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Sample Matrix Influence on GC/MS/MS Multiresidue Pesticide Analysis

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Abstract

The complexity of pesticide analysis lends itself well to gas chromatography/mass spectrometry (GC/MS). However, the quantitation limits and maximum residue limits (MRLs) drive the need for a multiresidue method with a reasonable linear range and low limits of detection (LODs). For this reason, tandem mass spectrometry (MS/MS) can be used for screening, confirming, and quantifying low-level pesticides. It not only provides low limits of quantitation (LOQs), but also minimizes matrix interferences. When low-level detections are needed, the cleanliness of the GC/MS system is key. Depending on the nature of the samples injected (as well as other factors), the frequency at which system maintenance is required varies (that is, replacement of the liner and septum, cutting the head of the column, source cleaning, and so on). When a sample is injected, the entire GC/MS flowpath (from the inlet, through the column, to the detector) is subjected to that sample. Over the course of repeated injections and multiple sequences, the GC inlet and column degrade, and the MS becomes contaminated. This Application Note examines the presence of matrix still introduced to a GC/MS/MS system after using appropriate sample preparation procedures, applying backflush, and using matrix-optimized multiple reaction monitoring (MRM).

Introduction

GC/MS maintenance[1-3]

GC/MS maintenance is a universal requirement for maintaining analytical targets during sample analysis. GC inlet maintenance is performed when consumables wear out (septa, syringes, nuts, ferrules, O-rings, and so on) or get dirty (liners, column, gas lines, traps, and so on). One of the key components of sample introduction is the inlet septum. All columns must have carrier gas head pressure to establish flow through the column. Septa maintain the leak-free seal and exclude air from the inlet. Septa are usually available according to their recommended upper temperature limits. Lower-temperature septa are usually softer, seal better, and can withstand more punctures (injections) than their high-temperature counterparts. After repeated injections, the septum deteriorates and is unable to maintain the inlet head pressure and a leak-free seal. Replace the septum when signs of deterioration are visible (gaping holes, fragments in inlet liner, poor chromatography, low column pressure, and so on). Agilent nonstick Advanced Green septa (p/n 5183-4759) are premium, nonstick septa that are functional for a range of applications.

In GC, the inlet is the first part of the instrument with which your sample can interact. The inlet's main purpose is to transfer the sample onto the GC column for analysis. This leads to the liner being a popular site for contamination. Liners become contaminated with use, collecting nonvolatiles, volatiles, salts, excess reagents, and so on. Using an Agilent 4 mm, Ultra Inert, splitless, single-taper liner with glass wool (p/n 5190-2293) enables trace analysis in dirty samples. The glass wool (located near the bottom of the liner) helps in volatilization of high molecular weight components, increases mixing of the sample, and protects the head of the column. Liners should be replaced when contamination is visible in the liner or if chromatography has become degraded.

Selecting the right capillary column for your application can be an uncertain and difficult task. There are four major column parameters to consider:

- Stationary phase
- Diameter
- Length
- Film thickness

The most critical decision is the choice of stationary phase, which is based on factors such as selectivity, polarity, and phenyl content. To meet the worldwide drive for lower detection levels, Agilent J&W HP-5ms Ultra Inert columns (such as 19091S-431UI) are tested for inertness for active compounds and feature improved signal-to-noise ratio for better sensitivity and mass spectral integrity. Columns tend to last longer than the inlet septum and liner, but they require maintenance when the septum and liner are replaced. The main indication of when to trim the head of the column is when chromatographic problems (peak tailing, change in response, retention time changes, and so on) become visible. Typically, 0.5 to 1 m from the front of the column should be removed to return to optimal chromatographic performance.

The Agilent 7010B triple quadrupole GC/MS has a high-efficiency electrospray ionization (EI) source, which produces at least 20 times as many ions as its previous generation, improving analytical efficiency. With a more sensitive detector, more precautions should be considered. During system operation, the GC/MS ion source requires cleaning. This is indicated by a loss in analyte response that is not improved by GC inlet and column maintenance (or in source tuning by poor calibrant ion peak shapes or escalating repeller or election multiplier voltages). Proper cleaning (slurry of aluminum oxide powder and methanol), assembly, and installation are essential to robust and reliable operation. The objective is to return the GC system performance to an established norm, which is usually considered the response of the target compounds in calibration standards. The metric for failing may not be response alone, but loss of peak shape (chromatographic criteria), selective loss of a few sensitive target compounds, or a combination of MS signal and chromatographic criteria.

Sample preparation[6]

Sample preparation is an essential part of successful chromatography. It extends column lifetime, reduces the need for repeated samples, and minimizes interferences that can jeopardize your separation, detection, and quantification. Many laboratories focused on pesticide residue analysis in food commodities routinely use the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method[4,5]. This straightforward sample preparation enables the analysis of hundreds of pesticides at low concentrations with a single extraction. However, sample preparation shortcuts are becoming more common within the laboratory. Not only will this influence the data quality, but it will also increase the frequency of GC/MS system maintenance.

Three diverse matrices were selected to demonstrate matrix differences. These commodities included organic honey, jasmine rice, and black loose-leaf tea. Each matrix was extracted with their specified QuEChERS methodology (Figure 1), in which various dispersive solid phase extractions (dSPE) were used for matrix cleanup. Even though the QuEChERS technique is a quick and dirty sample preparation procedure that provides excellent results, shortcuts are still taken. During the QuEChERS procedure, extracts were taken at the following steps:

- After the 10 mL acetonitrile (ACN) extraction (noted with LLE in the data file)
- After the first centrifuge step (noted with salt in the data file)
- After the final dSPE (noted with dSPE in the data file)

Matrix-optimized MRMs[6]

Matrix effects have been a common complaint among MRM acquisition methods in pesticides analysis. The usefulness of a given compound's MRMs can change depending on the matrix being measured. The ability to have multiple MRMs from which to choose aids in lab productivity, improved quant method generation, and achieving optimal analysis. The Agilent G9250AA Rev. A.04.02 Pesticides and Environmental Pollutants (P&EP) Standard MRM Database is the most comprehensive GC MRM database available. With over 1,100 compounds and up to 10 MRMs/compound, the database was used to obtain the matrix-optimized MRMs for each target compound.
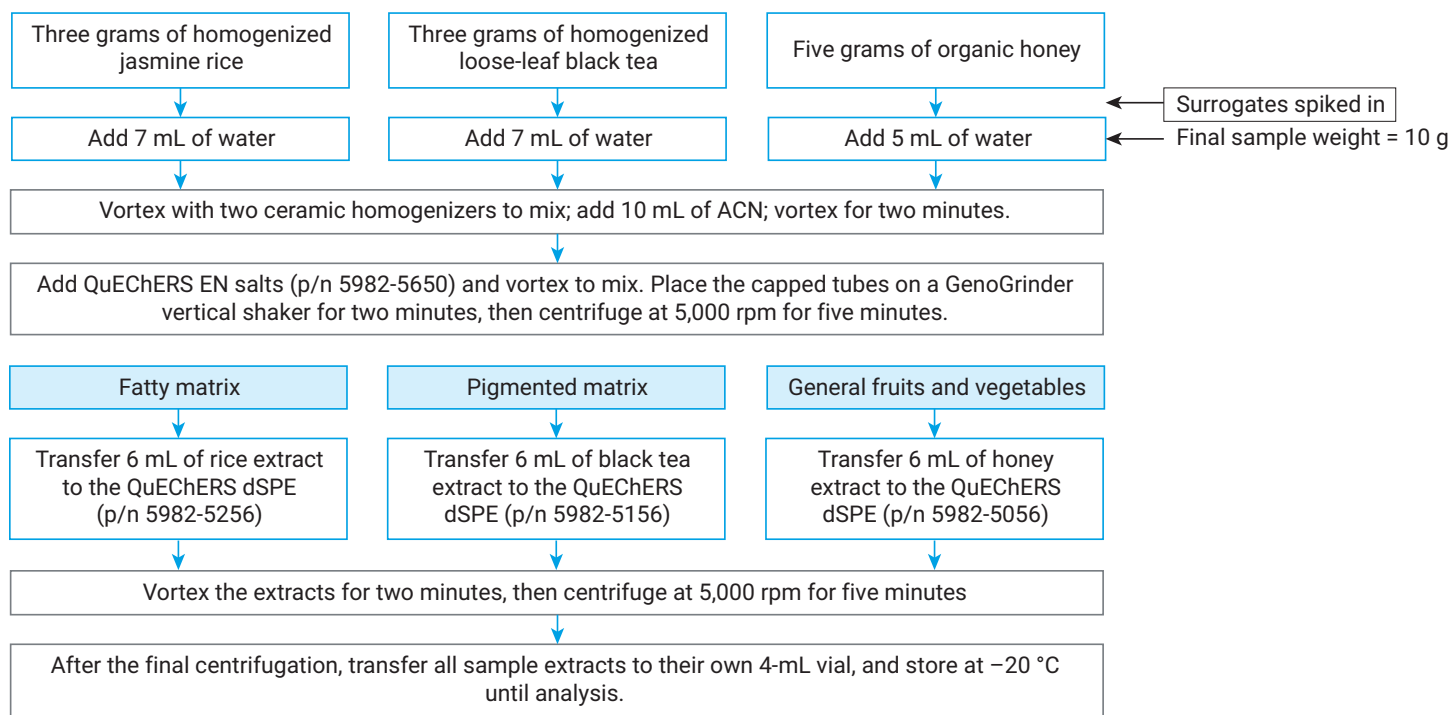

Figure 1. QuEChERS methodology for application.

Experimental

Instrumentation

All analyses were run on an Agilent 7890B GC equipped with an Agilent 7693B autosampler and an Agilent 7010A triple quadrupole GC/MS. Table 1 displays the GC and backflush parameters, and Tables 2 and 3 show the scan and dMRM method parameters, respectively. The GC was configured with a multimode inlet (MMI) equipped with a 4 mm Ultra Inert, splitless, single taper, glass wool liner (p/n 5190-2293). From the inlet, two Agilent J&W HP-5ms Ultra Inert columns (15 m × 0.25 mm, 0.25 µm; p/n 19091S-431 UI) were coupled to each other through a purged Ultimate union (PUU) for the use of midcolumn/post run backflushing (Figure 2).

Table 1. 7890B GC method conditions.

Parameter	Value
MMI injection mode	Hot-splitless
Injection volume	1 µL
Inlet temperature	280 °C
Carrier gas	He, constant flow 1.00 mL/min (column 2 = 1.20 mL/min)
MS transfer line temperature	280 °C
Oven program (20 minute method)	60 °C–1 minute 40 °C/min to 170 °C–0 minutes 10 °C/min to 310 °C–3 minutes
PUU Backflush settings*	
Timing	1.5 minutes duration during postrun
Oven temperature	310 °C
AUX EPC pressure	~50 psi
Inlet pressure	~2 psi

* Backflush conditions optimized for application method in an Agilent laboratory. A 1.5-minute backflush duration may be too short for other methods; recommendations can be made for a five-minute backflush duration.

Table 2. 7010A scan parameters.

Parameter	Value
Scan type	MS1 Scan
Electron energy	70 eV
Tune	atunes.eihs.tune.xml
EM gain	10*
MS1 Start mass–end mass	40 to 600
Collision cell	1.5 mL/min N_2 and 2.25 mL/min He
Source temperature	280 °C
Quad temperatures	150 °C
Scan time	200 ms
Step size	0.1 amu
Threshold	100

* Note that a gain of 10 for the 7010A is high. The gain recommendation for the 7010 is 0.2–1.

Table 3. 7010A dMRM parameters.

Parameter	Value
Scan type	dMRM
Electron energy	70 eV
Tune	atunes.eihs.tune.xml
EM gain	10*
MS1 and MS2 resolution	Unit
Collision cell gas flows	1.5 mL/min N_2 and 2.25 mL/min He
Source temperature	280 °C
Quad temperatures	150 °C
Quant/qual transitions	Matrix optimized
Right and left RT deltas	0.1 minutes
Dwell times	Optimized by dMRM
Minimum dwell time (ms)	10
Cycles per second	3.07

* Note that a gain of 10 for the 7010A is high. The gain recommendation for the 7010 is 0.2–1.

Backflush

Different actions can be used to maintain analytical performance and reduce the frequency of system maintenance. One enhancement Agilent introduced into the GC/MS maintenance process, column backflushing, has improved ion source and GC longevity, and made GC/MS column and inlet maintenance rapid and ventless (Figure 3). Backflush reverses the column flow in a GC system after the last compound of interest has eluted, removing high-boiling components. Backflush provides longer column life, quicker GC maintenance, and less MS maintenance. Midcolumn backflush was performed in this application.

Results and discussion

Using the appropriate sample preparation procedures, applying backflush, and using matrix-optimized MRMs all promote optimal GC/MS conditions. However, sample preparation shortcuts are becoming more common within the laboratory. This influences the data quality, and also increases the frequency of GC/MS system maintenance. Figures 4 through 11 explore different steps that were taken along the sample preparation procedure followed (see Experimental section) for each of the three matrices (organic honey, jasmine rice, loose-leaf black tea). Also included are figures that demonstrate the MRM acquisitions comparison to that of their scan chromatograms.
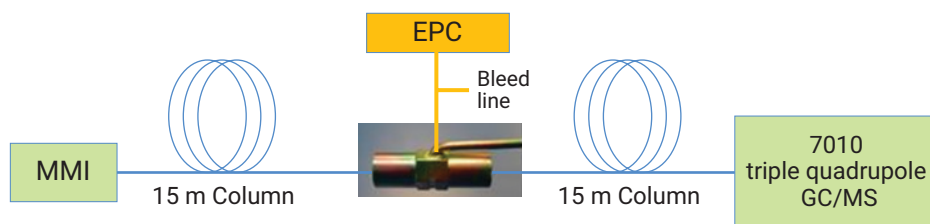

Figure 2. Column configuration for an optimal MRM application.

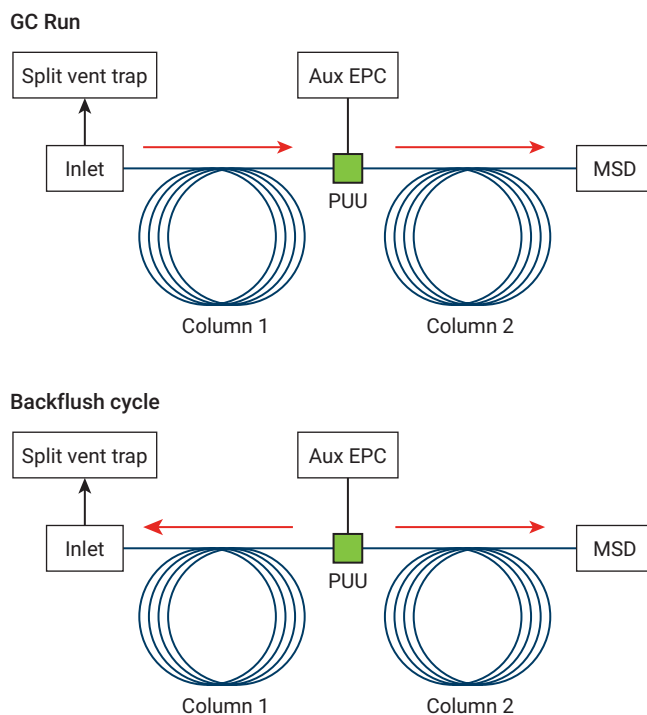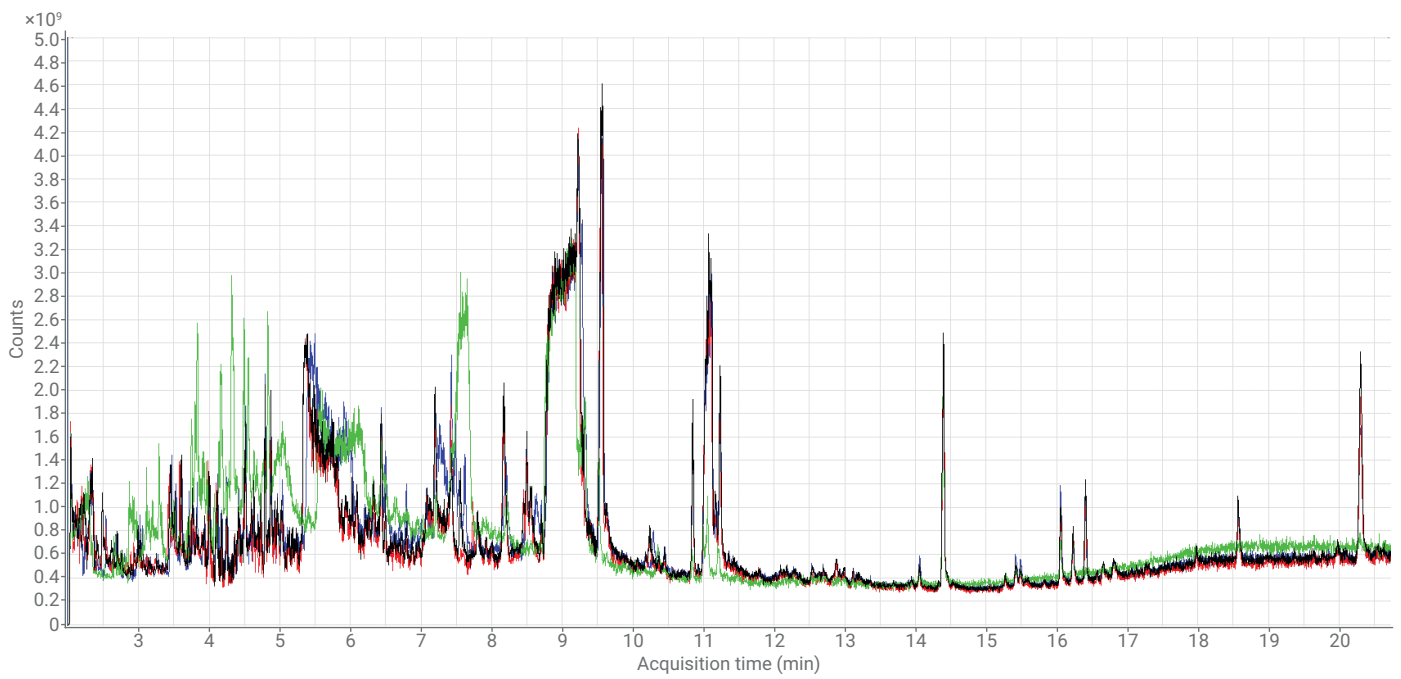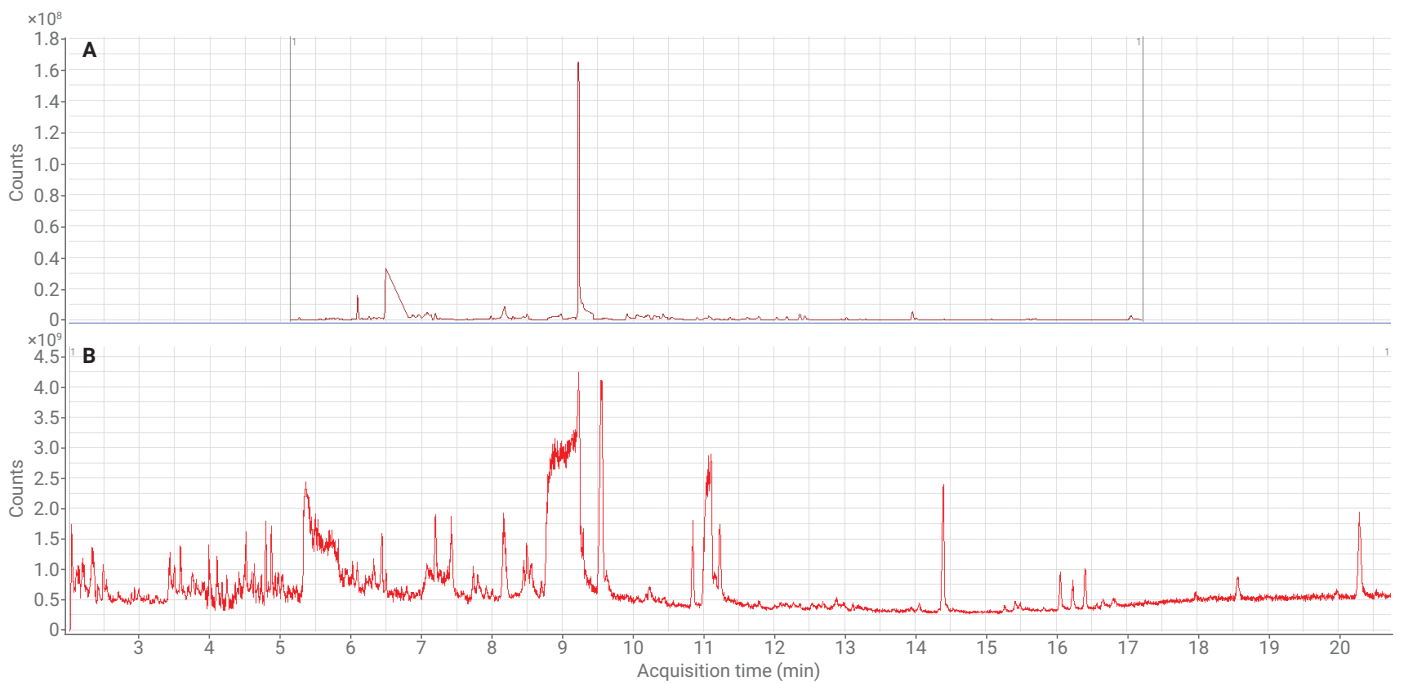**GC Run**

Backflush cycle

Figure 3. Illustration of midcolumn backflush flows during analytical run and backflush cycle.

Figure 4. Scans of black loose-leaf tea sample at various matrix extraction steps: dSPE Black Tea Blank_SCAN (black); dSPE Black Tea Spike_SCAN (red); LLE Black Tea Blank_SCAN (green); Salt Black Tea Blank_SCAN (blue).

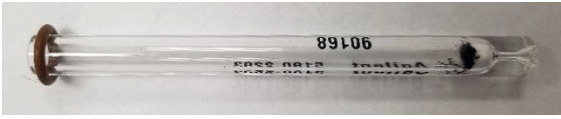**Figure 5.** MRM acquisition of the final dSPE black tea extract (A) and its scan acquisition (B).
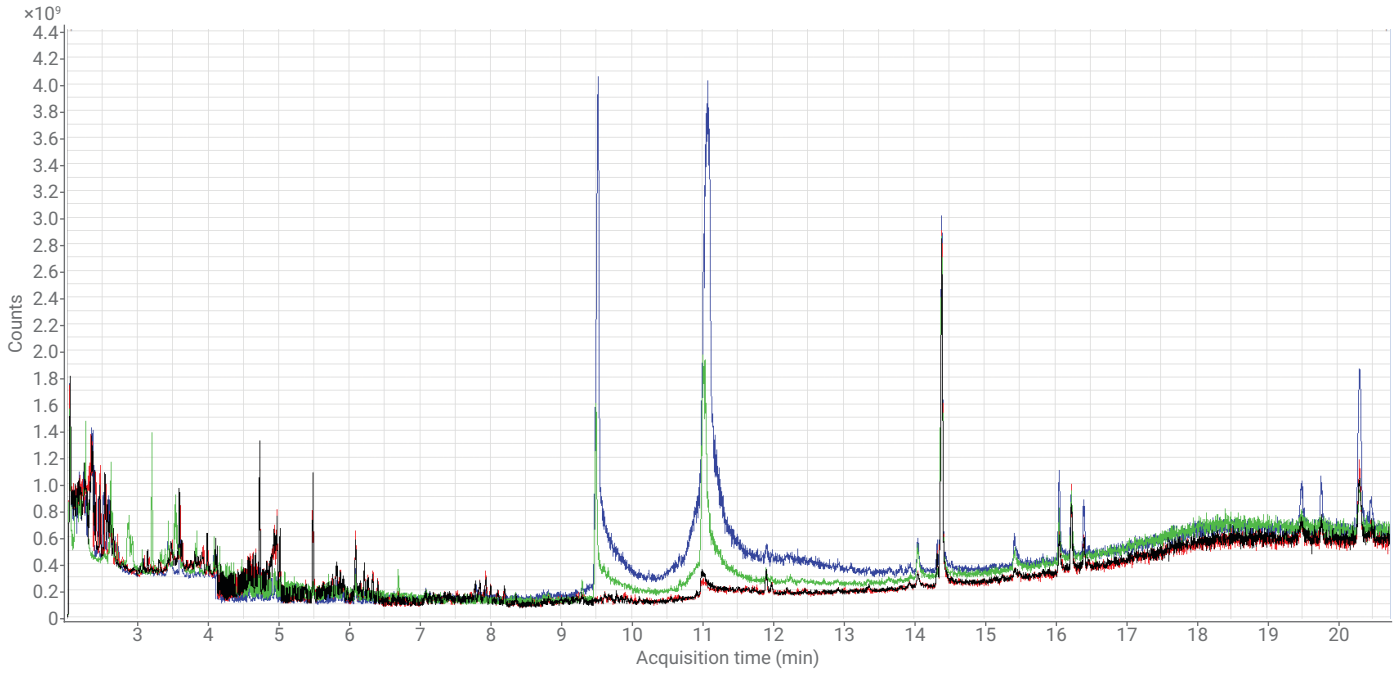
Figure 6. Liner after black tea analysis.

Figure 7. Scans of jasmine rice sample at various matrix extraction steps: dSPE Rice Blank_SCAN (black); dSPE Rice Spike_SCAN (red); LLE Rice Blank_SCAN (green); Salt Rice Blank_SCAN (blue).
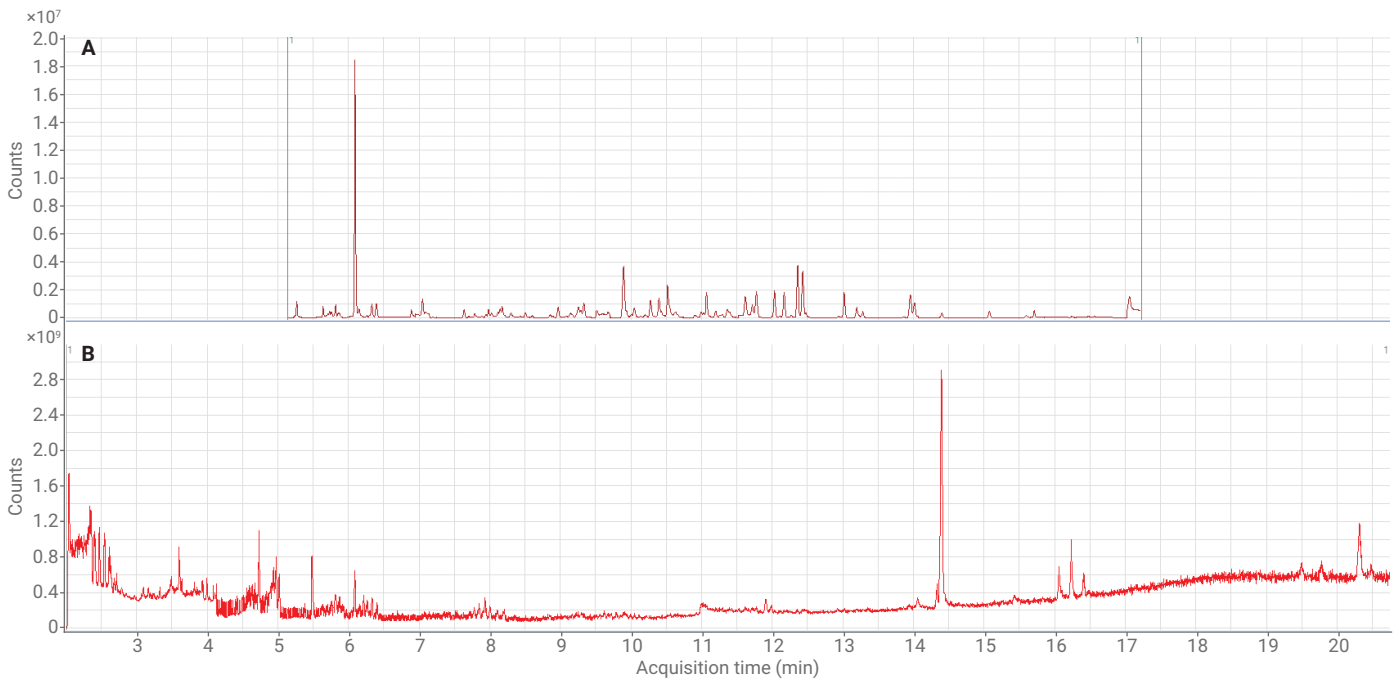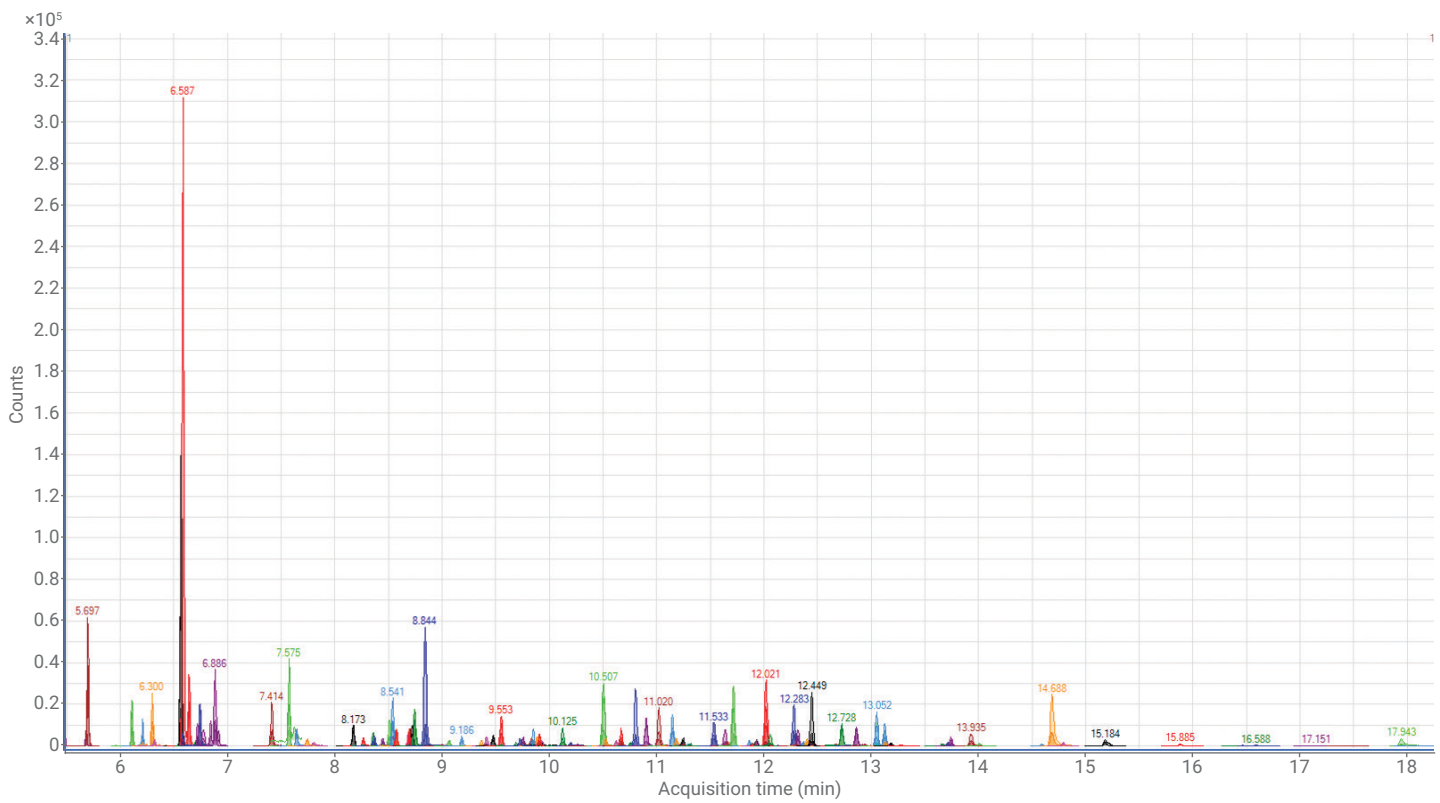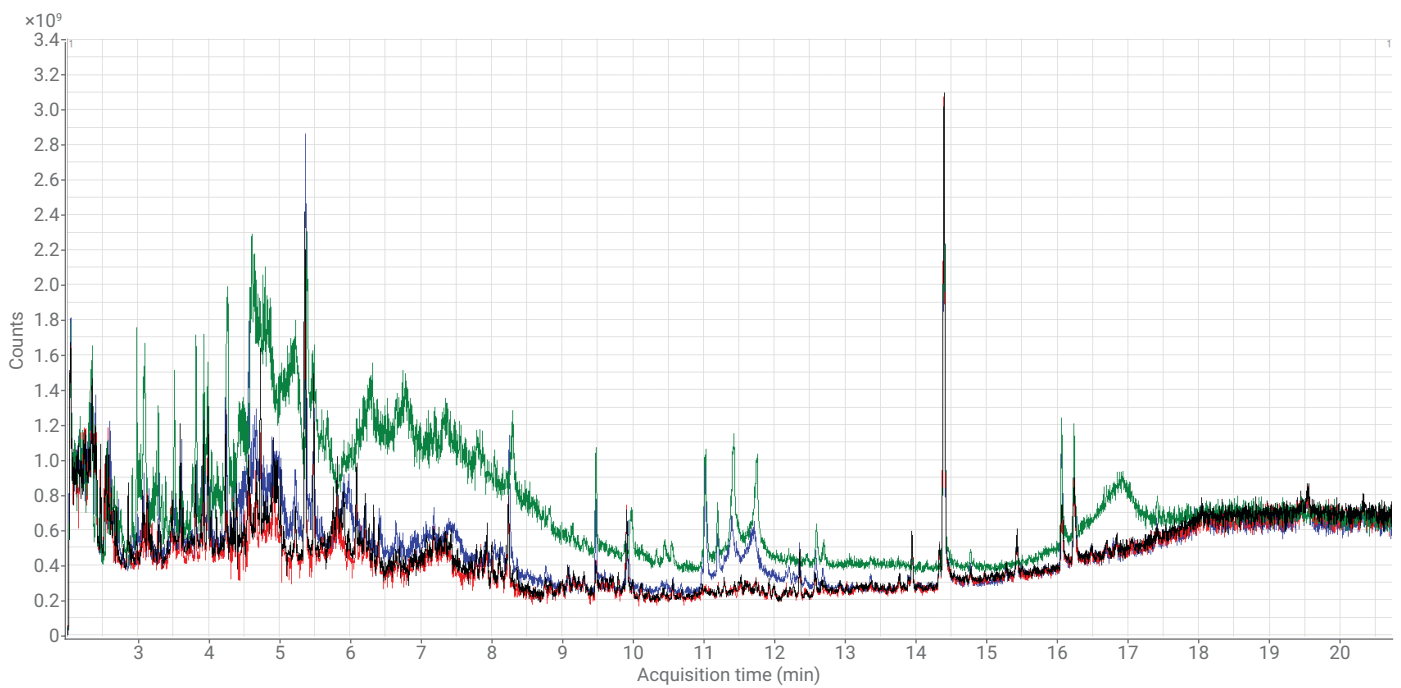

Figure 8. MRM acquisition of the final dSPE rice extract (A) and its scan acquisition (B).
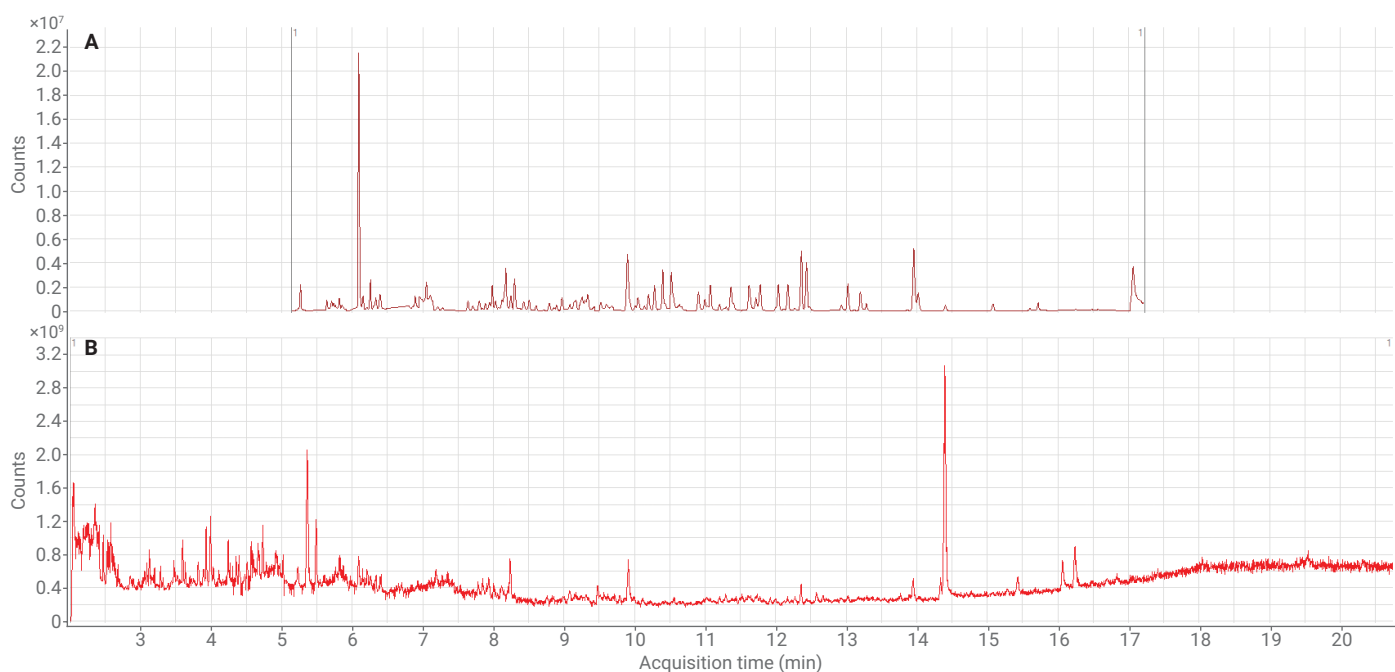
Figure 9. Extracted MRM chromatogram of final dSPE rice extract.

Figure 10. Scans of organic honey sample at various matrix extraction steps: dSPE Honey Blank_SCAN (black); dSPE Honey Spike_SCAN (red); LLE Honey Blank_SCAN (green) Salt Honey Blank_SCAN (blue).

Figure 11. MRM acquisition of the final dSPE honey extract (A) and its scan acquisition (B).

Table 4. Data results for organic honey analysis.

| Compound | Organic honey data | | | |
	%RSD	MDL (pg/µL)	iLOQ (pg/µL)	%Error
2,4-D butyl ester	8.09	0.26	0.94	7.08
Aminocarb	8.4	0.285	1.032	2.09
Azinphos-ethyl	13.77	0.436	1.579	8.53
Benthiavalicarb-isopropyl	14.22	0.437	1.583	10.49
BHC-*alpha*	7.83	0.261	0.943	4.01
BHC-*beta*	17.19	0.541	1.959	9.1
Chlordane-*cis*	13.08	0.411	1.486	9.42
Chlorpyrifos-methyl	7.76	0.252	0.91	6.42
Dazomet	4.38	0.152	0.552	0.45
DDT-*o,p'*	8.78	0.27	0.977	11.35
Diazinon	7.33	0.238	0.862	6.15
Difenoconazole II	19.99	0.663	2.399	4.12
Ethofenprox	16.5	0.539	1.951	6.88
Ethoprophos	8.72	0.293	1.061	3.11
Flurenol-butyl	6.85	0.219	0.793	6.32
Haloxyfop-r-methyl	6.74	0.217	0.786	7.2
Heptachlor endo-epoxide	7.75	0.493	1.783	8.13
Hexazinone	4.91	0.157	0.569	7.85
Iprobenfos	4.69	0.157	0.569	2.94
Permethrin, (1R)-*trans-*	10.25	0.335	1.211	5.57
Phenanthrene-D10	6.59	0.217	0.786	4.92
Phorate	29.01	1.023	3.699	2.01
Terbufos sulfone	3.46	0.112	0.404	6.54
Triadimefon	4.26	0.137	0.497	6.97

Conclusions

Multiresidue pesticide analysis by MS/MS is used for screening, confirming, and quantifying low-level pesticides. It provides low limits of quantitation, and minimizes interferences. When low-level detections are needed, the cleanliness of the GC/MS system is key. Over the course of repeated injections and multiple sequences, the GC inlet and column degrades, and the MS becomes contaminated. This Application Note examines the presence of matrix still introduced to a GC/MS/MS system after using appropriate sample preparation procedures, applying backflush, and using matrix-optimized MRMs.

The 7010 Series triple quadrupole GC/MS system can confirm pesticide residues at the low ppb level even in the most complex extracts. The calibration standards were prepared at concentrations ranging from 0.12 pg/µL to 50 pg/µL; for 90 % of compounds, a calibration curve with a R^2 ≥0.990 was produced. All analyzed pesticides obtained a %RSD of repeated measurements of ≤30 %, and 90 % have a LOQ ≤1.5 pg/µL.

References

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