked in at this point

Sample extract cleanup

QuEChERS tubes:	1mL of supernatant from the previous step was placed into several 2mL polypropylene centrifuge tubes, each containing one of the following adsorbent mixes: A. 50mg PSA + 150mg $MgSO_4$ (cat.# 26124) B. 50mg PSA + 150mg $MgSO_4$ + 50mg C18 (cat.# 26125) C. 50mg PSA + 150mg $MgSO_4$ + 50mg GCB (cat.# 26123)
Cleanup:	Samples were shaken with the adsorbents for 30 seconds (carbon for 2 minutes), then centrifuged to produce a clear supernatant for GC/MS analysis.
Internal standard:	Pentachloronitrobenzene in a formic acid solution, pH 5.

PSA = primary-secondary amine, GCB = graphitized carbon black

One microliter splitless injections of the extracts were performed by a Shimadzu AOC-20i autosampler using "mid" injection speed into a Shimadzu QP-2010 Plus GC/MS system operated under the conditions below.

Table II Instrument conditions

Column:	Rtx®-CLPesticides2 20m, 0.18mm ID, 0.14µm (cat.# 42302)
Sample:	custom pesticide mix 200µg/mL each pesticide, internal standards: 8140-8141 ISTD, 1000µg/mL (cat.# 32279), 508.1 ISTD 100µg/mL (cat.# 32091), triphenylphosphate 1000µg/mL (cat.# 32281)
Inj.:	1.0µL splitless (hold 1 min.)
Inj. temp.:	250°C
Carrier gas:	helium
Flow rate:	constant linear velocity @ 40cm/sec
Oven temp.:	40°C (hold 1 min.) to 320°C @ 12°C/min.
Det:	Shimadzu GCMS-QP2010 Plus
Transfer line temp.:	300°C
Ionization:	Electron ionization
Mode:	Selected ion monitoring

PSA = primary-secondary amine, GCB = graphitized carbon black

Results and Discussion

Primary and secondary amine exchange material (PSA) is the base sorbent used for dSPE cleanup of QuEChERS fruit and vegetable extracts because it removes many organic acids and sugars that might act as instrumental interferences. A pesticide-spiked strawberry extract (200ng/mL) subjected to dSPE with PSA was used to generate one-point calibration curves. Spiked strawberry extracts subjected to additional dSPE sorbents were analyzed and the results versus PSA dSPE are shown as percent recoveries in Table III. C18 is suggested for use when samples might contain fats, not an issue for a strawberry extract, but it was important to verify that gross losses of more hydrophobic pesticides (e.g. Endrin and DDT) would not occur. GCB is used to remove pigments, and when treated, the pink/red strawberry extract became clear. However, GCB can also have a negative effect on certain pesticides, especially those that can assume a planar shape like chlorothalonil and thiabendazole.

Restek dSPE products in a variety of standard sizes and formats make QuEChERS even simpler. The centrifuge tube format, available in 2mL and 15mL sizes, contains magnesium sulfate (to partition water from organic solvent) and a choice of SPE sorbents, including PSA (to remove sugars and fatty acids), C18 (to remove nonpolar interferences such as fats), and GCB (to remove pigments and sterols). Custom products also are available by request. If you are frustrated by the time and cost involved with your current approach to pesticide sample cleanup, we suggest you try this simple and economical new method.

Table III Pesticide percent recoveries in strawberry extracts treated with C18 or GCB dSPE, relative to PSA only.

Rt (min.)	Pesticide	CAS Number	Action/Use	Classification	C18[1]	GCB[2]
9.50	Dichlorvos	62-73-7	Insecticide	Organophosphorus	111	116
9.67	Methamidophos	10265-92-6	Insecticide	Organophosphorus	105	107
11.75	Mevinphos	7786-34-7	Insecticide	Organophosphorus	112	130
12.02	*o*-Phenylphenol	90-43-7	Fungicide	Unclassified	106	97
12.14	Acephate	30560-19-1	Insecticide	Organophosphorus	128	147
13.89	Omethoate	1113-02-6	Insecticide	Organophosphorus	120	119
14.74	Diazinon	333-41-5	Insecticide	Organophosphorus	108	127

14.98	Dimethoate	60-51-5	Insecticide	Organophosphorus	124	151
15.69	Chlorothalonil	1897-45-6	Fungicide	Organochlorine	125	13
15.86	Vinclozolin	50471-44-8	Fungicide	Organochlorine	102	98
16.21	Metalaxyl	57837-19-1	Fungicide	Organonitrogen	105	117
16.28	Carbaryl	63-25-2	Insecticide	Carbamate	114	111
16.60	Malathion	121-75-5	Insecticide	Organophosphorus	124	160
16.67	Dichlofluanid	1085-98-9	Fungicide	Organohalogen	122	103
17.51	Thiabendazole	148-79-8	Fungicide	Organonitrogen	88	14
17.70	Captan	133-06-2	Fungicide	Organochlorine	88	91
17.76	Folpet	133-07-3	Fungicide	Organochlorine	108	63
18.23	Imazalil	35554-44-0	Fungicide	Organonitrogen	115	95
18.39	Endrin	72-20-8	Insecticide	Organochlorine	104	101
18.62	Myclobutanil	88671-89-0	Fungicide	Organonitrogen	119	114
19.07	4,4-DDT	50-29-3	Insecticide	Organochlorine	102	95
19.22	Fenhexamid	126833-17-8	Fungicide	Organochlorine	118	77
19.40	Propargite 1	2312-35-8	Acaricide	Organosulfur	110	95
19.43	Propargite 2	2312-35-8	Acaricide	Organosulfur	121	114
19.75	Bifenthrin	82657-04-3	Insecticide	Pyrethroid	106	81
20.04	Dicofol	115-32-2	Acaricide	Organochlorine	98	54
20.05	Iprodione	36734-19-7	Fungicide	Organonitrogen	118	90
20.21	Fenpropathrin	39515-41-8	Insecticide	Pyrethroid	113	96
21.32	cis-Permethrin	52645-53-1	Insecticide	Pyrethroid	106	65
21.47	*trans*-Permethrin	51877-74-8	Insecticide	Pyrethroid	109	71
23.74	Deltamethrin	52918-63-5	Insecticide	Pyrethroid	97	52

1. 50mg PSA, 50mg C18, 2. 50mg PSA, 50mg GCB

% recovery = RRF C18 or GCB/RRF PSA X 100

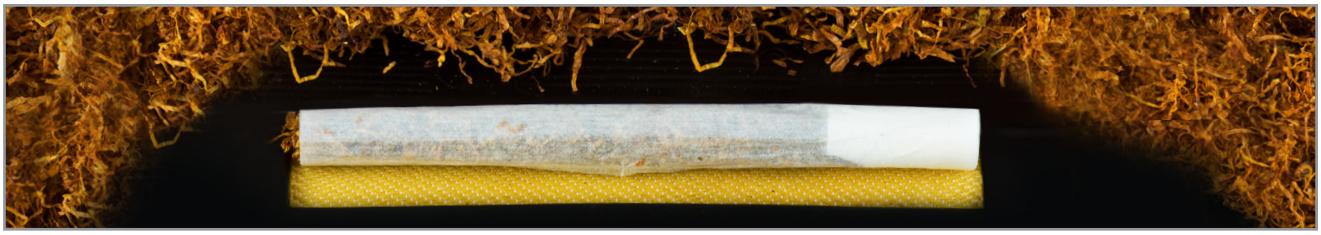
References

1. Michelangelo Anastassiades, Steven J. Lehotay, Darinka Štajnbaher, Frank J. Schenck, *Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and Dispersive Solid-Phase Extraction for the Determination of Pesticide Residues in Produce*, J AOAC International, 2003, vol. 86(22), pp.412-431.
2. AOAC Official Method 2007.01, *Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate.*
3. http://www.quechers.com/

References not available from Restek.

RELATED SEARCHES

QuEChERS, *dSPE*, *strawberries*, *multiresidue*, *pesticides*



Food Safety Applications

Evaluation of Dispersive and Cartridge Solid Phase Extraction (SPE) Cleanups for Multiresidue Pesticides in QuEChERS Extracts of Finished Tobacco Using GCxGC-TOFMS

By Michelle Misselwitz, Jack Cochran, and Julie Kowalski

Abstract

Tobacco is a high-value production crop for the United States and ranks 6th in the amount of pesticides applied per acre in American agriculture. Even after the processing of tobacco, some pesticide residues remain on the final product. We used the Quick–Easy–Cheap–Effective–Rugged–Safe (QuEChERS) sample preparation approach to isolate residues prior to analyzing pesticides in tobacco. We evaluated the cleanup efficacy and pesticide recoveries for different formulations of QuEChERS dispersive solid phase extraction (dSPE) cleanup and the more traditional cartridge solid phase extraction (cSPE) cleanup. Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS) was used to determine pesticide residues in the resulting extracts. The results of the cleanup evaluation indicated that the dSPE cleanup formulation with 7.5 mg of carbon (verses 50 mg) provided the best recovery of targeted pesticides. The average recoveries for the 500 ppb spike level and 50 ppb spike level were 92% (13% RSD) and 91% (22% RSD) respectively.

Introduction

Tobacco has a rich history in the United States and around the world. Christopher Columbus made notes in his journal about the custom of indigenous Americans smoking a "strange leaf" and within a century tobacco was in global use. Tobacco is now grown widely, with China, India, Brazil, the United States, and Turkey producing two-thirds of the world's supply [1].

Pesticides are used heavily on tobacco in order to increase crop production value. In fact, tobacco ranks 6th out of all crops in the U.S. in terms of pesticide application, falling only behind potatoes, tomatoes, citrus, grapes, and apples [2]. Although these fruits and vegetables are grown using more pesticides per acre, the final residue levels on these foods is regulated. No such controls exist for tobacco. Although the U.S. Environmental Protection Agency (EPA) regulates and approves the pesticides that can be applied to tobacco based on worker safety, environmental quality, and crop protection, it does not set allowable levels for pesticides in finished tobacco products. For some time, the U.S. Department of Agriculture (USDA) has analyzed pesticides in tobacco for non-EPA approved pesticides on both imported and domestic products, and recently it has also began including pesticides that are EPA-approved for application to the tobacco plant. In spite of this additional monitoring, the lack of set regulatory limits creates the potential for high levels of pesticide residues to remain on final tobacco products. A few countries that import U.S. tobacco products do have regulations on maximum residue levels of pesticides in either cigarettes or the tobacco leaf itself [2].

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) methodology was developed for the determination of multiresidue pesticides in fruits and vegetables [3]. This methodology uses a simple shake extraction where the pesticides are extracted and partitioned using acetonitrile and a salt/buffer solution. The resultant extract is then cleaned using a very quick dispersive solid phase extraction (dSPE) step that requires no additional solvent usage. While this method was originally developed for high water content produce, we have successfully adapted the method for dry commodities, such as dietary supplements [4]. This approach utilized a modified QuEChERS extraction and a cartridge solid phase extraction cleanup (cSPE). The cSPE cleanup provides the potential for enhanced cleanup capacity for complex matrices like dietary supplements and tobacco, but it requires additional solvent and extra time for sample elution and concentration. For the more complex dietary supplement finished products we employed comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS) as the determinative technique [5]. Analyzing tobacco using GCxGC proved to be a powerful technique in separating matrix interferences from the pesticides of interest.

Here we used the QuEChERS extraction approach and GCxGC-TOFMS and evaluated several cleanup methods for finished tobacco product. The tobacco extract can be very complex, so we explored both dSPE and cSPE cleanup approaches and monitored their performance for pesticide recovery and matrix reduction. The wide range of pesticides chosen for this study covered many, but not all, of the 37 pesticides that have been approved by the EPA for use on tobacco.

Experimental

Sample Preparation

Two types of bulk loose cigarette tobacco, a light and dark, were provided by Global Laboratory Services. A custom stock standard that included organochlorine, organonitrogen, and organophosphorus pesticides was prepared at Restek and diluted to 10 ng/µL and 1 ng/µL concentrations in acetonitrile. Recovery experiments were performed at two fortification levels, 500 ppb and 50 ppb. Fortified samples were prepared by adding 100 µL of the appropriate diluted standard (10 ng/µL or 1 ng/µL) to tobacco samples. Unfortified samples were also prepared in order to find potential incurred pesticides in tobacco and make matrix-matched standards for quantification.

Matrix-matched standards were prepared at 100 pg/µL and 10 pg/µL by adding 5 µL of a standard solution (1 ng/µL and 0.1 ng/µL) to 45 µL of the final cleaned extract of the unfortified tobacco samples for each type of cleanup.

QuEChERS Extraction

A 2 g sample of tobacco was weighed into a 50 mL polypropylene centrifuge tube (Restek, cat. # 26239). After the addition of 10 mL of organic-free water to the sample, 100 µL of QuEChERS internal standard mix for GC-MS analysis (Restek, cat. # 33267) was added to each sample. For samples that were fortified, 100 µL of the fortification standard was then added. Next, 10 mL of acetonitrile was added and the samples were vortexed for 30 min using a digital Vortex-Genie 2 (Scientific Industries, cat. # SI-A236). Immediately after vortexing, pre-packaged QuEChERS European EN 15662 method formulation extraction salts containing 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dehydrate, and 0.5 g disodium hydrogen citrate sesquihydrate (Restek, cat. # 26236) were added to each centrifuge tube. The tubes were immediately shaken for 1 min and then centrifuged in the Q-sep™ 3000 centrifuge (Restek, cat. # 26230) for 5 min at 3000 g. The top acetonitrile layer was collected and aliquots were taken for subsequent cleanup.

Extract Cleanup

Two formulations of pre-packaged dispersive solid phase extraction tubes were evaluated, the AOAC 2007.01 formulation containing 150 mg $MgSO_4$, 50 mg primary secondary amine (PSA), 50 mg graphitized carbon black (GCB), and 50 mg C18 (Restek, cat. # 26219); and the mini-multiresidue, European EN 15662 formulation containing 150 mg $MgSO_4$, 25 mg PSA and 7.5 mg GCB (Restek, cat.# 26218). For cleanup, a 1 mL aliquot of each extract was fortified with 5 µL of an anthracene standard (Restek, cat. # 33264) and added to the dSPE tubes. The tubes were gently shaken for 2 min and then centrifuged for 5 min using a Q-sep™ 3000 centrifuge (Restek, cat. # 26230). A 0.5 mL portion of the supernatant extract was removed and placed into an autosampler vial and 5 µL of a 5% formic acid solution in acetonitrile was added to each sample.

For the cartridge solid phase extraction cleanup, a 6 mL pesticide residue cleanup SPE cartridge packed with 500 mg CarboPrep® 90 material and 500 mg PSA (Restek, cat. # 26194) was used. Approximately 0.5 cm of anhydrous $MgSO_4$ was added to the top of the cartridge bed. The cartridge was rinsed with 20 mL of acetone prior to the sample being loaded. After the cartridge rinsing, 1 mL of tobacco extract was loaded onto the cartridge and eluted with 15 mL of a 3:1 acetone:toluene mixture. The eluent was collected and evaporated to 1 mL under a stream of nitrogen using a TurboVap® II concentration workstation (Biotage, cat. # 103187).

GCxGC-TOFMS Analysis

A LECO Pegasus 4D GCxGC-TOFMS equipped with an Agilent 6890 GC and 7683 autoinjector was used to determine pesticide recoveries and levels of incurred pesticides in tobacco. A 30 m x 0.25 mm x 0.25 µm Rxi®-5Sil MS column (Restek, cat. #13623) was installed in the primary oven and connected via a press-fit (BGB Analytik AG, cat. # 2525LD) to a 1.3 m x 0.25 mm x 0.25 µm Rtx®-200 column (Restek, cat. # 15124) installed in the secondary oven. The primary oven temperature conditions were 90 °C (hold 1 min) to 310 °C (hold 2 min) at 5 °C/min. The secondary oven temperature program tracked the primary oven with a +5 °C offset. The second dimension separation time was 3 sec with a +20 °C modulator temperature offset. The carrier gas was helium operated under corrected constant flow conditions at 2 mL/min. 1 µL fast autosampler splitless injections were made with a 1 min purge valve time and an inlet temperature of 250 °C. A 4 mm Sky® single taper inlet liner with wool (Restek, cat. # 23303.5) was used for all analyses. Data were acquired from 45 to 550 u with an acquisition rate of 100 spectra/sec. The transfer line was 300 °C and electron ionization at 70 eV was used with a source temperature of 225 °C.

QuEChERS Extract Cleanup Evaluation

An Agilent 6890 GC with a flame ionization detector (FID) was used to quickly evaluate the removal of the tobacco matrix for each type of cleanup. We used a 15 m x 0.25 mm x 0.25 µm Rxi®-5Sil MS column (Restek, cat. #13620) with helium carrier gas operated in constant flow at 2 mL/min. The oven temperature program was 80 °C (hold 1 min) to 350 °C (hold 5.5 min) at 20 °C/min and yielded a 20 min analysis time. A 4 mm Sky® single taper liner with wool was installed in the inlet, which was set to 250 °C. 1 µL fast splitless injections (0.75 min purge valve time) were performed with a 7683 autoinjector. The FID temperature was 350 °C and the makeup flow plus column flow was held constant at 50 mL/min. Data were collected at 5 Hz.

Gravimetric analyses of the nonvolatile residue remaining in the final extracts were performed for both the dSPE and cSPE cleanup procedures. Cleanups for the two formulations of dSPE tubes and the cSPE procedure were each performed in triplicate. The resultant replicate extracts were combined and added to tared conical vials that were placed on a 60 °C hotplate and evaporated under a stream of dry nitrogen gas (Thermo Scientific, Reacti-Therm I [cat.# TS-18821] and Reacti-Vap [cat. # TS-18825]). The vials were reweighed after all solvent was evaporated to determine the amount of nonvolatile material present after the extract cleanup.

All data were processed with the LECO ChromaTOF® software. Recoveries of pesticides in the fortified tobacco samples were quantified using the GCxGC-TOFMS data, matrix-matched standards, and the internal standard PCB 52 for each cleanup type. The matrix-matched standards represented 100% recovery and were used for single-point calibration and quantification. Evaluation of the tobacco extract prior to cleanup and following each cleanup type were performed by overlaying the FID traces to visually inspect gross differences in cleanup efficacy.

Results and Discussion

Removal of Matrix Interferences

The goal of this work was to use the quicker dSPE cleanup methodology for the extracts as long as it was effective when analyzing tobacco using GCxGC-TOFMS. From previous work with complex matrices, we have found that dSPE does not have the capacity to clean the extract enough for GC-MS analysis, including GC-TOFMS or even GCxGC-TOFMS analysis [5]. In order to quickly determine if the time- and solvent-intensive cSPE was necessary, we evaluated the extracts using GC-FID. Pigment reduction from extracts is important for GC work, since many pigments are nonvolatile and quickly degrade the performance of the GC inlet and column. With a first visual inspection of the resultant extracts, it was clear that as the amount of carbon was increased, the pigment in the extract decreased (Figure 1). When overlaying the GC-FID traces for each cleanup type, it was apparent that some matrix components that were not removed by either dSPE cleanup were significantly reduced by the cSPE cleanup (Figure 2). The most notable were the fatty acids that are eluting in the middle of the chromatogram. The PSA sorbent is a weak anion exchange material and will remove fatty acids. Therefore, the 500 mg of PSA in the cSPE cartridge provided a more effective cleanup of fatty acids in the tobacco matrix than either of the dSPE cleanups, which contained just 50 mg or 25 mg of PSA. However, not all matrix interferences were further removed by the cSPE, which limits the benefits of this technique over the much quicker dSPE cleanup.

The next step in evaluating the efficacy of the different types of cleanups in removing matrix interferences was to determine the amount of nonvolatile residue that was removed by each cleanup. While nonvolatile residue will not necessarily cause interference in the actual chromatogram, it is an important aspect to evaluate when developing a cleanup method. Large amounts of residue from injected samples will quickly collect in the inlet liner and at the front of the column, degrading method performance and requiring more frequent injection port and column maintenance. In this respect, the use of wool in the splitless liner is also important in further protecting the analytical column from the residue of the tobacco matrix. The gravimetric analysis determined that the 25 mg PSA and 7.5 mg GCB dSPE formulation provided a 50% reduction in nonvolatile material from the raw tobbacco extract. The other dSPE formulation that had 50 mg PSA, 50 mg C18, 50 mg GCB removed 70% of the nonvolatile residue. The cSPE, which contained 500 mg PSA and 500 mg carbon, also removed 70% of the matrix material. The cSPE procedure took approximately 3 hours compared to just 20 minutes for the dSPE process, so the lack of additional removal of nonvolatile matrix components and only minimal improvement in cleanup of matrix intereferences did not outweigh the extra time and solvent usage of cSPE. Only the dSPE extracts were further evaluated for recovery of pesticides in tobacco on the GCxGC-TOFMS system.

Figure 1: Tobacco extract (from left to right) with no cleanup; dSPE cleanup with 7.5 mg GCB and 25 mg PSA; dSPE cleanup with 50 mg GCB, 50 mg PSA, and 50 mg C18; and cSPE cleanup with 500 mg CarboPrep® 90 and 500 mg PSA.

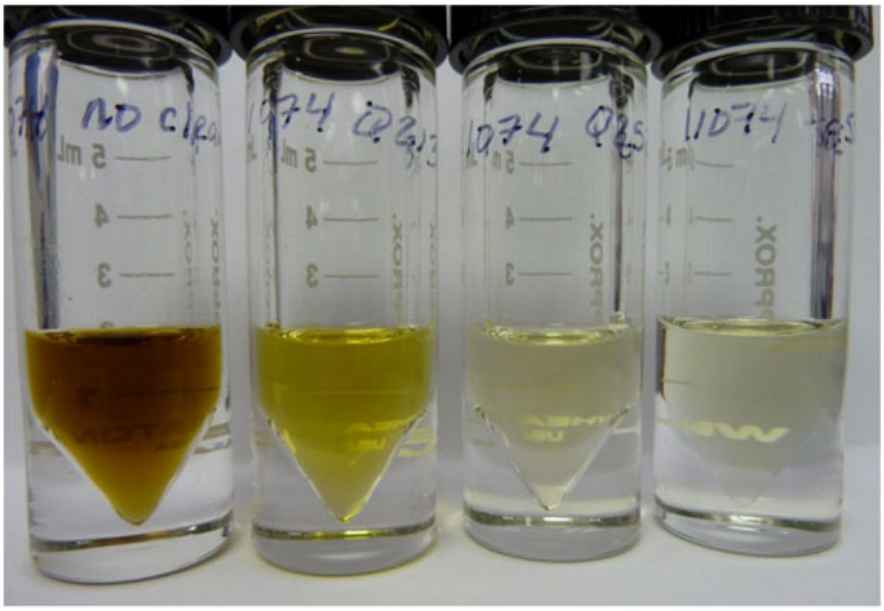
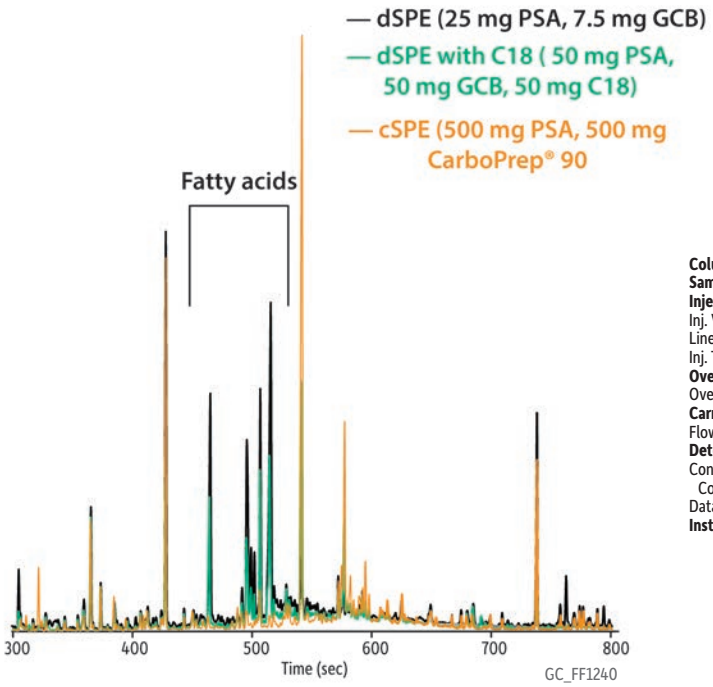


Figure 2: GC-FID overlay of cleaned extracts evaluated for the removal of semivolatile tobacco matrix. The cSPE provided a more effective cleanup for fatty acids, but did not perform better than dSPE for all potential matrix interferences.



Column	Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620)
Sample	Tobacco extracts
Injection	
Inj. Vol.:	1 µL splitless (hold 0.75 min)
Liner:	4 mm Sky® single taper w/wool (cat.# 23303.5)
Inj. Temp.:	250 °C
Oven	
Oven Temp.:	80 °C (hold 1 min) to 350 °C at 20 °C/min (hold 5.5 min)
Carrier Gas	He, constant flow
Flow Rate:	2 mL/min
Detector	FID @ 350 °C
Constant Column + Constant Make-up:	50 mL/min
Data Rate:	5 Hz
Instrument	Agilent/HP6890 GC

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Pesticide Recovery

Percent recoveries for the QuEChERS extraction and dSPE cleanups were calculated for both the high (500 ppb) and low (50 ppb) fortification levels using matrix-matched standards. The use of matrix-matched standards instead of solvent-only standards ensures more accurate quantitation. Percent recoveries calculated from a solvent-only standard can have a high bias due to matrix enhancement effects that originate primarily in the GC inlet. Many of the compounds from either the EPA approved list or the USDA monitoring list were included in our study (Table I). However, some pesticides from these lists were not included because either they are not amenable to GC or because they are functionally similar to pesticides that were evaluated, so similar results should be expected.

The dSPE formulation containing 50 mg PSA, 50 mg C18, and 50 mg GCB had good average percent recoveries of 82% and 81% for the high and low fortification levels respectively, with percent relative standard deviations (% RSDs) of 23% and 33%. The other dSPE formulation containing 25 mg PSA and 7.5 mg GCB had somewhat better average recoveries of 92% and 91% (13% and 22% RSD) for the high and low fortification levels respectively (Table I). The recovery values highlight that the QuEChERS extraction and dSPE cleanup approach that we employed for cigarette tobacco performs well for a wide range of pesticides. A closer inspection of the recovery values for individual pesticides reveals that there are several cases (e.g., pentachlorobenzene, hexachlorobenzene, pentachloroaniline, pentachlorothioanisole) where the dSPE formulation with 7.5 mg GCB clearly outperformed the formulation with 50 mg GCB. This is not surprising since we have previously reported that graphitized carbon black can reduce the recoveries of planar pesticides [6], including chlorinated fungicides such as those listed above. Unlike the cSPE cleanup, the dSPE cleanup used here does not employ any type of elution step that might help recover the planar pesticides, so the potential loss of planar pesticides increases as the amount of GCB increases.

Table I: Percent recoveries for pesticides included in the EPA approved list and USDA monitoring list for tobacco determined using QuEChERS extraction, dSPE cleanup, and GCxGC-TOFMS analysis.

		500 ppb Fortified Sample (100 pg on-column)		50 ppb Fortified Sample (10 pg on-column)	
Pesticide	Regulatory List*	50 mg PSA, 50 mg C18, 50 mg GCB	25 mg PSA, 7.5 mg GCB	50 mg PSA, 50 mg C18, 50 mg GCB	25 mg PSA, 7.5 mg GCB
Methamidophos	USDA	77	83	72	89
Dichlorvos		95	101	74	109
1,2,3,5-Tetrachlorobenzene		70	82	68	85
Mevinphos	USDA	89	101	103	112
Acephate	EPA/USDA	93	87	69	105
Pentachlorobenzene		49	75	45	76
o-Phenylphenol		94	100	91	96
Tetrachloronitrobenzene		74	93	90	93
Omethoate	USDA	97	91	76	96
2,3,5,6-Tetrachloroaniline		75	92	64	92
alpha-BHC		92	94	90	99
Hexachlorobenzene	USDA	21	61	18	63
Pentachloroanisole		59	80	58	84
Dimethoate	USDA	100	102	85	91
beta-BHC		96	94	82	92
Pentachloronitrobenzene		61	85	56	73
Pentachlorobenzonitrile		46	90	40	84
gamma-BHC		94	95	86	94
Chlorothalonil		59	80	53	77
Anthracene		96	107	103	106
Diazinon	EPA/USDA	88	96	98	77
delta-BHC		95	94	97	103
Pentachloroaniline		43	82	41	84
Vinclozolin		95	97	110	83
Carbaryl	EPA	94	95	79	100
Metalaxyl	EPA/USDA	100	98	166	78
Pentachlorothioanisole		21	67	30	70

Continued on page 6.

Table I: Continued

Pesticide	Regulatory List*	500 ppb Fortified Sample (100 pg on-column)		50 ppb Fortified Sample (10 pg on-column)	
		50 mg PSA, 50 mg C18, 50 mg GCB	25 mg PSA, 7.5 mg GCB	50 mg PSA, 50 mg C18, 50 mg GCB	25 mg PSA, 7.5 mg GCB
Pirimiphos methyl		93	93	78	85
Methiocarb		93	95	85	92
Dichlofluanid		86	90	77	88
Malathion	EPA	98	94	93	132
Chlorpyrifos	EPA/USDA	83	91	77	83
Fenthion	USDA	93	88	78	97
DCPA		94	97	76	85
Parathion	USDA	89	100	112	98
Cyprodinil		51	92	73	127
Heptachlor epoxide	USDA	87	91	91	88
Thiabendazole		50	94	51	150
Captan	USDA	91	77	ND	ND
Folpet		84	90	67	66
Procymidone		94	95	83	99
Endosulfan I	EPA/USDA	75	87	61	100
Imazalil		108	101	ND	ND
4,4'-DDE	USDA	67	76	58	71
Dieldrin	USDA	75	83	76	101
Myclobutanil		96	98	112	94
Endrin	USDA	75	87	87	106
Endosulfan II	EPA/USDA	92	93	101	91
Oxadixyl		97	91	83	172
4,4'-DDD	USDA	80	100	83	74
2,4'-DDT	USDA	71	76	66	78
Carfentrazone ethyl		99	122	117	114
Endosulfan sulfate	EPA/USDA	94	97	74	94
Fenhexamid		123	103	81	111
4,4'-DDT	USDA	76	79	72	74
Propargite		88	100	107	57
Piperonyl butoxide		86	99	99	86
Iprodione		96	105	81	88
Bifenthrin		73	72	68	75
Dicofol		75	57	ND	50
Fenpropathrin		82	98	107	75
Phosalone		83	95	78	79
Azinphos methyl		99	94	ND	ND
cis-Permethrin	USDA	70	84	66	71
Coumaphos		59	88	46	132
trans-Permethrin	USDA	85	90	74	74
Cypermethrin	USDA	85	105	ND	ND
Pyraclostrobin		75	95	66	62
Fluvalinate		84	97	143	94
Difenoconazole		88	89	110	104
Deltamethrin		79	84	53	63
Azoxystrobin		102	104	73	99

* EPA approved list and USDA monitoring list

GCxGC-TOFMS Analysis

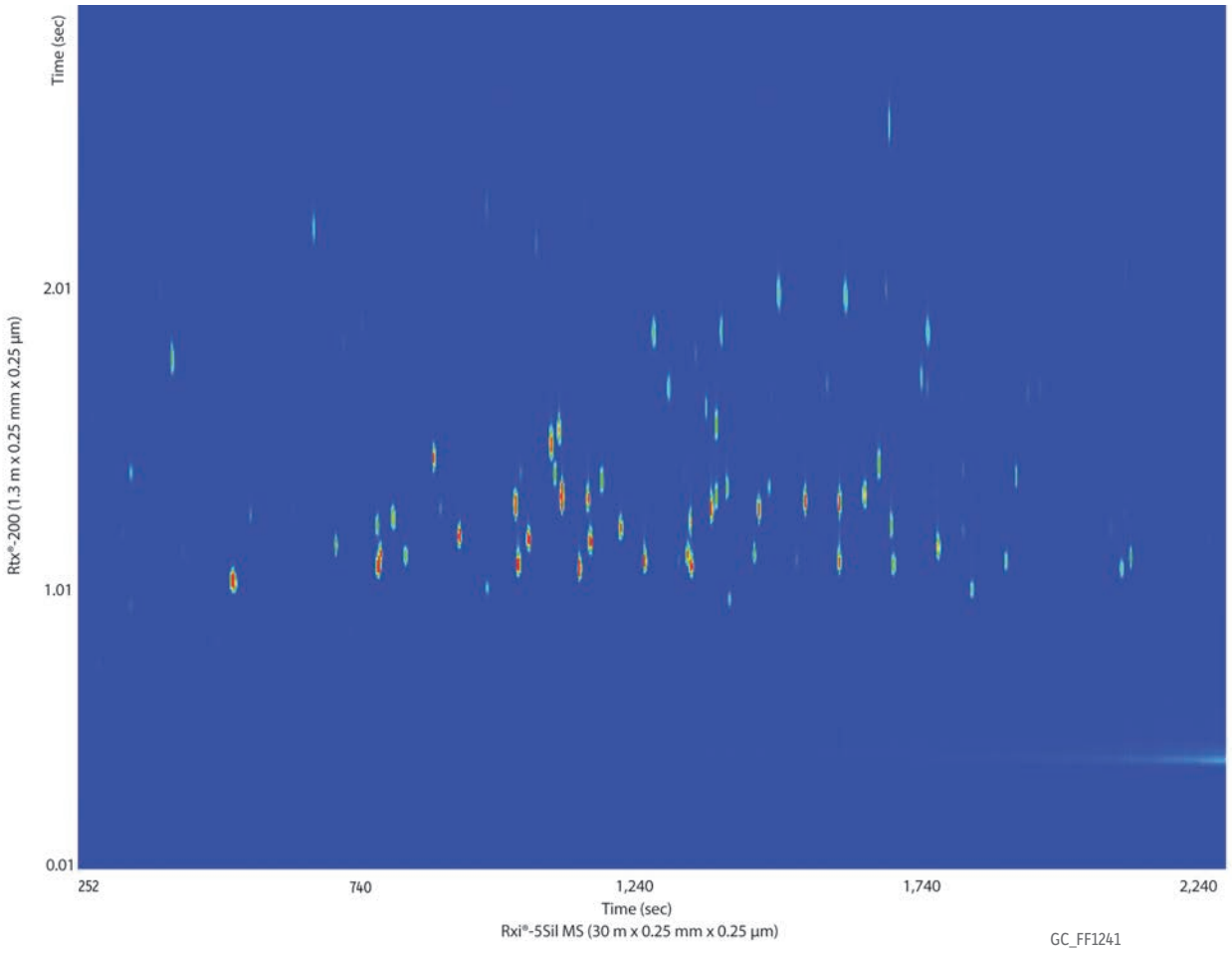
The fortification levels of the pesticides in the cigarette tobacco samples were equivalent to 100 pg and 10 pg on-column, assuming 100% recoveries were achieved with QuEChERS and the chosen cleanup. These detection levels would not be possible with full-scan GC-MS analysis with a quadrupole. A TOFMS has pg-level detectability for many pesticides and, by utilizing comprehensive two-dimensional GC, we are able to further decrease the detection limits due to the peak focusing of the modulator. The peaks eluting from the first dimension column are trapped and then immediately injected onto the very short and fast second dimension column. This yields peaks that are 100 ms wide, increasing the signal-to-noise ratio (S/N) and detectability. Only four of the targeted pesticides were not detected in the 50 ppb fortified tobacco sample using GCxGC-TOFMS.

The QuEChERS method utilizes dSPE because it provides a fast way to clean up extracts, while removing "just enough" of the matrix intereferences to accurately quantify pesticides of interest. For the most part, laboratories are performing targeted pesticide analysis and, therefore, using GC-MS operated in the selected ion monitoring (SIM) mode. While GC-MS (SIM) is somewhat selective, matrix components can still negatively impact quantification. Gross overload of matrix intereferences can shift peaks of interest, so the retention times do not match standards and can even shift them far enough that the SIM window no longer detects the analyte. Isobaric intereferences of matrix components can also impact data quality of target pesticides by skewing the ion ratios used for both qualification and quantification. This is especially problematic for analyzing pesticides in tobacco that do not have intense higher m/z ions that can be used for quantification and qualification ion ratios. The use of GC-MS (SIM) can require a more comprehensive cleanup, like cSPE, in order to avoid these issues.

By using GCxGC-TOFMS, matrix interferences can be chromatographically separated from the pesticides of interest. This can help alleviate some of the quantification issues with extracts that have not gone through a more extensive cleanup. By coupling the GCxGC to the time-of-flight mass spectrometer we have the sensitivity of a GC-MS (SIM) analysis and the added ability to perform non-target screening of pesticide residues. With all of the spectral information collected, the data can be archived and re-examined in the future for historical information on pesticides that were not targeted or not expected to be in the extracts.

In order to maximize the GCxGC separation space we chose two columns of different selectivities, a nonpolar Rxi®-5Sil MS column for the first dimension and a more polar selective Rtx®-200 column for the second dimension. The chemically diverse group of pesticides was nicely spread across the contour plot by using this column configuration (Figure 3).

Figure 3: The Rxi®-5Sil MS and Rtx®-200 columns have orthogonal selectivities that provide a good separation of a multi-pesticide standard.



Peaks

1. Methamidophos
2. Dichlorvos
3. 1,2,3,5-Tetrachlorobenzene
4. Mevinphos
5. Acephate
6. Pentachlorobenzene
7. *o*-Phenylphenol
8. Tetrachloronitrobenzene
9. Omethoate
10. 2,3,5,6-Tetrachloroaniline
11. Alpha-BHC
12. Hexachlorobenzene
13. Pentachloroanisole
14. Dimethoate
15. Beta-BHC
16. Pentachloronitrobenzene
17. Pentachlorobenzonitrile
18. Gamma-BHC
19. Chlorothalonil
20. Anthracene
21. Diazinon
22. Delta-BHC
23. Pentachloroaniline
24. Vinclozolin
25. Carbaryl
26. Metalaxyl
27. Pentachlorothioanisole
28. Pirimiphos methyl
29. Methiocarb
30. Dichlofluanid
31. Malathion
32. Chlorpyrifos
33. Fenthion
34. DCPA
35. Parathion
36. Cyprodinil
37. Heptachlor epoxide
38. Thiabendazole
39. Captan
40. Folpet
41. Procymidone
42. Endosulfan I
43. Imazalil
44. 4,4'-DDE
45. Dieldrin
46. Myclobutanil
47. Endrin
48. Endosulfan II
49. Oxadixyl
50. 4,4'-DDD
51. 2,4'-DDT
52. Carfentrazone ethyl
53. Endosulfan sulfate
54. Fenhexamid
55. 4,4'-DDT
56. Propargite
57. Piperonyl butoxide
58. Iprodione
59. Bifenthrin
60. Dicofol
61. Fenpropathrin
62. Phosalone
63. Azinphos-methyl
64. *cis*-Permethrin
65. Coumaphos
66. *trans*-permethrin
67. Cypermethrin
68. Pyraclostrobin
69. Fluvalinate
70. Difenoconazole
71. Deltamethrin
72. Azoxystrobin

Column Rxi®-5Sil MS 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)
Rtx®-200 1.3 m, 0.25 mm ID, 0.25 µm (cat.# 15124)
Sample Custom pesticide standard
Diluent: Acetonitrile
Conc.: 500 pg/µL
Injection
Inj. Vol.: 1.0 µL splitless (hold 1.0 min)
Liner: 4 mm Sky® single taper w/wool (cat.# 23303.5)
Inj. Temp.: 250 °C
Oven
Oven Temp.: Rxi®-5Sil MS: 90 °C (hold 1.0 min) to 310 °C at 5 °C/min (hold 2.0 min)
Rtx®-200: 95 °C (hold 1.0 min) to 315 °C at 5 °C/min (hold 2.0 min)
Carrier Gas He, corrected constant flow (2 mL/min)
Modulation
Modulator Temp. Offset: +20 °C
Second Dimension Separation Time: 3 sec
Hot Pulse Time: 0.9 sec
Cool Time between Stages: 0.6 sec
Detector MS
Mode:
Transfer Line Temp.: 300 °C
Analyzer Type: TOF
Source Temp.: 225 °C
Electron Energy: 70 eV
Mass Defect: -20 mu/100 u
Ionization Mode: EI
Acquisition Range: 45 to 550 amu
Spectral Acquisition Rate: 100 spectra/sec
Instrument LECO Pegasus 4D GCxGC-TOFMS
Notes Rtx®-200 (cat.# 15124) is a 2 m column. A 1.3 m section was cut off and used as the second dimension column.

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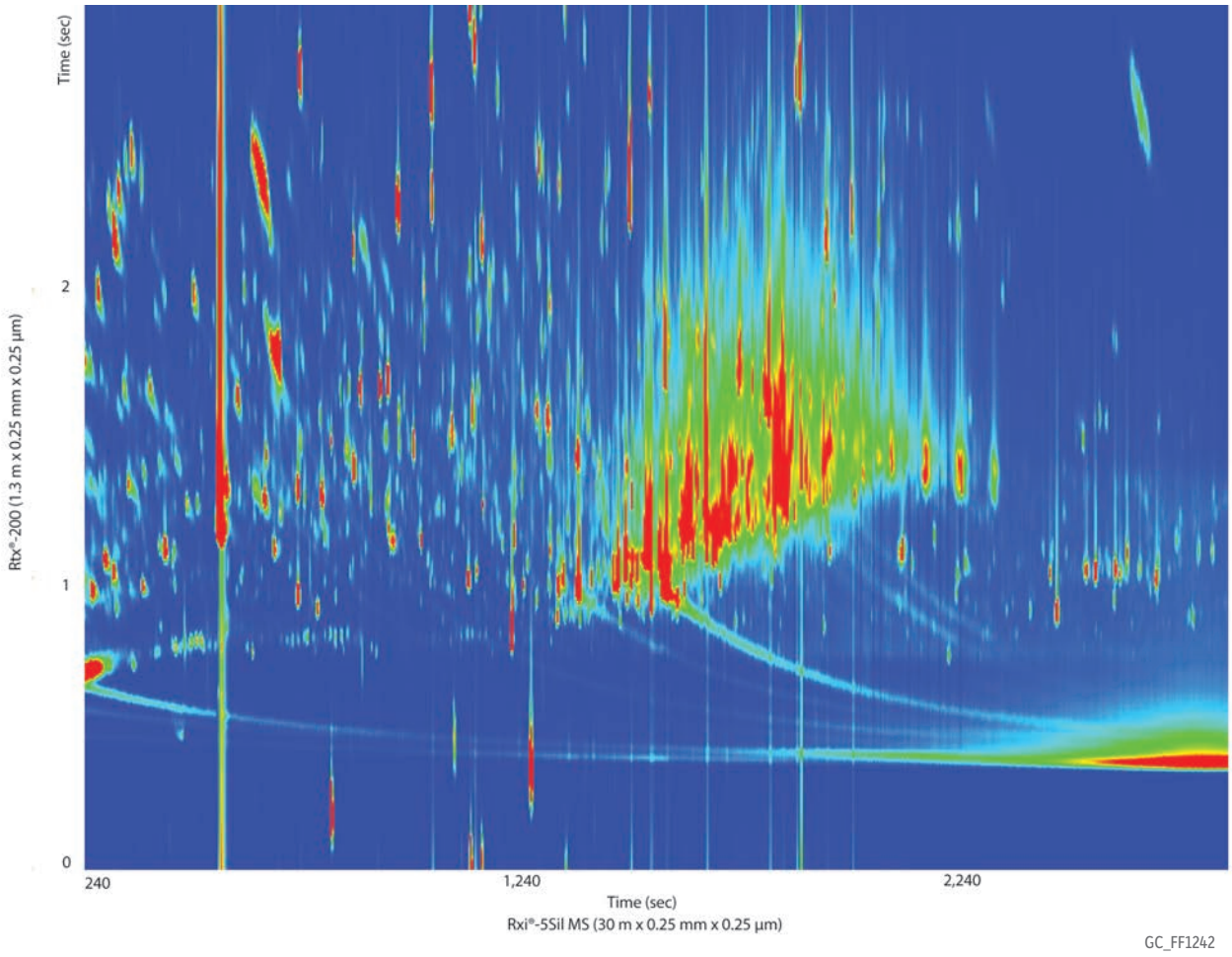
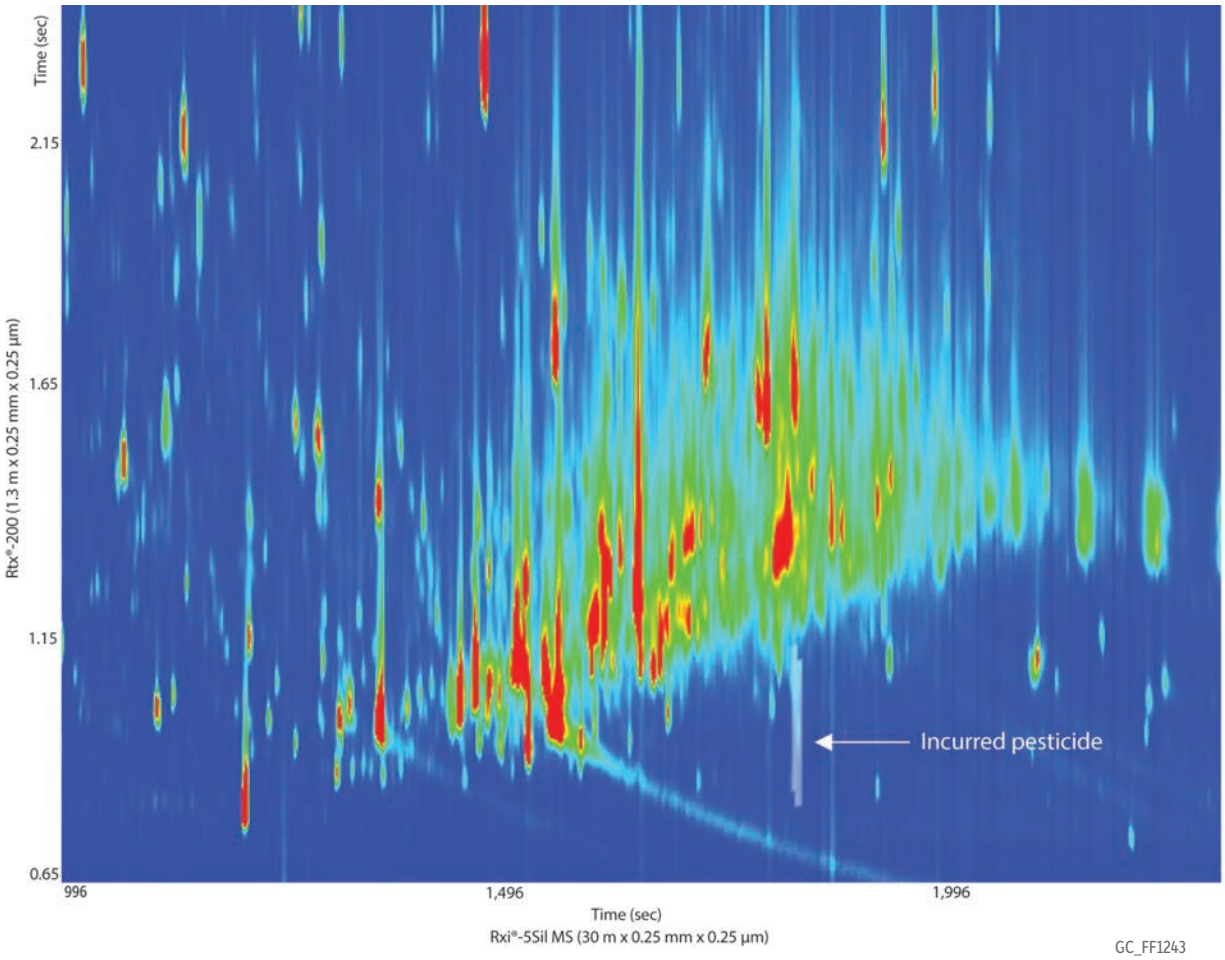
While the separation of pesticide standards is important, especially for isobaric interferences, separation of the matrix components from the pesticides of interest is even more important, especially given the concentration differential between gross matrix interferences and trace-level pesticides. The cigarette tobacco extract that we analyzed was a very complex sample, even after dSPE cleanup (Figure 4). The GCxGC separation of the tobacco matrix from the peaks of interest is what allowed us to use the faster, cheaper dSPE cleanup. This was especially apparent for one incurred pesticide that we found in the sample, piperonyl butoxide (Figure 5). In a 1D GC analysis, the peak of interest would have been completely obscured by matrix interferences that were not removed during cleanup.

Figure 4: GCxGC contour plot of an unfortified tobacco extract after dSPE cleanup with 25 mg PSA and 7.5 mg GCB highlights the complexity of the sample matrix.



See Figure 3 for instrument conditions.

Figure 5: Second dimension GCxGC separation of matrix components from the incurred pesticide piperonyl butoxide in an unfortified tobacco extract. The matrix would have coeluted and overwhelmed the piperonyl butoxide determination in a one-dimensional analysis.



See Figure 3 for instrument conditions.

Conclusion

When analyzing pesticides in tobacco, the QuEChERS extraction and dispersive solid phase extraction cleanup provided good recoveries for a wide range of residues at both the 500 ppb and 50 ppb fortification levels. We evaluated the more traditional cartridge solid phase extraction cleanup and found that it did not provide significantly greater removal of nonvolatile residues, except for fatty acids, compared to dSPE cleanup. GCxGC-TOFMS provided good separation of the complex matrix from the pesticides of interest. Without the use of GCxGC, determination of pesticide residues in such a complex matrix would have been difficult without a much more extensive cleanup.

Acknowledgments

The authors would like to acknowledge John Mathis from the U.S. FDA, previously with Global Laboratories, for valuable conversations and tobacco samples.

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Lit. Cat.# FFAN1823-UNV

Technical Article

Don't Overestimate Cannabidiol During Medical Cannabis Potency Testing by Gas Chromatography

By Jack Cochran

Accurate potency testing of medical cannabis with gas chromatography (GC) depends principally on choosing a column with the right selectivity; otherwise, coelutions between cannabinoids of interest may cause error in potency measurements. Cannabidiol is one of the chief cannabinoids with pharmacological value and provides relief against nausea, anxiety, and inflammation. Potency testing for medical marijuana is often done using "5-type" GC columns since they are commonly available in most labs. However, on 5-type columns cannabidiol can coelute with cannabichromene, a compound that likely also has medical value and is increasingly becoming part of potency testing. To identify and report both of these compounds accurately, a GC column with a different stationary phase is needed.

Proper Column Choice Results in More Accurate Potency Data

As shown in Figure 1, cannabinoids are aromatic compounds, meaning they will likely be better separated on a column that contains aromatics in the stationary phase because these stationary phases are more selective for aromatic-containing analytes. A fully non-aromatic stationary phase, like a "1-type" (100% dimethyl polysiloxane) column is not appropriate for this analysis since cannabichromene (CBC) and cannabidiol (CBD) will coelute completely. While 5-type columns (5% phenyl) contain some aromatic component, they generally also produce coelutions for cannabichromene and cannabidiol, depending on the conditions used. At best, CBC and CBD can be only partially resolved on 15 m 5% phenyl columns. Much better separations are obtained on higher phenyl-content phases, such as Rxi®-35Sil MS (35% phenyl type) and Rxi®-17Sil MS (50% phenyl type) columns, as they offer excellent selectivity for aromatic cannabinoids. Not only do both columns resolve cannabichromene and cannabidiol, the chromatograms in Figures 2 and 3 demonstrate that they also separate delta-8-tetrahydrocannabinol (d8-THC), delta-9-tetrahydrocannabinol (d9-THC), cannabigerol (CBG), and cannabinol (CBN). Although both columns perform well, the Rxi®-35Sil MS column is recommended because of the slightly faster analysis time and greater space overall between the peaks of interest.

While stationary phase selectivity is the most important factor in choosing a GC column for cannabinoid analysis, there are some additional aspects of this work that will benefit labs doing medical marijuana potency testing. First, cost savings were achieved by using a 15 m column. When a column with the proper selectivity is used, a 15 m column easily provides the separating power needed for this analysis at about half the cost of a 30 m column. Also, the 0.25 mm x 0.25 µm format has good sample loading capacity and is robust, especially when a proper split injection is used with a Sky® Precision® split liner with wool. Finally, hydrogen carrier gas was used here instead of helium. Using hydrogen provides a faster analysis, increasing sample throughput. Hydrogen carrier gas is a convenient way to speed up run times, increase productivity, and reduce the cost and availability concerns associated with using helium carrier gas.

Figure 1: Since cannabinoids are aromatic compounds, a GC column that contains aromatics in the stationary phase will provide much better separations than a column with a non-aromatic phase.

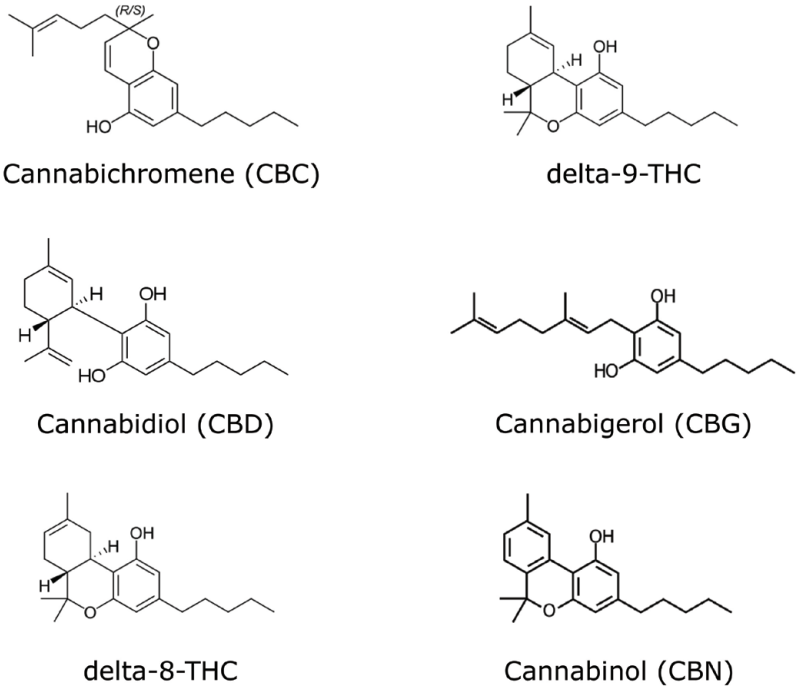


Figure 2: The Rxi®-35Sil MS column provides both the best separation and the fastest analysis time, making it the ideal GC column choice for medical cannabis potency testing.

Peaks
1. Cannabichromene
2. Cannabidiol
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

Column: Rxi®-35Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13820); **Sample:** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 225 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.9 min); **Carrier Gas;** H_2, constant flow; Flow Rate: 2.5 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument:** Agilent/HP6890 GC

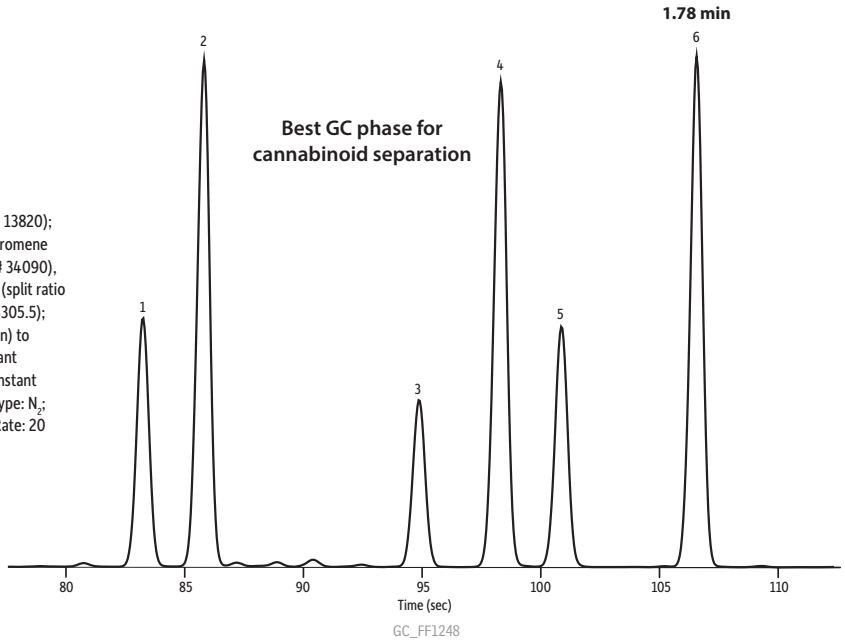
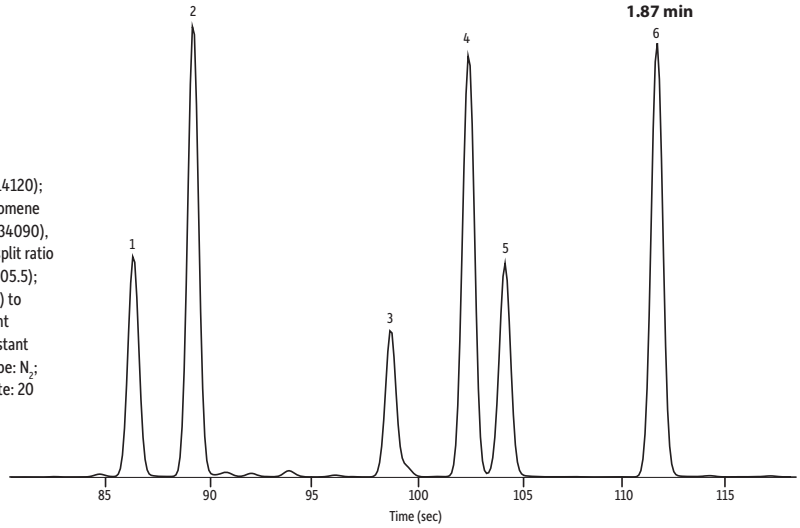


Figure 3: Cannabinoids can be effectively separated on an Rxi® 17Sil MS column, but with slightly less resolution than that obtained with the optimal selectivity of the Rxi®-35Sil MS column.

Peaks
1. Cannabichromene
2. Cannabidiol
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

Column: Rxi®-17Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 14120); **Sample;** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 225 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.9 min); **Carrier Gas:** H_2, constant flow; Flow Rate: 2.5 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument:** Agilent/HP6890 GC



Adjusting Conditions for 5-Type Columns

While using an Rxi®-35Sil MS column provides the best selectivity and speed for cannabinoid analysis, cannabidiol potency can be determined in medical cannabis using a 5-type column under certain conditions. If you already have a 5-type column for this work, you can vary the GC conditions, especially carrier flow and oven temperature program, and still separate cannabichromene and cannabidiol, just not as quickly or easily as with the Rxi®-35Sil MS column. Figures 4 and 5 show this analysis on Rxi®-5ms and Rxi®-5Sil MS columns, respectively. Again, the 0.25 mm x 0.25 µm format was used here because it offers better efficiency than wider bore columns (e.g., 0.32 mm and 0.53 mm IDs), which may not separate cannabichromene and cannabidiol under any operational conditions.

Figure 4: The selectivity of a 5-type column is not sufficient to fully separate cannabichromene and cannabidiol, resulting in less accurate medical marijuana potency testing.

Peaks
1. Cannabichromene
2. Cannabidiol
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

Column: Rxi®-5ms, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13420); **Sample;** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 250 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.6 min); **Carrier Gas:** H_2, constant flow; Flow Rate: 1.6 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument:** Agilent/HP6890 GC

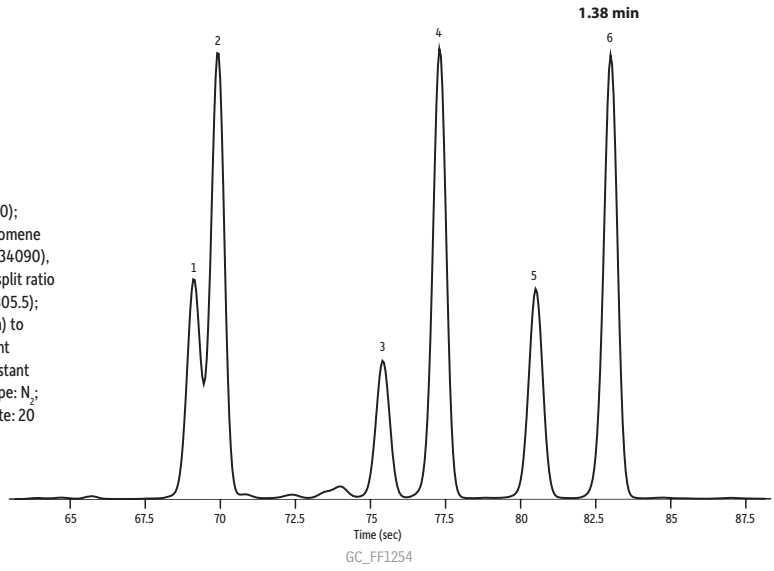
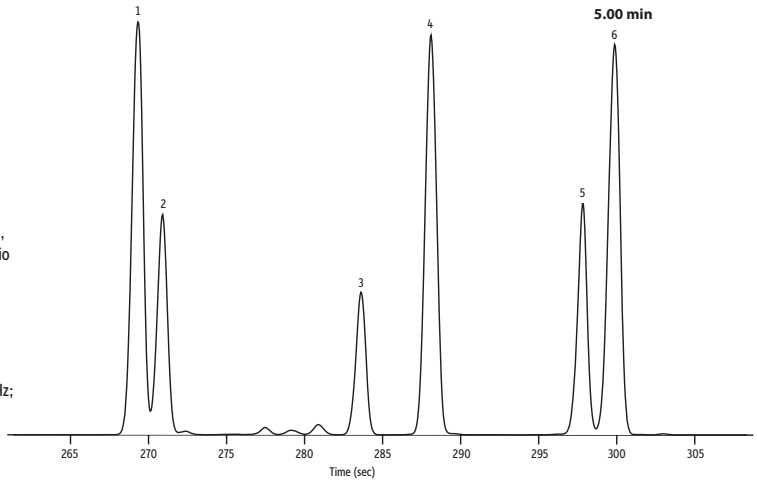


Figure 5: Rxi®-5Sil MS columns offer better resolution of key cannabinoids than standard 5-type columns, but the incomplete separation and longer analysis time mean further optimization is needed for accurate reporting.

Peaks
1. Cannabidiol
2. Cannabichromene
3. delta-8-Tetrahydrocannabinol
4. delta-9-Tetrahydrocannabinol
5. Cannabigerol
6. Cannabinol

Column: Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620); **Sample;** Cannabinoids standard (cat.# 34014), Cannabichromene (cat.# 34092), delta-8-Tetrahydrocannabinol (THC) (cat.# 34090), Cannabigerol (cat.# 34091); **Injection:** Inj. Vol.: 1 µL split (split ratio 50:1); Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5); Inj. Temp.: 250 °C; **Oven:** Oven Temp.: 150 °C (hold 0.1 min) to 330 °C at 35 °C/min (hold 0.7 min); **Carrier Gas:** H_2, constant flow; Flow Rate: 1.6 mL/min; **Detector:** FID @ 350 °C; Constant Column + Constant Make-up: 50 mL/min; Make-up Gas Type: N_2; Hydrogen flow: 40 mL/min; Air flow: 450 mL/min; Data Rate: 20 Hz; **Instrument:** Agilent/HP6890 GC



Note that even though these are both 5-type columns, the elution order of cannabichromene and cannabidiol changed. This is due to two things. The first is that Rxi®-5ms and Rxi®-5Sil MS columns differ slightly in selectivity for certain compounds; even though they are both considered 5-type columns, they contain different stationary phases that retain some compounds differently. The second reason is that the GC oven programs are different for the columns, which means that the compounds are eluting at different temperatures. You may be able to further optimize the separation of cannabichromene and cannabidiol on a 5-type column, but the selectivity and faster analysis that can be obtained using a high-phenyl content Rxi®-35Sil MS column make it ideal for potency determinations in medical cannabis.

To sum things up, proper column choice is essential for accurate and robust cannabis potency testing. Using the right column not only gives you more confidence in your potency values, but it also saves you time and money. Switching to hydrogen carrier gas can reduce your costs even further, while increasing sample throughput.

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Applications Note

Developing New Methods for Pesticides in Dietary Supplements

Advantages of the QuEChERS Approach

New requirements for dietary supplements to be manufactured under cGMP regulations have created a need for methods to detect pesticides in these complex, largely botanical products. QuEChERS offers a simple, cost-effective approach that can reduce matrix interferences as well as variation among technicians. Here we demonstrate a procedure that incorporates a QuEChERS extraction, cSPE cleanup and GC-TOFMS, resulting in good recoveries for a wide range of pesticide chemistries in dandelion root powder.

Introduction

Recently the FDA announced that makers of dietary supplements (e.g. vitamins, herbal and botanical pills, etc.) will have to adhere to current Good Manufacturing Practices (cGMPs), marking a major shift in regulatory oversight and testing for the industry. Previously, compliance was voluntary, but in 2003, due to public and industry concern, the FDA proposed requiring dietary supplement manufacturers to adhere to cGMP standards. The final rule was issued in June 2007 and is in full effect June 2010 [1]. Basic GMPs require implementing comprehensive procedures to ensure product quality and safety. Since many dietary supplements are largely derived from botanical sources, they must be tested for pesticide contaminants in order to meet cGMP regulations. As a result of this requirement, labs are working to develop and validate methods, an endeavor which is complicated by the wide range of pesticides and matrices to be tested.

Labs can begin method development with the FDA Pesticide Analytical Manual (PAM), which includes procedures for plant materials. While PAM Method 303 is an appropriate starting point, it has several disadvantages, including high solvent consumption, manual procedures that contribute to analytical variation, and the inability to extract polar pesticides. As an alternative, we developed a QuEChERS-based method for analyzing pesticides in dietary supplements that has several advantages over PAM 303 (Table I). QuEChERS is an approach that was developed by the USDA Eastern Regional Research Center as a simple, rapid, effective, yet inexpensive way to extract pesticide residues from fruits and vegetables, followed by a novel dispersive solid phase extraction (dSPE) cleanup of the extract. Because of these benefits, the approach has become popular and has been expanded to include numerous other matrices. We chose QuEChERS as an alternative to PAM 303 because of its speed, simplicity, and low solvent use, as well as its ability to produce good extraction efficiencies for relatively polar pesticides [2].

Based on preliminary studies, we knew that while the extraction part of QuEChERS would be successful, the dSPE cleanup step probably did not have the capacity to handle the matrix complexity of most dietary supplements. Thus, we compared dSPE to a cartridge solid phase extraction (cSPE) cleanup and established a procedure that uses a QuEChERS extraction, cSPE cleanup, and GC-TOFMS for accurate determinations of 46 pesticides in dandelion root powder. This approach saves time and can reduce analyst variation by minimizing manual preparation with prepackaged extraction salts and snap-and-shoot standards. As shown in Figure 1, it also uses much less solvent, salt, and sorbent, making it a greener, more cost-effective method than PAM 303.

Table I Decrease costs and increase reproducibility with a GMP-friendly QuEChERS approach to analyzing pesticides in dietary supplements.

	PAM 303 Method	QuEChERS + cSPE	Benefits of QuEChERS + cSPE
Solvent used (mL)	1,850	92	**20x less solvent;** cleaner, greener, & cost-effective
# of Solvents	4	3	
Salt and sorbent used (g)	35	6.6	**5x less salt/sorbent**
Glassware/lab equipment	• Separatory funnel (1L capacity) • Filter apparatus • Florisil column	• Centrifuge • SPE manifold	Fast, easy batch processing
Manual preparation	• Salt solution • Standards • Florisil column	None—prepackaged salts and cSPE cartridge are ready to use	**Highly reproducible; less manual prep means less human error**

Procedure

Sample Wetting and Fortiÿcation

Fully processed dandelion root powder obtained from a dietary supplement manufacturer was used for this work. The powder was wetted and then fortified with 46 pesticides representing different chemical classes that have been previously reported in dietary supplements [3]. Typically, QuEChERS methods use 10-15 grams of material with high water content (>80%). Therefore, to prepare for a QuEChERS extraction with a dry commodity, it is critical to use a reduced amount of material and wet it with water prior to extraction. In this work, 1 g of dietary supplement powder was combined with 9 mL of water. After shaking to mix well, the wetted supplement was fortified with 200 µL of a 2 ng/µL pesticides spiking solution resulting in a 400 ng/g spike level, relative to the original commodity. Also, 100 µL of QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) was added. The sample was then allowed to soak for 2 hours prior to extraction.

Figure 1 QuEChERS extraction and cSPE cleanup simpliÿes sample prep for pesticides in dietary supplements.

QuEChERS Extraction

1. **Wet** 1 g of matrix powder with 9 mL of water. Fortify as necessary, then soak 2 hours.
2. **Add** 10 mL acetonitrile.
3. **Shake** 1 min.
4. **Add** Q-sep™ Q110 extraction salts.
5. **Shake** 1 min.
6. **Centrifuge** 5 min. at 3,000 U/min.

cSPE Cleanup

1. **Prepare** 6mL Resprep® Combo SPE Cartridges as follows. Add magnesium sulfate to a level approximately half the height of either the GCB or PSA bed. Rinse cartridge with 20 mL of 3:1 acetonitrile:toluene.
2. **Load** 1 mL of extract on cartridge and elute with 50 mL 3:1 acetonitrile:toluene.
3. **Evaporate** to approximately 0.5-1 mL using dry nitrogen gas and a 35-40°C water bath.
4. **Add** 3 mL toluene and evaporate to just under 0.5 mL.
5. **Rinse** evaporation vessel with toluene and adjust final volume to 0.5 mL.

Solvent Usage: 92 mL, 3 solvents

PAM Extraction

1. **Weigh** 20-25 g and fortify as necessary.
2. **Add** 350 mL 65:35 acetonitrile:water.
3. **Blend** 5 min. and filter.
4. **Transfer** to a 1 L separatory funnel and add 100 mL petroleum ether (hexanes).
5. **Shake** 1-2 min.
6. **Add** 10 mL saturated sodium chloride and 600 mL water.
7. **Shake** 45 seconds and allow layers to separate.
8. **Wash** organic layer with 100 mL water and transfer to a graduated cylinder.
9. **Wash** organic layer again with another 100 mL water and transfer to cylinder.
10. **Add** 15 g sodium sulfate to organic fraction.
11. **Shake** vigorously, then evaporate to ~100 mL.

PAM Cleanup

1. **Prepare** a Florisil® cleanup column as follows. Add Florisil® to a 22 mm x 300 mm column to a height of 4 inches, then top with ½ inch sodium sulfate.
2. **Transfer** extract to column for cleanup.
3. **Elute** in 3 separate fractions as follows:
   a. 200 mL 6% diethyl ether in petroleum ether.
   b. 200 mL 15% diethyl ether in petroleum ether.
   c. 200 mL 50% diethyl ether in petroleum ether.
4. For each fraction: **evaporate** solvent, **adjust** final volume, and **add** internal standards as necessary for GC injection.

Solvent Usage: 1,850 mL, 4 solvents

QuEChERS Extraction

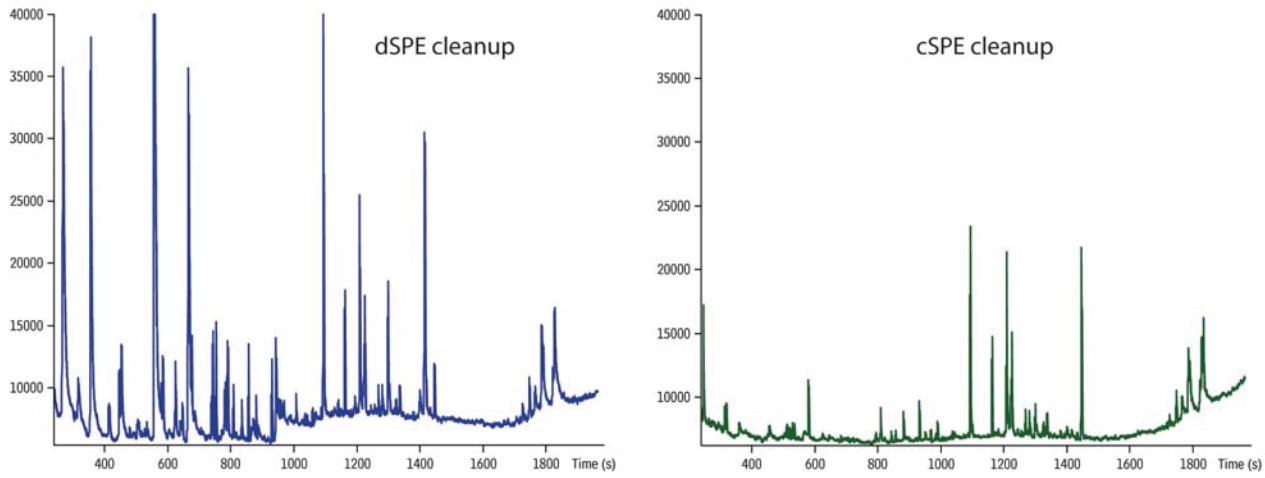
The EN 15662 QuEChERS method was used for sample extraction [4]. 10 mL of acetonitrile was added to the wetted sample. After a 1 minute shake, Q-sep™ Q110 buffering extraction salts (cat.# 26213, 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate) were 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). Lastly, 5 µL of quality control standard anthracene (cat.# 33264) was added to a 1 mL aliquot of extract to indicate fatal losses of planar compounds to Carboprep® 90 during cleanup.

Extract Cleanup

Two approaches were explored for extract cleanup: dSPE and cSPE. For dSPE, 1 mL of extract was added to a Q210 dSPE tube containing 150 mg $MgSO_4$ and 25 mg PSA (cat.# 26215), shaken for 2 minutes, and then centrifuged for 5 minutes. The resulting final extract was then analyzed by GC-TOFMS.

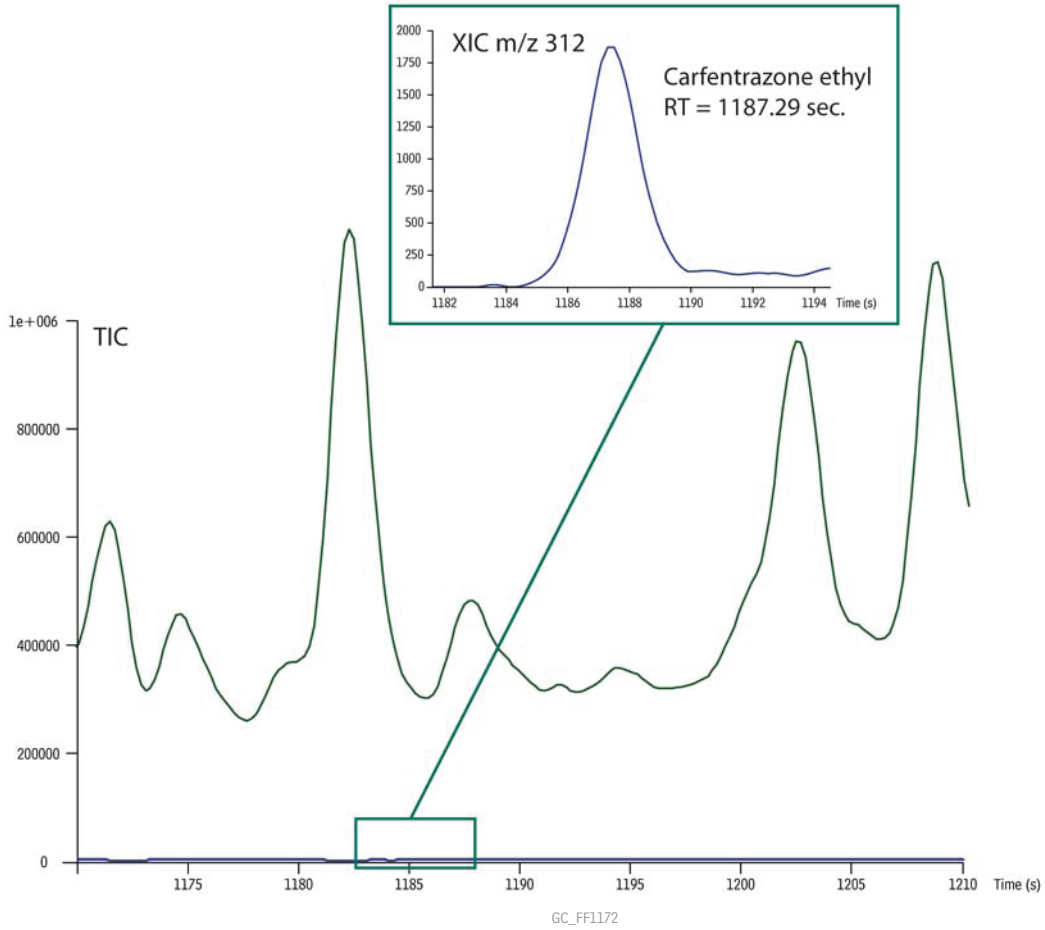
For cSPE cleanup [5], 1 mL of extract was processed with a 6 mL Resprep® Combo SPE Cartridge (cat.# 26194), which is designed for pesticide residue cleanup and contains 500 mg CarboPrep® 90 and 500 mg primary secondary amine (PSA). To prepare the SPE cartridge, magnesium sulfate was first added to a level approximately one-quarter height of the total bed; then the cartridge was rinsed with 20 mL of 3:1 acetonitrile: toluene, which was discarded. For cleanup, 1 mL of extract was loaded onto the prepared cartridge and then eluted with 50 mL 3:1 acetonitrile: toluene. The eluent was then evaporated and solvent exchanged using dry

Figure 2 QuEChERS extracts of pesticides in dietary supplements beneÿt from cSPE cleanup, which minimizes matrix interferences by removing more sugars and fatty acids than dSPE.



Column	Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)
Sample	Dandelion Root powder spiked with 46 pesticides QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) anthracene for quality control (cat.# 33264)
Conc.:	80 pg/µL
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 8 °C/min.
Carrier Gas	He, constant flow
Flow Rate:	1.5 mL/min.
Detector	MS
Mode:	
Analyzer Type:	TOF
Source Temp.:	225 °C
Electron Energy:	70 eV
Ionization Mode:	EI
Acquisition Range:	45-550 amu
Acquisition Rate:	5 spectra/sec.
Instrument	LECO Pegasus 4D GCxGC-TOFMS
Notes	Sample Preparation: Wetting:1 g of Dandelion Root powder combined with 9 mL of water, shaken well, fortified with pesticides and QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267), soak for 2 hours. Extraction: 10 mL acetonitrile added then addition of Q-sep™ Q110 (cat.# 26213), 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™ Q110 dSPE tube (cat.# 26213), shake, centrifuge. cSPE: add control standard anthracene (cat.# 33264) to 1 mL extract, add magnesium sulfate to cartridge, use 6mL Combo SPE Cartridge containing 500mg CarboPrep® 90/500mg PSA (cat.# 26194), concentrate via evaporation.

Figure 3 Using TOFMS allows deÿnitive identiÿcation and quantiÿcation, even when matrix components coelute with target analytes. (Inset: carfentrazone ethyl S/N = 105; extracted ion chromatogram, m/z 312.)



Column	Rxi®-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (cat.# 13623)
Sample	Dandelion root powder spiked with 46 pesticides QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) anthracene for quality control (cat.# 33264)
Solvent:	toluene
Conc.:	80 pg/µL
Injection	
Inj. Vol.:	1 µL splitless (purge valve time 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 8 °C/min.
Carrier Gas	He, constant flow
Flow Rate:	1.5 mL/min.
Detector	MS
Mode:	Full mass range
Analyzer Type:	TOF
Source Temp.:	225 °C
Electron Energy:	70 eV
Ionization Mode:	EI
Acquisition Range:	45-550 amu
Acquisition Rate:	5 spectra/sec.
Instrument	LECO Pegasus 4D GCxGC-TOFMS
Notes	**Sample Preparation:** Wetting: 1 g of dandelion root powder combined with 9 mL of water, shaken well, fortified with pesticides and QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267), soak for 2 hours. Extraction: 10 mL acetonitrile added, then Q-sep™ Q110 (cat.# 26213), centrifuge with Q-sep™ 3000 Centrifuge (cat.# 26230). cSPE: add control standard anthracene (cat.# 33264) to 1 mL extract, add magnesium sulfate to cartridge, use 6 mL Combo SPE Cartridge containing 500 mg CarboPrep® 90/500 mg PSA (cat.# 26194), concentrate via evaporation

Table II This QuEChERS-based method provides good recoveries for a variety of pesticides found in dietary supplements.

Compound	RT (sec.)	Recovery (%)	Class	Type
1,2,3,5-Tetrachlorobenzene	418.0	46	Organochlorine	Chemical intermediate
Pentachlorobenzene	587.0	51	Organochlorine	Metabolite
Tetrachloronitrobenzene	648.8	72	Organochlorine	Fungicide
2,3,5,6-Tetrachloroaniline	678.0	64	Organochlorine	Fungicide
alpha-BHC	739.4	69	Organochlorine	Insecticide
Hexachlorobenzene	744.4	56	Organochlorine	Impurity
Pentachloroanisole	754.6	62	Organochlorine	Metabolite
beta-BHC	780.5	88	Organochlorine	Insecticide
Pentachloronitrobenzene	784.2	62	Organochlorine	Fungicide
Pentachlorobenzonitrile	790.0	70	Organochlorine	Impurity
gamma-BHC	791.2	85	Organochlorine	Insecticide
Diazinon	816.6	71	Organophosphorus	Insecticide
Chlorothalonil	819.2	100	Organochlorine	Fungicide
delta-BHC	836.4	85	Organochlorine	Insecticide
Pentachloroaniline	857.6	75	Organochlorine	Metabolite
Pentachlorothioanisole	931.2	66	Organochlorine	Metabolite
PCB 52	932.0	-	Organochlorine	Internal standard
Chlorpyrifos	952.6	92	Organophosphorus	Insecticide
Dacthal	958.8	83	Organochlorine	Herbicide
Parathion	963.2	91	Organophosphorus	Insecticide
Heptachlor epoxide	1008.4	93	Organochlorine	Metabolite
Procymidone	1027.4	100	Organonitrogen	Fungicide
Endosulfan I	1059.8	70	Organochlorine	Insecticide
4,4'-DDE	1094.6	90	Organochlorine	Metabolite
Dieldrin	1097.8	91	Organochlorine	Insecticide
Myclobutanil	1100.6	100	Organonitrogen	Fungicide
Endosulfan II	1141.6	110	Organochlorine	Insecticide
Oxadixyl	1149.4	100	Organonitrogen	Fungicide
4,4'-DDD	1152.2	98	Organochlorine	Insecticide, Breakdown product
2,4'-DDT	1155.0	94	Organochlorine	Insecticide
Carfentrazone ethyl	1188.0	110	Organonitrogen	Herbicide
Endosulfan sulfate	1194.8	105	Organochlorine	Metabolite
Fenhexamid	1202.4	94	Organonitrogen	Fungicide
4,4'-DDT	1203.8	96	Organochlorine	Insecticide
Piperonyl butoxide	1237.6	93	Other	Insecticide synergist
Iprodione	1261.0	110	Organochlorine	Fungicide
Cypermethrin 1	1466.8	130	Pyrethroid	Insecticide
Cypermethrin 2	1474.8	86	Pyrethroid	Insecticide
Cypermethrin 3	1478.6	75	Pyrethroid	Insecticide
Cypermethrin 4	1481.8	100	Pyrethroid	Insecticide
Pyraclostrobin	1538.0	92	Organonitrogen	Fungicide
Fluvalinate 1	1541.4	100	Pyrethroid	Insecticide
Fluvalinate 2	1546.8	94	Pyrethroid	Insecticide
Difenoconazole 1	1562.0	99	Triazole	Fungicide
Difenoconazole 2	1566.6	81	Triazole	Fungicide
Azoxystrobin	1596.0	93	Organonitrogen	Fungicide

nitrogen gas and a 35-40 °C water bath. Evaporation was allowed to proceed until approximately 0.5-1 mL eluent was left, at which point about 3 mL of toluene was added. The mixture was evaporated to just under 0.5 mL, and then the evaporation vessel was rinsed with toluene to bring the sample to a final volume of 0.5 mL. The resulting final extract was then analyzed by GC-TOFMS.

Standards

Matrix-matched standards were prepared at 80 pg/µL, as 80 pg/µL is the expected final concentration in extract of the 400 ng/g matrix spikes (assuming 100% recoveries). Matrix-matched standards were prepared by adding standard solution to the final extract (post-cleanup) from a control sample. Actual recoveries were calculated by comparing peak areas for fortified samples that were extracted and cleaned, to areas of a matrix-matched standard, using the internal standard quantification method.

GC-TOFMS

A LECO Pegasus III GC-TOFMS instrument was used and all data were processed with LECO ChromaTOF™ software. Gas chromatography was performed using an Rxi®-5Sil MS column (30m x 0.25mm x 0.25µm, cat.# 13623). Instrument conditions are shown in Figure 1. Temperature and flow settings yielded an analysis time of 32.75 minutes.

Results

One aspect of this investigation was to compare the applicability of two sample cleanup methods, dSPE and cSPE for QuEChERS extracts of pesticides in dietary supplements. While dSPE has the advantage of improved speed and less solvent usage, it does not have the sorbent capacity to adequately clean up these samples (Figure 2). Since cSPE uses more sorbent, it is a better choice for dietary supplements (and other complex samples, e.g. spices, essential oils) as it can remove more matrix components, such as fatty acids, sugars, and pigments. QuEChERS methods developed for dietary supplements of botanical origin can benefit from the extra sorbent capacity of cSPE, which reduces GC inlet/column contamination and chromatographic interference from complex botanical matrices.

Even with effective extraction and cleanup techniques, dietary supplements can be challenging to analyze due to their complexity. Coelutions are common and pesticide residues can be overwhelmed by abundant matrix compounds not only qualitatively, but also by interfering with quantification masses. Figure 3 plots the total ion chromatogram (TIC) and extracted ion m/z 312 corresponding to the quantitation mass for carfentrazone ethyl. It is clear that target pesticide signals can be obscured in the TIC. LECO ChromaTOF™ software was able to identify target pesticides by comparison with reference spectra using automatic peak find and spectral deconvolution algorithms, along with calibration and quantification. TOFMS makes this powerful data processing possible with very fast acquisition rates and unbiased mass spectra, and by having pg level sensitivity in full mass range mode, which allows the potential for finding non-target pesticides. An alternate GC/MS approach for targeted pesticides in dietary supplements would be to use selected ion monitoring with a typical quadrupole mass spectrometer.

Overall, the combination of QuEChERS extraction, cSPE cleanup, and GC-TOFMS used in this method produced good recoveries for most compounds tested (Table II). Although early eluting compounds trended toward lower recoveries, most analytes, including more polar compounds, showed excellent recoveries. The potential for good recoveries of polar pesticides is a major advantage to QuEChERS methods; this difference is due to the use polar acetonitrile as the extraction solvent, rather than petroleum ether (hexanes) which is used in PAM 303. The lower recoveries here of early eluting compounds may be due to evaporative loss during concentration steps, due to their higher volatility. Additionally, in the case of planar compounds, reduced recoveries may be due to interaction with the CarboPrep® 90 sorbent used to remove pigments and other matrix compounds, although the planar quality control standard, anthracene, did not show drastic losses during cSPE Overall, the chromatography and recovery results seen for a broad range of pesticides in dandelion root demonstrate the utility of the QuEChERS approach for dietary supplement testing.

Conclusion

Demonstrated here is a QuEChERS approach that helps accomplish the pesticide testing now required for dietary supplements. The basic methodology presented here for dandelion root can be modified for other analytes and matrices and illustrates the advantages of the QuEChERS approach for labs developing cGMP methods. Analytical benefits include reduced interferences and good recoveries, even of polar compounds. Other benefits include an overall savings of both materials and prep time compared to the PAM 303 method, and better expected reproducibility due to the straight-forward procedure with fewer manual preparations.

References

[1] US Food and Drug Administration, Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements, Docket No. 1996N-0417 (formerly No. 96N-0417), CFSAN 200441 (2007) 34752.
[2] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC International 86 (2003) 412.
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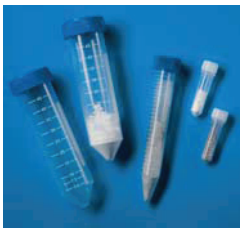
[4] Foods of Plant Origin—Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE (QuEChERS-method). (EN 15662 Version 2008.)
[5] M. Okihashi, Y. Kitagawa, K. Akutsu, H. Obana, Y. Tanaka, J. Pestic. Sci. 30 (2005) 368.

Pesticide Residue Cleanup SPE Cartridges

- Convenient, multiple adsorbent beds in a single cartridge.
- For use in multiple-residue pesticide analysis, to remove matrix interferences.

SPE Cartridge	qty.	cat#
6mL Combo SPE Cartridge Packed with 500mg CarboPrep 90/500mg Aminopropyl, Polyethylene Frits	30-pk.	26193
6mL Combo SPE Cartridge Packed with 500mg CarboPrep 90/500mg PSA, Polyethylene Frits	30-pk.	26194
6mL SPE Cartridge Packed with 500mg PSA, Polyethylene Frits	30-pk.	26195
6mL Combo SPE Cartridge Packed with 200mg CarboPrep 200 and 400mg PSA, PTFE Frits	30-pk.	26127
6mL Combo SPE Cartridge Packed with 250mg CarboPrep 200 and 500mg PSA, PTFE Frits	30-pk.	26128
6mL Combo SPE Cartridge Packed with 500mg CarboPrep 200 and 500mg PSA, PTFE Frits	30-pk.	26129



Q-sep™ QuEChERS Tubes

for Extraction and Clean-Up of Pesticide Residue Samples from Food Products

- Fast, simple sample extraction and cleanup using dSPE.
- Fourfold increases in sample throughput.
- Fourfold decreases in material cost.
- Convenient, ready to use centrifuge tubes with ultra pure, preweighed adsorbent mixes.

Description	Material	Methods	qty.	cat#
50mL Centrifuge Tubes for Sample Extraction				
Q110	4g $MgSO_4$, 1g NaCl, 1g trisodium citrate dihydrate, 0.5g disodium hydrogen citrate sesquihydrate	European EN 15662	50-pk.	26213
Q150	6g $MgSO_4$, 1.5g NaOAc	AOAC 2007.1	50-pk.	26214
Empty 50mL Centrifuge Tube	—	European EN 15662, AOAC 2007.1	25-pk.	26227
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
Q212	150mg $MgSO_4$, 25mg PSA, 2.5mg GCB	European EN 15662	100-pk.	26217
Q213	150mg $MgSO_4$, 25mg PSA, 7.5mg GCB	European EN 15662	100-pk.	26218
Q250	150mg $MgSO_4$, 50mg PSA	AOAC 2007.1	100-pk.	26124
Q251	150mg $MgSO_4$, 50mg PSA, 50mg C18	AOAC 2007.1	100-pk.	26125
Q253	150mg $MgSO_4$, 50mg PSA, 50mg GCB	—	100-pk.	26123
Q252	150mg $MgSO_4$, 50mg PSA, 50mg C18, 50mg GCB	AOAC 2007.1	100-pk.	26219
15mL Centrifuge Tubes for dSPE (clean-up of 6mL extract)				
Q350	1200mg $MgSO_4$, 400mg PSA	AOAC 2007.1	50-pk.	26220
Q351	1200mg $MgSO_4$, 400mg PSA, 400mg C18	AOAC 2007.1	50-pk.	26221
Q352	1200mg $MgSO_4$, 400mg PSA, 400mg C18, 400mg GCB	AOAC 2007.1	50-pk.	26222
Q370	900mg $MgSO_4$, 150mg PSA	European EN 15662	50-pk.	26223
Q371	900mg $MgSO_4$, 150mg PSA, 15mg GCB	European EN 15662	50-pk.	26224
Q372	900mg $MgSO_4$, 150mg PSA, 45mg GCB	European EN 15662	50-pk.	26225
Q373	900mg $MgSO_4$, 150mg PSA, 150mg C18	—	50-pk.	26226
Q374	900mg $MgSO_4$, 300mg PSA, 150mg GCB	—	50-pk.	26126

Sorbent Guide

Sorbent	Removes
$MgSO_4$	excess water
PSA*	sugars, fatty acids, organic acids, anthocyanine pigments
C18	lipids, nonpolar interferences
GCB**	pigments, sterols, nonpolar interferences

*PSA—primary and secondary amine exchange material
**GCB—graphitized carbon black

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Q-sep™ 3000 Centrifuge
for QuEChERS

- Meets requirements of AOAC and European QuEChERS methodology.
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- Small footprint requires less bench space.
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Priced to fit your laboratory's budget, the Q-sep™ 3000 Centrifuge is the first centrifuge specifically designed for QuEChERS methodology. This compact, quiet, yet powerful, unit spins at the 3,000g force required by the European method.

Centrifuge includes 50 mL tube carriers (6), 50 mL conical tube inserts (6), 4-place 15 mL tube carriers (6), and 2 mL tube adaptors (24).

Description	qty.	cat.#
Q-sep 3000 Centrifuge, 110V	ea.	26230
Q-sep 3000 Centrifuge, 220V	ea.	26231
Replacement Accessories		
50mL Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26232
50mL Conical Tube Insert for Q-sep 3000 Centrifuge	6-pk.	26249
4-Place Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26233
2mL Tube Adaptors for Q-sep 3000 Centrifuge	4-pk.	26234

Rxi®-5Sil MS Columns (fused silica)
(low polarity Crossbond® silarylene phase; selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

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

QuEChERS Quality Control Standards for GC/MS Analysis

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

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

QuEChERS Internal Standard Mix for GC/MS Analysis (6 components)

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

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

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General Applications

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.

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 µL of QuEChERS Internal Standard Mix for GC/MS Analysis (cat.# 33267) containing PCBs 18, 28, and 52 (50 µg/mL each), triphenylphosphate (20 µg/mL), tris-(1,3-dichloroisopropyl)phosphate (50 µg/mL), and triphenylmethane (10 µg/mL).

- *400 ng/g Spiked Dietary Supplement*
  Each spike was fortified with 200 µL of a 2 ng/µL standard that contained 46 pesticides, representing different chemical classes, previously reported in dietary supplements [3]. 100 µL 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 $MgSO_4$, 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 $MgSO_4$ 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 $MgSO_4$, 25 mg PSA, 25 mg C18; cat.# 26216), Q213 (150 mg $MgSO_4$, 25 mg PSA, 7.5 mg GCB; cat.# 26218), and Q252 (150 mg $MgSO_4$, 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 $MgSO_4$ 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 a 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 µm Rxi®-5Sil MS column (cat.# 13623) with a constant flow of helium at 1.5 mL/min. 1 µL 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 µm Rxi®-5Sil MS column (cat.# 13623) connected to a 1.5 m x 0.18 mm x 0.20 µm 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/µL, 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).

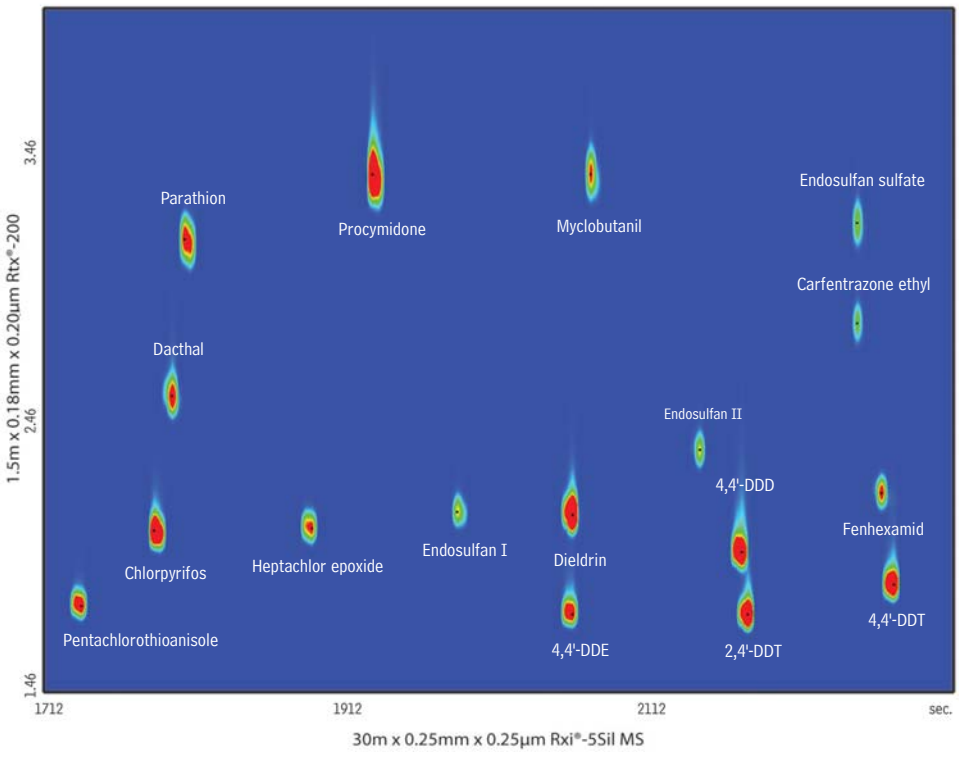
Table I GC-TOFMS and GCxGC-TOFMS recovery comparison for QuEChERS extracts and cartridge SPE cleanups of dietary supplements.

	Dandelion			Sage		Finished Product	
Compound	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).

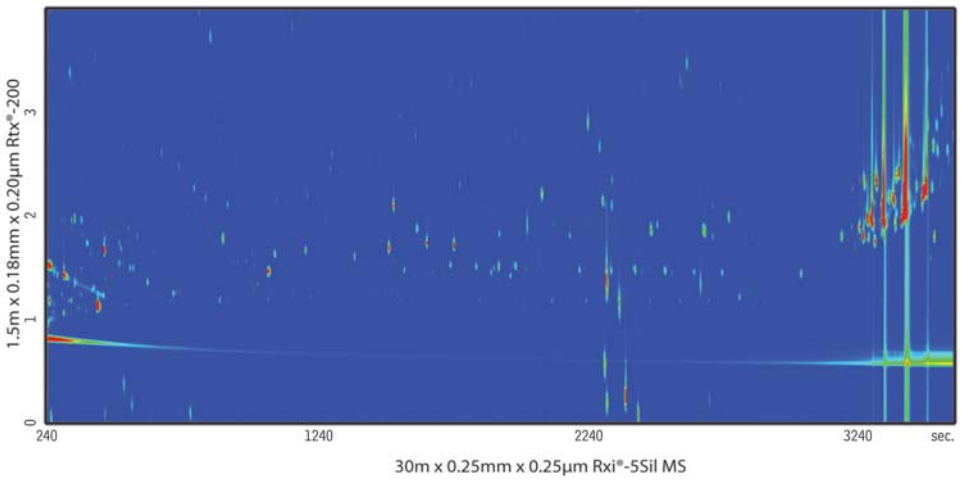
Figure 1 GCxGC-TOFMS separation of a dietary supplement pesticide standard. GCxGC-TOFMS allows the separation 2,4′-DDT and 4,4′-DDD along the second dimension (Rtx®-200 column). These compounds coelute in the first dimension (Rxi®-5Sil MS column) and have very similar mass spectra.



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

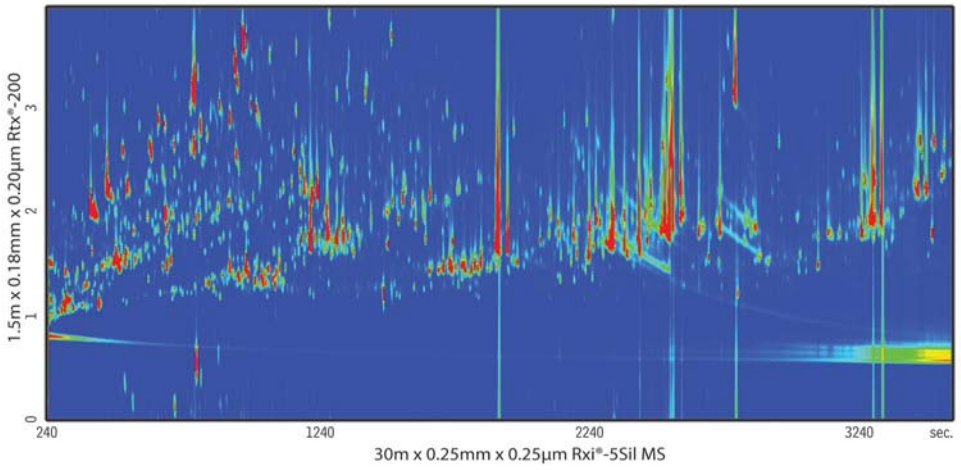
Figure 2 GCxGC-TOFMS can be used to separate compounds that coelute in complex dietary supplement matrices when analyzed by single dimension GC-TOFMS.

A. Dandelion root



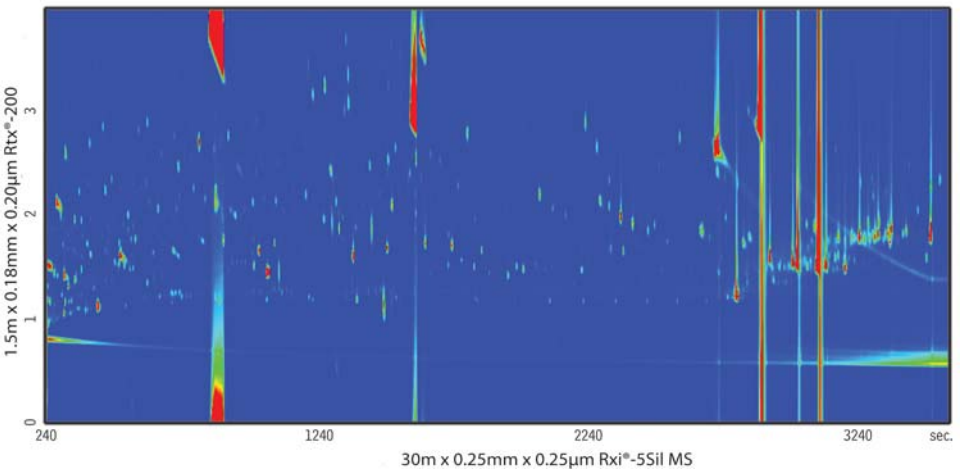
GC_FF1189

B. Sage



GC_FF1190

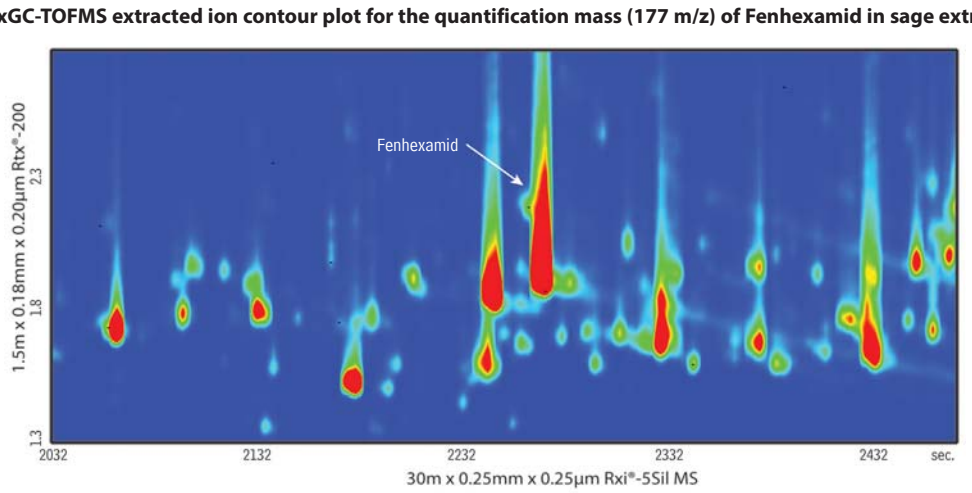
C. Finished Product



GC_FF1191

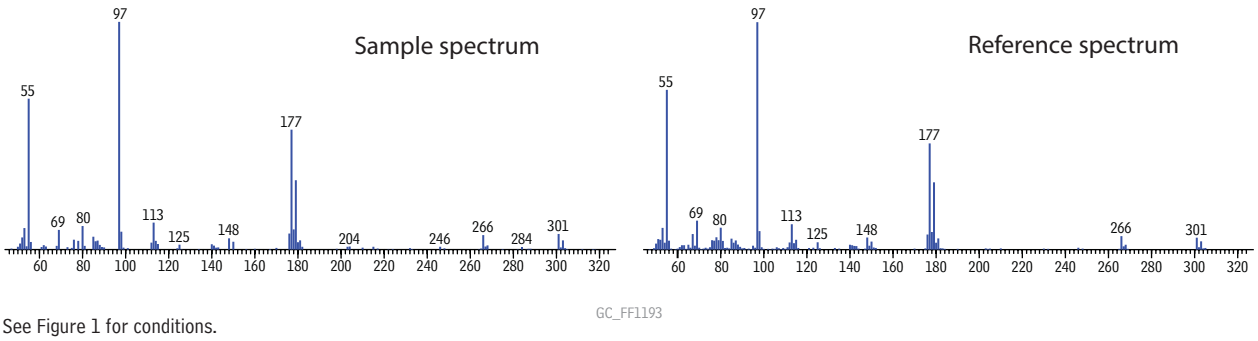
See Figure 1 for conditions.

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



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.

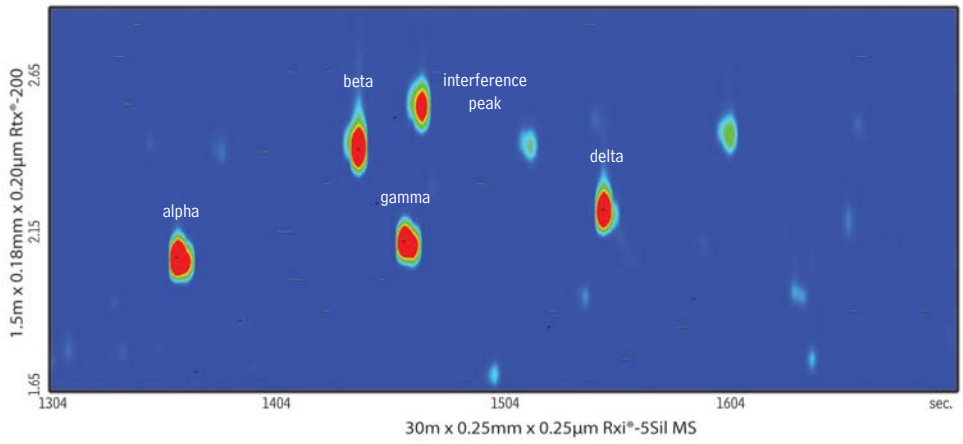


See Figure 1 for conditions.

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.

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.

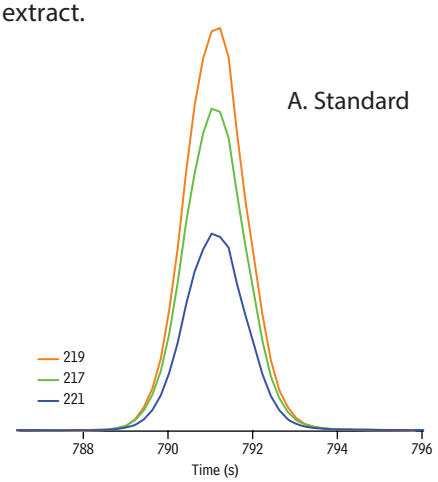
GCxGC-TOFMS extracted ion contour plot for the quantification mass (219 m/z) of the hexachlorocyclohexanes in sage extract.



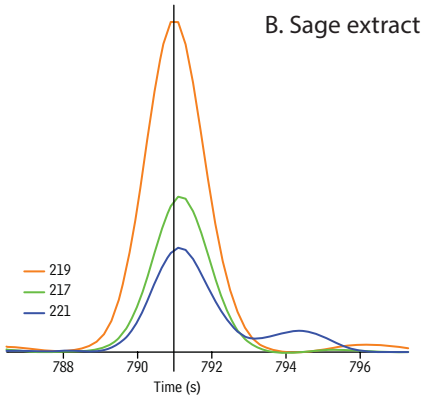
GC_FF1194

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.



GC_FF1196



GC_FF1195

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

1. Developing New Methods for Pesticides in Dietary Supplements Advantages of the QuEChERS Approach. http://www.restek.com/restek/images/external/PHAN1242.pdf (accessed June 21, 2010).
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.

Description	Material	Methods	qty.	cat#
Extraction Salt Packets and 50mL Centrifuge Tubes				
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

Q-sep™ 3000 Centrifuge

for QuEChERS

Description	qty.	cat.#
Q-sep 3000 Centrifuge, 110V	ea.	26230
Q-sep 3000 Centrifuge, 220V	ea.	26231
Replacement Accessories		
50mL Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26232
50mL Conical Tube Insert for Q-sep 3000 Centrifuge	6-pk.	26249
4-Place Tube Carrier for Q-sep 3000 Centrifuge	2-pk.	26233
2mL Tube Adaptors for Q-sep 3000 Centrifuge	4-pk.	26234

Pesticide Residue Cleanup SPE Cartridges

- Convenient, multiple adsorbent beds in a single cartridge.
- For use in multiple-residue pesticide analysis, to remove matrix interferences.

SPE Cartridge	qty.	cat#
6mL Combo SPE Cartridge Packed with 500mg CarboPrep 90/500mg PSA, Polyethylene Frits	30-pk.	26194

Rtx®-200 (fused silica)

(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

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

Press-Tight® Connectors

- Deactivated Press-Tight® connectors assure better recovery of polar and nonpolar compounds.
- Siltek® treated connectors are ideal for organochlorine pesticides analysis.
- Fit column ODs from 0.33–0.74mm (Restek 0.1mm–0.53mm ID).
- 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

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-dichloroisopropyl)phosphate	50
PCB 28	50		
PCB 52	50	triphenylmethane	10
triphenyl phosphate	20		

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

Visit **www.restek.com/quechers** for detailed technical literature and a complete line of QuEChERS products.

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Contact us or your local Restek® representative (www.restek.com/contact-us).

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Lit. Cat.# EL?L/116

Detecting Illegal Dyes in Foods

Identify Four Sudan Dyes in One HPLC Analysis, Using an Ultra Aqueous C18 Column

By Julie Kowalski, Innovations Chemist

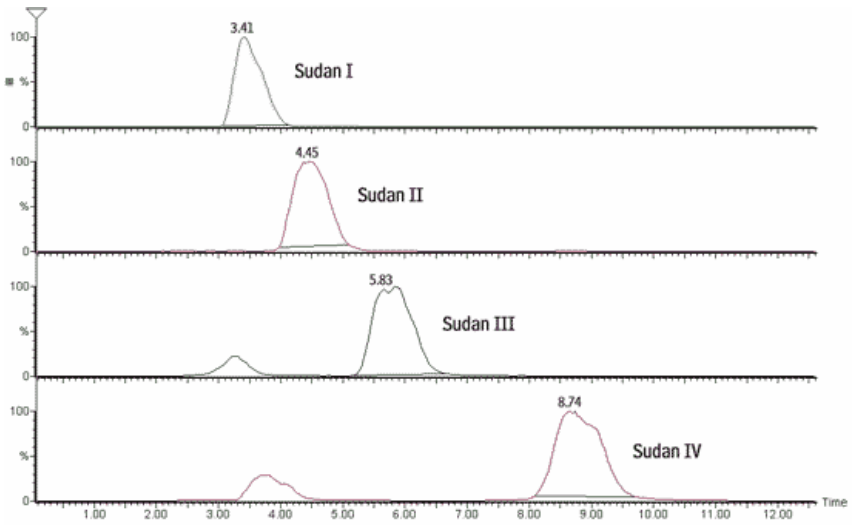
Sudan I, Sudan II, Sudan III and Sudan IV (Scarlet Red) are synthetic industrial azo-dyes used in waxes, plastics, oils and polishes. Sudan dyes sometimes are added to foods such as chili powder to mimic, intensify and prolong the appearance of natural red hues. The International Agency for Research on Cancer (IARC) classifies Sudan dyes as Class 3 carcinogens and, therefore, it is illegal to use them as food additives, according to the FDA and EU. (2,3) Since 2003, European nations have required random product testing and testing of suspected adulterated products, and Sudan dyes recently have been found in food products in some European countries. More than six hundred products containing Sudan dyes have been recalled in the UK—the largest food recall in British history. (1)

Laboratories performing analysis of Sudan dyes are not required to follow defined methods. However, the EU has set detection limits for these dyes at 0.5 - 1 mg/kg, and has stated that any foods or food ingredients found to contain more than the established limit should be withdrawn from the market. (1)

HPLC MS of Sudan I, Sudan II, Sudan III and Sudan IV

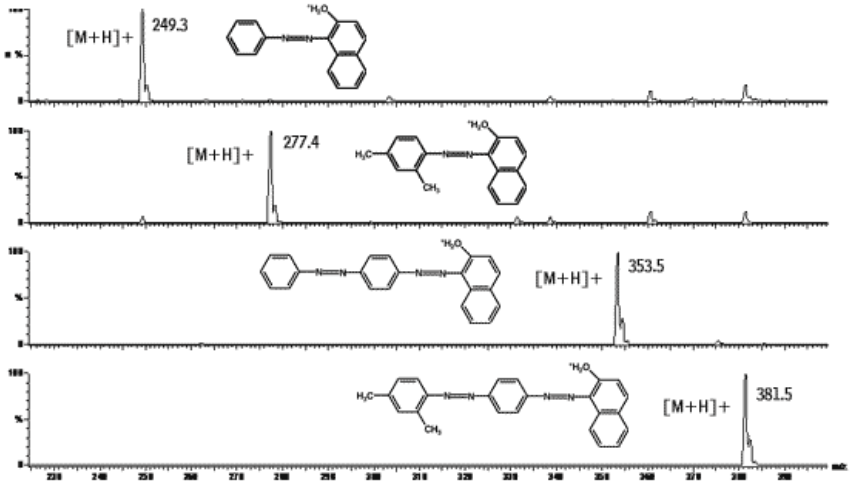
Sudan dyes were prepared as in *Simple HPLC Analysis for Sudan Dyes* and analyzed using an update of the method described there. Ten microliters of a 1ppm solution were injected, via a Waters 717plus autosampler. A 1525 binary pump was used to provide an isocratic flow of 0.15 mL/minute. The mobile phase was acetonitrile: methanol (70:30, v/v), each containing 0.1% formic acid. A Waters ZMD mass spectrometer was used in positive electrospray mode, 3.5 kVolts capillary voltage, 10 volts cone voltage, 150°C source temperature and 200°C desolvation temperature. An Ultra Aqueous C18 column (150 x 4.6mm, cat.# 9178565) and positive electrospray mass spectrometry were used to identify the four Sudan dyes in less than ten minutes. The cone voltage was optimized to discourage in-source fragmentation. Extracted ion chromatograms and mass spectra for the Sudan dyes are shown in Figure 1 and Figure 2.

LCMS DATA
Extracted m/z values for [M+H]+ of Sudan I, II, III, IV



This figure shows the extracted ion chromatograms for Sudan I (m/z 249), Sudan II (m/z 277), Sudan III (m/z 353) and Sudan IV (m/z 381).

Sudan Dyes Spectra



This figure shows the mass spectra for Sudan I (m/z 249), Sudan II (m/z 277), Sudan III (m/z 353) and Sudan IV (m/z 381). These were produced by manually combining spectra and subtracting background.

References

1. www.ift.org
2. Commission Decision of 20 June 2003 on emergency measures regarding hot chilli and hot chilli products, notified under document number C(2003) 1970, (2003/460/EC), OJ L. 154/114, 21.6.2003.
3. Implementation of Commission Decision 2003/460/EC of the 21st January 2004.

Other Sources

1. Journal of Chromatography A: 1042, 2004, 123-130
2. Journal of Chromatography A: 1058, 2004, 127-135

RELATED SEARCHES

Sudan I, *Sudan II*, *Sudan III*, *Sudan IV*, *Scarlet Red*, *azo-dyes*, *Ultra Aqueous C18*

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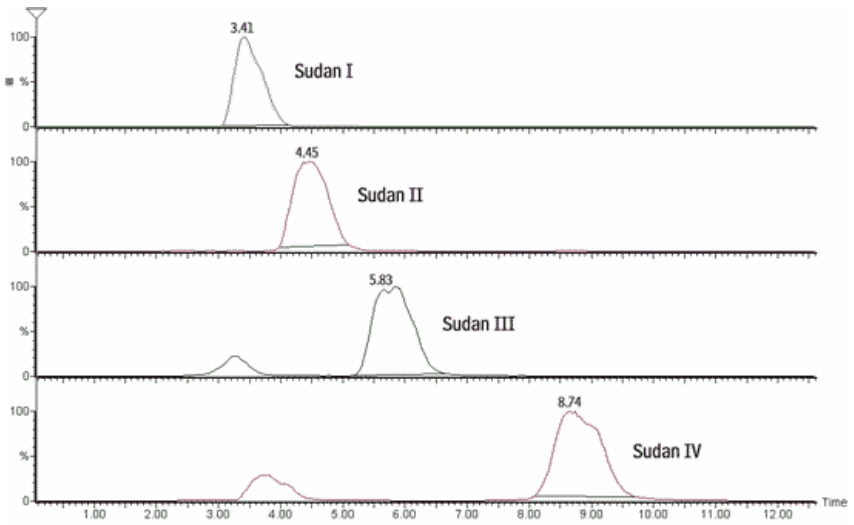
LCMS DATA
Extracted m/z values for [M+H]+ of Sudan I, II, III, IV



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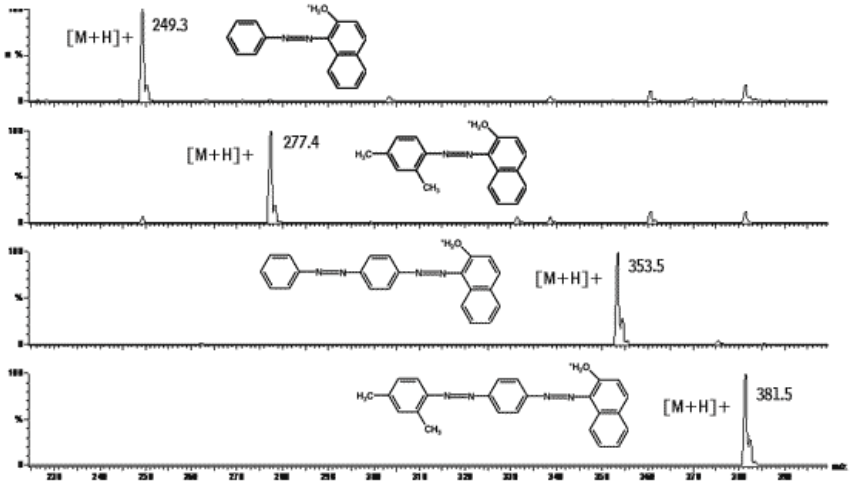
Sudan Dyes Spectra



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Other Sources

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2. Journal of Chromatography A: 1058, 2004, 127-135

RELATED SEARCHES

Sudan I, *Sudan II*, *Sudan III*, *Sudan IV*, *Scarlet Red*, *azo-dyes*, *Ultra Aqueous C18*

Foods, Flavors & Fragrances

Comprehensive Pesticide Residue Analysis by LC/MS/MS

Using an Ultra Aqueous C18 Column

By Becky Wittrig, Ph.D., Global HPLC Specialist, and André Schreiber, Ph.D., AB SCIEX

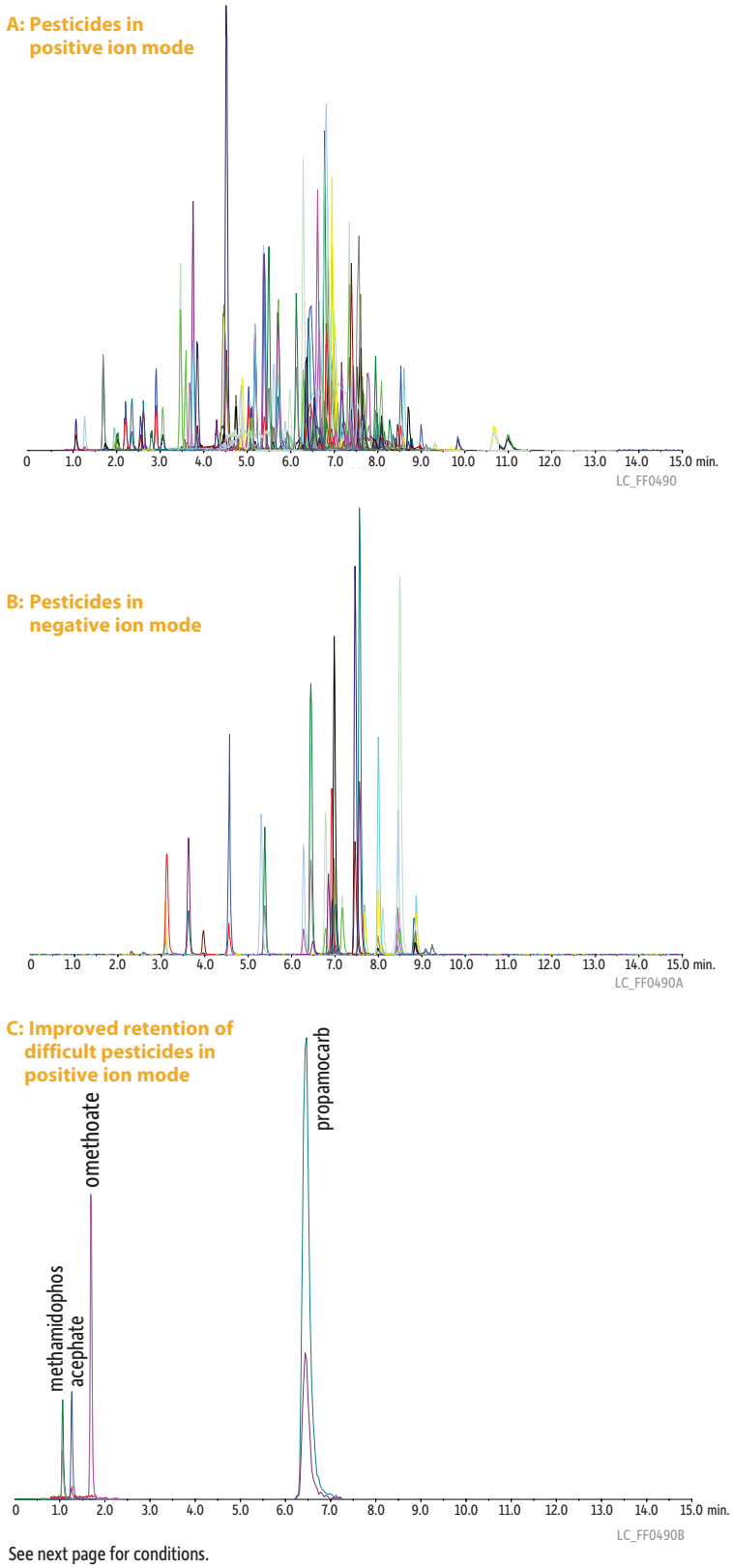
- Easily resolve and quantify more than 280 pesticide species.
- Use LC/MS/MS to reliably monitor difficult polar and/or thermally unstable species.
- Aqueous C18 phase offers optimal selectivity and retention.

Food safety is a topic of great interest globally. With recent contamination issues in a wide range of commodities, ensuring the quality of our food supply is becoming increasingly important. Pesticide residue content is one area of concern. While pesticides have typically been monitored by gas chromatography, polar and/or thermally unstable pesticides are difficult or impossible to monitor using this approach. Thus, traditional HPLC techniques are used for select pesticide classes, such as the carbamate and phenylurea pesticides.

With recent advances in LC/MS/MS instrumentation, this technique is quickly gaining acceptance for pesticide residue testing. LC/MS/MS can be used to simultaneously monitor hundreds of potential contaminants—including those difficult to detect by GC. Using both LC/MS/MS and GC approaches allows for a faster, more complete picture of pesticide residues. MS/MS technology also permits identification of the target pesticides through the selection of specific MRM transitions for each compound. For example, aldicarb, a carbamate pesticide, uses two MRM transitions of 208.2→89.1amu and 208.2→116.1amu.

While the MS/MS detector allows for specific, sensitive detection of the pesticide species, the LC separation is still important to ensure the highest quality data. Conventional C18 stationary phases are typically used for pesticide monitoring, but the selectivity and retention is poor for more polar species. In contrast, Ultra Aqueous C18 columns are ideal for multi-pesticide residue monitoring methods. In Figure 1, the analysis of more than 280 pesticides using the 3µm Ultra Aqueous C18 is shown. Optimized stationary phase selectivity

Figure 1 More than 280 pesticide residues—including difficult polar species—show excellent peak shape and retention on a 3µm Ultra Aqueous C18 column.



See next page for conditions.

Conditions for Figure 1 (previous page):

Sample:	multicomponent pesticide standard
Inj.:	10µL
Conc.:	1ppb each pesticide
Sample diluent:	water
Column:	Ultra Aqueous C18
Cat. #:	9178312
Dimensions:	100mm x 2.1mm
Particle size:	3µm
Pore size:	100Å
Conditions:	
Instrument:	Shimadzu Prominence® UFLCXR
Mobile phase:	A: 10 mM NH_4OAc in water B: 10 mM NH_4OAc in methanol

Time (min.)	%B
0.0	20
8.0	90
12.0	100
14.8	100
14.9	20

Flow:	500µL/min
Temp.:	35°C
Det.:	Applied Biosystems 4000 QTRAP® LC/MS/MS system
Ion Source:	TurboIonSpray® A & C: ESI+ B: ESI-
IonSpray Voltage:	5kV (ESI+), -4.2kV (ESI-)
Gas 1:	50psi
Gas 2:	60psi
Source Temp.:	600°C

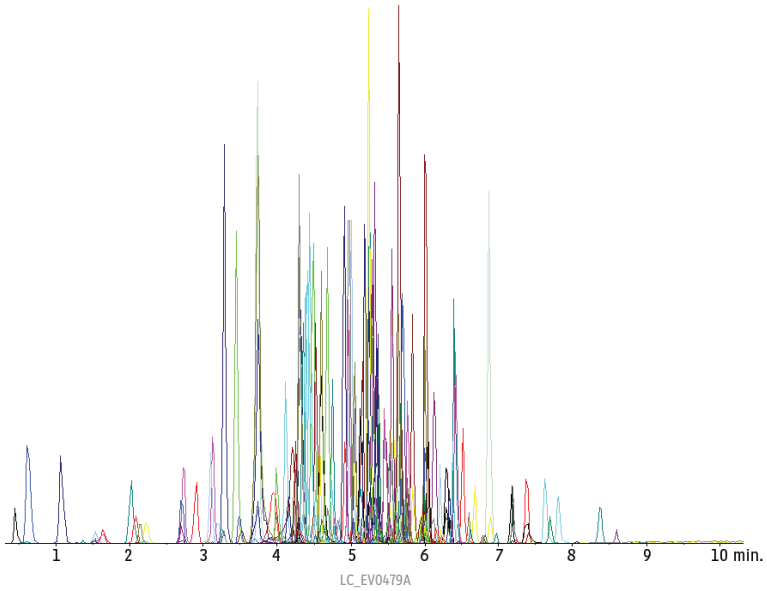
Table I Peak list for pesticides in negative ion mode.

Compound ID	Retention Time (min)	Transition 1	Transition 2
Acrinathrin	9.09	540 → 372	540 → 299.9
Chlorfluazuron	9.24	539.9 → 356.8	539.9 → 519.7
Clothianidin	3.63	249.9 → 58	247.9 → 59
Diflubenzuron	7.68	309 → 156.1	310 → 288.9
Diuron	6.78	230.9 → 185.8	230.9 → 149.8
Fluazinam	7.99	462.5 → 415.8	462.5 → 397.9
Fludioxonil	6.93	246.9 → 179.9	246.9 → 125.9
Forchlorfenuron	6.44	246 → 126.9	246 → 91.2
Hexaflumuron	8.45	459 → 438.8	459 → 174.9
Imibenconazol	8.82	409 → 250.9	411 → 253
Lufenuron	8.87	509 → 325.9	509 → 175
Metaflumizon	8.5	505.1 → 301.9	505.1 → 140.9
Metamitron	3.97	201 → 184.8	201 → 116.9
Methoxyfenozid	6.98	366.9 → 104.9	366.9 → 148.9
Nitenpyram	2.59	269.2 → 221.6	269.2 → 100.8
Novaluron	8.42	491.1 → 470.7	493.1 → 472.7
Profoxydim	7.57	464.3 → 277.9	464.3 → 126.8
Propoxycarbazon	3.09	396.9 → 156	396.9 → 112.9
Prothioconazol	7.16	342 → 100.1	343.9 → 99.9
Tebufenozid	7.46	350.9 → 149	350.9 → 105
Teflubenzuron	8.85	378.6 → 338.8	378.6 → 195.9
Tepraloxydim	4.57	340 → 248	340 → 220.1
Terbacil	5.38	214.9 → 158.9	216.9 → 160.9
Tralkoxydim	6.28	328.1 → 253.8	328.1 → 281.8
Triadimefon	7.01	292 → 67.9	292 → 234.9
Triflumuron	8	356.9 → 153.8	358.9 → 155.9

allows for an even distribution of the compounds throughout the retention time window (Tables I and II). As well, retention of more polar pesticides is greatly improved, as demonstrated in Figure 1C. The Ultra Aqueous C18 column, in a 100 x 2.1mm, 3µm configuration is the column of choice for LC/MS/MS pesticide monitoring methods.

Ultra-high pressure LC (UHPLC) can also be used with MS/MS detection for monitoring pesticide residues. UHPLC allows for higher sample throughput when used in conjunction with a highly efficient <2µm particle size column. The 1.9µm Pinnacle® DB Aqueous C18, in a 50 x 2.1mm configuration, is ideally suited for this application, as shown in Figure 2.

Figure 2 Higher sample throughput can be achieved using UHPLC and MS/MS with a 1.9µm Pinnacle® DB Aqueous C18 column.



Compounds analyzed are a subset of those in Figure 1; data are shown for a qualitative overall run time comparison only.

Sample:	multicomponent pesticide standard
Inj.:	5µL
Conc.:	33.3ppb each pesticide
Sample diluent:	water
Column:	Pinnacle® DB Aqueous C18
Cat.#:	9418252
Dimensions:	50mm x 2.1mm
Particle size:	1.9µm
Pore size:	140Å

Conditions:	
Mobile phase:	A: 10mM NH_4OAc in water B: 10mM NH_4OAc in methanol

Time (min.)	%B
0.0	10
1.0	10
8.0	90
10.0	90
11.0	10

Flow:	600µL/min.
Temp.:	35°C
Instrument:	Shimadzu Prominence® UFLCXR
Det.:	Applied Biosystems 4000 QTRAP® LC/MS/MS system Ion Source: TurboIonSpray®, ESI+ IonSpray Voltage, ESI voltage: 5kV (ESI+) Gas 1: 40psi Gas 2: 60psi Source Temp.: 500°C; max pressure ~7,200psi

Using LC/MS/MS technology and Aqueous C18 columns, in combination with gas chromatography, results in the most comprehensive monitoring of pesticide residues. Labs interested in more complete multi-residue analysis of pesticides in food matrices, including difficult polar or thermally unstable compounds, should consider adding LC/MS/MS and Aqueous C18 columns to routine testing procedures. The Aqueous C18 phase is also available on 1.9µm Pinnacle® DB silica for UHPLC platforms.

Acknowledgements
The authors wish to thank the US FDA for their collaboration and recognize the participation of multiple FDA labs in this work.

Table II Peak list for pesticides in positive ion mode (continued on next page).

Compound ID	Retention Time (min)	Transition 1	Transition 2
Acephate	1.27	184.1 → 143	184.1 → 125
Acetamiprid	4.44	223.2 → 126.1	223.2 → 99.1
Acibenzolar-S-methyl	7.22	211 → 136	211 → 140
Alanycarb	7.91	400.1 → 238.2	400.1 → 91.1
Aldicarb	4.49	208.2 → 116.1	208.2 → 89.1
Aldicarbsulfone	2.03	223.1 → 86.1	223.1 → 148
Aldicarbsulfoxide	2.2	207.1 → 132.1	207.1 → 89.1
Ametryn	6.96	228.1 → 186.1	228.1 → 96
Aminocarb	5.37	209.1 → 137.1	209.1 → 152.1
Avermectin B1a	11.2	890.5 → 305	890.5 → 145
Avermectin B1b	11.4	876.5 → 291	876.5 → 145
Azoxystrobin	6.78	404.1 → 372.1	404.1 → 344.1
Benalaxyl	7.78	326.2 → 148.2	326.2 → 91.1
Bendiocarb	5.1	224.2 → 109.2	224.2 → 167.2
Benfuracarb	8.33	411.2 → 195.1	411.2 → 252.1
Benthiavalicarb	6.87	382.1 → 116	382.1 → 180.1
Benthiavalicarb-isopropyl	6.87	382.1 → 196.8	382.1 → 179.9
Benzoximate	7.98	364 → 199	364 → 105
Bitertanol	7.98	338 → 70	338 → 269
Boscalid	6.98	343 → 307	343 → 140
Bromuconazole (isomer 1)	7.36	378 → 159.1	378 → 161
Bromuconazole (isomer 2)	8.05	378.1 → 159.1	378.1 → 161
Bufencarb	5.18	222.2 → 95.1	222.2 → 71
Bupirimate	7.65	317 → 166	317 → 108
Buprofezin	8.53	306.2 → 201.1	306.2 → 116.2
Butafenacil	7.08	492.2 → 331.1	492.2 → 180.1
Butocarboxim	4.4	191.1 → 75	191.1 → 116
Butocarboximsulfoxid	2.1	207 → 74.9	207 → 90
Butoxycarboxim	1.95	223.1 → 106	223.1 → 166
Carbaryl	5.63	202.1 → 145	202.1 → 127
Carbendazim	4.52	192.2 → 160.2	192.2 → 132.1
Carbetamide	4.74	237 → 192	237 → 118
Carbofuran	5.18	222.2 → 123.1	222.2 → 165.2
Carboxine	5.61	236 → 143	236 → 87
Carfentrazone-ethyl	7.53	412 → 346	412 → 366
Chlorfluazuron	9.18	540 → 158	540 → 383
Chloroxuron	7.53	291 → 72	291 → 218
Chlorpyrifos	8.35	350 → 198	350 → 96.9
Chlorpyrifos-methyl	7.88	324 → 125.1	322 → 125.1
Chlortoluron	6.31	213.1 → 72.2	213.1 → 46.2
Cinidon-ethyl	8.71	394.1 → 348.1	394.1 → 107
Clethodim	5.81	360 → 164	360 → 268
Clofentezine	8.27	303 → 138	303 → 102
Clomazone	7.04	240.1 → 125	240.1 → 89.1
Clothianidin	3.35	250 → 169.1	250 → 132
Cyanazine	5.51	241.2 → 214.2	241.2 → 104.1
Cyazofamid	7.4	325 → 108	325 → 261
Cycluron	6.71	199.1 → 89.1	199.1 → 89
Cymoxanil	3.91	199 → 128	199 → 111
Cyproconazole (isomer 1)	7.45	292.2 → 70.2	292.2 → 125.2
Cyproconazole (isomer 2)	7.44	292.1 → 70.2	292.1 → 125.2
Cyprodinil	8.15	226 → 93	226 → 77
Cyromazine	2.63	167.2 → 85.1	167.2 → 68.25
Demeton-S-methyl	2.9	230.9 → 89.1	230.9 → 61
Demeton-S-methyl-sulfon	5.6	262.9 → 108.9	262.9 → 169
Desethyl-atrazine	3.9	188.1 → 146.2	188.1 → 104.1
Desisopropyl-atrazine	3.2	174.1 → 104.1	174.1 → 96.1
Desmedipham	6.23	318 → 182	318 → 136
Desmethyl-pirimicarb	5.11	225 → 72	225 → 168.1
Diazinon	7.88	305.1 → 169.2	305.1 → 97
Dichlorvos	5.36	221 → 109.1	223.1 → 109.1
Diclobutrazol	7.58	328 → 70	328 → 160
Dicrotophos	3.47	238 → 112	238 → 193
Diethofencarb	6.56	268 → 226	268 → 180
Difenoconazole (isomer 1)	8.35	406.2 → 251.1	408.2 → 253.1
Difenoconazole (isomer 2)	8.35	406.1 → 251.1	408.1 → 253.1
Difenoxuron	6.78	287.2 → 123.2	287.2 → 72
Diflubenzuron	7.62	311 → 158.2	311 → 141.2
Dimethenamide	6.9	276.2 → 244.1	276.2 → 168.3
Dimethoate	3.58	230 → 125	230 → 199.1
Dimethomorph	7.22	388.2 → 301.1	388.2 → 165.2
Dimoxystrobin	7.6	327.1 → 205	327.1 → 116
Diniconazole	8.07	326 → 70	326 → 159
Dinotefuran	2.02	203.1 → 114.1	203.1 → 129
Dioxacarb	3.68	224 → 123	224 → 167.1
Diphenylamin	7.03	170.1 → 93	170.1 → 92
Disulfoton	6.08	275.1 → 89	275.1 → 61
Diuron	6.7	233.1 → 72	235.1 → 72.1
Edifenphos	6.66	311 → 283	328 → 283
EPN	6.72	324 → 157.1	324 → 296
Epoxiconazole	7.65	330 → 121	330 → 101
Etaconazole	7.57	328.2 → 159.1	328.2 → 123
Ethiofencarb	5.58	226.1 → 106.9	226.1 → 164.1
Ethiofencarbsulfoxid	3.48	242 → 107	242 → 185.1
Ethion	7.9	385 → 199	385 → 171
Ethiprole	6.62	397.3 → 351	397.3 → 255.5
Ethofenprox	9.66	394.1 → 107.1	394.1 → 177.2
Ethofumesate	6.54	304 → 121	304 → 161
Ethoprophos	5.98	243 → 131	243 → 97
Ethoxyquin	7.4	218.1 → 174	218.1 → 160
Etoxazole	9	360.1 → 141	360.1 → 57.2
Famoxadone	7.72	392 → 331	392 → 238
Fenamidone	6.65	312.1 → 92.1	312.1 → 65
Fenamiphos	7.5	304.2 → 217.1	304.2 → 202.1
Fenarimol	7.3	331 → 268	331 → 81
Fenazaquin	9.91	307 → 161	307 → 147
Fenbuconazole	7.57	337 → 125	337 → 70
Fenhexamid	7.04	302 → 97	302 → 55
Fenitrothion	6.4	278 → 125.2	278 → 109
Fenoxycarb	7.53	302.2 → 88.1	302.2 → 116.2
Fenpropimorph	9.84	304 → 147	304 → 117
Fenpyroximate	9.29	422 → 366.1	442 → 135.1
Fenthion	6.9	278.9 → 169	278.9 → 246.9
Fenuron	3.85	165.1 → 72.1	165.1 → 46
Flonicamid	2.25	230.1 → 203.1	230.1 → 174
Flucarbazone	2.81	397.1 → 130.1	397.1 → 115
Flufenacet	7.17	364.1 → 152.2	364.1 → 194.2
Flufenoxuron	8.98	489.1 → 158.2	489.1 → 141.2
Fluometuron	5.98	233.1 → 72.1	233.1 → 46
Fluopicolid	7.2	385 → 174.8	383 → 173
Fluoxastrobin	7.34	459.2 → 427.2	459.2 → 188
Fluquinconazole	7.31	376 → 349	376 → 307
Flusilazole	7.6	316 → 247	316 → 165
Flutolanil	6.81	341.1 → 242.1	341.1 → 262.1
Flutriafol	5.99	302 → 123	302 → 109
Fonophos	5.9	247 → 183	247 → 201
Forchlorfenuron	6.37	248 → 93.1	248 → 165.1
Formetanate	4.48	222 → 165.1	222 → 120
Fuberidazole	5.5	185 → 157	185 → 65
Furalaxyl	6.62	302.1 → 95.1	302.1 → 242.1
Furathiocarb	8.42	383.2 → 195.2	383.2 → 252.2
Hexaconazole	7.95	314 → 70	314 → 159
Hexaflumuron	8.42	461.1 → 158.2	461.1 → 141.1
Hexythiazox	8.77	353 → 228	353 → 168
Hydramethylnon	9.2	495.2 → 323.1	495.2 → 151
Imazalil	8.1	297.1 → 159.2	299.1 → 161.2
Imazapyr	7.3	262.2 → 217.2	262.2 → 202.2
Imidacloprid	3.82	256.2 → 209	256.2 → 175.2
Indoxacarb	8.03	528 → 203	528 → 56
Ipconazole	8.33	334.2 → 70	334.2 → 125
Iprodion	7.6	330.1 → 244.9	332.1 → 247
Iprovalicarb	6.98	321.2 → 119	321.2 → 203.2
Irgarol	7.72	254.2 → 198.1	254.2 → 83.2
Isofenphos	6.66	314 → 120	314 → 162.1
Isoprocarb	5.87	194.2 → 95.1	194.2 → 137.2
Isoproturon	6.42	207.2 → 72.1	207.2 → 46.1
Isoxaflutole	5.87	360.1 → 251.1	360.1 → 220.1
Kresoxim-methyl	7.53	314 → 116	314 → 206
Lenacil	6.5	235.3 → 153.2	235.3 → 136.2
Linuron	6.99	249.1 → 160	249.1 → 182.1
Lufenuron	8.83	511.1 → 158.1	511.1 → 141.2
Malathion	6.83	331 → 99.1	331 → 127.1
Mandipropamid	6.81	412.1 → 328.1	412.1 → 355.9
Mefenacet	7.35	299 → 148.1	299 → 120.1
Mepanipyrim	7.57	224 → 106	224 → 77
Mepronil	6.86	270.1 → 119.1	270.1 → 228
Metalaxyl	6.13	280.2 → 220.2	280.2 → 192.3
Metconazole	8.01	320 → 70	320 → 125
Methabenzthiazuron	6.56	222.1 → 165.2	222.1 → 150.3
Methamidophos	1.06	142 → 94	142 → 125
Methiocarb	6.82	226.1 → 169.2	226.1 → 121.1
Methomyl	2.62	163.1 → 88.1	163.1 → 106
Methoprotryne	6.95	272.2 → 240.2	272.2 → 198
Methoxyfenozide	6.9	369 → 149	369 → 133
Metobromuron	6.22	259 → 170.2	259 → 148.2
Metolachlor	7.5	284.2 → 252.2	284.2 → 176.2
Metoxuron	5.1	229 → 72.1	229 → 156.1
Metribuzin	5.14	215.1 → 187.2	215.1 → 84.1

Table II Peak list for pesticides in positive ion mode (continued from previous page).

Compound ID	Retention Time (min)	Transition 1	Transition 2
Mevinphos	4.29	225 → 127	225 → 193
Mexacarbate	7.02	223.2 → 166.2	223.2 → 151
Milbemectin A3	10.3	546.4 → 511.3	546.4 → 493.3
Milbemectin A4	10.5	560.4 → 525.4	560.4 → 55.2
Molinate	7.3	188.2 → 126.2	188.2 → 55.1
Monocrotophos	2.9	224 → 127	224 → 98
Monolinuron	5.93	215.1 → 126.1	215.1 → 99
Monuron	5.7	199.2 → 72.2	199.2 → 126.3
Myclobutanil	7.17	289 → 70	289 → 125
Neburon	7.65	275 → 88	275 → 114
Nitenpyram	2.55	271.2 → 126.1	271.2 → 237.2
Novaluron	8.38	493 → 158.1	493 → 141.1
Nuarimol	6.7	315 → 252	315 → 81
Omethoate	1.69	214 → 124.9	214 → 182.8
Oxadixyl	4.85	279.2 → 219.2	279.2 → 132.1
Oxamyl	2.35	237.1 → 72.1	237.1 → 90.1
Oxydemeton-methyl	3.1	247 → 169	247 → 109
Paclobutrazol	6.82	294 → 70	294 → 125
Parathion-ethyl	6.7	292.1 → 236.2	292.1 → 94.1
Parathion-methyl	7.6	263.9 → 232.1	263.9 → 125
Penconazole	8.01	284 → 159	284 → 70
Pencycuron	8.1	329.1 → 125.1	331.2 → 127
Phenmedipham	6.35	301.1 → 136	301.1 → 168.1
Phenthoate	7	321 → 163	321 → 79
Phosmet	6.7	318 → 160	318 → 133
Phoxim	7.9	299.1 → 129.1	299.1 → 77.1
Picoxystrobin	7.44	368 → 145	368 → 205
Pinoxaden	7.99	401.3 → 317.2	401.1 → 57
Piperonyl butoxide	8.62	356.2 → 177.2	356.2 → 119
Pirimicarb	6.29	239.2 → 72.1	239.2 → 182.2
Pirimicarb-desmethylformamido	6.4	253.2 → 72.1	253.2 → 225.3
Pirimiphos-ethyl	7.48	334 → 198.1	334 → 182.1
Prochloraz	8.29	376.1 → 308	376.1 → 70.1
Promecarb	6.86	208.2 → 109.1	208.2 → 151.1
Prometon	6.86	226.1 → 142.1	226.1 → 86
Prometryn	7.4	242.2 → 200.1	242.2 → 158.1
Propachlor	6.2	212.2 → 170.1	212.2 → 94.1
Propamocarb	6.61	189.2 → 102.2	189.2 → 73.9
Propargite	8.79	368 → 231	368 → 175
Propazine	6.9	230.1 → 146.1	230.1 → 188.1
Propham	5.78	180 → 138	180 → 120
Propiconazole	7.98	342.1 → 159.1	342.1 → 69.1
Propoxur	5.03	210.1 → 111	210.1 → 168.1
Prosulfocarb	8.5	252.3 → 91.1	252.3 → 128.1
py Cinerin I	9	317.2 → 149	317.2 → 106.9
py Cinerin II	8.2	361.2 → 149	361.2 → 106.9
Pymetrozin	3.61	218 → 105	218 → 78
Pyracarbolid	5.4	218.2 → 125	218.2 → 97
Pyraclostrobin	7.95	388 → 194	388 → 163
Pyrazophos	7.8	374 → 222	374 → 194
Pyrethrin I	8.9	329.2 → 160.9	329.2 → 132.9
Pyrethrin II	8.3	373.1 → 160.9	373.1 → 308.9
Pyridaben	9.33	365 → 147	365 → 309
Pyridaphenthion	7.8	341 → 189	341 → 205
Pyrimethanil	7.24	200 → 107	200 → 82
Pyriproxyfen	8.72	322 → 96	322 → 185
Quinalphos	6.7	299 → 147	299 → 163
Quinoxyfen	9.12	308 → 197	308 → 162
Rotenone	7.61	395 → 213	395 → 192
Secbumeton	6.85	226.2 → 170.1	226.2 → 100
Siduron	6.55	233.3 → 137.2	233.3 → 94
Simetryn	6.36	214 → 124	214 → 144
Spinosyn A	11.3	732.6 → 142.1	732.6 → 98
Spinosyn D	11.6	746.6 → 142.1	746.6 → 98
Spirodiclofen	8.96	411.3 → 313.2	411.3 → 213.1
Spiromesifen	8.8	371.3 → 273	371.3 → 255
Spiroxamine (isomer 1)	10.7; 11	298.4 → 144.2	298.4 → 100.2
Spiroxamine (isomer 2)	10.7; 11	298.3 → 144.2	298.3 → 100.2
Sulfentrazone	4.77	387 → 307.1	387 → 146
Sulfotep-ethyl	7	323 → 115	323 → 171.1
Sulprofos	7	323 → 219	323 → 247
Tebuconazole	7.8	308 → 70	308 → 125
Tebufenozide	7.39	353.1 → 133.1	353.1 → 297.1
Tebufenpyrad	8.56	334 → 117	334 → 145
Tebuthiuron	5.71	229.2 → 172.4	229.2 → 116.1
Teflubenzuron	8.81	381.1 → 141.2	381.1 → 158.2
Terbufos	6.5	289 → 103	289 → 57
Terbumeton	6.84	226 → 170	226 → 114
Terbutryn	7.57	242.2 → 186.1	242.2 → 68.1
Tetraconazole	7.3	372 → 159	372 → 70
Thiabendazole	5.71	202.1 → 175.1	202.1 → 131.2
Thiacloprid	4.89	253.1 → 126.1	253.1 → 99.1
Thiamethoxam	3.06	292 → 211	292 → 181
Thidiazuron	5.18	221.2 → 102.1	221.2 → 127.9
Thiobencarb	8.09	258.1 → 125	258.1 → 89
Thiofanox	5.7	219 → 57.1	219 → 60.9
Thiofanoxsulfon	3.4	251.1 → 75.9	251.1 → 57
Thiofanoxsulfoxid	3.6	235.1 → 104.1	235.1 → 57
Thiophanate-methyl	5.1	343 → 151	343 → 192
Tolclofos-methyl	8	301 → 175	301 → 268.9
Topramezone	1.73	364.1 → 334.1	364.1 → 125
Triadimefon	6.94	294 → 197	294 → 225
Triadimenol	7.04	296.1 → 70.1	296.1 → 227.2
Tricyclazole	5.18	190 → 163	190 → 136
Trifloxystrobin	8.09	409 → 186	409 → 206
Triflumizole	8.47	346 → 278	346 → 73
Triflumuron	7.94	359.1 → 156.2	359.1 → 139
Triticonazole	7.38	318 → 70	318 → 125
Uniconazole	7.4	292.2 → 70.1	292.2 → 43
Vamidothion	3.75	288 → 146	288 → 118
Zoxamide	7.7	336.1 → 186.9	338.1 → 188.7

HPLC Columns (USP L1)

Ultra Aqueous C18 Columns

pore size: 100Å
carbon load: 15%
endcap: no
pH range: 2.5 to 8
temperature limit: 80°C

3µm Column, 2.1mm	cat. #
100mm	9178312
100mm (with Trident Inlet Fitting)	9178312-700

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Pinnacle® DB Aqueous C18 Columns

pore size: 140Å
carbon load: 6%
pH range: 2.5 to 8
temperature limit: 80°C

1.9µm Column, 2.1mm	cat. #
50mm	9418252

Lit. Cat.# FFFF1185A-US

Foods, Flavors & Fragrances Applications

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

By Julie Kowalski, Innovations Chemist, and Jack Cochran, Director of New Business and Technology

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 $MgSO_4$, 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 $MgSO_4$
- Restek Q-sep™ Q212 (cat.# 26217), 25 mg PSA, 2.5 mg GCB, 150 mg $MgSO_4$
- Restek Q-sep™ Q213 (cat.# 26218), 25 mg PSA, 7.5 mg GCB, 150 mg $MgSO_4$
- Restek Q-sep™ Q252 (cat.# 26219), 50 mg PSA, 50 mg C18, 50 mg GCB, 150 mg $MgSO_4$

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

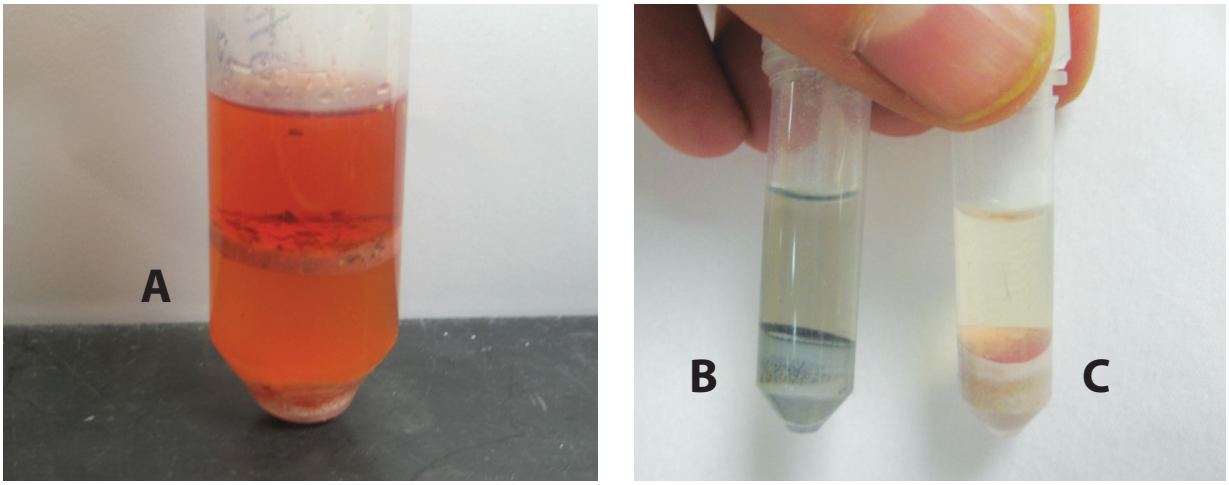
A LECO® Pegasus® 4D GCxGC-TOFMS was used for GC-TOFMS analysis and all data were processed with ChromaTOF® software. Gas chromatography was performed using a 30 m x 0.25 mm x 0.25 µm Rxi®-5Sil MS column (cat.# 13623) with a constant flow of helium at 2 mL/min. and a fast, autosampler, splitless injection of 1 µL, purge valve time of 1.5 minutes, into a 5 mm single gooseneck 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

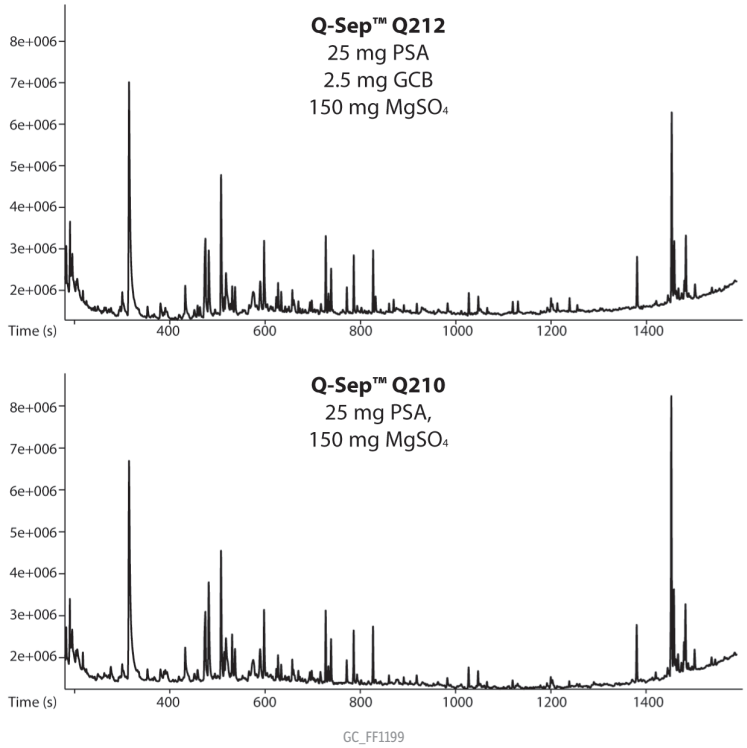
Strawberries produced a mid-intensity colored extract (Figure 1, A). The subsequent dSPE cleanup of the acetonitrile layer decreased levels of matrix co-extractives and removed some of the less volatile components, such as pigments. This reduces contamination of the GC inlet and front of the GC column 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 $MgSO_4$, and (C) Q210 tube, 25 mg PSA, 150 mg $MgSO_4$.



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.



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
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 fl w
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 Qu ChERS 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 diffe ent 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
o-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
cis-Permethrin	20.08	100	93	Organohalogen
trans-Permethrin	20.21	100	110	Organohalogen
Deltamethrin	22.53	120	88	Organohalogen

NQ = not quantifiable, due to high le el 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
o-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
cis-Permethrin	ND	ND	Organohalogen
trans-Permethrin	20.21	100	Organohalogen
Deltamethrin	ND	ND	Organohalogen

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

Incurred Pesticides in Amish and Commercially Grown Strawberries

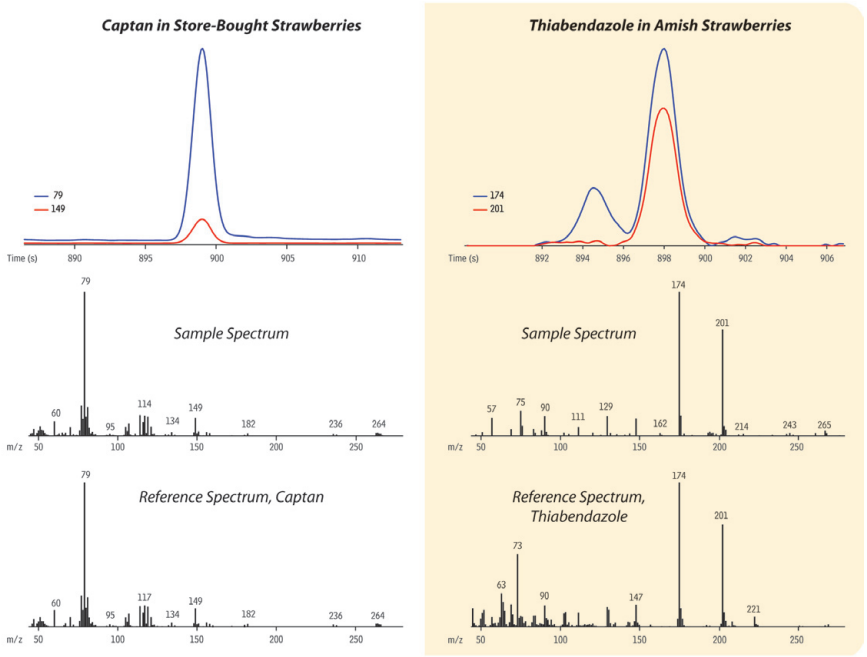
The QuEChERS dSPE sorbents discussed above both provided effective sample cleanup and good recovery values for most of the target pesticides. However, since GCB was not necessary for sample cleanup, Q-sep™ Q210 tubes were used to evaluate incurred samples in order to prevent the possible loss of low 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		
o-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.



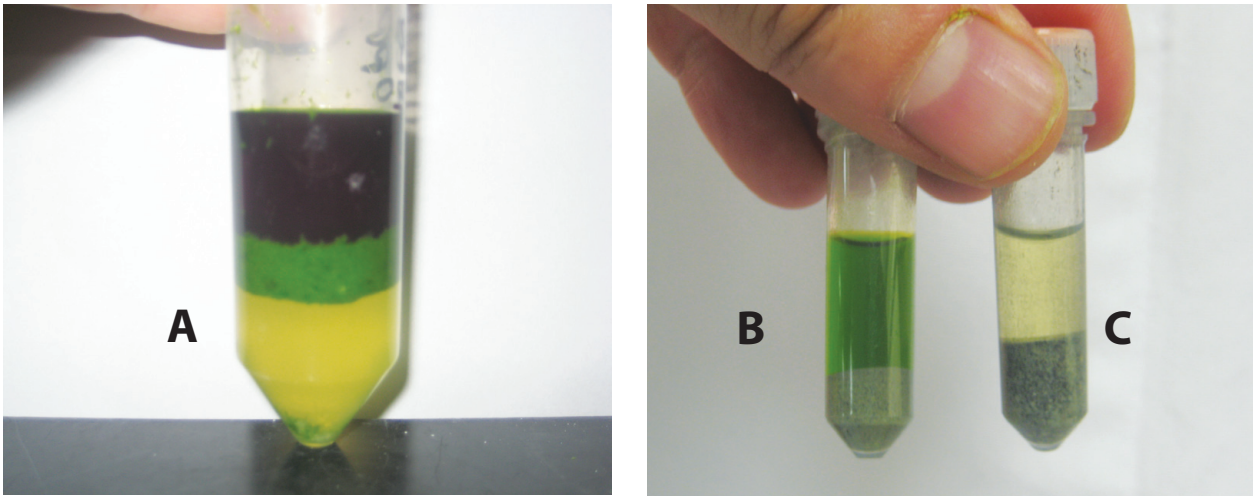
GC_FF1201

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 fl w
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

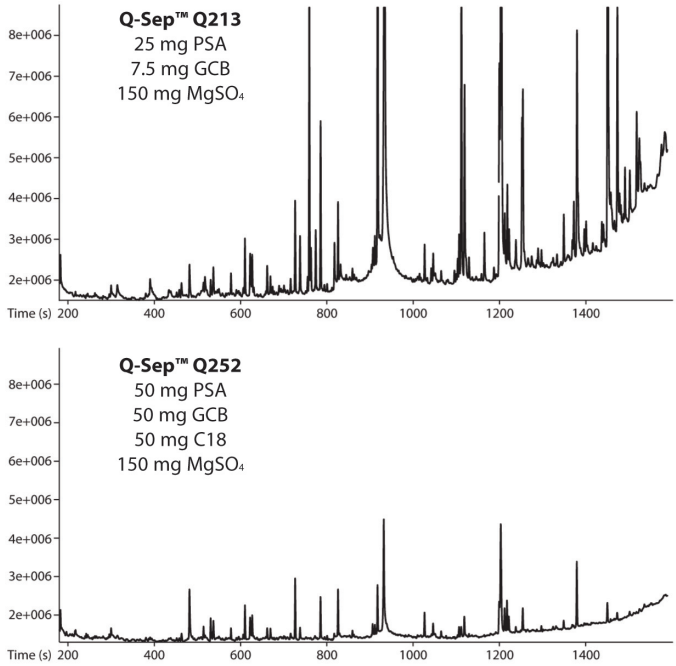
Spinach produced a very intensely colored extract (Figure 4, A). The concentrated color indicates that a large amount of pigment, chlorophyll, was extracted in acetonitrile along with the target analytes. Chlorophyll is not volatile enough to chromatograph, so it can deposit in the liner and column and 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 $MgSO_4$, and Q-sep™ Q252 tubes containing 50 mg PSA, 50 mg C18, 50 mg GCB, 150 mg $MgSO_4$. 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 $MgSO_4$, and (C) Q252 tube, 50 mg PSA, 50 mg C18, 50 mg GCB, 150 mg $MgSO_4$.



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.



GC_FF1200

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)
Diluent:	acetonitrile
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 fl w
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 Qu ChERS 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 diffe ent 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
o-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
cis-Permethrin	20.07	NQ	NQ	Organohalogen
trans-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 (50 mg PSA, 50 mg GCB, 50 mg C18)	Class
Methamidophos	ND	ND	Organonitrogen
Dichlorvos	5.58	110	Organophosphorus
Mevinphos	7.94	80	Organophosphorus
Acephate	ND	ND	Organophosphorus
o-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
Fenpropathrin	18.51	100	Organonitrogen
cis-Permethrin	20.07	100	Organohalogen
trans-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
		cis-Permethrin	1,900
		trans-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.

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Analyzing Alcoholic Beverages by Gas Chromatography

Inside:

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HOT tech tip

Fusel Alcohols

Fusel alcohols are higher-order (i.e., secondary or tertiary) alcohols, traces of which usually are present in all beers. They are produced through a pathway very similar to the pathway for ethanol, the preferred alcohol of beer. Fusel alcohols contribute a hot, spicy, solvent-like flavor and an alcohol "burn". Small amounts of these components can be desirable in a strong ale or barley wine, but they can be offensive, and therefore are unwanted, in a Pilsner or other lager. In addition to their influence on flavor, they usually cause low carbonation and poor head retention in bottle-conditioned beers, because they are deadly to yeast. Higher fermentation temperatures can produce excessively rapid yeast growth, and yeast mutations, which, in turn, stimulate the formation of these components.

Introduction

The volatile component profiles of alcoholic beverage products consist of a wide range of compounds, including acids, alcohols, aldehydes, and other trace level flavor compounds. Analysts trained in the sensory evaluation of distilled liquors, wines, or beers tell us no two products are exactly alike. The unique sensory properties of different types and brands of distilled liquor products often are due to minor differences among the volatile components present. By using instrumental methods for qualitatively or quantitatively evaluating these differences, in addition to sensory techniques, quality assurance analysts can obtain a wealth of information about their products.

In addition to alcohols and flavor compounds, impurities such as sulfur gases occasionally are present, and might lead to off odors or flavors in the product. Because even parts per billion (ppb) levels of sulfur compounds can impact product quality, a sensitive and selective method of analysis is needed to detect these impurities. The majority of these contaminants are present in the gas phase, necessitating a gas phase sampling and analysis system. Because sulfur compounds also can be very reactive, an inert analysis system is highly desirable.

Gas chromatography (GC) is a powerful tool in the analysis of alcoholic beverage products. Minimal sample preparation, in general, is required, since the samples are in the liquid state in an alcohol or alcohol/water matrix. The flavor compounds tend to be volatile in nature, which fulfills one of the main requirements of GC. General detectors, such as the flame ionization detector (FID), or more information-rich detectors, such as the mass selective detector (MSD), can be used. Additionally, the ability to automate the analysis makes GC a very practical tool in a QA/QC environment. In this guide, we will discuss how GC can be used to (1) monitor alcohol content in alcoholic beverages, (2) determine the volatile profile of a product, and (3) detect trace level impurities.

Analysis of Alcohols and Aldehydes in Alcoholic Beverages

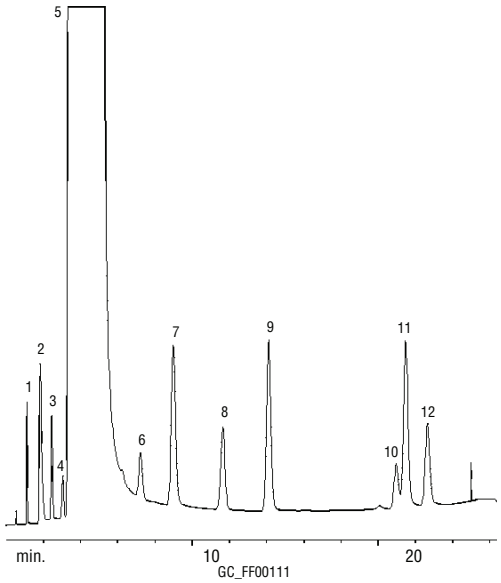
Alcoholic beverages contain a wide range of volatile compounds, including alcohols and short-chain aldehydes. Gas chromatography can be used to analyze these compounds without preliminary extractions. For example, AOAC International has published methods for the analysis of fusel oils, methanol, ethanol, and higher alcohols by GC.[1] Traditionally, packed columns prepared from glass tubing have been used for alcoholic beverage analysis, but these are prone to breakage and can adsorb some of the more reactive compounds. Restek's Silcosteel®-treated CarboBlack™ columns are made from stainless steel which has been treated to provide it with a deactivated silica surface. This conditioning significantly improves inertness and flexibility, relative to traditional glass packed columns.

CarboBlack™ packed columns can be used to quantify the various alcohols in alcoholic beverages. For example, ethanol can be monitored to determine the proof value of the beverage, while methanol and isopropanol can be quantified to determine the levels of denaturants present.[2] While poor methanol peak shapes often are associated with columns of limited sample capacity, a CarboBlack™ B packed column with 5% Carbowax® 20M provides an excellent peak shape for methanol, and completely resolves methanol from ethanol, as shown in Figure 1. In addition, the two predominant fusel oils, active amyl alcohol and isoamyl alcohol, can be resolved and monitored by using this column.

Alcohols and aldehydes in alcoholic beverages also can be monitored by capillary GC. Since capillary columns offer efficient separations, capillary GC is especially useful in analyses of structurally similar compounds, such as the fusel alcohols.The unique polarity of the Rtx®-1301 stationary phase ensures excellent resolution of a range of alcohols and fusel oils. An example of a rum analysis is shown in Figure 2.

Figure 1

Difficult-to-monitor alcoholic beverage components methanol, active amyl alcohol, and isoamyl alcohol can be quantified from a packed column analysis, using 5% Carbowax® on CarboBlack™ B.



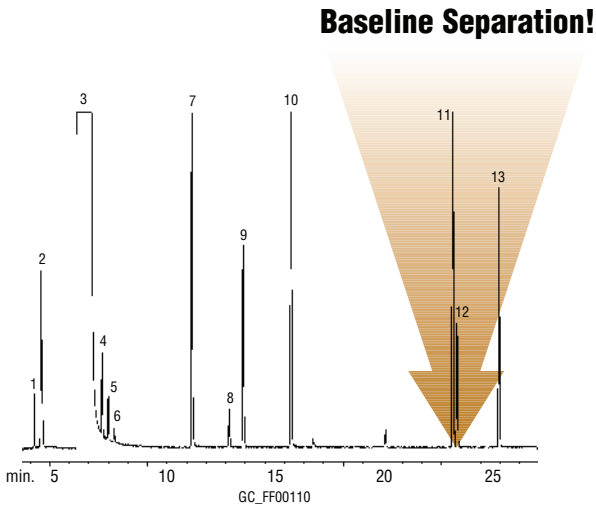
1. acetaldehyde
2. methanol
3. acetone
4. ethyl formate
5. ethanol
6. ethyl acetate
7. *n*-propanol
8. *sec*-butanol
9. isobutanol
10. active amyl alcohol
11. isoamyl alcohol
12. *n*-amyl alcohol

5% Carbowax® 20M 80/120 CarboBlack™ B (cat.# 80105)
2m, 1/8" OD x 2mm ID SilcoSmooth™ tubing
0.5µL on-column injection of fusel oils in rum,
Concentration: neat

Oven temp.: 65°C (hold 5 min.) to 150°C @ 4°C/min.
Inj./det. temp.: 200°C / 250°C
Det.: FID
Carrier gas: nitrogen
Column flow rate: 20mL/min.
FID sensitivity: 1.28 x 10^{-10} AFS

Figure 2

An Rtx®-1301 capillary column offers excellent resolution of alcohols and fusel oils.



1. acetaldehyde
2. methanol
3. ethanol
4. acetone
5. ethyl formate
6. isopropanol
7. *n*-propanol
8. ethyl acetate
9. *sec*-butanol
10. isobutanol
11. isoamyl alcohol
12. active amyl alcohol
13. *n*-amyl alcohol

60m, 0.25mm ID, 1.4µm Rtx®-1301 (cat.# 16016)
1.0µL split injection using a Cyclosplitter® sleeve (cat.# 20706).
Concentration: neat

Oven temp.: 35°C (hold 5 min.) to 100°C @ 1°C/min.
Inj./det. temp.: 150°C / 200°C
Carrier gas: hydrogen @ 40cm/sec.
Split ratio: 100:1

Additional Restek Literature

Performance information on six polyethylene glycol (PEG) columns—free on request.

Lit. Cat. #59890

Flavor Compounds in Distilled Liquor Products

Distilled liquor products contain a wide range of volatile and non-volatile compounds in an ethanol/water matrix. The most abundant fusel alcohols and esters can be determined by simple split injection, which also minimizes the amount of matrix ethanol and water transferred to the column. However, many trace-level fatty acids and their esters, which often are used to indicate product quality in alcoholic beverages such as whiskey and rum, cannot be determined by this approach. Capillary gas chromatography is a powerful tool for the analysis of these compounds, but the large ranges in volatilities and acidities can make it difficult to quantify all of the components in a single chromatographic separation. In addition, because the concentrations can vary widely, a splitless injection technique with some type of preconcentration step often is necessary. One example of this is large volume injection (LVI) with a venting step, which can be optimized to remove most of the matrix ethanol and water. Since some water will enter the chromatographic column, a stabilized phase, such as the Stabilwax®-DA phase, should be used.

By using a bonded polyethylene glycol (PEG) capillary column, flavor compounds in distilled liquor products can be quantified in a single splitless injection. A Stabilwax®-DA column was selected for this application, to improve peak shape and reproducibility for acidic components. An acidic functionality added to the PEG stationary phase reduces adsorption of acidic components and significantly reduces peak tailing. An optimized configuration of 30m, 0.18mm ID, and 0.18µm film thickness minimizes analysis times.

To optimize the chromatographic conditions for this analysis, we used a test mixture containing acids, esters, and flavor compounds typically found in alcoholic beverages (Figure 3). A computer modeling program, ezGC™, was used to optimize the column configuration, temperature program, and inlet flow for this system.

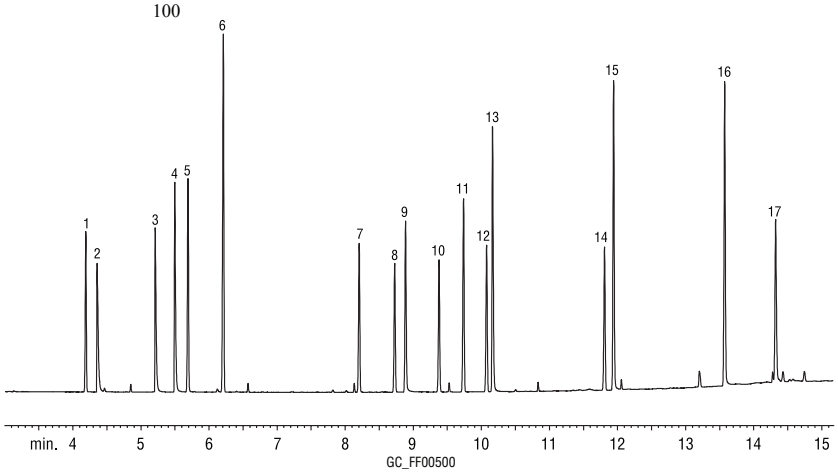
To test the applicability of this column in these dimensions, the critical pair of caproic acid and ethyl laurate was studied. These components can be very difficult to resolve on standard Carbowax®-type columns, especially if peak tailing or broadening occurs, or if one component is present at a significantly higher concentration. The Stabilwax®-DA column achieves baseline resolution of these two compounds in a reasonable analysis time (Figure 4).

Figure 3

Acids, esters, and flavor compounds typically found in alcoholic beverages are well resolved on a Stabilwax®-DA column.

Peak List	Conc. (ppm)
1. ethyl octanoate	100
2. acetic acid	100
3. propionic acid	100
4. isobutyric acid	100
5. decanol 3	50
6. ethyl decanoate	50
7. ethyl laurate	50
8. *cis*-lactone	100
9. 2-phenylethanol	50
10. *trans*-lactone	100
11. methyl myristate	50
12. ethyl myristate	50
13. octanoic acid	100
14. ethyl palmitate	50
15. decanoic acid	100
16. dodecanoic acid	100
17. vanillin	100

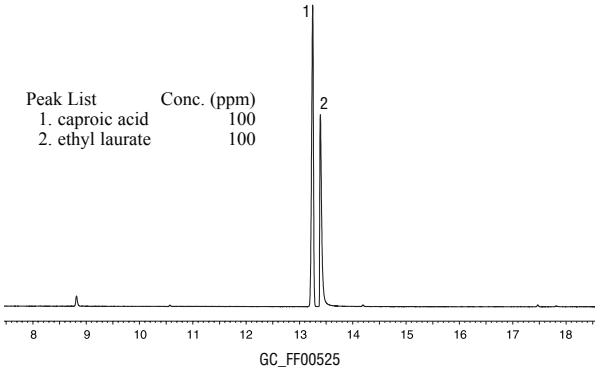
Stabilwax®-DA 30m, 0.18mm ID, 0.18µm (cat.# 550752)
Inj.: 1µL splitless (hold 0.5 min.) at conc. shown in peak list, in ethyl acetate, 4mm ID splitless liner w/wool (cat.# 20814-202.1)
Inj. temp.: 240°C
Carrier gas: hydrogen
Make-up gas: nitrogen
Linear velocity: 28psi @ 240°C
Oven temp.: 70°C to 240°C at 12°C/min. (hold 3 min.)
Det.: FID



Because alcoholic beverage samples often are injected via splitless mode, it was important to ascertain the stability of the Stabilwax®-DA column when exposed to aqueous injections. We verified stability by performing a splitless injection of the alcoholic beverage test mix, followed by five 1µL injections of water. We repeated this process 10 times, then made a final injection of the test mix. The chromatogram for the final test mix injection is shown in Figure 5. Even after repeated splitless injections of 100% water, there is very little degradation in the peak shapes for the test mix components. Over the course of the study, the variation in the peak retention times was 0.08-0.22% RSD. These data include retention times for the polar free fatty acids, which can be difficult to analyze under ideal conditions. The excellent stability of this stationary phase is demonstrated by the reproducibility of the retention times.

Figure 4

A Stabilwax®-DA column resolves the caproic acid / ethyl laurate critical pair to baseline.

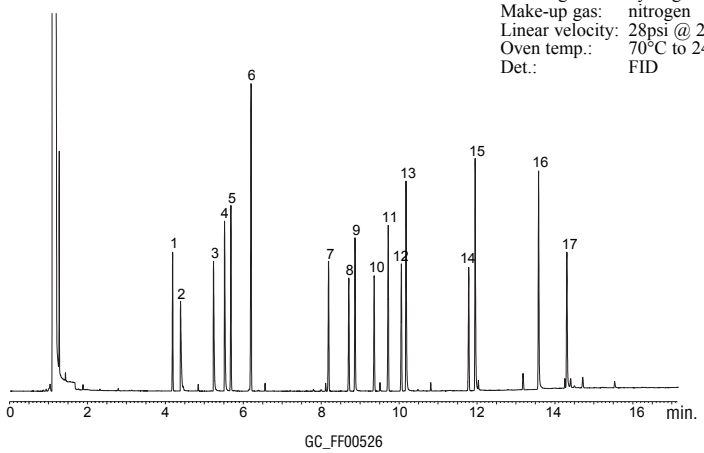


Peak List	Conc. (ppm)
1. caproic acid	100
2. ethyl laurate	100

Stabilwax®-DA 30m, 0.18mm ID, 0.18µm (cat.# 550752)
Inj.: 1µL splitless (hold 0.5 min.) at conc. shown in peak list, in ethyl acetate, 4mm ID splitless liner w/wool (cat.# 20814-202.1)
Inj. temp.: 240°C
Carrier gas: hydrogen
Make-up gas: nitrogen
Linear velocity: 28psi @ 240°C
Oven temp.: 80°C to 230°C at 5°C/min.
Det.: FID

Figure 5

Stabilwax®-DA columns are well named: repeated injections of water produce very little degradation in the peak shapes for alcoholic beverage test mix components.



Stabilwax®-DA 30m, 0.18mm ID, 0.18µm (cat.# 550752)
Inj.: 1µL splitless (hold 0.5 min.) at conc. shown in peak list, in ethyl acetate, 4mm ID splitless liner w/wool (cat.# 20814-202.1)
Inj. temp.: 240°C
Carrier gas: hydrogen
Make-up gas: nitrogen
Linear velocity: 28psi @ 240°C
Oven temp.: 70°C to 240°C at 12°C/min. (hold 3 min.)
Det.: FID

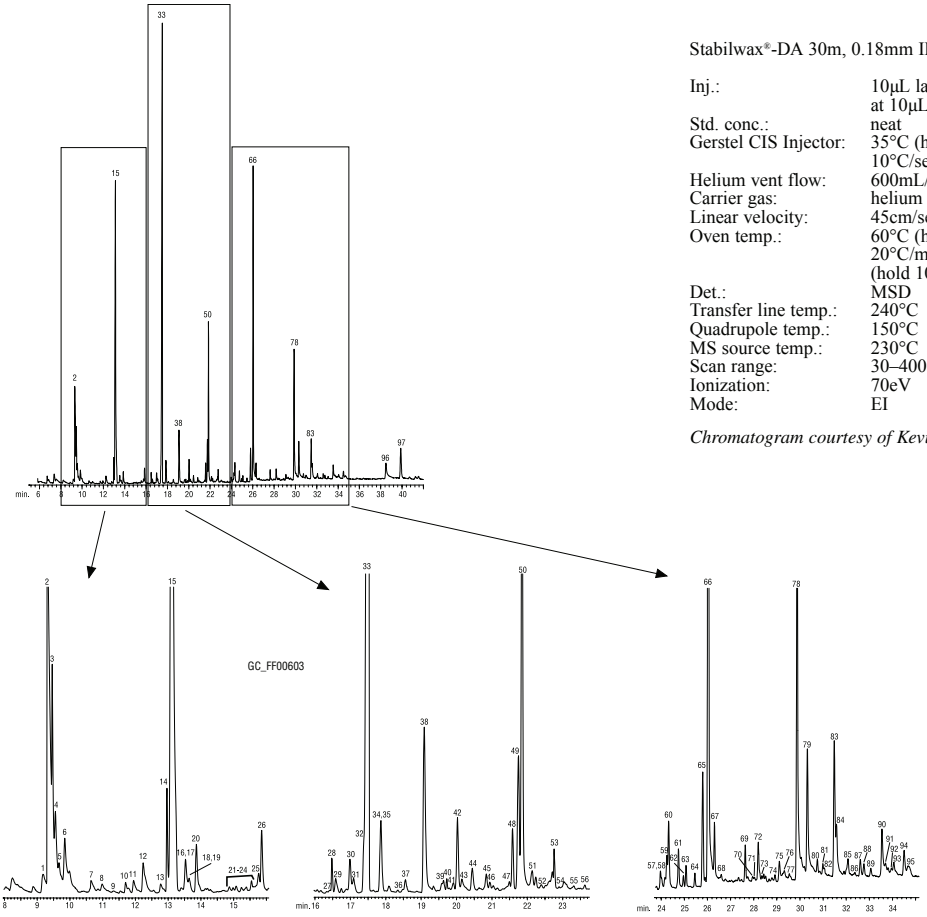
Peak List	Conc. (ppm)
1. ethyl octanoate	100
2. acetic acid	100
3. propionic acid	100
4. isobutyric acid	100
5. decanol 3	50
6. ethyl decanoate	50
7. ethyl laurate	50
8. *cis*-lactone	100
9. 2-phenylethanol	50
10. *trans*-lactone	100
11. methyl myristate	50
12. ethyl myristate	50
13. octanoic acid	100
14. ethyl palmitate	50
15. decanoic acid	100
16. dodecanoic acid	100
17. vanillin	100

6

Large volume injections (LVI) can be used to determine flavor compounds in alcoholic beverages such as malt whiskeys and grappas. Whiskey is distilled from a fermented mash of grain, such as corn, rye, barley, or wheat. The whiskey is aged in barrels or casks, and it is during the aging process that whiskey obtains its characteristic color, flavor, and aroma. Factors that influence the flavor of the final product include the characteristics of the grain, the recipe, and how the whiskey is distilled. The flavor profiles of whiskeys contain hundreds of compounds, including fatty acids, esters, alcohols, and aldehydes, in a wide range of concentrations. An example of a malt whiskey profile, determined by GC/MS, is shown in Figure 6.

Figure 6

Malt whiskey profile, determined by GC/MS, using a Stabilwax®-DA column and a large volume injection technique.



Stabilwax®-DA 30m, 0.18mm ID, 0.18µm (cat.# 550752)

Inj.:	10µL large volume injection (splitless), at 10µL/min.
Std. conc.:	neat
Gerstel CIS Injector:	35°C (hold 2 min.), to 300°C @ 10°C/sec. (hold 5 min.)
Helium vent flow:	600mL/min with 1.8 min. vent end time
Carrier gas:	helium
Linear velocity:	45cm/sec.
Oven temp.:	60°C (hold 2 min.) to 100°C @ 20°C/min., to 240°C @ 5°C/min. (hold 10 min.)
Det.:	MSD
Transfer line temp.:	240°C
Quadrupole temp.:	150°C
MS source temp.:	230°C
Scan range:	30–400amu
Ionization:	70eV
Mode:	EI

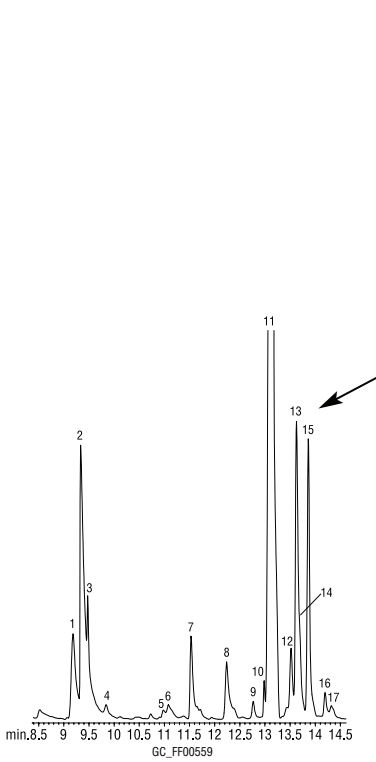
Chromatogram courtesy of Kevin MacNamara, Ph.D., Irish Distilleries, Ltd.

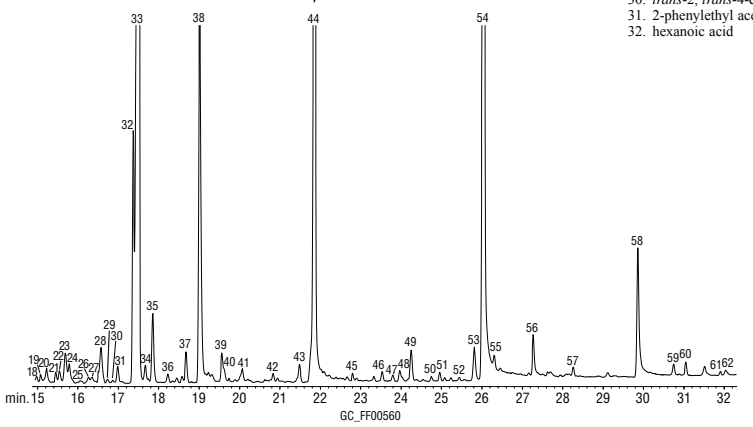
1. ethyl octanoate
2. acetic acid
3. siloxane
4. 1-hydroxy-2,3-butadione
5. 1-hydroxy-2-propanone acetate
6. furfural
7. formic acid
8. propionic acid
9. isobutyric acid
10. dimethyl sulfoxide
11. 5-methyl furfural
12. methyl decanoate + unknown
13. butyric acid
14. siloxane
15. ethyl decanoate
16. furfuryl alcohol
17. isoamyl octanoate
18. isovaleric acid
19. 2-methylbutyric acid
20. diethyl succinate
21. 3-methyl-2(5H)-furanone (possible)
22. valeric acid
23. ethyl undecanoate
24. isobutyl decanoate
25. 2(5H)-furanone
26. unknown
27. di(ethyleneglycol) butyl ether
28. siloxane
29. methyl dodecanoate
30. 2-phenylethyl acetate
31. methylcyclopentenolone
32. hexanoic acid
33. ethyl dodecanoate
34. isoamyl decanoate
35. guaiacol
36. dodecyl acetate (possible)
37. whiskey lactone (1)
38. 2-phenylethanol
39. heptanoic acid
40. siloxane
41. dimethoxybenzene or 4-methylguaiacol
42. whiskey lactone (2)
43. dodecanol
44. unknown
45. phenol
46. methyl tetradecanoate
47. nerolidol
48. diethyl malate
49. ethyl tetradecanoate
50. octanoic acid
51. unknown
52. *p*-cresol
53. siloxane
54. diethyl octanedioate
55. monomethyl succinate (possible)
56. 3,5-dimethyl-2,4(5H) furandione
57. nonanoic acid
58. diethyl 2-hydroxyglutarate
59. unknown
60. tetradecanol
61. 4-vinylguaiacol
62. diethyl nonanedioate
63. methyl hexadecanoate
64. ethyl γ-lactone 2-hydroxyglutarate
65. ethyl hexadecanoate
66. decanoic acid
67. ethyl 9-hexadecenoate
68. triacosan
69. unknown
70. phthalide
71. diethylphthalate
72. hexadecanol
73. 4-hydroxycinnamic acid (decomp.)
74. methyl stearate
75. benzoic acid
76. methyl 8-octadecenoate
77. ethyl stearate
78. dodecanoic acid
79. hydroxymethylfurfural
80. ethyl linoleate
81. 4-allyl-2,6-dimethoxyphenol
82. diisobutyl phthalate
83. vanillin
84. sinapic acid (decomp.)
85. 2-phenylethyl decanoate + 2 unknowns
86. 4-propenyl-2,6-dimethoxyphenol
87. ethyl vanillate
88. acetovanillone
89. vanillin methyl ketone
90. tetradecanoic acid
91. ethyl homovanillate
92. propiovanillone
93. fatty acid ester
94. (similar to 4-allyl-2,6-dimethoxyphenol)
95. unknown
96. hexadecanoic acid
97. syringealdehyde

Grappa is the spirit produced from grape marc, or the skins of the grapes after they have been pressed during wine production. Grape marc is fermented and distilled either directly or by water vapor. Grappas generally do not require the same amount of aging as other alcoholic beverages, although, for example, Italian law requires at least six months of aging. Flavored grappas can be produced by adding ingredients such as herbs and fruits. Flavor profiles of grappas contain hundreds of compounds at a wide range of concentrations. The chromatographic profile of an example grappa is shown in Figure 7.

Figure 7

Grappa profile, determined by GC/MS, using a Stabilwax®-DA column and a large volume injection.





1. ethyl octanoate
2. acetic acid
3. siloxane
4. furfural
5. propionic acid
6. benzaldehyde + linalool
7. isobutyric acid
8. methyl decanoate
9. butyric acid
10. siloxane
11. ethyl decanoate
12. isoamyl octanoate
13. isovaleric acid + 2-methylbutyric acid
14. susquiterpene (shoulder)
15. diethyl succinate
16. ethyl 9-decenoate
17. α-terpineol
18. (possible 3-methyl-2(5H)- furanone)
19. valeric acid + susquiterpene
20. linalool oxide
21. susquiterpene
22. isobutyl decanoate
23. 1-decanol
24. β-citronellol
25. susquiterpene
26. methyl salicylate + unknown
27. siloxane
28. methyl dodecanoate
29. hexyl octanoate + 2-tridecanone
30. *trans*-2, *trans*-4-decadienol
31. 2-phenylethyl acetate
32. hexanoic acid
33. ethyl dodecanoate
34. unknown
35. isoamyl decanoate
36. benzyl alcohol
37. unknown
38. 2-phenylethanol
39. 2-ethylhexanoic acid
40. heptanoic acid
41. dodecanol
42. phenol
43. γ-nonalactone
44. octanoic acid
45. siloxane
46. ethyl cinnamate
47. γ-decalactone + unknown
48. nonanoic acid
49. phenylethyl hexanoate
50. diethyl nonanedioate
51. methyl hexadecanoate
52. dimethyl naphthalene
53. ethyl hexadecanoate
54. decanoic acid
55. ethyl 9-hexadecenoate
56. geranic acid
57. 2-phenylethyl octanoate
58. dodecanoic acid
59. ethyl linoleate
60. diisobutyl phthalate
61. ethyl linolenate
62. phenylethyl decanoate
63. dibutyl phthalate

Stabilwax®-DA 30m, 0.18mm ID, 0.18µm (cat.# 550752)

Inj.:	10µL large volume injection (split less), at 10µL/min.
Std. conc.:	neat
Gerstel CIS Injector:	35°C (hold 2 min.), to 300°C @ 10°C/sec. (hold 5 min.)
Helium vent flow:	600mL/min with 1.8 min. vent end time
Carrier gas:	helium
Linear velocity:	45cm/sec.
Oven temp.:	60°C (hold 2 min.) to 100°C @ 20°C/min., to 240°C @ 5°C/min. (hold 10 min.)
Det.:	MSD
Transfer line temp.:	240°C
Quadrupole temp.:	150°C
MS source temp.:	230°C
Scan range:	30–400amu
Ionization:	70eV
Mode:	EI

Chromatogram courtesy of Kevin MacNamara, Ph.D., Irish Distilleries, Ltd.

HOT tech tip

Sample, Transfer, and Analyze Sulfur Compounds at Parts-per-Billion Levels

Our exclusive Sulfinert™ process is the next generation of metals passivation treatments, developed specifically for deactivating metal surfaces that contact organo-sulfur compounds. Untreated stainless steel adsorbs or reacts with hydrogen sulfide, mercaptans, and other active sulfur-containing compounds. Applied to a stainless steel surface, a Sulfinert™ layer prevents these compounds, and other active compounds (e.g., amines), from contacting the reactive metal surface. Combine custom-deactivated sample storage and transfer components with stock Sulfinert™-treated parts to passivate your entire system, and obtain highly accurate information about sulfur compounds in your samples.

Additional Important Features

Durable and flexible - will not crack or flake.
Stable to 400°C.
No memory effects, as seen with polymeric surfaces.

Determining Trace Sulfur Compounds In Beer

Trace sulfur compounds that are generated during the fermentation process can affect the taste and aroma of malted products such as beers. Several common volatile sulfur compounds might be present in beer at ppb or ppm levels (Table 1).

Table 1

Volatile sulfur-containing compounds found in beer at ppm to ppb levels.

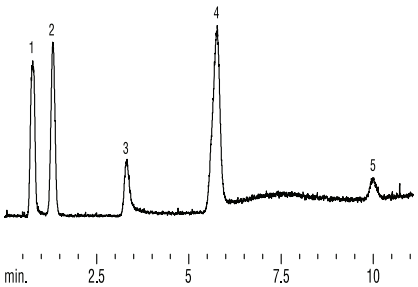
hydrogen sulfide	ethyl mercaptan	dimethyl disulfide
isopropyl mercaptan	t-butyl mercaptan	isobutyl mercaptan
carbonyl sulfide	sulfur dioxide	carbon disulfide
methyl ethyl sulfide	sec-butyl mercaptan	n-butyl mercaptan
methyl mercaptan	dimethyl sulfide	t-amyl mercaptan
n-propyl mercaptan	diethyl sulfide	

Accurate measurement of reactive sulfur compounds at these levels requires a highly inert chromatographic system. Restek's Rt-XLSulfur™ micropacked column contains a modified divinyl benzene polymer packed into Sulfinert™ tubing, and is specifically designed for monitoring ppb levels of active sulfur compounds. The Rt-XLSulfur™ column exhibits low bleed and thermal stability to 300°C. This column provides excellent resolution of hydrogen sulfide, carbonyl sulfide and sulfur dioxide.

Sample introduction into the column is a critical step in obtaining accurate analytical results for sulfur compounds. In this application, a beer headspace sample was introduced onto the column using a Valco six-port sampling valve fitted with a 1mL sample loop. The valve, sample loop, and all other surfaces in the sample pathway were deactivated using our Sulfinert™ deactivation process. The use of Sulfinert™-treated hardware is critical to achieving a 20ppb detection level for sulfur dioxide and the other target sulfur compounds (Figure 8).

Figure 8

Low levels of reactive sulfur compounds in CO_2 (i.e., 20ppb) easily can be detected using an Rt-XLSulfur™ micropacked column and a Sulfinert™ treated sample pathway.



GC_FF00491

1. hydrogen sulfide
2. carbonyl sulfide
3. methyl mercaptan
4. ethyl mercaptan and/or dimethyl sulfide
5. dimethyl disulfide

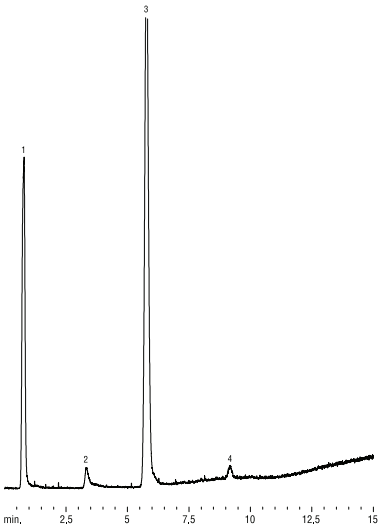
1m, 0.75mm ID Sulfinert™ tubing
Rt-XLSulfur™ 100/120 mesh (cat.# 19806)
Conc.: sulfur standard @ 20ppb each in CO_2
Inj.: 1cc sample loop, 6-port Valco® valve
Carrier gas: helium
Flow rate: 10mL/ min. @ ambient temp.
Oven temp.: 60°C to 260°C @ 15°C/min. (hold 5 min.)
Det. sensitivity: SCD, attn. x 1
Det. temp.: 800°C

We evaluated the effectiveness of the Rt-XLSulfur™ column by measuring trace sulfur compounds in one domestic (US) and two imported brands of beer. The results from headspace sampling of these products demonstrate the capability of the RT-XLSulfur™ column and the Sulfinert™ deactivated GC system to easily detect sulfur compounds at the 20ppb level (Figure 9).

Figure 9

ppb levels of hydrogen sulfide, dimethyl sulfide, and/or ethyl mercaptan and methyl mercaptan in beer.

Domestic Beer



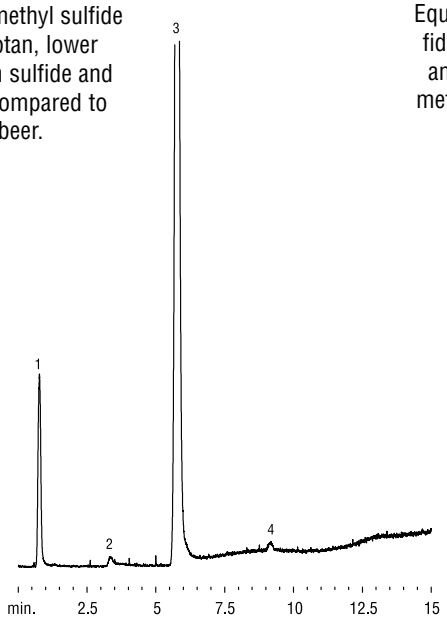
GC_FF00494

1. hydrogen sulfide
2. methyl mercaptan
3. dimethyl sulfide and/or ethyl mercaptan
4. unknown

1m, 0.75mm ID Sulfinert™ tubing
Rt-XLSulfur™ 100/120 mesh (cat.# 19806)
Conc.: headspace of a domestic (US) or imported beer sample
Inj.: 1cc sample loop, 6-port Valco® valve
Carrier gas: helium
Flow rate: 10mL/ min. @ ambient temp.
Oven temp.: 60°C to 260°C @ 15°C/min. (hold 5 min.)
Det. sensitivity: SCD, attn. x 1
Det. temp.: 800°C

Imported Beer (Mexico)

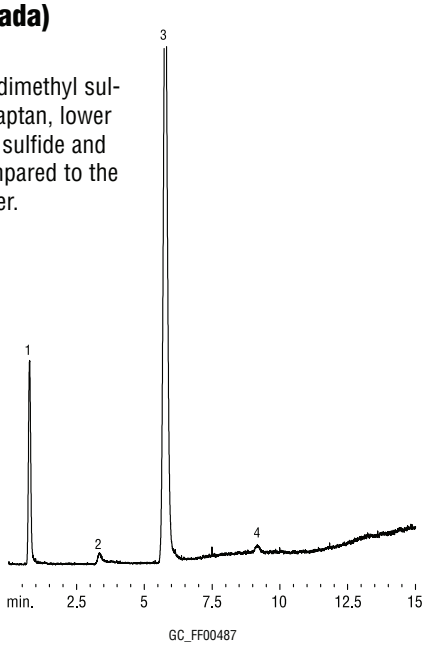
Higher amounts of dimethyl sulfide and/or ethyl mercaptan, lower amounts of hydrogen sulfide and methyl mercaptan, compared to the domestic beer.



GC_FF00483

Imported Beer (Canada)

Equivalent amounts of dimethyl sulfide and/or ethyl mercaptan, lower amounts of hydrogen sulfide and methyl mercaptan, compared to the domestic beer.



GC_FF00487

Summary

Gas chromatography is a simple, sensitive way to characterize the volatile compounds in alcoholic beverage products. Alcohols and aldehydes in alcoholic beverages can be analyzed by packed column GC or capillary GC, depending on the target analytes and their concentrations. Capillary GC provides very efficient separations, thereby resolving closely-related compounds, but the higher capacity of packed column GC systems sometimes makes it easier to detect trace levels of alcohols and short-chain aldehydes in the presence of high levels of ethanol. Ultimately, the choice of technique will depend on the needs of the analyst and the equipment available.

A Stabilwax®-DA capillary column is an excellent choice for analyses of acids, esters, and other flavor components in alcoholic beverage products. This highly stable column has been optimized for analyses of acidic compounds, making it possible to analyze a wide range of compounds. Large volume injection (LVI) techniques accomodate a wide range of concentrations in a single run. As shown in this guide, analytes at higher concentrations, such as alcohols and esters, and trace level flavor compounds can be analyzed simultaneously. The venting step during the large volume injection can be optimized to remove most of the ethanol/water matrix.

Low levels of reactive sulfur compounds in malted beverages also can be monitored reliably by gas chromatography. The combination of an Rt-XLSulfur™ micropacked column and a Sulfinert™ deactivated sample introduction system provides a state-of-the-art, robust, sampling and analysis approach for monitoring trace levels of volatile sulfur compounds in beer. This system also can be used to detect sulfur compounds in carbon dioxide used for artificial carbonation of carbonated beverages, such as soda waters and soft drinks. For information about this application, and example chromatograms, visit the following page on our website: www.restekcorp.com/advntage/d01four.htm

References

1. *AOAC Official Methods of Analysis (2000)*, 17th edition, AOAC International.

2. Deman, *Principles of Food Chemistry* (1990), 2nd edition, Van Nostrand Reinhold, New York.

Additional Restek Literature

Foods Flavors Fragrances minicatalog

Lit. Cat. #59260

Preservatives by HPLC

Lit. Cat. #59398

Genuine Restek Replacement Parts

Lit. Cat. #59627C

Flavor Volatiles in Alcoholic Beverages

Lit. Cat. #59579

Capillary Columns for Alcoholic Beverage Analysis

Ordering Information | Rtx®-1301 (G43) Capillary GC Columns (Fused Silica)

(Crossbond® 6% cyanopropylphenyl/94% dimethyl polysiloxane)

ID	df (µm)	temp. limits*	15-Meter	30-Meter	60-Meter
0.25mm	0.10	-20 to 280°C	16005	16008	16011
	0.25	-20 to 280°C	16020	16023	16026
	0.50	-20 to 270°C	16035	16038	16041
	1.00	-20 to 260°C	16050	16053	16056
	1.40	-20 to 240°C			16016
0.32mm	0.10	-20 to 280°C	16006	16009	16012
	0.25	-20 to 280°C	16021	16024	16027
	0.50	-20 to 270°C	16036	16039	16042
	1.00	-20 to 260°C	16051	16054	16057
	1.50	-20 to 250°C	16066	16069	16072
0.53mm	0.10	-20 to 280°C	16007	16010	16013
	0.25	-20 to 280°C	16022	16025	16028
	0.50	-20 to 270°C	16037	16040	16043
	1.00	-20 to 260°C	16052	16055	16058
	1.50	-20 to 250°C	16067	16070	16073
	3.00	-20 to 240°C	16082	16085	16088

Ordering Information | Stabilwax®-DA Capillary GC Columns (Fused Silica)

(Crossbond® Carbowax® for acidic compounds)

ID	df (µm)	temp. limits	15-Meter	30-Meter	60-Meter
0.18mm	0.18	40 to 250°C		550752	
0.25mm	0.10	40 to 250°C	11005	11008	11011
	0.25	40 to 250°C	11020	11023	11026
	0.50	40 to 250°C	11035	11038	11041
0.32mm	0.10	40 to 250°C	11006	11009	11012
	0.25	40 to 250°C	11021	11024	11027
	0.50	40 to 250°C	11036	11039	11042
	1.00	40 to 240/250°C	11051	11054	11057
0.53mm	0.10	40 to 250°C	11007	11010	11013
	0.25	40 to 250°C	11022	11025	11028
	0.50	40 to 250°C	11037	11040	11043
	1.00	40 to 240/250°C	11052	11055	11058
	1.50	40 to 230/240°C	11062	11065	11068

CarboBlack™ Solid Supports

Graphitized carbon black offers unique selectivity and very little adsorption for alcohol analyses. Two CarboBlack supports are available, CarboBlack™ B and CarboBlack™ C. CarboBlack™ B support, with its higher surface area, can support up to a 10% loading of a non-silicone liquid phase. CarboBlack™ C support can hold up to a 1% loading of a non-silicone liquid phase.

Ordering Information | CarboBlack™ Packed Columns

		Stainless Steel Tubing				SilcoSmooth™ Tubing			
On CarboBlack™ B	Mesh	L (ft.)	OD (in.)	ID (mm)	cat.#*	L (m)	OD (in.)	ID (mm)	cat.#*
5% Carbowax® 20M	80/120	—	—	—	—	2	1/8	2	80105-
5% Carbowax® 20M	60/80	6	1/8	2.1	88012-	1.8	1/8	2	80106-
6.6% Carbowax® 20M	80/120	6	1/8	2.1	80451-	2	1/8	2	80107-

** Please include configuration suffix number when ordering.*

Leak Detective™ II Leak Detector*

- Affordable thermal conductivity leak detector—every analyst can have one.
- Compact, ergonomic design is easy to hold and operate with one hand.
- Helium, hydrogen, and nitrogen can be detected at $1x10^{-4}$cc/sec. or at an absolute concentration as low as 100ppm.**
- Fast results—responds in less than 2 seconds to trace leaks of gases with thermal conductivities different than air.
- Micro-chip design improves sensitivity and response timover previous models.
- Auto zeroing with the touch of a button.
- Battery-operated for increased portability (one 9-volt).

Description	qty.	cat.#
Leak Detective™ II Leak Detector	ea.	20413

**Never use liquid leak detectors on a capillary system because liquids can be drawn into the system.*

***Caution: NOT designed for determining leaks of combustible gases. A combustible gas detector should be used for determining combustible gas leaks in possibly hazardous conditions.*

Configurations

General Configuration
Suffix -800

Agilent 5880, 5890, 5987, 6890:
Suffix -810

Varian 3700, Vista Series, FID:
Suffix -820

PE 900-3920 Sigma 1,2,3:
Suffix -830

PE Auto System 8300, 8400, 8700 (Not On-Column):
Suffix -840

See our catalog for custom configurations

www.restek.com

Micropacked Columns

- Higher efficiency than packed columns.
- Higher capacity than capillary columns.
- Made from inert, flexible Silcosteel® tubing.

Micropacked columns are inexpensive, rugged, and easy to install and to operate. With our inert Silcosteel® treatment, micropacked columns are a powerful tool for solving many difficult application problems. Because the Silcosteel® coating is thin, the column can be flexed and coiled without any fear of damage to the inert surface.

Micropacked columns fit packed or capillary injection systems. 1mm ID, ($^1/_{16}$-inch OD) micropacked columns improve efficiency of packed column instruments, without the expense of converting to a capillary injection system. 0.75mm ID (0.95mm OD) micropacked columns install easily into a capillary injector, using slightly larger ferrules. Micropacked columns operate at flows exceeding 10cc/min., for trouble-free operation. Packed with 100/120 mesh particles.

Ordering Information | Rt-XLSulfur™ Micropacked Columns

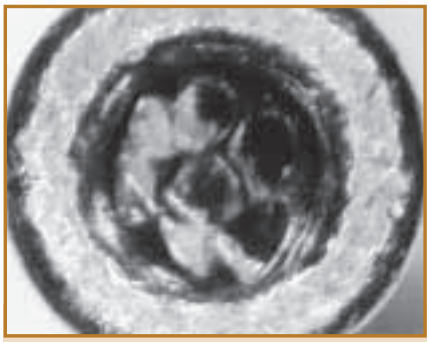
Purchase installation kit separately.

OD	ID (mm)	1-Meter	2-Meter
$^1/_{16}$"	1.0mm	19804	19805
0.95mm	0.75mm	19806	19807

Ordering Information | Micropacked Columns Installation Kits

	for 0.75mm ID col.	for 1mm ID col.	for 2mm ID col.
For valve applications	21062	21065	21067
For split applications	21063	—	—
For all Agilent GCs	21064	—	—
For direct injections	—	21066	—

HOT tech tip



A common problem with micropacked columns is the integrity of the end plug. Glass wool is difficult to insert into an opening less than 1mm wide and can be dislodged easily by carrier gas pressure surges that occur during valve switching. Restek's chemists insert braided wire into the column bore, then make a small crimp near the column outlet. End plugs are Silcosteel®-treated to ensure that the sample contacts only inert surfaces.

Headspace Vials

6.0mL Headspace Vial

Headspace Autosampler Vials

Description	100-pk.	1000-pk.
6mL Clear Vial	21166	21167
10mL Clear Vial, Flat Bottom	24683	24684
10mL Clear Vial, Rounded Bottom	21164	21165
20mL Clear Vial, Flat Bottom	24685	24686
20mL Clear Vial, Rounded Bottom	21162	21163
27mL Clear Vial	21160	21161

Silver Seal with PTFE/Gray Butyl Rubber Septum

20mm Aluminum Seals w/Septa, Assembled

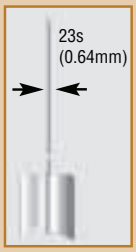
Description	100-pk.	1000-pk.
Silver Seal w/ PTFE/Gray Butyl Rubber	21761	21762
Silver Seal w/ PTFE/Silicone	21763	21764
Pressure Release Silver Seal w/ PTFE/Gray Butyl Rubber Septum <125°C	21765	21766
Pressure Release Silver Seal w/ PTFE/Silicone Septum >125°C	21767	21768

Syringes

Restek offers complementing lines of syringes from Hamilton & SGE.

- Hamilton: The historical leader in precision fluid measuring devices for over 40 years, with a commitment to precision, quality, and accuracy.
- SGE: Over 25 years of providing a comprehensive range of analytical syringes unsurpassed in design, quality, and performance.

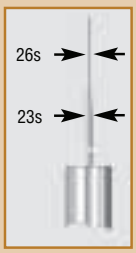
Needle Gauge for Agilent 7673 Syringes



23s—Single Gauge Needle

- The most popular gauge for Agilent 7673.
- Stocked for same-day shipment.
- Best for Merlin Microseal® septum and standard septum-equipped GCs.
- Packed column injection ports.
- Split/splitless injection ports.

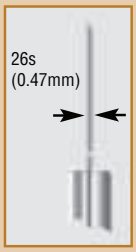
SuperfleX™ Flexible Plunger Syringe
Agilent 7673 Syringe
Gas-Tight Luer-lock Syringe



23s-26s—Dual Gauge (tapered) Needle

- Durability of a 23s gauge needle.
- Ability of a 26s gauge needle to perform split/splitless and on-column injections.

SGE Removable Needle for Agilent 7673 Autosampler



26s—Single Gauge Needle

- On-column injection ports.
- Split/splitless injection ports.

Hamilton 10µL, Autosampler Cemented Needle for Agilent 7673 Autosampler

Drawings reproduced with permission from Hamilton.

Hamilton Syringes

Volume	Needle Term.	Needle Gauge	Needle Length	Point Style	Hamilton Model	Hamilton cat.#	Restek qty.	Restek cat.#
5µL	ASN*	23s	1.71"	Agilent	75	87990	6-pk.	20170
5µL	ASN	26s	1.71"	Agilent	75	87989	6-pk.	21230
5µL	ASN	23s–26s	1.71"	Agilent	75	87994	6-pk.	24594
10µL	ASN	23s	1.71"	Agilent	701	80390**	6-pk.	20169
10µL	ASN	26s	1.71"	Agilent	701	80389	6-pk.	24599
10µL	ASN	23s–26s	1.71"	Agilent	701	80391	6-pk.	24600

** Autosampler cemented needle.*
*** Designated by Agilent as #80397.*

SGE Syringes

Volume	Needle Term.	Needle Gauge	Needle Length	Point Style	SGE Model	SGE cat.#	Restek qty.	Restek cat.#
5µL	F*	23	42mm	Cone	SK-5F-HP-0.63	001814	6-pk.	24783
5µL	F	26	42mm	Cone	SK-5F-HP-0.47	001804	6-pk.	24782
5µL	F	23-26s	42mm	Cone	SK-5F-HP-0.63/0.47	001822	6-pk.	21214
10µL	F	23	42mm	Cone	SK-10F-HP-0.63	002814	6-pk.	24787
10µL	F	26	42mm	Cone	SK-10F-HP-0.47	002804	6-pk.	24786
10µL	F	23–26s	42mm	Cone	SK-10F-HP-.063/0.47	002822	6-pk.	21215

** Fixed needle.*

Siltek™ Deactivation—The Next Generation

- Maximizes the inertness of the sample pathway.
- Minimizes breakdown.
- Low bleed.
- Thermally stable.
- "Clean and green"—manufactured without the use of harmful organic solvents.

Restek offers the next generation of deactivation. The Siltek™ deactivation process (patent pending) produces a highly-inert glass surface, which features high temperature stability, extreme durability, and low bleed. Try Siltek™ liners, guard columns, wool, and connectors for better recovery of sample analytes.

For Siltek™ inlet liners, add the corresponding suffix number to your liner catalog number.

Siltek™ Inlet Liners

qty.	Siltek™	Siltek™ with Siltek™ wool	Siltek™ with CarboFrit™
each	-214.1	-213.1	-216.1
5-pk.	-214.5	-213.5	-216.5
25-pk.	-214.25	-213.25	-216.25

Benefits of wool-packed Precision™ Liners

- Wool minimizes vaporization and helps wipe the needle during injection.
- No guessing where the wool should be placed; easy to change wool.
- Wool stays in position during pressure pulses in the inlet during an injection.
- 100% deactivation ensures inertness.*

** Not Siltek™ deactivation.*

Precision™ Liners	ID**/OD & Length (mm)	ea.	5-pk.	25-pk.
 Agilent 4mm Split Precision™ Liner	4.0 ID 6.3 OD x 78.5	21022	21023	20979
 Varian 1078/1079 Split Precision™ Liner	3.4 ID 5.0 OD x 54	21024	21025	
 Shimadzu 17A Split Precision™ Liner	3.5 ID 5.0 OD x 95	21020	21021	
 Varian 1075/1077 Split Precision™ Liner	4.0 ID 6.3 OD x 72	21030	21031	
 Thermo Finnigan 5mm Split Precision™ Liner	5.0 ID 8.0 OD x 105	21028	21029	
 PerkinElmer Auto SYS Split Precision™ Liner	4.0 ID 6.2 OD x 92.1	21026	21027	

Inlet Liners for APEX ProSep™ 800 & ProSep™ 800 Plus GCs

	Benefits/Uses:	ID**/OD & Length (mm)	Similar to APEX part #	cat.# ea.
Mega IV (4.0mm ID)	injections <125µL	4.0 ID 6.0 OD x 243	L-00410	21075
Micro I (1.0mm ID)	injections <5µL	1.0 ID 6.0 OD x 243	L-00110	21073
MIDI II (2.0mm ID)	injections <25µL	2.0 ID 6.0 OD x 243	L-00210	21074

***Nominal ID at syringe needle expulsion point.*

Vespel® Ring Inlet Seals for Agilent 5890/6890 and 6850 GCs

- Easy-to-use, patent-pending design saves time.
- Vespel® material seals the first time, every time.
- Very little torque is required to make a seal—reduces operator variability.
- Lower leak rate versus OEM metal inlet seals—reduces detector noise.
- Increases column lifetime by preventing oxygen from leaking into the carrier gas.
- Soft sealing area reduces wear on the critical seal of the injection port base.

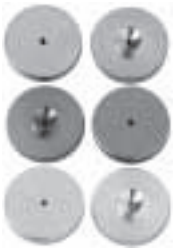
Vespel® ring ensures a leak-tight seal the first time, every time.

0.8mm ID Vespel® Ring Inlet Seal	2-pk.	10-pk.
Gold-Plated	21562	21563
Silcosteel®	21564	21565
Stainless Steel	21560	21561
1.2mm ID Vespel® Ring Inlet Seal	**2-pk.**	**10-pk.**
Gold-Plated	21568	21569
Silcosteel®	21570	21571
Stainless Steel	21566	21567

Washers included.

Replacement Inlet Seals for Agilent 5890/6890/6850 Split/Splitless Injection Ports

- Special grade of stainless steel that is softer and deforms more easily, ensuring a completely leak-tight seal.
- Increases column lifetime because oxygen cannot leak into the carrier gas.
- Reduced noise benefits high-sensitivity detectors (e.g., ECDs, MSDs).
- Silcosteel® seal offers the inertness of glass.



Single-Column Installation, 0.8mm Opening*		0.25/0.32mm ID Dual-Column Installation, 1.2mm Opening		0.53mm ID Dual-Column Installation 1/16-inch Opening	
2-pk.	10-pk.	2-pk.	10-pk.	2-pk.	10-pk.
		Stainless Steel Inlet Seal			
21315	21316	20390	20391	20392	20393
		Gold-Plated Inlet Seal			
21317	21318	21305	21306	—	—
		Silcosteel® Inlet Seal			
21319	21320	21307	21308	—	—

**0.8mm ID stainless steel inlet seal is equivalent to Agilent part #18740-20880, 0.8mm ID gold-plated inlet seal is equivalent to Agilent part #18740-20885.*
Note: All seals include washers.

Replacement FID Jets

- Standard Version: Engineered with a fluted tip to guide the capillary column into the jet.
- High-Performance Version: Identical to the standard version, except that it has been Silcosteel®-treated. Extremely inert, use with active compounds.

Capillary Adaptable FID Jet for Agilent 5890/6890/6850 GCs (0.011-inch ID tip)

(Similar to Agilent part # 19244-80560.)

Description	qty.	cat.#	qty.	cat.#
Standard	ea.	20670	3-pk.	20671
High-Performance Silcosteel®	ea.	20672	3-pk.	20673

Capillary Dedicated FID Jet for Agilent 6890/6850 GCs

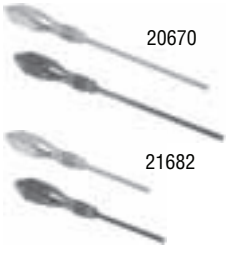
(Similar to Agilent part # G1531-80560.)

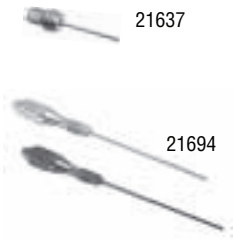
Description	qty.	cat.#	qty.	cat.#
Standard	ea.	21621	3-pk.	21682
High-Performance Silcosteel®	ea.	21620	3-pk.	21683

Capillary FID Jet for Agilent 5880 GCs

(Similar to Agilent part # 19301-80500.)

Description	qty.	cat.#
Standard	ea.	21637
	ea.	21638





Packed Column FID Jets for Agilent 5890/6890/6850 GCs

0.018-Inch ID (Similar to Agilent part # 18710-20119.)	qty.	cat.#	qty.	cat.#
Standard	ea.	21694	3-pk.	21695
	ea.	21696	3-pk.	21697
0.030-Inch ID (Similar to Agilent part # 18789-80070.)	**qty.**	**cat.#**	**qty.**	**cat.#**
Standard	ea.	21688	3-pk.	21689
	ea.	21686	3-pk.	21687

Foods Safety Applications

Analytical Method for Polycyclic Aromatic Hydrocarbons (PAHs) in Yerba Mate Tea Using Modified QuEChERS, Solid Phase Extraction and GC-TOFMS and GC-MS/MS

By Julie Kowalski, Amanda Rigdon, and Jack Cochran

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are toxic compounds found in some foods, especially those that are smoked, roasted, grilled, or dried during preparation. Yerba mate (*Ilex paraguariensis*) tea is of particular interest because of relatively high PAH levels and proposed links between yerba mate tea and health problems. While classic sample extraction methods yield excellent results for PAHs in tea, these techniques are time consuming and costly. A much less resource-intensive modified QuEChERS extraction and silica solid phase extraction (SPE) sample cleanup method was developed and yielded good quantitative recoveries for PAHs in yerba mate tea. Chromatographic separation of EFSA PAH4 compounds and isobaric interferences was optimized on a high-phenyl stationary phase using both GC-TOFMS with hydrogen carrier gas and GC-MS/MS. Incurred values of PAHs determined via GC-TOFMS and GC-MS/MS compared favorably. Total levels of EFSA PAH4 compounds were relatively high with respect to other foods and ranged from approximately 200 to 800 ng/g in dry tea.

Introduction

Traditionally, mate tea is brewed from loose yerba mate (*Ilex paraguariensis*) leaves and stems in hot water and drunk from a gourd through a metal straw called a bombilla. Yerba mate is especially popular in Argentina, Brazil, Paraguay, and Uruguay and has enjoyed a long history in some cultures and is still often shared by passing the gourd among groups of people to show hospitality [1]. Mate's economic importance is growing as products manufactured from mate and the tea itself are introduced worldwide. Growing popularity is partially due to the reputation of providing numerous health benefits, including increased energy and weight loss, as well as for treatment of many health problems from headaches to hypertension [2-7]. However, high incidence of esophageal cancer in populations with high mate tea consumption suggests a possible link between mate and cancer [8-20]. One important consideration is the relatively high levels of toxic PAHs in mate tea, likely due to processing with wood fires or other drying processes [8].

Polycyclic aromatic hydrocarbons are formed during combustion processes and are of concern because some are toxic to humans. Food is a common route of exposure for humans and some regulations exist for some foods and specific PAHs [21]. Historically, benzo[a]pyrene was used as the sole toxicity marker; however, data showed foods contain toxic PAHs without the presence of benzo[a]pyrene. The EFSA reevaluation suggested at least a subset of four PAHs, PAH4, as well as a subset of eight PAHs, PAH8, should be monitored in foods [21]. The PAH4 compounds are benzo[a]pyrene, chrysene, benz[a]anthracene, and benzo[b]fluoranthene. The PAH8 subset consists of the PAH4 plus benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, dibenz[ah]anthracene, and benzo[ghi]perylene. Development of analytical methods for PAHs should now focus on these subsets.

PAH analysis is challenging because there are isobaric PAHs that interfere with these PAHs of interest making accurate quantitation difficult, if not impossible. For example, chrysene is a toxic PAH and part of PAH4, but analysis of chrysene is complicated by the presence of triphenylene, which is an isobaric interference that completely or partially coelutes when using gas chromatography. This causes biasing of chrysene concentration or forces the compounds to be reported together. This is problematic because chrysene is toxic while triphenylene is not, often causing an overestimation of the toxicity in food items. Optimizing this separation and resolving other isobaric compounds is critical to providing correct quantitative data for the PAH4 and PAH8 compounds that are used as toxicity markers.

In addition, analysis of PAHs in foodstuffs is challenging because the compounds have to be determined at trace levels. Often, rigorous and time-consuming sample preparation is used to extract PAHs and clean up the sample before analysis. The complex nature of mate tea has led researchers to employ exhaustive sample preparation, including supercritical fluid extraction [22], pressurized fluid extraction [8], and gel permeation chromatography [23-25].

QuEChERS sample preparation methods are a desirable alternative because they are quick and easy, but still provide quality results. These methods typically work because they are paired with mass spectrometry based techniques like tandem mass spectrometry. Traditionally, QuEChERS involves a sample extraction followed by dispersive solid phase extraction cleanup [26, 27]. Although QuEChERS was originally designed for pesticide residues in fruit and vegetables [26], many modifications have been explored to expand the approach beyond the original scope. Compounds other than pesticides, like PAHs, veterinary drugs, and persistent organic pollutants, are now tested using QuEChERS approaches and difficult commodities like tea, spices, and tobacco have been tested using QuEChERS type methods [28-40].

This work describes the development of an analytical method for PAHs in tea that allows analysts to more quickly and accurately characterize target PAHs. Sample preparation is based on a modified QuEChERS extraction and solid phase extraction sample cleanup. Both GC-TOFMS and GC-MS/MS techniques were used and analyses were optimized for resolution of isobaric compounds, as well as for maintaining a reasonable analysis time.

Experimental

Materials

Development of this analytical method for PAHs in tea used six commercially available brands of dried yerba mate tea. All solvents were LC-MS grade or higher. The reference standards and sources were: EPA Method 8310 PAH mixture which contains 18 PAHs (cat.# 31874, Restek Corporation); 5-methylchrysene, benzo[c]phenanthrene, benzo[j]fluoranthene, cyclopenta[cd]pyrene, and dibenzo[a,e]pyrene (Cerilliant); coronene, dibenz[a,c]anthracene, perylene, and triphenylene (Sigma-Aldrich); benzo[e]pyrene (Ultra Scientific); and benzo(ghi)fluoranthene and benzo[a]fluoranthene (Santa Cruz Biotechnology). Although 30 PAHs were tested, special attention was paid to the PAH4* and PAH8 groups. The internal standard mix used was the SV internal standard mix (cat.# 31206, Restek Corporation). Original unbuffered QuEChERS extraction salts (cat.# 23992) and solid phase extraction cartridges with PTFE frits containing 500 mg silica (cat.# 24036) were also obtained from Restek Corporation.

*Since the completion of this work, Restek has developed an EFSA PAH4 certified reference material (cat.# 32469) prepared at 1,000 µg/mL in toluene that is both convenient and compatible with QuEChERS solvents.

Sample Preparation and Modified QuEChERS Extraction

Dried tea was powdered using a hand-held blender. QuEChERS extraction requires a sample with high water content (>80%). To prepare the dried tea material for a QuEChERS extraction, 1 g of powdered tea was combined with 10 mL of water in a FEP tube. After shaking to mix well, PAHs and internal standards were added. PAHs were fortified at 50 and 500 ng/g dry tea and internal standards were added at 100 ng/g. The sample was allowed to soak for 10 minutes and then 10 mL of hexane:acetone (1:1, v/v) were added. Samples were then vortexed for 30 minutes. The prepackaged unbuffered QuEChERS salts (4 g $MgSO_4$ and 1 g NaCl) were added slowly. The samples were shaken by hand for 1 minute and then centrifuged for 5 minutes at 3,000 x g.

An investigation of extraction solvents was performed to determine whether using acetonitrile or hexane:acetone (1:1, v/v) produced better recoveries. The previously described procedure was used with some modifications. Two grams of powdered tea were fortified with 18 PAHs at 50 µg/g (in dry tea) using the EPA method 8310 PAH mixture (cat.# 31874). The PAH-fortified tea samples were soaked overnight at approximately 4 °C to maximize introduction of PAHs into the tea itself prior to their attempted extraction. The samples were then processed with the QuEChERS extraction and cleanup described in this work. The final optimized extraction procedure is summarized in Table I.

Solid Phase Extraction Cleanup

Two milliliters of extract was exchanged to hexane by evaporating to less than 1 mL using a gentle stream of nitrogen gas in a heating block at 50 °C, and then adding hexane for a total volume of 2 mL. This process was performed twice. The silica cartridge was conditioned with 3 mL of methanol followed by 3 mL of acetone under high vacuum, then with 3 mL of hexane:methylene chloride (1:1, v/v) and 6 mL of hexane at a rate of approximately 1 drop per second. One milliliter of extract was loaded onto the cartridge and eluted with 5 mL of various percentages of methylene chloride in hexane. Elution solvents tested were 0, 15, 25, 50, and 75 percent volume methylene chloride in hexane. Eluted samples were concentrated to 1 mL final volume with a gentle stream of nitrogen gas at 50 °C. The final cleanup procedure is summarized in Table I.

Table I: Final Extraction and Cleanup Procedure

Modified QuEChERS Extraction
1. Homogenize dry tea into a powder.
2. Soak 1 g tea powder in 10 mL water for 10 min in an FEP centrifuge tube.
3. Add 10 mL hexane:acetone (1:1) and vortex 30 min.
4. Add Q-sep® QuEChERS unbuffered salts (cat.# 23991), shake 1 min, and then spin for 5 min in a Q-sep® 3000 centrifuge.
5. Evaporate 2 mL of extract down to 1 mL, then adjust final volume to 2 mL with hexane. Perform this step twice.
Silica SPE Cleanup
1. Rinse Resprep® SPE cartridges (3 mL, 0.5 g silica; cat.#24036) with 3 mL methanol followed by 3 mL acetone.
2. Condition cartridges with 3 mL hexane:methylene chloride (1:1), followed by 6 mL hexane.
3. Load 1 mL of extract onto cartridge and elute with 5 mL hexane:methylene chloride (7:3).
4. Evaporate to 1 mL.

Gas Chromatography Methods
Optimized chromatographic methods were used on three formats: GC-FID, GC-TOFMS, and GC-MS/MS. GC-FID was only used for extraction solvent evaluations. GC-TOFMS and GC-MS/MS platforms were used for spike recovery and quantitative analysis of incurred PAHs.

Gas Chromatograph-Flame Ionization Detection
GC-FID (GCxGC-FID instrument, LECO Corporation) was used to evaluate extraction solvents. A 30 m x 0.25 mm x 0.25 µm Rxi®-5Sil MS column (cat.# 13623, Restek Corporation) was installed and operated with a constant flow of helium at 2 mL/min and an oven program of 80 °C, hold 0.1 min, then ramp at 8.5 °C/min to 330 °C and hold for 0.49 min. The inlet was held at 300 °C and outfitted with a Sky® 4.0 mm ID Precision® inlet liner with wool (cat.# 23305, Restek Corporation). One microliter was injected using a split ratio of 10:1. The FID was held at 350 °C.

Gas Chromatography-Time of Flight Mass Spectrometry
A LECO® Pegasus III GC-TOFMS instrument (LECO Corporation) was used for separation and quantification. Gas chromatography was performed using a high phenyl content Rxi®-PAH GC column in a 60 m x 0.25 mm x 0.10 µm configuration (cat.# 49317, Restek Corporation). A splitless injection of 2.5 µL was performed using a Sky® 4 mm single taper inlet liner with wool (cat.# 23303, Restek Corporation). The inlet temperature was 275 °C. The splitless purge valve time was set to 1 min. A constant flow of hydrogen at 2.4 mL/min and oven temperature program of 80 °C (hold 1 min) ramping at 40 °C/min to 210 °C, then 3 °C/min to 260 °C, then 11.5 °C/min to 350 °C (hold 6.26 min) was used. The LECO® Pegasus III TOFMS had a source temperature of 300 °C, used electron ionization at 70 eV, and stored a mass range of 45 to 550 u with an acquisition rate of 5 spectra/sec.

Gas Chromatography-Tandem Mass Spectrometry
GC-MS/MS analysis was performed using a Thermo TSQ™ 8000. (Thermo Fisher Scientific) equipped with a 40 m x 0.18 mm x 0.07 µm Rxi®-PAH column (cat.# 49316, Restek Corporation), constant flow helium at 1.4 mL/min and a 2 mm single taper with wool inlet liner (cat.# 23316, Restek Corporation). The inlet was held at 275 °C and the oven program was 80 °C (hold 1 min), then 37 °C/min to 210 °C, then 3 °C/min to 260 °C, then 11 °C/min to 350 °C (hold 5.0 min). A splitless injection of 0.5 µL with a splitless time of 0.58 min and surge duration of 0.6 min was used. The transfer line was held at 330 °C. Three SRM transitions for each compound were collected. The SRM mode was not used in the typical manner where fragments are monitored. Instead, the SRM mode was used for the benefit of reduced background interferences. PAHs have a strong molecular ion, so transitions between the molecular ion, $[M]^{+\bullet}$, in Q1 and ions $[M]^{+\bullet}$, $[M\text{-}H]^{+\bullet}$, and $[M\text{-}2H]^{+\bullet}$ in Q3 are used. Quantitation was based on one transition. The emission energy was 90 µA and the collision energy was set to 10.

Percent Recovery and Incurred PAH Determination
Fortified samples at 500 ng/g (ppb) were prepared with the optimized sample preparation procedure described in Table I. Acenaphthene-d10, chrysene-d12, naphthalene-d8, perylene-d12, and phenanthrene-d10 were used as internal standards. Internal standards were assigned to analytes by closest retention time to target analytes. Quantitation of fortified and unfortified samples was performed using a solvent calibration curve with levels of 50, 150, 500, 1,000 and 2,000 ppb.

Results and Discussion

Sample Extraction Solvent Investigation
QuEChERS is a desirable method for processing samples because it is quick, easy, and inexpensive. The initial QuEChERS approach used for this work started by hydrating the dry tea then extracting with acetonitrile followed by partitioning via the addition of salts. The hydration step is important for QuEChERS extractions because it is required for the proper partitioning to occur [41, 42]. Acetonitrile typically is used for QuEChERS extractions because it is an effective solvent for pesticides and can result in lower coextracted material for some matrices [41, 43]. However, solubility and recovery of PAHs with acetonitrile as the extraction solvent proved to be problematic. Based on the results of the extraction solvent investigation, hexane:acetone (1:1, v/v) is a stronger extraction solvent for PAHs and, thus, it was used instead of acetonitrile for the remaining experiments.

Table II shows recovery values for PAHs in mate tea samples fortified at 50 µg/g and processed with acetonitrile and hexane:acetone. There is a general trend of lower PAH recovery with acetonitrile compared to recovery using hexane:acetone (1:1, v/v). This is not unexpected as there is a polarity mismatch between PAHs, nonpolar compounds, and acetonitrile which is a polar solvent. Recovery values produced using acetonitrile also show a trend of progressively lower recovery for higher molecular weight PAHs. This can be attributed to the lower solubility of high molecular weight PAHs compared to lower molecular analogs. Recovery values of target PAHs with hexane:acetone (1:1, v/v) as the extraction solvent are in the acceptable range and no bias for high molecular weight PAHs is observed. Hexane:acetone (1:1, v/v) was used as the extraction solvent in all subsequent experiments.

Table II: Percent recovery values for PAHs fortified at 50 µg/g in one yerba mate tea. Values shown compare acetonitrile and hexane:acetone (1:1, v/v) as the extraction solvents used during the initial sample extraction of wetted mate tea.

PAH	Acetonitrile (% Recovery)	Hexane:Acetone (1:1, v/v) % Recovery
Naphthalene	73	91
2-Methylnaphthalene	74	91
1-Methylnaphthalene	73	99
Acenaphthylene	56	25
Acenaphthene	96	96
Fluorene	65	140
Phenanthrene	64	93
Anthracene	79	98
Fluoranthene	58	83
Pyrene	130	220
Benz[a]anthracene	55	89
Chrysene	55	80
Benzo[b]fluoranthene	53	110
Benzo[k]fluoranthene	55	140
Benzo[a]pyrene	51	83
Indeno[1,2,3-cd]pyrene	53	90
Dibenz[a,h]anthracene	56	98
Benzo[ghi]perylene	52	94

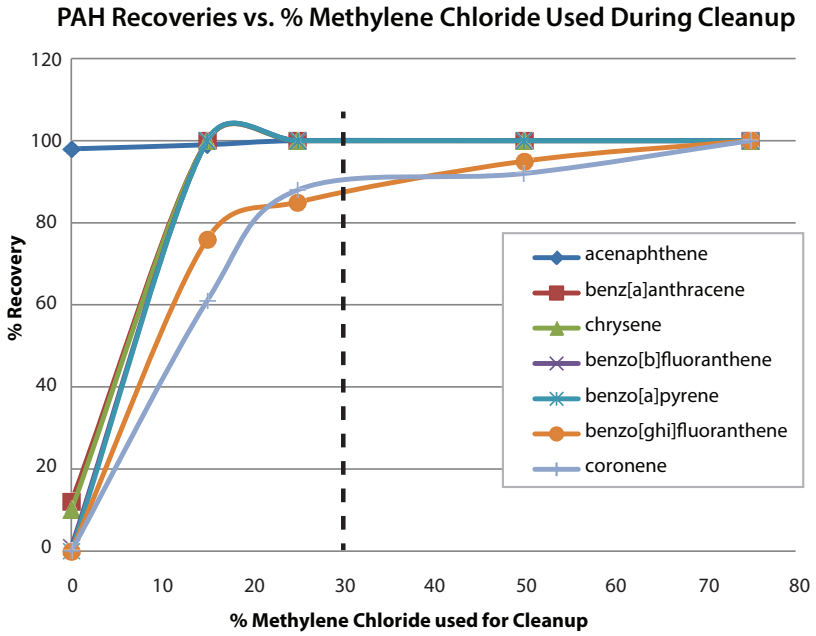
Evaluation of Methylene Chloride Percentage in Hexane Solvent for Sample Cleanup

Commodities like tea and spices can be particularly challenging because these matrices are very complex. Sample cleanup is needed to remove coextracted material to prepare samples for analysis. Extensive clean up techniques can greatly reduce the number and amount of coextracted compounds [44, 45]. However, these methods are often laborious, time-intensive processes.

The strategy for this work was to develop a simple cleanup process that removes enough coextracted material to allow for successful analysis, but also minimizes the resources needed to perform the cleanup. Gas chromatographic systems can be quickly fouled by introducing samples with a significant amount of nonvolatile material. This material resides in the GC inlet, dirtying the inlet liner and seals. The head of the column can also become contaminated with nonvolatile material that cannot be eluted from the column. This scenario necessitates instrument and column maintenance.

The silica SPE cleanup was optimized with respect to the elution solvent and volume. PAHs can be eluted with a combination of hexane and methylene chloride. Fortified tea samples at 50 µg/g were tested using different elution solvents with low to high methylene chloride percentages. Figure 1 displays the percent recovery values with respect to percent methylene chloride in hexane for low, mid, and high molecular weight PAHs. Acenaphthene is low molecular weight and was easily eluted with hexane; however, larger molecular weight compounds required a stronger solvent to elute from the silica. The plots of individual PAHs show increased recovery as the ratio of methylene chloride increases. At 15% methylene chloride in hexane, mid-sized PAHs, like the PAH4 shown in Figure 1, are recovered well with values around 100%. However, large PAHs like coronene are only recovered to approximately 60%. Based on the recovery values, a compromise of 30% methylene chloride in hexane was chosen for subsequent work. This elution solvent composition yielded acceptable recovery of all target PAHs. A higher ratio of methylene chloride would improve recovery of large PAHs, but would also increase the amount of coextracted material.

Figure 1: Percent recovery values of low, mid, and high molecular weight PAHs with respect to percent of methylene chloride in hexane used for silica SPE elution are plotted. PAHs were fortified at 50 µg/g.



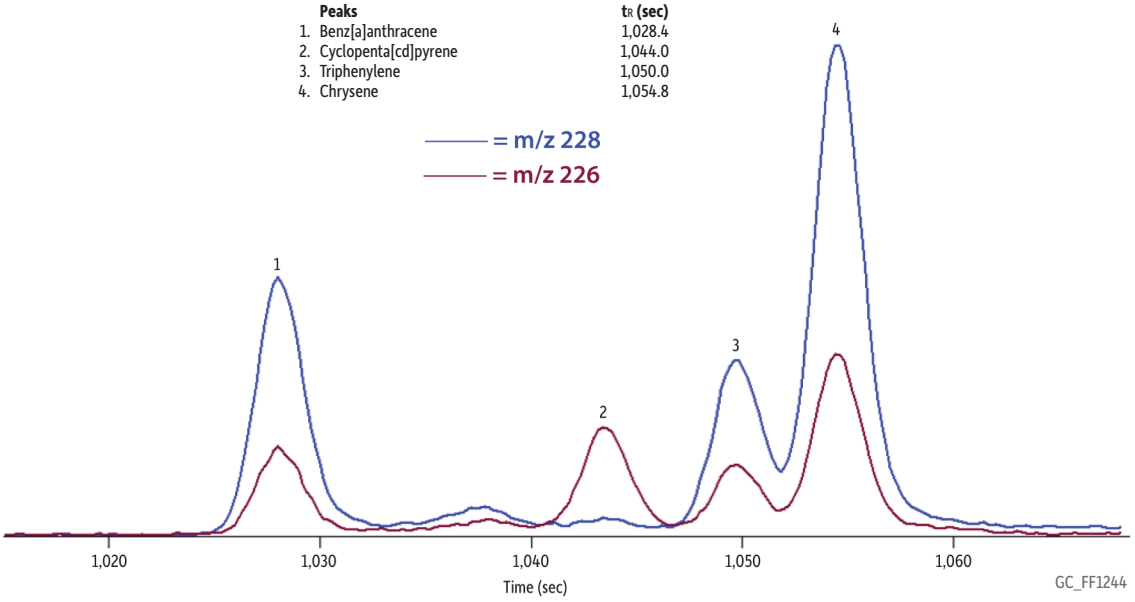
Chromatographic Method

Optimizing the chromatographic separation is critical for PAH analysis due to the isobaric compounds that commonly coelute making quantitation difficult. An optimized GC method was developed using a high phenyl content Rxi®-PAH column that is selective for the highly aromatic PAHs. Due to the selectivity of the column stationary phase and the column formats, analysis of the 30 PAHs used in the study, including the dibenzopyrenes, was accomplished in 35 minutes which is a relatively fast analysis time. Figure 2A shows an extracted ion chromatogram of m/z 226 and 228 for commercial tea 1, which was produced using the GC-TOFMS method described above. The separation of triphenylene and chrysene shows distinct peaks. This allowed for reliable peak integration for both compounds, which are isobaric congeners that usually elute at or near the same retention time. Similarly, benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene are notoriously challenging to separate, but these compounds also are well resolved as shown in Figure 2B. In total, the optimized method used for this work separated critical pairs and allowed accurate, independent quantitation of important toxicity markers including chrysene and benzo[b]fluoranthene which are EFSA PAH4 compounds and dibenz[a,h]anthracene which is a PAH8 compound.

Figure 2: A) GC-TOFMS extracted ion chromatogram of m/z 226 and 228 showing the separation of incurred PAHs, including triphenylene and chrysene, from commercial tea 1. B) GC-TOFMS extracted ion chromatogram of m/z 252 showing the separation of incurred benzofluoranthenes from commercial tea 1.

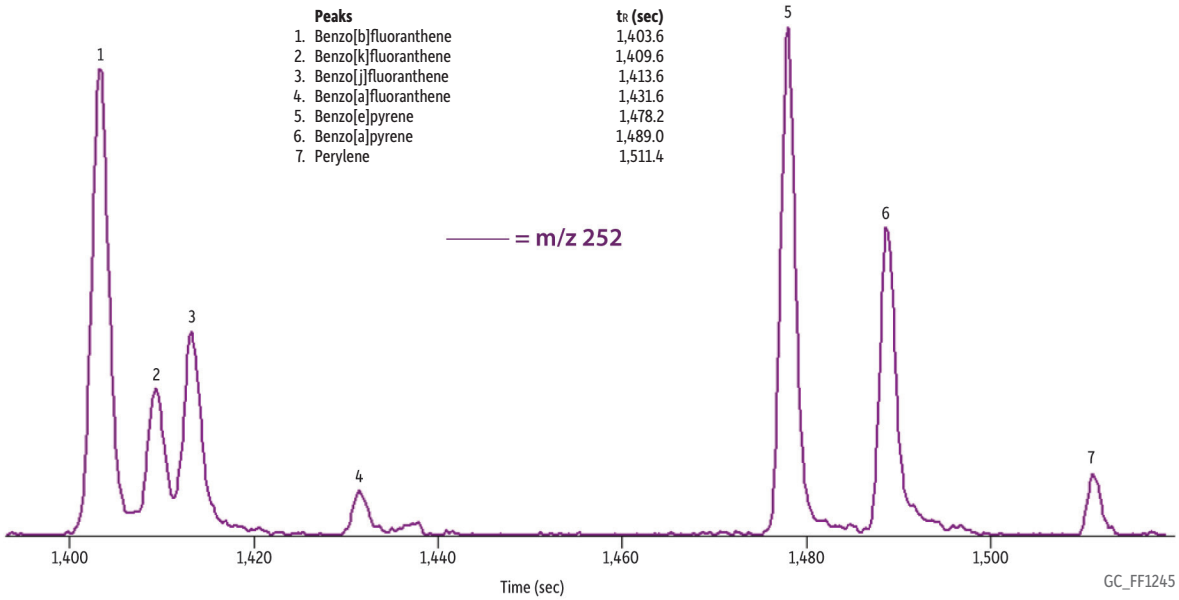
A

Peaks	t_R (sec)
1. Benz[a]anthracene	1,028.4
2. Cyclopenta[cd]pyrene	1,044.0
3. Triphenylene	1,050.0
4. Chrysene	1,054.8



B

Peaks	t_R (sec)
1. Benzo[b]fluoranthene	1,403.6
2. Benzo[k]fluoranthene	1,409.6
3. Benzo[j]fluoranthene	1,413.6
4. Benzo[a]fluoranthene	1,431.6
5. Benzo[e]pyrene	1,478.2
6. Benzo[a]pyrene	1,489.0
7. Perylene	1,511.4



Column	Rxi®-PAH, 60 m, 0.25 mm ID, 0.10 µm (cat.# 49317)
Injection	
Inj. Vol.:	2.5 µL splitless (hold 1 min)
Liner:	Sky® 4 mm single taper w/wool (cat.# 23303.5)
Inj. Temp.:	275 °C
Purge Flow:	40 mL/min
Oven	
Oven Temp.:	80 °C (hold 1 min) to 210 °C at 40 °C/min to 260 °C at 3 °C/min to 350 °C at 11.5 °C/min (hold 6.25 min)
Carrier Gas	H_2, constant flow
Flow Rate:	2.4 mL/min
Detector	TOFMS
Transfer Line Temp.:	320 °C
Analyzer Type:	TOF
Source Temp.:	300 °C
Electron Energy:	70 eV
Mass Defect:	0 mu/100 u
Solvent Delay Time:	3.67 min
Tune Type:	PFTBA
Ionization Mode:	EI
Acquisition Range:	45-550 amu
Spectral Acquisition Rate:	5 spectra/sec
Instrument	LECO Pegasus® 4D GCxGC-TOFMS

Incurred PAHs in Teas

Quantitative analysis was easily accomplished using this optimized chromatographic method. Incurred values of isobaric compounds were evaluated in six commercial mate teas and results are displayed in Table III. Quantitative bias is demonstrated by comparing the concentrations of compounds in each isobaric pair, which are grouped between grey rows. Based on values in Table III, chrysene would be biased about 20%, benzo[b]fluoranthene by approximately 50%, and dibenzo[a,h]anthracene by as much as 60% if the combined area of the pairs had to be reported. The ability to separate these compounds is critical to determining the presence and concentration of toxicity marker PAHs.

Recovery of PAHs in Fortified Teas

The optimized method used a modified QuEChERS extraction with hexane:acetone (1:1, v/v), silica SPE cleanup, and a selective Rxi®-PAH GC column. Both GC-MS/MS and GC-TOFMS were able to perform the method and yielded satisfactory recovery values, but GC-MS/MS offered better sensitivity. Recovery values are shown in Table IV. The recovery values for all PAHs in this study range between 72 and 130% with only four compounds in the 70 to 80% range. Recoveries for the EFSA PAH4 compounds were 81-100% and recoveries for the EFSA PAH8 compounds were 81-110%. The recovery values indicate that this analytical method for PAHs in tea is suitable for PAHs with a wide range of volatility and molecular weight.

Comparison of GC-TOFMS and GC-MS/MS for EFSA PAH4 Compounds

Samples of six yerba mate teas were processed with the optimized sample preparation method and two GC-MS based methods. The GC-TOFMS method used a selective Rxi®-PAH GC column in a 60 m x 0.25 mm x 0.10 µm configuration (cat.# 49317) format that increases the separation of isobaric compounds as well as sample loading capacity. Peak resolution was enhanced by use of hydrogen carrier gas. PAHs are ideal for analysis by hydrogen carrier GC-MS because they form strong molecular ions and do not suffer from hydrogen reactivity, thus mitigating potential sensitivity loss when using hydrogen in GC-MS. The GC-MS/MS also used the selective Rxi®-PAH GC column, but in a 40 m x 0.18 mm x 0.07 µm format (cat.# 49316) that balances separation with analysis time. In addition, sample loading is smaller because the stationary phase film thickness is relatively thin. Thus, it was important to minimize the sample injection volume for this method. Mate tea samples had relatively high incurred PAHs concentrations, so using a 0.5 µL injection was not detrimental to overall detectability. However, for other commodities with trace levels of PAHs, using a highly sensitive tandem MS can compensate for lower injection volumes.

The concentrations of incurred PAH4 compounds were determined by both GC-TOFMS and GC-MS/MS and are shown in Table V. The combined levels for the PAH4 are shown in the last row. The values determined by both techniques agree well with each other. This indicates that both the sample preparation and analysis methods are suitable for PAH analysis in mate tea.

Table III: Values of incurred PAHs in six different mate teas determined using the final extraction/cleanup method and GC-TOFMS. Isobaric pairs are grouped and separated by a grey-colored row in the table.

PAH	Tea 1 (ng/g)	Tea 2 (ng/g)	Tea 3 (ng/g)	Tea 4 (ng/g)	Tea 5 (ng/g)	Tea 6 (ng/g)
Triphenylene	82	14	18	54	34	14
Chrysene	320	81	85	260	140	130
Benzo[b]fluoranthene	150	67	35	150	49	52
Benzo[j]fluoranthene	65	39	20	75	27	31
Dibenz[a,c]anthracene	11	9.3	10	12	10	6.9
Dibenz[a,h]anthracene	18	15	10	21	12	12

Table IV: Percent recovery values for PAHs in a commercially available tea. PAHs were fortified at 500 µg/g dry tea.

PAH	Tea 1 % Recovery
Naphthalene	90
Acenaphthylene	110
Acenaphthene	99
Fluorene	110
Phenanthrene	81
Anthracene	130
Fluoranthene	72
Pyrene	74
Benzo[c]phenanthrene	75
Benz[a]anthracene	81
Triphenylene	80
Chrysene	82
5-Methylchrysene	76
Benzo[b]fluoranthene	92
Benzo[k]fluoranthene	96
Benzo[j]fluoranthene	89
Benzo[a]fluoranthene	97
Benzo[e]pyrene	89
Benzo[a]pyrene	100
Perylene	94
Dibenz[a,c]anthracene	100
Indeno[1,2,3-cd]pyrene	110
Dibenz[a,h]anthracene	98
Benzo[ghi]perylene	88
Dibenzo[a,e]pyrene	93
Coronene	86

Table V: Incurred concentrations, ng/g dry tea, for the PAH4 compounds in six brands of yerba mate tea. For each tea, values are reported for the GC-MS/MS and GC-TOFMS methods. The combined concentrations of the PAH4 are reported in the last row.

PAH	Tea 1 (ng/g)		Tea 2 (ng/g)		Tea 3 (ng/g)		Tea 4 (ng/g)		Tea 5 (ng/g)		Tea 6 (ng/g)	
	MS/MS	TOF	MS/MS	TOF	MS/MS	TOF	MS/MS	TOF	MS/MS	TOF	MS/MS	TOF
Benz[a]anthracene	190	190	33	45	43	52	150	170	94	100	52	62
Chrysene	320	320	46	81	70	85	250	260	140	140	110	130
Benzo[b]fluoranthene	150	150	78	67	57	35	140	150	70	49	79	52
Benzo[a]pyrene	120	140	66	82	24	36	160	160	42	42	80	81
Combined EFSA PAH4	780	800	220	270	190	210	700	740	350	340	320	320

Conclusion

The mate teas tested have high levels of PAHs when compared to typical residue limits of between 1 and 10 ng/g. The EFSA PAH4 sums are shown in the last row of Table V and range from 190-800 ng/g in dry tea. The streamlined sample preparation method for PAHs in yerba mate tea provided satisfactory recovery of all PAHs tested. The selective chromatographic methods were paired with MS-based detection. Sufficient separation of isobaric PAHs was accomplished using Rxi®-PAH columns with a PAH selective stationary phase making quantitation of individual PAHs straightforward. Overall, this analytical method for PAHs in tea required less resources and time than typically needed for analysis of a difficult matrix like mate tea while providing improved data quality.

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Analysis of Nitrofurans in Honey

Using LC/MS/MS and an Ultra C18 Column

By Eberhardt Kuhn, Ph.D., International Marketing Specialist; and Becky Wittrig, Ph.D., HPLC Product Marketing Manager

- Sensitive detection of antibiotic metabolites in a complex matrix.
- Ultra C18 column assures the resolution needed for the LC-MS/MS method.
- Excellent peak shape at sub-ppb levels.

Nitrofurans are a class of veterinary antibiotics used to increase growth rate and prevent or treat disease in animals. Animals have been treated with antibiotics since the 1950s and, currently, about 45% of the antibiotics produced each year in the U.S. are administered to livestock. In Europe, this practice is illegal, because the inadvertent consumption of residual antibiotics in animal tissue, such as meat or liver, can lead to increased drug resistance or allergies in humans.

Nitrofurans have been detected not only in treated animals, but also in animal products, including honey. The low levels of these compounds and the complexity of honey as a matrix present challenges for the analysis of nitrofurans. In addition, nitrofurans are unstable and metabolize rapidly in vivo. Any analysis method for nitrofurans, therefore, must be able to separate and detect these metabolites. In the analysis of honey, it is of interest to quantify four nitrofurans: furazolidone, furaltadone, nitrofurazone, and nitrofurantoin, through their respective metabolites, 3-amino-2-oxazolidone (AOZ), 5-mofolinomethylmethyl-3-amino-2-oxazolidone (AMOZ), semicarbazide (SC) and 1-aminohydantoin (AHD). The method of choice for the analysis of nitrofuran and nitrofuran metabolites in honey is LC/MS/MS,with separation on a C18 column.

In this study, honey samples treated with the four nitrofuran metabolites were dissolved in water, then extracted with ethyl acetate. After centrifugation, the extract was evaporated and reconstituted in 125mM HCl, then derivatized with 2-nitrobenzaldehyde. After two liquid-liquid extractions with ethyl acetate, the extract was evaporated and reconstituted with mobile phase, filtered, and injected into the LC-MS/MS system. The column used for the analysis was a 100mm x 2.1mm, 3µm Ultra C18 column. For maximum sensitivity and specificity, a triple quadrupole analyzer was used, with electrospray ionization and selected reaction monitoring (SRM).

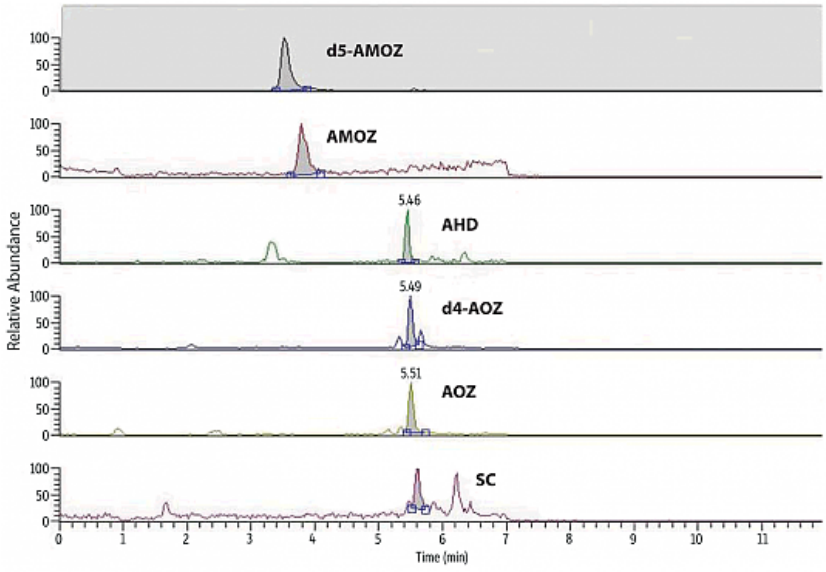
Results from the analysis of 0.3ppb nitrofuran metabolites in honey are shown in Figure 1. The Ultra C18 HPLC column is an excellent choice for this analysis. As a reliable general purpose column based on a high-purity, base-deactivated silica, its utility extends to other compounds that might be present in animal derived matrixes, such as steroids and vitamins.

In analyses for nitrofuran antibiotics, an Ultra C18 HPLC column is an excellent choice, especially for analyzing trace levels of these compounds in a complex sample matrix.

Figure 1 Nitrofuran metabolites in honey detected at 0.3ppb by LC/MS/MS, using an Ultra C18 column.

Peaks	t_R (min)	Prec. Ion	Prod. Ion	Collision E (V)	Tube Lens
1. d5-AMOZ	3.7	--	--	--	--
2. AMOZ	3.8	335	291	10	100
3. AHD	5.46	249	134	12	110
4. d4-AOZ	5.49	--	--	--	--
5. AOZ	5.51	236	134	12	120
6. SC	5.6	209	166	12	80

AMOZ = 3-amino-5-morpholinomethyl-2-oxazolidinone; AHD = 1-aminohydantoin hydrochloride; AOZ = 3-amino-2-oxazolidinone; SC = semicarbazide



LC_0323

Column	Ultra C18 (cat.# 9174312)
Dimensions:	100 mm x 2.1 mm ID
Particle Size:	3 µm
Pore Size:	100 Å
Temp.:	30 °C
Sample	
Conc.:	0.3 ppb each analyte
Mobile Phase	
A:	0.05% formic acid in methanol
B:	0.05% formic acid – 5 mM NH_4 formate in water

Time (min)	%B
0	90
2.5	90
5	10
10	10
12	90
15	90

Flow:	0.2 mL/min
Detector	MS/MS triple quadrupole (Thermo Scientific Discovery)
Ion Source:	Electrospray
Ion Mode:	ESI+
Notes	**Analyzer Parameters:** Only segment: 15 min Data type: centroid Scan mode: SRM product Scan width (m/z): 0.7 Scan time (s): 0.25 Peak width: Q1: within 0.7; Q2: 0.7 Collision gas pressure (mTorr): 1.5 (argon) Divert valve: active, with 3 positions Positions-1° 2 min, 2° 8 min, 3° 5 min

Acknowledgements

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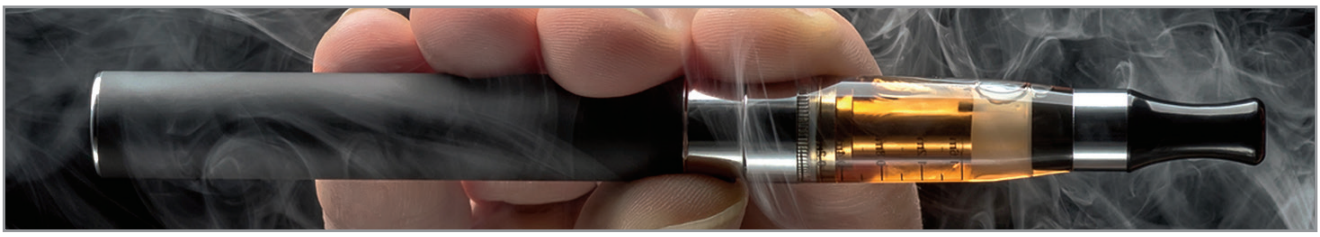
RELATED SEARCHES

nitrofurans, *honey*, *nitrofuran metabolites*, *food contaminants*, *Ultra C18*, *LC/MS/MS*



Foods, Flavors & Fragrances

Analysis of Nicotine and Impurities in Electronic Cigarette Solutions and Vapor

By Jason S. Herrington, Colton Myers, and Amanda Rigdon

Abstract

Electronic cigarettes (e-cigarettes) are growing in popularity exponentially. Despite their ever-growing acceptance, relatively little work has been done to characterize their vapor. To date, the majority of e-cigarette research has focused on characterizing the solutions, which are ultimately vaporized for the end user to inhale. The current study focused on developing a complete analytical package for the quick and simple analysis of electronic cigarette solutions and vapor to determine nicotine content and impurity profiles. Rapid (<5 min) gas chromatography–flame ionization detector (GC-FID) methods (using both helium and hydrogen carrier gas) were developed for the determination of nicotine content in e-cigarette solutions. In addition, a straightforward GC mass spectrometry (GC-MS) method was developed for the determination of impurities in e-cigarette liquids. Lastly, a simple sampling device was developed to draw e-cigarette vapor into a thermal desorption (TD) tube, which was then thermally extracted and analyzed via the same GC-MS method. This novel approach was able to provide detectable levels of volatile organic compounds (VOCs) and semivolatile organic compounds (SVOCs), which were not detected in the liquids, from a single 40 mL puff. All three of the methods may be done with one GC, two detectors, and one analytical column (Rtx®-VMS), thereby reducing required resources and affording easy comparison of results.

Introduction

Electronic cigarettes (e-cigarettes) do not burn tobacco, rather they produce an aerosol (without flame or smoke) from a battery-powered, metal heating element and liquid-containing cartridge [1]. The liquid typically consists of humectants (propylene glycol [1,2-propanediol] and/or glycerin), flavorants, and nicotine [2]. When an e-cigarette's power source is activated, the heating element vaporizes the liquid to form a mist, which the end user then may inhale (often referred to as "vape") [3]. The smoke-like vapor imitates tobacco smoke visually and replicates the burning sensation in the throat and lungs (often referred to as "throat hit"). These similarities to tobacco smoke, combined with the same hand-to-mouth behaviors, have contributed to the rapid adaptation of electronic cigarettes [4-6]. Despite their increasing use on a global scale [3], relatively little is known about the e-cigarette chemical components. The majority of studies have focused primarily on the nicotine content and impurities (e.g., nitrosamines) of e-cigarette liquid (e-juice) [7]. More important, relatively little is known about the chemical composition of the vapor, which is ultimately what end users are exposed to [7, 8].

Only a few researchers (e.g., Goniewicz et al. [7], Kosmider [9], and Schober et al. [8]) have attempted to characterize e-cigarette vapor by analyzing it for the presence of volatile organic compounds (VOCs), nitrosamines, heavy metals, and polycyclic aromatic hydrocarbons (PAHs); however, their study designs have been relatively complex and/or required the use of a specialized smoking machine and/or an array of specialized analytical instruments. Such requirements are often not practical for routine contract laboratory testing. The current study evaluates the nicotine content and impurities of several commercially available e-cigarettes and their respective solutions via simple and rapid GC-FID and GC-MS methods. In addition, the primary e-cigarette emissions were analyzed for VOCs and semivolatile organic compounds (SVOCs) via a simple and novel technique that pairs thermal desorption (TD) with GC-MS. Results, analytical techniques, obstacles, and solutions are discussed.

Experimental

Electronic Cigarettes and Liquids

Four commercially available electronic cigarettes (Table I) were chosen from the "Best E-Cigarettes of 2014," which is a top 10 list of e-cigarettes as viewed by "experts and users" [10]. It is important to note that these four chosen e-cigarettes also routinely appeared on other web-based review sites as "top 10" performers. In addition, these four brands were readily obtained from local stores. All four e-cigarettes were "1st generation" cigarettes (i.e., generally mimicking the size and look of regular cigarettes) [11] and, with the exception of vendor D, were disposable. In addition to the e-cigarettes, their respective e-liquids (i.e., same brand, flavor, and nicotine content) were obtained.

Vendors A, B, and C indicated their claimed nicotine percentage was based on wt/wt analysis. Vendor D indicated their labeled value was based on vol/vol analysis; however, one side of the D refill solution bottle denoted 1,000 mg of nicotine, which is in keeping with a wt/wt analysis (which appears to be the industry standard) or a wt/vol analysis. Therefore, it was not entirely clear how vendor D determined their nicotine concentrations. Upon receipt, 1 mL of each e-cigarette solution was pipetted with a calibrated syringe onto a calibrated scale to determine the density of each solution. Measured densities were later used to convert wt/wt label claims to wt/vol values for direct comparison to the analytically determined wt/vol values using the following equation:

$$Nicotine\ Concentration\ (mg/mL) = Solution\ Density\left(\frac{mg}{mL}\right) \times Vendor\ Claimed\ Concentration\left(\frac{mg}{mg}\right)$$

Table I: Characteristics of Electronic Cigarettes and Liquids

Vendor	Claimed Nicotine % (wt /wt)	Style	Measured Density (g/mL)
A	1.8 (18 mg/1,000 mg)	Classic Tobacco	1.1179
B	1.2 (12 mg/1,000 mg)	Classic Tobacco	1.1843
C	1.2 (12 mg/1,000 mg)	Menthol	1.2006
D	1.8 (18 mL/1,000 mL)*	Classic Tobacco	1.1271

*One label on the solution refill bottle indicated the % nicotine was based on % vol /vol; however, the other side of the bottle denoted 1,000 mg, which is in keeping with wt/wt analysis.

Nicotine

The following system was used to analyze electronic cigarette e-liquid nicotine concentrations: an Agilent 7890A GC equipped with an Agilent FID. An Rtx®-VMS column was chosen as the analytical column based on its unique ability to separate volatile compounds. The GC-FID parameters for both helium and hydrogen carrier gases are presented in Table II. The nicotine levels of the e-cigarette solutions were determined by calibrating the GC-FID with a National Institute of Standards and Technology (NIST) traceable nicotine standard (cat.# 34085). The 1,000 µg/mL nicotine standard was serially diluted with methylene chloride to generate a 7-point external calibration curve (Table III). Although not shown, a United States (U.S.) Environmental Protection Agency (EPA) Method 8260 internal standard (cat.# 30074) was found to be suitable for the current work.

All electronic cigarette solutions were diluted with methylene chloride by one hundred fold. This dilution was carried out for the following reasons: 1) Initial work with the e-cigarette solutions indicated the liquids were relatively viscous in nature. This viscosity resulted in the formation of air bubbles in the autosampler syringe. A 100:1 dilution remedied any viscosity issues. 2) The e-cigarette solutions chosen for this study appeared to have nicotine concentrations of ~15–25 mg/mL, which was outside the concentration range of the calibration curve (Table III). A 100:1 dilution resulted in nicotine levels that fell between the upper and lower limits of the calibration curve. It is important to note that methylene chloride was chosen as the diluent instead of methanol because the methanol solvent peak coeluted with the ethanol (one of the major constituents of e-cigarette solutions) peak.

Table II: Analytical system and parameters utilized for quantifying the nicotine content of electronic cigarette liquids.

Agilent 7890A GC-FID			
Column	Rtx-VMS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 19915)		
Injection	Diluted (100:1) electronic cigarette liquid		
Inj. Vol.	1.0 µL split (200:1)		
Liner	Sky 4 mm Precision liner w/wool (cat.# 23305.5)		
Inj. Temp.	250 °C		
Septum Purge Flow	3 mL/min		
Detector	FID @ 250 °C		
Carrier Gas	He, constant flow	H_2, constant flow*	H_2, constant flow*
Flow Rate	2.0 mL/min	2.50 mL/min	2.50 mL/min
Linear Velocity	44.4 cm/sec	67.2 cm/sec	67.2 cm/sec
Oven	100 °C to 260 °C at 35 °C/min (hold 0.25 min)	100 °C to 260 °C at 54 °C/min (hold 0.15 min)	100 °C to 240 °C at 35 °C/min
*Requires a fast ramping oven			

Table III: External nicotine calibration curve for quantifying the nicotine content of electronic cigarette liquids.

1.00 mg/mL Nicotine Standard (cat.# 34085)				
Level	**µL of Previous Level**	**µL of Methylene Chloride**	**Total Volume (µL)**	**Concentration (mg/mL)**
1	NA	NA	NA	1.00
2	100	100	200	0.500
3	100	100	200	0.250
4	100	100	200	0.125
5	100	100	200	0.063
6	100	100	200	0.031
7	100	100	200	0.016

Impurities

The following analytical system was used for the qualitative determination of any impurities found in the electronic cigarette solutions: an Agilent 7890B GC coupled with an Agilent 5977A MS detector. The GC-MS parameters are presented in Table IV. This analysis also utilized the Rtx®-VMS column based on its proven performance for volatile compounds.

Table IV: Analytical system and parameters utilized for determination of electronic cigarette solution impurities.

Agilent 7890B/5977A GC-MS Parameters	
Column	Rtx-VMS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 19915)
Injection	Diluted (2:1) electronic cigarette liquid
Inj. Vol.	1.0 µL split (10:1)
Liner	Sky 4 mm Precision liner w/wool (cat.# 23305.5)
Inj. Temp.	250 °C
Purge Flow	3 mL/min
Oven	35 °C (hold 1 min) to 250 °C at 11 °C/min (hold 4 min)
Carrier Gas	He, constant flow
Flow Rate	2.0 mL/min
Linear Velocity	51.15 cm/sec
Detector	MS
Mode	Scan
Transfer Line Temp.	250 °C
Analyzer Type	Single quadrupole
Source Temp.	230 °C
Quad Temp.	150 °C
Electron Energy	70 eV
Tune Type	BFB
Ionization Mode	EI
Acquisition Range	15 – 550 amu
Rate	5.2 scans/sec

Vapor

Electronic cigarette vapor was analyzed for nicotine and impurities by trapping the vapor on thermal desorption tubes. Goniewicz et al. and other researchers have used smoking machines (e.g., Teague TE-2, Borgwaldt RM20S) to generate and collect e-cigarette aerosols; however, access to such an apparatus was not available for this study [7]. Therefore, in order to provide reproducible and quantitative results, a simple sampling device (Figure 1) was adapted from a 50 mL gas-tight syringe (cat.# 24761). The syringe was used to draw 40 mL of vapor in ~4 seconds from the e-cigarettes across a stainless steel thermal desorption tube packed with Tenax TA, Carbograph TD, and Carboxen 1003 (unconditioned [cat.# 26469] or conditioned [cat.# 26470]). This tube was chosen based on the optimized combination of three sorbents to screen for VOCs in the C2-3 range up to SVOCs in the C30-32 range. Although this method was manual, a ~4-second puff was utilized, as suggested based on Farsalinos et al.'s observations on e-cigarette topography [12]. In addition to the single puff sample, a 10-puff sample was also taken in order to mimic a smoking regime. This sample was taken by manually drawing ten 4-second puffs separated by 10-second intervals between puffs. The desorption tube was then transferred to the following analytical system for determining the VOCs and SVOCs directly emitted from an e-cigarette: a Markes UNITY™ thermal desorption system paired with an Agilent 7890B GC coupled to an Agilent 5977A MS detector. The UNITY™ system and GC-MS parameters are presented in Table V and Table IV, respectively.

The vapor concentrations of selected VOCs were calculated from a 5-point calibration curve generated by analyzing a series of volumes of a 10.0 ppbv primary standard (Table VI). The 10.0 ppbv primary standard was generated by injecting 180 mL of a 1.00 ppmv component TO-15 + IV mix (cat.# 34396) and 180 mL of a 1.00 ppmv ozone precursor mixture/PAMS (cat.# 34420) into an evacuated 6-liter SilcoCan® air monitoring canister (cat.# 24142-650) and pressurizing the canister to 30 psig with 50% RH nitrogen. Ochiai et al. [13] determined 50% RH to be optimal for stability. The standard was allowed to age for 7 days.

Figure 1: Gas-tight syringe sampling apparatus for quantitatively drawing electronic cigarette vapor into a thermal desorption tube.

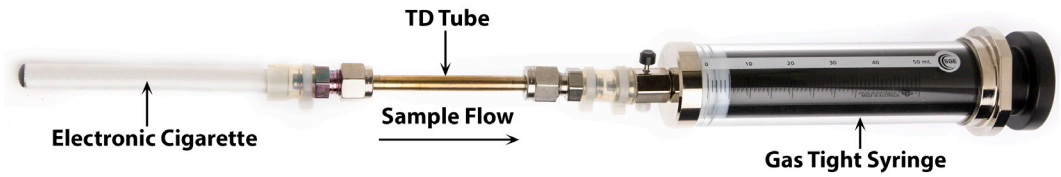


Table V: Markes UNITY™ thermal desorption system and parameters utilized for thermally extracting electronic cigarette aerosols for the qualitative and quantitative determination of VOCs and SVOCs emitted from electronic cigarettes.

Markes UNITY Parameters			
General Settings		**Trap Settings**	
Operating Mode	Standard two stage	Pre-Trap Fire Purge	1.0 min
Standby Split	True	Flow	20.0 mL/min
Standby Flow	5 mL/min	Trap Low	0 °C
Flow Path Temperature	210 °C	Heating Rate	Max
Minimum Carrier Pressure	5.0 psi	Trap High	320 °C
GC Cycle Time	0.0	Trap Hold	5 min
		Split On	True
Pre-Desorption		Split On	20 mL/min
Prepurge Time	1.0 min		
Trap in Line	False		
Split On	True		
Flow	20 mL/min		
Tube/Sample Desorption			
Time 1	10.0 min		
Temperature 1	320 °C		
Trap in Line	True		
Split On	False		

Table VI: Calibration curve for calculating vapor concentrations determined on a Markes UNITY™ thermal desorption system.

Standard (ppbv)	Injection Volume (mL)	Calibration Concentration (ppbv)
10.0	720	180
10.0	360	90
10.0	120	30
10.0	40	10
10.0	4	1.00

Blanks

The Markes UNITY™ system was operated with helium carrier gas for desorbing the thermal desorption tubes and the cryogenic trap during ballistic heating for analyte focusing on the head of the analytical column. The combination of helium gas (devoid of oxygen) and elevated temperatures may have established conditions that were ideal for pyrolysis of propylene glycol and/or glycerin. The pyrolysis of propylene glycol and glycerin has been demonstrated to produce formaldehyde, acetaldehyde, and acrolein. Therefore, the following experiments were conducted to evaluate any compound contributions from the TD-GC-MS process itself: empty stainless steel tubes (i.e., no sorbents) and packed thermal desorption tubes (i.e., multi-bed sorbents) were injected with 1 µL aliquots of the electronic cigarette solutions and run through the TD-GC-MS analysis. In addition, the air drawn through the electronic cigarettes during sampling came from the laboratory. Due to the ubiquitous nature of VOCs such as formaldehyde and benzene, it was imperative to determine the background contributions of VOCs to the vapor analysis. Therefore, 40 mL samples of the laboratory air were periodically collected with thermal desorption tubes and analyzed with the same TD-GC-MS method.

Results and Discussion

Nicotine

Analyses of electronic cigarette solutions, as shown in Figure 2 (helium), Figure 3 (hydrogen, fast ramp), and Figure 4 (hydrogen, standard ramp), using the GC-FID conditions in Table II afforded the rapid (i.e., <5 minute GC run time) determination of the major chemical components. All four vendors' e-cigarette liquids appeared to contain ethanol, propylene glycol, glycerin, and nicotine. It is important to note that all four vendors listed propylene glycol, glycerin, and nicotine; however, none of the vendors listed ethanol as an ingredient. Blank analyses indicated that ethanol was not from laboratory contamination. Methylene chloride was used as the diluent to solve viscosity and concentrations issues, hence the abundant presence of methylene chloride. As shown in Figure 5, the rapid GC-FID method produced an acceptable external calibration of nicotine from 0.016 to 1.00 mg/mL (r > 0.995).

As shown in Table VII, the vendor claimed nicotine concentrations were lower than the actual measured nicotine concentrations by 4 to 28%. Recall the wt/wt label claims were converted to wt/vol values using the measured density of each solution in order to allow direct comparison to the actual values determined analytically using the calibration curve. The observation of increased nicotine content was consistent with what Schober et al. [8] and others have observed as well.

Figure 2: Analysis of major electronic cigarette solution components via GC-FID (helium).

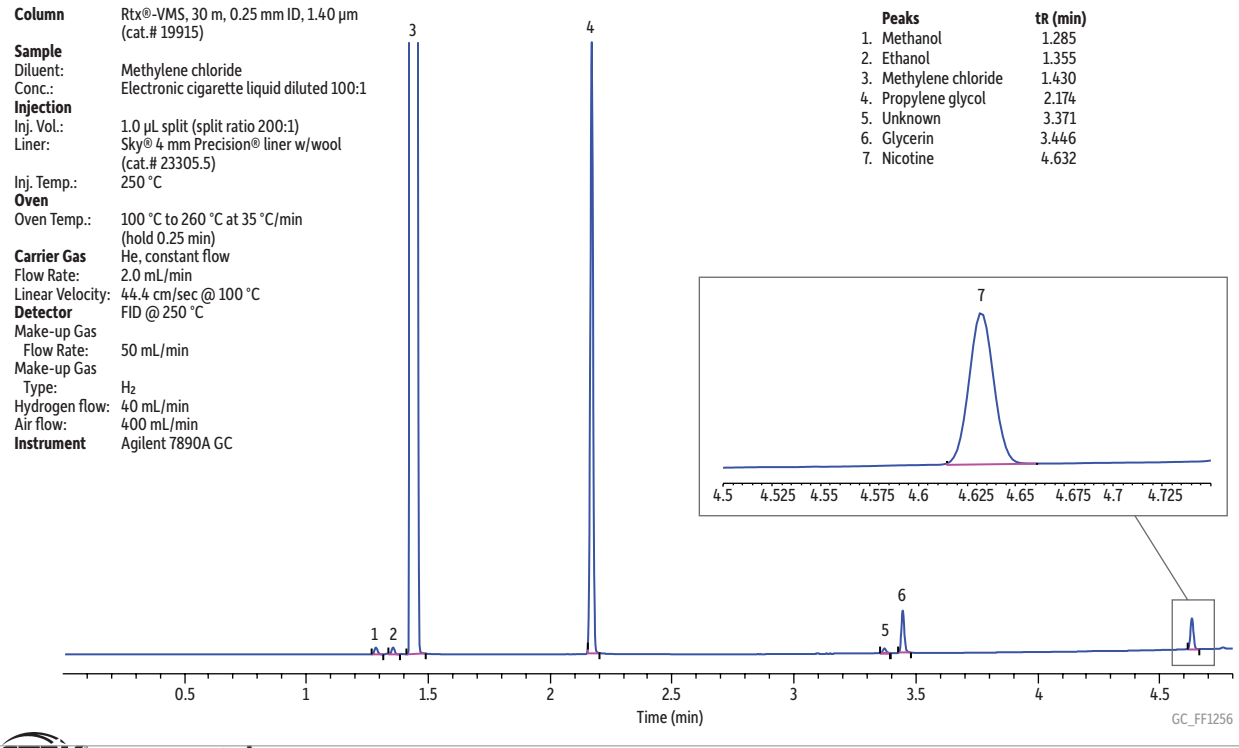


Figure 3: Analysis of major electronic cigarette solution components via GC-FID (hydrogen, fast ramp).

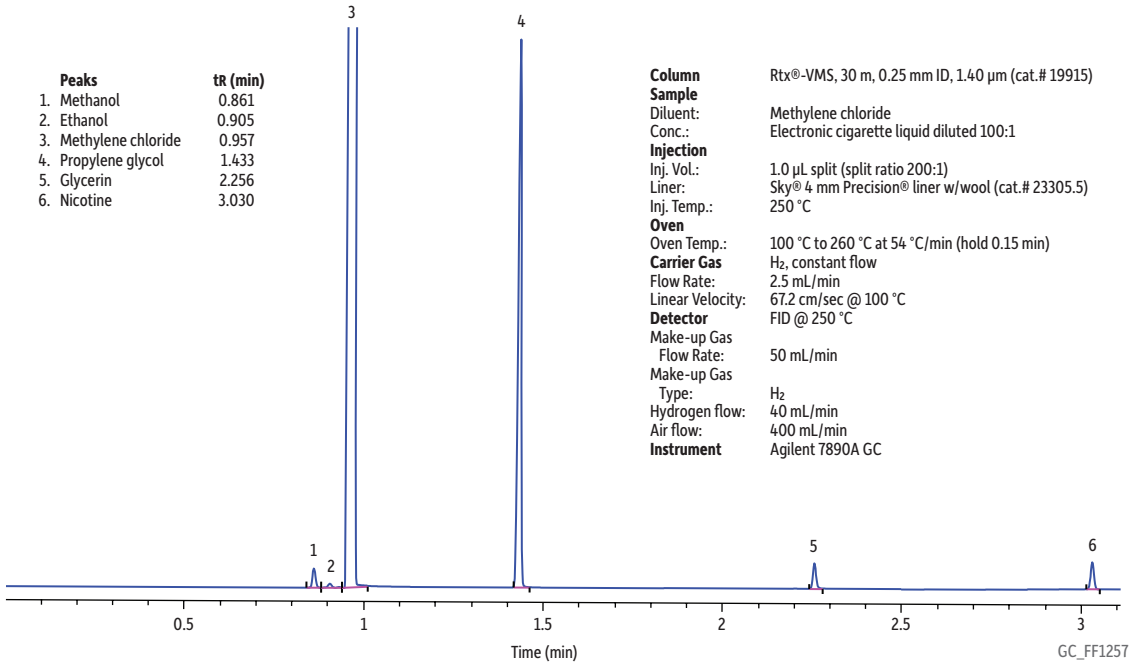


Figure 4: Analysis of major electronic cigarette solution components via GC-FID (hydrogen, standard ramp).

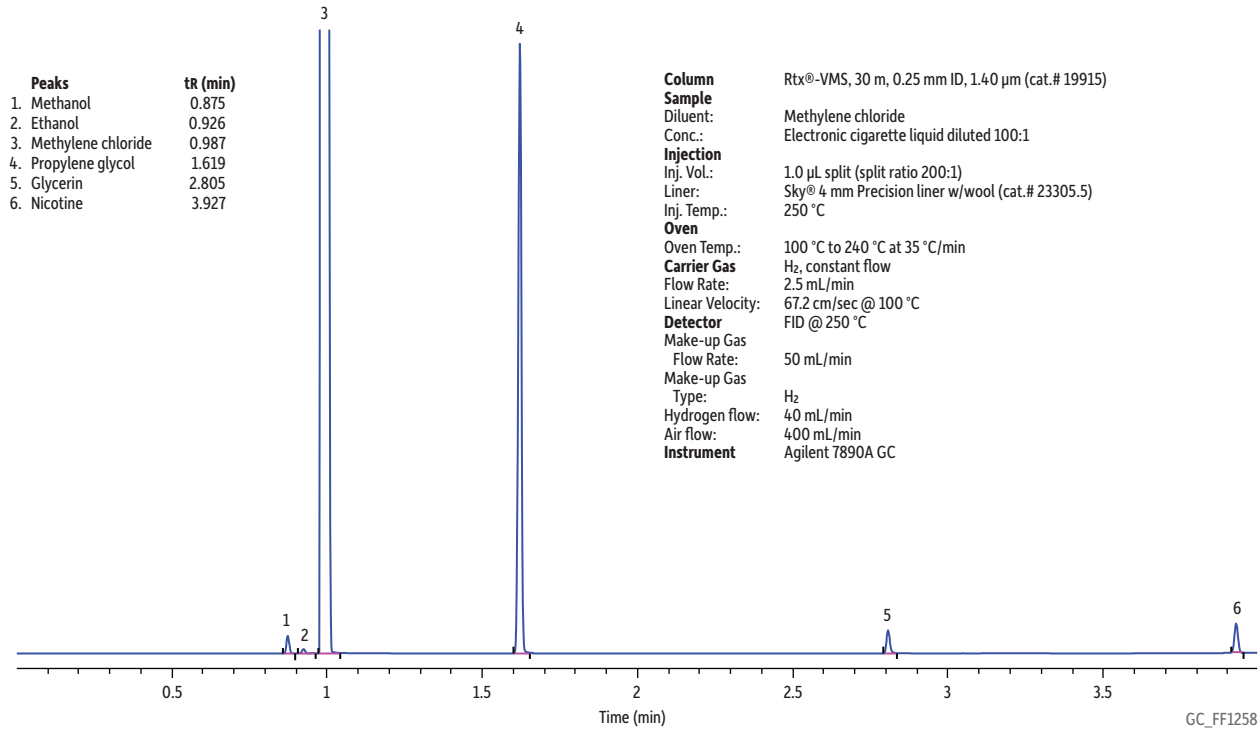


Figure 5: A linear response was obtained for nicotine over a concentration range of 0.06–1.00 mg/mL using the GC-FID method as demonstrated by the external calibration curve (r > 0.995).

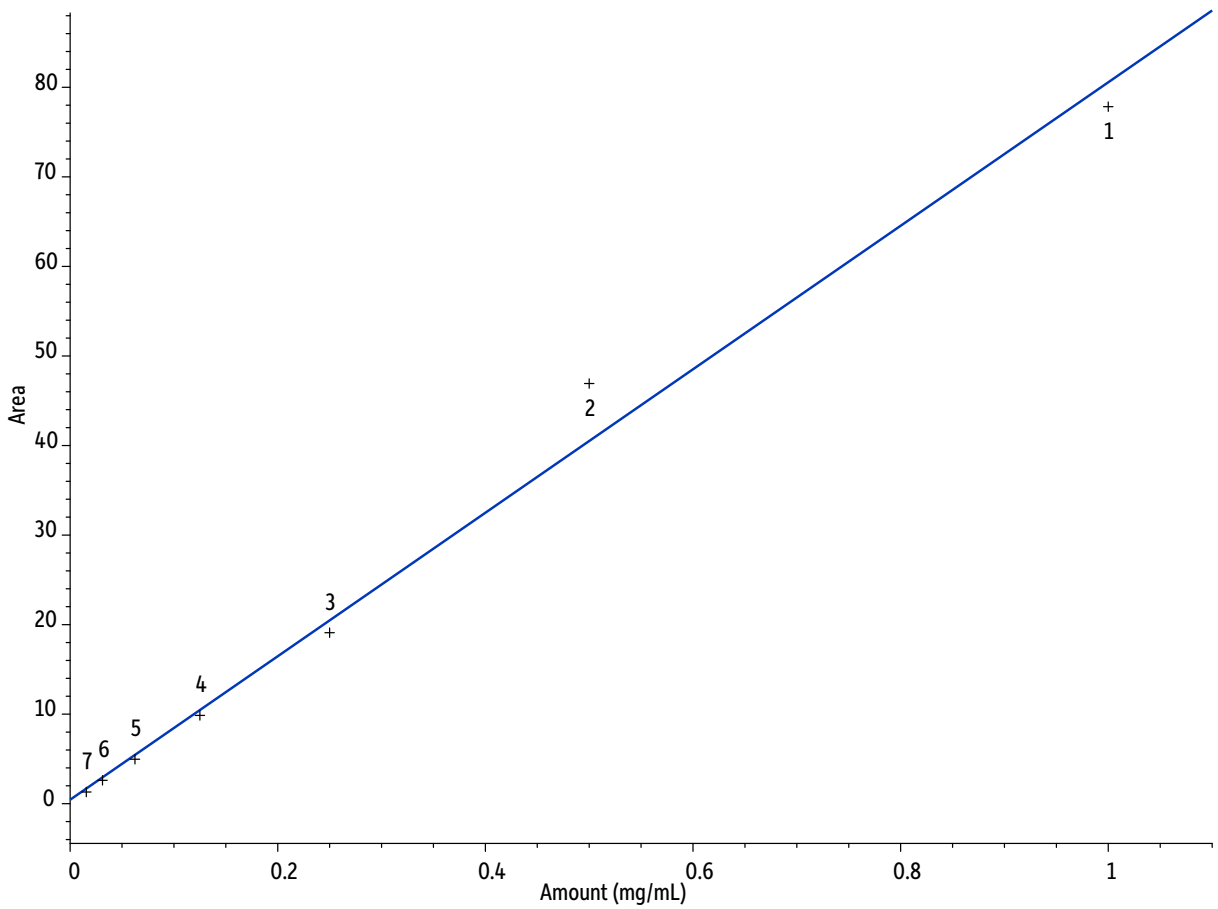


Table VII: Vendor nicotine concentrations as claimed and as determined in the current study by direct comparison with pure nicotine standards via GC-FID.

Vendor	Vendor Claimed Nicotine (mg/mL)^	Nicotine (mg/mL) Determined*	% Difference
A	20.1	23.4	17%
B	14.2	14.8	4%
C	14.4	17.4	21%
D	20.3	26.0	28%

^ Calculated based on determined density.
* Average of 3 analyses.

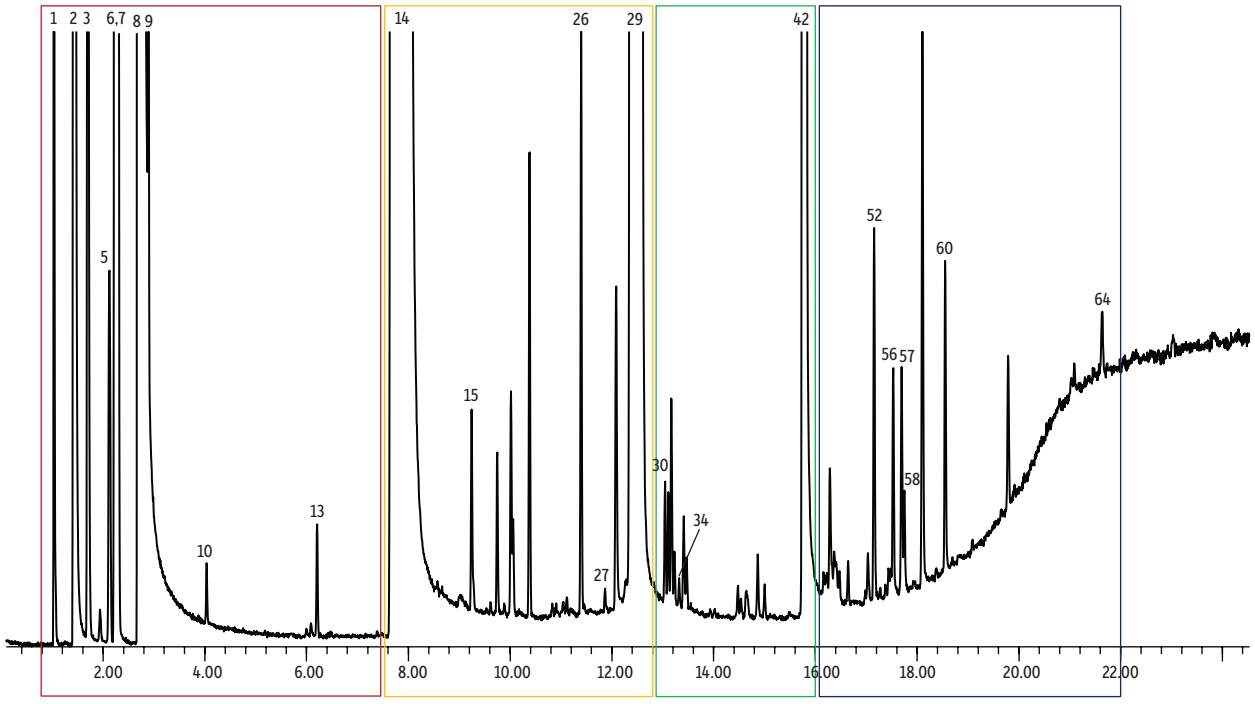
Impurities in E-Cigarette Solutions

As shown in Figure 6, the analysis of electronic cigarette solutions revealed that they contained numerous compounds besides the vendor-listed propylene glycol, glycerin, and nicotine. For the solution shown in Figure 6 (vendor A) there were 64 unidentified and identified (some only tentatively) compounds found in the e-cigarette solution. Compounds were deemed "identified" when verified with a subsequent run of an external standard with matching retention times and mass spectral data. Compounds were deemed "tentatively identified" when the mass spectral quality was 80% or greater according to the NIST 2011 database [14]. Several pyrazines were tentatively identified, which is consistent with manufacturer-added flavorings. For example, acetylpyrazine, which was tentatively identified, is a flavorant well known for producing "nutty" flavors/aromas. In addition, several pyridines were identified, which is consistent with tobacco-derived nicotine. For example, 3-(3,4-dihydro-2H-pyrrol-5-yl)- pyridine(myosmine) was also tentatively identified and this compound is an alkaloid found in tobacco [15]. It is important to note that almost half (36) of the compounds were unidentified; future work should focus on identifying these compounds.

Figure 6: Analysis of electronic cigarette solution (e-juice) by GC-MS revealed the presence of numerous components in addition to the compounds listed on the product labels.

Peaks	tR (min)	Match Quality	EC Liquid	Blank	Region
1. Nitrogen/oxygen/carbon dioxide	1.051	100	x	x	Red
2. Water	1.441	100	x	x	Red
3. Methanol	1.709	100	x	x	Red
4. Unidentified	1.934		x	x	Red
5. *cis*-1,2-Dimethylcyclopropane	2.117	94	x	x	Red
6. Ethanol	2.239	100	x		Red
7. 1,1-Dichloroethene	2.282	94	x	x	Red
8. Methylene chloride	2.757	100	x	x	Red
9. 1,2-Dichloroethene	2.891	94	x	x	Red
10. Ethyl acetate	4.037	91	x		Red
11. Unidentified	6.000		x		Red
12. Unidentified	6.085		x		Red
13. Toluene	6.207	100	x		Red
14. Propylene glycol	7.853	100	x		Orange
15. 2,3-Dimethylpyrazine	9.243	91	x		Orange
16. Unidentified	9.615		x		Orange
17. Unidentified	9.713		x		Orange
18. Unidentified	9.889		x		Orange
19. Unidentified	10.017		x		Orange
20. Unidentified	10.060		x		Orange
21. Trimethylpyrazine	10.383	94	x		Orange
22. Unidentified	10.828		x		Orange
23. Unidentified	10.907		x		Orange
24. Unidentified	11.047		x		Orange
25. Unidentified	11.114		x		Orange
26. Acetylpyrazine	11.394	95	x		Orange
27. N-(1-Methylethyl)benzenamine	11.864	80	x		Orange
28. Dipropylene glycol	12.071	91	x		Orange
29. Glycerin	12.473	100	x		Orange
30. Dipropylene glycol methyl ether	13.040	80	x		Green
31. Unidentified	13.107		x		Green
32. Unidentified	13.168		x		Green

Peaks	tR (min)	Match Quality	EC Liquid	Blank	Region
33. Unidentified	13.229		x		Green
34. 1-(3-Pyridinyl)ethanone	13.321	94	x		Green
35. Unidentified	13.412		x		Green
36. Unidentified	13.463		x		Green
37. Unidentified	14.479		x		Green
38. Unidentified	14.534		x		Green
39. Unidentified	14.643		x		Green
40. Unidentified	14.863		x		Green
41. Unidentified	15.003		x		Green
42. Nicotine	15.800	100	x		Green
43. Unidentified	16.161		x		Blue
44. Unidentified	16.222		x		Blue
45. α-Damascone	16.289	95	x		Blue
46. Unidentified	16.374		x		Blue
47. Unidentified	16.417		x		Blue
48. Unidentified	16.478		x		Blue
49. Unidentified	16.643		x		Blue
50. Unidentified	16.984		x		Blue
51. Unidentified	17.033		x		Blue
52. Myosmine	17.155	95	x		Blue
53. Unidentified	17.276		x		Blue
54. Unidentified	17.380		x		Blue
55. Unidentified	17.441		x		Blue
56. Nicotine 1-N-oxide	17.533	93	x		Blue
57. Anabasine	17.697	98	x		Blue
58. Nicotyrine	17.752	91	x		Blue
59. Unidentified	18.105		x		Blue
60. 2,3-Dipyridyl	18.550	97	x		Blue
61. Unidentified	19.788		x		Blue
62. Unidentified	21.025		x		Blue
63. Unidentified	21.092		x		Blue
64. Cotinine	21.635	91	x		Blue



Column Rtx®-VMS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 19915)
Sample
Diluent: Methylene chloride
Conc.: Electronic cigarette liquid diluted 2:1
Injection
Inj. Vol.: 1 µL split (split ratio 10:1)
Liner: Sky® 4 mm Precision® liner w/wool (cat.# 23305.5)
Inj. Temp.: 250 °C

Oven
Oven Temp.: 35 °C (hold 1 min) to 250 °C at 11 °C/min (hold 4 min)
Carrier Gas He, constant flow
Flow Rate: 2.0 mL/min
Linear Velocity: 51.15 cm/sec @ 35 °C
Detector MS
Mode: Scan
Scan Program:

Group	Start Time (min)	Scan Range (amu)	Scan Rate (scans/sec)
1	0	15-550	5.2

Transfer Line Temp.: 250 °C
Analyzer Type: Quadrupole
Source Type: Extractor
Extractor Lens: 6mm ID
Source Temp.: 230 °C
Quad Temp.: 150 °C
Electron Energy: 70 eV
Tune Type: BFB
Ionization Mode: EI
Instrument Agilent 7890B GC & 5977A MSD

Vapor

As shown in Figure 7, the simple sampling device (Figure 1) was able to successfully draw electronic cigarette vapor into a thermal desorption tube and provide detectable levels of VOCs and SVOCs from a single 40 mL puff. As observed in the impurities study, there clearly were numerous compounds (i.e., 82 unidentified and identified [some only tentatively]) in the e-cigarette vapor beyond propylene glycol, glycerin, and nicotine. However, the analysis of the vapor revealed the presence of 18 more compounds in addition to those found in the liquid analysis. Of particular interest was the presence of formaldehyde, acetaldehyde, acrolein, and xylenes, as well as several siloxanes. The current observation of these three carbonyls (formaldehyde, acetaldehyde, and acrolein) was consistent with Goniewicz et al.'s [7] and Kosmider et al.'s [9] observations.

These observations are significant for the two following reasons: 1. All three of these carbonyls are acutely toxic; in addition, formaldehyde is a known human carcinogen [16] and acetaldehyde is a probable human carcinogen [17]. 2. These compounds were not present in the e-juice, which indicates they were generated during the vaporization process and/or from the e-cigarette materials. This is consistent with the fact that pyrolysis of glycerin results in the formation of formaldehyde, acetaldehyde, and acrolein [18]. This is also consistent with the fact that polysiloxanes are often used as plastic additives and the majority of the first generation e-cigarettes, like those evaluated in this study, are made with plastic bodies. All of the aforementioned have profound implications for how e-cigarettes should be evaluated, especially when considering that end users are ultimately exposed to the e-cigarette vapor rather than the liquid.

To expound upon this further, acrolein was not found in the electronic cigarette solutions. However, acrolein was found in the vapor from all four of the e-cigarettes evaluated in the current study. The acrolein concentrations ranged from 1.5 to 6.7 ppmv per 40 mL puff (0.003–0.015 µg/mL), which is comparable to the 0.004 µg/mL Goniewicz et al. reported [7]. To put these concentrations into perspective, these levels exceeded the National Institute of Occupational Safety and Health (NIOSH) short-term exposure limit (STEL) of 350 ppbv. Furthermore, assuming 40 mL per puff and 400 to 500 puffs per e-cigarette (values suggested by several e-cigarette manufacturers), each e-cigarette would generate ~20 to 230 µg of acrolein. From a human health perspective, the acrolein emissions observed in the current study appear to be on par with what has previously been reported for conventional tobacco cigarettes (3 to 220 µg of acrolein/cigarette) [19]. Formaldehyde and acetaldehyde standards were not available at the time of publishing this application note. However, their peak areas were on the same order of magnitude as acrolein, thereby suggesting their concentrations were comparable, which is also consistent with what Goniewicz et al. reported [7].

Currently, the U.S. Food and Drug Administration (FDA) does not have any regulatory authority over electronic cigarettes. However, the FDA does acknowledge that e-cigarettes, their associated risks, nicotine levels, and any potentially harmful chemicals inhaled are "not fully studied." Therefore, the FDA has issued a proposed rule to extend their authority to include e-cigarettes [20]. Regardless of the status of the FDA's authority over e-cigarettes, it is clear from the current research and the research of others that the e-cigarette landscape is not fully understood. However, it appears that e-cigarettes are not without human health risks. Most important, and as demonstrated by the current work, when designing future e-cigarette studies investigators should strongly consider the difference between analyzing electronic cigarette solutions and analyzing electronic cigarette vapor, as it very clear that their chemical profiles are different.

Figure 7: A single 40 mL puff of electronic cigarette vapor collected on a thermal desorption tube and analyzed via GC-MS.

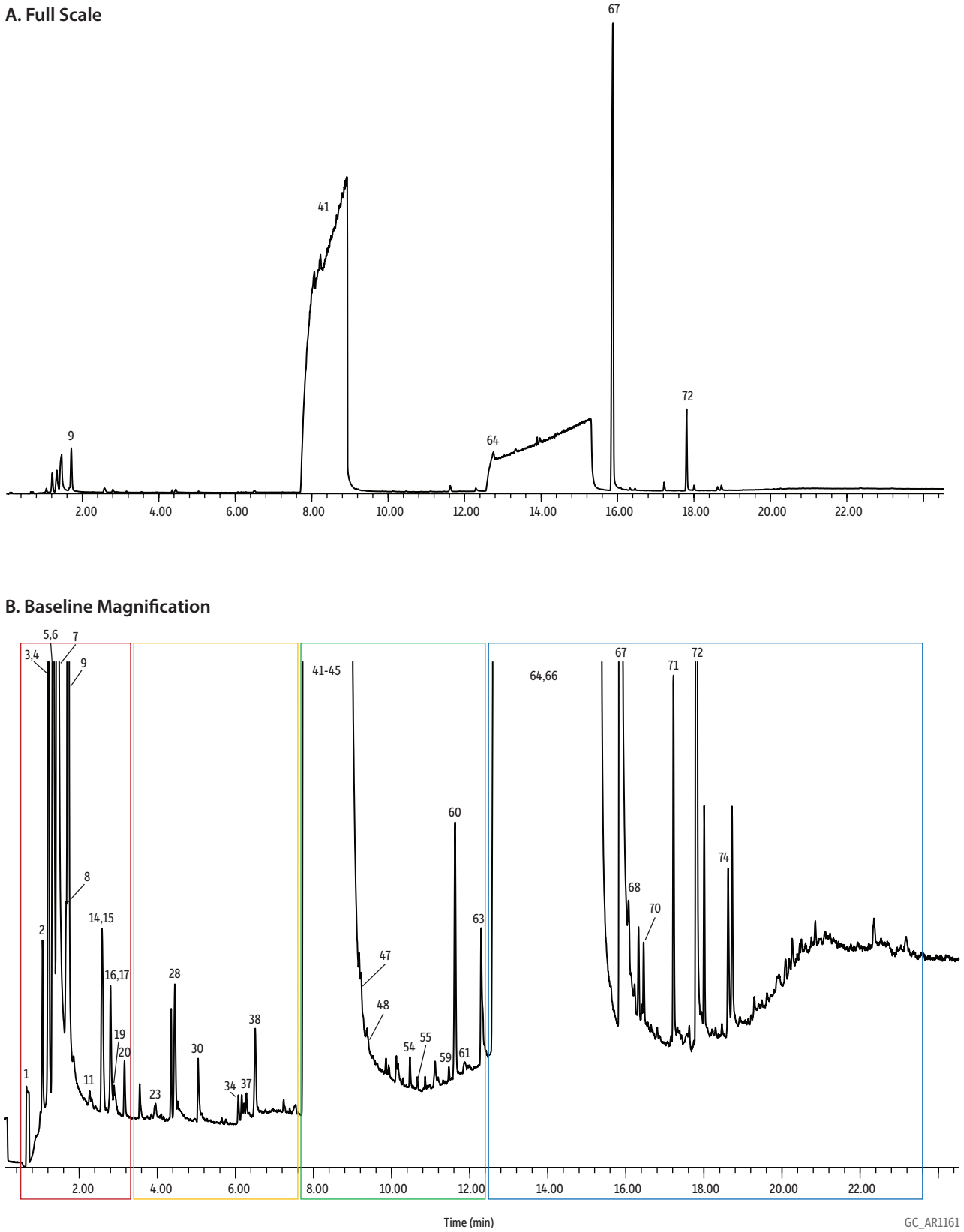


Figure 7: Peak List

	Peaks	tR (min)	Match Quality	Vapor	Blank*	Region
1.	Nitrogen/oxygen	0.685	100	x	x	Red
2.	Carbon dioxide	1.063	100	x	x	Red
3.	Propene	1.200	100	x		Red
4.	Formaldehyde	1.227	100	x		Red
5.	Sulfur dioxide	1.313	90	x		Red
6.	Chloromethane	1.380	100	x		Red
7.	Water	1.453	100	x	x	Red
8.	Acetaldehyde	1.672	100	x		Red
9.	Methanol	1.715	100	x	x	Red
10.	Unidentified	1.885		x		Red
11.	Ethanol	2.270	100	x		Red
12.	Unidentified	2.331		x		Red
13.	Unidentified	2.410		x		Red
14.	Acrolein	2.581	100	x		Red
15.	Propanal	2.629	100	x		Red
16.	Methylene chloride	2.770	100	x	x	Red
17.	Acetone	2.843	100	x		Red
18.	Unidentified	2.892		x		Red
19.	Hexane	2.928	100	x		Red
20.	Acetonitrile	3.160	100	x	x	Red
21.	Unidentified	3.544		x		Orange
22.	Unidentified	3.842		x		Orange
23.	Trimethylsilanol	3.928	100	x		Orange
24.	Unidentified	4.092		x		Orange
25.	Unidentified	4.159		x		Orange
26.	Unidentified	4.245		x		Orange
27.	Unidentified	4.354		x		Orange
28.	Benzene	4.452	100	x	x	Orange
29.	Unidentified	4.519		x		Orange
30.	Acetic acid	5.055	86	x		Orange
31.	Unidentified	5.141		x		Orange
32.	Unidentified	5.647		x		Orange
33.	Unidentified	5.756		x		Orange
34.	1-Hydroxy-2-propanone	6.073	80	x		Orange
35.	Unidentified	6.165		x		Orange
36.	Unidentified	6.220		x		Orange
37.	Toluene	6.280	100	x	x	Orange
38.	Hexamethylcyclotrisiloxane	6.506	91	x		Orange
39.	Unidentified	7.231		x		Orange
40.	Unidentified	7.530		x		Orange
41.	Propylene glycol	7.737	100	x		Green
42.	*m*-Xylene	8.048	100	x		Green
43.	*p*-Xylene	8.048	100	x		Green
44.	*o*-Xylene	8.530	100	x		Green
45.	Styrene	8.597	100	x		Green
46.	Unidentified	9.158		x		Green
47.	Octamethylcyclotetrasiloxane	9.218	91	x		Green
48.	4-Methyl-1-(1-methylethyl)cyclohexene	9.371	95	x		Green
49.	Unidentified	9.639		x		Green
50.	Unidentified	9.852		x		Green
51.	Unidentified	9.932		x		Green
52.	Unidentified	10.121		x		Green
53.	Unidentified	10.219		x		Green
54.	Trimethylpyrazine	10.468	80	x		Green
55.	Benzaldehyde	10.657	100	x		Green
56.	Unidentified	10.858		x		Green
57.	Unidentified	11.120		x		Green
58.	Unidentified	11.187		x		Green
59.	Acetylpyrazine	11.541	93	x		Green
60.	Decamethylcyclopentasiloxane	11.620	91	x		Green
61.	Phenol	11.870	94	x		Green
62.	Unidentified	12.272		x		Green
63.	1,1'-Oxybis-2-propanol	12.333	90	x		Green
64.	Glycerin	12.748	100	x		Blue
65.	Unidentified	13.327		x		Blue
66.	Dodecamethylcyclohexasiloxane	13.979	94	x		Blue
67.	Nicotine	15.862	100	x		Blue
68.	Tetradecamethylhexasiloxane	16.082	91	x		Blue
69.	Unidentified	16.326		x		Blue
70.	Unidentified	16.460		x		Blue
71.	Myosmine	17.216	94	x		Blue
72.	Nicotyrine	17.807	90	x		Blue
73.	Unidentified	18.002		x		Blue
74.	2,3'-Dipyridyl	18.618	94	x		Blue
75.	Unidentified	18.721		x		Blue
76.	Unidentified	19.294		x		Blue
77.	Unidentified	19.611		x		Blue
78.	Unidentified	20.093		x		Blue
79.	Unidentified	20.190		x		Blue
80.	Unidentified	20.269		x		Blue
81.	Unidentified	20.501		x		Blue
82.	Unidentified	20.855		x		Blue

*The concentrations of these compounds in e-cigarette vapor were too close to blank and/or laboratory air concentrations to definitively state they were emitted from the e-cigarettes.

Column Rtx®-VMS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 19915)
Sample
Conc.: One 40 mL puff of electronic cigarette vapor drawn via a gas-tight syringe to replicate vaping
Injection Direct
Oven
Oven Temp.: 35 °C (hold 1 min) to 250 °C at 11 °C/min (hold 4 min)
Carrier Gas He, constant flow
Flow Rate: 2.0 mL/min @ 35 °C
Detector MS
Mode: Scan
Scan Program:

Group	Start Time (min)	Scan Range (amu)	Scan Rate (scans/sec)
1	0	15-550	5.2

Transfer Line Temp.: 250 °C
Analyzer Type: Quadrupole
Source Type: Extractor
Extractor Lens: 6mm ID
Source Temp.: 230 °C
Quad Temp.: 150 °C
Electron Energy: 70 eV
Tune Type: BFB
Ionization Mode: EI
Preconcentrator Markes UNITY™
Instrument Agilent 7890B GC & 5977A MSD
Acknowledgement Markes

Blanks

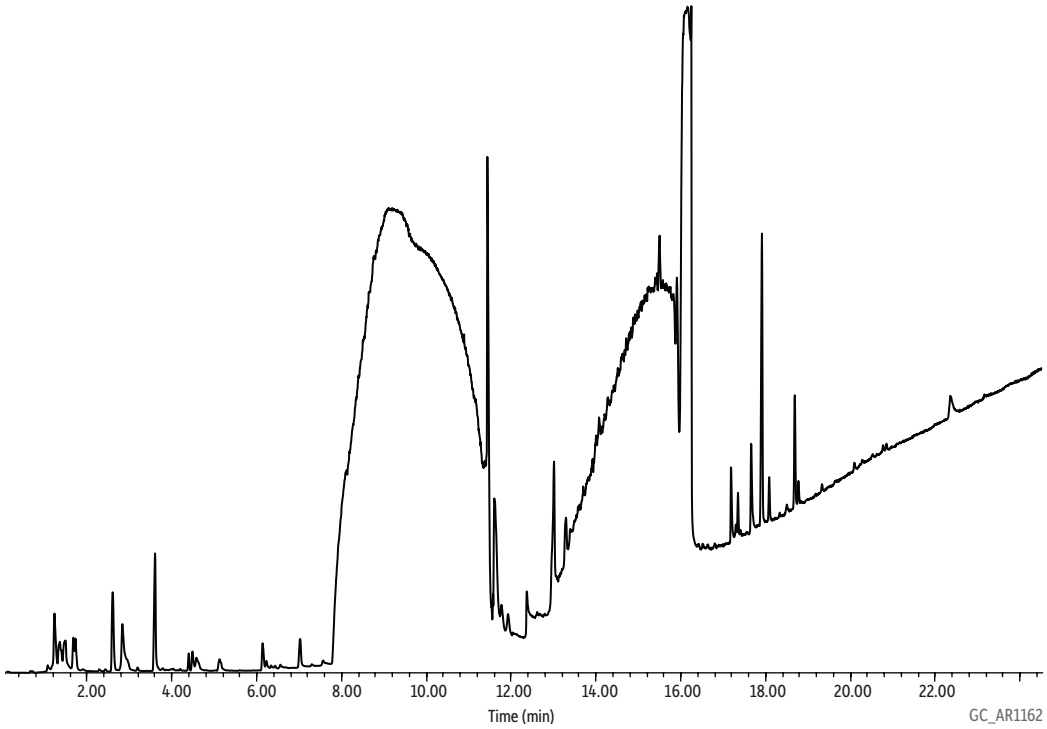
The 1 µL aliquots of electronic cigarette solutions injected into empty stainless steel tubes (i.e., no sorbents) and analyzed via the TD-GC-MS method resulted in the formation of formaldehyde, acetaldehyde, and acrolein. However, the concentrations of these three compounds did not increase when 1 µL aliquots of the e-cigarette solutions were injected into packed thermal desorption tubes (i.e., multi-bed sorbents) and analyzed via the TD-GC-MS method. The two aforementioned observations are consistent with the hypothesis that pyrolysis of propylene glycol and/or glycerin was taking place within the TD-GC-MS system itself and not in the thermal desorption tube media (i.e., the multi-sorbent bed). However, it was unclear as to where the pyrolysis was taking place (i.e., on the cryogenic trap during ballistic heating versus in the heated transfer lines) within the TD-GC-MS system. Regardless, the pyrolysis was responsible for 14 to 23% of the vapor concentrations of formaldehyde, acetaldehyde, and acrolein observed in the current study. The aforesaid percent contributions were approximated by comparing the carbonyl/nicotine ratios obtained from the empty stainless steel tubes and packed thermal desorption tubes to the 40 mL puff samples. In addition, the laboratory air was sometimes a source for certain VOCs; however, these levels (i.e., low ppbv) were often well below the e-cigarette levels (i.e., low to mid ppmv). Future investigators should be aware of their laboratory air concentrations and the potential pyrolysis within the TD-GC-MS system and make necessary adjustments in their reporting limits and/or background corrections. It was outside the scope of the current work; however, future work should focus on reducing pyrolysis contribution by adjusting line temperatures, heating rates, flow rates, etc.

Advantages/Limitations/Future Research

Researchers like Goniewicz et al. had access to specialized smoking machines, which enabled "realistic" smoking regimes (e.g., a 1.8 second puff with 10 second intervals between puffs). These smoking regimes may reveal more about e-cigarette vapor and/or be more accurate than the simple sampling device (Figure 1) utilized in the current study. However, the current work is significant in that multiple puffs were not needed because the present analytical techniques demonstrated detectability from a single 40 mL puff. In fact, it is important to note that a smoking regime of a 4-second puff with 10-second intervals between 10 puffs was executed manually with the simple sampling device (Figure 1). The results of this 10-puff sample are shown in Figure 8. The 10-puff sample did reveal some early eluting compounds (i.e., identified, tentatively identified, and unidentified), which were not identified in the single-puff (Figure 7). However, the propylene glycol and glycerin peaks, which were already overloaded in the single-puff sample, became so large in the 10-puff sample that most of the peaks previously identified in the single-puff sample were lost due to interference with propylene glycol and glycerin. In addition, this overloading of propylene glycol and glycerin contaminated the Markes UNITY™ thermal desorption system, thereby requiring a time-consuming cleaning to avoid carryover.

As previously mentioned in the discussion of the blanks results, future researchers should be aware of the potential pyrolysis conditions within the TD-GC-MS system and how that may affect their formaldehyde, acetaldehyde, and acrolein vapor concentrations. Alternative sampling/analytical approaches (e.g., DNPH-coated solid sorbents) are available for these carbonyls, which would circumvent the pyrolysis issues; however, they come at the significant disadvantage of time-consuming solvent extractions and the inability to scan for a large number of compounds (e.g., the 82 VOCs/SVOCs observed in the current study) in a single 40 mL puff. Future TD-GC-MS work on e-cigarette vapor should focus on optimizing the thermal desorption parameters in order to reduce pyrolysis contributions by adjusting line temperatures, heating rates, flow rates, etc. Overall, the current method may be well suited for the easy and rapid screening of e-cigarette vapor for a large number of VOCs and SVOCs.

Figure 8: Ten 40 mL puffs of electronic cigarette vapor collected on a thermal desorption tube and analyzed via GC-MS.



Column	Rtx®-VMS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 19915)
Sample	
Conc.:	Ten 40 mL puffs of electronic cigarette vapor drawn via a gas-tight syringe to replicate vaping
Injection	Direct
Oven	
Oven Temp.:	35 °C (hold 1 min) to 250 °C at 11 °C/min (hold 4 min)
Carrier Gas	He, constant flow
Flow Rate:	2.0 mL/min @ 35 °C
Detector	MS
Mode:	Scan

Scan Program:

Group	Start Time (min)	Scan Range (amu)	Scan Rate (scans/sec)
1	0	15-550	5.2

Transfer Line Temp.:	250 °C
Analyzer Type:	Quadrupole
Source Type:	Extractor
Extractor Lens:	6mm ID
Source Temp.:	230 °C
Quad Temp.:	150 °C
Electron Energy:	70 eV
Tune Type:	BFB
Ionization Mode:	EI
Preconcentrator	Markes UNITY™
Instrument	Agilent 7890B GC & 5977A MSD
Acknowledgement	Markes

Conclusions

As electronic cigarettes explode in popularity, public attention is rapidly turning toward consumer safety. While research to date has focused primarily on the components of e-cigarette solutions, data presented here indicate a need for substantially more research into the chemical profile of vapor samples. To that end, this study included development of analytical methods for both solution and vapor samples. All three methods developed in the current study used an Rtx®-VMS column—a proprietary phase to Restek—which was chosen to reduce required resources and afford easy comparison of results.

For e-cigarette solutions, rapid GC-FID methods using helium or hydrogen carrier gas were established for the determination of nicotine content. These methods would be suitable for fast quality control testing of electronic cigarette solutions. In addition, a straightforward GC-MS method was developed for the determination of impurities in e-cigarette solutions. Results showed that electronic cigarette solutions contained numerous compounds in addition to the compounds listed on the label by the vendor (propylene glycol, glycerin, and nicotine). In this study, e-cigarette solution profiles revealed 64 identified (some only tentatively) and unidentified compounds, far more than the three that were listed on the product label.

In order to analyze vapor samples, a simple yet novel sampling device was developed to draw electronic cigarette vapor into a thermal desorption tube, which was then thermally extracted and analyzed via a GC-MS method. This approach provided detectable levels of 82 VOCs and SVOCs from a single 40 mL puff and can be easily implemented by labs that do not have access to a smoking machine. Notably, some of compounds found are known to be detrimental to human health. These compounds were detected in the vapor, but not in the e-cigarette solution, which indicates they were produced during the vaporization process.

It is unequivocal that electronic cigarette solutions, and more important—vapor—have numerous compounds beyond the ingredients listed on the product label. As these compounds have potential implications for human health, the scientific community needs to place more emphasis on vapor testing in order to definitively identify the chemicals present and to determine how typical usage patterns relate to human health exposure limits.

Acknowledgements

Markes International Inc., 11126-D Kenwood Road, Cincinnati, OH 45242

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Accurately Determine Mineral Oil Hydrocarbons in Food and Packaging

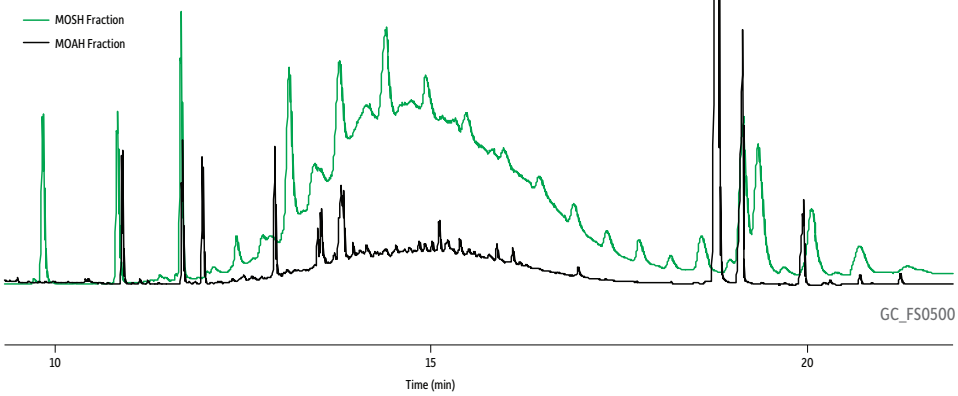
According to the German Federal Institute for Risk Assessment (BfR), mineral oil hydrocarbons (MOHs) may damage the liver, heart valves, and lymph nodes. Because the structure of some mineral oil hydrocarbons resembles that of PAHs, they may also be carcinogenic/mutagenic. These dangerous compounds can enter the food supply through farm and production equipment as well as through printer ink. In addition, current technology is unable to sufficiently remove MOHs from recycled cardboard. Once the cardboard is used for packaging, volatile hydrocarbons in mineral oils are then able to make their way into our food through gas diffusion.

In short, accurate testing for mineral oil hydrocarbons in food and packaging is imperative to the safety of our food supply; however, because MOHs include both aliphatic *and* aromatic hydrocarbons, analysis is difficult when employing standard methods. In response, a Swiss governmental lab, Kantonales Labor Zurich, devised a technique using LC fractionation coupled with GC-FID—and this technique has become the BfR reference method for the determination of MOHs in substances intended to come in contact with food. The R&D laboratory of Axel Semrau, a German solutions provider and equipment supplier, has been collaborating with DANI Instruments and leading food labs in Germany to drive the development of this methodology. With their input, Restek and its German subsidiary have created a unique solution for the analysis of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in food and packaging (Figure 1).

Turn the page to find the answers to your **MOSH/MOAH** challenges!

Figure 1: MOSH/MOAH Compounds on Rxi®-5Sil MS and Hydroguard®-Treated MXT® With Allure® Silica HPLC Column (Online LC/GC Coupling)

Column: Rxi®-5Sil MS, 15 m, 0.25 mm ID, 0.25 µm (cat.# 13620) using Hydroguard®-Treated MXT® Guard Column & Transfer Line 10 m, 0.53 mm ID (cat.# 70084); **Sample**: 10 g rice extracted with 10 mL hexane, evaporated to a final volume of 0.5 mL; **Injection:** Direct; **Oven:** Oven Temp.: 60 °C (hold 8 min) to 85 °C at 5 °C/min (hold 2 min) to 325 °C at 25 °C/min (hold 6.4 min); **Carrier Gas:** He, constant pressure (29.01 psi, 200.0 kPa); **Detector**: FID; **Instrument**: DANI Master GC; **Notes**: **Sample Preparation:** The sample for this chromatogram was fractionated using HPLC under the following conditions: **Column:** Allure® Silica (cat.# 9160572); Dimensions: 250 mm x 2.1 mm ID; Particle Size: 5 µm; Pore Size: 60 Å; **Mobile Phase:** A: Hexane, **B: Dichloromethane; Gradient (%B):** 0.0 min (0% @ 0.3 mL/min), 1.5 min (35% @ 0.3 mL/min), 6.2 min (35% @ 0.3 mL/min), 6.3 min (100% @ 0.5 mL/min), 15.0 min (100% @ 0.5 mL/min), 15.1 min (0% @ 0.5 mL/min), 25.0 min (0% @ 0.5 mL/min), 25.1 min (0% @ 0.3 mL/min), 30.0 min (0% @ 0.3 mL/min); **Instrument:** Agilent 1260 Infinity.; **Acknowledgement**: Restek thanks Axel Semrau GmbH & Co. KG with their collaborator, DANI Instruments S.p.A., for this chromatogram.



World-Class Mineral Oil Hydrocarbon (MOH) Analysis by Restek

Certified Reference Material (CRM):

MOSH/MOAH Standard

This 9-component mix contains non-interfering internal standards as well as both MOSH and MOAH markers to correctly cut fractions for reliable results. Like all of the certified reference materials (CRMs) manufactured and QC-tested in Restek's ISO-accredited labs, it can also help you satisfy your ISO requirements with ease.

Compound (CAS #)	Conc.
Bicyclohexyl (92-51-3)	300 µg/mL
Cholestane (5-alpha-cholestane) (481-21-0)	600
1-Methylnaphthalene (90-12-0)	300
2-Methylnaphthalene (91-57-6)	300
n-Pentylbenzene (538-68-1)	300
Perylene (198-55-0)	600
1,3,5-Tri-*tert*-butylbenzene (1460-02-2)	300
n-Tridecane (C13) (629-50-5)	150
n-Undecane (C11) (1120-21-4)	300
150-600 µg/mL each in toluene, 1 mL/ampul	cat.# 31070 (ea.)

HPLC Column:

Allure® Silica Column

This high-capacity 250 mm x 2.1 mm ID column is packed with ultra-pure 5 µm Allure® silica particles, which have small 60 Å pores and a surface area of 650 m^2/g. The high purity and surface area facilitate MOSH/MOAH fractionation, while the robust chemistry and design boost column life.

Description		cat.#
5 µm Columns	250 mm, 2.1 mm ID	9160572

GC Guard Column:

Hydroguard®-Treated MXT® Guard Column

The extremely nonpolar nature of this water-resistant guard ensures efficient solvent trapping as well as complete and uniform wetting during injection from the HPLC system, minimizing peak splitting and maximizing MOH resolution. Additionally, the MXT® tubing adds amazing ruggedness, so your guard lasts longer under even harsh conditions.

Nominal ID	Nominal OD	10-Meter cat.#
0.53 mm	0.74 ± 0.025 mm	70084

Diameters greater than 0.10 mm are tested with the Grob test mix to ensure high inertness.

GC Analytical Column:

Rxi®-5Sil MS Column

Restek's elite line of Rxi® gas chromatography columns is manufactured and tested to offer industry-leading performance and reproducibility. The low-polarity Rxi®-5Sil MS stationary phase incorporates phenyl groups in the polymer backbone to increase stability and reduce bleed, making it ideal for use in demanding analyses like the determination of mineral oil hydrocarbons.

ID	df	temp. limits	15-Meter cat.#
0.25 mm	0.25 µm	-60 to 320/350 °C	13620

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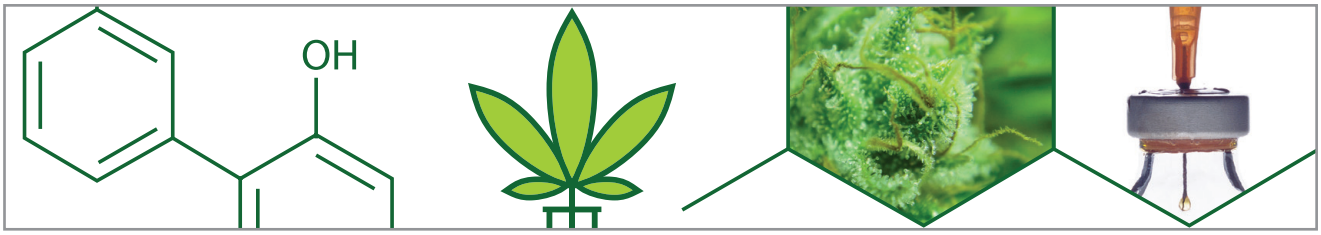


Foods, Flavors & Fragrances Applications

A Preliminary FET Headspace GC-FID Method for Comprehensive Terpene Profiling in Cannabis

By Amanda Rigdon, Corby Hilliard, and Jack Cochran

Abstract

This application note describes an FET headspace GC-FID method that was developed in hops for the analysis of terpenes in cannabis. Good chromatographic separation allowed quantification of critical compounds across the volatility range, including α-pinene, β-myrcene, α-humulene, β-caryophyllene, and caryophyllene oxide.

Introduction

In addition to cannabinoids, cannabis contains a suite of compounds known as terpenes. Terpenes are not only responsible for the characteristic aromas of cannabis strains, but they also are suspected to contribute to the therapeutic properties of cannabis. By themselves, terpenes have anti-inflammatory and anti-microbial properties, and they also reportedly contribute to an "entourage effect" with cannabinoids, modulating and/or enhancing their activity [1,2].

Because terpenes may contribute to the therapeutic effects of cannabis, there is a growing demand for analytical methods that profile terpenes in marijuana samples. In addition to analyzing terpenes for therapeutic purposes, terpenes can also be used as differentiators among cannabis strains and terpene profiles can be used for strain identification.

While relatively few terpenes have been studied for therapeutic purposes, cannabis strains can contain dozens of terpenes in varying levels. Of these, the primary compounds of interest include α-pinene, β-myrcene, α-humulene, and β-caryophyllene [2,3]. Accurately profiling these analytes and other emerging terpenes of interest depends heavily on separating them from potentially interfering compounds. When an interfering terpene, or other compound, coelutes with a terpene of interest, quantification will be compromised and, since many terpenes have the same molecular weight and share fragment ions, mass spectrometry cannot be relied upon to distinguish a terpene of interest from a coeluting interference terpene. The only way to accurately identify and quantify terpenes is to ensure that the terpenes of interest are chromatographically separated from all interfering compounds. GC is an excellent technique for accomplishing this.

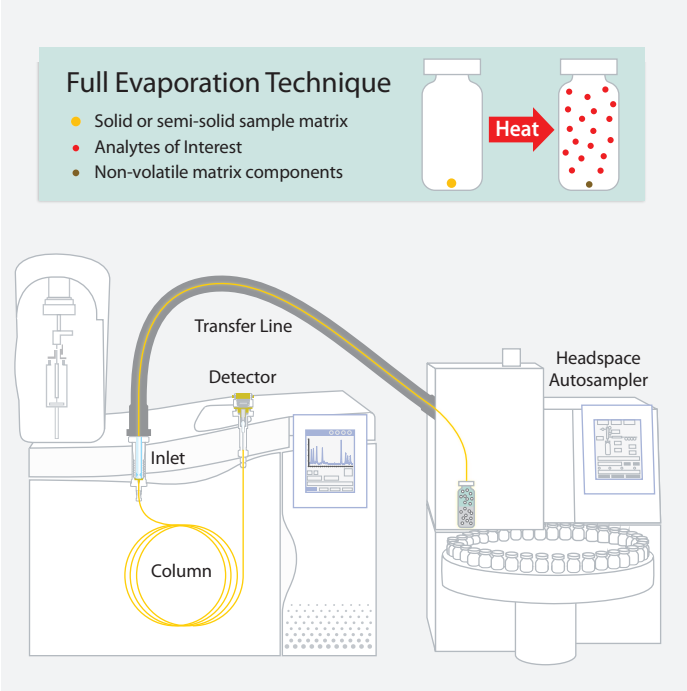
Here we present a headspace gas chromatography–flame ionization detection (GC-FID) method for a comprehensive set of 38 terpenes found in cannabis. Since cannabis is illegal in Pennsylvania where this work was done, we developed the method using hops as a model system since they are related to cannabis and contain a similar suite of terpenes [2,3,4]. The headspace method presented here utilizes full evaporation technique (FET) sample preparation because cannabis product matrices are extremely varied and plant material will not dissolve in solvent. FET involves the use of a very small sample amount (10–50 mg), which effectively creates a single phase gas system in the headspace vial at equilibrium, making it ideal for this application [5,6,7]. Figure 1 illustrates the basic principle of headspace gas chromatography using FET. To achieve chromatographic separation, a 30 m x 0.25 mm x 1.4 µm Rxi®-624Sil MS column was used. This column was chosen based on several factors. First, and most importantly, the cyano-based stationary phase of the Rxi®-624Sil MS has excellent selectivity for terpenes, making it ideal to effect a good separation for a large suite of these compounds. Second, in addition to its excellent selectivity for terpenes, the maximum temperature of this column is 320 °C, which allows for elution of some of the less volatile terpenes and matrix compounds that may be present in the headspace sample. Third, this GC column phase is also well-suited for residual solvent analysis, potentially minimizing the number of columns and instruments required by labs to test cannabis.

Experimental

Sample Preparation

Pelletized hops from three strains (UK East Kent Golding, Citra, and Cascade) were purchased from HopUnion. The pelletized hops were first ground to a fine powder using an IKA® mill. Because the hops were already ground and pelletized, very little grinding was necessary. For cannabis plant material, it is recommended that samples be frozen prior to grinding or that grinding occur under liquid nitrogen. This keeps the samples cold during the grinding process, reducing loss of the more volatile terpenes such as α-pinene. 10 mg samples of each strain were then placed in headspace vials (Figure 2). An incubation temperature of 140 °C was used to ensure volatilization of all terpenes and terpenoids in the sample. This temperature was chosen because it is also sufficient to melt samples of cannabis concentrates. An incubation time of 30 minutes was used to ensure the establishment of equilibrium during incubation, which is required for reproducible, quantitative results.

Figure 1: Setup and Basic Principle of FET Headspace Injection Coupled With GC-FID Analysis



Gas Chromatographic Conditions

Samples were analyzed on an Agilent® 6890 gas chromatograph equipped with a Tekmar® HT-3 headspace autosampler. A 30 m x 0.25 mm x 1.4 µm Rxi®-624Sil MS column was installed based on its selectivity for terpenes and because it could also be used for analysis of residual solvents in cannabis concentrates. A 1 mm straight Sky® inlet liner was used to limit the volume in the GC inlet. For headspace instruments, reducing the inlet volume increases efficiency by reducing band broadening during sample introduction. Greater efficiency maximizes peak separation, which is essential for this analysis. Complete chromatographic conditions are presented in Figure 4.

Figure 2: Grinding samples maximizes and normalizes surface area from sample to sample, increasing sensitivity and reproducibility.

Quantification

To aid in peak identification, a multi-component terpene standard was prepared with each compound at approximately 0.02% wt/vol. 10 µL of this standard solution was injected into a capped headspace vial and analyzed by FET headspace GC-FID. Standards were analyzed under the same conditions as the samples in order to eliminate the potential for discrimination across the volatility range (e.g., more volatile terpenes may show higher responses than less volatile terpenes). Since any discrimination effect would be the same in both the sample and standard, analytes were quantified based on their relative response factor compared to the standard as shown in Equation 1. This normalizes the values between sample and standard, ensuring accurate quantification across the full range of volatility for terpenes. Note that while the relative response factor technique improves accuracy, the semi-quantitative preparation of the standard and lack of well-characterized certified reference materials for terpenes limits the overall quantitative accuracy that can be obtained for this analysis. Additionally, the lack of pure, neat standards available to prepare a more concentrated standard resulted in a standard well below the level of many of the terpenes detected in this work. For accurate quantification, a calibration curve encompassing the expected concentration range of all analytes is required. The data presented in this article should be considered semi-quantitative.

Equation 1: Sample Concentration Calculation

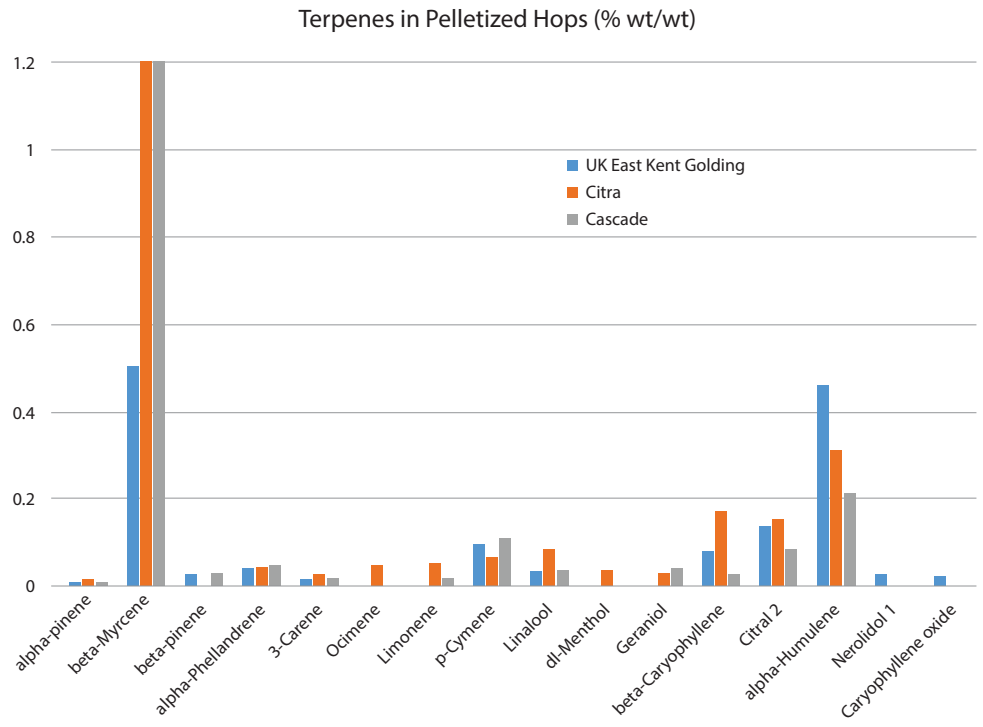
$$\text{Given: } \frac{\text{Standard Area}}{\text{Standard Concentration}} = \frac{\text{Sample Area}}{\text{Sample Concentration}}$$

$$\text{Sample Concentration} = \frac{(\text{Sample Area} \times \text{Standard Concentration})}{\text{Standard Area}}$$

Results and Discussion

The purpose of this study was to develop an FET headspace GC-FID method for the analysis of terpenes in cannabis using hops as a model system. The terpenes found in our samples matched well with literature descriptions of the terpenes present in hops [4]. High levels of terpenes were found across the volatility range, indicating that the FET headspace GC-FID technique was appropriate and that analysis of the standard adequately normalized any discrimination between the more and less volatile terpenes (Figure 3). Due to the starting concentration of some of the commercially available terpene standards, the maximum concentration at which the mixed terpene standard used for quantification could be prepared was 0.02% wt/vol, which is significantly lower than the concentration of some of the more prevalent terpenes in hops and cannabis. The use of a more concentrated standard solution is recommended to improve quantification of the higher concentrations found in these samples.

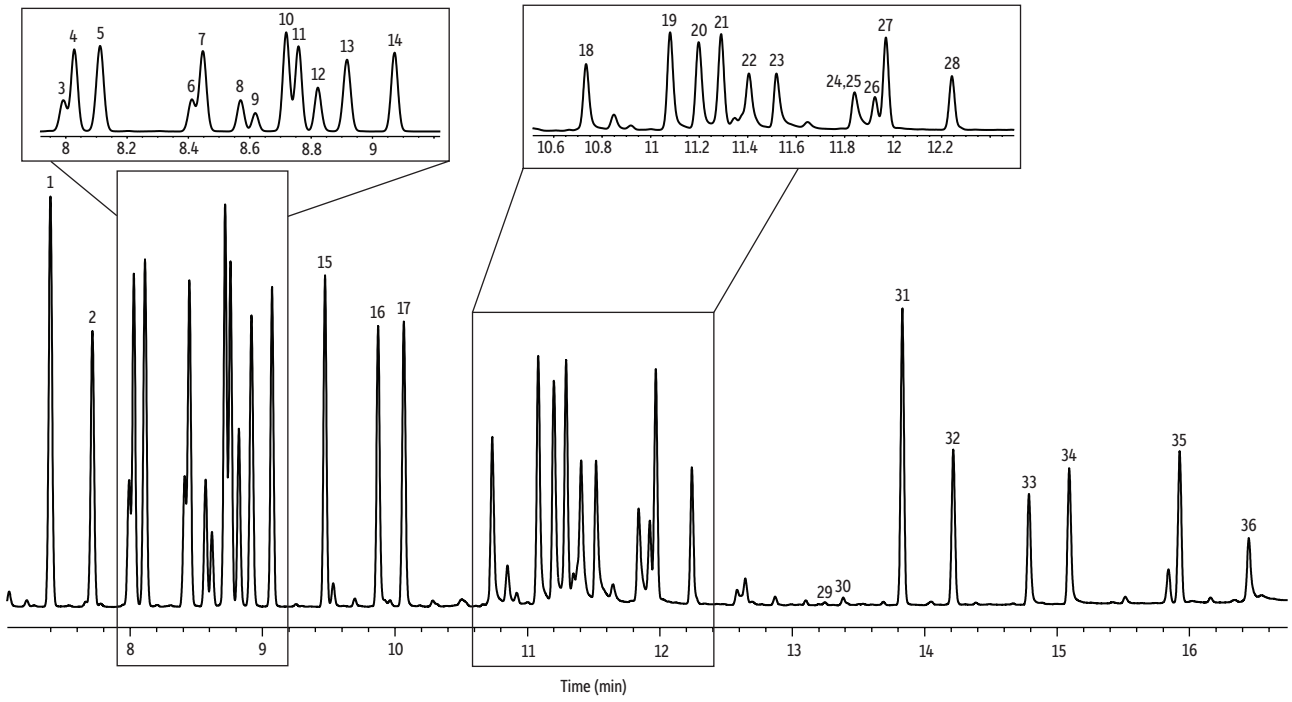
Figure 3: Terpene Profiles of Pelletized Hops



Figures 4–7 show individual chromatograms for the standard and each sample profiled for terpenes. Note that α-pinene, β-myrcene, α-humulene, β-caryophyllene, and caryophyllene oxide are well separated from interferences. For complex matrices, such as hops and marijuana, excellent chromatographic efficiency and selectivity are required to separate terpenes from one another and from other volatile matrix components in order to obtain accurate quantification. The selectivity of the Rxi®-624Sil MS column used here provided good separation of most terpenes and the small bore configuration (0.25 mm internal diameter) improved column efficiency, ultimately resulting in greater resolution between closely eluting terpenes than would be obtained using a wider bore column.

Figure 4: A 0.02% wt/vol multi-component terpenes standard analyzed on an Rxi®-624Sil MS column (30 m x 0.25 mm x 1.4 µm) demonstrates that this column provides the selectivity and efficiency needed to separate key terpenes using a simple FET headspace GC-FID method.

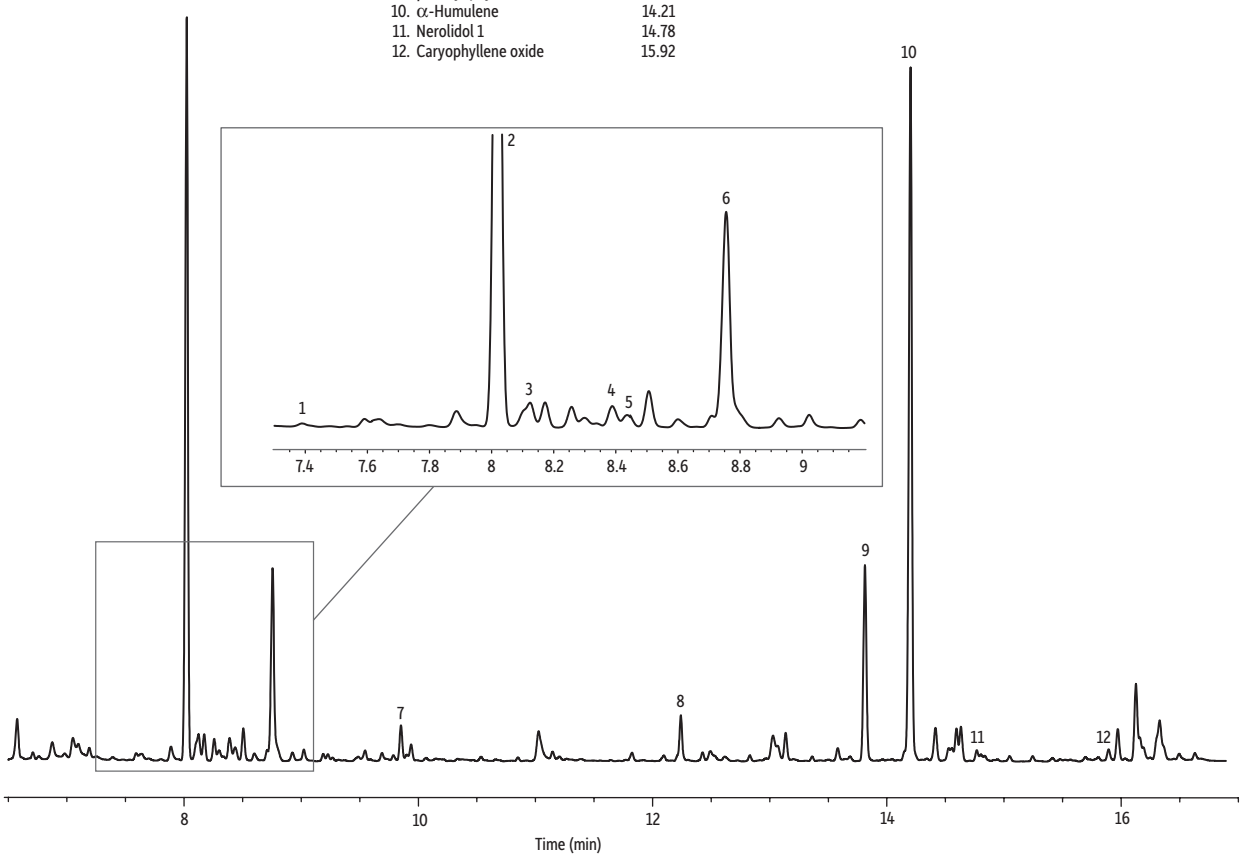
Peaks	t_R (min)	Peaks	t_R (min)	Peaks	t_R (min)	Peaks	t_R (min)
1. α-Pinene	7.39	10. Limonene	8.71	19. dl-Menthol	11.08	28. Citral 2	12.24
2. Camphene	7.71	11. *p*-Cymene	8.75	20. Borneol	11.19	29. Citral 3	13.19
3. β-Myrcene	7.98	12. β-Ocimene	8.82	21. α-Terpineol	11.29	30. Citral 4	13.43
4. Sabinene	8.02	13. Eucalyptol	8.91	22. Dihydrocarveol	11.40	31. β-caryophyllene	13.83
5. β-Pinene	8.11	14. γ-Terpinene	9.06	23. Citronellol	11.51	32. α-Humulene	14.21
6. α-Phellandrene	8.4	15. Terpinolene	9.47	24. Geraniol	11.82	33. Nerolidol 1	14.78
7. δ 3-Carene	8.44	16. Linalool	9.87	25. 2-Piperidinone	11.88	34. Nerolidol 2	15.08
8. α-Terpinene	8.57	17. Fenchone	10.06	26. Citral 1	11.92	35. Caryophyllene oxide	15.92
9. Ocimene	8.61	18. Isopulegol	10.73	27. Pulegone	11.97	36. α-Bisabolol	16.43



Column	Rxi® -624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868)
Sample	Terpenes mix
Diluent:	Isopropyl alcohol
Conc.:	200 ng/µL (0.02% wt/vol). The sample was prepared by placing 10 µL into the headspace vial.
Injection	headspace-loop split (split ratio 10:1)
Liner:	Sky® 1.0 mm ID straight inlet liner (cat.# 23333.1)
Headspace-Loop	
Inj. Port Temp.:	250 °C
Instrument:	Tekmar HT-3
Inj. Time:	1.0 min
Transfer Line Temp.:	160 °C
Valve Oven Temp.:	160 °C
Needle Temp.:	140 °C
Sample Temp.:	140 °C
Sample Equil. Time:	30.0 min
Vial Pressure:	20 psi
Loop Pressure:	15 psi
Oven	
Oven Temp.:	60 °C (hold 0.10 min) to 300 °C at 12.50 °C/min (hold 3.0 min)
Carrier Gas	He, constant flow
Linear Velocity:	33 cm/sec
Detector	FID @ 320 °C
Make-up Gas Flow Rate:	45 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	40 mL/min
Air flow:	450 mL/min
Data Rate:	20 Hz
Instrument	Agilent/HP6890 GC

Figure 5: Chromatographic Terpene Profile of Pelletized UK East Kent Golding Hops

Peaks	t_R (min)
1. α-Pinene	7.39
2. β-Myrcene	8.02
3. β-Pinene	8.11
4. α-Phellandrene	8.40
5. δ 3-Carene	8.44
6. *p*-Cymene	8.75
7. Linalool	9.87
8. Citral-2	12.24
9. β-Caryophyllene	13.83
10. α-Humulene	14.21
11. Nerolidol 1	14.78
12. Caryophyllene oxide	15.92



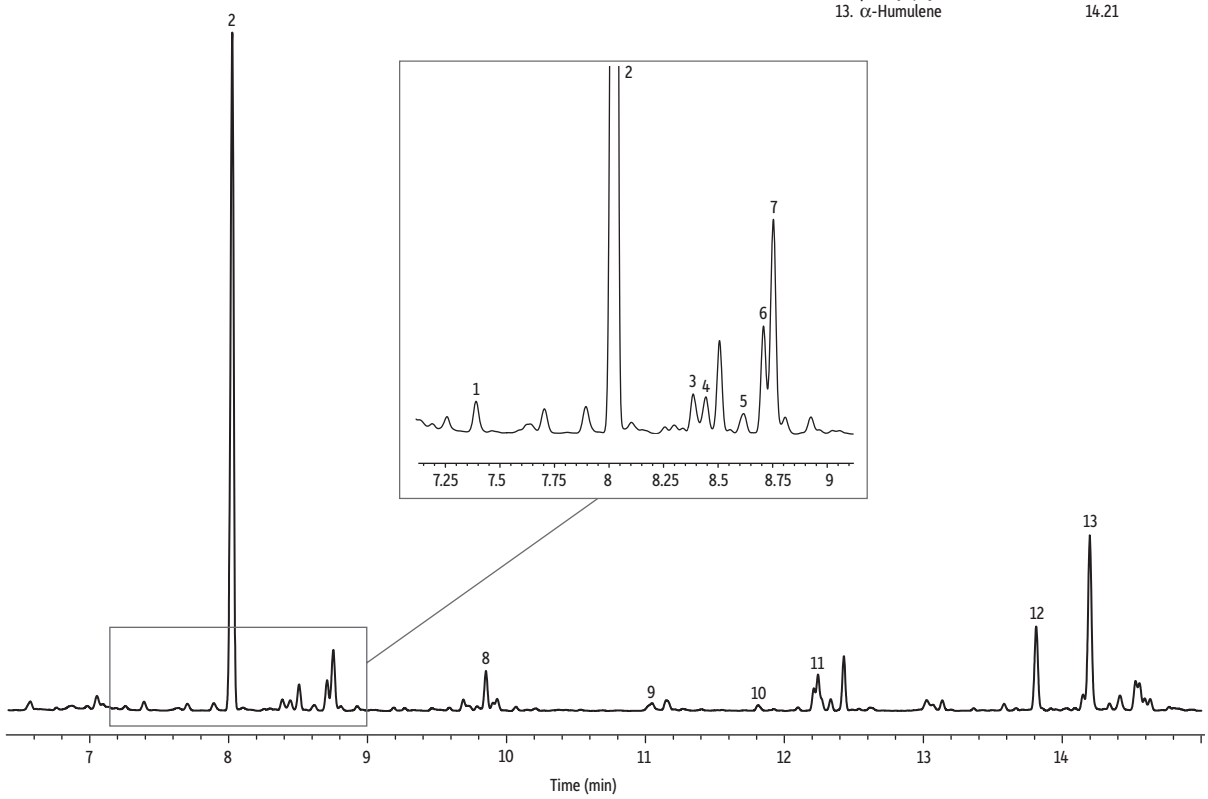
GC_FS0522

Column Rxi®-624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868)
Sample
Conc.: 10 mg of ground UK East Kent Goldings hops
Injection headspace-loop split (split ratio 10:1)
Liner: Sky® 1.0 mm ID straight inlet liner (cat.# 23333.1)
Headspace-Loop
Inj. Port Temp.: 250 °C
Instrument: Tekmar HT-3
Inj. Time: 1.0 min
Transfer Line Temp.: 160 °C
Valve Oven Temp.: 160 °C
Needle Temp.: 140 °C
Sample Temp.: 140 °C
Sample Equil. Time: 30.0 min
Vial Pressure: 20 psi
Loop Pressure: 15 psi

Oven
Oven Temp.: 60 °C (hold 0.10 min) to 300 °C at 12.50 °C/min (hold 3.0 min)
Carrier Gas He, constant flow
Linear Velocity: 33 cm/sec
Detector FID @ 320 °C
Make-up Gas Flow Rate: 45 mL/min
Make-up Gas Type: N_2
Hydrogen flow: 40 mL/min
Air flow: 450 mL/min
Data Rate: 20 Hz
Instrument Agilent/HP6890 GC

Figure 6: Chromatographic Terpene Profile of Pelletized Citra Hops

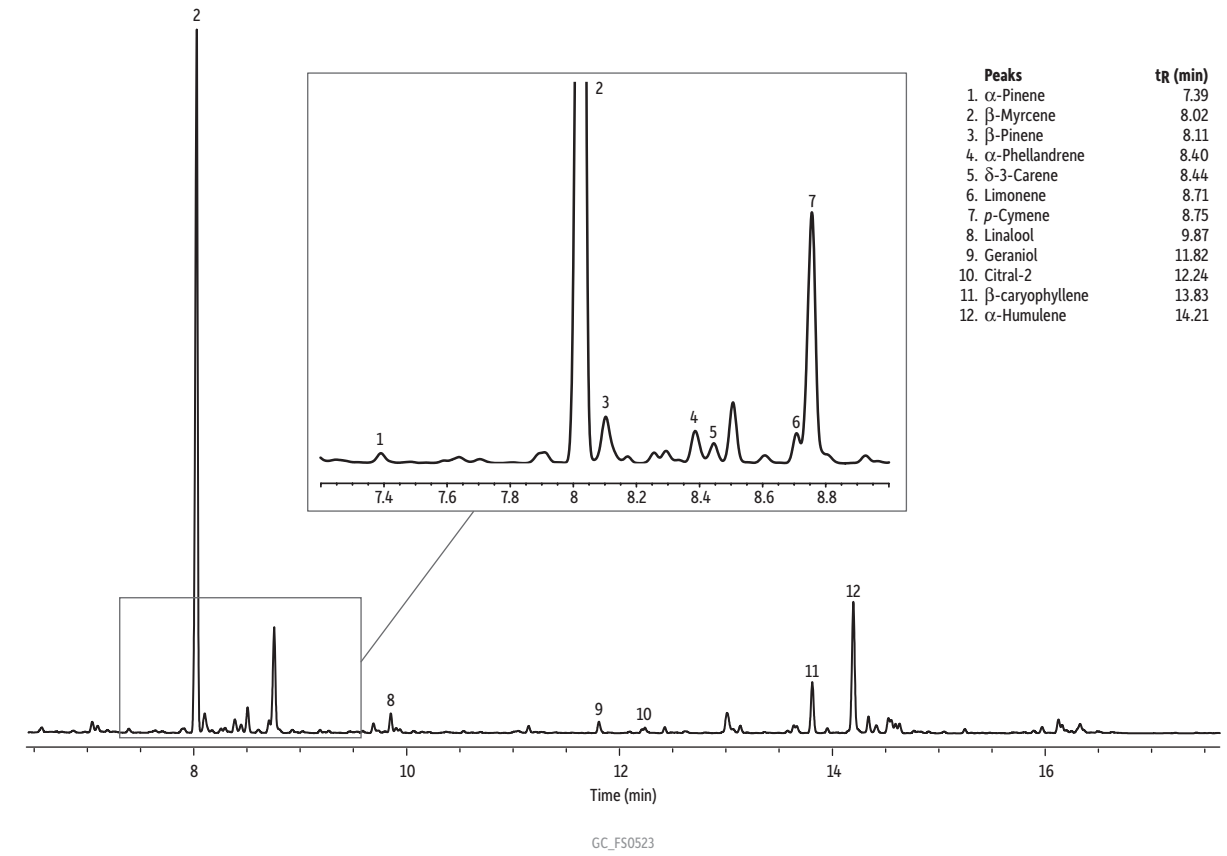
Peaks	t_R (min)
1. α-Pinene	7.39
2. β-Myrcene	8.02
3. α-Phellandrene	8.40
4. δ 3-Carene	8.44
5. Ocimene	8.61
6. Limonene	8.71
7. *p*-Cymene	8.75
8. Linalool	9.87
9. dl-Menthol	11.08
10. Geraniol	11.82
11. Citral-2	12.24
12. β-Caryophyllene	13.83
13. α-Humulene	14.21



GC_FS0525

Column	Rxi®-624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868)
Sample	
Conc.:	10 mg of ground Citra hops
Injection	headspace-loop split (split ratio 10:1)
Liner:	Sky® 1.0 mm ID straight inlet liner (cat.# 23333.1)
Headspace-Loop	
Inj. Port Temp.:	250 °C
Instrument:	Tekmar HT-3
Inj. Time:	1.0 min
Transfer Line Temp.:	160 °C
Valve Oven Temp.:	160 °C
Needle Temp.:	140 °C
Standby flow rate:	50 mL/min
Sample Temp.:	140 °C
Platen temp equil. time:	1.0 min
Sample Equil. Time:	30.0 min
Vial Pressure:	20 psi
Pressurize Time:	5.0 min
Pressure Equilibration Time:	0.20 min
Loop Pressure:	15 psi
Loop Fill Time:	2.0 min
Oven	
Oven Temp.:	60 °C (hold 0.10 min) to 300 °C at 12.50 °C/min (hold 3.0 min)
Carrier Gas	He, constant flow
Flow Rate:	1.4 mL/min
Linear Velocity:	33 cm/sec
Detector	FID @ 320 °C
Make-up Gas Flow Rate:	45 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	40 mL/min
Air flow:	450 mL/min
Data Rate:	20 Hz
Instrument	Agilent/HP6890 GC

Figure 7: Chromatographic Terpene Profile of Pelletized Cascade Hops



Peaks	t_R (min)
1. α-Pinene	7.39
2. β-Myrcene	8.02
3. β-Pinene	8.11
4. α-Phellandrene	8.40
5. δ-3-Carene	8.44
6. Limonene	8.71
7. *p*-Cymene	8.75
8. Linalool	9.87
9. Geraniol	11.82
10. Citral-2	12.24
11. β-caryophyllene	13.83
12. α-Humulene	14.21

Column	Rxi®-624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868)
Sample	
Conc.:	10 mg of ground Cascade hops
Injection	headspace-loop split (split ratio 10:1)
Liner:	Sky® 1.0 mm ID straight inlet liner (cat.# 23333.1)
Headspace-Loop	
Inj. Port Temp.:	250 °C
Instrument:	Tekmar HT-3
Inj. Time:	1.0 min
Transfer Line Temp.:	160 °C
Valve Oven Temp.:	160 °C
Needle Temp.:	140 °C
Sample Temp.:	140 °C
Sample Equil. Time:	30.0 min
Vial Pressure:	20 psi
Loop Pressure:	15 psi

Oven	
Oven Temp.:	60 °C (hold 0.10 min) to 300 °C at 12.50 °C/min (hold 3.0 min)
Carrier Gas	He, constant flow
Linear Velocity:	33 cm/sec
Detector	FID @ 320 °C
Make-up Gas Flow Rate:	45 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	40 mL/min
Air flow:	450 mL/min
Data Rate:	20 Hz
Instrument	Agilent/HP6890 GC

While many cyano-based columns are commercially available, the Rxi®-624Sil MS column is recommended for terpene analysis because, in addition to offering optimized selectivity, the stationary phase is stabilized with silarylene, which significantly increases the operational temperature range of the column and improves its robustness. This is important for terpene analysis because some of the less-volatile terpenes require relatively high elution temperatures that would tax non-silarylene cyano stationary phases, resulting in shorter column lifetimes.

Although the Rxi®-624Sil MS column performs exceptionally well for the analysis of terpenes and residual solvents, it is too retentive for cannabinoids. In fact, cannabinoids do not elute from the Rxi®-624Sil MS column even at its 320 °C maximum

temperature. Injection of cannabinoids on this column can potentially result in reduced column lifetime, selectivity changes, or baseline disturbances due to cannabinoids "bleeding" off of the stationary phase over time. Since both cannabinoids and terpenes will be present in cannabis samples, the sample preparation method must minimize the introduction of cannabinoids onto the analytical column. The full evaporation technique headspace sampling approach used here is ideal for terpene profiling because it introduces the volatile terpenes onto the GC column while eliminating the introduction of less volatile cannabinoids and nonvolatile matrix components into the system. This results in longer column lifetime and reduced inlet maintenance. Headspace sampling in general is simple to perform and requires no extraction or cleanup. While other methods exist that could remove cannabinoids from the sample while leaving the terpenes behind, these sample preparation methods are more time- and labor-intensive, and the increased amount of sample handling could result in loss of some of the more volatile terpenes, such as α-pinene. Grinding samples under dry ice is an additional measure that could be taken to minimize the loss of more volatile terpenes as it reduces the heat generated during the grinding process.

Conclusion

An FET headspace GC-FID method was used to analyze a comprehensive suite of terpenes in hops that are also found in cannabis samples. Compounds of interest across the volatility range were chromatographically separated and quantified. This method utilizes straightforward FET sample preparation, which minimizes manual labor and sample handling time. In addition, because it prevents nonvolatile material from entering the GC system, using the FET approach can increase column lifetime and reduce inlet maintenance. This technique, column, and instrument setup can also be used to analyze residual solvents in cannabis concentrates, eliminating the need for additional capital investment for different instrumentation and/or columns.

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Foods, Flavors & Fragrances Applications

A Fast, Simple FET Headspace GC-FID Technique for Determining Residual Solvents in Cannabis Concentrates

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Abstract

Due to rapid growth in the medical cannabis industry, demand is increasing for analysis of residual solvents in cannabis concentrates in order to protect consumer safety. This application note details a simple, fast test for common residual solvents using full evaporation technique headspace GC-FID and an Rxi®-624Sil MS column.

Introduction

As the popularity of cannabis concentrates increases, consumer safety concerns are resulting in the establishment of new regulations to control the level of residual solvents in commercial cannabis concentrates. The State of Colorado, for example, published allowable concentrations of certain residual solvents in Rule R 712. This is because, although cannabis concentrates can be produced in numerous ways, one of the most common means of extracting therapeutic compounds, like tetrahydrocannabinol (THC), cannabidiol (CBD), and terpenes, from cannabis is through extraction with an organic solvent, such as butane. After the cannabinoids and terpenes are extracted from the plant material, the organic solvent is allowed to evaporate and then is purged off using heat and/or vacuum. These extraction solvents can be difficult to purge completely, so the finished product needs to be tested to ensure that residual solvents are only present at or below safe levels. For consumer safety, especially with medicinal products, accurate and comprehensive analysis of residual solvents is necessary for concentrates and extracts.

Since residual solvents are extremely volatile, they cannot be analyzed by HPLC and lend themselves nicely to GC analysis. One of the most common and reliable ways to quantify residual solvents is through headspace gas chromatography–flame ionization detection (GC-FID). Headspace injection works by driving volatile compounds of interest from the sample into a gas phase in the headspace of the vial above the sample. An aliquot is then withdrawn from the headspace of the vial and analyzed by GC-FID in order to determine the volatile components of the sample. One approach for headspace GC-FID that is particularly useful for analyzing cannabis concentrates is the full evaporation technique (FET). FET sample preparation involves the use of a very small sample amount (e.g., 20–50 mg), which effectively creates a single-phase gas system in the headspace vial at equilibrium [1]. FET is ideal for difficult and varied matrices like cannabis concentrates because it eliminates matrix interferences that can cause inaccurate quantification, and it also has the advantages of little to no manual sample handling and a very small sample size. Additionally, high sensitivity can be achieved through the creation of a single-phase system in the headspace vial. Figure 1 illustrates the basic principle of headspace GC using the full evaporation technique.

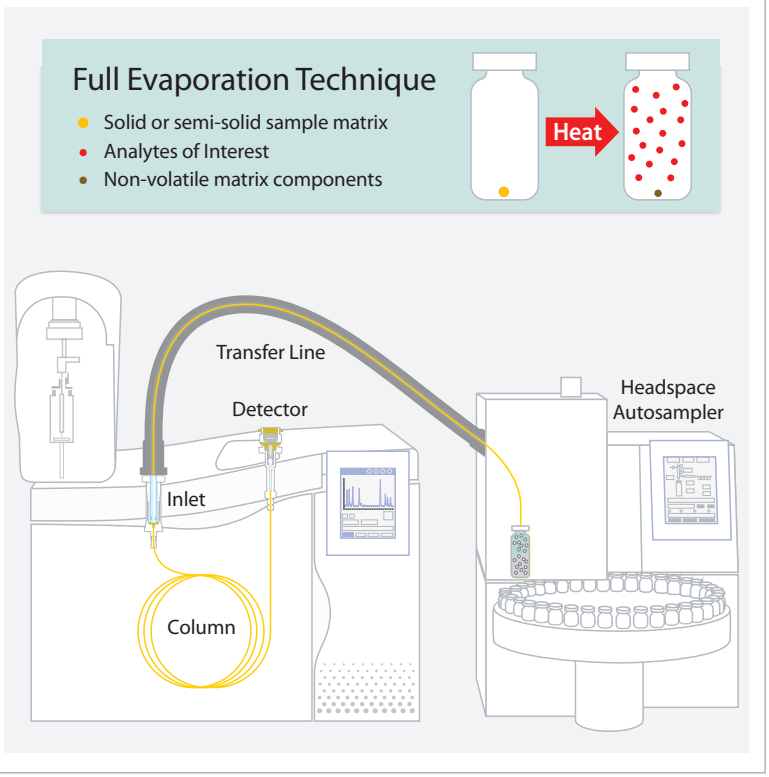
The work described here demonstrates the viability of FET headspace injection and GC-FID analysis of residual solvents in cannabis concentrates. The method is simple to implement, quick to run, and does not require expensive dynamic headspace equipment or mass spectrometric detectors. While the methodology presented here is suitable for residual solvents in cannabis concentrates, it is not applicable for finished tinctures in alcohol. Finished alcohol tinctures contain large amounts of alcohol which will severely interfere with quantification of other residual solvents in the sample. Therefore, an alternate approach is required for alcohol tinctures. This technique also may be applicable for oil or glycerin tinctures; however, it has not been evaluated for that use.

Experimental

Headspace and GC Method Optimization

An Rxi®-624Sil MS column was selected for this work as it is designed specifically for volatiles analysis and is widely used for the analysis of residual solvents in pharmaceutical products. Final FET headspace injector and GC-FID operating conditions are presented in Figure 3. Initially, modeled conditions for analyzing the specific compounds of interest were generated using Restek's *EZ*GC™ chromatogram modeler. The method from the modeler was then optimized to account for headspace analysis employing a headspace instrument with a transfer line.

Figure 1: Setup and Basic Principle of FET Headspace Injection Coupled With GC-FID Analysis



The following parameters were optimized for this method:

- *Linear velocity:* Linear velocity was increased to 80 cm/sec to allow for fast sample transfer through the headspace instrument transfer line. Fast sample transfer minimizes band broadening, which maximizes efficiency, resolution, and sensitivity. The original GC oven program generated by the *EZ*GC™ chromatogram modeler was translated using the *EZ*GC™ method translator to give a new oven program optimized for the new carrier flow. Method translation is required when changing flow rates in order to keep elution temperatures constant. Changes in elution temperatures between the original and the translated method will sometimes result in drastically different separations or even coelutions, especially on highly selective phases like the Rxi®-624Sil MS column.

- *GC inlet liner choice:* The liner used for this work was a 1 mm straight Sky® inlet liner (cat.# 23333.1). The use of a small internal diameter liner minimizes band broadening by reducing the overall volume of the inlet, again resulting in higher efficiency, resolution, and sensitivity.

- *Split ratio:* A split ratio of 10:1 was used for this work. Although maximum sensitivity is required due to very low expected levels of target analytes, using a split ratio of at least 10:1 ensures high sample velocity through the GC inlet, which minimizes band broadening, increasing resolution without compromising sensitivity. Sharper peaks are taller peaks, so any loss in sensitivity is mitigated through an increase in signal-to-noise ratio.

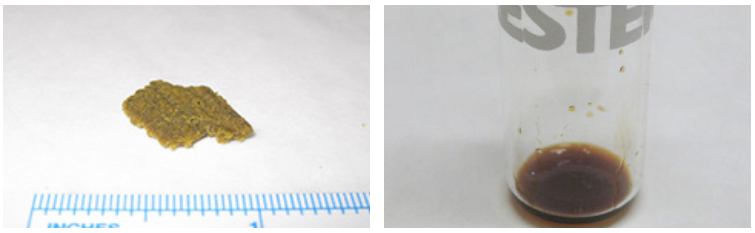
- *Equilibration temperature:* Samples were equilibrated at 140 °C to encourage complete melting of waxy concentrates. By melting the extracts, the ratio of surface area to volume is maximized, ensuring 100% transfer of the analytes of interest into the headspace. The use of a larger sample size will compromise this ratio; therefore, sample sizes should be kept as small as possible to ensure accurate quantification (20 mg is recommended for this application). Representative concentrates are shown in Figure 2. Small samples (20–25 mg) of each concentrate type were placed in a capped headspace vial and incubated for 30 minutes at 140 °C. All concentrates melted completely at the 140 °C incubation temperature, forming a thin film at the bottom of the headspace vial.

- *Equilibration time:* The equilibration time for this method was 30 minutes. This allows enough time for waxy concentrates to melt completely and ensures equilibrium is reached in the headspace vial. Equilibrium is required for accurate and reproducible quantification.

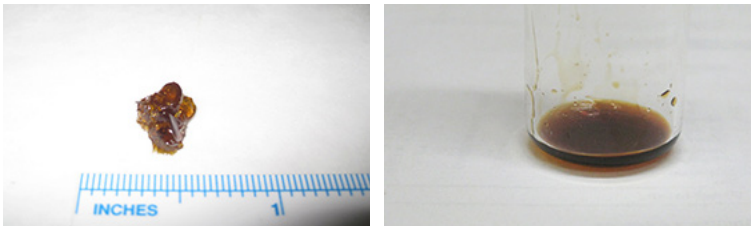
- *Oven program:* The oven program was optimized for speed for this application. In samples that contain terpenes, it is recommended that the oven ramp be extended to 320 °C and the isothermal hold time be extended to 5 minutes in order to ensure complete elution of any terpenes that may be present in the sample.

Figure 2: Cannabis concentrate samples are solid before FET incubation (left) and then melt completely into a thin liquid layer after a 30-minute incubation at 140 °C (right).

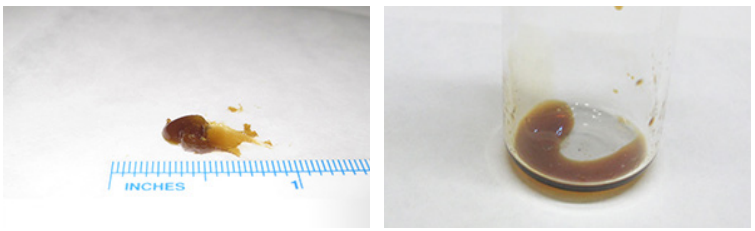
Crumble - Melting point = ~115 °C



Shatter - Melting point = 108 °C



Taffy - Melting point = 102 °C



Photos and melting point data courtesy Cal-Green Solutions

Table I: Commodity and Calibration Standard Curve Equivalency Levels

Concentration in Commodity (ppm)	Amount in 20 mg Sample (µg)	Concentration in 10 µL Standard (µg/mL)
500	10	1,000
250	5	500
100	2	200
50	1	100
25	0.5	50
10	0.2	20
5	0.1	10

Calibration Curve Preparation

When preparing standards for FET headspace GC-FID, it is necessary to calculate the total mass of analyte that will be present in a representative sample, since the equilibrium state results in a single-phase system. For example, a 20 mg sample containing a residual solvent at 50 ppm contains 1 µg of that residual solvent. Therefore, the 50 ppm point in the calibration curve should contain 1 µg of each compound of interest. Since FET headspace GC-FID depends on the establishment of a single phase system, very small volumes are required for standards. The volume used for standards in this application was 10 µL, which was placed directly into a capped headspace vial by injecting it through the vial septum with a clean syringe. Table I presents the 7-point calibration curve standards and their corresponding concentrations in commodity samples.

Standards were prepared in dimethyl sulfoxide (DMSO), which is a less-volatile, later-eluting solvent that does not interfere with the residual solvents of interest. Because FET establishes a single-phase system in the headspace vial without partitioning, it is not necessary to matrix-match standards and samples, which simplifies standard preparation for varied matrices.

The calibration curve was prepared by first making a 1,000 µg/mL stock solution for dilution. The stock solution was prepared as follows:

- Prepare a 5,000 µg/mL stock solution of butane by bubbling butane standard through DMSO on a balance in a fume hood. The butane used for this work was a mixture of butane and isobutane.
- Prepare a 1,000 µg/mL stock solution by adding 2 mL of 5,000 µg/mL butane stock to a 10 mL volumetric flask, adding ~4 mL DMSO, and then volumetrically adding each neat solvent to the flask using a syringe. Volumes required for the 1,000 µg/mL stock standard were adjusted to account for the density of each solvent as shown in Table II.
- After the addition of neat solvents, fill the flask to the line with DMSO and mix by gently inverting the flask three times and rotating to swirl the contents between inversions.

Table II: Density-Adjusted Volumes Used to Prepare 10 mL of the 1,000 µg/mL Stock Solution

Compound	Density (g/mL)	Volume Required (µL)
Butane	measured gravimetrically	2,000
Chloroform	1.48	6.7
Isobutane	NA	2,000
Acetone	0.79	12.6
Methanol	0.79	12.6
Ethanol	0.79	12.7
IPA	0.79	12.7
Benzene	0.88	11.4
Toluene	0.87	11.5
Pentane	0.63	16.0
Hexane	0.65	15.3
Heptane	0.68	14.7

The 1,000 µg/mL stock solution prepared using Table II was used as the highest calibration standard. All other calibration points were prepared in 5 mL volumetric flasks with separate dilutions of the 1,000 µg/mL stock solution. Serial dilution was not used for this work in order to minimize time-consuming syringe rinsing during calibration curve preparation. Because the compounds used here are volatile, work needed to be completed as quickly as possible to prepare the calibration standards. In addition, volumetric flasks were kept capped to minimize evaporative loss. Table III details the preparation of the calibration curve standards.

Table III: Calibration Curve Preparation

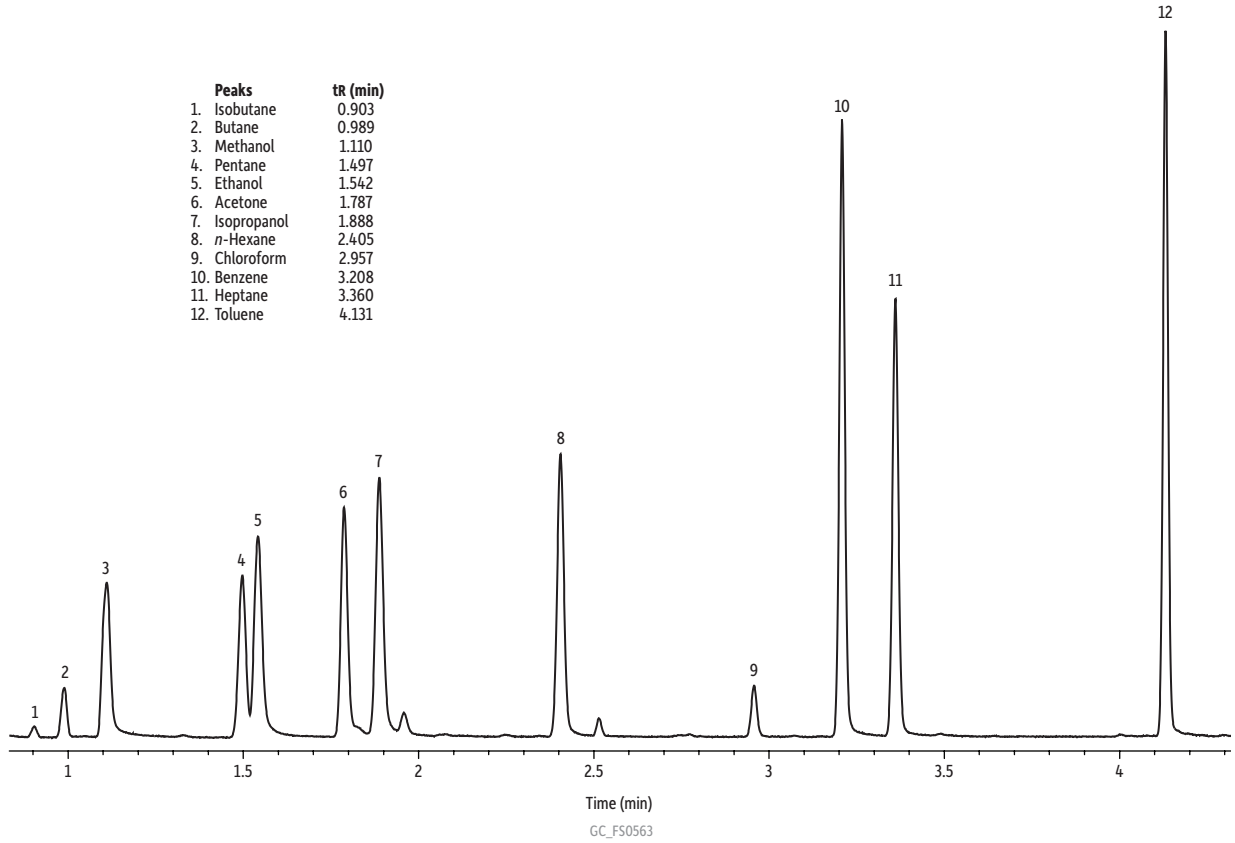
Calibration Level (ppm in Commodity)	Volume of 1,000 µg/mL Stock Solution (mL)	Final Volume (mL)	Final Calibration Standard Concentration (µg/mL)
500	5	5	1,000
250	2.5	5	500
100	1	5	200
50	0.5	5	100
25	0.25	5	50
10	0.1	5	20
5	0.05	5	10

After preparation, all calibration standards were divided into 2.5 mL aliquots and stored in the refrigerator at 5 °C. Since DMSO freezes under refrigeration, calibration standards were allowed to thaw completely prior to use. By aliquoting the calibration standards into separate vials, freeze/thaw cycles were reduced for the entire volume of the calibration solution, allowing for longer storage life of calibration and stock solutions. If desired, calibration standards may be split into aliquots smaller than 2.5 mL to further reduce freeze/thaw cycles. This can be accomplished by pipetting aliquots into gas-tight vials using a glass pipet and immediately capping the vials.

Results and Discussion

Good chromatographic peak shape, separation, and sensitivity were achieved for all analytes of interest. Figure 3 shows the 25 ppm calibration standard. Use of the Restek® Rxi®-624Sil MS column allowed for the separation of the wide variety of solvents that may be present in cannabis concentrates in a short analysis time, while retaining and resolving highly volatile butane isomers. This column was selected for the FET headspace GC-FID method because it was designed specifically for volatiles analysis and is widely used for the analysis of residual solvents in pharmaceutical products. Additionally, the column's unique selectivity also resolves dozens of terpenes [2]. This allows cannabis terpene profiling to be done without changing columns or injection technique, which decreases downtime between methods and improves lab productivity.

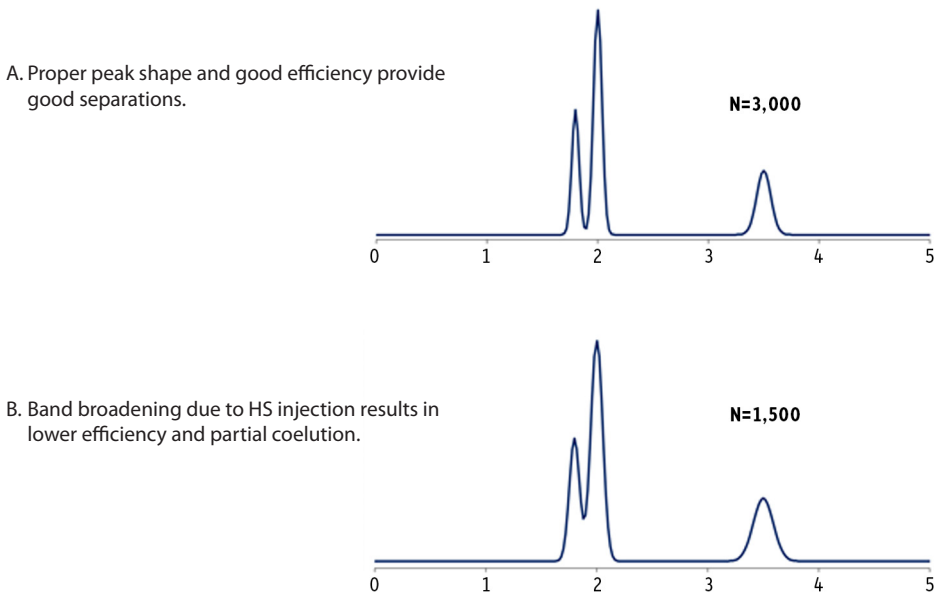
Figure 3: Calibration standard corresponding to a 20 mg cannabis concentrate sample containing 25 ppm of residual solvents. Good chromatographic separation and sensitivity were achieved for common residual solvents.



Column	Rxi®-624Sil MS, 30 m, 0.25 mm ID, 1.40 µm (cat.# 13868)
Sample	Residual solvent mix
Diluent:	Dimethyl sulfoxide (DMSO)
Conc.:	25 ppm (For the HS-FET technique, 10 µL of a 50 µg/mL standard was placed into a 20 mL headspace vial to represent a 25 ppm sample concentration, assuming a 20 mg sample weight.)
Injection	headspace-loop split (split ratio 10:1)
Liner:	Sky® 1.0 mm ID straight inlet liner (cat.# 23333.1)
Headspace-Loop	
Inj. Port Temp.:	250 °C
Instrument:	Tekmar HT3
Inj. Time:	1.0 min
Transfer Line Temp.:	160 °C
Valve Oven Temp.:	160 °C
Needle Temp.:	140 °C
Sample Temp.:	140 °C
Platen temp equil. time:	1.0 min
Sample Equil. Time:	30.0 min
Vial Pressure:	20 psi
Pressurize Time:	5.0 min
Loop Pressure:	15 psi
Loop Fill Time:	2.0 min
Oven	
Oven Temp.:	35 °C (hold 1.5 min) to 300 °C at 30 °C/min (hold 2.0 min)
Carrier Gas	He, constant flow
Linear Velocity:	80 cm/sec
Detector	FID @ 320 °C
Make-up Gas Flow Rate:	45 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	40 mL/min
Air flow:	450 mL/min
Data Rate:	20 Hz
Instrument	Agilent/HP6890 GC
Notes	The butane used for standard preparation was a mixture of butane and isobutane in an unknown ratio. The concentrations should be considered approximate, but do not exceed 50 ppm for any component.

In addition to using a highly efficient, selective Rxi®-624Sil MS column, it is critical to optimize several GC parameters for headspace analyses in order to prevent band broadening. Early-eluting compounds such as isobutane and butane do not focus on the head of the analytical column, so band broadening through the headspace system and injection port can reduce efficiency, severely impacting sensitivity and resolution for these compounds (Figure 4). As detailed in the Experimental section, band broadening was controlled by using a fast linear velocity, narrow bore inlet liner, and a 10:1 split ratio. This approach speeds up sample transfer and ensures good chromatographic peak shape and response.

Figure 4: Lower efficiency (N) due to band broadening during headspace sample introduction can reduce both resolution and sensitivity (modeled chromatogram).



Analysis of calibration standards resulted in good sensitivity and linear responses for all analytes of interest. Table IV shows the signal-to-noise ratios at 10 ppm and 50 ppm (current Colorado regulatory cutoff values), as well as the correlation coefficients (r values) and coefficients of determination (r^2 values) for all analytes. All compounds exhibited adequate signal-to-noise ratios (> 10:1) at their respective Colorado state regulatory limits. Signal-to-noise ratios were > 10:1 for all compounds at 10 ppm, with the exception of isobutane. The Colorado cutoff for isobutane was 50 ppm at the time of this study; however, prior to publication, Colorado changed the limits and solvents of interest for residual solvent testing. This method will be suitable for the new regulations as well as the older ones.

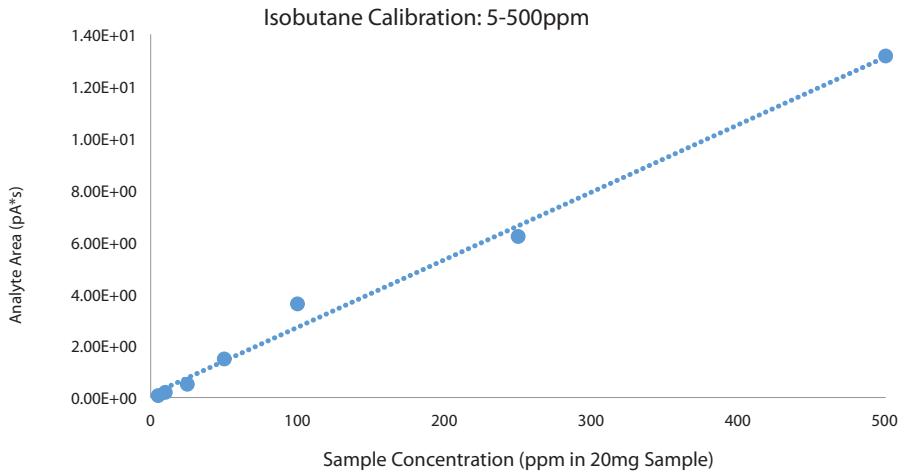
Figure 5 shows plots of the most linear (heptane) and least linear (isobutane) calibration curves. All calibration curves exhibited acceptable linearity without the use of an internal standard. The use of an internal standard may improve linearity and reproducibility, if desired.

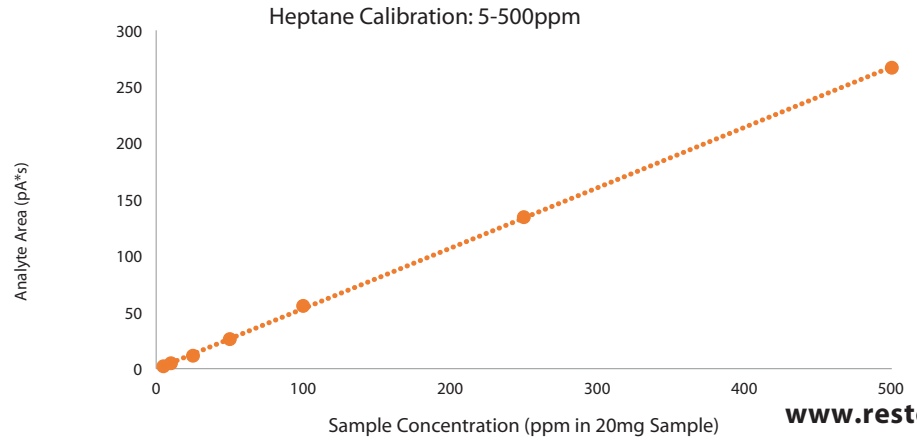
Table IV: Using full evaporation technique sample introduction for headspace GC-FID resulted in good sensitivity and linearity for all residual solvents as shown by peak response and correlation data for the calibration standards.

Compound	S:N 10 ppm	S:N 50 ppm	r	r^2
Isobutane	5.30	30.7	0.996	0.992
Butane	18.8	119	0.997	0.994
Methanol	48.1	189	0.999	0.999
Pentane	19.0	50.0	0.998	0.995
Ethanol	45.2	88.1	0.999	0.998
Acetone	49.9	97.0	0.999	0.999
Isopropanol	56.4	107	0.998	0.996
Hexane	45.6	109	0.999	0.998
Chloroform	11.5	22.5	0.999	0.998
Benzene	150	293	0.999	0.998
Heptane	88.4	193	1.00	1.00
Toluene	166	317	0.999	0.998

*Signal-to-noise ratios were calculated using Chemstation® software. Noise ranges were set at 0.2–0.6 minutes and 2.1–2.3 minutes.

Figure 5: Representative Calibration Curves from 5–500 ppm for Heptane and Isobutane





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Conclusion

By combining a selective Rxi®-624Sil MS GC column with the FET headspace GC-FID technique, excellent sensitivity and linearity were achieved for residual solvent compounds applicable to cannabis concentrates. The use of FET headspace GC-FID should allow quantification without the use of matrix-matched standards by creating a single non-partitioning phase system in the headspace vial. This technique also has the added benefit of needing very little sample and is applicable for the analysis of other volatile compounds, such as terpenes, in cannabis products.

References

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