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Intelligent Agilent GC/MS/MS

Conquering Analytical Challenges

Application Compendium

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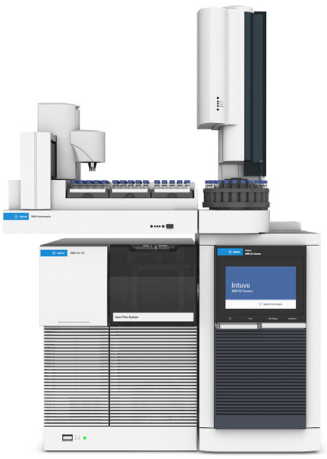
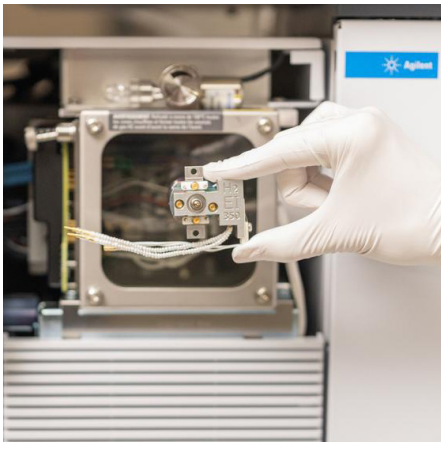
Introduction

The Agilent 7000E and 7010C GC/TQ systems lead the way in analytical excellence and mass spec intelligence. With smart features like SWARM Autotune, these systems can intelligently and automatically optimize their performance, providing users with unparalleled efficiency and accuracy in their analyses. While helium remains the gold standard carrier gas for GC/MS, there are growing concerns regarding its unstable supply chain and rising costs. To address this, Agilent offers a compelling alternative with the use of hydrogen carrier gas in combination with the innovative HydroInert source. The HydroInert source, compatible with the 7000E GC/TQ, significantly improves performance with hydrogen as a carrier gas, mitigating potential concerns and providing a highly reliable and cost-effective solution for analytical laboratories. By embracing the Agilent 7000E and 7010C GC/TQ systems, researchers can confidently conquer their analytical challenges while ensuring maximum instrument productivity and throughput.



Agilent Intuvo 9000/7000E GC/TQ

Agilent 8890/7000E GC/TQ



HydroInert Source

Application Note

Food & Beverage Testing

Hydrogen Carrier Gas for Analyzing Pesticides in Pigmented Foods with GC/MS/MS

Authors

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Abstract

This application note describes the key strategies for pesticide analysis with gas chromatography/triple quadrupole mass spectrometry (GC/TQ) using hydrogen as the carrier gas while maintaining sensitivity to meet maximum residue limits (MRLs). The key aspects addressed in this work include the recommended column configuration, the optimized injection conditions, and the appropriate choice of the mass spectrometer (MS) electron ionization (EI) source hardware developed for use with hydrogen carrier gas. The 20 m × 20 m (0.18 mm × 0.18 µm) Agilent HP-5ms UI midcolumn backflush configuration allowed for maintaining the same retention times as with helium, leading to time savings associated with method translation. The resulting chromatographic resolution achieved under the optimal conditions with hydrogen surpassed that with helium. The optimized injection conditions included solvent vent mode, a 2 mm dimpled liner, and the use of analyte protectants. The analyte response with hydrogen was enhanced on average 10-fold when using the optimized conditions compared to using hydrogen carrier gas with the injection conditions, commonly used with helium. Both the Agilent HydroInert and the Agilent High Efficiency Source (HES) resulted in nearly identical spectra observed with hydrogen and helium, which allowed using the same multiple reaction monitoring (MRM) transitions and collision energies as with helium. The ability to use the same MRMs, collision energies, and retention times greatly simplified the transition from helium to hydrogen.

The resulting method allowed for quantitation of over 90% of the 203 target pesticides at or below the default MRL of 10 parts per billion (ppb) in the pigmented spinach matrix with both the HydroInert and the HES sources. The method detection limits (MDLs) for compounds susceptible to reactions with hydrogen, and hence, presenting the biggest challenge to the analysis with hydrogen, were in the sub-ppb range, with the HES enabling higher sensitivity and lower MDLs. The calibration performance was demonstrated over a broad range of concentrations, meeting the SANTE/11312/2021 guidelines. The relative standard error (RSE) for over 94% of 203 targets was below 20%. Even the compounds most prone to reacting with hydrogen, such as tecnazene, could be accurately quantitated over the ranges of 0.5 to 5,000 ppb and 0.1 to 1,000 ppb with the HydroInert and HES sources, respectively. Finally, simultaneous dynamic MRM and full scan data acquisition mode was demonstrated for accurate quantitation and reliable compound identification. The identification was based on spectral matching with the Agilent 8890/7000E and the Agilent 8890/7010C GC/TQ systems using hydrogen carrier gas.

Introduction

Due to recurring helium shortages and increased prices experienced in the recent years, there is an intensified demand for adapting the GC/MS analysis to hydrogen carrier gas. While helium is the optimal carrier gas for GC/MS, hydrogen has emerged as a viable alternative. Hydrogen brings chromatographic benefits to the analysis if proper measures are taken to translate the method.[1,2] Additionally, hydrogen emerges as a renewable and cost-effective alternative for sustainable laboratory practices. However, unlike helium, hydrogen is not chemically inert. This lack of inertness raises concerns as hydrogen can potentially react with target analytes, matrix components, or solvents. Such reactions can lead to compound degradation, chromatographic issues like peak tailing, distorted ion ratios in the mass spectrum, compromised library matching, and decreased sensitivity. Therefore, the transition from helium to hydrogen carrier gas requires due diligence. The EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion Guide[1] provides detailed instructions for method conversion from helium to hydrogen carrier gas. The user guide outlines considerations and procedures for hydrogen safety necessary to make the transition to hydrogen carrier gas successful.

Since the introduction of the HydroInert source, several applications have been implemented successfully with hydrogen carrier gas. Those applications included analysis of semivolatile organic compounds with GC/MS[3] and GC/MS/MS[4], volatile organic compounds[5], polycyclic aromatic hydrocarbons (PAHs) in environmental samples with GC/MS[6] and GC/MS/MS[7] and PAHs in infant formula with GC/MS[8], flavor and fragrance GC/MS analysis[9], and the EPA TO-15 analysis.[10] Analyzing pesticides poses its own set of challenges, even when using helium as the carrier gas, due to the diverse and labile nature of many pesticides and the complex matrices they are found in. The best practices in sample preparation and GC/MS/MS analysis of pesticides with helium carrier gas have been described in previous work.[11] This application note describes the key strategies for analyzing pesticides with hydrogen carrier gas while delivering high-quality uncompromised results. The components enabling successful analysis of pesticides with hydrogen in foods include:

- Effective sample extraction and matrix cleanup, such as QuEChERS extraction followed by Agilent Captiva enhanced matrix removal (EMR) pass-through cleanup
- Solvent vent injection mode with the 2 mm dimpled liner and the temperature-programmable multimode inlet (MMI)
- Use of the analyte protectants
- Minibore columns with the same phase ratio as those with the helium method (20 m × 20 m, 0.18 mm × 0.18 µm)
- Midcolumn backflush
- Method translation and retention time-locking techniques
- EI sources with reduced or eliminated source reactivity with hydrogen

The novelty of the work involved the evaluation of several EI sources, including the standard Inert Plus Extractor EI source, the HydroInert source, and the HES for pesticides analysis with hydrogen carrier gas. Both the Agilent 8890/7000E and Agilent 8890/7010C gas chromatography/triple quadrupole mass spectrometry (GC/TQ) systems were ideally suited to meet the analytical needs with hydrogen carrier gas.

The resulting method was applied to analyzing a broad panel of 203 GC-amenable pesticides in a spinach QuEChERS extract to demonstrate method sensitivity. The achieved sensitivity was sufficient for quantitating pesticides at the MRLs. Calibration performance was demonstrated over the concentration range up to four orders of magnitude while meeting SANTE 11312/2021 guidelines.[12] Simultaneous dynamic multiple reaction monitoring (dMRM) and scan (dMRM/scan) data acquisition mode was demonstrated for

compound screening via spectral deconvolution and search against spectral libraries, while the dMRM data were used for accurate quantitation. The reduced/eliminated in-source hydrogen reactions with the HydroInert and HES sources significantly improved library match scores and, hence, identity confirmations for untargeted compounds.

Experimental

GC/TQ analysis

The 8890/7000E and 8890/7010C GC/TQ systems (Figure 1A) were used and configured to achieve the best performance with hydrogen carrier gas. The GC was configured with the Agilent 7693A automatic liquid sampler (ALS) and 150-position tray, an MMI operated in solvent vent mode, midcolumn backflush capability provided by an Agilent purged Ultimate union (PUU) installed between two identical 20 m columns (0.18 mm × 0.18 µm), and the 8890 GC pneumatic switching device (PSD) module (Figure 1B). A 40 m column can be used in lieu of two 20 m columns, although without the backflushing capability. Several EI source configurations including the optional 3 mm and 6 mm lenses were evaluated with the 7000E GC/TQ. The best performance with the 7000E GC/TQ was achieved when using the HydroInert source with the default 9 mm lens. The 7010C GC/TQ delivered excellent performance with hydrogen carrier gas when using the standard HES. The best practices when converting the GC/TQ from helium to hydrogen carrier gas described in the Helium to Hydrogen Conversion Guide[1] were followed to ensure safe and successful conversion. The instrument operating parameters are listed in Table 1.

The Method Translation software allows users to port a current GC method to another GC column configuration and/or carrier gas while ensuring that relative retention order is maintained, i.e., peaks elute in the same order.[13,14] It is available among the downloadable GC Tools from the Agilent GC calculators and method translation software page.[15] In this work, the method translation technique was used to determine the approximate hydrogen carrier flow rate for a 20 m × 20 m column configuration. This method translation was used to obtain nominally the same retention times as with the conventional 15 m × 15 m method with helium carrier gas i.e., speed gain of 1. Those flows were 1 and 1.2 mL/min for columns 1 and 2, respectively. Next, retention time locking to chlorpyrifos methyl at 9.143 minutes resulted in precise matching of the retention times between the hydrogen and the conventional 20-minute helium method described in other application notes[11] and in the GC/MS/MS pesticide residue analysis reference guide.[16] Chlorpyrifos methyl is selected as a retention time locking compound because it typically does not present analytical challenges, elutes in the middle of the pesticide chromatographic range, and is commonly used as a process control compound for GC-amenable pesticides used in the Pesticide Data Program laboratories.[16] Retention time locking is a technique that allows a new column or instrument to have retention times that match the retention times provided in the databases, including the MRM database used in this work, or an existing method precisely, allowing methods to be easily ported from one instrument to another and across the Agilent GC/MS and GC/MS/MS instruments globally.

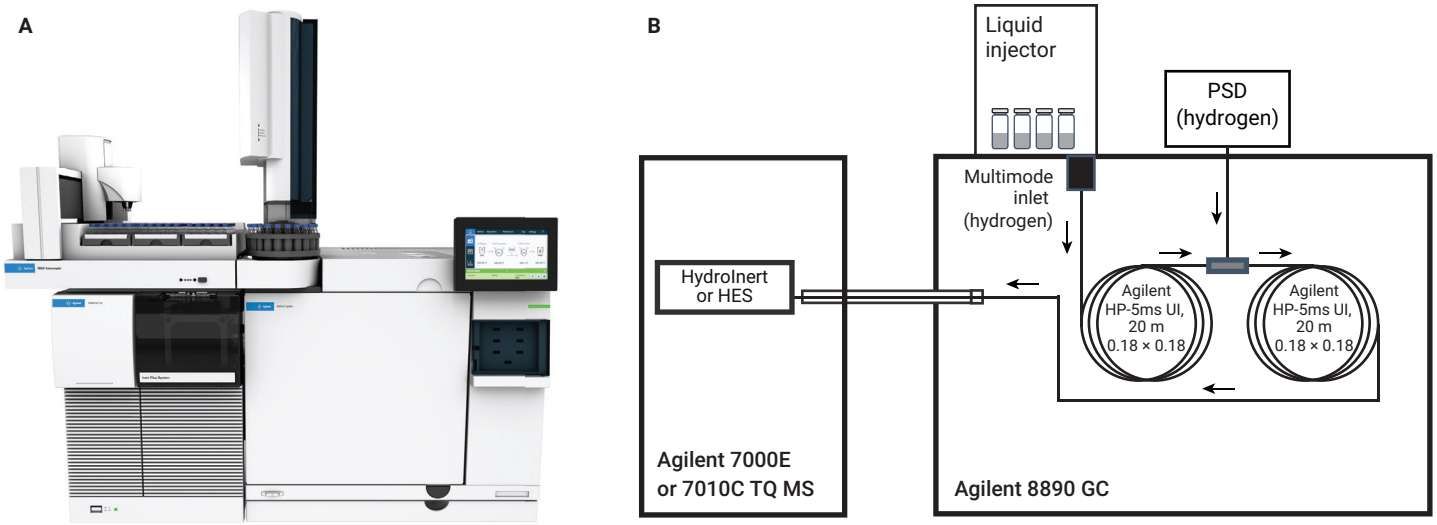

Figure 1. The Agilent 8890/7000E and 8890/7010C GC/TQ system (A) and system configuration (B).

Table 1. Agilent 8890/7000E and 8890/7010C gas chromatograph and mass spectrometer conditions for pesticide analysis with hydrogen carrier gas.

GC	
Model	Agilent 8890 with Fast Oven, Auto Injector and Tray
Inlet	Multimode Inlet (MMI)
Mode	Solvent Vent
Purge Flow to Split Vent	60 mL/min at 2.56 min
Septum Purge Flow	3 mL/min
Vent Flow	100 mL/min
Vent Pressure	5 psi until 0.06 min
Septum Purge Flow Mode	Switched
Cryo	On (Air)
Cryo Use Temperature	200 °C
Injection Volume	2.0 µL
L1 Airgap	0.2 µL
Gas Saver	Off
Inlet Temp	60 °C for 0.06 min, then to 280 °C at 600 °C/min
Postrun Inlet Temperature	310 °C
Postrun Total Flow	25 mL/min
Carrier Gas	Hydrogen
Inlet Liner	Agilent Ultra Inert 2 mm dimpled liner
Inlet Liner Part Number	5190-2297
Oven	
Initial Oven Temperature	60 °C
Initial Oven Hold	1 min
Ramp Rate 1	40 °C/min
Final Temp 1	170 °C
Final Hold 1	0 min
Ramp Rate 2	10 °C /min
Final Temp 2	310 °C
Final Hold 2	2.25 min
Total Run Time	20 min
Postrun Time (Backflush Duration)	1.5 min
Equilibration Time	0.5 min
Column 1	
Type	Agilent HP-5ms UI (p/n 19091S-577UI)
Length	20 m
Diameter	0.18 mm
Film Thickness	0.18 µm
Control Mode	Constant flow
Flow	1.0 mL/min (nominal before retention time locking)
Inlet Connection	Multimode inlet (MMI)
Outlet Connection	PSD (PUU)
PSD Purge Flow	5 mL/min
Postrun Flow (Backflushing)	–6.260 mL/min

Column 2	
Type	Agilent HP-5ms UI (p/n 19091S-577UI)
Length	20 m
Diameter	0.18 mm
Film Thickness	0.18 µm
Control Mode	Constant flow
Flow	1.2 mL/min (nominal before retention time locking)
Inlet Connection	PSD (PUU)
Outlet Connection	MSD
Postrun Flow (Backflush Duration)	6.406 mL/min
MSD	
Model	Agilent 7000E or 7010C
Source	HydroInert (G7006-67930) or HES
Vacuum Pump	Performance turbo
Tune File	Atunes.eiex.jtune.xml or Atunes.eihs.jtune.xml
Solvent Delay	3.75 min
Quad Temperature (MS1 and MS2)	150 °C
Source Temperature	280 °C
Mode	dMRM; Scan (45-450 *m/z*; 220 ms); dMRM/Scan (200 ms)
He Quench Gas	Off
N_2 Collision Gas	1.5 mL/min
Collision Energies	Same as listed for helium in P&EP 4.0
MRM Statistics	
Total MRMs (dMRM mode)	614
Minimum Dwell Time	3 ms
Minimum Cycle Time	69.8 ms
Maximum Concurrent MRMs	52
EM Voltage Gain Mode	10
Scan Parameters	
Scan Type	MS1 Scan
Scan Range	45 to 450 *m/z*
Scan Time	220 ms
Step Size	0.1 amu
Threshold	0
EM Voltage Gain Mode	1
Agilent MassHunter Workstation	– MassHunter Acquisition software for GC/MS systems 10.2 – MassHunter Quantitative 10.1 – Unknowns Analysis Quantitative Analysis 10.1 – MassHunter Qualitative 10

The precise matching of the retention times between the hydrogen carrier method and the Agilent MassHunter Pesticide & Environmental Pollutant MRM database (P&EP 4, part number G9250AA) allowed for creating the MS method seamlessly and enabled great time savings. The database includes up to 9 MRM transitions for each of over 1,100 compounds and their retention times for the 20-minute analysis with helium or hydrogen. The use of P&EP 4 increased the ease and speed of setting up a targeted dynamic MRM (dMRM) method.

Acquiring data in dMRM mode enabled the capability for large multi-analyte assays and accurate quantitation of narrow peaks by an automated and most efficient dwell time distribution. The dMRM capability resulted in successful analysis for a large panel of 203 pesticides with 614 total MRM transitions and up to 52 concurrent MRMs. Furthermore, dMRM allowed the analyst to add and remove additional analytes with ease.

Full scan data acquisition mode was used for evaluating mass spectra with hydrogen carrier gas and for the initial screening of the matrix extract. This screening was used to evaluate the in-source loading and for monitoring the efficiency of the sample cleanup procedure that followed the QuEChERS extraction. Either a blank matrix, a representative sample, or a matrix-matched calibration standard can be used for initial screening.

Additionally, simultaneous dMRM/scan data acquisition mode enabled simultaneous targeted quantitation of a large multi-analyte assay and full scan data acquisition for unknown identification and retrospective analysis within one analytical run.

Agilent MassHunter Workstation revisions 10.1 and 10.2 including MassHunter Acquisition for GC/MS 10.2, MassHunter Quantitative Analysis 10.1, including Unknowns Analysis, and MassHunter Qualitative Analysis 10.0 packages were used in this work.

Sample preparation

A sample preparation workflow chart is shown in Figure 2. The sample preparation included two major steps: sample extraction by traditional QuEChERS extraction, followed by Captiva enhanced matrix removal (EMR) pass-through cleanup. The Captiva EMR–High Chlorophyll Fresh with NH_2 (Captiva EMR–HCF1) cartridge was used for high chlorophyll fresh matrix (spinach). The new sample preparation workflow demonstrated as a simplified procedure with improvement on both sample matrix removal and targets quantitation data quality.

As shown in Figure 2, samples were first extracted using the traditional Agilent Bond Elut QuEChERS EN extraction kit (part number 5892-5650CH). Homogenized fresh spinach

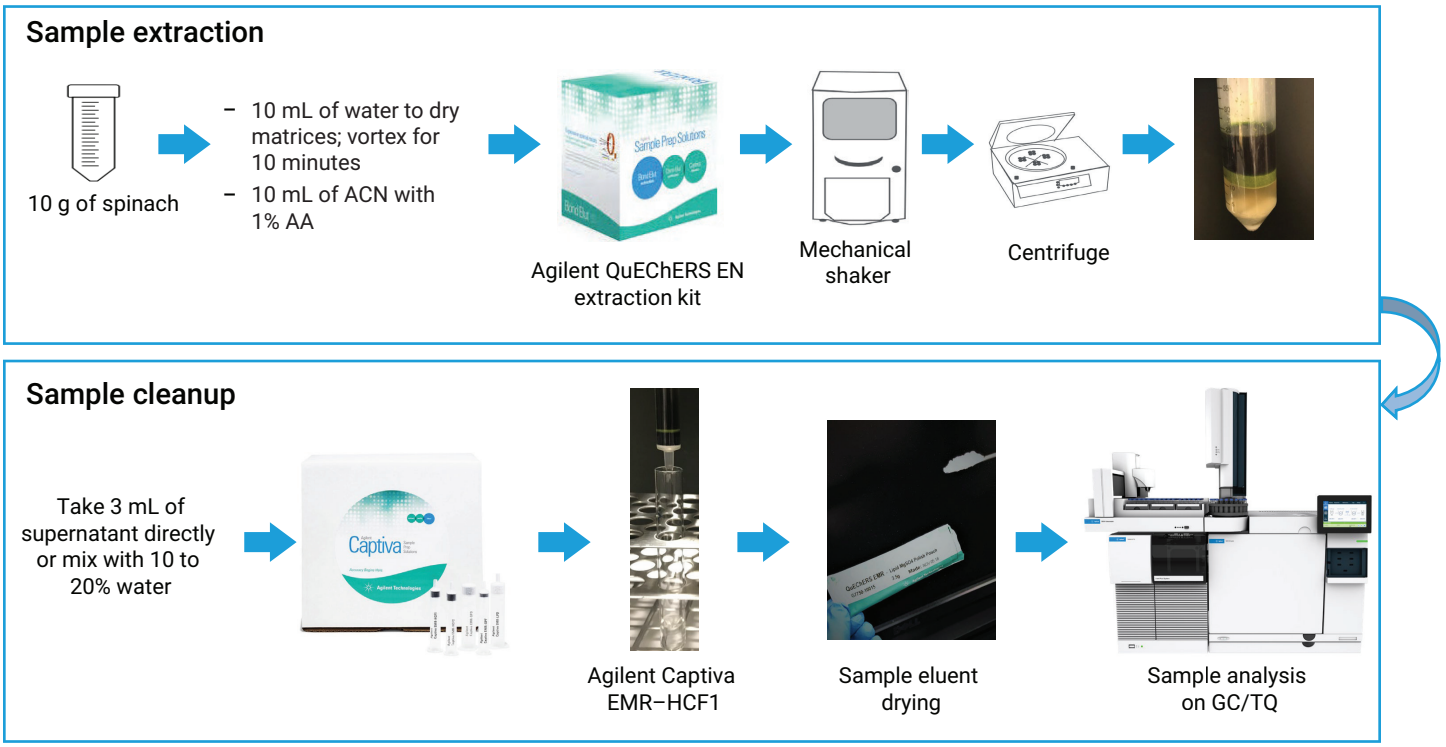

Figure 2. Sample preparation flowchart including traditional Agilent QuEChERS extraction, followed by Agilent Captiva EMR pass-through cleanup.

(10 g) was used for extraction. The 10 mL of acetonitrile (ACN) with 1% acetic acid was then added, followed by extraction. After extraction, 3 mL of crude extract was transferred to an Agilent Captiva EMR–HCF1 cartridge (part number 5610-2088) for pass-through cleanup. The Agilent positive pressure manifold 48 processor (PPM-48; part number 5191-4101) was used for Captiva EMR pass-through cleanup processing. The sample eluent was collected and further dried by anhydrous $MgSO_4$ (Agilent part number 5982-0102). Samples were then ready for GC/TQ analysis.

Analyte protectants

Analyte protectants (APs) were added to all the samples so that the stock solution of the APs comprised 10% of the injected sample volume. The stock solution of the APs consisted of 3-ethoxy-1,2-propanediol (ethylglycerol) at 10 mg/mL, D-sorbitol at 1 mg/mL, L-gulonolactone at 1 mg/mL dissolved in ACN with 1% acetic acid and 12% of water (v/v). This mixture was found to be the most promising AP combination as reported in the peer-reviewed literature.[17] The APs can be added via sandwich injection using the Agilent 7693A automatic liquid sampler as described in the previously published application notes.[18,19] When using the APs, it is recommended that one of the syringe wash solvents comprises of ACN/isopropanol mixture 1:1 (v/v) to prevent syringe plunger stickiness. The polytetrafluoroethylene (PTFE) tipped plunger syringes (10 µL) also helped in this respect (Agilent part number G4513-80220).

Matrix-matched calibration

Calibration performance was evaluated using a series of matrix-matched calibration standards ranging from 0.1 to 5,000 ppb (w/v), including 0.1, 0.5, 1, 5, 10, 50, 100, 250, 500, 1,000, and 5,000 ppb. The GC multiresidue pesticide kit (part number 32562, Restek, Bellefonte, PA, USA) containing 203 compounds, regulated by the FDA, USDA, and other global governmental agencies, was used for preparing matrix-matched calibration standards. The concentrations expressed in ppb (w/v) correspond to the pesticide concentration in the injected sample. The QuEChERS sample preparation procedure resulted in a dilution factor of 1. Hence, the reported concertation in ppb in the sample corresponds to µg/kg in the original commodity. The standard α-BHC-d6 (Agilent QuEChERS IS standard number 6, part number PPS-610-1) at a final concentration of 50 ppb in vial was used as the internal standard for quantitation of the target pesticides.

The developed method calibration performance was validated with both HydroInert and HES sources according to the analytical method validation and performance criteria outlined in SANTE 11312/2021.[12] A multilevel calibration that included up to 11 levels was used. An appropriate calibration function, either linear or quadratic, guided by the lower value of the relative standard error (RSE) was used. A weighting factor of 1/x allowed for maintaining accuracy across the entire calibration range. The deviation of the back-calculated concentrations of the calibration standards from the true concentrations, using the calibration curve in the relevant region, did not exceed ±20%.

Method detection limits

There are many alternative procedures to estimate the MDL. The approach used in this study was to perform eight injections of a matrix-matched calibration standard to assess the uncertainty in the measuring system.[20] This approach is recommended by The Official Journal of the European Communities, Commission Decision of 12 August 2002; Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results in the EU[21] and the EPA Guidelines Establishing Test Procedures for the Analysis of Pollutants in the US.[22] The concertation selected for the multiple injection trials was 1 ppb for most compounds. For compounds with higher limits of quantitation, eight trials were performed at the concentration of 5 ppb. The calculated MDLs were obtained by applying the formula shown in Equation 1.

$$MDL = s \cdot t(n - 1, 1 - alpha = 99) = s \cdot 2.998$$

Equation 1.

Where:

t (n – 1, 1 – alpha) = t value for the 99%, which is 2.998

Confidence level with n – 1 degrees of freedom

n = number of trials (8)

s = standard deviation of the eight trials.

The calculated MDL < spike level < 10x calculated MDL equation was used to evaluate the empirically determined MDL and ensure its validity.

Results and discussion

Increased chromatographic resolution while maintaining retention times with hydrogen

To assess the feasibility of analyzing pesticides using hydrogen carrier gas, a panel of 203 GC-amenable pesticides was evaluated in a pigmented spinach matrix. Increased chromatographic resolution was achieved when using the recommended minibore column configuration. The configuration was comprised of the two 20 m columns (0.18 mm × 0.18 µm) with hydrogen carrier gas, resulting in a 20-minute analysis compared to the conventional 20-minute analysis with helium carrier gas (Figure 3). It is noted that the oven program used with hydrogen was the same as with helium. The combination of method translation followed by retention time locking allowed for transferring the conventional 20-minute analysis with helium carrier gas to hydrogen carrier gas, while maintaining the relative elution order and precisely matching the retention times. The magnified part of the chromatogram shown in Figure 3 demonstrates the increased chromatographic resolution for cyfluthrins and cypermethrins.

The advantages provided by chromatographic resolution include reduced matrix interferences and minimized interference between coeluting analytes, therefore streamlining a complex pesticide residue analysis that often spans over several hundreds of targets.

The ability to precisely predict and match the retention times observed with helium resulted in great time savings and significantly simplified the transition from helium to hydrogen. This prediction provides an advantage in simplifying the conversion of the existing MRM methods from helium and allows for using the retention times from the databases created with helium, such as P&EP 4.

Proof of concept: fast 10-minute analysis with hydrogen

In addition to translating the method from helium to hydrogen, with a speed gain of 1 as presented in Figure 3, it was shown that a faster analysis can be performed with hydrogen. Previously, a fast 10-minute analysis has been demonstrated with helium as published elsewhere.[23] The chromatographic resolution with hydrogen and fast analysis was similar to that observed with the conventional 20-minute analysis with helium. The same minibore 10 m × 10 m (0.18 mm × 0.18 µm) HP-5ms UI column configuration as discussed in the application note on the fast analysis of pesticides with helium[23] was used with hydrogen.

The retention times observed with the 10-minute analysis using hydrogen and a 10 m × 10 m column configuration precisely matched the retention times observed with the 10-minute analysis with helium when using the 10 m × 10 m column configuration reported in the corresponding application note.[23] The method was precisely scaled from the conventional 20-minute analysis using the method translation tool, providing a speed gain of 2. New retention times (RT) were calculated using the following empirical equation: $RT_{new} = RT_{old}/2 + 0.09$ minutes. This formula is only applicable to the 10 m × 10 m method described in this application note.

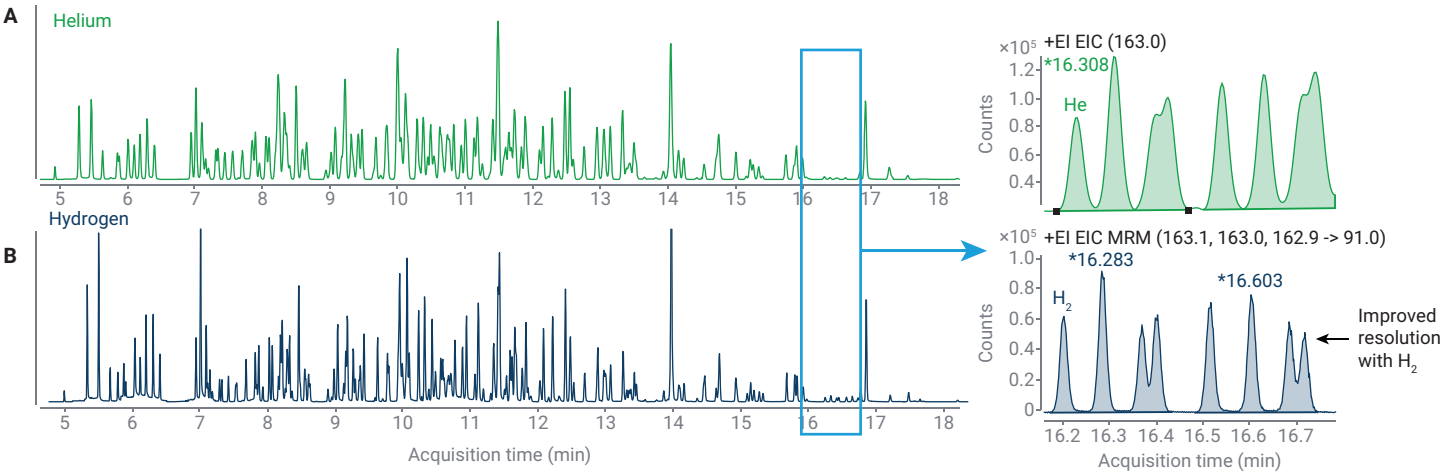

Figure 3. MRM chromatograms for a mixture of (A) 203 pesticides acquired with helium carrier gas with the conventional 20-minute method, (B) 203 pesticides acquired with hydrogen with the 20-minute method using a 20 m × 20 m minibore configuration.

Figure 4 shows an MRM chromatogram acquired for a subset panel consisting of 103 compounds out of 203. The resolution for cyfluthrins and cypermethrins was comparable to that observed with helium and the conventional 20-minute analysis (Figure 3A). Increased chromatographic resolution with hydrogen carrier gas resulted in narrower peaks. Thus, data rate needed to be increased with the fast hydrogen method resulting in shorter dwell times. A fast 10-minute analysis is recommended only when targeting panels of fewer than 200 compounds.

The best practices for pesticide analysis described elsewhere[11] unlocked high analysis ruggedness as demonstrated with 700 consecutive injection of spinach QuEChERS extract using the 10-minute analysis with helium as shown in application note 5991-4967EN.[23] As a result, no additional system maintenance, aside from liner and septum change every 100 injections was needed. The same best practices were implemented in this work ensuring the analysis ruggedness and robustness.

The 20-minute analysis with hydrogen carrier using the 20 m × 20 m (0.18 mm × 0.18 µm) column configuration was used in the rest of this work.

Optimized injection with hydrogen

The injection step is often considered among the most critical and vulnerable stage in the GC/MS analysis of pesticide residue, especially at trace levels. The multimode inlet (MMI) with the programmable temperature injection is commonly used to significantly reduce thermal degradation. It enables effective analyte transfer to the column through rapid temperature and flow programming.[16,24] The solvent vent mode used with the MMI resulted in the elimination of most of the injection solvent through the split vent at a low temperature, permitting the introduction of a larger injection volume. The solvent vent mode resulted in improved peak shapes of early eluting analytes when injecting 2 µL of ACN.

The optimized injection conditions are summarized in Table 1. Starting the injection at a lower temperature of 60 °C and ramping up to 280 °C allowed volatilization of all the target analytes while maintaining their chemical integrity upon introduction to the GC column. A high vent flow of 100 mL/min enabled solvent elimination resulting in improved peak shape, which could be distorted when injecting larger volumes of ACN. Also, in the postrun, the inlet was further heated to 310 °C while backflushing to bake out any matrix residue that may remain in the inlet. This increases maintenance-free operation of the system.

The use of APs provided GC system deactivation in each injection. This resulted in improved ruggedness, that is, long-term repeatability of analyte peak intensities, shapes, and retention times. Moreover, the use of APs helped with equalization of both the matrix-induced response enhancement and matrix-induced response diminishment effects.[16]

The combination of solvent vent injection with the injection volume of 2 µL, an Ultra Inert 2 mm dimpled liner (Agilent part number 5190-2297), and the use of APs resulted in high sensitivity even for challenging pesticides. For example, for tolclofos-methyl, the response was increased 22-fold when comparing the injection of 1 µL in cold splitless mode in solvent to a 2 µL injection in solvent vent mode in the QuEChERS extract with the APs. The average response increase over 203 compounds was 10.9-fold when comparing the optimized 2 µL injection in solvent vent mode in the QuEChERS extract with the APs using the 2 mm dimpled liner to the cold splitless injection with 1 µL injection volume.

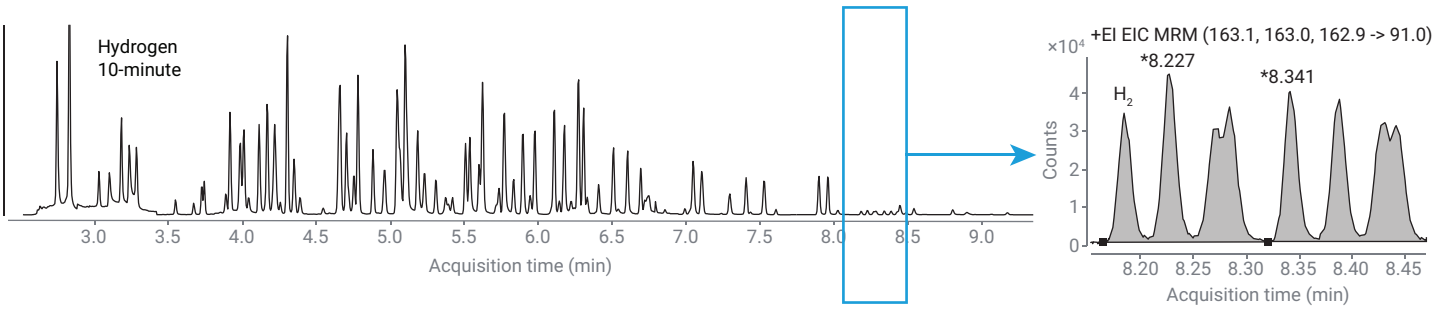

Figure 4. MRM chromatograms for a mixture of 103 pesticides acquired with hydrogen with the 10-minute method using a 10 m × 10 m minibore configuration.

EI source considerations with hydrogen: eliminating in-source reactions to preserve sensitivity and spectral fidelity

Hydrogen carrier gas is expected to provide advantages for chromatographic separation. However, hydrogen could present a challenge for detection when a mass spectrometer is used. Because hydrogen is not inert, it can react with compounds susceptible to hydrogen reduction in the EI source. If an EI source that does not eliminate source-induced reactivity is used, then chemical transformations will take place leading to:

- Spectral changes with hydrogen compared to helium
  - The existing spectral libraries cannot be used for compound identification
  - Previously developed acquisition methods, including SIM ions and MRM transitions, cannot be consistently used with hydrogen
- Undesirable and uncontrollable reactions
  - Quantitation accuracy and precision could be compromised if in-source reactions occur
  - Calibration linearity is affected
- A need to verify each compound for potential reactivity with hydrogen

When using GC/TQ in the MRM data acquisition mode, minimizing or eliminating the undesirable in-source reaction is important because the ions that are diminished in the spectrum with hydrogen and the ions where abundance increases should not be used as precursor ions in the MRM transitions. The reduced ions will lead to substantially sacrificed sensitivity. The newly formed ions are products of uncontrolled chemical reactions occurring in the source, whose rate may depend upon concentration. Therefore, such ions should not be used for quantitation. This means that the MRMs developed with helium and those available in the databases cannot be used for those compounds that react with hydrogen. Finding suitable precursors for compounds reacting with hydrogen in the source can be extremely challenging because of the unpredictable and uncontrollable nature of the in-source reactions.

For this reason, using the EI sources with reduced or eliminated source reactivity such as HydroInert and HES is essential to minimize or prevent the undesirable in-source reactions when using hydrogen.

It is commonly known and expected that hydrogen carrier gas often reduces sensitivity 2 to 5-fold of standard EI sources.[25] The reduced sensitivity can be a combination of a decreased signal and increased noise and is anticipated even for the compounds that do not interact with hydrogen in the EI source.

For example, chlorpyrifos-methyl does not undergo pronounced reaction with hydrogen in the EI source as evidenced by its mass spectrum unchanged with hydrogen carrier gas. Figures 5A and B show the mass spectra acquired for chlorpyrifos-methyl with helium and hydrogen using the standard Inert Plus Extractor EI source, equipped with the 3 mm extractor lens. In both cases, the spectra largely resemble the library spectrum shown in the mirror plot resulting in good library match scores. Figure 5C shows the quantifying and qualifying MRM transitions for chlorpyrifos-methyl acquired with helium (on the top) and with hydrogen. With the 7000E GC/TQ, chlorpyrifos-methyl can be reliably detected at 5 ppb in spinach extract with hydrogen carrier gas using either the conventional or the HydroInert EI sources. The observed sensitivity in terms of signal-to-noise ratio is comparable to that observed with helium, although slightly decreased. The detection limits with the 7010C GC/TQ were lower than with the 7000E, enabling the detection of chlorpyrifos-methyl at 0.5 ppb with both helium and hydrogen. With every MS EI source tested, a decrease in signal-to-noise ratio was observed with hydrogen near the limit of detection and was less pronounced at higher concentrations. With the HES, the slight decrease in sensitivity towards chlorpyrifos-methyl was noted at 0.5 ppb (Figure 5C). Similar performance was observed for other compounds, whose spectra with hydrogen looked like the spectra with helium.

In summary, the compounds that did not react with hydrogen in the EI source, could be detected with hydrogen. A decrease in signal-to-noise ratio at the low levels, close to the detection limits, was 2 to 5-fold with hydrogen when compared to helium. The 7010 GC/TQ equipped with the HES was more sensitive than the 7000E GC/TQ.

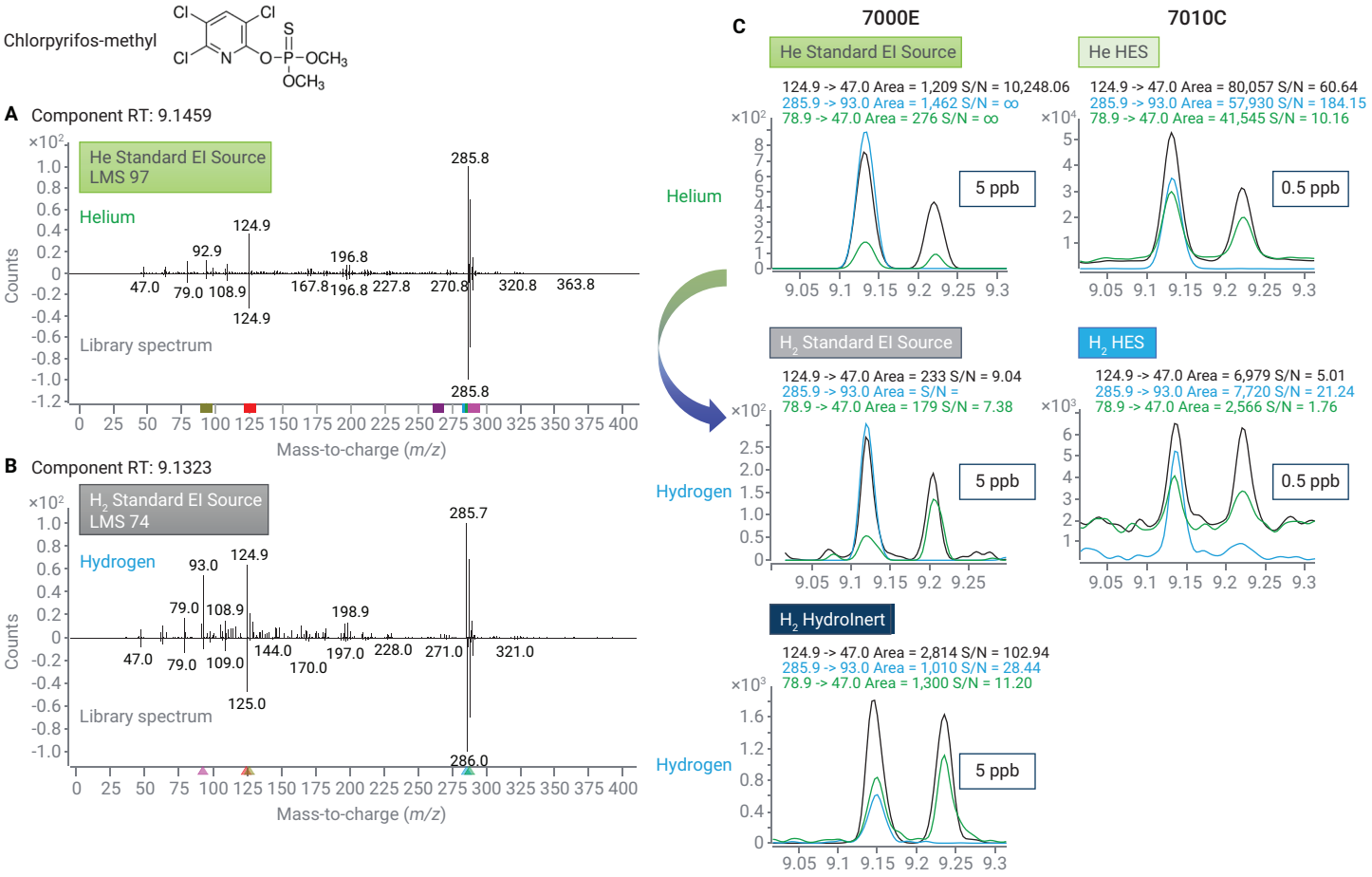

Figure 5. Mass spectra and MRM chromatograms for chlorpyrifos-methyl acquired with helium and hydrogen using the standard EI source, the HydroInert, and the HES.

Unlike chlorpyrifos-methyl, quantitation of compounds susceptible to reacting with hydrogen carrier gas is hindered with a traditional EI source. For example, tecnazene undergoes hydrogenation in a traditional EI source as evidenced by the changed ion ratios of 261 *m/z*, 231 *m/z*, 215 *m/z*, 203 *m/z*, 161 *m/z* (Figure 6B compared to Figure 6A) and the low library match score of 59. Nitro compounds are known to be susceptible to hydrogenation when in the presence of heat, hydrogen, and metal surfaces, and all these factors are present in the standard EI source. There is a large abundance of 231 *m/z* and low 261 *m/z*, indicating conversion of tecnazene to tetrachloroaniline in the source. This conversion is confirmed to occur in the source because the mass spectrum is observed at the retention time of tecnazene, which is well separated from tetrachloroaniline. More information on the in-source conversions is provided in the technical overview of HydroInert source.[26] The resulting diminishment of 261 *m/z*, 259 *m/z*, and 215 *m/z* results in the 100-fold loss of sensitivity when using the standard EI source with hydrogen carrier gas compared to helium. Figure 6E shows that 50 ppb was the lowest concentration at which tecnazene could be detected with hydrogen if using the standard EI source. Substantial sensitivity reduction makes it impossible to analyze tecnazene at the default MRL of 10 ppb with the standard EI source.

Unlike the standard EI source, HydroInert, and HES sources reduce or eliminate source reactivity, hence, minimizing or preventing the undesirable in-source reactions with hydrogen. This is evidenced by the excellent matching of the spectra observed with hydrogen using HydroInert (Figure 6C) and HES (Figure 6D) and the library spectrum acquired with helium resulting in the high library match scores of 94 and 93, respectively. The ability to preserve the intact mass spectrum allowed for using the same MRM transitions as with helium. The sensitivity with hydrogen was sufficient to detect tecnazene at 0.5 ppb in spinach QuEChERS extract with HydroInert and 0.1 ppb with HES (Figure 6E).

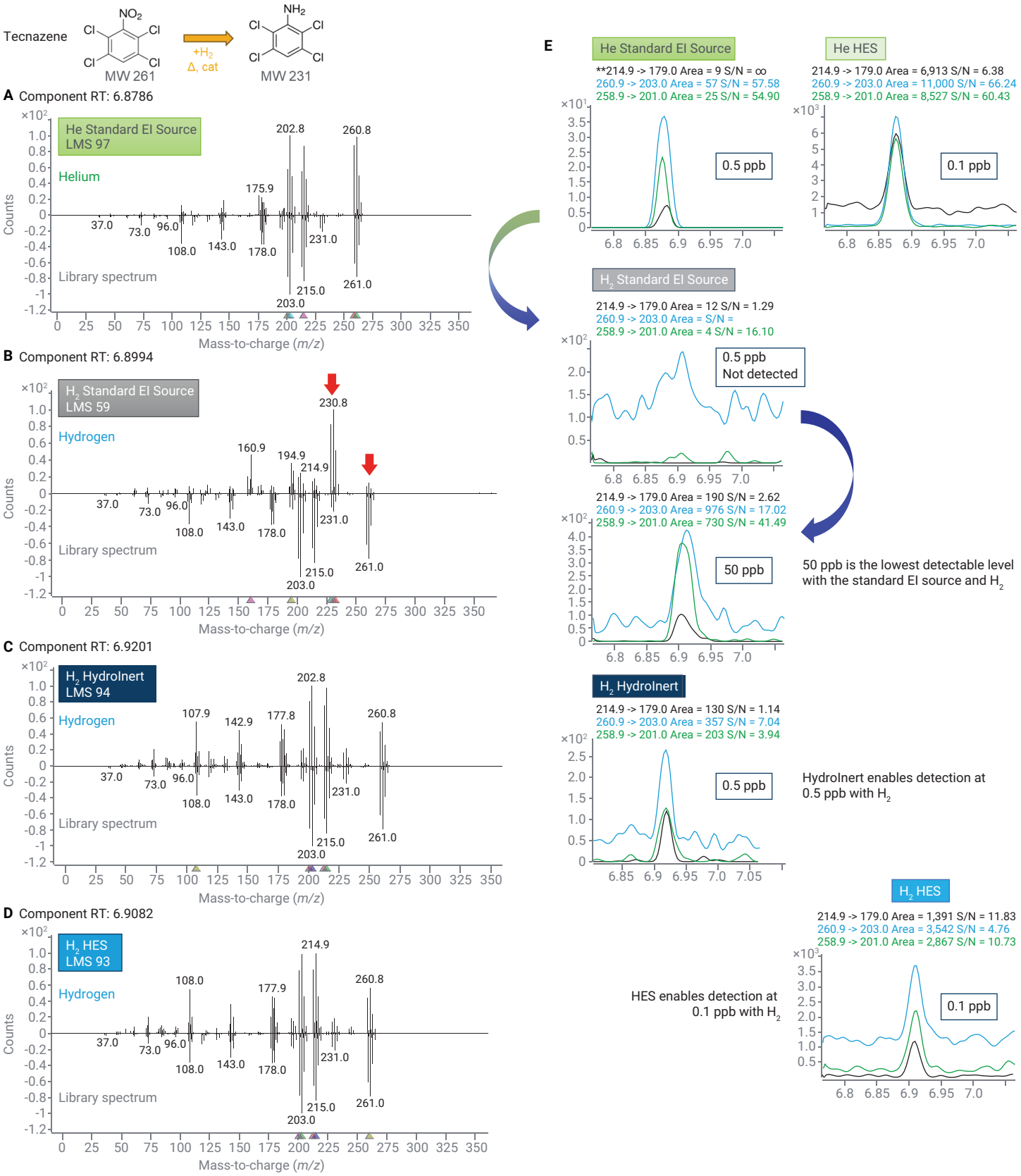

Figure 6. Mass spectra and MRM chromatograms for tecnazene acquired with helium and hydrogen using the standard EI source, the HydroInert, and the HES.

Various EI source configurations were evaluated in this study. It was found that the optional larger diameter extractor lenses (6 and 9 mm) in the standard Inert Plus Extractor EI source did not provide benefits as pronounced as HydroInert source. Among the portfolio of lenses available for HydroInert, the default 9 mm lens was shown to provide the best performance with hydrogen carrier gas in terms of spectral fidelity and sensitivity when compared to the 3 and 6 mm HydroInert lenses. For HES, no source modification was needed.

Both HydroInert and HES demonstrated the capability for successful pesticide analysis by GC/TQ, largely due to prevention of in-source reactions when hydrogen carrier gas is used. The HydroInert source was specifically developed to work with hydrogen carrier gas as it is manufactured from a material more inert than the standard EI source. HydroInert is available with the 7000E GC/TQ and can also be purchased as a replacement source for the 7000C, D, and E GC/TQs. HydroInert should not be used with helium carrier gas as discussed in the technical overview Agilent Inert Plus GC/MS System with HydroInert Source.[26] The HES source was found to minimize the in-source reactions similarly to HydroInert. However, unlike the Inert Plus extractor source design, the standard HES can be used in the GC/TQ with hydrogen providing the inert benefits, maintaining the spectrum fidelity, and enabling best sensitivity with hydrogen carrier gas.

The compounds that, like tecnazene, undergo chemical reaction with hydrogen when a traditional EI source is used could be easily identified by the compromised spectral fidelity expressed in the low library match scores. Fifteen compounds, for which spectra were noticeably distorted with hydrogen and the standard source, are listed in Table 2. These compounds feature diverse functional groups that can undergo hydrogenation, dehydrohalogenation, dehalogenation, double bond reduction, and other undesirable in-source reactions. The library match scores for these compounds were substantially lower with the standard source using hydrogen carrier when compared to helium. This is reflected with yellow shading in Table 2. The spectra for these compounds were restored when using HydroInert and HES sources. The restored spectra allowed for, first, using the MRM transitions developed with helium, and second, preserving sensitivity so that its decrease did not exceed 2 to 5-fold at the levels close to the detection limits. It is of note that like the compounds that did not react with hydrogen, sensitivity decrease for the compounds that undergo hydrogen reduction was most pronounced at low concentration, close to the detection limits. Appendix Figure 1 shows the comparison of the MRM chromatograms for the compounds susceptible to the in-source reactions acquired with helium and hydrogen at their detection limits. The evaluated sources shown in the Appendix Figure 1 are the standard Inert Plus Extractor EI source with a 3 mm lens, HydroInert, and HES with hydrogen, and the standard EI and HES with helium. Appendix Figure 1 provides a comprehensive comparison revealing:

- Substantial sensitivity losses with the standard EI source and hydrogen for the compounds susceptible to reacting with hydrogen
- Sensitivity recovered with HydroInert and HES using hydrogen when compared to the standard EI source
- Sensitivity comparison when transitioning from the standard EI source and helium to HydroInert with hydrogen or from HES with helium to HES with hydrogen
- Comparison of sensitivity between HydroInert and HES with hydrogen

The advantage of preserving the mass spectrum with hydrogen observed with HydroInert and HES resulted in the MDL levels below 1 ppb for the majority of the compounds most susceptible to reacting with hydrogen. The MDLs for those compounds observed with 7000E GC/TQ equipped with HydroInert and the 7010C equipped with HES are provided in Table 2. The MDL measurements were performed using a 1 ppb (w/v) matrix-matched standard for all compounds, except for prothiofos and profenofos, for which a 5 ppb (w/v) matrix-matched standard was used. Using HES enables lower MDLs than HydroInert with hydrogen. Higher sensitivity observed with HES is also demonstrated in Appendix Figure 1, where lower concentrations, often as low as 0.1 ppb, could be detected in spinach extract with HES even for the compounds most susceptible to reacting with hydrogen.

Table 2. Library match scores (LMS) observed for the pesticides most susceptible to reacting with hydrogen observed with helium and hydrogen carrier gasses with GC/TQ operating in scan data acquisition mode. Method detection limits (MDLs) observed with hydrogen using HydroInert and HES in dMRM mode.

Compound	Retention Time (min)	Library Match Scores in Scan MS1					Method Detection Limits (ppb)	
		Helium Carrier Gas		Hydrogen Carrier Gas			Hydrogen Carrier Gas	
		Agilent 7000E, Standard EI Source	Agilent 7010C, HES	Agilent 7000E, Standard EI Source	Agilent 7000E, HydroInert	Agilent 7010C, HES	Agilent 7000E, HydroInert	Agilent 7010C, HES
Tecnazene	6.915	82	84	59	94	93	0.49	0.24
BHC-alpha (benzene hexachloride)	7.623	98	98	81	93	96	0.69	0.20
Dichloran	7.783	89	93	67	90	89	1.00	0.31
BHC-beta	8.019	97	97	77	92	96	0.68	0.24
BHC-gamma (Lindane, gamma HCH)	8.133	80	82	73	69*	91	0.95	0.19
Pentachloronitrobenzene	8.212	91	93	67	91	95	0.31	0.38
BHC-delta	8.502	90	94	74	87	94	0.74	0.31
Heptachlor	9.328	91	88	74	87	93	0.74	0.29
Malathion	9.742	90	90	56	84	76	0.65	0.44
Bromophos-ethyl	11.037	93	90	62	87	92	0.63	0.26
Prothiofos	11.510	95	94	65	92	91	2.52	1.02
Profenofos	11.561	91	87	66	90	85	3.48	2.27
Sulprofos	12.666	98	88	61	87	91	0.87	0.39
Tebuconazole	13.292	93	92	66	89	76	0.58	0.30
Piperonyl butoxide	13.402	92	94	68	92	79	0.84	0.59

* Complete coelution of lindane with terbufos (<1 scan apart) resulted in a lower LMS.

Calibration performance

The developed method calibration performance was validated with both HydroInert and HES sources in accordance the analytical method validation and performance criteria outlined in SANTE 11312/2021.[12] The multilevel calibration was used so that the deviation of the back-calculated concentrations of the calibration standards from the true concentrations using the calibration curve in the relevant region did not exceed ±20%.

It has been demonstrated in literature[27,28] that the correlation coefficient R^2 by itself can be an inconsistent measure of the calibration accuracy. Instead, the residual error at each calibration point can be characterized using percent relative standard error (%RSE) defined as shown in Equation 2:

$$\%RSE = 100 \times \sqrt{\sum_{i=1}^{n} \frac{\left[\frac{x'_i - x_i}{x_i}\right]^2}{n - p}}$$

Equation 2.

Where x_i is the true concentration of each calibration standard

x'_i is the measured concentration of each calibration standard

$\frac{x'_i - x_i}{x_i}$ is the relative error in calculated concentration for each calibration point

n is the number of calibration points used in the curve

(n – p) is the degree of freedom

p is determined by the type of the curve. For linear equations, p = 2 and for quadratic, p = 3.

For 203 evaluated compounds, the calibration RSE values were ≤20 for 190 and 194 compounds corresponding to 94 and 96% of the evaluated compounds with HydroInert and HES, respectively. The accuracy of the back-calculated concentrations of the calibration standards along with the RSE value guided the choice of linear versus quadratic curve fit. For example, tecnazene, the compound that could be severely affected by the in-source reaction with the standard EI source was accurately quantitated over the extended calibration range from 0.5 to 5,000 ppb in spinach QuEChERS extract with a linear calibration fit and the RSE value of 12.8 using the 7000E GC/TQ equipped with HydroInert (Figure 7A). The use of the 7010C GC/TQ with HES resulted in higher sensitivity at lower concentrations, enabling quantitation from 0.1 to 1,000 ppb with a quadratic fit and RSE of 14.4 (Figure 7B) or alternatively from 0.1 to 250 ppb with a linear fit and RSE of 16.6. The calibration ranges reported in this work (Appendix Tables 1 and 2) were selected to cover the widest concentration range because the MRLs may vary over a broad concentration range depending on different pesticides and food commodities. Encompassing a broader calibration range minimizes the need to reinject the samples if the MRLs of the target compounds vary several-fold. If the linearity of calibration is a priority, a narrower concentration range can be considered as discussed with tecnazene.

Less than 5% of the evaluated pesticides, eleven and nine compounds, were found to be problematic to quantitate using hydrogen carrier gas with HydroInert and HES, respectively. Those compounds are marked as not applicable (N/A) in Appendix Tables 1 and 2. Among those compounds were chlorothalonil, dichlofluanid, tolylfluanid, allethrin, captan, folpet, captafol, fenamiphos, iprodione, triflumizole, acequinocyl, and fluvalinate-tau I. Quantitation was not possible either due to insufficient signal or matrix interferences. Additional method optimization, including sample preparation aimed at removing the coeluting matrix interferences would be needed for quantitating these compounds in spinach matrix with hydrogen carrier gas. Other application notes provide the conditions suitable for quantitating these pesticides with either GC/MS/MS using helium carrier gas[11] or LC/MS/MS.[29]

The calibration performance for the evaluated compounds with the 7000E and the 7010C GC/TQ is summarized in Figure 8. The details are provided in Appendix Tables 1 and 2, including the calibration ranges, the calibration function type, the correlation coefficients, and the RSE values. Over 92% of the compounds could be quantitated at or below 10 ppb, which corresponded to the default MRL. This makes the developed method suitable for analyzing the evaluated pesticides at the MRL levels in the pigmented spinach matrix.

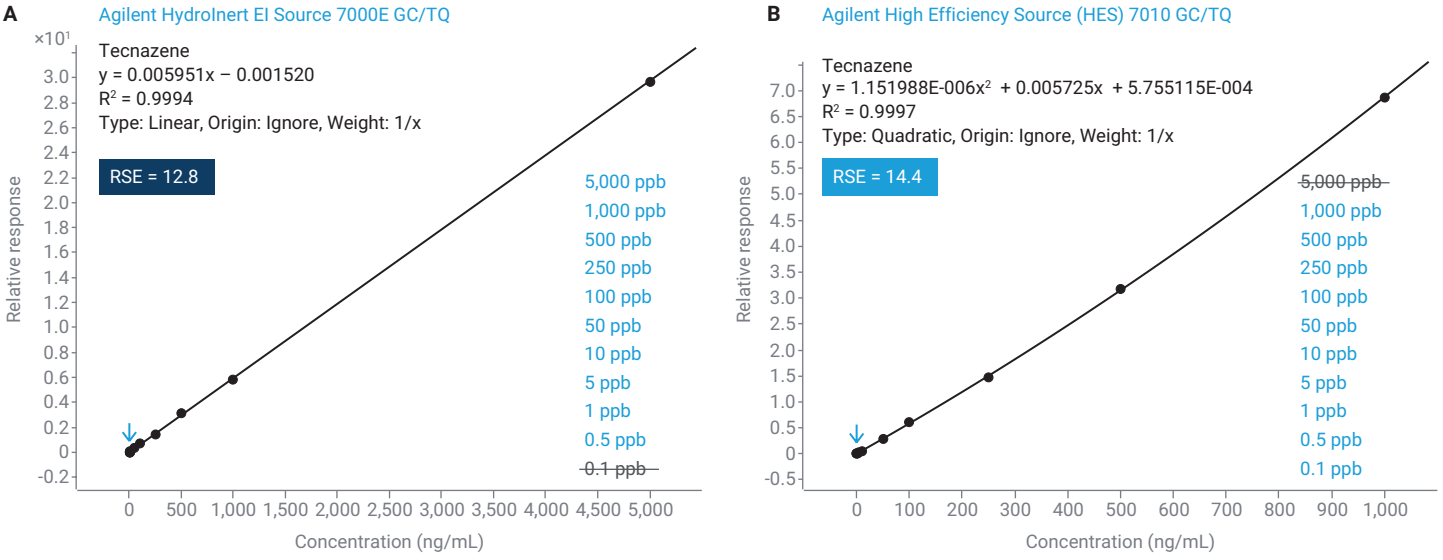

Figure 7. Matrix-matched calibration curves for tecnazene in spinach with the Agilent 7000E and Agilent 7010C GC/TQ with hydrogen carrier gas.

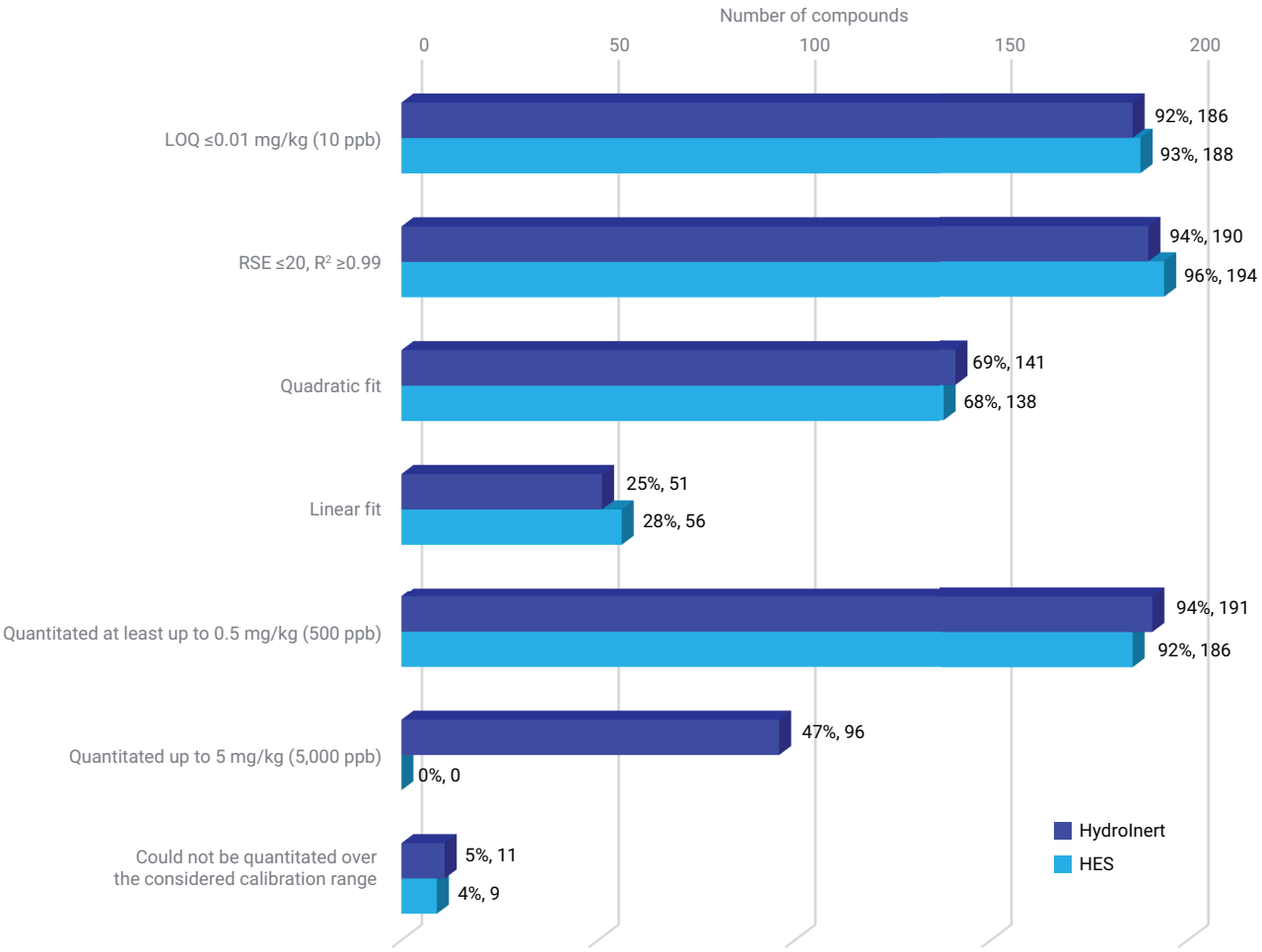

Figure 8. Calibration performance summary for 203 GC-amenable pesticides with the Agilent 8890/7000E and 8890/7010C GC/TQ in spinach using hydrogen carrier gas.

Effect of matrix-derived interferences and in-source loading

Evaluating samples in full scan data acquisition mode facilitates the evaluation of in-source matrix loading. This practice is among five keys to unlocking maximum performance in the analysis of pesticides described in the corresponding application note.[11] Either with helium or hydrogen, every MS source has a limitation on the amount of material present in the source, at any point of time, to maintain the optimal performance. Quantitation accuracy of the analysis can be significantly compromised if the source is overloaded with matrix. Hence, it is essential to analyze matrix in full scan mode to evaluate the total ion chromatogram (TIC) and maintain the optimal GC/TQ performance. The recommendation is to ensure that for the regions where targets elute, the maximum abundance of the base peak chromatogram (BPC) does not exceed 7×10^7 counts when acquiring data in full scan data acquisition mode with gain set to 1. Figure 9 demonstrates the comparison of the spinach and cayenne pepper QuEChERS extracts. The cayenne pepper sample features a higher matrix background compared to spinach, especially eluting between 11 and 14 minutes.

Figure 9B provides the example of quantitating two pesticides, tecnazene and flutolanil in spinach and cayenne pepper extract at 0.5 ppb with the 7010C GC/TQ. Tecnazene, although prone to reacting with hydrogen, had a stable measurable response at 0.5 ppb in the extract. It eluted at 6.91 minutes, with some matrix components coeluting. Flutolanil eluted at 11.42 minutes, during the part of the chromatogram when a lot of background derived from the

matrix was observed in the cayenne pepper extract. As a result, two out of the three ions had detectable interferences in the cayenne pepper extract for flutolanil at 0.5 ppb even in the selective MRM data acquisition mode. Some of the practices that can be used to lower the matrix background include adequate sample cleanup, sample dilution, and smaller injection volume. The latter two approaches often result in better limit of quantitation (LOQs), especially with the HES equipped 7010C GC/TQ system.

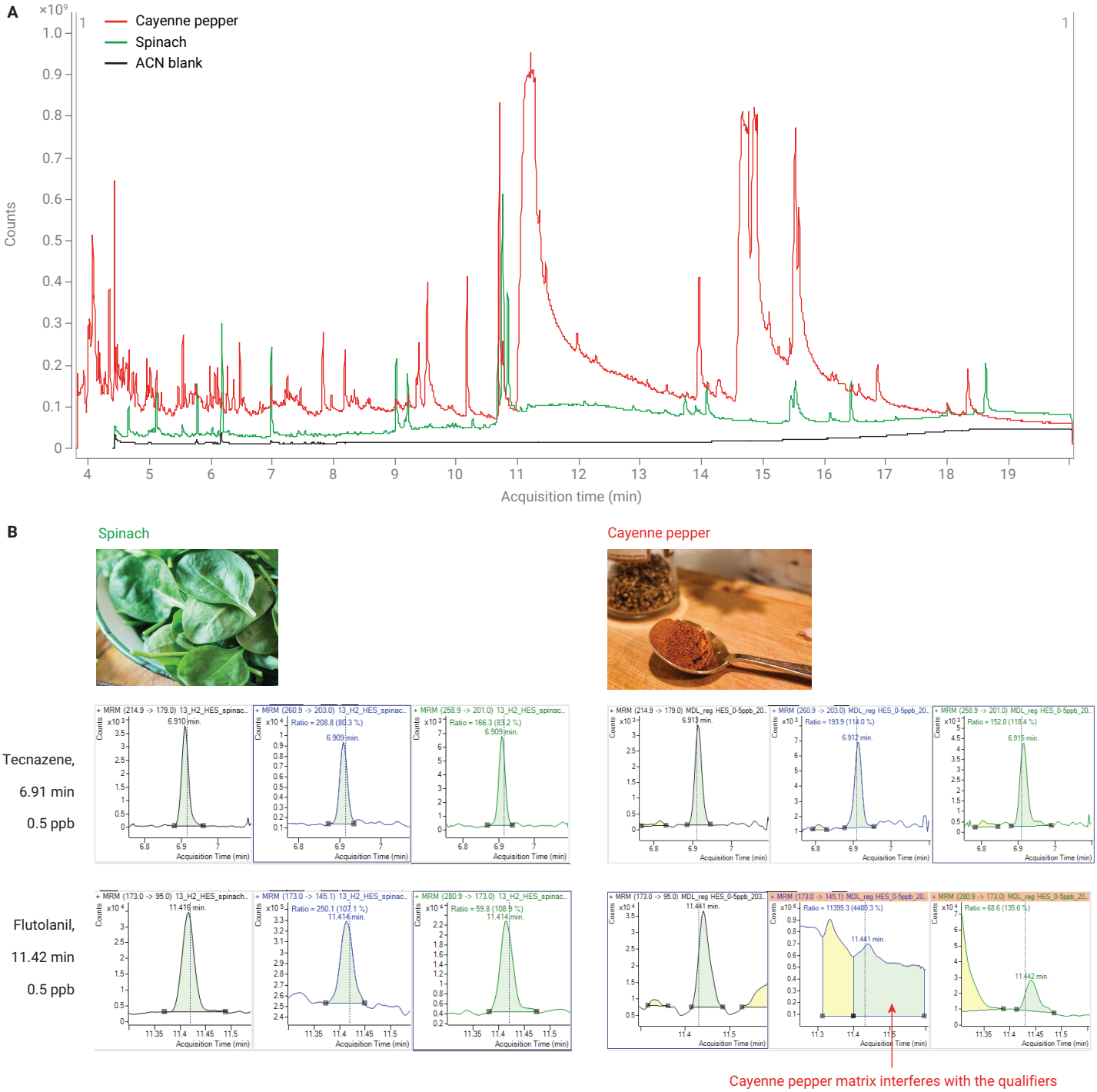

Figure 9. Scan total ion chromatogram (TIC) of the spinach and cayenne pepper QuEChERS extracts, and acetonitrile blank (A). MRM chromatograms for tecnazene and flutolanil in spinach and cayenne pepper extracts at 0.5 ppb acquired with the Agilent 7010C GC/TQ with hydrogen carrier gas (B).

Dynamic MRM/Scan mode: sensitive quantitation with more confidence

The simultaneous dMRM/scan capability available with the 7000E and the 7010C GC/TQs enables identification of the unknown compounds and retrospective analysis, while maintaining sensitivity and dynamic range of the method comparable to a conventional dMRM analysis as described in the application note 5994-4966EN.[30] Full scan data unlocks the opportunity to perform compound screening via spectral deconvolution and component search against GC/MS spectral libraries such as NIST. This functionality is valuable for retrospective analysis, eliminating the need to reanalyze the sample.

The benefit of preserving spectral fidelity provided with HydroInert and HES allowed for identifying the compound based on the spectral match and confirming its identity. Figure 10A illustrates the screening results for spinach extract spiked with a pesticide mixture at 500 ppb with the 7000E GC/TQ equipped with HydroInert using hydrogen carrier gas. The compounds susceptible to reduction with hydrogen presented in Table 2 were among the hits identified in the sample shown in Figure 10A, including prothiofos (LMS 83), sulprofos (LMS 80), tebuconazole (LMS 83), and tecnazene (LMS 82), as shown in the components table. The LMS for tecnazene was 82 and the delta between the observed retention time and the retention time provided in the spectral library was –0.016 minutes. The lower right of Figure 10A shows the spectral information displayed in MassHunter Unknowns Analysis for the hit. The raw mass spectrum appears on the lower right and a mirror plot compares the deconvoluted mass spectrum to the library spectrum. The ratio between 261 *m/z*, 215 *m/z*, and 203 *m/z* in the observed spectrum is similar to how these ions appear in the reference library spectrum confirming that tecnazene does not undergo chemical transformation in the HydroInert EI source with the 7000E GC/TQ.

Figure 10B shows the deconvoluted mass spectrum of tecnazene acquired in the dMRM/scan mode with the 7010C GC/TQ. As with the 7000E and HydroInert, tecnazene's spectrum was preserved intact resulting in a high LMS of 92.

The advantage brought by the simultaneous dMRM/scan functionality is the ability to quantitate the targets within the same run with the screening. Figures 10C and 10D demonstrate the MRM chromatograms for tecnazene at 10 ppb acquired with the 7000E and the 7010C in spinach extract when operating in the simultaneous dMRM/scan using hydrogen carrier gas. In both cases, accurate quantitation resulting in the calculated concentrations of 9.20 and 10.03 ppb was achieved.

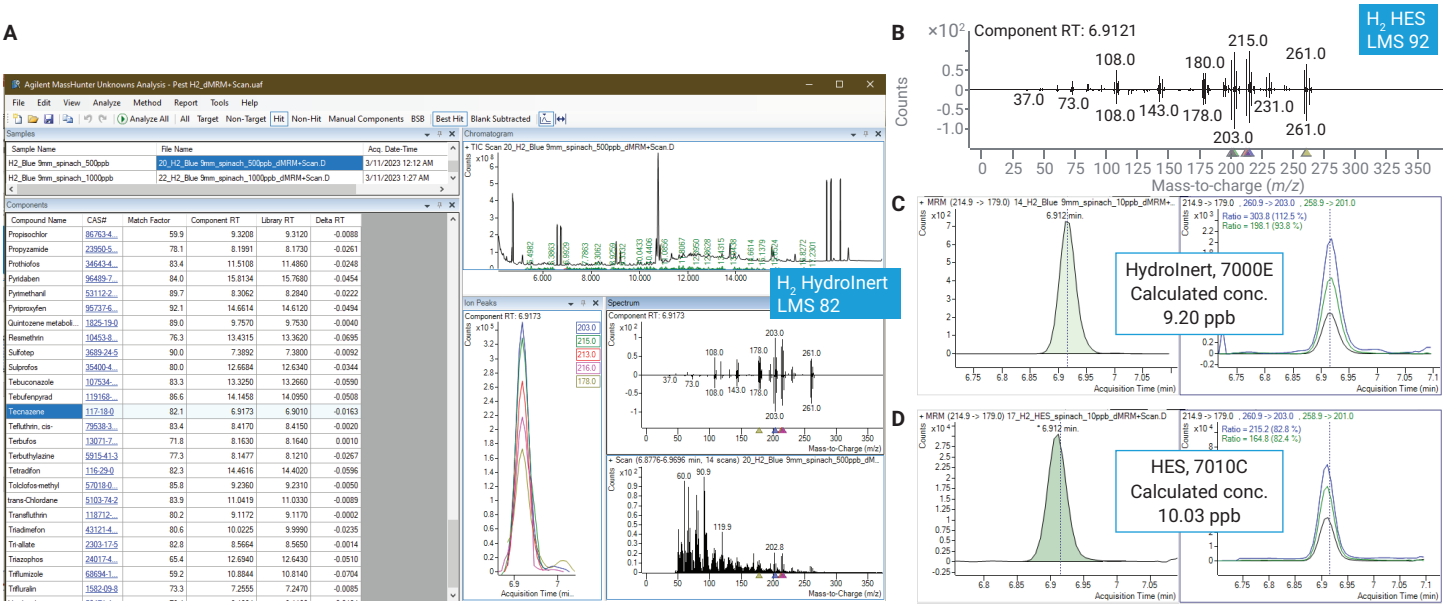

Figure 10. Analysis in simultaneous dMRM/Scan: tecnazene at 500 ppb in the spinach QuEChERS extract analyzed with the HydroInert source (A) and the HES source (B); MRM chromatograms at 10 ppb with the HydroInert (C) and the HES (D).

Conclusion

This application note presents key strategies for pesticide analysis using GC/MS/MS with hydrogen as the carrier gas, while maintaining sensitivity to meet MRLs. The optimized method includes a minibore 20 m × 20 m (0.18 mm × 0.18 µm) column configuration, solvent vent injection mode with the 2 mm dimpled liner, addition of the analyte protectant, and the use of hydrogen compatible electron ionization sources, namely the Agilent HydroInert source and the Agilent High Efficiency Source (HES). The optimized setup with hydrogen showed improved chromatographic resolution and allowed for precisely matching the retention times with helium. The HydroInert and HES sources were shown to provide best sensitivity and preserve spectral fidelity even for the compounds highly prone to reacting with hydrogen in the source by minimizing or preventing such undesirable reactions. As a result, the same MRM transitions, with the same collision energies for the targets eluting at the same retention times as with helium could be used with hydrogen carrier gas, streamlining the transition from helium to hydrogen.

The presented method allowed for quantitation of 92% and 93% of target pesticides at or below 10 ppb in spinach with hydrogen when using the Agilent 8890/7000E and the 8890/7010C GC/TQ systems, respectively. These results were compared to quantitation of 98.5% with helium when using the Agilent 8890/7000E GC/TQ system. The remaining compounds could be successfully analyzed with LC/MS/MS. Sub-ppb level detection limits were achieved, with higher sensitivity using the HES. The method demonstrated accurate quantitation over a broad calibration range with both the 7000E and the 7010C GC/TQ systems. Finally, simultaneous dynamic MRM and full scan data acquisition mode was demonstrated for accurate quantitation and reliable compound identification based on spectral matching.

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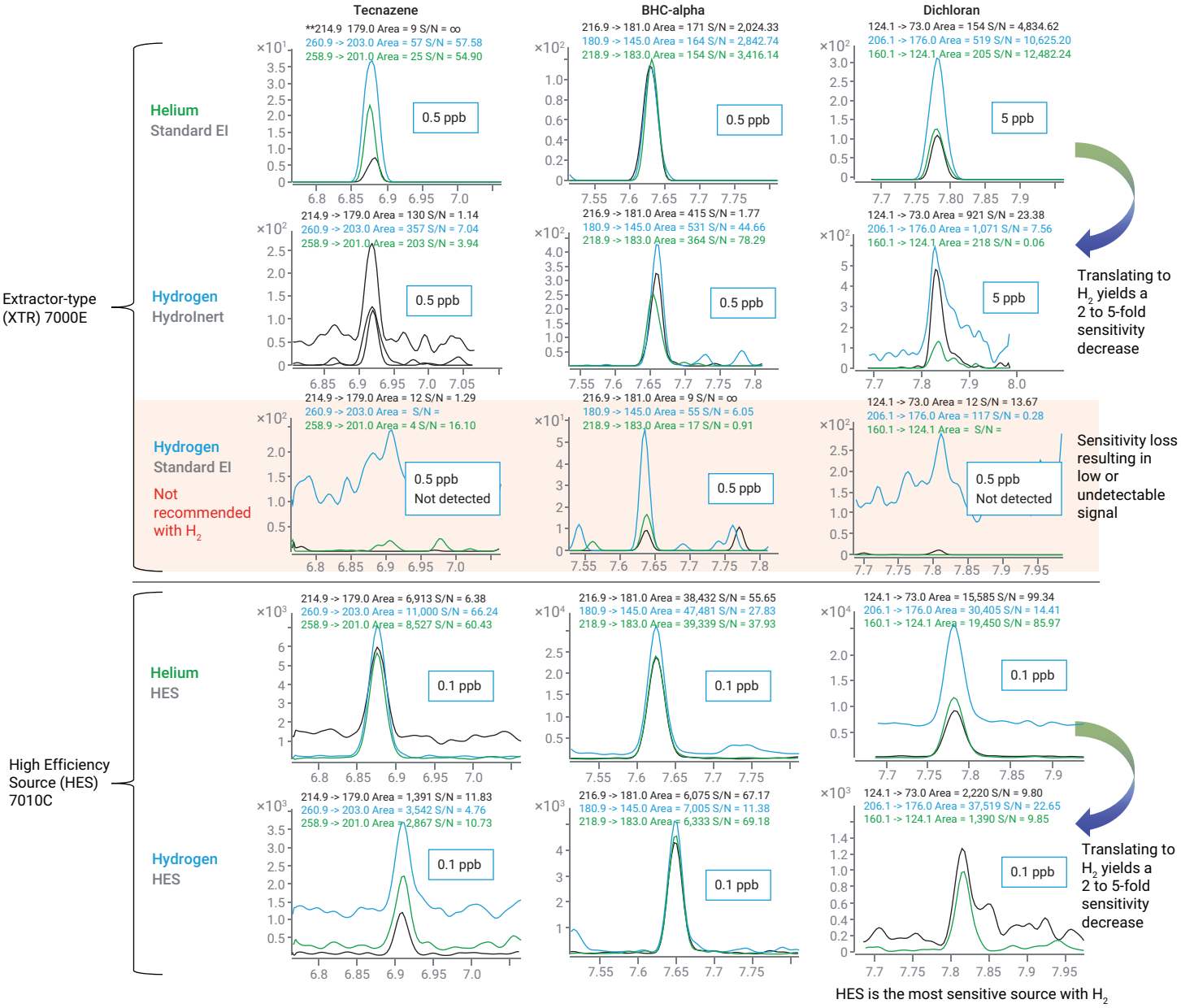
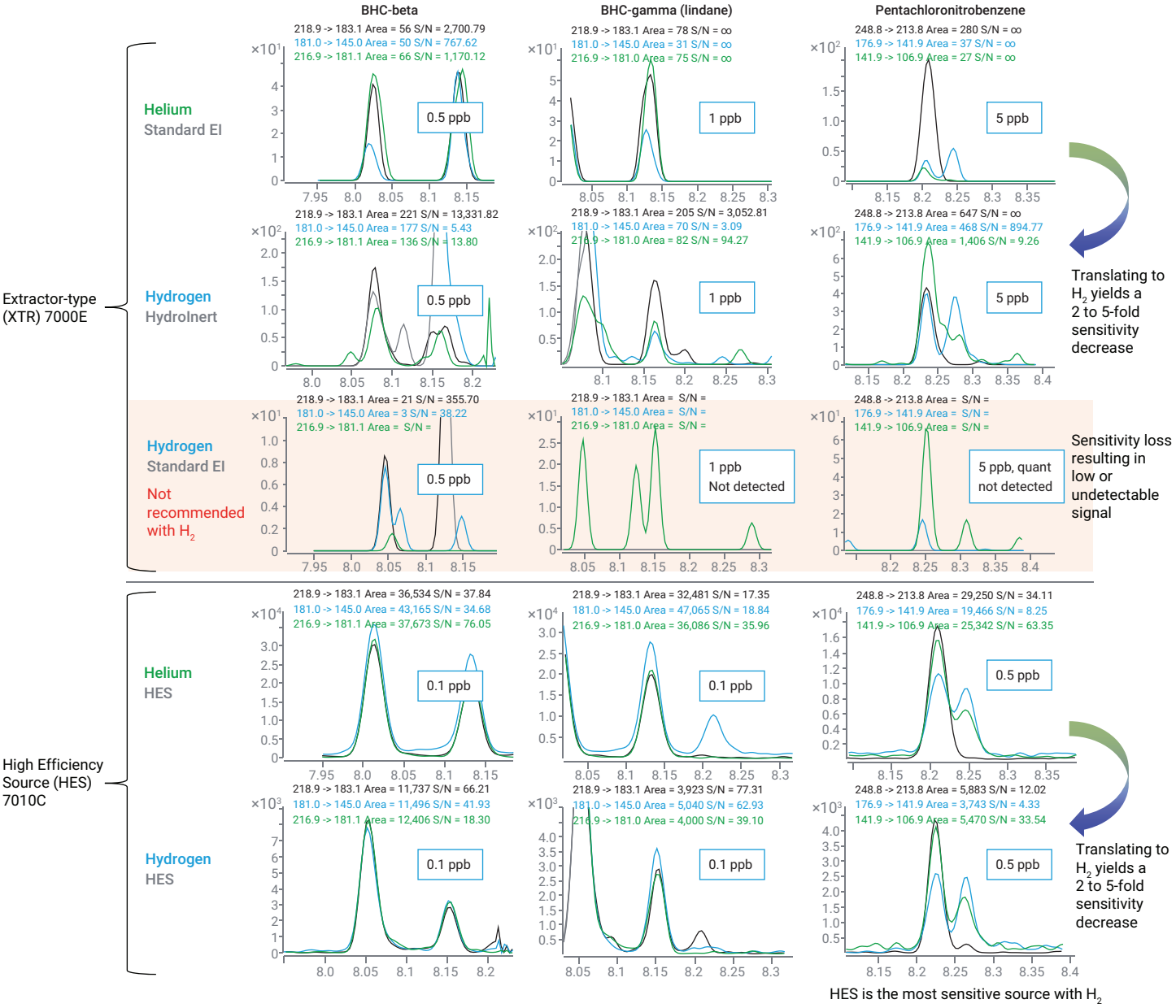
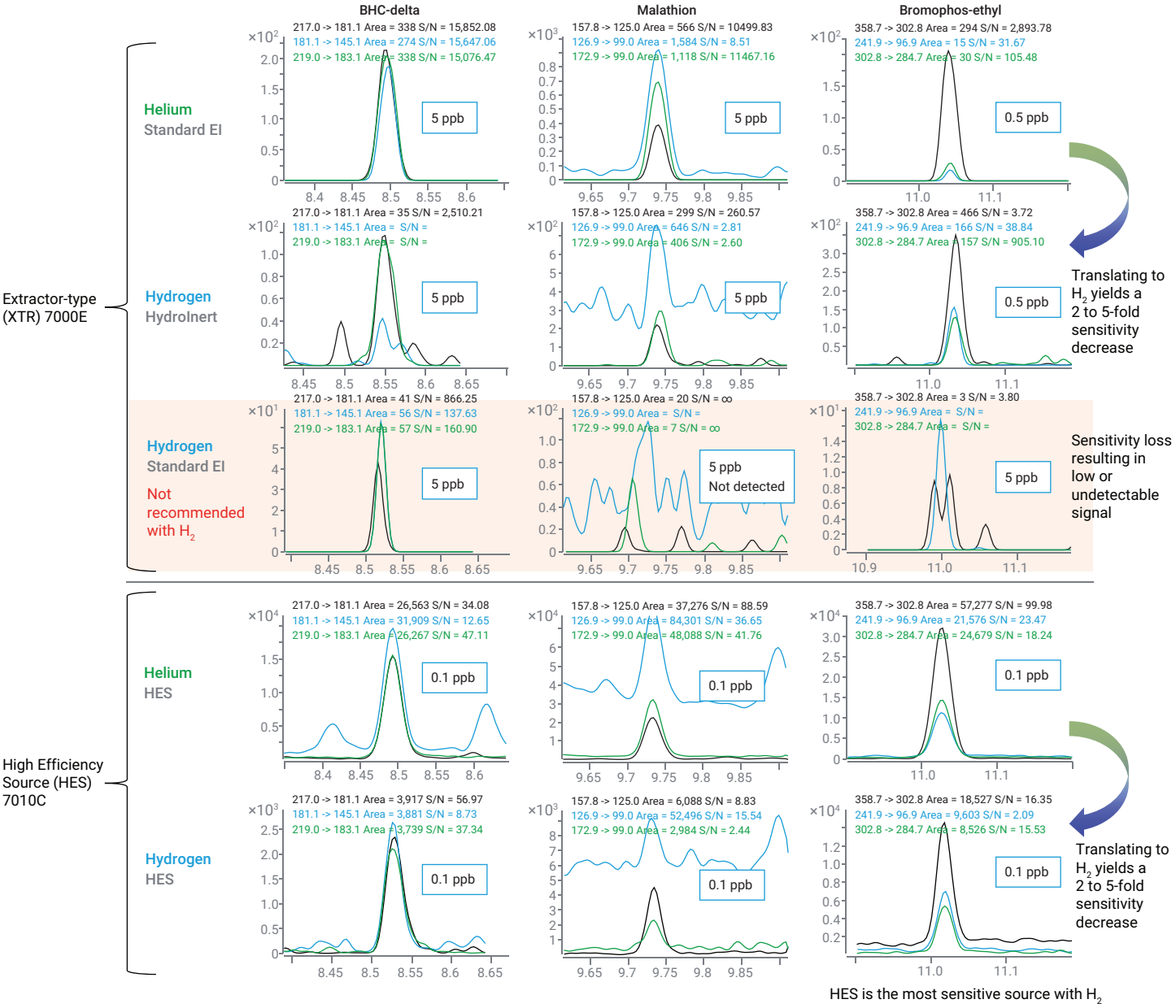
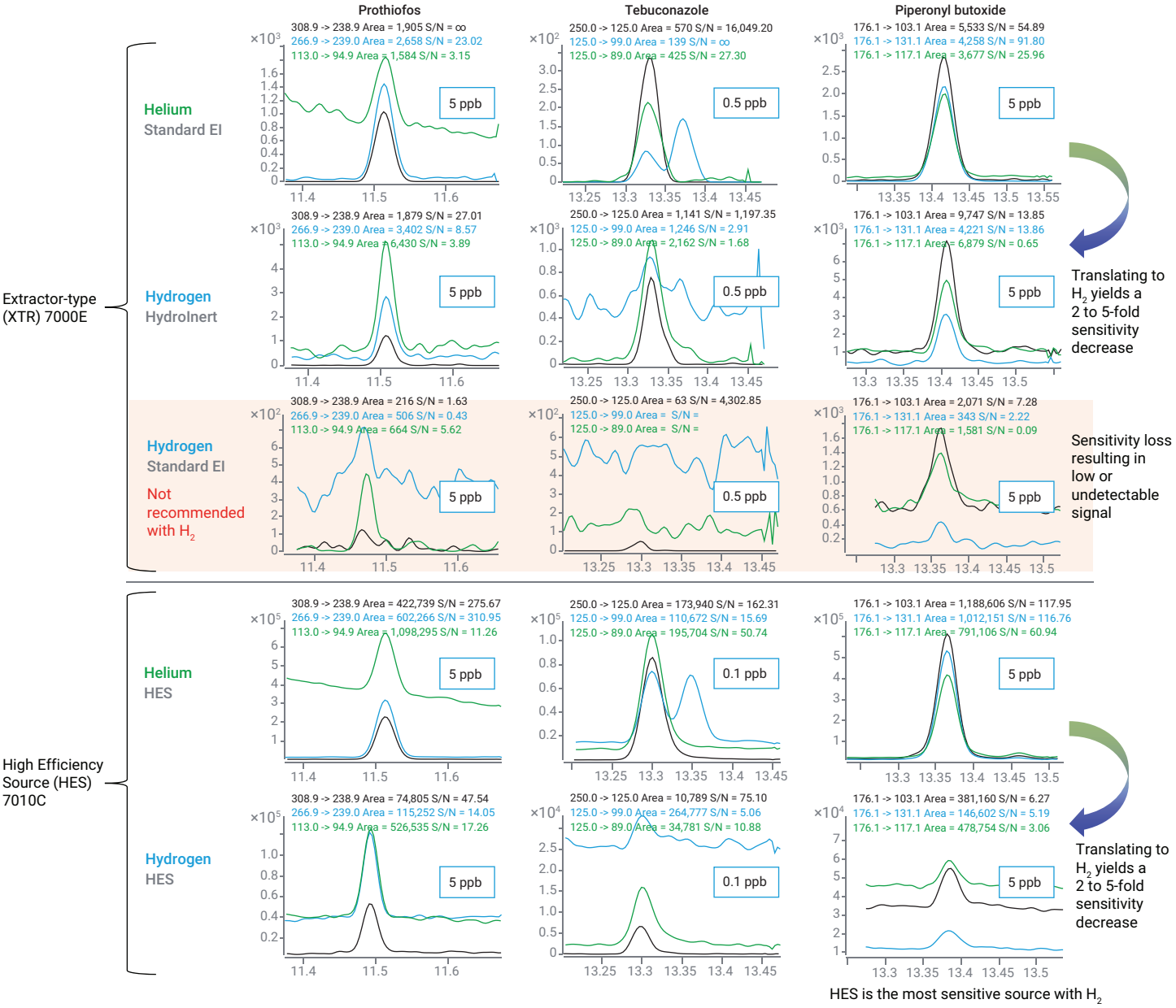
Appendix

Appendix Figure 1. MRM chromatograms for pesticides susceptible to reacting with hydrogen acquired in spinach QuEChERS extract under the optimized injection conditions (2 µL, solvent vent, analyte protectants) with helium and hydrogen carrier gasses using the Agilent 7000E and Agilent 7010C GC/TQ. Identically prepared samples were used for comparison. Black traces correspond to the quantifying MRM transition. The qualifying MRM transitions are blue and green. Continued on subsequent pages.




BHC-beta
BHC-gamma (lindane)
Pentachloronitrobenzene
Extractor-type (XTR) 7000E
Helium
Standard EI
Hydrogen
HydroInert
Hydrogen
Standard EI
Not recommended with H2
High Efficiency Source (HES) 7010C
Helium
HES
Hydrogen
HES
0.5 ppb
1 ppb
5 ppb
0.1 ppb
1 ppb Not detected
5 ppb, quant not detected
Translating to H2 yields a 2 to 5-fold sensitivity decrease
Sensitivity loss resulting in low or undetectable signal
Translating to H2 yields a 2 to 5-fold sensitivity decrease
HES is the most sensitive source with H2


BHC-delta
Malathion
Bromophos-ethyl
Extractor-type (XTR) 7000E
Helium
Standard EI
217.0 -> 181.1 Area = 338 S/N = 15,852.08
181.1 -> 145.1 Area = 274 S/N = 15,647.06
219.0 -> 183.1 Area = 338 S/N = 15,076.47
5 ppb
157.8 -> 125.0 Area = 566 S/N = 10499.83
126.9 -> 99.0 Area = 1,584 S/N = 8.51
172.9 -> 99.0 Area = 1,118 S/N = 11467.16
5 ppb
358.7 -> 302.8 Area = 294 S/N = 2,893.78
241.9 -> 96.9 Area = 15 S/N = 31.67
302.8 -> 284.7 Area = 30 S/N = 105.48
0.5 ppb
Hydrogen
HydroInert
217.0 -> 181.1 Area = 35 S/N = 2,510.21
181.1 -> 145.1 Area = S/N =
219.0 -> 183.1 Area = S/N =
5 ppb
157.8 -> 125.0 Area = 299 S/N = 260.57
126.9 -> 99.0 Area = 646 S/N = 2.81
172.9 -> 99.0 Area = 406 S/N = 2.60
5 ppb
358.7 -> 302.8 Area = 466 S/N = 3.72
241.9 -> 96.9 Area = 166 S/N = 38.84
302.8 -> 284.7 Area = 157 S/N = 905.10
0.5 ppb
Translating to H_2 yields a 2 to 5-fold sensitivity decrease
Hydrogen
Standard EI
Not recommended with H_2
217.0 -> 181.1 Area = 41 S/N = 866.25
181.1 -> 145.1 Area = 56 S/N = 137.63
219.0 -> 183.1 Area = 57 S/N = 160.90
5 ppb
157.8 -> 125.0 Area = 20 S/N = ∞
126.9 -> 99.0 Area = S/N =
172.9 -> 99.0 Area = 7 S/N = ∞
5 ppb
Not detected
358.7 -> 302.8 Area = 3 S/N = 3.80
241.9 -> 96.9 Area = S/N =
302.8 -> 284.7 Area = S/N =
5 ppb
Sensitivity loss resulting in low or undetectable signal
High Efficiency Source (HES) 7010C
Helium
HES
217.0 -> 181.1 Area = 26,563 S/N = 34.08
181.1 -> 145.1 Area = 31,909 S/N = 12.65
219.0 -> 183.1 Area = 26,267 S/N = 47.11
0.1 ppb
157.8 -> 125.0 Area = 37,276 S/N = 88.59
126.9 -> 99.0 Area = 84,301 S/N = 36.65
172.9 -> 99.0 Area = 48,088 S/N = 41.76
0.1 ppb
358.7 -> 302.8 Area = 57,277 S/N = 99.98
241.9 -> 96.9 Area = 21,576 S/N = 23.47
302.8 -> 284.7 Area = 24,679 S/N = 18.24
0.1 ppb
Hydrogen
HES
217.0 -> 181.1 Area = 3,917 S/N = 56.97
181.1 -> 145.1 Area = 3,881 S/N = 8.73
219.0 -> 183.1 Area = 3,739 S/N = 37.34
0.1 ppb
157.8 -> 125.0 Area = 6,088 S/N = 8.83
126.9 -> 99.0 Area = 52,496 S/N = 15.54
172.9 -> 99.0 Area = 2,984 S/N = 2.44
0.1 ppb
358.7 -> 302.8 Area = 18,527 S/N = 16.35
241.9 -> 96.9 Area = 9,603 S/N = 2.09
302.8 -> 284.7 Area = 8,526 S/N = 15.53
0.1 ppb
Translating to H_2 yields a 2 to 5-fold sensitivity decrease
HES is the most sensitive source with H_2


Prothiofos
Tebuconazole
Piperonyl butoxide
Extractor-type (XTR) 7000E
Helium
Standard EI
Hydrogen
HydroInert
Hydrogen
Standard EI
Not recommended with H2
High Efficiency Source (HES) 7010C
Helium
HES
Hydrogen
HES
5 ppb
0.5 ppb
0.1 ppb
Translating to H2 yields a 2 to 5-fold sensitivity decrease
Sensitivity loss resulting in low or undetectable signal
Translating to H2 yields a 2 to 5-fold sensitivity decrease
HES is the most sensitive source with H2

Appendix Table 1. Calibration performance for 203 pesticides in spinach with hydrogen carrier gas using the Agilent 7000E GC/TQ equipped with HydroInert.

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
Allidochlor	4.992	138.0 → 96.0	1	5,000	Quadratic	0.9997	10.6
Dichlorobenzonitrile, 2,6-	5.320	171.0 → 100.0	0.1	5,000	Quadratic	0.9992	17.1
Biphenyl	5.481	154.1 → 153.1	0.1	5,000	Quadratic	0.9992	12.8
Mevinphos, E-	5.671	127.0 → 109.0	1	1,000	Linear	0.9971	19.7
3,4-Dichloroaniline	5.781	160.9 → 99.0	0.1	5,000	Quadratic	0.9995	19.3
Pebulate	5.842	128.0 → 57.1	5	5,000	Quadratic	0.9985	6.2
Etridiazole	5.871	211.1 → 183.0	5	5,000	Quadratic	0.9994	19.2
N-(2,4-dimethylphenyl)formamide	6.073	120.0 → 77.0	10	1,000	Quadratic	0.9978	10.9
cis-1,2,3,6-Tetrahydrophthalimide	6.076	79.0 → 77.0	10	5,000	Quadratic	0.9957	17.9
Methacrifos	6.096	124.9 → 47.1	1	5,000	Linear	0.9997	13.1
Chloroneb	6.179	191.0 → 113.0	0.1	1,000	Quadratic	0.9991	7.6
2-Phenylphenol	6.299	169.1 → 115.1	0.1	1,000	Quadratic	0.9984	18.0
Pentachlorobenzene	6.378	249.9 → 215.0	0.1	5,000	Quadratic	0.9988	16.8
Tecnazene	6.915	214.9 → 179.0	0.5	5,000	Linear	0.9994	12.8
Propachlor	6.925	120.0 → 77.1	5	5,000	Quadratic	0.9995	14.6
Diphenylamine	6.991	169.0 → 168.2	0.1	1,000	Quadratic	0.9992	6.1
Cycloate	7.067	154.1 → 72.1	0.5	1,000	Quadratic	0.9989	19.8
2,3,5,6-Tetrachloroaniline	7.096	230.9 → 159.9	0.5	5,000	Quadratic	0.9939	16.7
Chlorpropham	7.142	127.0 → 65.1	0.5	1,000	Quadratic	0.9987	17.3
Trifluralin	7.261	264.0 → 160.1	0.5	5,000	Quadratic	0.9990	17.1
Ethalfluralin	7.293	275.9 → 202.1	1	1,000	Linear	0.9940	16.3
Benfluralin	7.295	292.0 → 264.0	0.5	5,000	Quadratic	0.9984	17.1
Sulfotep	7.394	237.8 → 145.9	0.5	5,000	Linear	0.9996	15.3
Phorate	7.396	121.0 → 47.0	1	5,000	Linear	0.9997	16.8
Diallate I	7.499	234.1 → 150.0	0.5	1,000	Quadratic	0.9993	14.2
BHC-alpha (Benzene Hexachloride)	7.662	216.9 → 181.0	1	5,000	Quadratic	0.9997	12.4
Hexachlorobenzene	7.789	283.8 → 248.8	0.1	1,000	Quadratic	0.9989	14.3
Dichloran	7.836	124.1 → 73.0	5	5,000	Quadratic	0.9978	11.7
Pentachloroanisole	7.844	264.8 → 236.8	0.1	5,000	Quadratic	0.9985	15.8
Atrazine	7.943	214.9 → 58.1	1	5,000	Quadratic	0.9995	10.0
Clomazone	8.010	125.0 → 89.0	0.5	1,000	Quadratic	0.9994	15.5
BHC-beta	8.099	218.9 → 183.1	0.5	1,000	Quadratic	0.9995	17.4
Profluralin	8.123	318.1 → 199.1	5	5,000	Quadratic	0.9972	15.7
BHC-gamma (Lindane, gamma-HCH)	8.169	218.9 → 183.1	1	1,000	Quadratic	0.9997	13.1
Terbufos	8.172	230.9 → 129.0	1	1,000	Quadratic	0.9999	11.2
Terbuthylazine	8.173	172.9 → 138.1	1	5,000	Quadratic	0.9993	12.9
Propyzamide	8.218	173.0 → 109.0	0.1	1,000	Quadratic	0.9997	16.0
Pentachloronitrobenzene	8.240	248.8 → 213.8	1	5,000	Quadratic	0.9987	13.6
Fonofos	8.267	246.1 → 137.0	1	1,000	Quadratic	0.9995	10.0
Pentachlorobenzonitrile	8.285	274.9 → 239.9	0.5	5,000	Quadratic	0.9977	13.1
Diazinon	8.298	137.1 → 84.0	1	1,000	Quadratic	0.9995	11.0
Pyrimethanil	8.320	198.0 → 118.1	0.5	1,000	Quadratic	0.9994	10.0
Fluchloralin	8.337	264.0 → 160.0	10	1,000	Quadratic	0.9929	17.9
Tefluthrin	8.428	177.1 → 87.0	0.5	1,000	Quadratic	0.9997	15.3
Disulfoton	8.440	88.0 → 60.0	0.5	1,000	Linear	0.9990	13.0

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
Isazofos	8.545	256.9 → 162.0	5	5,000	Linear	0.9997	5.6
BHC-delta	8.571	217.0 → 181.1	5	1,000	Quadratic	0.9963	16.2
Triallate	8.576	142.9 → 83.0	0.5	5,000	Quadratic	0.9966	14.6
Terbacil	8.579	160.0 → 76.0	50	1,000	Quadratic	0.9985	14.6
Chlorothalonil	8.628	265.9 -> 230.9	N/A				
Endosulfan Ether	8.865	240.9 → 205.9	0.1	5,000	Quadratic	0.9932	16.2
Acetochlor	9.023	222.9 → 132.2	5	5,000	Linear	0.9994	7.4
Dimethachlor	9.023	196.9 → 148.2	1	5,000	Quadratic	0.9997	11.7
Propanil	9.026	161.0 → 99.0	0.1	5,000	Quadratic	0.9963	15.9
Pentachloroaniline	9.026	191.9 → 82.9	10	1,000	Quadratic	0.9959	14.8
Transfluthrin	9.131	163.1 → 143.1	0.1	5,000	Quadratic	0.9971	13.6
Vinclozolin	9.145	187.0 → 124.0	0.5	5,000	Quadratic	0.9980	13.8
Parathion-methyl	9.163	262.9 → 109.0	5	5,000	Quadratic	0.9999	11.2
Tolclofos-methyl	9.163	267.0 → 93.0	1	5,000	Quadratic	0.9991	12.7
Chlorpyrifos-methyl	9.165	124.9 → 47.0	1	5,000	Quadratic	0.9998	12.2
Alachlor	9.281	188.1 → 160.1	5	5,000	Linear	0.9989	6.7
Heptachlor	9.342	271.7 → 236.9	0.1	1,000	Linear	0.9983	16.3
Metalaxyl	9.367	234.0 → 146.1	1	1,000	Linear	0.9990	10.7
Propisochlor	9.368	162.0 → 120.1	5	5,000	Linear	0.9991	5.1
Ronnel	9.402	125.0 → 47.1	1	5,000	Quadratic	0.9987	12.8
Prodiamine	9.581	275.1 → 255.1	5	5,000	Quadratic	0.9976	11.9
Pirimiphos-methyl	9.604	290.0 → 125.0	0.5	1,000	Quadratic	0.9999	15.0
Fenitrothion	9.609	277.0 → 260.1	5	5,000	Quadratic	0.9999	8.3
Linuron	9.680	187.1 → 124.1	5	500	Quadratic	0.9931	12.0
Malathion	9.763	157.8 → 125.0	5	5,000	Quadratic	0.9999	16.0
Pentachlorothioanisole	9.768	295.8 → 245.8	1	5,000	Quadratic	0.9961	10.0
Dichlofluanid	9.784	123.0 → 77.0	N/A				
Metolachlor	9.927	238.0 → 162.2	0.1	1,000	Linear	0.9979	16.8
Aldrin	9.940	254.9 → 220.0	1	1,000	Linear	0.9972	6.2
Fenthion	9.950	278.0 → 109.0	1	1,000	Linear	0.9980	9.5
Anthraquinone	9.958	208.0 → 152.2	0.5	1,000	Quadratic	0.9987	14.1
Chlorpyrifos	9.975	196.9 → 169.0	5	5,000	Quadratic	0.9987	8.7
Parathion	10.005	291.0 → 109.0	5	5,000	Linear	0.9997	7.6
Triadimefon	10.047	208.0 → 111.0	0.5	1,000	Quadratic	0.9991	6.2
Dichlorobenzophenone, 4,4'-	10.065	139.0 → 111.0	0.5	1,000	Quadratic	0.9986	9.2
DCPA (Dacthal, Chlorthal-dimethyl)	10.076	298.9 → 221.0	1	1,000	Linear	0.9996	4.3
Fenson	10.232	141.0 → 77.1	0.5	1,000	Linear	0.9988	8.8
MGK-264	10.254	164.2 → 67.1	10	1,000	Linear	0.9949	12.4
Bromophos	10.304	330.9 → 315.9	1	5,000	Quadratic	0.9996	15.9
Pirimiphos-ethyl	10.312	318.1 → 166.1	1	1,000	Quadratic	0.9996	4.4
Diphenamid	10.334	239.0 → 167.1	1	1,000	Linear	0.9979	7.9
Isopropalin	10.363	280.1 → 238.1	5	1,000	Linear	0.9993	7.7
Isodrin	10.461	193.0 → 157.0	0.1	1,000	Linear	0.9977	14.7
Cyprodinil	10.464	225.2 → 224.3	1	1,000	Linear	0.9972	5.9
Pendimethalin	10.546	251.8 → 161.1	5	5,000	Quadratic	0.9997	8.2
Metazachlor	10.572	209.0 → 132.2	0.5	5,000	Quadratic	0.9996	15.0
Fipronil	10.591	350.8 → 254.8	10	500	Linear	0.9902	16.2

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
Penconazole	10.610	248.0 → 157.1	1	1,000	Linear	0.9967	7.9
Chlozolinate	10.613	186.0 → 109.0	1	5,000	Quadratic	0.9992	12.7
Heptachlor Exo-Epoxide	10.633	352.8 → 262.9	1	1,000	Linear	0.9988	10.1
Tolylfluanid	10.662	238.0 → 137.0	N/A				
Allethrin	10.670	91.0 → 65.0	N/A				
Chlorfenvinphos	10.719	266.9 → 159.0	0.5	5,000	Quadratic	0.9997	14.5
Bromfenvinfos-methyl	10.733	295.0 → 108.9	10	1,000	Quadratic	0.9995	6.6
Quinalphos	10.768	146.0 → 118.0	5	1,000	Linear	0.9995	4.0
Captan	10.772	149.0 → 70.0	N/A				
Triflumizole	10.774	91.0 → 65.0	N/A				
Triadimenol	10.806	168.0 → 70.0	1	1,000	Linear	0.9991	9.1
Folpet	10.891	261.8 → 130.1	N/A				
Procymidone	10.894	282.8 → 96.0	1	1,000	Linear	0.9988	13.7
Chlorbenside	10.941	125.0 → 89.0	1	1,000	Linear	0.9981	10.0
Tetrachlorvinphos	10.945	78.9 → 47.0	10	5,000	Quadratic	0.9973	16.2
Bromophos-ethyl	11.051	358.7 → 302.8	1	1,000	Linear	0.9980	9.2
Chlordane-*trans*	11.055	271.7 → 236.9	0.1	5,000	Quadratic	0.9990	11.2
DDE-*o,p'*	11.100	246.0 → 176.2	0.5	1,000	Quadratic	0.9993	9.6
Paclobutrazol	11.155	125.1 → 89.0	0.1	1,000	Linear	0.9983	14.2
Endosulfan I (Alpha Isomer)	11.285	194.9 → 125.0	5	5,000	Quadratic	0.9989	10.9
Chlordane-*cis*	11.287	372.8 → 265.9	1	5,000	Quadratic	0.9992	8.6
Flutriafol	11.386	123.1 → 75.1	0.1	5,000	Quadratic	0.9997	12.0
Nonachlor, *trans*-	11.400	271.8 → 236.9	0.5	5,000	Quadratic	0.9988	10.4
Chlorfenson	11.416	175.0 → 111.0	0.1	5,000	Quadratic	0.9997	16.0
Fenamiphos	11.457	154.0 → 139.0	5	5,000	Quadratic	0.9991	16.3
Bromfenvinfos	11.459	266.9 → 159.1	1	1,000	Linear	0.9944	17.7
Flutolanil	11.475	173.0 → 95.0	0.1	5,000	Quadratic	0.9987	16.5
Iodofenphos	11.496	376.8 → 361.8	5	5,000	Quadratic	0.9997	14.2
Prothiofos	11.524	308.9 → 238.9	1	1,000	Linear	0.9996	11.8
Profenofos	11.603	338.8 → 268.7	5	1,000	Quadratic	0.9947	15.7
Pretilachlor	11.630	262.0 → 202.2	1	5,000	Quadratic	0.9997	6.9
DDE-*p,p'*	11.653	246.1 → 176.2	1	1,000	Quadratic	0.9991	10.5
Oxadiazon	11.685	174.9 → 112.0	0.5	1,000	Quadratic	0.9996	11.7
Fludioxonil	11.704	248.0 → 127.1	0.5	5,000	Quadratic	0.9982	18.8
Tricyclazole	11.750	189.0 → 161.1	5	500	Quadratic	0.9963	18.1
Dieldrin	11.751	262.9 → 193.0	1	5,000	Quadratic	0.9996	13.5
Oxyfluorfen	11.773	252.0 → 146.0	1	5,000	Quadratic	0.9957	18.6
DDD-*o,p'*	11.825	235.0 → 165.1	0.1	1,000	Linear	0.9983	12.0
Myclobutanil	11.853	179.0 → 125.1	0.1	1,000	Linear	0.9991	11.1
Flusilazole	11.886	233.0 → 165.1	0.5	500	Quadratic	0.9990	16.1
Bupirimate	11.902	272.9 → 193.1	1	1,000	Linear	0.9992	8.0
Fluazifop-*p*-butyl	12.035	281.9 → 91.0	0.1	1,000	Quadratic	0.9985	7.9
Nitrofen	12.060	202.0 → 139.1	1	5,000	Linear	0.9987	7.8
Ethylan	12.080	223.1 → 167.1	1	5,000	Quadratic	0.9995	12.5
Chlorfenapyr	12.105	247.1 → 227.1	5	5,000	Quadratic	0.9943	13.3
Endrin	12.150	262.8 → 193.0	1	5,000	Quadratic	0.9997	10.7
Chlorobenzilate	12.230	139.1 → 111.0	0.1	1,000	Linear	0.9987	9.6

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
Endosulfan II (Beta Isomer)	12.321	206.9 → 172.0	1	5,000	Quadratic	0.9999	15.9
DDD-*p,p'*	12.419	237.0 → 165.1	0.5	5,000	Quadratic	0.9988	12.7
Ethion	12.471	230.9 → 175.0	0.5	1,000	Linear	0.9974	15.1
DDT-*o,p'*	12.473	237.0 → 165.2	1	5,000	Quadratic	0.9998	14.5
Chlorthiophos	12.520	324.8 → 268.9	0.5	5,000	Quadratic	0.9996	15.5
Nonachlor, *cis-*	12.529	408.8 → 299.8	1	5,000	Quadratic	0.9996	11.1
Endrin Aldehyde	12.598	344.9 → 244.9	5	250	Quadratic	0.9961	19.3
Sulprofos	12.685	140.0 → 125.1	0.5	5,000	Quadratic	0.9997	10.7
Triazophos	12.722	161.2 → 134.2	10	5,000	Quadratic	0.9995	12.0
Carbophenothion	12.872	153.0 → 96.9	5	5,000	Quadratic	0.9995	7.2
Carfentrazone-ethyl	12.876	329.9 → 309.9	0.5	1,000	Linear	0.9981	16.2
Methoxychlor Olefin	12.881	238.0 → 195.1	0.5	5,000	Quadratic	0.9995	20.0
Edifenphos	12.966	172.9 → 109.0	10	500	Linear	0.9959	9.4
Norflurazon	13.039	145.0 → 75.0	1	1,000	Quadratic	0.9964	12.1
DDT-*p,p'*	13.074	235.0 → 165.2	5	5,000	Linear	0.9992	6.6
Endosulfan Sulfate	13.080	271.9 → 237.0	5	1,000	Quadratic	0.9992	11.2
Lenacil	13.092	153.1 → 136.1	0.5	500	Linear	0.9903	14.3
Methoxychlor, *o,p'-*	13.247	227.1 → 121.1	0.1	5,000	Quadratic	0.9987	17.6
Hexazinone	13.309	171.0 → 71.1	1	500	Quadratic	0.9970	10.0
Tebuconazole	13.352	250.0 → 125.0	0.5	1,000	Quadratic	0.9986	9.6
Piperonyl Butoxide	13.424	176.1 → 103.1	0.5	1,000	Quadratic	0.9989	12.2
Propargite	13.425	135.0 → 77.1	10	5,000	Quadratic	0.9986	17.4
Captafol	13.428	150.0 → 79.0	N/A				
Resmethrin	13.448	171.0 → 128.0	5	1,000	Linear	0.9912	18.6
Nitralin	13.606	315.9 → 274.0	100	5,000	Quadratic	0.9992	69.8
Iprodione	13.772	313.8 → 55.9	N/A				
Tetramethrin I	13.860	164.0 → 107.1	5	1,000	Quadratic	0.9992	12.3
Pyridaphenthion	13.874	340.0 → 199.0	5	5,000	Quadratic	0.9999	10.1
Endrin Ketone	13.928	147.0 → 111.0	5	5,000	Quadratic	0.9970	23.7
Bifenthrin	13.957	181.2 → 165.2	0.5	5,000	Quadratic	0.9978	18.0
Phosmet	13.958	160.0 → 133.1	100	5,000	Quadratic	0.9987	16.3
Bromopropylate	13.977	338.8 → 182.9	0.5	5,000	Quadratic	0.9986	14.8
EPN	13.981	169.0 → 141.1	10	5,000	Quadratic	0.9997	10.9
Methoxychlor, *p,p'-*	14.082	227.0 → 169.1	1	5,000	Quadratic	0.9993	13.2
Fenpropathrin	14.098	207.9 → 181.0	0.5	5,000	Quadratic	0.9946	14.4
Tebufenpyrad	14.163	332.9 → 171.0	0.5	1,000	Quadratic	0.9980	10.3
Azinphos-methyl	14.438	160.0 → 132.1	50	1,000	Linear	0.9968	6.1
Phenothrin I	14.438	122.9 → 81.1	5	1,000	Linear	0.9948	9.3
Tetradifon	14.481	158.9 → 111.0	0.5	5,000	Quadratic	0.9988	14.3
Phosalone	14.641	182.0 → 111.0	5	5,000	Quadratic	0.9991	19.7
Pyriproxyfen	14.675	136.1 → 78.1	0.5	1,000	Linear	0.9974	15.8
Leptophos	14.685	171.0 → 51.0	5	5,000	Quadratic	0.9997	8.0
Cyhalothrin (Lambda)	14.734	181.1 → 152.1	10	500	Linear	0.9844	12.9
Mirex	14.906	271.8 → 236.8	1	5,000	Quadratic	0.9996	6.2
Acrinathrin	14.928	207.8 → 181.1	10	500	Quadratic	0.9938	13.9
Fenarimol	15.154	139.0 → 75.0	1	1,000	Quadratic	0.9940	15.0
Pyrazophos	15.183	221.0 → 193.1	10	5,000	Quadratic	0.9998	8.0

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
Azinphos-ethyl	15.273	132.0 → 77.1	50	5,000	Quadratic	0.9994	12.2
Pyraclofos	15.311	194.0 → 138.0	50	1,000	Quadratic	0.9973	17.3
Permethrin, (1R)-*cis*-	15.663	183.1 → 168.1	5	1,000	Quadratic	0.9961	13.0
Permethrin, (1R)-*trans*-	15.790	163.0 → 127.0	1	5,000	Quadratic	0.9904	18.4
Pyridaben	15.831	147.2 → 117.1	1	1,000	Quadratic	0.9949	14.0
Fluquinconazole	15.909	108.0 → 57.0	0.5	1,000	Quadratic	0.9990	17.2
Coumaphos	15.934	225.9 → 163.1	10	500	Linear	0.9858	18.3
Prochloraz	15.982	180.0 → 138.0	10	1,000	Quadratic	0.9993	11.2
Cyfluthrin I	16.232	162.9 → 127.0	10	1,000	Quadratic	0.9943	18.4
Cypermethrin I	16.539	163.0 → 127.0	10	1,000	Quadratic	0.9966	17.5
Acequinocyl	16.575	187.9 → 160.0	N/A				
Flucythrinate I	16.763	156.9 → 107.1	1	1,000	Quadratic	0.9998	11.1
Ethofenprox	16.840	163.0 → 107.1	1	1,000	Quadratic	0.9956	13.7
Fluridone	17.241	328.9 → 328.1	1	1,000	Quadratic	0.9999	16.2
Fenvalerate I	17.470	167.0 → 125.1	5	1,000	Quadratic	0.9998	16.0
Fluvalinate-tau I	17.663	250.0 → 200.0	N/A				
Deltamethrin	17.984	250.7 → 172.0	10	5,000	Quadratic	1.0000	11.7

Appendix Table 2. Calibration performance for 203 pesticides in spinach with hydrogen carrier gas using the Agilent 7010C GC/TQ.

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
Allidochlor	4.992	132.0 → 56.1	1	1,000	Quadratic	0.9995	16.3
Dichlorobenzonitrile, 2,6-	5.320	171.0 → 100.0	0.1	1,000	Quadratic	0.9996	14.6
Biphenyl	5.481	154.1 → 153.1	0.1	500	Quadratic	0.9991	19.1
Mevinphos, E-	5.671	127.0 → 109.0	0.1	500	Quadratic	0.9984	18.4
3,4-Dichloroaniline	5.781	160.9 → 99.0	0.1	1,000	Quadratic	0.9983	15.8
Pebulate	5.842	128.0 → 57.1	1	1,000	Quadratic	0.9999	10.4
Etridiazole	5.871	211.1 → 183.0	0.5	500	Quadratic	0.9997	16.5
N-(2,4-dimethylphenyl)formamide	6.073	120.0 → 77.0	5	500	Quadratic	0.9987	8.3
cis-1,2,3,6-Tetrahydrophthalimide	6.076	151.1 → 80.0	1	1,000	Quadratic	0.9996	6.6
Methacrifos	6.096	124.9 → 47.1	0.1	500	Quadratic	0.9990	19.8
Chloroneb	6.179	191.0 → 113.0	0.1	1,000	Linear	0.9995	11.7
2-Phenylphenol	6.299	169.1 → 115.1	1	1,000	Linear	0.9995	14.7
Pentachlorobenzene	6.378	249.9 → 215.0	0.1	1,000	Quadratic	0.9992	16.3
Tecnazene	6.915	214.9 → 179.0	0.1	1,000	Quadratic	0.9997	14.4
Propachlor	6.925	176.1 → 57.1	0.1	500	Quadratic	0.9964	15.7
Diphenylamine	6.991	169.0 → 168.2	1	1,000	Quadratic	0.9988	12.4
Cycloate	7.067	154.1 → 72.1	0.1	500	Quadratic	0.9972	18.0
2,3,5,6-Tetrachloroaniline	7.096	230.9 → 159.9	0.1	1,000	Quadratic	0.9990	14.4
Chlorpropham	7.142	127.0 → 65.1	0.1	1,000	Quadratic	0.9990	16.7
Trifluralin	7.261	306.1 → 264.0	0.1	500	Quadratic	0.9994	17.0
Ethalfluralin	7.293	275.9 → 202.1	0.5	500	Quadratic	0.9994	16.8
Benfluralin	7.295	292.0 → 264.0	0.1	500	Quadratic	0.9995	17.0
Sulfotep	7.394	237.8 → 145.9	0.1	500	Quadratic	0.9987	15.7
Phorate	7.396	121.0 → 47.0	0.5	500	Quadratic	0.9988	11.1
Diallate I	7.499	234.1 → 150.0	0.1	500	Quadratic	0.9985	17.8

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
BHC-alpha (Benzene Hexachloride)	7.662	216.9 → 181.0	0.1	500	Quadratic	0.9997	18.8
Hexachlorobenzene	7.789	283.8 → 248.8	0.1	1,000	Linear	0.9988	16.2
Dichloran	7.836	124.1 → 73.0	0.1	500	Quadratic	0.9993	18.2
Pentachloroanisole	7.844	264.8 → 236.8	0.1	1,000	Linear	0.9988	16.9
Atrazine	7.943	214.9 → 58.1	0.1	1,000	Quadratic	0.9998	19.8
Clomazone	8.010	125.0 → 89.0	0.1	1,000	Quadratic	0.9997	14.1
BHC-beta	8.099	218.9 → 183.1	0.1	1,000	Quadratic	0.9996	16.9
Profluralin	8.123	318.1 → 199.1	5	1,000	Quadratic	0.9995	8.7
BHC-gamma (Lindane, gamma-HCH)	8.169	218.9 → 183.1	1	500	Quadratic	0.9999	3.0
Terbufos	8.172	230.9 → 129.0	1	1,000	Quadratic	0.9997	13.1
Terbuthylazine	8.173	228.9 → 173.1	0.1	1,000	Quadratic	0.9998	9.1
Propyzamide	8.218	173.0 → 109.0	1	1,000	Quadratic	0.9996	17.2
Pentachloronitrobenzene	8.240	248.8 → 213.8	0.1	1,000	Quadratic	0.9992	13.7
Fonofos	8.267	246.1 → 137.0	0.5	500	Quadratic	0.9994	19.8
Pentachlorobenzonitrile	8.285	274.9 → 239.9	0.1	1,000	Linear	0.9995	16.6
Diazinon	8.298	137.1 → 84.0	0.5	1,000	Quadratic	0.9999	12.7
Pyrimethanil	8.320	198.0 → 118.1	0.1	1,000	Quadratic	0.9997	18.6
Fluchloralin	8.337	325.8 → 62.9	0.5	1,000	Quadratic	0.9998	16.9
Tefluthrin	8.428	177.1 → 87.0	0.1	500	Linear	0.9974	16.1
Disulfoton	8.440	88.0 → 60.0	0.5	1,000	Quadratic	0.9996	7.4
Isazofos	8.545	256.9 → 162.0	1	500	Quadratic	0.9981	13.9
BHC-delta	8.571	217.0 → 181.1	1	500	Quadratic	0.9992	8.2
Triallate	8.576	268.0 → 184.1	0.5	500	Linear	0.9993	13.2
Terbacil	8.579	160.0 → 76.0	50	1,000	Quadratic	0.9935	13.0
Chlorothalonil	8.628	265.9 → 230.9	10	500	Quadratic	0.9955	17.4
Endosulfan Ether	8.865	240.9 → 205.9	0.5	500	Quadratic	0.9975	18.5
Acetochlor	9.023	222.9 → 132.2	0.1	1,000	Quadratic	0.9986	15.4
Dimethachlor	9.023	196.9 → 148.2	0.1	500	Quadratic	0.9981	18.1
Propanil	9.026	161.0 → 99.0	0.5	1,000	Linear	0.9991	6.1
Pentachloroaniline	9.026	191.9 → 82.9	5	1,000	Quadratic	0.9965	11.6
Transfluthrin	9.131	163.1 → 143.1	5	1,000	Linear	0.9975	12.5
Vinclozolin	9.145	187.0 → 124.0	0.5	250	Quadratic	0.9973	18.5
Parathion-methyl	9.163	125.0 → 47.0	0.5	1,000	Quadratic	0.9984	18.3
Tolclofos-methyl	9.163	267.0 → 93.0	0.5	1,000	Linear	0.9983	17.1
Chlorpyrifos-methyl	9.165	124.9 → 47.0	0.5	1,000	Quadratic	0.9983	16.8
Alachlor	9.281	188.1 → 160.1	5	1,000	Linear	0.9946	19.0
Heptachlor	9.342	271.7 → 236.9	5	1,000	Linear	0.9981	8.2
Metalaxyl	9.367	234.0 → 146.1	0.1	1,000	Quadratic	0.9995	17.4
Propisochlor	9.368	162.0 → 120.1	1	1,000	Linear	0.9956	12.7
Ronnel	9.402	125.0 → 47.1	0.5	1,000	Quadratic	0.9987	18.6
Prodiamine	9.581	321.0 → 203.0	0.5	500	Quadratic	0.9997	15.5
Pirimiphos-methyl	9.604	290.0 → 125.0	0.5	1,000	Quadratic	0.9996	19.4
Fenitrothion	9.609	125.1 → 47.0	0.5	1,000	Quadratic	0.9996	15.4
Linuron	9.680	187.1 → 124.1	1	500	Linear	0.9990	8.1
Malathion	9.763	157.8 → 125.0	0.1	1,000	Quadratic	0.9953	13.9
Pentachlorothioanisole	9.768	295.8 → 245.8	5	1,000	Quadratic	0.9960	12.4
Dichlofluanid	9.784	123.0 → 77.0	N/A				

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
Metolachlor	9.927	238.0 → 162.2	0.1	1,000	Linear	0.9992	12.2
Aldrin	9.940	254.9 → 220.0	0.5	250	Quadratic	0.9917	17.9
Fenthion	9.950	278.0 → 109.0	0.1	1,000	Quadratic	0.9999	3.6
Anthraquinone	9.958	208.0 → 152.2	0.1	1,000	Linear	0.9991	8.4
Chlorpyrifos	9.975	313.8 → 257.8	0.1	1,000	Linear	0.9998	4.3
Parathion	10.005	291.0 → 109.0	1	1,000	Quadratic	0.9998	14.5
Triadimefon	10.047	208.0 → 111.0	1	1,000	Linear	0.9971	13.0
Dichlorobenzophenone, 4,4'-	10.065	139.0 → 111.0	1	1,000	Quadratic	0.9994	9.2
DCPA (Dacthal, Chlorthal-dimethyl)	10.076	298.9 → 221.0	0.1	1,000	Quadratic	0.9988	19.9
Fenson	10.232	141.0 → 77.1	1	1,000	Quadratic	0.9984	8.0
MGK-264	10.254	164.2 → 67.1	10	1,000	Linear	0.9947	10.8
Bromophos	10.304	330.9 → 315.9	0.5	1,000	Quadratic	0.9985	14.6
Pirimiphos-ethyl	10.312	318.1 → 166.1	1	1,000	Linear	0.9982	8.1
Diphenamid	10.334	239.0 → 167.1	5	1,000	Linear	0.9990	12.1
Isopropalin	10.363	280.1 → 238.1	1	1,000	Linear	0.9991	18.4
Isodrin	10.461	193.0 → 157.0	0.5	500	Quadratic	0.9943	17.5
Cyprodinil	10.464	225.2 → 224.3	0.1	1,000	Linear	0.9971	14.5
Pendimethalin	10.546	251.8 → 161.1	0.1	100	Quadratic	0.9999	10.9
Metazachlor	10.572	209.0 → 132.2	5	1,000	Quadratic	0.9982	9.8
Fipronil	10.591	350.8 → 254.8	10	1,000	Quadratic	0.9932	18.3
Penconazole	10.610	248.0 → 157.1	5	1,000	Linear	0.9992	8.8
Chlozolinate	10.613	186.0 → 109.0	0.5	1,000	Quadratic	0.9994	19.1
Heptachlor Exo-Epoxide	10.633	352.8 → 262.9	0.5	500	Quadratic	0.9942	19.0
Tolylfluanid	10.662	238.0 → 137.0	10	500	Quadratic	0.9988	18.1
Allethrin	10.670	91.0 → 65.0	N/A				
Chlorfenvinphos	10.719	266.9 → 159.0	5	1,000	Quadratic	0.9983	12.5
Bromfenvinfos-methyl	10.733	169.9 → 99.0	10	500	Quadratic	0.9998	3.8
Quinalphos	10.768	146.0 → 118.0	5	1,000	Quadratic	0.9998	6.8
Captan	10.772	149.0 → 70.0	N/A				
Triflumizole	10.774	91.0 → 65.0	N/A				
Triadimenol	10.806	128.0 → 100.0	0.5	500	Quadratic	0.9922	14.3
Folpet	10.891	261.8 → 130.1	N/A				
Procymidone	10.894	282.8 → 96.0	1	500	Quadratic	0.9951	18.0
Chlorbenside	10.941	125.0 → 89.0	5	1,000	Quadratic	0.9964	12.8
Tetrachlorvinphos	10.945	78.9 → 47.0	5	500	Quadratic	0.9948	13.8
Bromophos-ethyl	11.051	358.7 → 302.8	5	1,000	Linear	0.9951	14.4
Chlordane-*trans*	11.055	271.7 → 236.9	5	1,000	Linear	0.9935	16.5
DDE-*o,p'*	11.100	246.0 → 176.2	5	1,000	Quadratic	0.9926	20.0
Paclobutrazol	11.155	125.1 → 89.0	0.5	500	Quadratic	0.9959	19.7
Endosulfan I (Alpha Isomer)	11.285	194.9 → 125.0	5	1,000	Linear	0.9932	18.1
Chlordane-*cis*	11.287	372.8 → 265.9	5	1,000	Quadratic	0.9948	17.9
Flutriafol	11.386	123.1 → 75.1	10	1,000	Quadratic	0.9969	19.7
Nonachlor, *trans*-	11.400	406.8 → 299.8	10	1,000	Quadratic	0.9988	18.5
Chlorfenson	11.416	175.0 → 111.0	0.1	10	Quadratic	0.9949	17.0
Fenamiphos	11.457	154.0 → 139.0	N/A				
Bromfenvinfos	11.459	266.9 → 159.1	1	1,000	Quadratic	0.9979	10.9
Flutolanil	11.475	173.0 → 95.0	0.5	1,000	Linear	0.9955	15.9

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
Iodofenphos	11.496	376.8 → 361.8	10	1,000	Quadratic	0.9957	19.6
Prothiofos	11.524	308.9 → 238.9	10	1,000	Quadratic	0.9996	7.4
Profenofos	11.603	207.9 → 63.0	1	500	Quadratic	0.9979	12.7
Pretilachlor	11.630	262.0 → 202.2	0.5	1,000	Quadratic	0.9986	14.7
DDE-*p,p'*	11.653	246.1 → 176.2	10	1,000	Linear	0.9922	19.9
Oxadiazon	11.685	174.9 → 112.0	1	250	Quadratic	0.9902	15.9
Fludioxonil	11.704	248.0 → 127.1	0.5	1,000	Linear	0.9984	10.1
Tricyclazole	11.750	189.0 → 161.1	10	500	Quadratic	0.9988	15.1
Dieldrin	11.751	277.0 → 241.0	5	1,000	Linear	0.9950	15.4
Oxyfluorfen	11.773	252.0 → 146.0	5	250	Linear	0.9956	15.6
DDD-*o,p'*	11.825	235.0 → 165.1	5	500	Linear	0.9974	17.7
Myclobutanil	11.853	179.0 → 125.1	0.5	1,000	Linear	0.9977	12.4
Flusilazole	11.886	233.0 → 165.1	0.5	500	Quadratic	0.9974	16.7
Bupirimate	11.902	272.9 → 193.1	0.1	500	Quadratic	0.9934	17.9
Fluazifop-*p*-butyl	12.035	281.9 → 91.0	0.1	500	Quadratic	0.9966	17.3
Nitrofen	12.060	202.0 → 139.1	0.5	500	Linear	0.9940	17.6
Ethylan	12.080	223.1 → 167.1	5	1,000	Quadratic	0.9947	15.4
Chlorfenapyr	12.105	247.1 → 227.1	0.5	1,000	Quadratic	0.9976	15.0
Endrin	12.150	262.8 → 193.0	5	1,000	Quadratic	0.9963	11.2
Chlorobenzilate	12.230	139.1 → 111.0	5	1,000	Quadratic	0.9964	11.3
Endosulfan II (Beta Isomer)	12.321	206.9 → 172.0	1	1,000	Quadratic	0.9987	10.2
DDD-*p,p'*	12.378	237.0 → 165.1	5	1,000	Quadratic	0.9917	19.1
Ethion	12.471	230.9 → 175.0	5	1,000	Linear	0.9971	12.2
DDT-*o,p'*	12.473	237.0 → 165.2	0.1	1,000	Quadratic	0.9990	14.1
Chlorthiophos	12.520	324.8 → 268.9	5	1,000	Linear	0.9966	13.6
Nonachlor, *cis*-	12.529	408.8 → 299.8	0.1	50	Quadratic	0.9968	15.7
Endrin Aldehyde	12.598	249.9 → 214.9	10	1,000	Quadratic	0.9992	7.6
Sulprofos	12.685	140.0 → 125.1	0.1	1,000	Linear	0.9974	16.0
Triazophos	12.722	161.2 → 134.2	5	1,000	Quadratic	0.9976	9.0
Carbophenothion	12.872	342.0 → 157.0	0.1	1,000	Linear	0.9973	9.2
Carfentrazone-ethyl	12.876	329.9 → 309.9	0.1	1,000	Quadratic	0.9987	16.9
Methoxychlor Olefin	12.881	238.0 → 195.1	5	1,000	Linear	0.9966	12.5
Edifenphos	12.966	172.9 → 109.0	5	500	Quadratic	0.9998	16.3
Norflurazon	13.039	145.0 → 75.0	5	1,000	Linear	0.9988	7.3
DDT-*p,p'*	13.074	235.0 → 165.2	0.1	1,000	Quadratic	0.9983	19.1
Endosulfan Sulfate	13.080	271.9 → 237.0	0.1	1,000	Quadratic	0.9980	18.5
Lenacil	13.092	153.1 → 136.1	5	500	Quadratic	0.9980	14.2
Methoxychlor, *o,p'*-	13.247	227.1 → 121.1	0.5	1,000	Quadratic	0.9989	15.6
Hexazinone	13.309	171.0 → 71.1	0.5	1,000	Quadratic	0.9996	11.0
Tebuconazole	13.352	250.0 → 125.0	1	1,000	Quadratic	0.9997	3.1
Piperonyl Butoxide	13.424	176.1 → 103.1	5	1,000	Quadratic	0.9957	14.3
Propargite	13.425	135.0 → 107.1	5	1,000	Quadratic	0.9991	9.2
Captafol	13.428	150.0 → 79.0	N/A				
Resmethrin	13.448	171.0 → 128.0	5	1,000	Quadratic	0.9993	6.5
Nitralin	13.606	315.9 → 274.0	100	1,000	Quadratic	0.9962	11.8
Iprodione	13.772	313.8 → 55.9	N/A				
Tetramethrin I	13.860	164.0 → 107.1	5	1,000	Quadratic	0.9994	9.6

Name	RT	Transition	CF Limit Low	CF Limit High	CF	CF R^2	Relative Standard Error
Pyridaphenthion	13.874	340.0 → 199.0	5	1,000	Quadratic	0.9968	7.6
Endrin Ketone	13.928	316.9 → 280.9	5	1,000	Quadratic	0.9994	9.3
Bifenthrin	13.957	181.2 → 165.2	5	1,000	Quadratic	0.9978	8.9
Phosmet	13.958	160.0 → 133.1	100	1,000	Quadratic	0.9994	16.5
Bromopropylate	13.977	338.8 → 182.9	0.1	1,000	Linear	0.9960	12.6
EPN	13.981	169.0 → 77.1	5	1,000	Quadratic	0.9974	8.0
Methoxychlor, *p,p'*-	14.082	227.0 → 169.1	1	1,000	Quadratic	0.9986	6.9
Fenpropathrin	14.098	207.9 → 181.0	5	1,000	Linear	0.9971	16.7
Tebufenpyrad	14.163	332.9 → 171.0	1	500	Linear	0.9986	14.5
Azinphos-methyl	14.438	160.0 → 132.1	50	1,000	Quadratic	0.9982	9.5
Phenothrin I	14.438	122.9 → 81.1	50	1,000	Linear	0.9967	13.0
Tetradifon	14.481	158.9 → 111.0	0.5	1,000	Quadratic	0.9995	18.2
Phosalone	14.641	182.0 → 111.0	1	1,000	Linear	0.9933	18.4
Pyriproxyfen	14.675	136.1 → 78.1	5	1,000	Linear	0.9993	8.9
Leptophos	14.685	171.0 → 51.0	5	1,000	Quadratic	0.9977	14.4
Cyhalothrin (Lambda)	14.734	208.1 → 181.1	10	1,000	Linear	0.9983	12.0
Mirex	14.906	271.8 → 236.8	5	1,000	Linear	0.9974	9.8
Acrinathrin	14.928	207.8 → 181.1	0.5	1,000	Linear	0.9971	9.6
Fenarimol	15.154	139.0 → 75.0	0.5	1,000	Linear	0.9952	9.5
Pyrazophos	15.183	221.0 → 193.1	5	1,000	Quadratic	0.9968	19.0
Azinphos-ethyl	15.273	132.0 → 77.1	10	1,000	Quadratic	0.9959	12.1
Pyraclofos	15.311	194.0 → 138.0	10	500	Quadratic	0.9988	12.0
Permethrin, (1R)-*cis*-	15.663	183.1 → 168.1	5	500	Quadratic	0.9974	8.8
Permethrin, (1R)-*trans*-	15.790	163.0 → 127.0	1	1,000	Quadratic	0.9994	12.9
Pyridaben	15.831	147.2 → 117.1	1	1,000	Quadratic	0.9996	5.9
Fluquinconazole	15.909	108.0 → 57.0	0.5	1,000	Linear	0.9980	15.2
Coumaphos	15.934	361.9 → 109.0	10	500	Quadratic	0.9961	14.1
Prochloraz	15.982	310.0 → 69.8	1	1,000	Quadratic	0.9975	13.0
Cyfluthrin I	16.232	162.9 → 127.0	5	1,000	Linear	0.9938	14.8
Cypermethrin I	16.539	163.0 → 127.0	5	1,000	Linear	0.9959	12.6
Acequinocyl	16.575	187.9 → 160.0	N/A				
Flucythrinate I	16.763	156.9 → 107.1	1	250	Quadratic	0.9962	18.1
Ethofenprox	16.840	163.0 → 107.1	0.5	500	Quadratic	0.9992	19.4
Fluridone	17.241	328.0 → 258.9	5	1,000	Quadratic	0.9987	18.9
Fenvalerate I	17.470	167.0 → 125.1	0.5	1,000	Linear	0.9961	15.3
Fluvalinate-tau I	17.663	181.0 → 152.0	50	1,000	Linear	0.9937	8.2
Deltamethrin	18.141	250.7 → 172.0	10	1,000	Quadratic	0.9904	18.6

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Application Note

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A Fast and Robust GC/MS/MS Analysis of 203 Pesticides in 10 Minutes in Spinach

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Abstract

This application note describes two approaches for achieving robust, multiresidue pesticide analysis in 10 minutes by GC/MS/MS, while maintaining sufficient chromatographic resolution for the analysis of over 200 pesticides in spinach; a challenging high chlorophyll, fresh matrix. First, the conventional 15 × 15 m (0.25 mm × 0.25 µm) midcolumn backflush configuration was used with an accelerated oven ramp, yielding an analysis time of 10 minutes. Second, a minibore 10 × 10 m (0.18 mm × 0.18 µm) midcolumn backflush configuration was used, enabling a fast 10-minute analysis time. The latter method was precisely scaled using the Agilent GC method translation technique. It was shown that midcolumn backflushing enabled method robustness and extended maintenance-free operation of the system by minimizing column trimming and source cleaning. Results demonstrate that the Agilent 7000E and 7010C triple quadrupole GC/MS systems delivered excellent linearity over a concentration range of 0.1 to 1,000 parts per billion (ppb). Method robustness was shown with 700 consecutive injections of a spinach extract, spiked with pesticides at 20 ppb, that spanned over 175 hours of continuous running of the GC/TQ.

Introduction

There is a growing demand for more rapid methods for the identification and quantitation of chemical residues in food analysis without sacrificing method robustness and chromatographic performance. Conventional methods for multiresidue pesticide analysis typically take at least 20 minutes, resulting in longer sample cycle times. As a result, the GC/MS analysis time for a batch of samples could easily span over several days. This causes a sample analysis bottleneck and limits lab productivity. Therefore, shortening the GC/MS analysis time will undoubtedly improve sample analysis throughput and eventually laboratory productivity. However, shortened GC methods usually involve trade-offs in method robustness or performance. This application note focuses on demonstrating two fast GC/MS/MS methods using (a) the Agilent 8890 GC and 7000E triple quadrupole GC/MS system and (b) the Agilent 8890 GC and 7010C triple quadrupole GC/MS system. The presented methods provide a shortened run time of 10 minutes, while maintaining robust system performance in the challenging spinach extract, without loss in sensitivity or method performance.

Two GC/TQ system midcolumn backflush configurations described in this application note provide analysis times of 10 minutes, while maintaining sufficient chromatographic resolution and MS selectivity for the analysis of 203 compounds. The conventional 20-minute GC/MS/MS method, retention time locked to the Agilent MassHunter pesticides and environmental pollutants MRM database (P&EP MRM database), was used as a benchmark for the optimized, fast analyses.

First, the conventional 15 × 15 m (0.25 mm × 0.25 µm) midcolumn backflush configuration was used with an accelerated oven ramp, yielding an analysis time of 10 minutes. This configuration did not require any hardware changes. Second, a minibore 10 × 10 m (0.18 mm × 0.18 µm) midcolumn backflush configuration was used enabling a 10-minute analysis time. This configuration required a new set of columns when compared to the conventional 15 × 15 m setup and a GC oven insert (a pillow). However, the second configuration allowed for more accurate prediction of the retention times and preserved the elution order for all tested compounds.

With both fast methods, the retention times were accurately predicted using the retention times available in the P&EP MRM database.[1] Using the GC method translation technique and maintaining the same column phase ratio allowed for accurately predicting the retention times and maintaining elution order for the 203 analyzed pesticides with the 10 × 10 m configuration. To update the retention times for the 10-minute method with the conventional 15 × 15 m configuration, a combination of pesticides and n-alkanes were used.

Midcolumn backflushing with both column configurations improved method robustness by reducing the regular maintenance frequency, such as column head trimming and source cleaning. Also, when used with a temperature-programmable multimode inlet (MMI), the liner change and other inlet maintenance procedures can be conducted much more rapidly without cooling down and venting the MS source, compared to a conventional configuration with a column connecting the inlet directly to the mass spectrometer.

The developed methods were applicable for analyzing pesticides to cover the broad range of maximum residue limits (MRLs) for different pesticides in spinach and to deliver excellent calibration performance over a dynamic range of 0.1 to 1,000 ppb.

To evaluate method robustness, a test of 700 continuous injections of the spinach extract spiked with low-level pesticides was performed. Relative standard deviation (RSD) for the response of many challenging analytes was under 15% over 700 injections. There was no need to trim the column, clean the source, or tune the MS over the test. The maintenance was limited to liner and septum replacement every 100 injections.

Experimental

GC/TQ analysis

Two column configurations used with the 8890/7000E and 8890/7010C GC/TQ combinations are shown in Figure 1. The GC was configured with the Agilent 7693A automatic liquid sampler (ALS) and 150-position tray; an MMI, operated in temperature-programmed splitless injection mode (cold splitless); a midcolumn backflush capability provided by the Agilent purged Ultimate union (PUU), installed between two identical 15 or 10 m columns; and the 8890 GC pneumatic switching device (PSD) module. The instrument operating parameters are listed in Table 1. Data were acquired in dynamic MRM (dMRM) mode, which enables the capability for large multi-analyte assays and to accurately quantitate narrow peaks by an automated and most-efficient dwell time distribution.

The dMRM capability enabled a successful analysis for a large panel of 203 pesticides with 614 total MRM transitions. The maximum number of concurrent MRM transitions with the conventional 15 × 15 m configuration and a traditional 20-minute analysis was 52. For the 10-minute analysis, the maximum number of concurrent MRM transitions with the conventional 15 × 15 m and the minibore 10 × 10 m configurations were 127 and 83, respectively (Figure 2). Furthermore, dMRM enables the analyst to add and

remove additional analytes with ease. The use of the P&EP MRM database increased the ease and speed of setting up a targeted dMRM method.

Agilent MassHunter Workstation revisions 10.1 and 10.2 including MassHunter Acquisition software for GC/MS systems 10.2, MassHunter Quantitative Analysis software 10.1, and MassHunter Qualitative Analysis software 10 packages were used in this work.

Calibration performance was evaluated using a series of matrix-matched calibration standards ranging from 0.1 to 1,000 ppb, including 0.1, 0.5, 1, 5, 10, 50, 100, 250, 500, and 1,000 ppb (w/v). The GC multiresidue pesticide kit containing 203 compounds (Restek, Bellefonte, PA, USA), regulated by the FDA, USDA, and other global governmental agencies, was used for preparing matrix-matched calibration standards. α-BHC-d6, at a final concentration of 20 ppb in vial, was used as the internal standard for quantitation of the target pesticides (Agilent Bond Elut QuEChERS IS standard number 6; part number PPS-610-1). A weighting factor of 1/x was applied to all calibration curves.

Retention time locking the 10-minute methods

Retention time locking allows a new column or instrument to have retention times that match the MRM database or an existing method exactly, allowing methods to be easily ported from one instrument to another and across instruments globally. This simplifies method maintenance and system setup. The retention times for the conventional 20-minute pesticide analysis are provided in the P&EP MRM database. The same GC column flow at which the 20-minute analysis was locked to the P&EP MRM database was used with the 10-minute method with the conventional 15 × 15 m configuration. This resulted in the new locking retention time for chlorpyrifos-methyl at 5.520 minutes. To update the retention times for the rest of the analytes, a combination of pesticides and n-alkanes were used to predict retention times for the new method based on the retention times for a 20-minute method from the P&EP MRM database.

The 10-minute analysis using the minibore 10 × 10 m configuration was precisely scaled using the method translation tool, providing a speed gain of 2. The fine tuning of the method enabled the best match between predicted and observed retention times across the elution range of 203 pesticides, which resulted in the 0.09 minutes offset. New retention times (RT) were calculated using the following equation:

$RT_{new} = RT_{old}/2 + 0.09$ minutes.

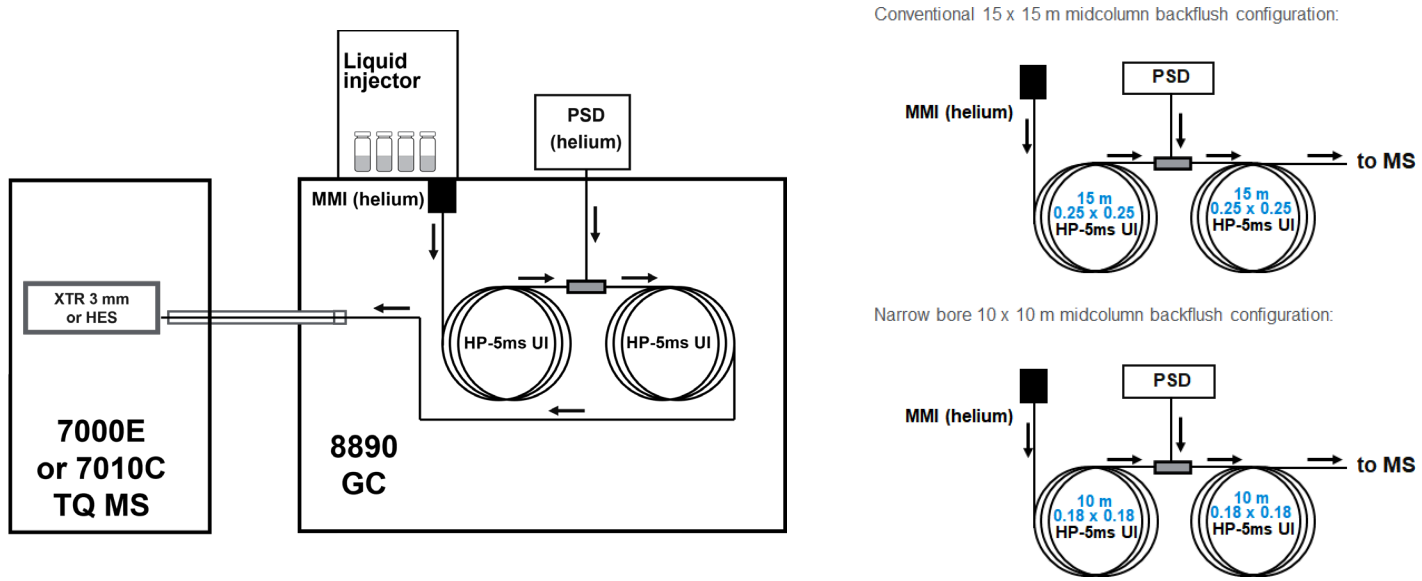


Figure 1. The Agilent GC/TQ system featuring two utilized midcolumn backflush configurations (right).

Table 1. Agilent 8890 GC and 7000 Series GC/TQ and the Agilent 8890 GC and 7010C GC/TQ system conditions enabling 10-minute pesticide analysis.

GC		
Agilent 8890 GC (220 V oven) with fast oven, auto injector, and tray		
Inlet	Multimode inlet (MMI)	
Mode	Cold splitless	
Purge Flow to Split Vent	60 mL/min at 0.75 min	
Septum Purge Flow	3 mL/min	
Septum Purge Flow Mode	Switched	
Injection Volume	1.0 µL	
Injection Type	Standard	
L1 Airgap	0.2 µL	
Gas Saver	On at 30 mL/min after 3 min	
Inlet Temperature	60 °C for 0.1 min, then to 280 °C at 600 °C/min	
Post Run Inlet Temperature	310 °C	
Post Run Total Flow	25 mL/min	
Carrier Gas	Helium	
Inlet Liner	Agilent Ultra Inert 2 mm dimpled liner	
Inlet Liner Part Number	5190-2297	
Oven		
	With 15 × 15 m	**With 10 × 10 m**
Initial Oven Temperature	60 °C	60 °C
Initial Oven Hold	1 min	0.5 min
Ramp Rate 1	80 °C/min	80 °C/min
Final Temp 1	170 °C	170 °C
Final Hold 1	0 min	0 min
Ramp Rate 2	35 °C /min	20 °C /min
Final Temp 2	310 °C	310 °C
Final Hold 2	3.625 min	1.125 min
Total Run Time	10 min	10 min
Post Run Time	1.5 min	1.5 min
Equilibration Time	0.25 min	0.25 min
		High-speed oven insert (pillow)

Column 1		
	With 15 × 15 m	**With 10 × 10 m**
Type	Agilent J&W HP-5ms Ultra Insert	Agilent J&W HP-5ms Ultra Inert
Agilent Part Number	19091S-431UI-KEY	19091S-571UI
Length	15 m	10 m
Diameter	0.25 mm	0.18 mm
Film Thickness	0.25 µm	0.18 µm
Control Mode	Constant flow	Constant flow
Flow	1.016 mL/min	1.3 mL/min
Inlet Connection	Multimode inlet (MMI)	Multimode inlet (MMI)
Outlet Connection	PSD (PUU)	PSD (PUU)
PSD Purge Flow	5 mL/min	5 mL/min
Post Run Flow (Backflushing)	–7.873	–3.174
Column 2		
	With 15 × 15 m	**With 10 × 10 m**
Type	Agilent J&W HP-5ms Ultra Inert	Agilent J&W HP-5ms Ultra Inert
Agilent Part Number	19091S-431UI-KEY	19091S-571UI
Length	15 m	10 m
Diameter	0.25 mm	0.18 mm
Film Thickness	0.25 µm	0.18 µm
Control Mode	Constant flow	Constant flow
Flow	1.216 mL/min	1.5 mL/min
Inlet Connection	PSD (PUU)	PSD (PUU)
Outlet Connection	MSD	MSD
Post Run Flow (Backflushing)	8.202	3.290

MSD		
Model	Agilent 7000 series (7000D and 7000E) or 7010C triple quadrupole GC/MS	
Source	Inert Extractor Source with a 3 mm lens or HES	
Vacuum Pump	Performance turbo	
Tune File	Atunes.eiex.jtune.xml or Atunes.eihs.jtune.xml	
Solvent Delay	3 min	
Quad Temperature (MS1 and MS2)	150 °C	
Source Temperature	280 °C	
Mode	dMRM	
He Quench Gas	2.25 mL/min	
N_2 Collision Gas	1.5 mL/min	
MRM Statistics		
	With 15 × 15 m	**With 10 × 10 m**
Total MRMs (dMRM Mode)	614	614
Minimum Dwell Time	2.33 ms	3.99 ms
Minimum Cycle Time	167.86 ms	110.38 ms
Maximum Concurrent MRMs	127	83
EM Voltage Gain Mode	10	10

Sample preparation

A sample preparation workflow chart is shown in Figure 3. The sample preparation included two major steps: sample extraction by traditional QuEChERS extraction, followed by Captiva enhanced matrix removal (EMR) pass-through cleanup. The Agilent Captiva EMR–High Chlorophyll Fresh with NH_2 (Captiva EMR–HCF1) cartridge was used for high chlorophyll fresh matrix (spinach). The new sample preparation workflow demonstrates as a simplified procedure with improvement on both sample matrix removal and targets quantitation data quality.

As shown in Figure 3, samples were first extracted using the traditional Agilent Bond Elut QuEChERS EN extraction kit (part number 5982-5650CH). Homogenized fresh spinach (10 g) was used for extraction. The 10 mL of ACN with 1% acetic acid was then added, followed by extraction. After extraction, 3 mL of crude extract was transferred to a Captiva EMR–HCF1 cartridge (part number 5610-2088) for pass-through cleanup. The sample eluent was collected and further dried by anhydrous $MgSO_4$ (part number 5982-0102). Samples were then ready for GC/TQ analysis. The Agilent positive pressure manifold 48 processor (PPM-48; part number 5191-4101) was used for Captiva EMR pass-through cleanup processing.

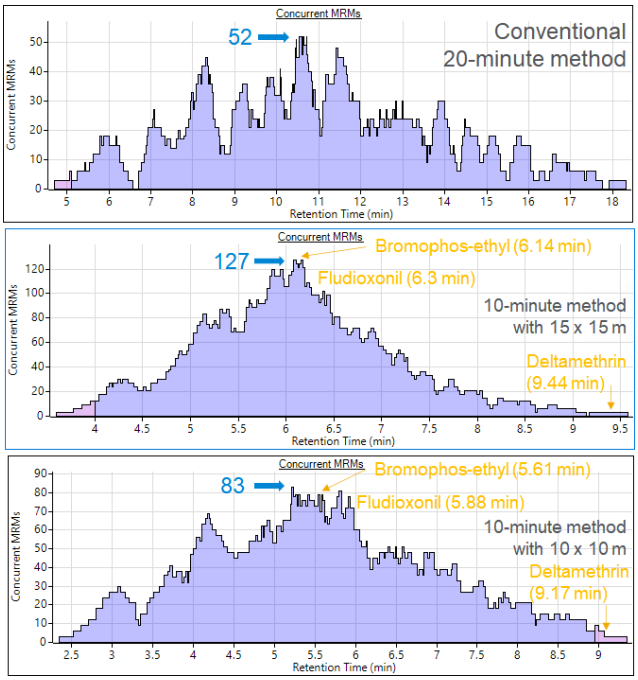


Figure 2. The distribution of 614 dMRM transitions with the 20-minute conventional pesticide analysis, the 10-minute analysis employing the conventional 15 × 15 m configuration, and the 10-minute method employing the minibore 10 × 10 m column configuration.

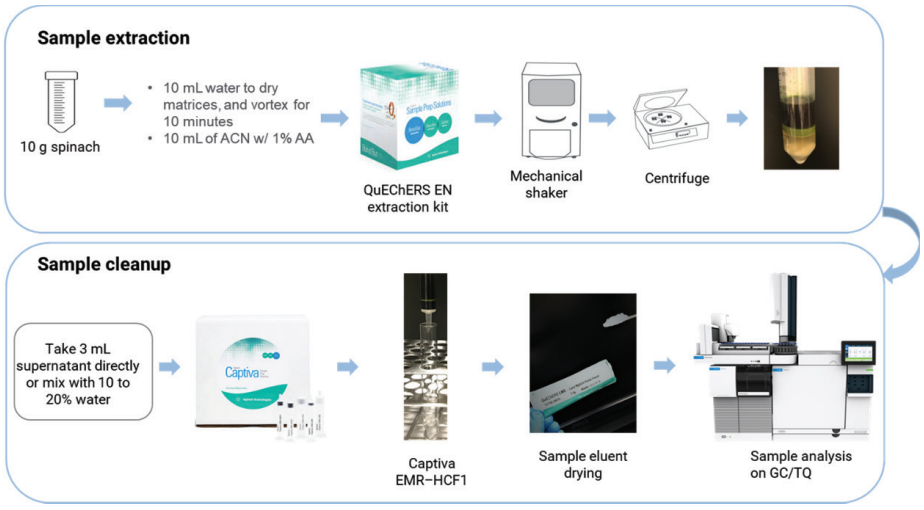


Figure 3. Sample preparation flowchart including traditional Agilent QuEChERS extraction, followed by Agilent Captiva EMR pass-through cleanup.

Results and discussion

Maintaining chromatographic resolution with the 10-minute analysis of over 200 pesticides

The presented GC midcolumn backflush configurations, including the conventional 15 × 15 m and the minibore 10 × 10 m configurations, enabled the 10-minute analysis of 203 pesticides with three MRM transitions acquired per each compound. Figure 4 demonstrates that the chromatographic resolution with the fast, 10-minute method was largely maintained with the conventional 15 × 15 m setup (Figure 4A) and completely preserved with the minibore 10 × 10 m setup (Figure 4B). The GC method translation technique used for transferring the method to the 10 × 10 m configuration allowed for preserving the relative elution order of the compounds.

Sensitivity and calibration performance over a wide dynamic range with the 10-minute separations

The method sensitivity achieved with the different column configurations and 10-minute separations was comparable to that observed with the conventional 20-minute method. Both 10-minute methods with the 15 × 15 m and the 10 × 10 m column configurations allowed for detecting all the targeted pesticides below their regulated MRLs, even for the most challenging ones. For example, deltamethrin, a challenging compound for GC/MS, was shown to be accurately quantitated in spinach down to 0.1 ppb with the 7010C GC/TQ and 1 to 5 ppb with the 7000 series GC/TQ (Figure 5A). While deltamethrin does not have an established MRL in spinach, it is regulated in many other food commodities including vegetable groups 8 and 9, and subgroups IB and IC, with the MRLs at 40 to 300 ppb.[2] The observed calibration ranges with the 7010 GC/TQ and the 7000 series GC/TQ would allow analysts to meet their analytical needs for the analysis of deltamethrin in various food matrices.

While deltamethrin is known to be challenging for GC/MS analysis, its elution at the end of the 10-minute analysis results in few concurrent MRM transitions. With only a few concurrent MRM transitions, the MRMs monitored for deltamethrin have relatively long dwell times (above 50 ms) even with the fast 10-minute methods (Figure 2). On the contrary, fludioxonil, a fungicide with an established MRL of 10 ppb in spinach[3], elutes during the crowded segment of the MRM methods with 120 and 80 concurrent MRM transitions in the 15 × 15 m method and the 10 × 10 m method configurations, respectively. Despite relatively short dwell times of 3 and 4.9 ms with the two configurations, fludioxonil was accurately quantitated down to 0.1 ppb with both the 7010C and the 7000 series GC/TQ systems with at least ten data points across the peak (Figure 5B). The 7010C GC/TQ equipped with the high efficiency source (HES) demonstrated superior sensitivity compared to the 7000 series GC/TQ. It allows for accurate quantitation below 0.1 ppb, even though this was not required in this work, as the MRLs for pesticides regulated in most food commodities by US EPA do not require sub-0.1 ppb quantitation. Similarly, bromophos-ethyl eluted in a crowded retention time window with a high number of concurrently monitored MRM transitions, leading to a short dwell time of 2.7 and 4.7 ms with the 15 × 15 m and the 10 × 10 m configurations, respectively. Bromophos-ethyl has recommended tolerances ranging from 20 to 2,000 ppb in various commodities.[4] Figures 5B and 5C demonstrate that fludioxonil and bromophos-ethyl were accurately quantitated over the wide concentration range of 0.1 to 1,000 ppb with excellent sensitivity and linearity in the challenging spinach matrix and at least nine data points across the peak.

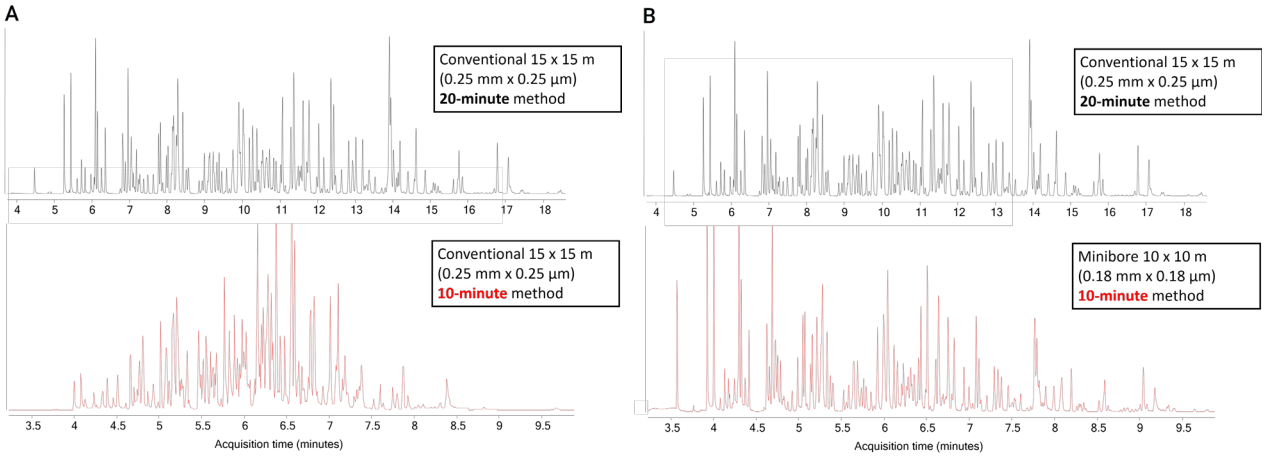
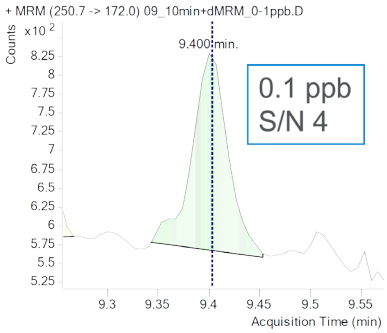
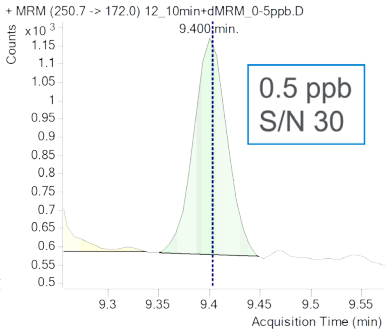


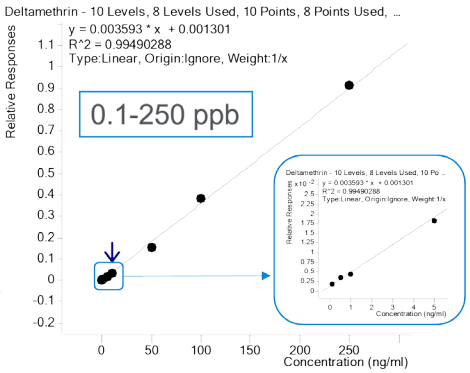
Figure 4. MRM total ion current chromatograms (TIC) or a mixture of 203 pesticides acquired with (A) the conventional 15 × 15 m configuration and (B) with the minibore 10 × 10 m configuration.

A) Deltamethrin

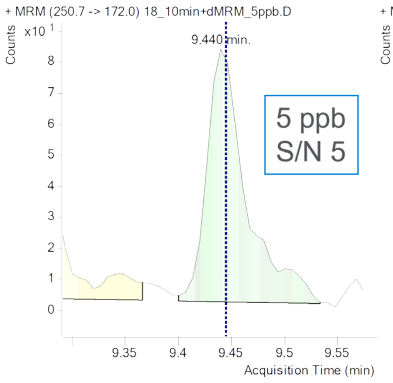
7010C
15 x 15 m

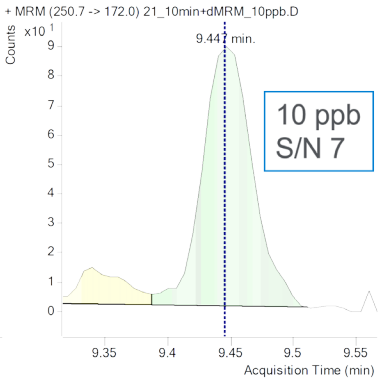


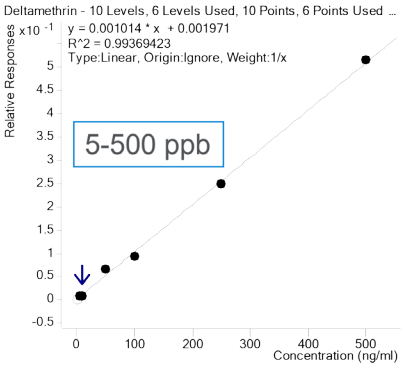




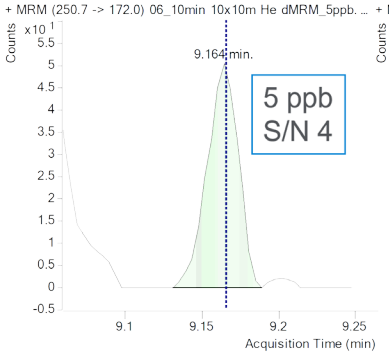
7000E
15 x 15 m

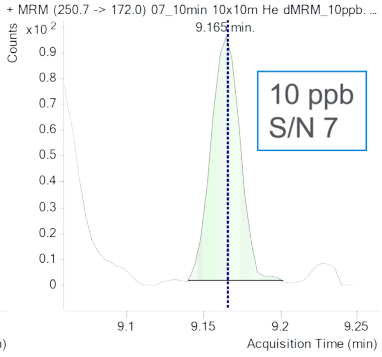


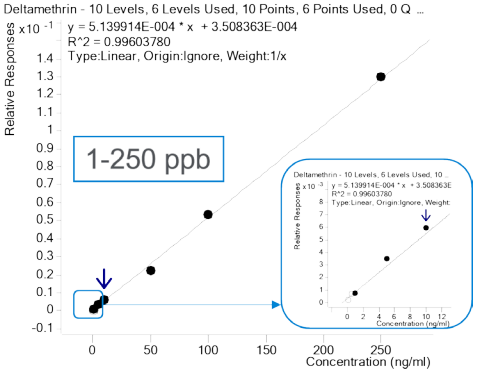




7000 series
10 x 10 m

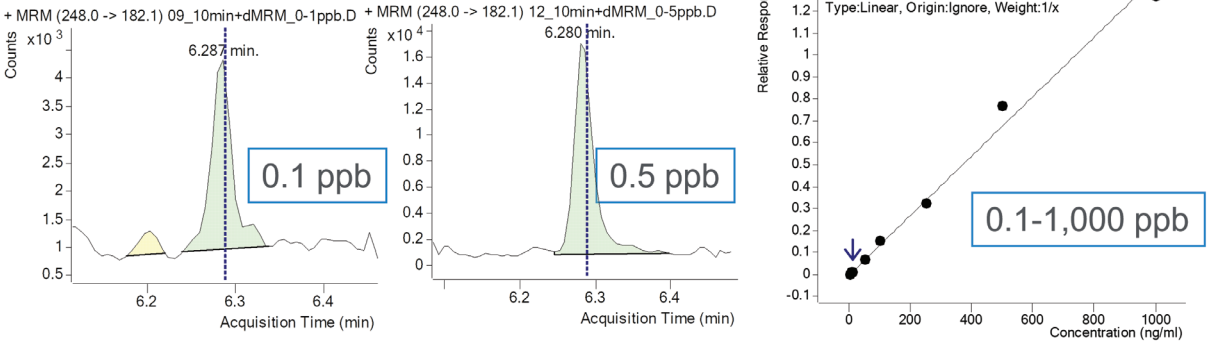




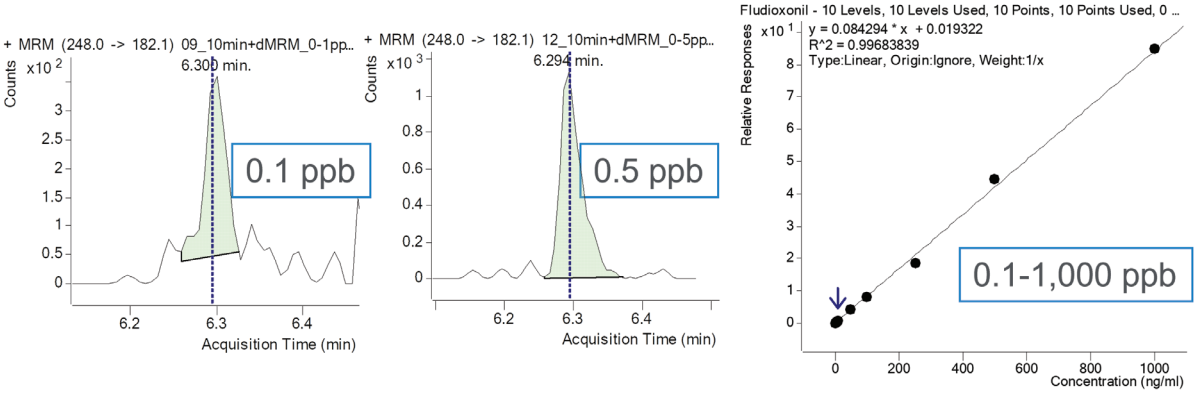


B) Fludioxonil

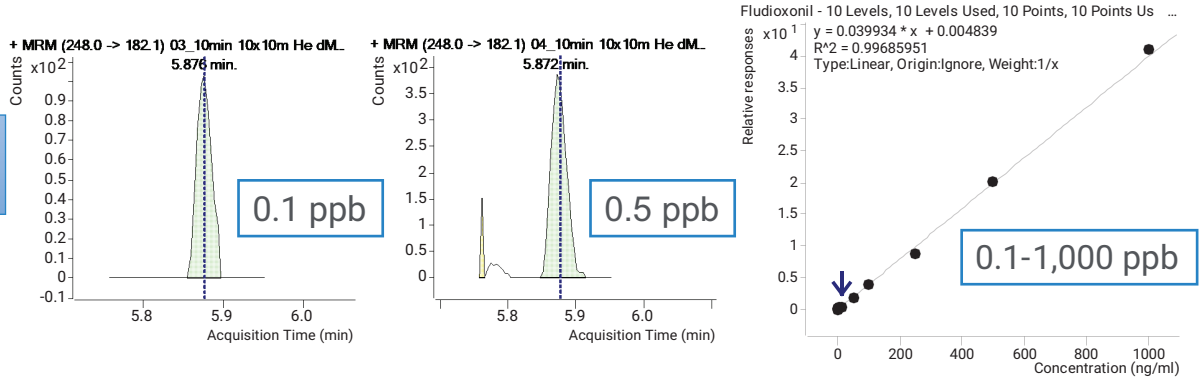
7010C
15 x 15 m



7000E
15 x 15 m



7000 series
10 x 10 m



C) Bromophos-ethyl

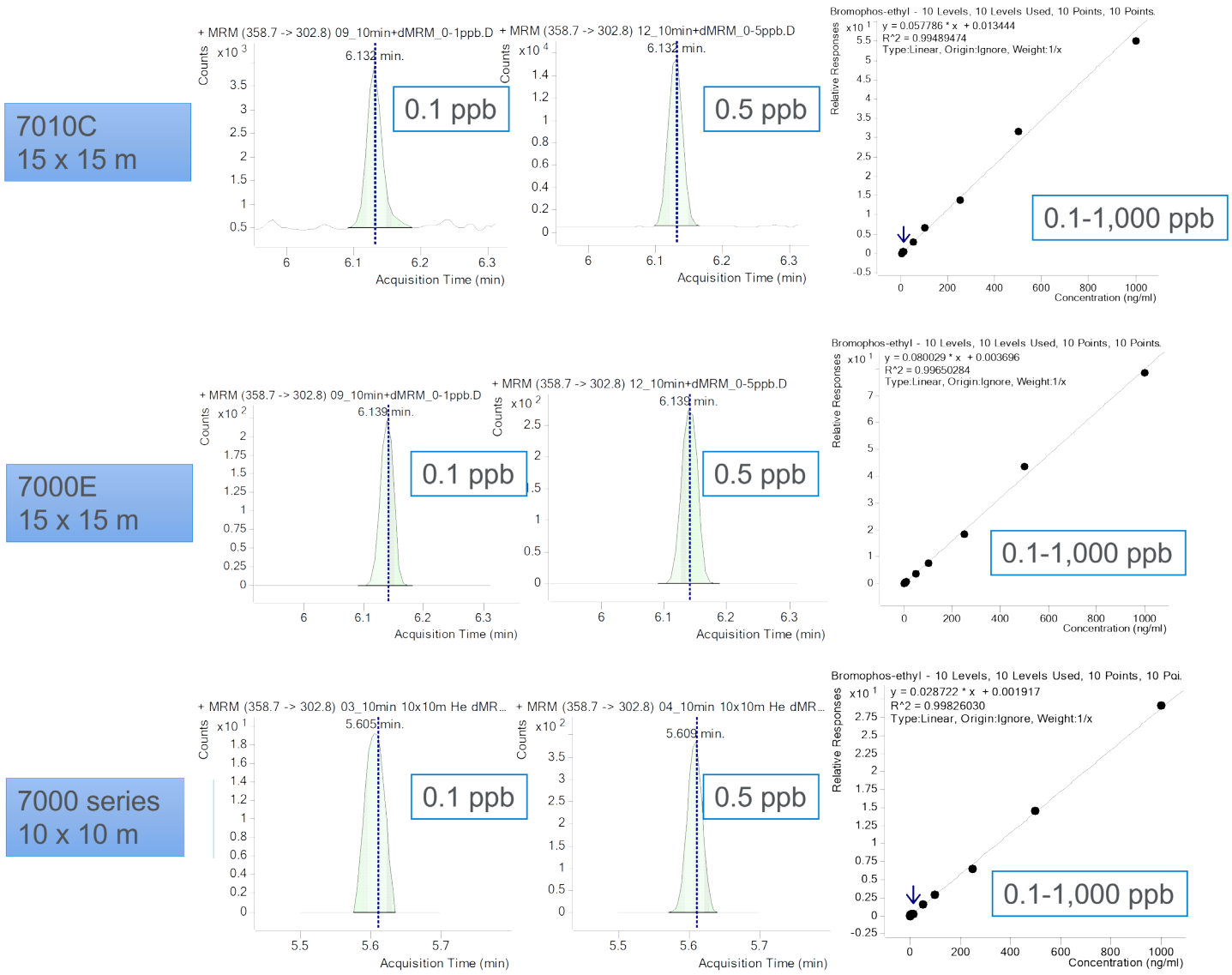


Figure 5. MRM chromatograms and matrix-matched calibration curves in spinach for (A) deltamethrin, (B) fludioxonil, and (C) bromophos-ethyl observed with different column configurations and 10-minute separations using the Agilent 7010C and 7000 series triple quadrupole GC/MS systems.

The biggest challenge with multiresidue pesticide analysis is that the MRLs established for pesticides in different food commodities vary significantly. This may require undesirable sample re-injection if the method calibration ranges do not encompass all the MRLs for the compounds of interest. A broad dynamic calibration range is desirable to use the more generic quantitation method for analyzing different pesticides in the commodity and for various foods and to simplify the sample pretreatment before instrument detection, such as further dilution. Figure 6 summarizes the calibration performance for the 203 pesticides that were analyzed in spinach with the 10-minute separations using the conventional 15 × 15 m configuration coupled with the 7010C and the 7000E GC/TQ, and the minibore 10 × 10 m configuration coupled with the 7000 series GC/TQ. The graph shows the number of compounds with the calibration correlation coefficient $R^2 > 0.99$, using the different regression fit (linear or quadratic), within the different calibration ranges.

Most of the target compounds demonstrated linear calibration curves over a wide range of either 0.1 to 1,000 ppb or 0.5 to 1,000 ppb, enabling their reliable quantitation at the varying MRLs established for different compounds.

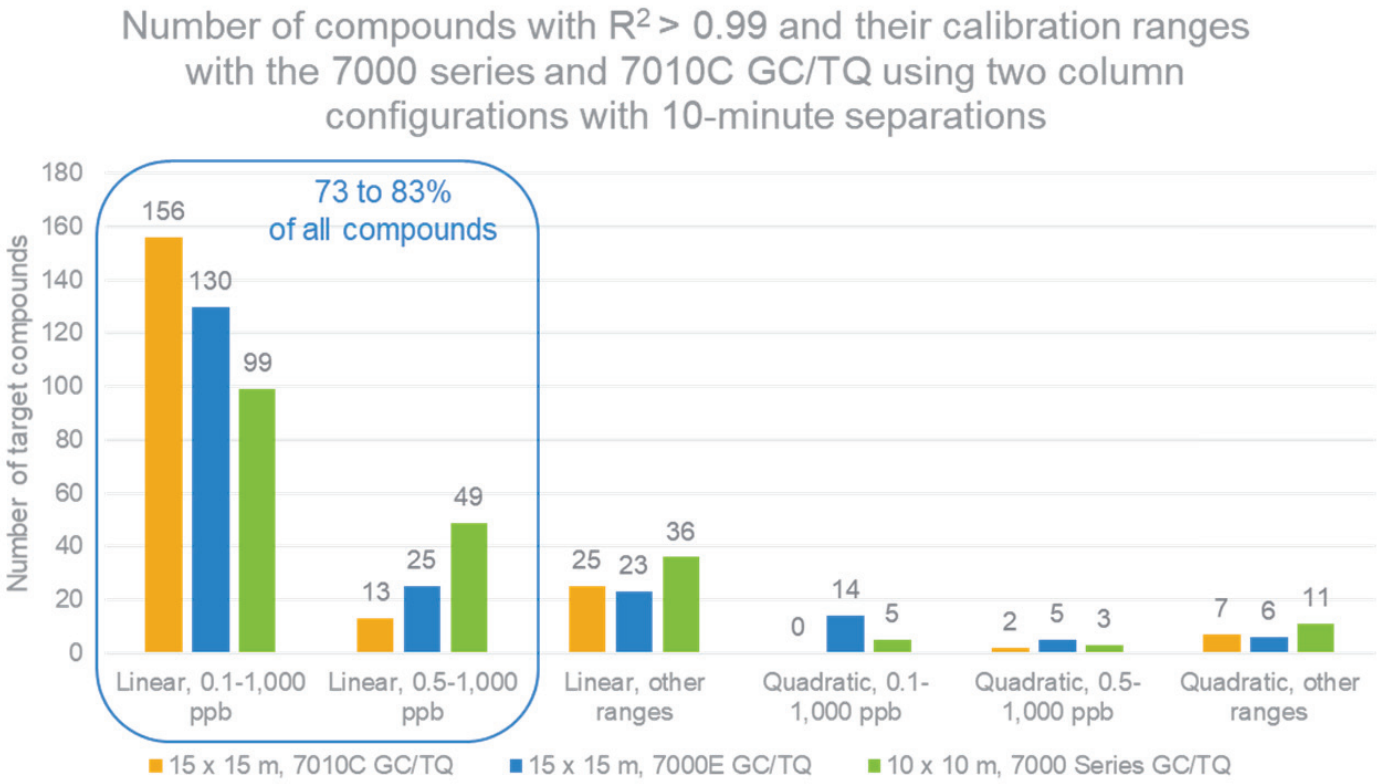


Figure 6. Calibration performance for the 203 pesticides with the 10-minute methods using the conventional 15 × 15 m configuration, coupled with the Agilent 7010C and 7000E triple quadrupole GC/MS systems, and the minibore 10 × 10 m configuration, coupled with the Agilent 7000 series triple quadrupole GC/MS in spinach. The graph shows the number of compounds and their calibration ranges.

Method robustness with 700 injections of a spinach extract

The robustness of the 10-minute analysis was demonstrated by analyzing a challenging, highly pigmented spinach extract spiked with pesticides at 20 ppb. The area of the analytes was monitored over 700 consecutive injections. Analyte response, normalized by the internal standards (ISTD), remained consistent over 700 injections that spanned over 175 hours of continuous running with the 10-minute method, using the conventional 15 × 15 m column configuration coupled with the 7000E GC/TQ. The only maintenance procedure performed during the robustness testing involved septum and liner replacement every 100 injections.

There was no need to perform inlet cleaning, GC column trimming, or MS source cleaning, or retune the MS during the entire study that involved over 1,000 injections (robustness testing over 700 runs and additional analyses performed for system evaluation and calibration).

The keys to successful and robust pesticide analysis that enables stable GC/TQ performance for over 700 injections are described in the application note 5994-4965EN.[5] The best practices used in this work included:

- Simplified and improved sample preparation achieved with the novel and improved Captiva EMR pass-through cleanup following traditional QuEChERS extraction
- Evaluation of in-source loading of the matrix in full scan data acquisition mode
- Postrun backflushing enabled with the conventional 15 × 15 m and the minibore 10 × 10 m midcolumn backflush configurations
- Leak-free GC/TQ system enabled with the self-tightening collared column nuts and CFT gold-plated flexible metal ferrules
- Use of temperature-programmed MMI with a 2 mm Ultra Inert dimpled liner (no glass wool)

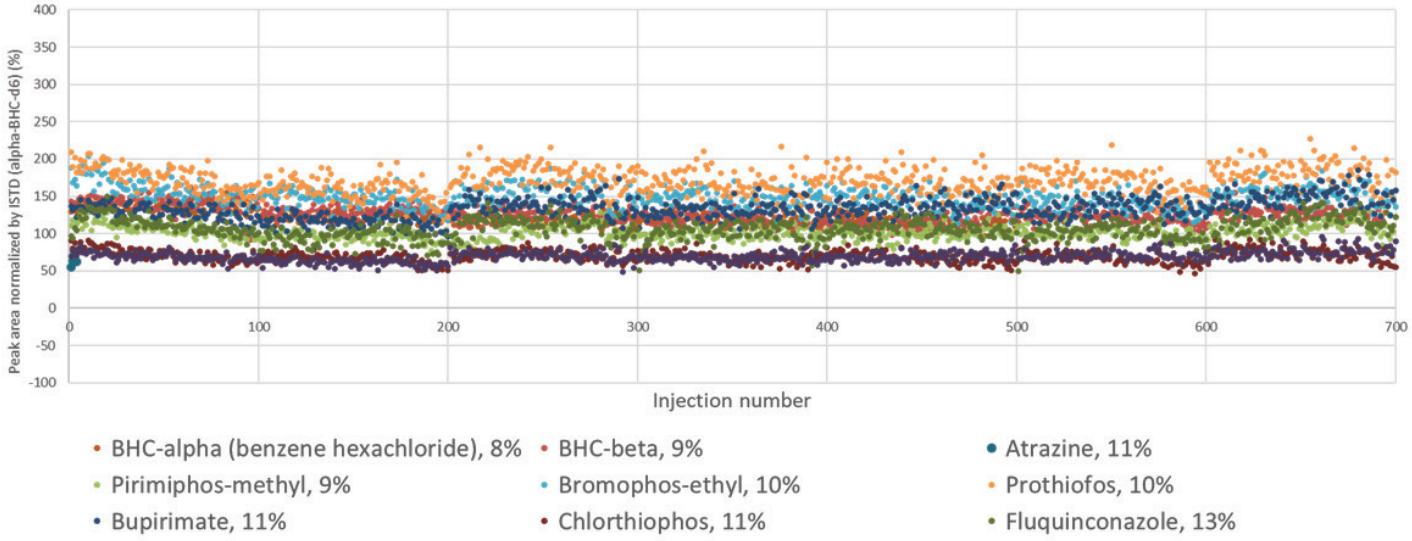


Figure 7. Stability of the peak area for pesticides spiked at 20 ppb into spinach extract, normalized by the ISTD, over 700 consecutive injections. The 10-minute analysis using the conventional 15 × 15 m column configuration coupled with the Agilent 7000E triple quadrupole GC/MS.

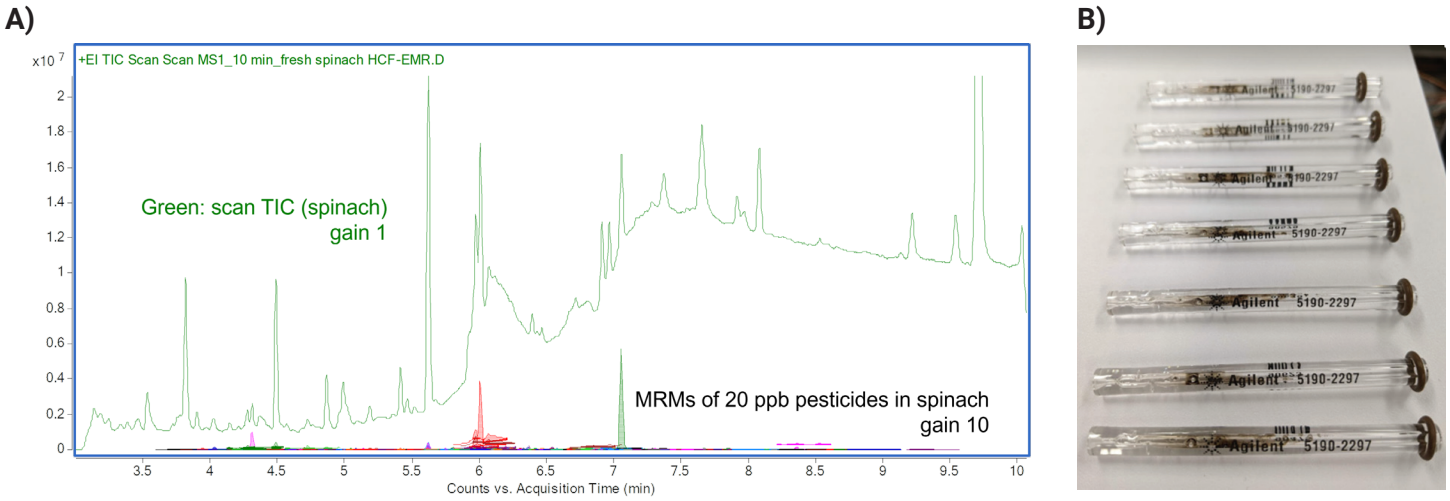


Figure 8. (A) TIC of a full scan chromatogram acquired for spinach extract and the MRM TIC for 20 ppb pesticides. (B) The GC inlet liners replaced after 100 injections when analyzing spinach extract during the robustness evaluation.

Highly pigmented spinach extract selected for the robustness testing was demonstrated to have a relatively high background in full scan data acquisition mode, as shown in Figure 8A, compared to the abundance of the MRM signal for pesticides at 20 ppb. The liners replaced after 100 injections, seven times during the robustness study, are shown in Figure 8B. This indicates that spinach extract truly presents a challenge for GC/MS analysis, hence, served as a suitable matrix for robustness performance evaluation.

Conclusion

This application note described two GC/TQ system configurations using midcolumn backflush that both enable robust pesticide analysis in 10 minutes, while maintaining sufficient chromatographic resolution for 203 compounds. The conventional 15 × 15 m (0.25 mm × 0.25 µm) and the minibore 10 × 10 m (0.18 mm × 0.18 µm) midcolumn backflush configurations were used to achieve a 10-minute analysis time. Results demonstrate that excellent linearity, over a calibration dynamic range of 0.1 to 1,000 ppb or 0.5 to 1,000 ppb, was achieved with the Agilent 7010C and 7000 series triple quadrupole GC/MS systems. Method robustness was shown with 700 consecutive injections of spinach extract spiked with pesticides at 20 ppb.

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Appendix 1

Compounds analyzed in this work and their observed retention times with two-column configurations and 10-minute separations.

Name	Retenion Time (min)		Name	Retenion Time (min)	
	15 × 15 m	10 × 10 m		15 × 15 m	10 × 10 m
Allidochlor	3.773	2.542	BHC-gamma (Lindane, gamma HCH)	5.201	4.174
Dichlorobenzonitrile, 2,6-	3.972	2.720	Pyrimethanil	5.222	4.246
Biphenyl	4.055	2.812	Tefluthrin	5.223	4.310
Mevinphos, E-	4.110	2.901	Fonofos	5.225	4.223
3,4-Dichloroaniline	4.193	2.954	Pentachloronitrobenzene	5.227	4.210
Pebulate	4.223	3.006	Pentachlorobenzonitrile	5.247	4.228
Etridiazole	4.246	3.016	Disulfoton	5.273	4.312
N-(2,4-dimethylphenyl)formamide	4.305	3.091	Isazofos	5.285	4.361
cis-1,2,3,6-Tetrahydrophthalimide	4.312	3.090	Terbacil	5.285	4.323
Methacrifos	4.321	3.129	Triallate	5.322	4.379
Chloroneb	4.375	3.171	BHC-delta	5.330	4.351
2-Phenylphenol	4.444	3.228	Chlorothalonil	5.350	4.392
Pentachlorobenzene	4.495	3.276	Propanil	5.463	4.570
Propachlor	4.702	3.546	Endosulfan ether	5.466	4.523
Tecnazene	4.712	3.547	Transfluthrin	5.476	4.658
Diphenylamine	4.734	3.582	Dimethachlor	5.477	4.596
Cycloate	4.757	3.626	Pentachloroaniline	5.482	4.552
Chlorpropham	4.769	3.656	Acetochlor	5.502	4.641
2,3,5,6-Tetrachloroaniline	4.793	3.633	Vinclozolin	5.503	4.654
Trifluralin	4.798	3.724	Parathion-methyl	5.526	4.668
Benfluralin	4.811	3.740	Chlorpyrifos-methyl	5.526	4.668
Ethalfluralin	4.812	3.670	Tolclofos-methyl	5.559	4.710
Sulfotep	4.869	3.789	Alachlor	5.564	4.725
Diallate I	4.928	3.846	Propisochlor	5.579	4.765
Phorate	4.932	3.852	Metalaxyl	5.583	4.763
BHC-beta	5.010	4.115	Ronnel	5.614	4.791
BHC-alpha (benzene hexachloride)	5.011	3.918	Prodiamine	5.622	4.871
Hexachlorobenzene	5.069	3.987	Heptachlor	5.630	4.763
Atrazine	5.072	4.048	Pirimiphos-methyl	5.650	4.892
Dichloran	5.072	3.998	Fenitrothion	5.676	4.891
Pentachloroanisole	5.083	4.013	Malathion	5.696	4.962
Clomazone	5.122	4.092	Linuron	5.708	4.927
Profluralin	5.123	4.156	Dichlofluanid	5.745	4.980
Terbuthylazine	5.155	4.163	Pentachlorothioanisole	5.767	4.972
Terbufos	5.173	4.178	Aldrin	5.768	5.061
Propyzamide	5.175	4.188	Fenthion	5.779	5.057
Diazinon	5.191	4.244	Metolachlor	5.783	5.046
Fluchloralin	5.199	4.261	Chlorpyrifos	5.790	5.075

Name	Retenion Time (min)		Name	Retenion Time (min)	
	15 × 15 m	10 × 10 m		15 × 15 m	10 × 10 m
Parathion	5.793	5.081	Chlorfenson	6.275	5.784
Triadimefon	5.811	5.100	Nonachlor, trans-	6.279	5.787
DCPA (Dacthal, Chlorthal-dimethyl)	5.829	5.124	Dieldrin	6.279	5.955
Anthraquinone	5.831	5.053	Fludioxonil	6.294	5.876
Dichlorobenzophenone, 4,4'-	5.840	5.110	Prothiofos	6.300	5.844
Pirimiphos-ethyl	5.869	5.241	Oxadiazon	6.303	5.920
MGK-264	5.881	5.315	Pretilachlor	6.303	5.895
Isopropalin	5.898	5.267	Iodofenphos	6.304	5.828
Fenson	5.902	5.194	Profenofos	6.312	5.877
Diphenamid	5.908	5.235	Oxyfluorfen	6.314	5.960
Bromophos	5.918	5.237	DDE-p,p'	6.342	5.906
Cyprodinil	5.941	5.314	Bupirimate	6.361	6.014
Pendimethalin	5.975	5.356	Myclobutanil	6.364	5.970
Chlozolinate	5.976	5.378	Chlorfenapyr	6.365	6.122
Allethrin	5.979	5.393	Flusilazole	6.370	5.995
Triflumizole	5.979	5.473	Fluazifop-p-butyl	6.388	6.090
Fipronil	5.993	5.431	DDD-o,p'	6.404	5.990
Penconazole	5.998	5.375	Tricyclazole	6.412	5.932
Metazachlor	5.999	5.358	Endrin	6.423	6.153
Chlorfenvinphos	6.016	5.436	Ethylan	6.453	6.121
Heptachlor exo-epoxide	6.016	5.402	Nitrofen	6.477	6.101
Isodrin	6.018	5.319	Chlorobenzilate	6.506	6.189
Captan	6.020	5.472	Ethion	6.571	6.315
Tolylfluanid	6.026	5.413	DDD-p,p'	6.582	6.280
Bromfenvinfos-methyl	6.036	5.436	DDT-o,p'	6.582	6.318
Quinalphos	6.047	5.463	Chlorthiophos	6.587	6.338
Triadimenol	6.053	5.476	Endosulfan II (beta isomer)	6.603	6.235
Procymidone	6.090	5.515	Triazophos	6.644	6.428
Folpet	6.127	5.513	Sulprofos	6.659	6.420
Paclobutrazol	6.137	5.653	Nonachlor, cis-	6.667	6.341
Chlorbenside	6.137	5.549	Carfentrazone-ethyl	6.668	6.509
Bromophos-ethyl	6.139	5.609	Methoxychlor olefin	6.702	6.519
DDE-o,p'	6.176	5.631	Endrin aldehyde	6.709	6.402
Tetrachlorvinphos	6.181	5.680	Carbophenothion	6.726	6.513
Chlordane-trans	6.187	5.610	Norflurazon	6.754	6.576
Chlordane-cis	6.196	5.744	Edifenphos	6.786	6.566
Fenamiphos	6.227	5.797	Lenacil	6.787	6.588
Flutolanil	6.233	5.801	DDT-p,p'	6.805	6.615
Bromfenvinfos	6.252	5.800	Iprodione	6.826	6.947
Flutriafol	6.255	5.764	Methoxychlor, o,p'-	6.846	6.703
Endosulfan I (alpha isomer)	6.274	5.724	Endosulfan sulfate	6.852	6.610

Name	Retenion Time (min)		Name	Retenion Time (min)	
	15 × 15 m	10 × 10 m		15 × 15 m	10 × 10 m
Piperonyl butoxide	6.854	6.788	Acrinathrin	7.415	7.607
Propargite	6.856	6.760	Leptophos	7.417	7.413
Resmethrin	6.857	6.756	Pyrazophos	7.556	7.660
Hexazinone	6.861	6.708	Fenarimol	7.631	7.641
Tebuconazole	6.886	6.739	Mirex	7.636	7.533
Captafol	6.890	6.805	Pyraclofos	7.645	7.728
Nitralin	6.913	6.862	Azinphos-ethyl	7.675	7.700
Bifenthrin	7.044	7.057	Permethrin, (1R)-cis-	7.785	7.901
Pyridaphenthion	7.048	7.004	Permethrin, (1R)-trans-	7.842	7.962
Tetramethrin I	7.052	6.999	Pyridaben	7.916	7.980
Fenpropathrin	7.106	7.121	Coumaphos	7.964	8.028
Bromopropylate	7.109	7.061	Fluquinconazole	7.964	8.023
EPN	7.112	7.061	Prochloraz	7.988	8.058
Tebufenpyrad	7.130	7.152	Cyfluthrin I	8.157	8.184
Methoxychlor, p,p'-	7.131	7.111	Cypermethrin I	8.250	8.339
Phosmet	7.135	7.054	Flucythrinate I	8.359	8.444
Endrin ketone	7.189	7.033	Acequinocyl	8.409	8.534
Phenothrin I	7.230	7.243	Ethofenprox	8.431	8.485
Azinphos-methyl	7.330	7.405	Fluridone	8.708	8.662
Tetradifon	7.330	7.305	Fenvalerate I	8.881	8.799
Cyhalothrin (Lambda)	7.334	7.438	Fluvalinate-tau I	8.970	8.894
Pyriproxyfen	7.358	7.406	Deltamethrin	9.444	9.166
Phosalone	7.389	7.387			

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Application Note

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Dynamic MRM/Scan Mode: Adding More Confidence to Sensitive Quantitation in Complex Foods by Triple Quadrupole GC/MS (GC/TQ)

Authors

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Abstract

This application note describes the use of the novel simultaneous dynamic multiple reaction monitoring (dMRM) and scan (dMRM/scan) data acquisition mode for triple quadrupole gas chromatography mass spectrometry (GC/TQ) analysis of pesticides in challenging food matrices. The simultaneous dMRM/scan capability enables identification of the unknown compounds and retrospective analysis, while maintaining sensitivity and dynamic range of the method comparable to a conventional dMRM analysis. Additionally, scan data enables more confidence in compound identification by library spectrum matching. Finally, the full scan data allow the analyst to evaluate the sample matrix to ensure the most efficient performance of the GC/TQ system.

This work demonstrates the application of dMRM/scan to the analysis of extracts, using Agilent QuEChERS sample preparation, of spinach, walnut, and cayenne pepper spiked with over 200 pesticides. The calibration results and method sensitivity for 203 evaluated compounds were comparable to results observed with conventional dMRM data acquisition mode with the Agilent 8890/7000E GC/TQ and the Agilent 8890/7010C GC/TQ.

The unknown identification workflow based on the spectral library matching using a retention time locked library was carried out with Agilent MassHunter Unknowns Analysis. Many of the compounds with the established maximum residue limits (MRLs) were identified with full scan data at concentrations below their MRLs even in the challenging cayenne pepper extract.

Introduction

Concern about trace-level food contaminants is driving the demand for robust, rapid, and reliable methods for identification and quantitation of chemical residues and contaminants in food matrices. Usually, the detection methods such as triple quadrupole GC/MS and triple quadrupole LC/MS are aimed at a specific list of targets that are commonly found in food samples. These methods can be effective but may overlook any residues that are not specifically targeted. The approach to overcome this challenge is to perform untargeted screening of the sample intending to find as many compounds of concern as possible and allowing for retrospective analysis. Untargeted screening can be accomplished by analyzing the sample in full scan data acquisition mode.[1,2] However, targeted triple quadrupole GC/MS (GC/TQ) analysis has an advantage of higher sensitivity and selectivity for the target analytes when compared to full scan analysis. The novel simultaneous dynamic MRM and scan (dMRM/scan) allows for acquiring both targeted dMRM GC/TQ data for target quantitation as well as full scan data for unknowns screening. Also, the simultaneous dynamic MRM and scan (dMRM/scan) deliver confident identification based on spectral library matching.

In this work, three challenging matrices, including a high-chlorophyll fresh spinach matrix, an oily dry walnut matrix, and a complex dry cayenne pepper matrix were used. The matrix blank extracts were post spiked with over 200 GC-amenable pesticides. The samples at various concentration levels were analyzed in dMRM/scan data acquisition mode enabling target quantitation with dMRM data and unknown identification with the simultaneously acquired full scan data. The performance of the targeted GC/TQ method component was evaluated based on the method sensitivity and the calibration performance over a dynamic range. The screening component of the method was evaluated based on the number of identified compounds and the concentration at which they could be reliably detected in full scan.

Experimental

GC/TQ analysis

The 8890/7000E and 8890/7010C triple quadrupole GC/MS systems (GC/TQ) were used and configured to achieve the best performance over a wide calibration range (Figure 1A). This calibration range encompassed the varying maximum residue limits (MRLs) for pesticides regulated in the analyzed commodities. The GC was configured with the Agilent 7693A automatic liquid sampler (ALS) and 150-position tray. The system used a multimode inlet (MMI) operated in temperature-programmed splitless injection mode. Midcolumn backflush capability was provided by the Agilent Purged Ultimate Union (PUU) installed between two identical 15 m columns, and the 8890 pneumatic switching device (PSD) module (Figure 1B).

The instrument method parameters are listed in Table 1 and Figure 2 demonstrates how dMRM/scan mode is set up in the triple quadrupole MS Method Editor of Agilent MassHunter Workstation software and the

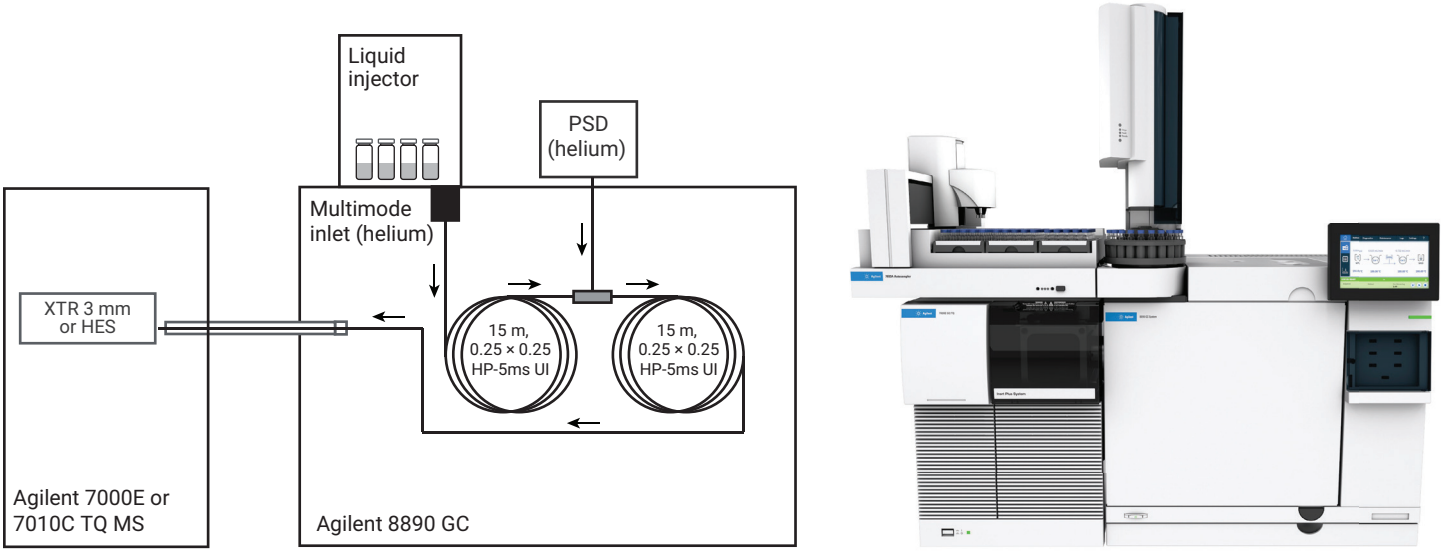

Figure 1. The Agilent 8890/7000E and 8890/7010C GC/TQ system (A) and system configuration (B).

Table 1. Agilent 8890/7000E and 8890/7010C GC/TQ conditions for simultaneous dynamic MRM and scan (dMRM/scan) pesticide analysis.

Parameter	Value
GC	Agilent 8890 with fast oven, auto injector and tray
Inlet	Multimode Inlet (MMI)
Mode	Splitless
Purge Flow to Split Vent	60 mL/min at 0.75 min
Septum Purge Flow	3 mL/min
Septum Purge Flow Mode	Switched
Injection Volume	1.0 µL
Injection Type	Standard
L1 Airgap	0.2 µL
Gas Saver	On at 30 mL/min after 3 min
Inlet Temperature	60 °C for 0.1 min, then to 280 °C at 600 °C/min
Post Run Inlet Temperature	310 °C
Post Run Total Flow	25 mL/min
Carrier Gas	Helium
Inlet Liner	Agilent Ultra Inert 2 mm dimpled liner, splitless
Inlet Liner Part Number	5190-2297
Oven	
Initial Oven Temperature	60 °C
Initial Oven Hold	1 min
Ramp Rate 1	40 °C/min
Final Temperature 1	170 °C
Final Hold 1	0 min
Ramp Rate 2	10 °C /min
Final Temperature 2	310 °C
Final Hold 2	2.25 min
Total Run Time	20 min
Post Run Time	1.5 min
Equilibration Time	0.25 min
Column 1	
Type	Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431UI-KEY)
Control Mode	Constant flow
Flow	1.016 mL/min
Inlet Connection	Multimode inlet (MMI)
Outlet Connection	PSD (PUU)
PSD Purge Flow	5 mL/min
Post Run Flow (Backflushing)	–7.873

Parameter	Value
Column 2	
Type	Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431UI-KEY)
Control Mode	Constant flow
Flow	1.216 mL/min
Inlet Connection	PSD (PUU)
Outlet Connection	MSD
Post Run Flow (Backflushing)	8.202
MSD	
Model	Agilent 7000E or 7010C
Source	Inert extractor source with a 3 mm lens or high efficiency source (HES)
Vacuum Pump	Performance turbo
Tune File	Atunes.eiex.jtune.xml or Atunes.eihs.jtune.xml
Solvent Delay	3 min
Quad Temperature (MS1 and MS2)	150 °C
Source Temperature	280 °C
Mode	Simultaneous dMRM/scan
He Quench Gas	2.25 mL/min
N_2 Collision Gas	1.5 mL/min
MRM Statistics	
Total MRMs (dMRM Mode)	614
Minimum Dwell Time (ms)	6.85
Minimum Cycle Time (ms)	69.8
Maximum Concurrent MRMs	52
EM voltage Gain Mode	10
Full Scan Parameters	
Scan Type	MS1 scan
Scan Range	45 to 450 *m/z*
Scan Time (ms)	220
Step Size	0.1 amu
Profile Data	No
Threshold	0

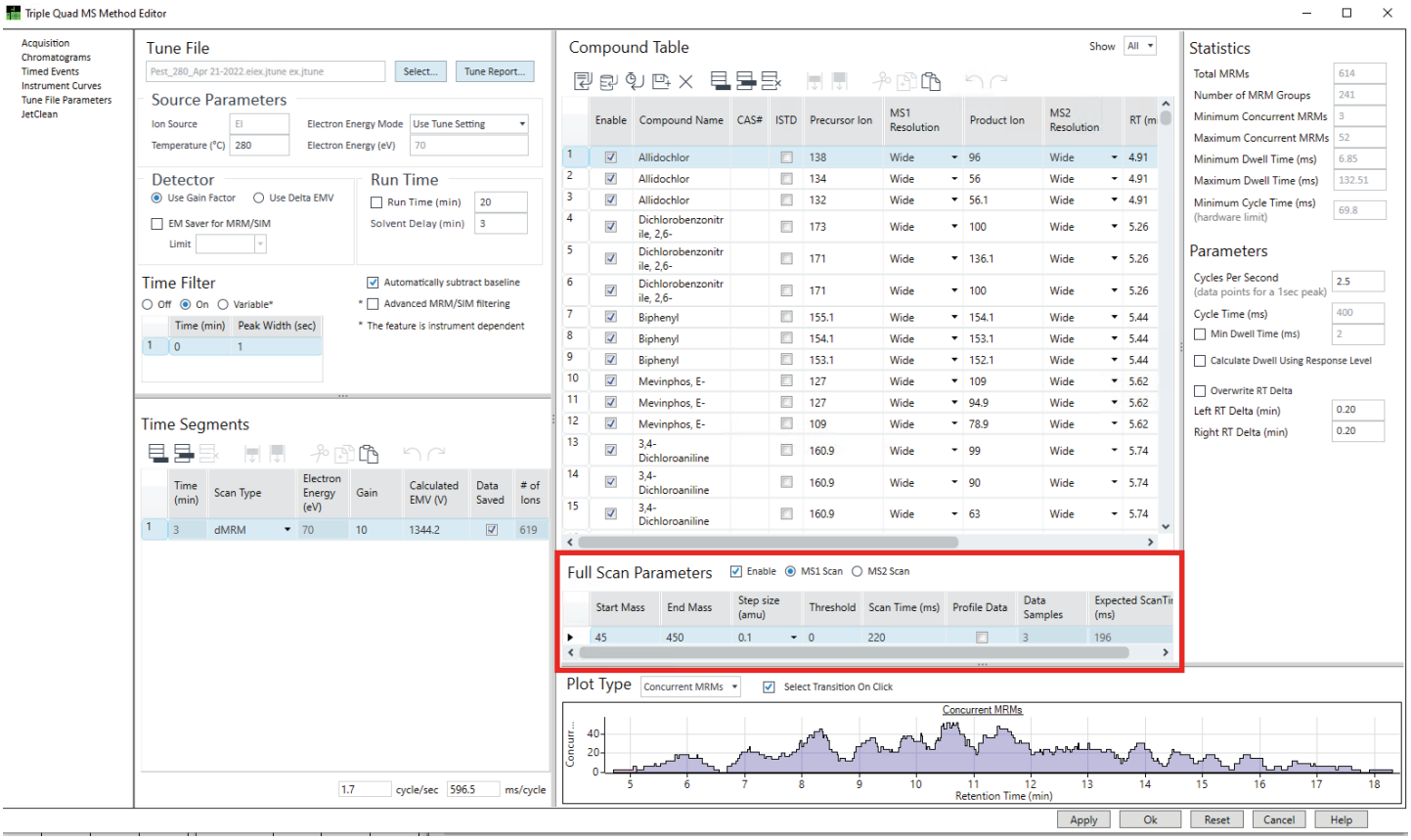

Figure 2. Triple quadrupole MS Method Editor showing the full scan acquisition parameters used for simultaneous dMRM/scan in this work.

recommended parameters used for sample screening. Additional details on the best practices for full scan data acquisition and processing using GC/TQ can be found in the application note 5994-3859EN.[1]

Data were acquired in dMRM/scan mode with one analytical run, enabling simultaneous targeted large multi-analyte assays and full scan data acquisition for unknown identification and retrospective analysis. The acquisition method was retention time-locked to match the retention times in the Agilent MassHunter Pesticide & Environmental Pollutant MRM Database (P&EP 4). The data file size difference of dMRM/scan for a 20-minute analysis compared to dMRM only was ~20 MB. For example, the file size for cayenne pepper extract analyzed in dMRM/scan mode that included 614 MRM transitions and full scan over 45 to 450 *m/z* is 30 MB. The same sample analyzed in dMRM only mode results in the file size of 11 MB.

Data acquisition and processing was performed with the Agilent MassHunter Workstation versions 10.1 and higher.

Calibration performance was evaluated using a series of matrix-matched calibration standards ranging from 0.1 to 1,000 ppb (w/v), including 0.1, 0.5, 1, 5, 10, 50, 100, 250, 500, 1,000, and 5,000 ppb. The GC multiresidue pesticide kit containing 203 compounds (Restek, Bellefonte, PA, USA), regulated by the FDA, USDA, and other global governmental agencies, was used for preparing matrix-matched calibration standards. A standard, α-BHC-d6, at a final concentration of 20 ppb in vial, was used as the internal standard for quantitation of the target pesticides (Agilent Bond Elut QuEChERS IS standard number 6, part number PPS-610-1). A weighting factor of 1/x was applied to all calibration curves.

Sample preparation

Sample preparation workflow chart is shown in Figure 3. The sample preparation included two major steps: Sample extraction by traditional QuEChERS extraction, followed with Agilent Captiva EMR pass-through cleanup. Different Captiva EMR products were used for different matrices based on different matrix challenges. Captiva EMR–HCF1 (part number 5610-2088) cartridge was used for high-chlorophyll fresh matrix spinach. Captiva EMR–LPD (part number 5610-2092) was used for the low pigmented but oily dry matrix walnut. Captiva EMR–GPD (part number 5610-2091) was used for a very challenging dry matrix cayenne pepper. The positive pressure manifold 48 processor (PPM-48, part number 5191-4101) was used for Captiva EMR pass-through cleanup processing. The new sample preparation workflow demonstrates a simplified procedure with improvement on both sample matrix removal and targets quantitation data quality. Figure 3 shows the sample preparation workflow. More details on the sample preparation workflow can be found in the application note 5994-4965EN.[3]

Results and discussion

The data acquired in simultaneous dMRM/scan mode can serve several important functions that are summarized in Figure 4.

The approach to handling and using the dMRM data remains unchanged when comparing to a conventional targeted GC/MS/MS analysis in dMRM data acquisition mode (highlighted in green in Figure 4). Simultaneous acquisition of full scan data provides three additional functionalities highlighted in blue in Figure 4.

Evaluation of the matrix in full scan

First, performing matrix screening in full scan data acquisition mode facilitates the evaluation of in-source matrix loading. The application note 5994-4965EN[4] describes the importance of analyzing matrix in full scan mode. This analysis allows users to evaluate the absolute abundance of the total ion chromatogram (TIC), which is recommended not to exceed 7×10^7 counts for GC/TQ. Evaluation of the TIC in full scan mode can signal that the EI source might be overloaded with matrix at any retention time. Source overloading could lead to compromised sensitivity and quantitation accuracy of coeluting analytes.

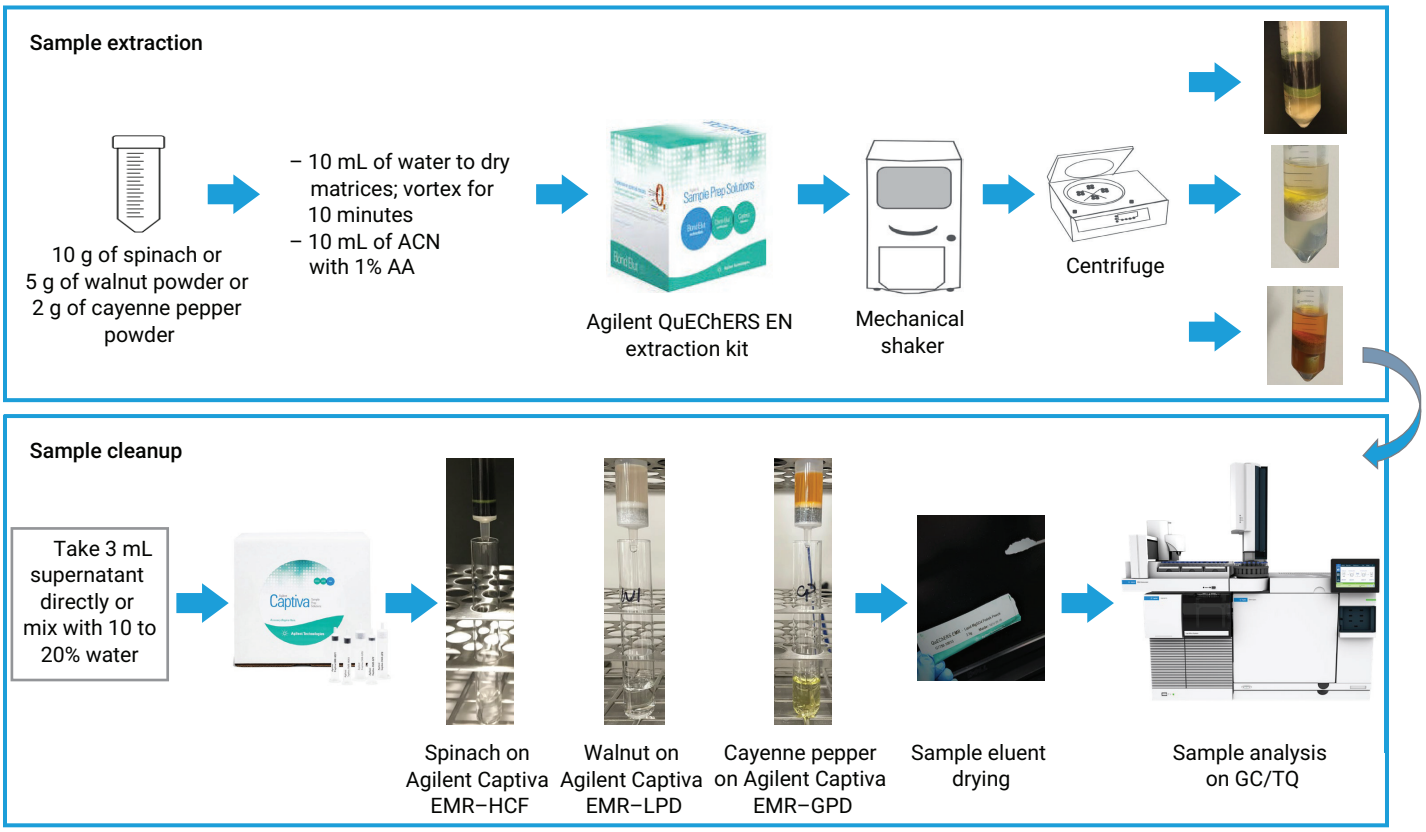

Figure 3. Sample preparation flowchart including traditional QuEChERS extraction, followed with Captiva EMR pass-through clean up.

Out of the three analyzed matrices, cayenne pepper featured the highest matrix background, with the TIC in scan exceeding 7 × 10^7 counts, as shown in Figure 5. Also, The MRM TIC on the bottom of Figure 5C shows that more MRM transitions were disturbed or had a higher background in cayenne pepper extract when compared to spinach and walnut extracts. This evaluation revealed that pesticides eluting between 11 and 12.5 minutes were expected to have compromised performance in the cayenne pepper matrix when evaluating sensitivity and the dynamic range.

For example, endosulfan I (α-endosulfan) eluted at 11.273 minutes and could be quantitated only starting at 5 ppb in the cayenne pepper matrix. However, endosulfan I could be quantitated down to 0.1 ppb in spinach and walnut extracts with both 7000E and 7010C GC/TQ systems. Evaluation of TIC in full scan reveals that cayenne pepper extract has more interferences originating from matrix interferences coeluting with endosulfan I than the other two matrices. However, the stereoisomer endosulfan II (β-endosulfan) eluted at 12.291 minutes, could be quantitated down to 0.1 ppb in all three matrices with fewer coeluting components arising from the cayenne pepper matrix.

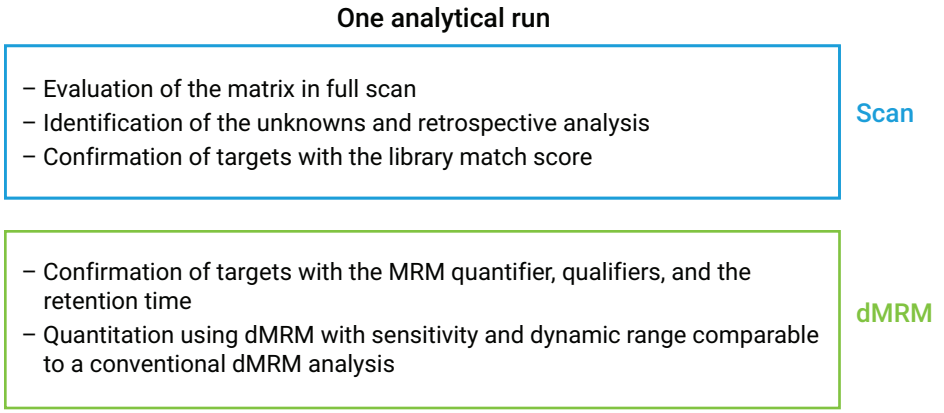

Figure 4. Functionality enabled with simultaneous dMRM/scan data acquisition mode within one analytical run.

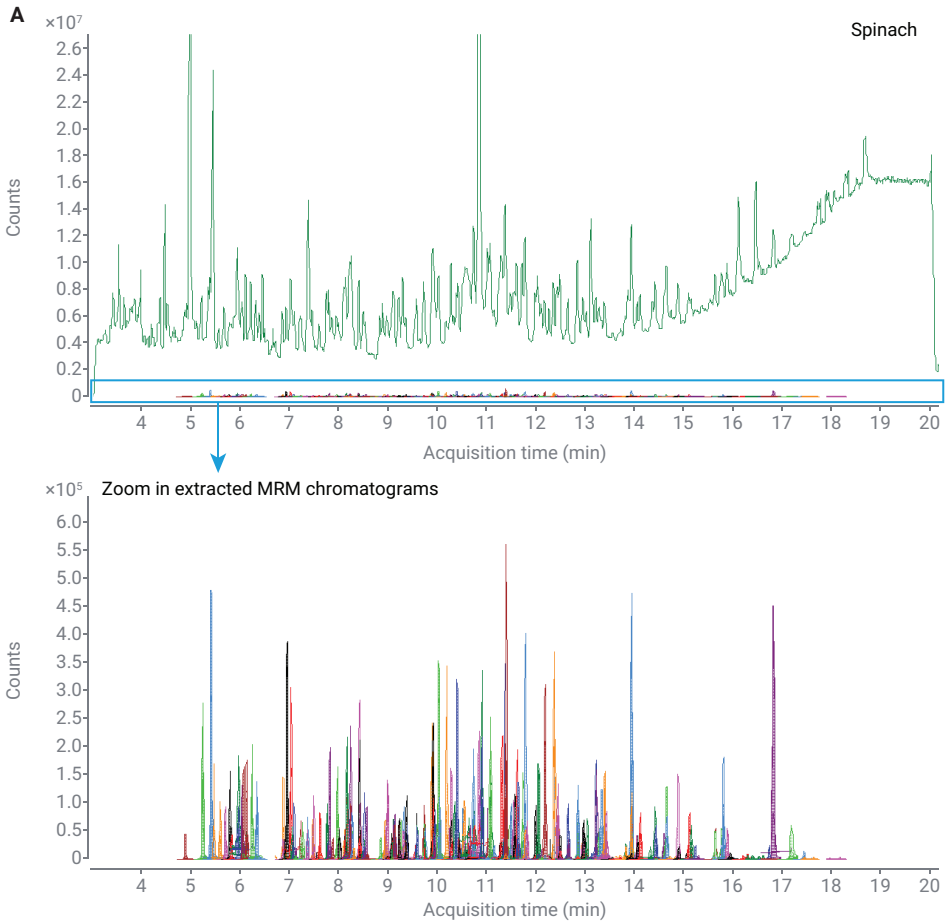

Figure 5A. Scan (on top) and dMRM (magnified on the bottom) TIC acquired in simultaneous dMRM/scan data acquisition mode for spinach extract.

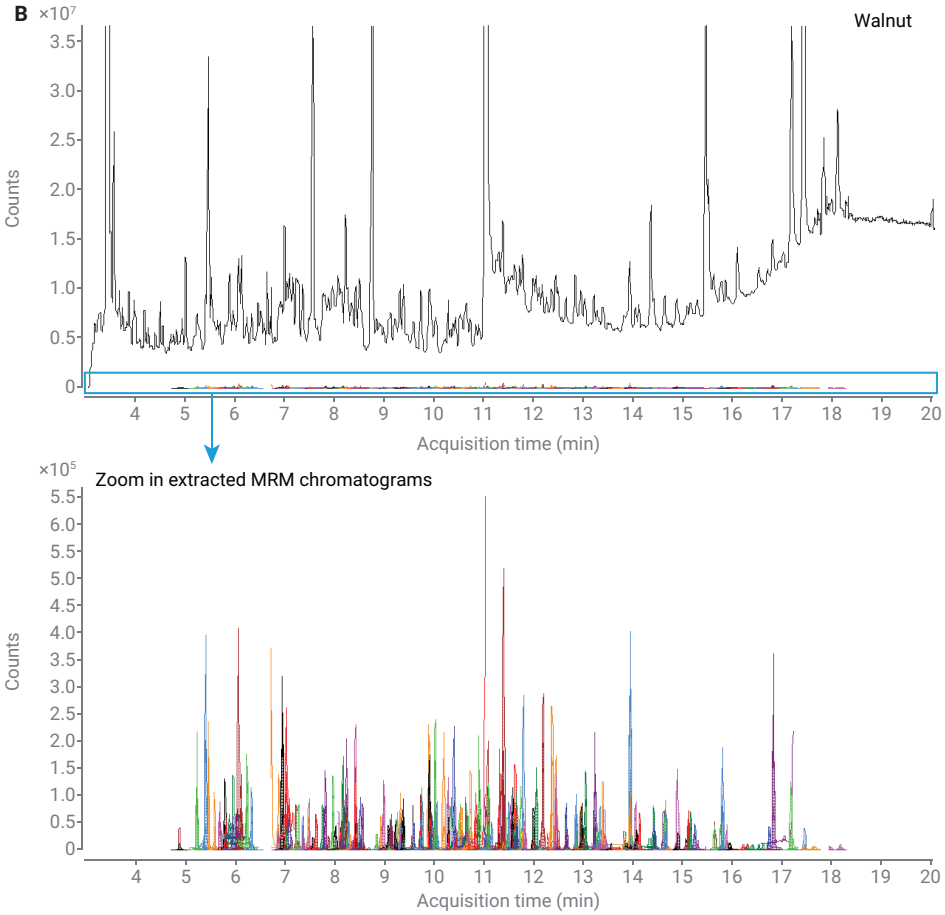

Figure 5B. Scan (on top) and dMRM (magnified on the bottom) TIC acquired in simultaneous dMRM/scan data acquisition mode for walnut extract.

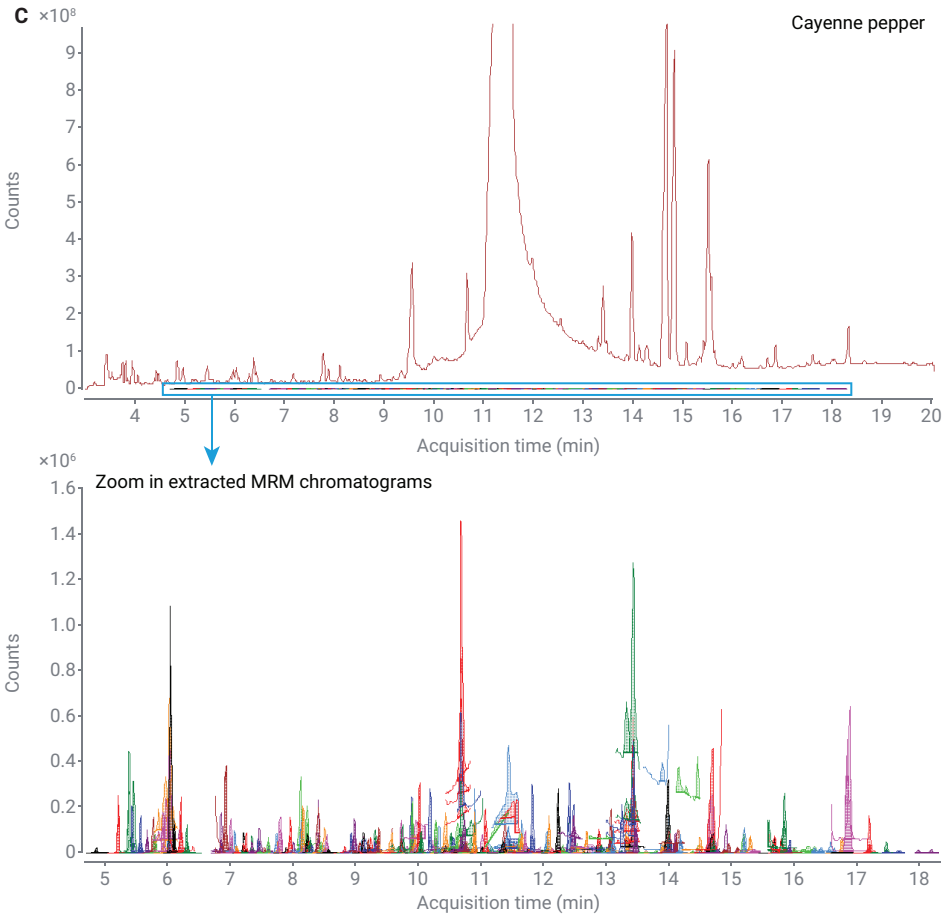

Figure 5C. Scan (on top) and dMRM (magnified on the bottom) TIC acquired in simultaneous dMRM/scan data acquisition mode for cayenne pepper extract.

Identification of the unknowns and retrospective analysis

Simultaneous dMRM/scan data acquisition mode allows for acquisition and storage of the full scan data for each analyzed sample. Full scan data unlock the opportunity to perform compound screening via spectral deconvolution and component search against GC/MS spectral libraries such as NIST. This functionality is valuable for retrospective analysis, eliminating the need to reanalyze the sample.

The 2016 Pesticide Data Program Annual Summary presented by USDA[4] revealed that chlorpropham was detected in one of the 707 analyzed spinach samples, while this herbicide does not have a tolerance established by EPA for use on spinach.[5] Since there is no tolerance established for chlorpropham, it is likely that this analyte is not on the target list for the GC/MS/MS method when analyzing spinach samples. Figure 6 demonstrates that chlorpropham was identified in the spinach QuEChERS extract with MassHunter Unknowns Analysis with a screening workflow against a retention time locked pesticide library. In this work, chlorpropham was spiked into spinach matrix to verify the ability to identify the compound using full scan data acquired simultaneously with the dMRM data in dMRM/scan data acquisition mode. Chlorpropham was successfully identified in spinach QuEChERS extract at a concentration of 50 ppb and above with the 7000E and the 7010C GC/TQ systems.

Figure 6 illustrates the screening results for spinach extract spiked with a pesticide mixture at 100 ppb. Chlorpropham was among the identified components and is highlighted in blue in the components table. The library match score (LMS) was 72 and the delta between the observed retention time and the retention time provided in the spectral library was 0.009 minutes. The lower right of Figure 6 shows the spectral information displayed in MassHunter Unknowns Analysis for the hit. The raw mass spectrum appears on the lower right and a mirror plot compares the deconvoluted mass spectrum to the library spectrum. The magnified chromatogram on the upper right highlights the component corresponding to chlorpropham in red. Other identified components are shown in green, and the TIC scan profile in black.

Note that some identified compounds such as alachlor, aldrin, and carfentrazone-ethyl had low LMS <60. However, small retention time delta and presence of the unique ions in the mass spectrum increased confidence in their identification.

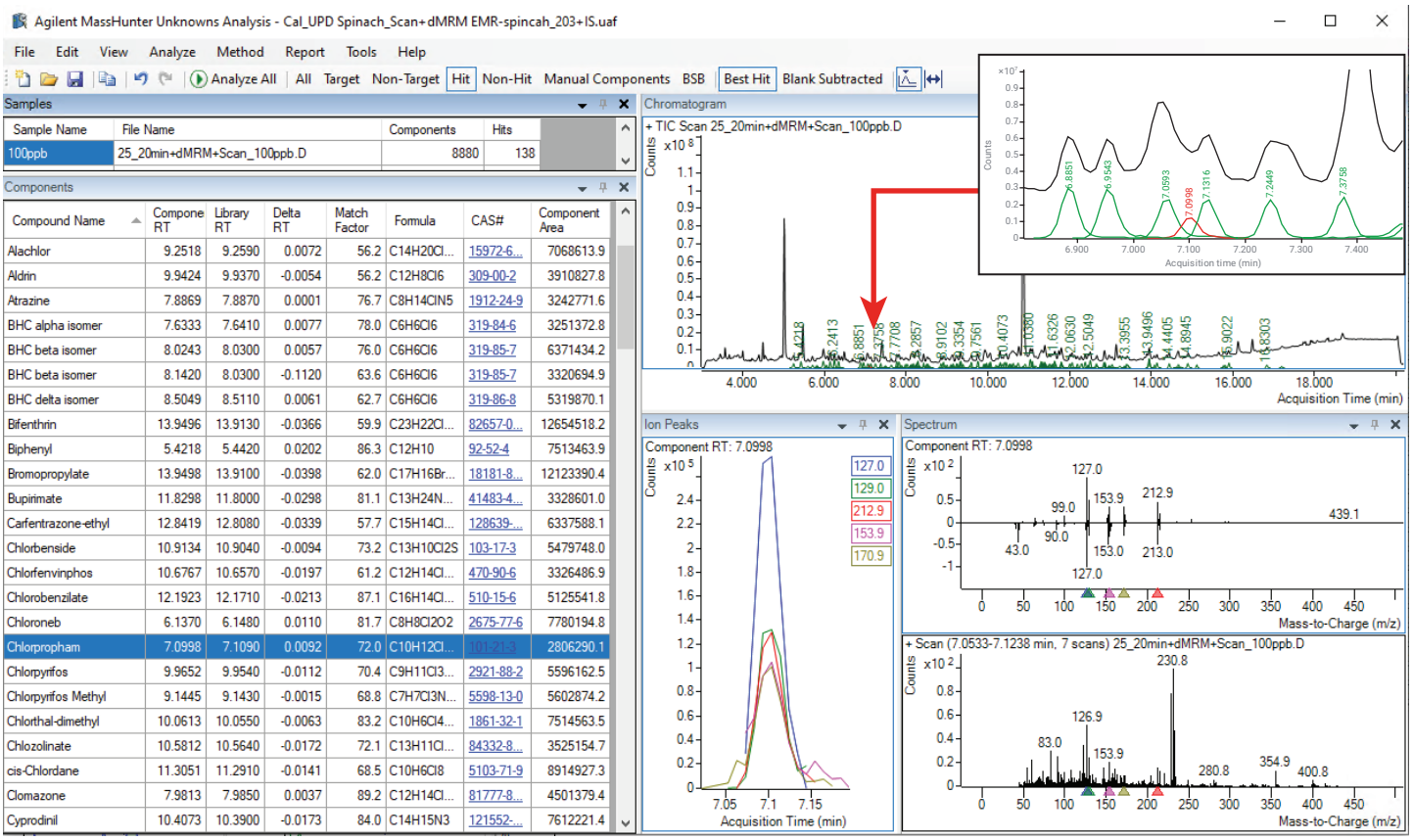

Figure 6. A partial list of search results for spinach extract spiked with a pesticide mixture at 100 ppb against a retention time-locked spectral library. Chlorpropham is selected in the components table and its extracted ion chromatograms and corresponding spectral information are shown on the lower right. The data were acquired with the 7000E GC/TQ in simultaneous dMRM/scan mode.

Confirmation of targets with library match score

The third functionality enabled with scan data acquired simultaneously with dMRM data is confirmation of targets with LMS. This functionality allows for increased confidence in compound identification that is especially important when reporting compounds quantitated above their MRLs. For example, if a compound is quantitated with dMRM at a concentration exceeding the MRL, the scan data can be evaluated to further confirm the finding.

Table 2 lists several pesticides among those spiked into the cayenne pepper extract that have established tolerances in non-bell pepper and spices applicable to cayenne pepper. Out of ten compounds, eight were identified with the 7000E GC/TQ based on spectral matching at concentrations less than or equal to the established MRL (highlighted in green in Table 2).

Figure 7 demonstrates the mirror plot of the deconvoluted mass spectrum from MassHunter Unknowns Analysis screening against the library spectrum at 100 ppb in cayenne pepper for bifenthrin (Figure 7A), chlorpyrifos (Figure 7B), and metolachlor (Figure 7C). These pesticides could be identified below their MRL level with scan data. They are highlighted in bold in Table 2. LMS at 100 ppb and at the MRL level are specified in the figure. The LMS values at 100 ppb and at the established MRL levels are noted in Figure 7. Typically, LMS values below 65 should trigger inspection of a hit. Based only on spectral match, this hits with LMS <65 might be rejected. For example, for bifenthrin and chlorpyrifos, there are three of the principal ions present in approximately the right ratios, and the RTs are within 0.074 and 0.033 minutes of those in the RTL library. The expected ion ratios and close RT matching increase confidence in correct compound identification.

Table 2. Pesticides among those spiked into the cayenne pepper extract that have established MRLs and the concentration required to identify them with the 7000E GC/TQ in simultaneous dMRM/scan.

Electronic Code of Federal Regulations (eCFR)	Commodity	Compound	Tolerance/MRL (ppb)	Scan identification limit on 7000E GC/TQ (ppb)
180.442	Pepper, non-bell	**Bifenthrin**	500	100
180.515	Herbs and spice, group 19	Carfentrazone-ethyl	2,000	250
180.342	Pepper	**Chlorpyrifos**	1,000	50
180.425	Pepper	Clomazone	50	50
180.436	Pepper	Cyfluthrin and beta-cyfluthrin	500	1,000
180.153	Pepper	Diazinon	500	250
180.182	Pepper	Endosulfan	2,000	500
180.516	Herbs and spice, group 19	Fludioxonil	20	5,000
180.111	Pepper	Malathion	8,000	250
180.368	Pepper, non-bell	**Metolachlor**	500	100

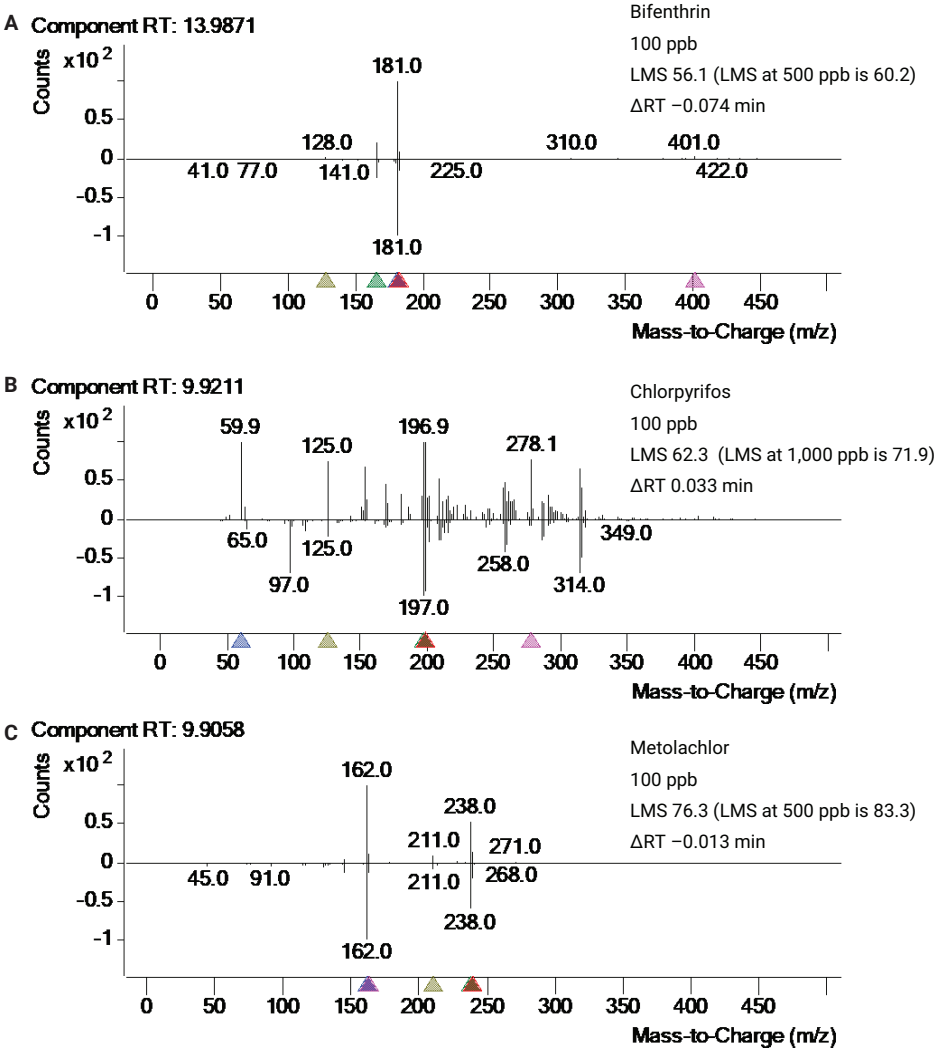

Figure 7. Spectral confirmation with library match score for bifenthrin (A), chlorpyrifos (B), and metolachlor (C) spiked at 100 ppb in cayenne pepper with the Agilent 7000E GC/TQ in simultaneous dMRM/scan data acquisition mode.

Pesticide quantitation with dMRM acquired in simultaneous dMRM/scan

Figure 8 provides the comparative quantitation results for three pesticides that have established MRLs in cayenne pepper. The samples were analyzed in simultaneous dMRM/scan and dMRM only data acquisition modes with the 7000E GC/TQ. The quantifier and the qualifier MRM chromatograms demonstrate comparable sensitivity at 0.1 ppb with anticipated slight sensitivity loss observed in dMRM/scan resulting from decreased dwell time due to simultaneous scanning. With both acquisition methods, excellent calibration linearity over the range 0.1 to 5,000 ppb for matrix-matched calibration standards in cayenne pepper was observed. The quantitation accuracy at the MRL level is noted in the figure.

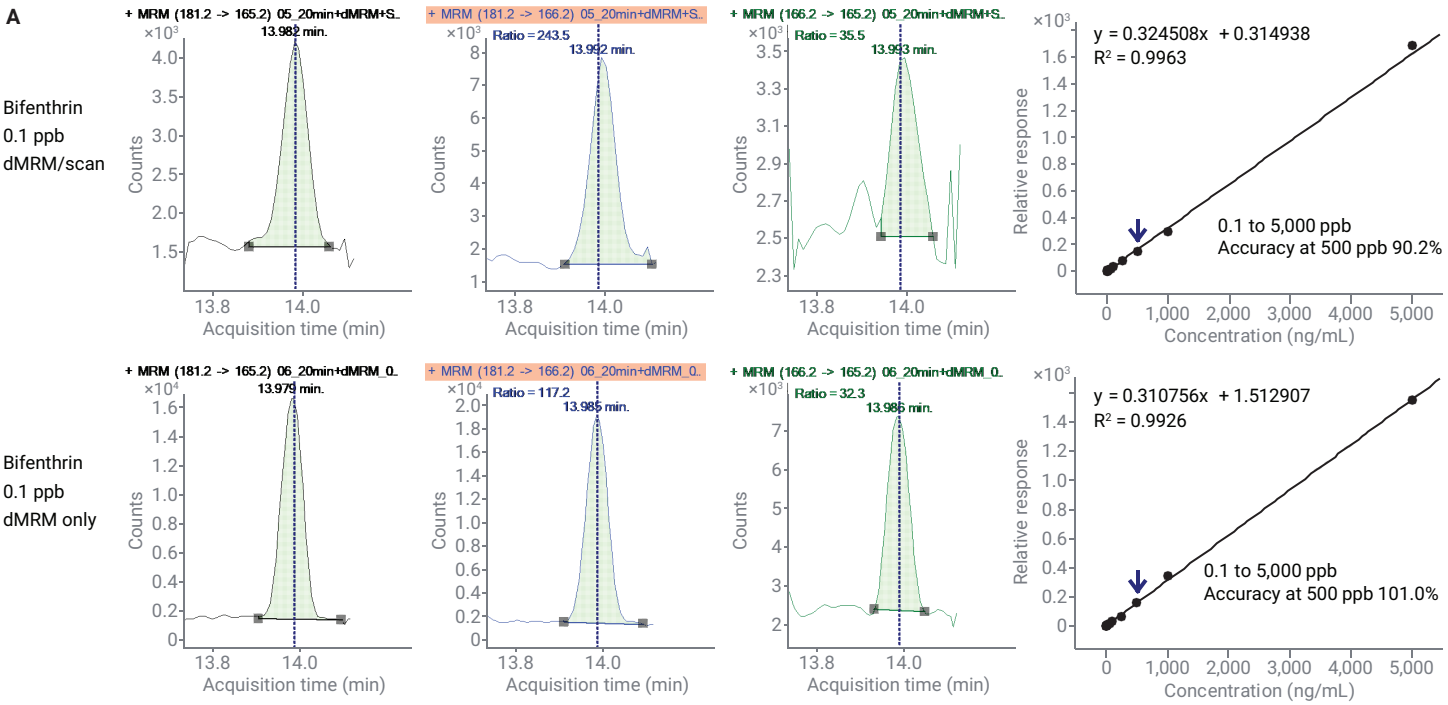

Figure 8A. Quantifier and qualifier ion profiles and matrix-matched calibration curves over 0.1 to 5,000 ppb for bifenthrin spiked at 100 ppb in cayenne pepper with the Agilent 7000E GC/TQ in simultaneous dMRM/scan and dMRM only data acquisition modes.

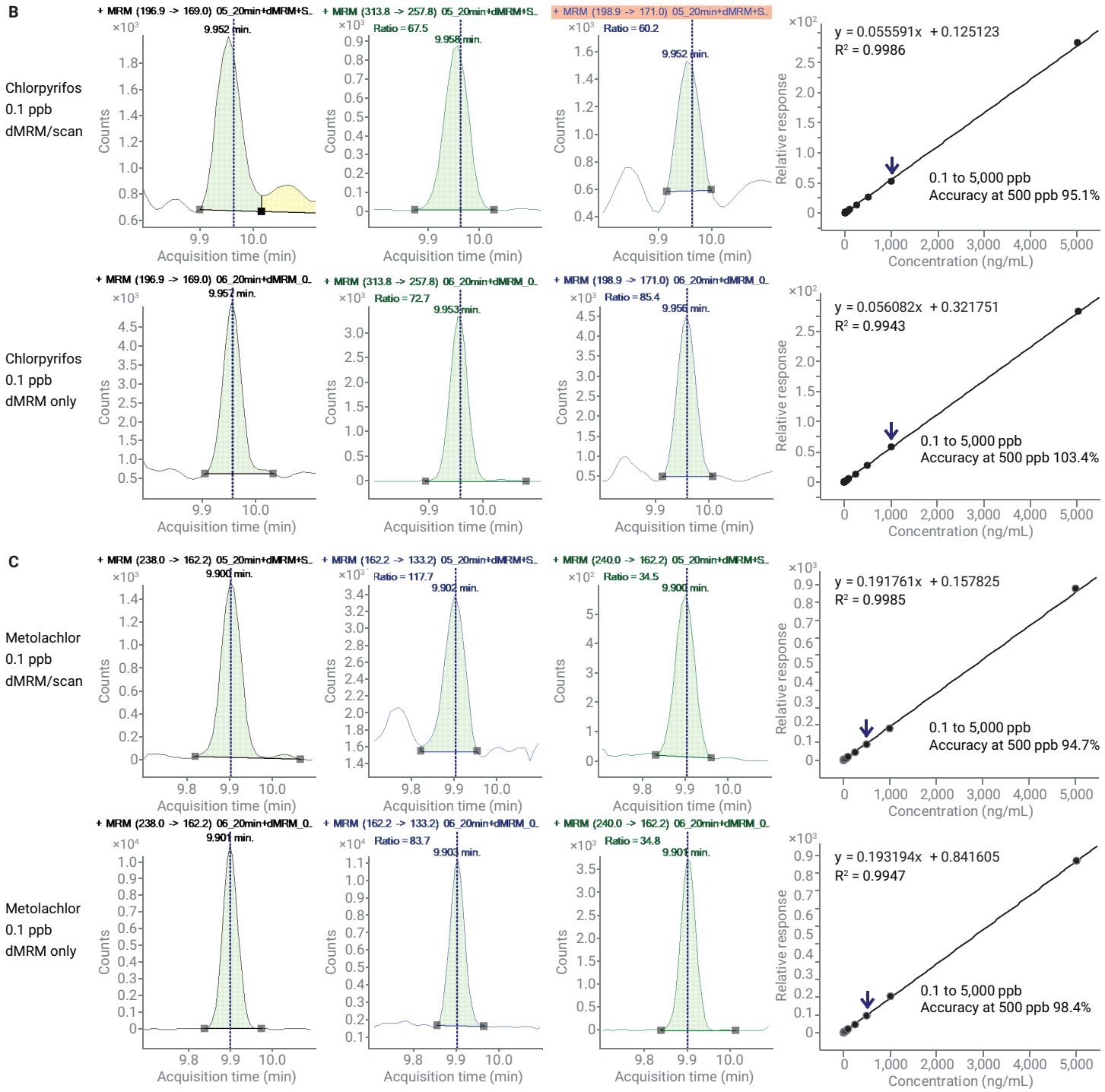

Figure 8B,C. Quantifier and qualifier ion profiles and matrix-matched calibration curves over 0.1 to 5,000 ppb for chlorpyrifos (B) and metolachlor (C) spiked at 100 ppb in cayenne pepper with the Agilent 7000E GC/TQ in simultaneous dMRM/scan and dMRM only data acquisition modes.

A summary in Figure 9 shows the calibration performance using dMRM data acquired in simultaneous dMRM/scan mode for the 203 pesticides that were analyzed in spinach, walnut, and cayenne pepper extracts with the 7000E and 7010C GC/TQ systems. The figure illustrates the number of compounds successfully meeting the correlation coefficient R^2 >0.99, the calibration fit (linear or quadratic), and the calibration range. The calibration results and method sensitivity were comparable to those observed with conventional dMRM data acquisition mode as shown in the application note 5994-4965EN.[3]

As expected, considering the recommended loading for the high efficiency source (HES) not to exceed 1 ng per analyte, the upper calibration limit for the 7010C was lower when compared to the 7000E (1,000 ppb versus 5,000 ppb). However, the calibration range achieved with the 7010C was up to four orders of magnitude with a linear fit for most of the analyzed compounds. The 7010C GC/TQ equipped with the HES enables superior sensitivity yielding high signal-to-noise (S/N) at low concentrations and allows for accurate quantitation at concentrations below 0.1 ppb. However, this sensitivity was not required in this work as the MRLs for pesticides regulated in the commodities of interest did not require sub 0.1 ppb quantitation. Alternatively, samples with the MRLs above 1,000 ppb can be further diluted before the analysis with the 7010C GC/TQ. The HES enables maintaining high sensitivity at the LOQ level even in the diluted samples.

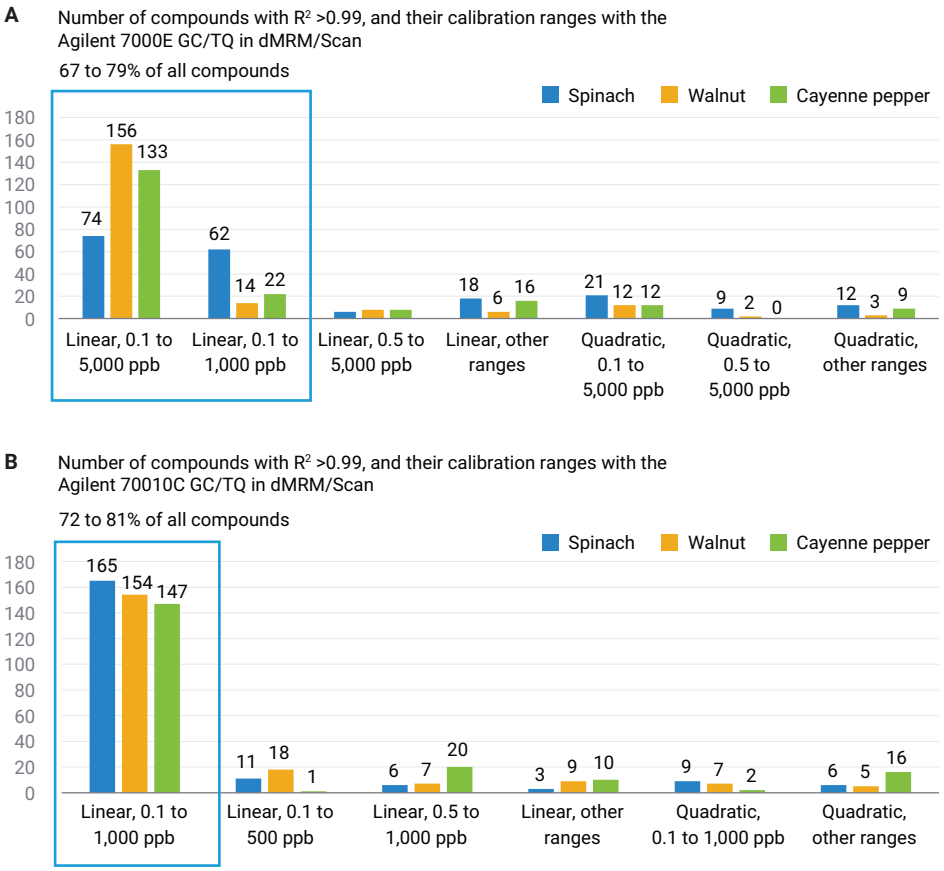

Figure 9. Calibration performance for the 203 pesticides with an Agilent 7000E (A) and Agilent 7010C (B) GC/TQ in spinach, walnut, and cayenne pepper QuEChERS extracts. The graph shows the number of compounds and their calibration ranges.

Conclusion

This application note described the use of the novel simultaneous dMRM/scan data acquisition mode for reliable identification and quantitation of pesticides in challenging food matrices with the Agilent 8890/7000E and 8890/7010C triple quadrupole GC/MS systems (GC/TQ). Simultaneous dMRM/scan mode eliminates the need to reanalyze the sample in each data acquisition mode separately. This mode enables retrospective analysis and demonstrates comparable performance for quantitation to dMRM only mode.

The data acquired in simultaneous dMRM/scan mode can serve several important functions including:

- Evaluation of the matrix in full scan
- Identification of the unknowns and retrospective analysis
- Confirmation of targets with the library match score
- Confirmation of targets with the MRM quantifier, qualifiers, and the retention time
- Quantitation using dMRM with sensitivity and dynamic range comparable to a conventional dMRM analysis.

This application note demonstrates the use of the acquired scan data for spinach, walnut, and cayenne pepper extracts for evaluating matrix blanks and performing screening based on spectral deconvolution with MassHunter Unknowns Analysis. The scan data allowed identifying compounds without established tolerances that may potentially be missed by the targeted GC/TQ dMRM method. Scan data were also used to confirm the identifications of the compounds with established tolerances included in the targeted dMRM method as was demonstrated with cayenne pepper. Finally, method sensitivity and calibration performance were comparable to those achieved with the conventional dMRM method making simultaneous dMRM/scan an attractive tool for reliable quantitation and compound identification within one analytical run.

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3. Andrianova, A.; Zhao, L. Five Keys to Unlock Maximum Performance in the Analysis of Over 200 Pesticides in Challenging Food Matrices by GC/MS/MS. *Agilent Technologies application note*, publication number 5994-4965EN, **2022**.
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Application Note

Food & Beverage Testing

Five Keys to Unlock Maximum Performance in the Analysis of Over 200 Pesticides in Challenging Food Matrices by GC/MS/MS

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Abstract

This application note describes five best practices to enhance analytical performance in the analysis of over 200 pesticides in challenging matrices including spinach, walnut, and cayenne pepper. The novel Agilent Captiva EMR passthrough cleanup procedure following the Agilent QuEChERS extraction enabled a cleaner matrix background. The cleanup and extraction reduced matrix interferences with target analytes and extended the maintenance-free operation time of the instrument. Calibration performance was demonstrated over a wide dynamic range to over four orders of magnitude. It was shown that the Agilent 8890/7000E triple quadrupole GC/MS system achieved excellent linearity over a concentration range of 0.1 to 5,000 ppb. The Agilent 8890/7010C triple quadrupole GC/MS system demonstrated superior sensitivity yielding a higher signal-to-noise ratio at lower concentrations.

Introduction

The global agriculture industry uses over a thousand different pesticides in the production of food. Producers require pesticides to meet the increasing demand for reasonably priced food. This growing demand has increased the use of pesticides and encouraged problematic agricultural practices that have elevated risks in the food supply and the environment. Concerns about trace level chemical pollutants in food are driving the demand for more rapid and reliable methods for the identification and quantitation of chemical residues. The Agilent 8890/7000E and 8890/7010C triple quadrupole GC/MS systems (GC/TQ) are ideally suited to meet this need.

The US Environmental Protection Agency (EPA) sets tolerances as part of the food safety equation.[1] The tolerance corresponds to the maximum residue limit (MRL), which is the maximal level of pesticide residue allowed to remain in or on the treated food commodity. The MRLs may vary over a broad concentration range depending on different pesticides and food commodities. For example, the MRLs established for 68 pesticides regulated in spinach vary from 10 ppb for fludioxonil to 60,000 ppb for boscalid.[2] This range of limits presents a challenge for the analysis, requiring both high sensitivity and the ability to calibrate over a wide dynamic range.

Five key components of successful pesticide analysis discussed in this application note are:

1. Effective sample extraction and matrix cleanup, which allow for minimal matrix background and interferences while maintaining high pesticide recoveries. Also, a robust analytical method that achieves the required method performance while increasing maintenance-free uptime.
2. Evaluation of the matrix in full scan data acquisition mode to ensure the most efficient performance, especially with the high efficiency source (HES).
3. Midcolumn backflushing to extend maintenance-free operation of the system. This technique minimizes column trimming and source cleaning while also allowing reduced analysis time.
4. A leak-free GC/TQ system enables extended GC column life and facilitates maintenance-free consistent and reliable MS performance.
5. Use of the temperature-programmed Agilent multimode inlet (MMI) with a 2 mm dimpled liner (no glass wool) to ensure efficient volatilization of even the most thermally labile compounds.

This application note demonstrates the analysis of over 200 pesticides in three challenging matrices, including a high chlorophyll fresh matrix spinach, a complex dry matrix cayenne pepper, and an oily dry matrix walnut. The achieved wide dynamic ranges with high method sensitivity enabled accurate quantification of pesticides in these matrices, at their MRLs.

Matrix-matched calibrations with R^2 >0.99 over a dynamic range as wide as 0.1 to 5,000 ppb were achieved with the 7000E GC/TQ and 0.1 to 1,000 ppb with the 7010C GC/TQ. The 7010C GC/TQ equipped with the HES enabled superior sensitivity yielding high signal-to-noise ratio even at low concentrations and allowed for accurate quantification at concentrations below 0.1 ppb. However, this was not required in this work as the MRLs for pesticides regulated in the commodities of interest did not require sub-0.1 ppb quantification.

Experimental

GC/TQ analysis

The 8890/7000E and 8890/7010C GC/TQ systems (Figure 1A) were used and configured to achieve the best performance over a wide calibration range. This calibration range encompassed the varying MRLs for pesticides regulated in the analyzed commodities. The GC was configured with the Agilent 7693A automatic liquid sampler (ALS) and 150-position tray. The system used a multimode inlet (MMI) operated in temperature-programmed splitless injection mode. Midcolumn backflush capability was provided by the Agilent Purged Ultimate Union (PUU) installed between two identical 15 m columns, and the 8890 pneumatic switching device (PSD) module (Figure 1B). The instrument operating parameters are listed in Table 1.

Data were acquired in dynamic MRM (dMRM) mode, which enables the capability for large multi-analyte assays and to accurately quantitate narrow peaks by an automated and most-efficient dwell time distribution. The dMRM capability enabled a successful analysis for a large panel of 203 pesticide with 614 total MRM transitions with up to 52 concurrent MRMs (Figure 2). Furthermore, dMRM enables the analyst to add and remove additional analytes with ease. The acquisition method was retention time-locked to match the retention times in the Agilent MassHunter Pesticide & Environmental Pollutant MRM Database (P&EP 4), which was used to seamlessly create the MS method. The use of P&EP 4 increased the ease and speed of setting up a targeted dMRM method. The acquisition method was retention time locked to the P&EP library.

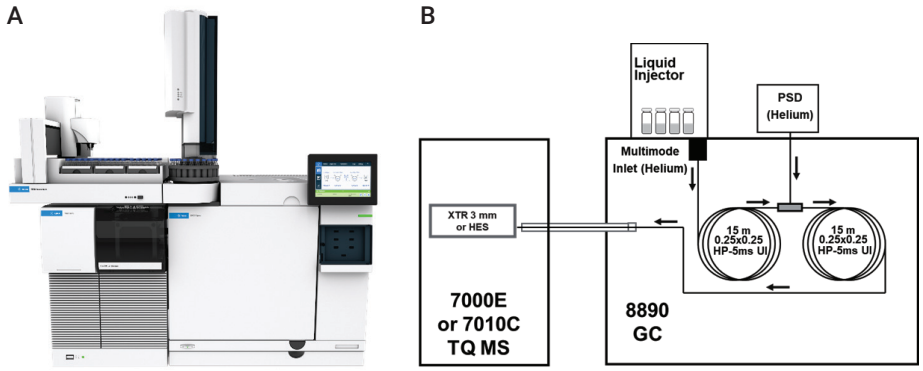

Figure 1. The Agilent 8890/7000E and 8890/7010C GC/TQ system (A) and system configuration (B).

Table 1. Agilent 8890/7000E and 8890/7010C gas chromatograph and mass spectrometer conditions for pesticide analysis.

GC	
Agilent 8890 with fast oven, auto injector, and tray	
Inlet	Multimode inlet (MMI)
Mode	Splitless
Purge Flow to Split Vent	60 mL/min at 0.75 min
Septum Purge Flow	3 mL/min
Septum Purge Flow Mode	Switched
Injection Volume	1.0 µL
Injection Type	Standard
L1 Airgap	0.2 µL
Gas Saver	On at 30 mL/min after 3 min
Inlet Temperature	60 °C for 0.1 min, then to 280 °C at 600 °C/min
Post Run Inlet Temperature	310 °C
Post Run Total Flow	25 mL/min
Carrier Gas	Helium
Inlet Liner	Agilent Ultra Inert 2 mm dimpled liner (p/n 5190-2297)
Oven	
Initial Oven Temperature	60 °C
Initial Oven Hold	1 min
Ramp Rate 1	40 °C/min
Final Temp 1	170 °C
Final Hold 1	0 min
Ramp Rate 2	10 °C /min
Final Temp 2	310 °C
Final Hold 2	2.25 min
Total Run Time	20 min
Post Run Time	1.5 min
Equilibration Time	0.25 min

Column 1	
Type	Agilent HP-5ms UI (p/n 19091S-431UI-KEY)
Length	15 m
Diameter	0.25 mm
Film Thickness	0.25 µm
Control Mode	Constant flow
Flow	1.016 mL/min
Inlet Connection	Multimode inlet (MMI)
Outlet Connection	PSD (PUU)
PSD Purge Flow	5 mL/min
Post Run Flow (Backflushing)	–7.873
Column 2	
Type	Agilent HP-5ms UI (p/n 19091S-431UI-KEY)
Length	15 m
Diameter	0.25 mm
Film Thickness	0.25 µm
Control Mode	Constant flow
Flow	1.216 mL/min
Inlet Connection	PSD (PUU)
Outlet Connection	MSD
Post Run Flow (Backflushing)	8.202

MSD	
Model	Agilent 7000E or 7010C
Source	Inert Extractor Source with a 3 mm lens or HES
Vacuum Pump	Performance turbo
Tune File	Atunes.eiex.jtune.xml or Atunes.eihs.jtune.xml
Solvent Delay	3 min
Quad Temperature (MS1 and MS2)	150 °C
Source Temperature	280 °C
Mode	dMRM or Scan
He Quench Gas	2.25 mL/min
N_2 Collision Gas	1.5 mL/min
MRM Statistics	
Total MRMs (dMRM Mode)	614
Minimum Dwell Time	6.85 ms
Minimum Cycle Time	69.8 ms
Maximum Concurrent MRMs	52
EM Voltage Gain Mode	10
Scan Parameters	
Scan Type	MS1 Scan
Scan Range	45 to 450 *m/z*
Scan Time (ms)	220
Step Size	0.1 amu
Threshold	0
EM Voltage Gain Mode	1

Full scan data acquisition mode was used for the preliminary screening of the matrix extract. This screening was used to evaluate the in-source loading and for monitoring the efficiency of the sample cleanup.

Agilent MassHunter Workstation revisions 10.1 and 10.2 including MassHunter Acquisition software for GC/MS systems 10.2, MassHunter Quantitative 10.1, and MassHunter Qualitative 10 packages were used in this work.

Calibration performance was evaluated using a series of matrix-matched calibration standards ranging from 0.1 to 5,000 ppb, including 0.1, 0.5, 1, 5, 10, 50, 100, 250, 500, 1,000, and 5,000 ppb. The standard α-BHC-d_6 at a final concentration of 20 ppb in vial was used as the internal standard for quantitation of the target pesticides. A linear or quadratic regression fit with a weighting factor of 1/x was applied to all calibration curves.

Sample preparation

A sample preparation workflow chart is shown in Figure 3. The sample preparation included two major steps: sample extraction by traditional QuEChERS extraction, followed with Captiva EMR pass-through clean up. Different Captiva EMR products were used for different matrices based on different matrix challenges. A Captiva EMR–HCF cartridge was used for high-chlorophyll fresh matrix spinach. Captiva EMR–LPD was used for the low pigmented but oily dry matrix walnut. Captiva EMR–GPD was used for a very challenging dry matrix cayenne pepper. The new sample preparation workflow demonstrates a simplified procedure with improvement on both sample matrix removal and targets quantitation data quality.

As shown in Figure 3, samples were first extracted by the traditional QuEChERS EN extraction kit (part number 5892-5650). For fresh spinach, 10 g of homogenized spinach sample was used for extraction. For walnut, 5 g of walnut powder was used, followed with the addition of 10 mL of water and 10 minutes of vortexing. For cayenne pepper, 2 g of cayenne pepper powder was used, followed with the addition of 10 mL water and 10 minutes vortexing. The 10 mL of ACN with 1% acetic acid was then added for extraction, followed with QuEChERS EN extraction. After extraction, 3 mL of crude extract or with 10% of water mixture was transferred to Captiva EMR cartridges for pass-through cleanup.

The following cartridges were used: Captiva Enhanced Matrix Removal High Chlorophyll Fresh, with NH_2, (Captiva EMR–HCF1, part number 5610-2088) for spinach, the Captiva Enhanced Matrix Removal Low Pigment Dry (Captiva EMR–LPD, part number 5610-2092) for walnut, and the Captiva Enhanced Matrix Removal General Pigmented Dry (Captiva EMR–GPD, part number 5610-2091) for cayenne pepper. The sample eluent was collected and further dried by anhydrous $MgSO_4$, (part number 5982-0102) and samples were then ready for GC/TQ analysis. The positive pressure manifold 48 processor (PPM-48, part number 5191-4101) was used for Captiva EMR pass-through clean up processing.

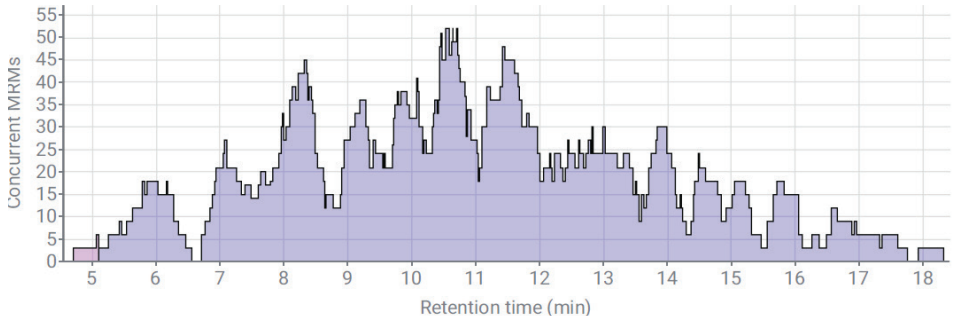

Figure 2. The distribution of 614 MRM transitions with up to 52 concurrent MRMs monitored during the analysis enabling most efficient dwell time distribution.

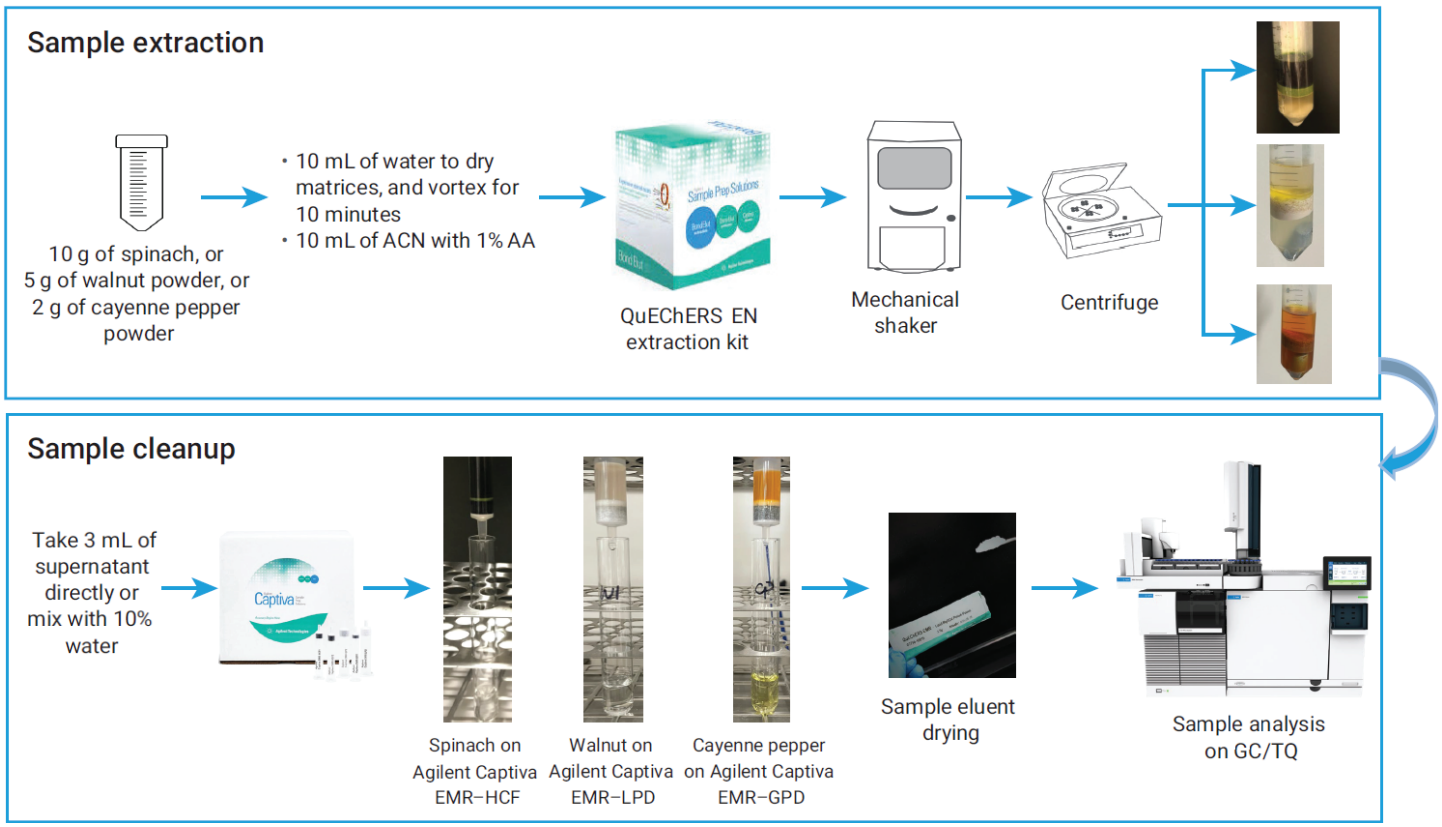

Figure 3. Sample preparation flowchart including traditional QuEChERS extraction, followed with Captiva EMR pass-through cleanup.

Results and discussion

Robust pesticide analysis that supports a high-throughput workflow must provide an extended maintenance-free operation with minimal downtime. The workflow must also meet the required sensitivity that can be at sub-ppb level. It must also enable calibration performance over a wide dynamic range that would encompass the MRLs for the compounds monitored in the commodity, which often vary over a wide dynamic range. The five key strategies outlined in this application note allowed achieving limits of quantification (LOQs) of up to 0.1 ppb while maintaining the calibration performance over a range up to 5,000 ppb for the 7000E and 1,000 ppb for the 7010C. In addition, the strategies would enable minimal instrument downtime limited to liner and septum replacement every ~100 injections.

The work presented in this application note and the system robustness study with 700 consecutive injections described elsewhere[3] resulted in over 1,000 injections of complex matrix extracts including spinach, walnut, and cayenne pepper. During this time, there was no need to perform TQ MS tuning, source cleaning, or GC column trimming.

Sample preparation

Efficient sample extraction and matrix cleanup are the keys to successful pesticide analysis. Analysis of crude QuEChERS extracts, especially of complex pigmented and oily matrices, can significantly increase the need for liner replacement, inlet cleaning, GC column trimming, and MS source cleaning. Such maintenance procedures decrease throughput of the analysis.

Performing an efficient matrix cleanup following QuEChERS extraction reduces in-source matrix loading and interferences with targets, while improving signal-to-noise ratio, accuracy, and reproducibility for target pesticides. Captiva EMR passthrough clean up following the traditional QuEChERS extraction was used in this work. The new sample cleanup protocol is a simplified procedure that demonstrates an improvement on both sample matrix removal and targets overall recovery and reproducibility. As shown in Figure 4, the abundance of TIC signal in full scan data acquisition mode was noticeably reduced for spinach, walnut, and cayenne pepper extracts after clean up when comparing the crude extracts before cleanup.

Matrix screening in full scan data acquisition mode

Performing sample screening in full scan data acquisition mode facilitates the evaluation of in-source matrix loading. Every MS source has a limitation on the amount of material present in the source, at any point of time, to maintain the optimal performance. Quantitation accuracy of the analysis can be significantly compromised if the source is overloaded with matrix. Therefore, it is essential to analyze matrix in full scan mode to evaluate TIC and maintain the optimal GC/TQ performance. The abundance of TIC in full scan mode is recommended not to exceed 7 $\times 10^7$ counts when analyzing with an EM gain set to 1. Out of the three analyzed matrices, cayenne pepper featured the highest matrix background, although noticeably reduced after the clean up procedure. This evaluation revealed that pesticides that elute between 11 and 12.5 minutes were expected to have sacrificed performance in the cayenne pepper matrix when evaluating sensitivity and the dynamic range. For example, Endosulfan I eluted at 11.273 minutes, and could be quantitated only starting at 5 ppb in the cayenne pepper matrix with both 7000E and 7010C, while spinach and walnut matrices had significantly lower matrix levels coeluting with Endosulfan I, with 0.1 ppb LOQ observed. Best practices on using the Agilent GC/TQ system in full scan data acquisition mode can be found in the application note 5994-3859EN.[4]

Some of the practices that can be employed to lower the matrix background include adequate sample cleanup, sample dilution, and smaller injection volume. The latter two approaches often result in better LOQs, especially with the HES-equipped 7010C GC/TQ system.

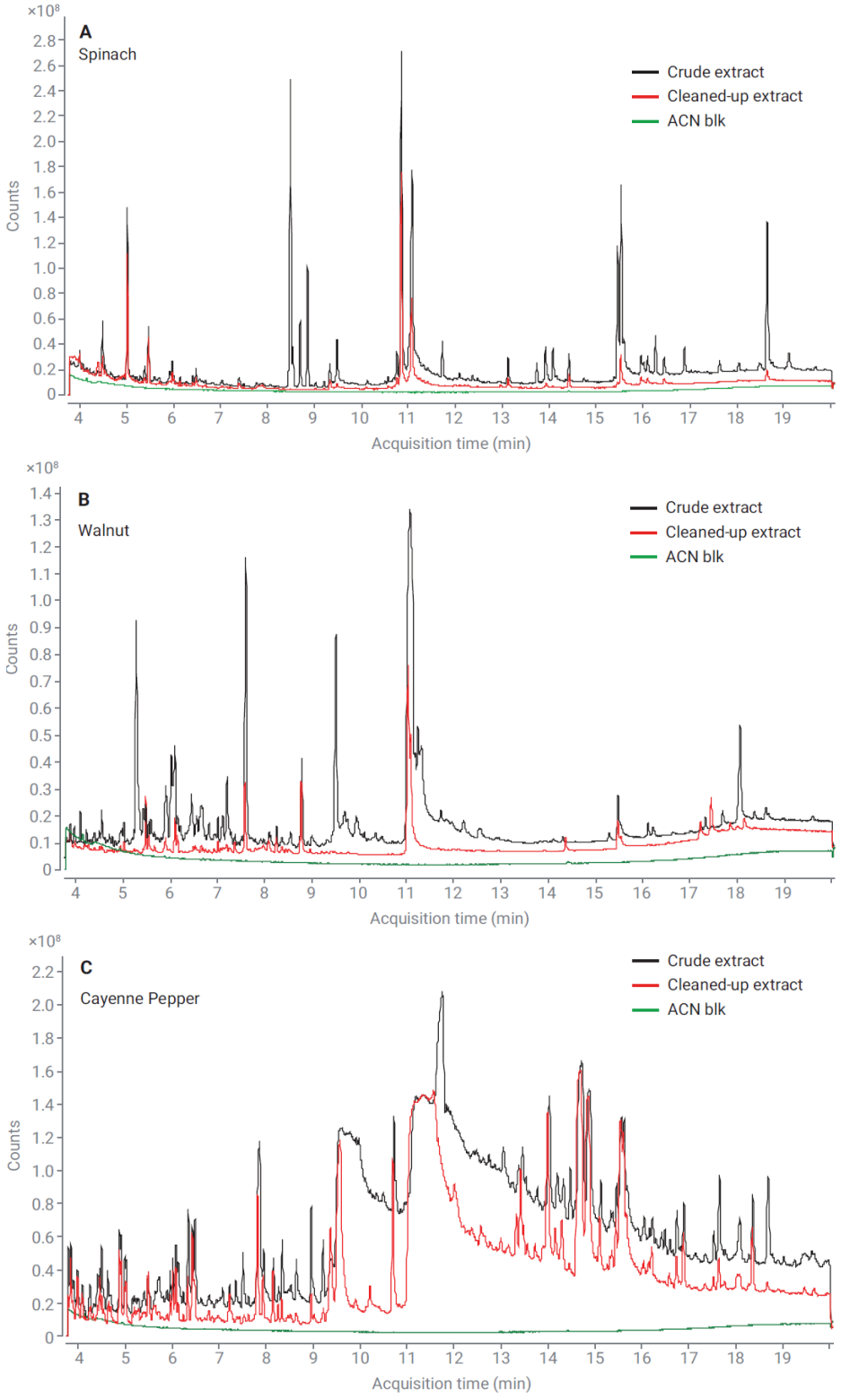

Figure 4. Scan TIC of the spinach (A), walnut (B), and cayenne pepper (C) extracts. The red trace corresponds to matrix sample with Captiva EMR cleanup, and the black trace corresponds to matrix sample without clean up. The green trace corresponds to the acetonitrile solvent blank.

Midcolumn backflushing

The use of the midcolumn backflushing configuration allows the analyst to limit the analysis time to the retention time of the last-eluting compound of interest. Challenging matrices, especially the oily ones, such as walnut, are rich in high-boiling components, with long retention times. These retention times often exceed that for the target pesticides. A common way to avoid ghost-peaks in the subsequent runs was to use an extended column bake-out after the last target analyte eluted from the column. However, this approach has several disadvantages including the deposition of high-boilers and GC column stationary phase into the EI source, contamination of the head of the GC column, a decrease of the column lifetime, and a longer cycle time due to the extended bake-out.

Midcolumn backflush allows the elution of the high boiling matrix components from the column without the sacrifices encountered with the bake-out approach. Midcolumn backflushing is a technique in which the carrier gas flow is reversed after the last analyte has exited the column. After the MS data are collected, the oven is held at the final temperature in post run mode, and the carrier gas flow through the first column is reversed. This reversed flow carries any high boilers that were in the column at the end of data collection. The high boilers are carried out of the head of the column and into the split vent trap (Figure 5A). The ability to reverse the flow is provided by the Agilent Purged Ultimate Union (PUU). The PUU is a tee that is inserted, in this case, between two identical 15 m columns.

During the analysis, a small makeup flow of carrier gas from the 8890 pneumatic switching device (PSD) module is used to sweep the connection. During backflushing, the makeup flow from the PSD is raised to a much higher value, sweeping high boilers backward out of the first column while simultaneously providing forward flow in the second column. For the configuration in this application, the backflushing time was 1.5 minutes. More details about using PSD for backflushing in the 8890 GC system can be found in the application note 5994-0550EN.[5]

The chromatograms shown in Figure 5B illustrate the effectiveness of the backflush technique in reducing cycle time sample carryover. The cycle time was reduced by 50% and the columns did not have to be exposed to the higher bake-out temperatures for an extended time. Using backflush, excess column bleed and heavy residues are not introduced into the MSD, thereby reducing ion source contamination.

In addition, the midcolumn backflushing configuration provides a significant time saving benefit when coupled with the MMI inlet. Maintenance procedures, such as septum and liner change, and column trimming can be performed without the need to cool down MS transfer line and source. When the septum is removed, the PSD provides the carrier gas flowing backward through column 1. The PSD also prevents air from entering the GC columns and the MS. MMI fast cooling capability enables more time savings. As a result, liner and septum replacement, which are the most common maintenance procedures, can be performed in a few minutes.

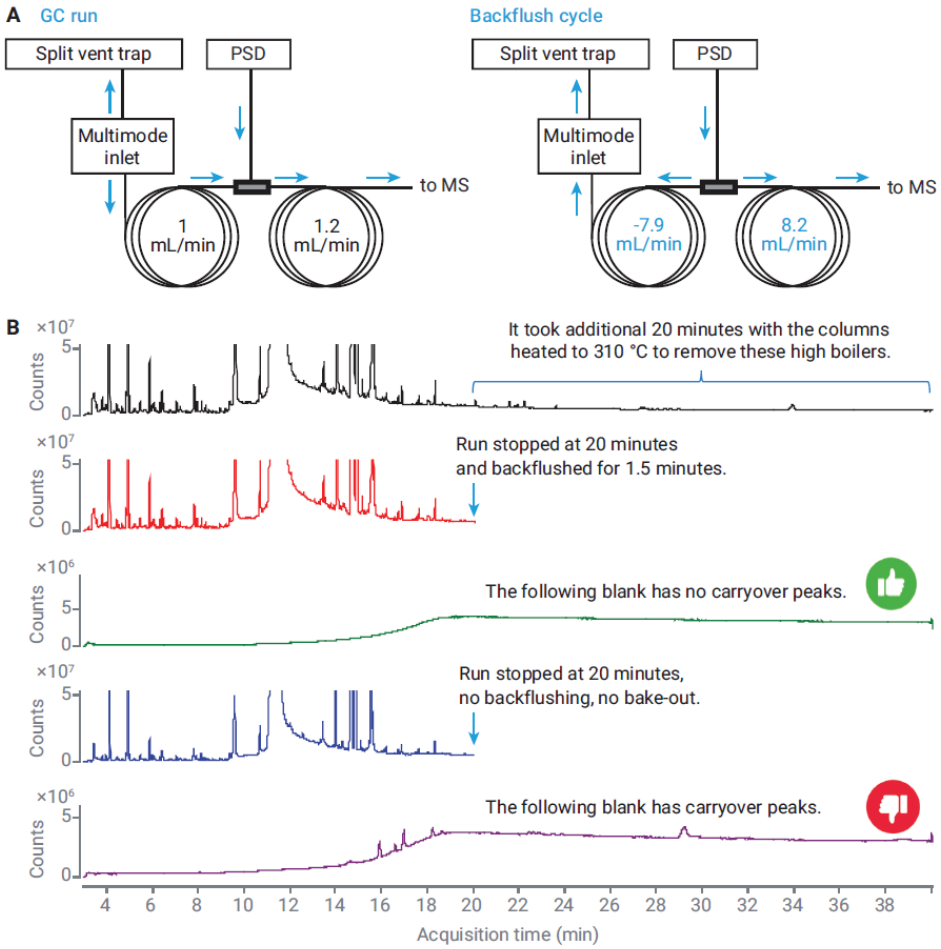

Figure 5. Midcolumn backflush configuration and gas flow during the GC run and the backflush cycle (A); TIC Scan chromatograms of a cayenne pepper extract followed by the analysis of an instrument blank with column bake-out, with backflush and without backflush or bake-out (B).

Leak-free GC/TQ system

Maintaining the GC/MS system leak-free is essential for the long-term performance of the instrument. Undesired leaks reduce the GC column lifetime and lead to oxidation of the EI source degrading its performance. The tools that enable tight connection make installation easy and reproducible and include the self-tightening collared column nuts for GC (Figures 6A and 6B part numbers G3440-81011 and G3440-81013) and CFT gold-plated flexible metal ferrules (Figure 6C, part number G2855-28501).

The self-tightening collared column nuts have an innovative spring-driven piston. The piston continuously presses against the short graphite/polyimide ferrule, maintaining a leak-free seal even after hundreds of temperature cycles of the oven. The addition of the collar makes column installation into the GC inlet and MS transfer line easy and reduces the possibility of variation. The locking collar allows locking the column in place, for accurate and repeatable installation results, time after time. The simplicity of the column installation process with the self-tightening collared column nuts is demonstrated in these videos.[6,7] When MS source maintenance is not required, the collared nut in combination with the column installation tool (part number G1099-20030) allows installation of the column into the MS without opening the side door.

Gold-plated flexible metal ferrules are inert and provide exceptionally reliable sealing. They prevent formation of microleaks at the CFT (PUU) connection and allow for maintaining high sensitivity of the GC/TQ.

Figure 6. Self-tightening collared column nuts for the inlet (A) and MS transfer line connection (B) and gold-plated flexible metal ferrules (C).

To confirm the leak-free status of the system, the air/water check, or autotune report, are often evaluated to determine how much of a leak is detected by the MS. However, this approach does not help to identify the source of the leak. Additionally, it may miss microleaks like those that may be present at user connections.

The novel leak test functionality is available with the 7000E and 7010C GC/TQ with MassHunter Data Acquisition 10.2 and above. The leak test can identify the source, and monitor the magnitude, of the leak. The tool monitors up to 10 user-specified ions (Figure 7A), including ions from a leak testing gas such as air duster (*m/z* 69 and 83, Figure 7B). The tool plots the corresponding chromatograms including EICs and TIC (Figure 7C).

Optimized injection with the temperature-programmable multimode inlet (MMI)

Efficiently volatilizing the sample in the GC inlet is an essential component of a successful GC/MS analysis. Some pesticides, such as captafol, captan, dicofol, folpet, and deltamethrin, are known to be thermally labile. They are anticipated to suffer thermal degradation during injection. Starting the injection at lower temperature of 60 °C and ramping up to 280 °C allows for volatilizing all the target analytes while maintaining their chemical integrity upon introduction to the GC column. Moreover, the ability to program the inlet temperature allows heating up the inlet further to 310 °C during the post run while backflushing. This heating enables the system to bake-out any matrix residue that may remain in the inlet.

Figure 7. The novel leak testing tool that enables monitoring of the user-specified ions to identify the source and the amount of leak.

The combination of temperature-programmable injection with an Ultra Inert 2 mm dimpled liner resulted in high sensitivity even for challenging pesticides like deltamethrin in a complex walnut matrix. Figure 8A demonstrates the response of deltamethrin, a pesticide with an established MRL in walnut, at 0.5 ppb with the 7000E and the 7010C GC/TQ. The 7010C GC/TQ is equipped with the HES that yields a higher sensitivity resulting in higher signal-to-noise ratio (S/N).

Pentachloronitrobenzene is a pesticide that is commonly analyzed by GC/MS in various food commodities as it has established MRLs in many vegetables and fruits (Crop Group 8 Fruiting Vegetables Group), peanuts, and soybean seeds that vary from 20 ppb to 1 ppm.[8] Pentachloronitrobenzene presents a challenge for LC/MS analysis, so GC/MS analysis is the technique of choice. Figure 8B demonstrates the chromatograms for a selective MRM transition for pentachloronitrobenzene in a walnut extract with the 7000E and the 7010C.

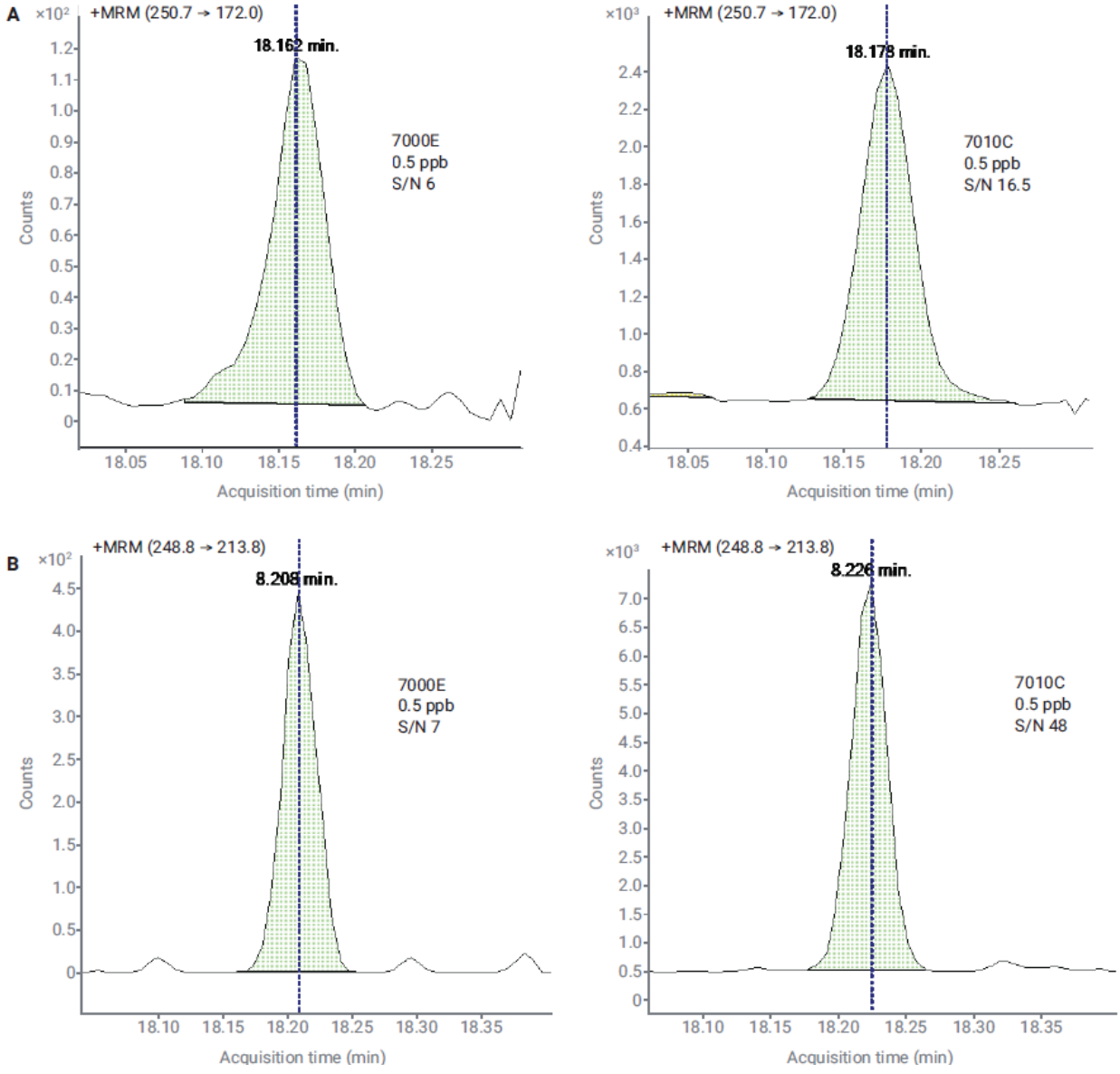

Figure 8. MRM chromatograms for deltamethrin (A) and pentachloronitrobenzene (B) at 0.5 ppb in walnut extract analyzed with the 7000E and the 7010C GC/TQ.

Calibration performance over a wide dynamic range with the 7000E and 7010C GC/TQ

The biggest challenge with the multiresidue analysis of food commodities is that the MRLs established for the pesticides vary over a wide range that may require undesirable sample reinjection. Achieving a broad dynamic calibration range can greatly reduce the need for diluting the sample and repeating the analysis.

Bifenthrin has established MRLs in spinach, walnut, and cayenne pepper that are 200, 50, and 500 ppb, respectively. Figure 9 demonstrates the linear calibration curves acquired with the 7000E over the calibration ranges of 0.1 to 1,000 ppb (R^2 = 0.996) in spinach, 0.1 to 5,000 ppb (R^2 = 0.991) in walnut, and 0.1 to 5,000 ppb (R^2 = 0.995) in cayenne pepper, encompassing the established MRL values.

MRLs for pesticide vary significantly not only across various commodities, but also for various pesticides regulated in one commodity. For example, pyriproxyfen and fludioxonil are monitored in spinach with the MRLs of 3,000 and 10 ppb, respectively. Figure 10A demonstrates that the 7000E GC/TQ maintained linear calibration performance for both pyriproxyfen and fludioxonil in spinach extract from 0.1 to 5,000 ppb, while demonstrating excellent accuracy even at low concentrations (see the zoomed in calibration for fludioxonil).

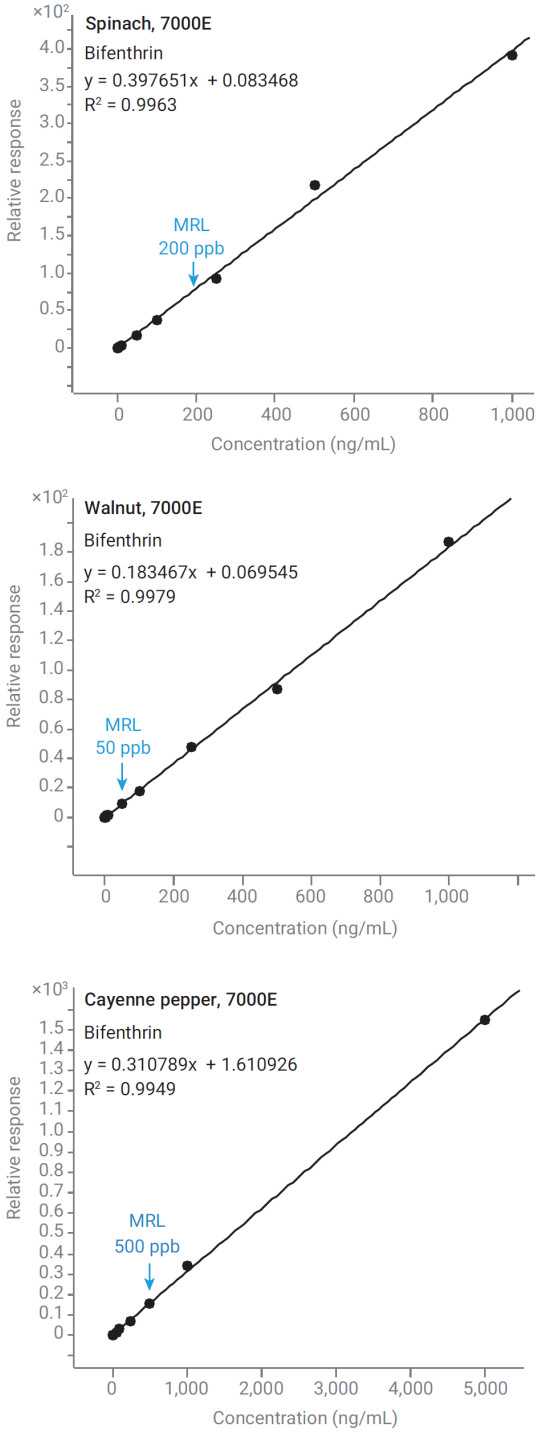

Figure 9. Matrix-matched calibration curves for bifenthrin in spinach, walnut, and cayenne pepper extracts with the 7000E GC/TQ.

As shown in Figure 10B, the 7010C GC/TQ also allowed for achieving a linear calibration curve over a broad range for both pesticides (0.1 to 1,000 ppb). However, the dynamic range of the 7010C would require an extra injection of a diluted sample to accommodate accurate quantitation of pyriproxyfen at its MRL of 3,000 ppb. While the upper limit of the calibration range achieved with the 7010C for pyriproxyfen and fludioxonil is lower than that with the 7000E, the 7010C delivers a higher sensitivity at lower concentrations. This is shown in Figure 10C and can be critical for the analysis of these pesticides in the commodities with lower established MRLs.

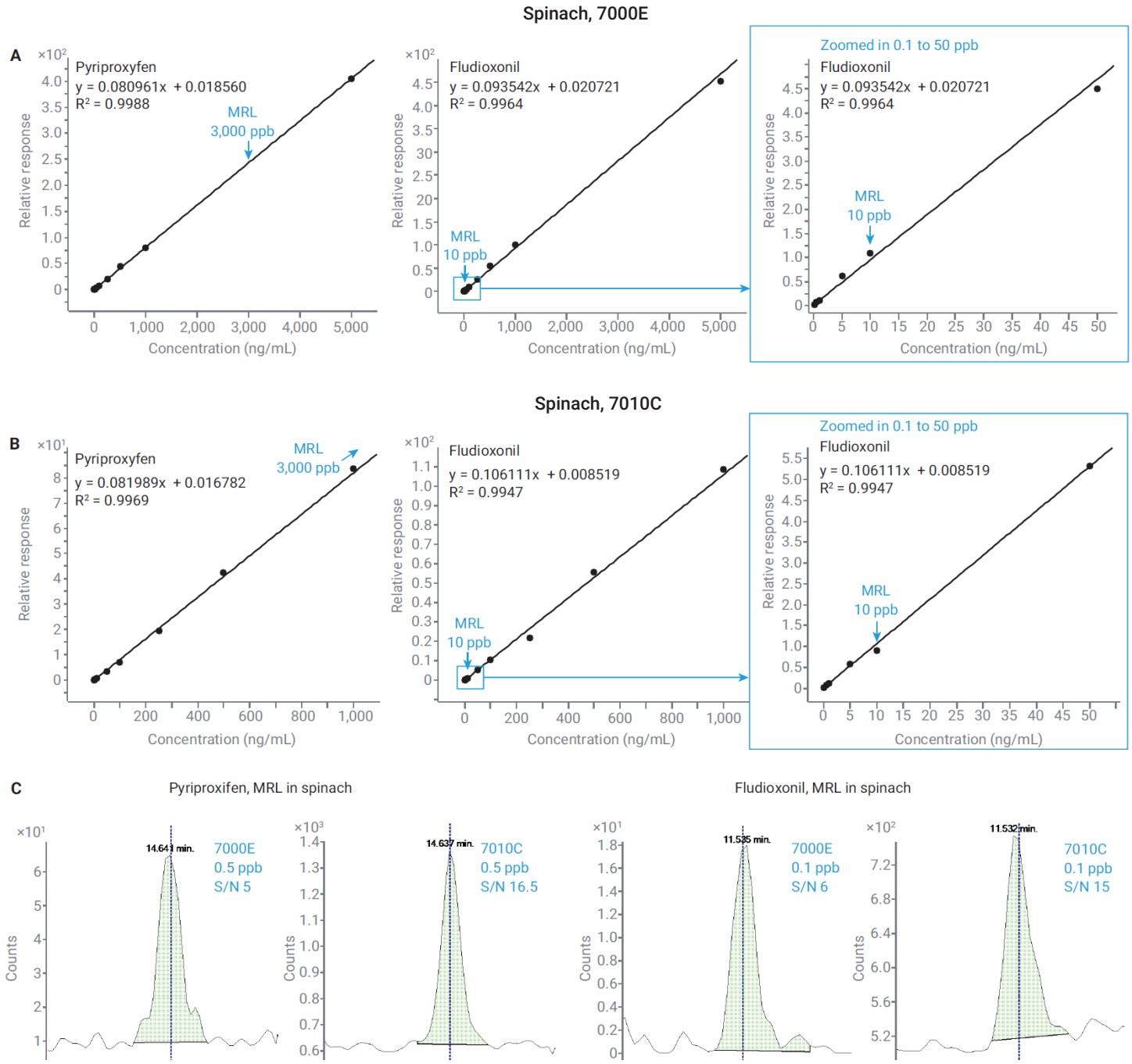

Figure 10. Matrix-matched calibration curves for pyriproxyfen and fludioxonil in spinach QuEChERS extracts with the 7000E GC/TQ (A) and with the 7010C GC/TQ (B); MRM chromatograms for pyriproxyfen and fludioxonil at 0.5 and 0.1 ppb in spinach QuEChERS extract analyzed with the 7000E and the 7010C GC/TQ (C).

Alternatively, samples with the MRLs above 1,000 ppb can be further diluted before the analysis with the 7010C GC/TQ. Superior sensitivity enabled with the HES allows for precise quantitation maintaining low LOQs even in the diluted sample. Additionally, injection of the dilutes samples increased maintenance-free operating time increased the number of injections that could be performed before the GC inlet liner needs replacement.

A summary in Figure 11 shows the calibration performance for the 203 pesticides that were analyzed in spinach, walnut, and cayenne pepper extracts with the 7000E and 7010C GC/TQ systems. The graph illustrates the number of compounds with the calibration correlation coefficient R^2 >0.99, the calibration fit (linear or quadratic), and the calibration range.

As expected, considering the recommended loading for the HES not to exceed 1 ng per analyte, the upper calibration limit for the 7010C was lower when compared to the 7000E (1,000 ppb versus 5,000 ppb). However, the calibration range achieved with the 7010C was up to four orders of magnitude with a linear fit for most of the analyzed compounds. The 7010C GC/TQ equipped with the HES enables superior sensitivity yielding high S/N at low concentrations and allows for accurate quantitation at concentrations below 0.1 ppb. However, this was not required in this work as the MRLs for pesticides regulated in the commodities of interest did not require sub 0.1 ppb quantitation. Alternatively, samples with the MRLs above 1,000 ppb can be further diluted before the analysis with the 7010C GC/TQ. The HES enables maintaining high sensitivity at the LOQ level even in the dilutes sample.

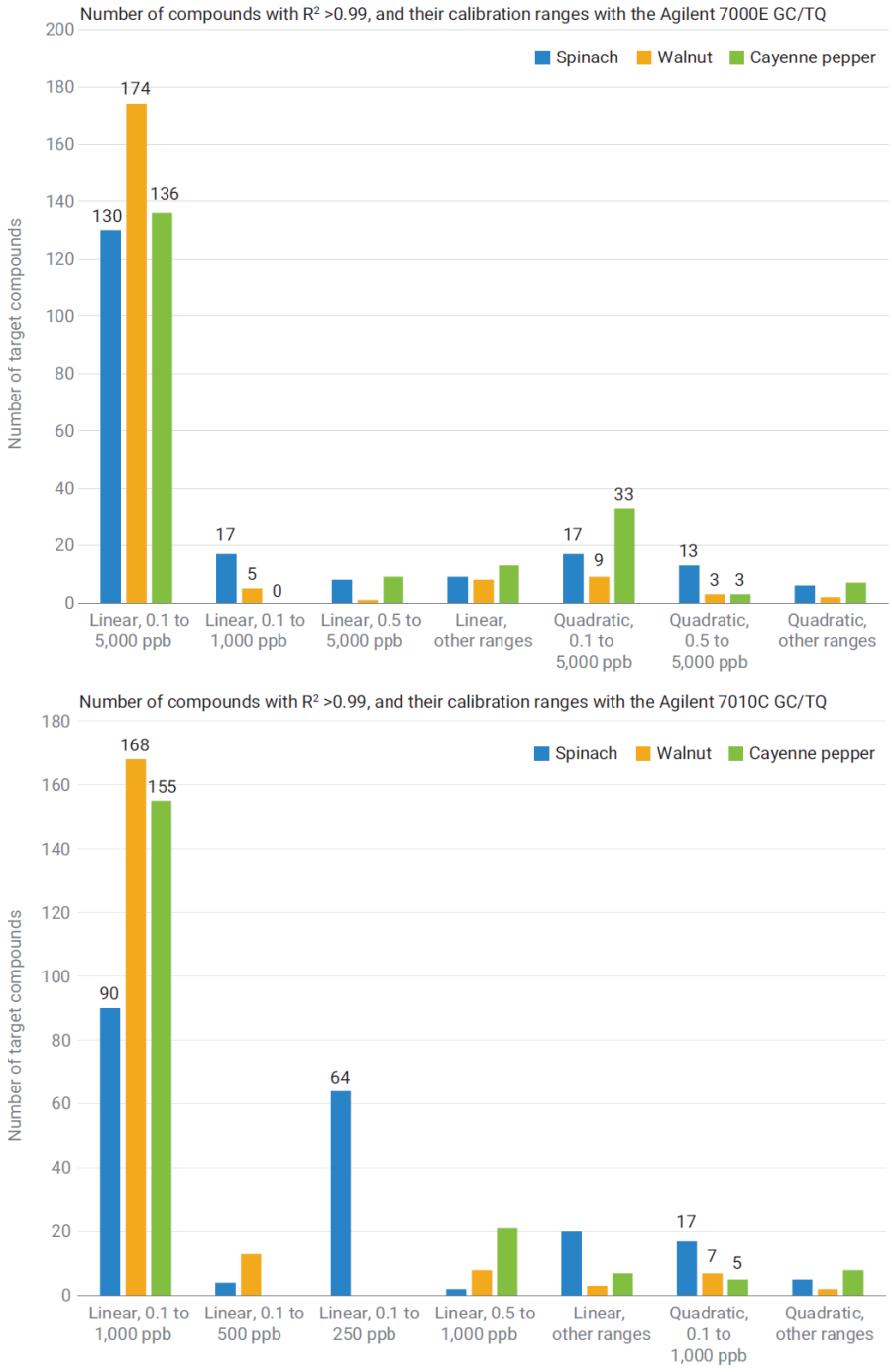

Figure 11. Calibration performance for the 203 pesticides with the 7000E and 7010C GC/TQ in spinach. The graph shows the number of compounds and their calibration ranges.

Conclusion

This application note described five best practices in sample preparation and Agilent 8890/7000E and 8890/7010C triple quadrupole GC/MS system analysis applied to 203 pesticides in challenging food matrices, including spinach, walnut, and cayenne pepper. These practices included:

- Simplified and improved sample preparation achieved with the novel and improved Agilent Captiva EMR pass-through clean up following the traditional Agilent QuEChERS extraction
- Evaluation of in-source loading of the matrix in full scan data acquisition mode
- Midcolumn backflushing
- Leak-free GC/triple quadrupole system enabled with the self-tightening collared column nuts and CFT gold-plated flexible metal ferrules
- Use of temperature-programmed multimode inlet with a 2 mm dimpled liner (no glass wool)

The resulting method allowed for excellent calibration performance over a wide dynamic range up to over four orders of magnitude. The calibration performance was as wide as 0.1 to 5,000 ppb and 0.1 to 1,000 for most of the compounds with the 7000E and the 7010C, respectively. The 7010C demonstrated superior sensitivity yielding a higher signal-to-noise ratio at lower concentrations. The wide dynamic ranges in combination with high sensitivity make the 7000E and the 7010C the ideal tools for analyzing pesticides at their MRLs in various commodities, including those with complex highly pigmented and oily matrices.

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Appendix 1

Compounds analyzed in this work and their observed retention times.

Name	Retenion Time (min)	Name	Retenion Time (min)	Name	Retention Time (min)
Allidochlor	4.893	Pyrimethanil	8.282	DCPA (Dacthal, Chlorthal-dimethyl)	10.062
Dichlorobenzonitrile, 2,6-	5.244	Diazinon	8.291	Fenson	10.201
Biphenyl	5.423	Fluchloralin	8.326	Diphenamid	10.288
Mevinphos, E-	5.597	Disulfoton	8.427	Bromophos	10.297
3,4-Dichloroaniline	5.708	Tefluthrin	8.431	Pirimiphos-ethyl	10.304
Pebulate	5.803	Terbacil	8.432	Isopropalin	10.358
Etridiazole	5.833	BHC-*delta*	8.504	Cyprodinil	10.407
cis-1,2,3,6-Tetrahydrophthalimide	5.966	Isazofos	8.527	MGK-264	10.443
N-(2,4-dimethylphenyl)formamide	5.973	Triallate	8.569	Isodrin	10.455
Methacrifos	6.055	Chlorothalonil	8.584	Metazachlor	10.532
Chloroneb	6.136	Endosulfan ether	8.857	Pendimethalin	10.535
2-Phenylphenol	6.246	Pentachloroaniline	8.913	Penconazole	10.562
Pentachlorobenzene	6.343	Propanil	8.942	Chlozolinate	10.584
Propachlor	6.888	Dimethachlor	8.996	Heptachlor exo-epoxide	10.621
Tecnazene	6.889	Acetochlor	9.093	Tolylfluanid	10.646
Diphenylamine	6.959	Vinclozolin	9.115	Allethrin	10.648
Cycloate	7.043	Transfluthrin	9.129	Fipronil	10.662
2,3,5,6-Tetrachloroaniline	7.059	Parathion-methyl	9.145	Chlorfenvinphos	10.676
Chlorpropham	7.102	Chlorpyrifos-methyl	9.146	Bromfenvinfos-methyl	10.683
Ethalfluralin	7.139	Tolclofos-methyl	9.233	Captan	10.732
Trifluralin	7.245	Alachlor	9.263	Triadimenol	10.746
Benfluralin	7.279	Propisochlor	9.333	Quinalphos	10.747
Sulfotep	7.376	Heptachlor	9.336	Triflumizole	10.77
Diallate I	7.481	Metalaxyl	9.337	Folpet	10.847
Phorate	7.498	Ronnel	9.396	Procymidone	10.858
BHC-*alpha* (benzene hexachloride)	7.636	Prodiamine	9.556	Chlorbenside	10.918
Hexachlorobenzene	7.768	Fenitrothion	9.596	Bromophos-ethyl	11.041
Dichloran	7.798	Pirimiphos-methyl	9.598	Chlordane-*trans*	11.043
Pentachloroanisole	7.823	Linuron	9.668	DDE-o,p'	11.09
Atrazine	7.885	Malathion	9.743	Paclobutrazol	11.106
Clomazone	7.982	Pentachlorothioanisole	9.758	Tetrachlorvinphos	11.169
BHC-beta	8.025	Dichlofluanid	9.764	Endosulfan I (*alpha* isomer)	11.273
Profluralin	8.117	Metolachlor	9.902	Chlordane-*cis*	11.305
Terbuthylazine	8.119	Anthraquinone	9.916	Flutriafol	11.322
BHC-gamma (Lindane, *gamma* HCH)	8.146	Fenthion	9.928	Fenamiphos	11.355
Terbufos	8.159	Aldrin	9.942	Chlorfenson	11.382
Propyzamide	8.175	Chlorpyrifos	9.964	Nonachlor, *trans*-	11.392
Pentachloronitrobenzene	8.219	Parathion	9.98	Bromfenvinfos	11.4
Fonofos	8.251	Triadimefon	10.011	Flutolanil	11.402
Pentachlorobenzonitrile	8.259	Dichlorobenzophenone, 4,4'-	10.033	Iodofenphos	11.479

Name	Retenion Time (min)	Name	Retenion Time (min)	Name	Retention Time (min)
Prothiofos	11.514	Carbophenothion	12.849	Phenothrin I	14.334
Fludioxonil	11.556	Carfentrazone-ethyl	12.851	Tetradifon	14.445
Profenofos	11.56	Methoxychlor olefin	12.865	Phosalone	14.61
Pretilachlor	11.592	Edifenphos	12.949	Azinphos-methyl	14.64
DDE-p,p'	11.637	Norflurazon	12.964	Pyriproxyfen	14.662
Tricyclazole	11.645	Lenacil	12.976	Leptophos	14.666
Oxadiazon	11.659	Endosulfan sulfate	13.04	Cyhalothrin (*Lambda*)	14.731
Dieldrin	11.73	DDT-p,p'	13.054	Mirex	14.898
Oxyfluorfen	11.737	Hexazinone	13.23	Acrinathrin	15.076
Myclobutanil	11.747	Methoxychlor, o,p'-	13.241	Fenarimol	15.121
DDD-o,p'	11.799	Tebuconazole	13.294	Pyrazophos	15.168
Flusilazole	11.8	Propargite	13.352	Azinphos-ethyl	15.252
Bupirimate	11.831	Piperonyl butoxide	13.404	Pyraclofos	15.303
Fluazifop-p-butyl	12.007	Resmethrin	13.44	Permethrin, (1R)-*cis*-	15.656
Nitrofen	12.023	Captafol	13.466	Permethrin, (1R)-*trans*-	15.772
Ethylan	12.063	Nitralin	13.563	Pyridaben	15.807
Chlorfenapyr	12.064	Iprodione	13.726	Fluquinconazole	15.895
Endrin	12.127	Tetramethrin I	13.836	Coumaphos	15.902
Chlorobenzilate	12.194	Pyridaphenthion	13.838	Prochloraz	15.958
Endosulfan II (beta isomer)	12.291	Endrin ketone	13.898	Cyfluthrin I	16.207
DDD-p,p'	12.383	Phosmet	13.931	Cypermethrin I	16.421
Ethion	12.453	Bromopropylate	13.952	Flucythrinate I	16.75
DDT-o,p'	12.457	EPN	13.955	Ethofenprox	16.829
Chlorthiophos	12.503	Bifenthrin	13.956	Fluridone	17.034
Nonachlor, *cis*-	12.508	Methoxychlor, p,p'-	14.062	Fenvalerate I	17.459
Endrin aldehyde	12.618	Fenpropathrin	14.077	Fluvalinate-tau I	17.646
Sulprofos	12.669	Tebufenpyrad	14.142	Deltamethrin	18.177
Triazophos	12.674				

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GC/MS/MS Analysis of PAHs with Hydrogen Carrier Gas

Using the Agilent HydroInert source in a challenging soil matrix

Authors

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Abstract

The Agilent 8890 GC and 7000E triple quadrupole GC/MS system (GC/TQ) with a novel electron ionization (EI) source—the Agilent HydroInert source, which is optimized for hydrogen carrier gas—were used for the analysis of polycyclic aromatic hydrocarbons (PAHs). The optimized method using the HydroInert source provides excellent peak shape, sensitivity, and linearity of R^2 ≥0.999, which was observed for all 27 analytes over their respective calibration ranges (0.1 to 1,000 pg for 26 analytes and 0.25 to 1,000 pg for one analyte). Method detection limits (MDLs) ranged from 0.03 to 0.16 pg with an average of 0.09 pg. The stability of calculated concentrations over 500 injections is presented, where, with routine maintenance and backflush, injection RSDs were <12% for all analytes. Further, the ability of the Agilent universal Ultra Inert (UI) mid-frit inlet liner to handle a complex matrix is demonstrated. By proper selection of instrument configuration and operating conditions, the system with hydrogen carrier gas can generate results comparable to or better than those with helium.

Introduction

PAHs are a group of chemical compounds that are composed of two or more fused conjugated benzene rings with a pair of carbon atoms shared between rings in their molecules. Further, PAHs originate from multiple sources and are widely distributed as contaminants throughout the world. Given the ubiquitous nature of this compound class, trace contamination is monitored in food products (i.e., edible oils, smoked meats, and seafood) and in the environment (i.e., air, water, and soil). The most common way to detect PAHs is with GC/MS on the single or triple quadrupole instrument. Helium is the preferred carrier gas for GC/MS analysis; however, its reoccurring shortages and mounting costs have increased demand for applications using hydrogen as the carrier gas.

This application note focuses on the analysis of PAHs on a triple quadrupole GC/MS in multiple reaction monitoring (MRM) mode using hydrogen as the GC carrier gas. When adopting hydrogen for GC/MS analysis, there are several factors to consider. First, hydrogen is a reactive gas, and may potentially cause chemical reactions in the inlet, column, and sometimes the MS EI source, which can change analysis results. To address potential issues in the source of the MS, the Agilent HydroInert source was used. Additional information can be found in the Agilent technical overview of the HydroInert source.[1] Second, for GC/MS applications, hardware changes in the gas chromatograph and mass spectrometer may be required when switching to hydrogen carrier gas. The Agilent Helium to Hydrogen Carrier Gas Conversion Guide[2] describes in detail the steps for conversion from helium to hydrogen carrier gas. Lastly, it is recommended that anyone working with flammable or explosive gases take a lab safety course covering proper gas handling and use. Further information on the safe use of hydrogen can be found in the Agilent Hydrogen Safety Manual[3] and Hydrogen Safety for the Agilent 8890 GC System Guide.[4]

In addition to the challenges of hydrogen carrier, there are often matrix-related problems with the analysis of PAHs. For example, in food and soil analyses, high-boiling matrix contaminants that elute after the analytes can require extended bake-out times to prevent ghost peaks in subsequent runs, hence decreasing column lifetime. The highest boiling contaminants can deposit in the head of the column, requiring more frequent column trimming and adjustment of MRM and data analysis time windows from the resulting retention time shift. Thus, this application note uses mid-column backflush to address some of the matrix-related factors. Backflushing is a technique where the carrier gas flow is reversed after the last analyte has exited the column. After the MS data are collected, the oven is held at the final temperature in postrun mode, and the carrier gas flow through the first column is reversed. Any high-boiling contaminants that were in the column at the end of data collection are carried out of the head of the column and into the split vent trap by this reversed flow.

This application note presents an optimized MRM method for analyzing 27 PAHs using hydrogen carrier gas, the HydroInert source, and mid-column backflush to address heavy matrix. A liquid-extracted soil sample was used as a worst-case scenario to test the Ultra Inert mid-frit inlet liner and the method for PAH analysis. Liner, column, and system robustness were demonstrated by 500 repeat injections of extracted soil sample.

Experimental

Chemicals and reagents

PAH calibration standards were diluted from the Agilent PAH analyzer calibration sample kit (part number G3440-85009) using isooctane. The kit contains a stock solution of 27 PAHs at 10 µg/mL and a stock solution of five internal standards (ISTDs) at 50 µg/mL. Twelve calibration levels were prepared: 0.1, 0.25, 0.5, 1, 2, 10, 20, 100, 200, 400, 750, and 1,000 ng/mL. Each level also contained 500 ng/mL of the ISTDs.

Instrumentation

The system used in this experiment (Figure 1) was configured to minimize the potential problems with hydrogen carrier gas and complex sample matrix in PAH analysis. The instrument operating parameters are listed in Table 1, and MRMs in Table 2. Table 3 contains a list of consumable items used for the current application. Important techniques to consider are outlined in Table 4.

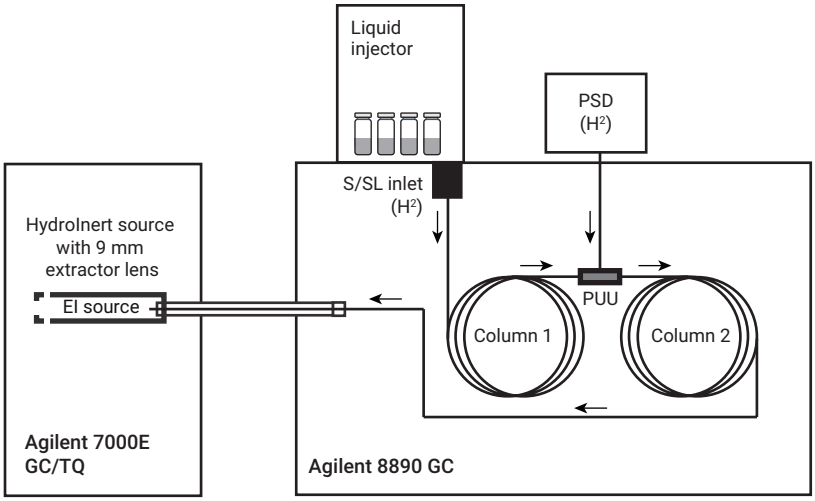

Figure 1. System configuration.

Table 1. GC and MS conditions for PAH analysis.

Agilent 8890 GC with Fast Oven, Auto Injector, and Tray	
Injection Volume	1.0 µL
Inlet	EPC split/splitless
Mode	Pulsed splitless
Injection Pulse Pressure	40 psi until 0.7 min
Purge Flow to Split Vent	50 mL/min at 0.75 min
Septum Purge Flow Mode	Standard, 3 mL/min
Inlet Temperature	320 °C
Oven	Initial: 60 °C (1 min hold) Ramp 1: 25 °C/min to 200 °C Ramp 2: 10 °C/min to 335 (4.4 min hold)
Column 1	Agilent J&W DB-EUPAH, 20 m × 0.18 mm, 0.14 µm
Control Mode	Constant flow, 0.9 mL/min
Inlet Connection	Split/Splitless
Outlet Connection	PSD (PUU)
Postrun Flow (Backflushing)	–5.274 mL/min
Column 2	Agilent J&W DB-EUPAH, 20 m × 0.18 mm, 0.14 µm
Control Mode	Constant flow, 1.1 mL/min
PSD Purge Flow	3 mL/min
Inlet Connection	PSD (PUU)
Outlet Connection	Agilent 7000E GC/TQ
Postrun Flow (Backflushing)	5.443 mL/min

Agilent 8890 GC Backflush Parameters	
Inlet Pressure (Backflushing)	2 psi
Backflush Pressure	80 psi
Void Volumes	7.2
Backflush Time	1.5 min
Agilent 7000E GC/TQ	
Source	Agilent HydroInert source
Drawout Lens	9 mm
Transferline Temperature	320 °C
Source Temperature	325 °C
Quadrupole Temperature	150 °C
Mode	Dynamic MRM
EM Voltage Gain	10
Solvent Delay	5.5 min
Collision Gas	Nitrogen (only), 1.5 mL/min
Automatically Subtract Baseline	Yes
Advanced SIM/MRM Thresholding	Yes
Tune File	atunes.eiex.jtune.xml

Table 2. MRM transitions used for quantifiers and qualifiers, with hydrogen carrier optimized collision energy.

Analyte	Retention Time (minutes)	Quantifier	Collision Energy	Qualifier	Collision Energy
Naphthalene-d_8 (ISTD)	5.902	136.0 → 136.0	5	136.0 → 108.0	15
Naphthalene	5.922	128.0 → 102.0	20	128.0 → 127.0	20
1-Methylnaphthalene	6.514	142.0 → 115.0	35	142.0 → 141.0	20
2-Methylnaphthalene	6.675	142.0 → 115.0	30	142.0 → 141.0	20
Biphenyl	7.049	154.0 → 152.0	30	154.0 → 153.0	20
2,6-Dimethylnaphthalene	7.081	156.0 → 115.0	35	156.0 → 141.0	20
Acenaphthylene	7.738	152.0 → 151.0	20	152.0 → 150.0	35
Acenaphthene-d_{10} (ISTD)	7.841	162.0 → 160.0	15	164.0 → 162.0	15
Acenaphthene	7.889	154.0 → 152.0	35	153.0 → 152.0	40
2,3,5-Trimethylnaphthalene	8.085	170.0 → 155.0	20	170.0 → 153.0	30
Fluorene	8.539	166.0 → 165.0	25	166.0 → 163.0	25
Dibenzothiophene	10.1	184.0 → 139.0	40	184.0 → 152.0	25
Phenanthrene-d_{10} (ISTD)	10.265	188.0 → 188.0	5	188.0 → 184.0	25
Phenanthrene	10.313	178.0 → 176.0	35	178.0 → 152.0	30
Anthracene	10.367	178.0 → 152.0	25	178.0 → 156.0	35
1-Methylphenanthrene	11.452	192.0 → 191.0	20	192.0 → 165.0	40
Fluoranthene	12.842	202.0 → 200.0	40	202.0 → 201.0	25
Pyrene	13.51	202.0 → 200.0	40	202.0 → 201.0	30
Benz[a]anthracene	16.327	228.0 → 226.0	35	228.0 → 224.0	55
Chrysene-d_{12} (ISTD)	16.46	240.0 → 236.0	35	240.0 → 240.0	5
Chrysene	16.531	228.0 → 226.0	35	228.0 → 224.0	55
Benzo[b]fluoranthene	18.953	252.0 → 250.0	40	250.0 → 248.0	40
Benzo[k]fluoranthene	19.003	252.0 → 250.0	40	250.0 → 248.0	40
Benzo[j]fluoranthene	19.087	252.0 → 250.0	40	250.0 → 248.0	45
Benzo[e]pyrene	19.793	252.0 → 250.0	40	250.0 → 248.0	45
Benzo[a]pyrene	19.903	252.0 → 250.0	40	250.0 → 248.0	40
Perylene-d_{12} (ISTD)	20.115	264.0 → 260.0	35	264.0 → 236.0	35
Perylene	20.177	252.0 → 250.0	40	250.0 → 248.0	45
Dibenz[a,c]anthracene	22.386	278.0 → 276.0	42	276.0 → 274.0	40
Dibenz[a,h]anthracene	22.488	278.0 → 276.0	40	276.0 → 274.0	40
Indeno[1,2,3-cd]pyrene	22.526	276.0 → 274.0	42	138.0 → 124.0	42
Benzo[ghi]perylene	23.562	276.0 → 274.0	42	274.0 → 272.0	45

Table 3. Agilent consumables and part numbers used in the method for PAH analysis.

Consumable	Description	Part Number
Injector Syringe	Blue Line autosampler syringe, 10 µL, fixed needle	G4513-80220
Inlet Septum	Advanced Green septum, nonstick, 11 mm	5183-4759
Inlet Liner	Universal Ultra Inert mid-frit inlet liner	5190-5105
Gold Seal	GC inlet seal, gold plated with washer, Ultra Inert	5190-6144
Column	DB-EUPAH, 20 m × 0.18 mm, 0.14 µm (quantity: 2)	121-9627
Backflush Union	Purged Ultimate union assy	G3186-80580
Backflush Ferules	CFT Ferrule Flex Gold flexible metal ferrule, gold plated, 0.4 mm id, for 0.1 to 0.25 mm id fused silica tubing	G2855-28501
Steel Tubing	Install kit for GCs, stainless steel	19199S
GC/MS Source	HydroInert complete source assembly for 7000 GC/TQ	G7006-67930

Table 4. Important techniques to consider in this study.

Consideration	Description
Hydrogen Gas	In-house hydrogen, with 99.9999% purity specification and low individual specifications on water and oxygen, was used as a carrier gas. It is essential to use a reliable source of clean hydrogen gas. For long-term use, generators with a >99.9999% specification and low individual specifications on water and oxygen are recommended. Moisture filters are recommended for use with hydrogen generators. For short-term use, cylinders with chromatographic or research-grade hydrogen are acceptable.
Pulsed Splitless Injection	Used to maximize transfer of the PAHs, especially the heavy ones, from the GC inlet into the column.
Inlet Liner	The Agilent universal UI mid-frit inlet liner was found to give good peak shape, inertness, and longevity with the soil extracts described later. The frit transfers heat to the PAHs and blocks the line of sight to the inlet base. If the PAHs condense on the inlet base, they are difficult to vaporize and sweep back into the column.
Column Dimensions	Two Agilent J&W DB-EUPAH columns (20 m × 0.18 mm id, 0.14 µm) were used to maintain optimal gas flow and inlet pressure in the backflush configuration.
8890 PSD Module and Midcolumn Backflushing	The pneumatic switching device (PSD) is an Agilent 8890 GC pneumatics module optimized for backflushing applications and provides for seamless pulsed injections. The capability to reverse the flow is provided by the Agilent purged Ultimate union (PUU). The PUU is a tee, inserted, in this case, between two identical 20 m columns. During the analysis, a small make-up flow of carrier gas from the 8890 PSD module is used to sweep the connection. During backflushing, the make-up flow from the PSD is raised to a much higher value, sweeping high-boiling contaminants backward out of the first of column and forward from the second.
HydroInert EI Source	The Agilent HydroInert source is a substitute for the extractor source when hydrogen carrier is used. It is constructed with materials that greatly reduce undesirable reactions in the source to maintain spectral fidelity when used with hydrogen. As commonly known, PAHs present unique challenges regarding the MS EI source, even with helium as the carrier gas.[5] With hydrogen carrier gas, the performance of PAHs is improved, especially with the HydroInert source. The 9 mm extractor lens is the default included with the HydroInert source and the best choice for PAH analysis[6,7] as it provides the best calibration linearity, precision of response, and peak shape.
Collision Gas	Only nitrogen should be used as collision gas in GC/TQ when hydrogen is the carrier gas. The collision cell helium inlet fitting must be capped. The optimal nitrogen gas flow was shown to be 1.5 mL/min, which agreed with the user manual recommendation. This flow was also demonstrated to be optimal in previous work on PAHs in hydrogen carrier.[8]
MS/MS	The added selectivity of MRM mode in GC/TQ simplifies the data review of high-matrix samples relative to GC/MS by reducing or eliminating interfering responses from the matrix. Interfering responses often require manual integration of quantifier or qualifier ions.

Matrix sample preparation

A sample of commercial topsoil (Weaver Mulch, Coatesville, PA, U.S.) was chosen to perform a response stability and robustness test. Extraction is described briefly. Topsoil was dried at 120 °C overnight. A 5 g sample of the dried soil was extracted with 30 mL dichloromethane/acetone (1:1 v/v) with agitation overnight. The extract was filtered, and the filtrate was reduced 7.5 fold in volume by evaporation. The resulting extract was spiked with 100 ppb of the 27 PAH analytes and 500 ppb of the five ISTD compounds.

Robustness testing

Calculated concentration stability was tested over 500 replicate injections using soil extract spiked with PAHs at 100 ppb. For this test, the MS was tuned at the beginning of the test only with no need to retune it throughout the robustness testing experiment. After every 100 injections, the liner and septa were replaced and the EM gain was updated. After every 300 injections, the split/splitless inlet gold seal was replaced. The column was not trimmed or replaced throughout the entire 500 injections. This test was designed to demonstrate the robustness of the system over continuous injections of an intentionally challenging matrix.

Results and discussion

GC/MS methodology

Figure 2 shows the MRM total ion chromatogram (TIC) of the 100 pg/µL calibration standard with 500 pg/µL ISTDs. Using these parameters, the peak shapes for PAHs—especially the latest ones—are excellent, and are comparable to previous hydrogen work.[8] In general, the HydroInert source provided the best peak shapes for PAHs when using hydrogen carrier gas. The chromatographic resolution obtained with the current setup is also better than that obtained with helium.[5] Due to the combination of hydrogen carrier and a smaller diameter column, the run time with the current method is 24 versus 26 minutes used in the helium method. The run time of the current method could have been reduced further and still maintained similar resolution. However, the current method conditions were chosen to achieve the best resolution of dibenz[a,c]anthracene, indeno[1,2,3-cd]pyrene, and dibenz[a,h] anthracene, because a more aggressive temperature ramp in the latter half of the method can reduce the resolution of this challenging cluster.

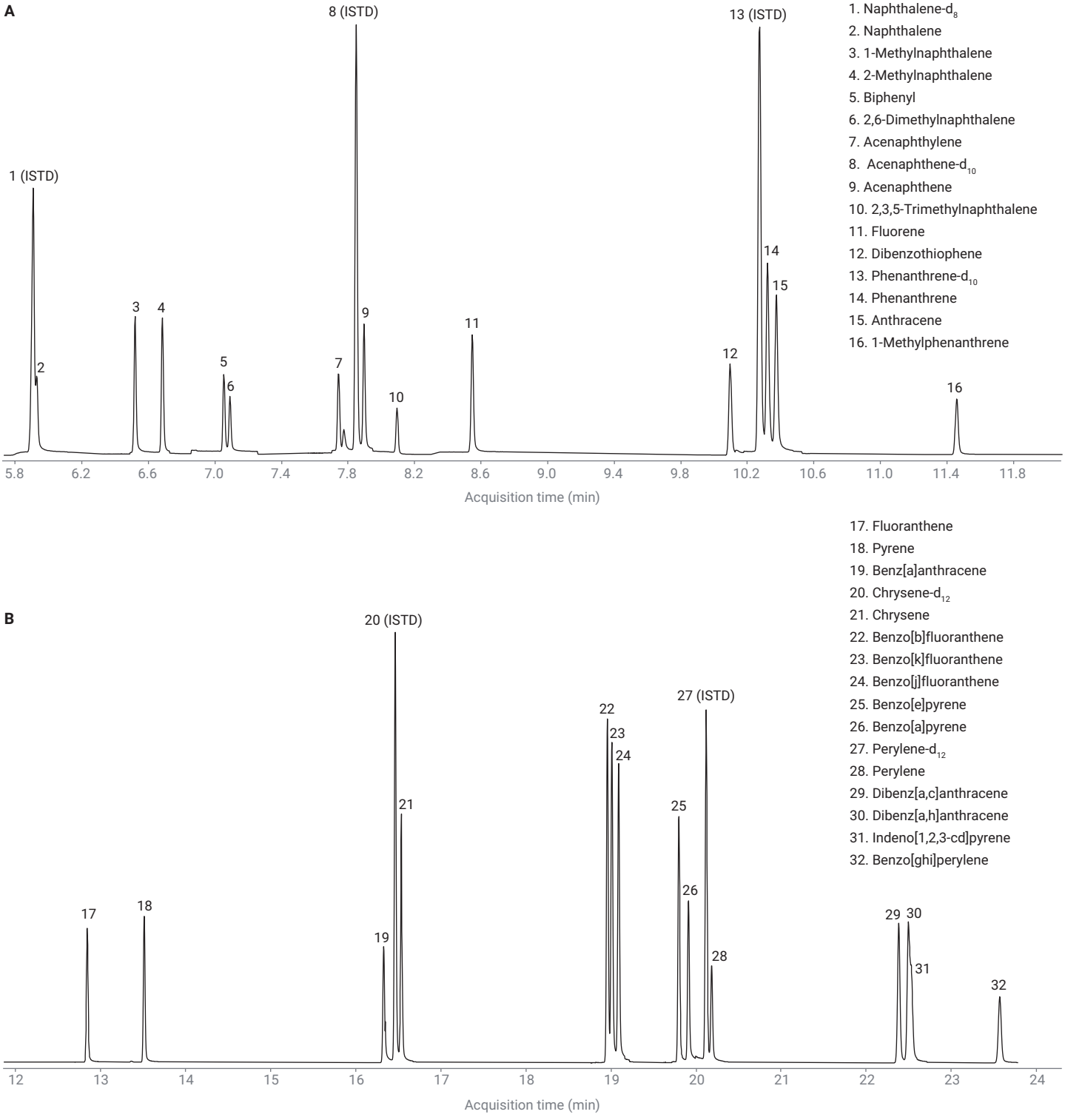

Figure 2. MRM TIC of 27 PAHs at 100 pg/µL and five ISTDs at 500 pg/µL.

Table 5 shows the calibration results of the system with 12 calibration levels from 0.1 to 1,000 pg. All analytes show excellent linearity across the entire range. Using the HydroInert source also resulted in excellent signal-to-noise ratios, allowing the calibration range to be extended to subpicogram levels. Of the 27 analytes, 26 had sufficient signal for calibration from 0.1 to 1,000 pg. One was calibrated from 0.25 to 1,000 pg. The calibration ranges and signal-to-noise observations demonstrated high sensitivity at the lowest calibration level, similar to previous PAH work performed with hydrogen.[8]

One of the problems encountered when using helium carrier gas and the standard 3 mm EI source extractor lens for the analysis of PAHs is that the response of ISTDs climbed with increasing concentration of the analytes. This effect can cause the response of perylene-d_{12} to increase by as much as 60% over the calibration range and cause significant errors in quantitation. This problem has been addressed previously using the Agilent JetClean self-cleaning ion source and a 9 mm extractor lens.[3,4] With JetClean, helium is used as the carrier gas, but hydrogen is continuously added to the source at a flow typically in the range of 0.16 to 0.33 mL/min. This approach reduces the creeping ISTD effect and results in excellent calibration linearity and quantitation.

Figure 3 shows the ISTD response stability over the calibration range with the current method. As demonstrated in Figure 3, the use of hydrogen carrier gas with the HydroInert source and a 9 mm extractor lens also eliminates the creeping ISTD response problem. The %RSD for the raw area responses across the calibration range are all 6.4% or less. This is important for achieving the excellent calibration linearity shown in Table 5.

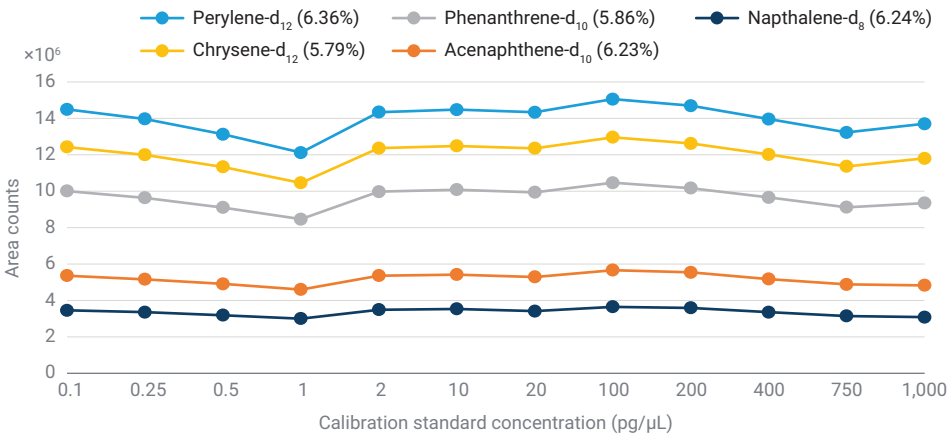

Figure 3. ISTD response over the calibration range.

Table 5. Results of a 12-level MRM ISTD calibration curve with a range of 0.1 to 1,000 pg. All calibration curves were linear, ignoring the origin, and weighted 1/x. MDLs were defined as MDL = t (n – 1,0.99) × SD, where t (n – 1,0.99) is the one-sided Student's t-statistic at the 99% confidence limit for n – 1 degrees of freedom, (2.998 for n = 8), and SD is the standard deviation of replicate solvent samples spiked at 0.25 pg.

Analyte	Linear Range (pg)	Correlation Coefficient (R^2)	MDL (pg)
Naphthalene	0.1 to 1000	0.9999	0.07
1-Methylnaphthalene	0.1 to 1000	0.9995	0.09
2-Methylnaphthalene	0.1 to 1000	0.9995	0.06
Biphenyl	0.1 to 1000	0.9994	0.16
2,6-Dimethylnaphthalene	0.1 to 1000	0.9994	0.10
Acenaphthylene	0.25 to 1000	0.9996	0.15
Acenaphthene	0.1 to 1000	0.9996	0.13
2,3,5-Trimethylnaphthalene	0.1 to 1000	0.9994	0.10
Fluorene	0.1 to 1000	0.9996	0.05
Dibenzothiophene	0.1 to 1000	0.9995	0.10
Phenanthrene	0.1 to 1000	0.9997	0.09
Anthracene	0.1 to 1000	0.9996	0.15
1-Methylphenanthrene	0.1 to 1000	0.9996	0.08
Fluoranthene	0.1 to 1000	0.9995	0.03
Pyrene	0.1 to 1000	0.9998	0.08
Benz[a]anthracene	0.1 to 1000	0.9995	0.13
Chrysene	0.1 to 1000	0.9996	0.11
Benzo[b]fluoranthene	0.1 to 1000	0.9995	0.06
Benzo[k]fluoranthene	0.1 to 1000	0.9999	0.09
Benzo[j]fluoranthene	0.1 to 1000	0.9999	0.12
Benzo[e]pyrene	0.1 to 1000	0.9997	0.07
Benzo[a]pyrene	0.1 to 1000	0.9998	0.11
Perylene	0.1 to 1000	0.9996	0.11
Dibenz[a,c]anthracene	0.1 to 1000	0.9997	0.05
Dibenz[a,h]anthracene	0.1 to 1000	0.9994	0.09
Indeno[1,2,3-cd]pyrene	0.1 to 1000	0.9996	0.08
Benzo[ghi]perylene	0.1 to 1000	0.9997	0.06

Method robustness in complex matrix

The soil extract used for the robustness test was deliberately chosen to have a high matrix content to challenge the system. Figure 4 shows the scan TIC of the spiked extract and the MRM TIC for comparison. As shown, the soil extract had a high level of matrix. When using MRM on the 7000E GC/TQ, the background is greatly reduced, allowing for low-level quantitation of PAHs using the current method.

Also, note that for soils with this level of organic content, further sample cleanup should be considered for routine analysis. The sample preparation used here was for test purposes only to deliberately challenge the system. Also, the extraction solvent (1:1 v/v dichloromethane/acetone) is not recommended for routine analysis with hydrogen carrier gas. Halogenated solvents like dichloromethane may react with hydrogen in the hot injection port and form low levels of HCl, which can degrade the liner and column head over time.

The stability of calculated concentration over 500 injections is presented in Figure 5. For 23 of 27 analytes, the response is stable, as shown in Table 6, where the RSDs for each set of 100 injections are under 5%. However, the calculated concentrations start to decline for dibenz[a,c]anthracene, dibenz[a,h]anthracene, indeno[1,2,3-cd] pyrene, and benzo[ghi]perylene around injection 70 (in a sequence of 100) and RSDs are slightly higher than 5% for each set of 100 injections. Over all 500 injections, with routine maintenance and backflush, injection RSDs were <12% for all analytes. This demonstrates excellent quantitation stability while continuously challenging the system with a complex soil extract.

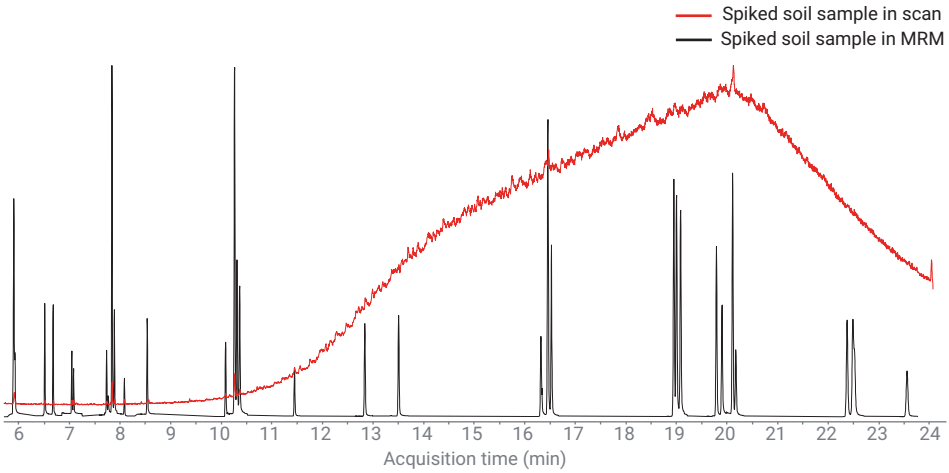

Figure 4. Spiked soil sample comparison of scan TIC and MRM TIC. The MRM trace is scaled up by an order of magnitude for visibility.

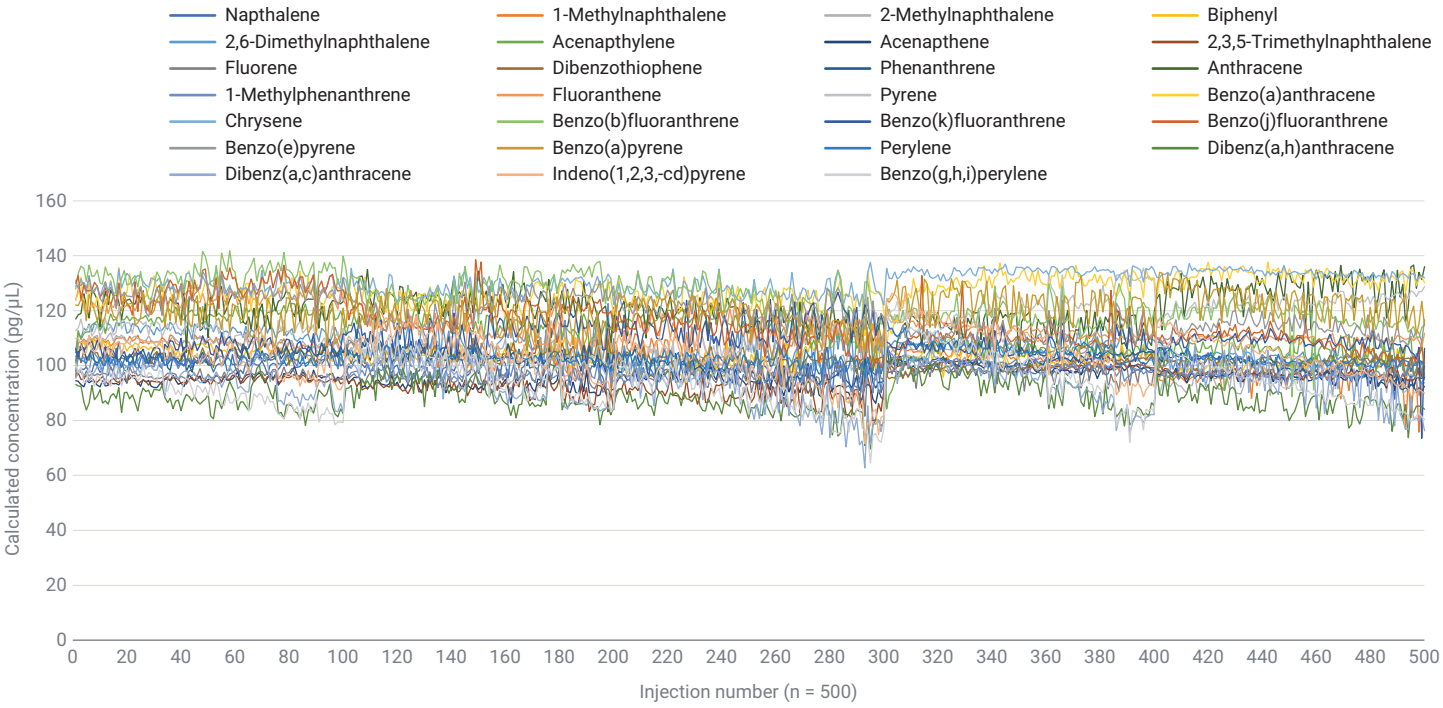

Figure 5. Stability of calculated concentrations over 500 injections of soil matrix spiked with 100 pg PAH standard and 500 pg of ISTD.

Table 6. Calculated concentration RSD% for every 100 injections and total 500 injections of extracted soil matrix spiked with 100 pg of PAH standard and 500 pg of ISTD standard.

Analyte	Injection RSD (%)					
	1 to 100	101 to 200	201 to 300	301 to 400	401 to 500	All (1 to 500)
Naphthalene	2.17	2.86	3.54	1.32	3.18	2.92
1-Methylnaphthalene	1.83	3.53	4.15	2.36	4.00	5.77
2-Methylnaphthalene	1.91	3.18	3.62	2.39	3.85	5.23
Biphenyl	1.94	2.74	4.86	2.30	2.56	3.55
2,6-Dimethylnaphthalene	1.97	4.08	4.56	2.28	1.87	4.50
Acenaphthylene	2.43	2.97	3.55	4.07	4.85	5.82
Acenaphthene	1.65	2.37	3.28	1.70	1.74	3.25
2,3,5-Trimethylnaphthalene	1.09	3.03	4.17	1.09	1.36	4.59
Fluorene	1.25	2.61	3.76	2.98	2.17	3.07
Dibenzothiophene	1.78	2.39	2.19	1.95	1.12	2.58
Phenanthrene	2.04	2.55	3.56	1.68	4.01	3.74
Anthracene	3.68	3.54	3.58	4.29	4.05	5.58
1-Methylphenanthrene	1.80	2.15	3.11	2.03	1.16	3.29
Fluoranthene	2.02	4.19	3.96	2.09	0.97	5.08
Pyrene	2.71	2.63	4.84	4.71	2.25	7.93
Benz[a]anthracene	2.82	2.92	2.93	3.81	1.91	3.71
Chrysene	1.96	2.41	2.59	1.14	1.00	2.86
Benzo[b]fluoranthene	2.97	3.74	4.22	3.48	4.40	6.07
Benzo[k]fluoranthene	2.29	4.24	4.67	2.90	3.78	4.68
Benzo[j]fluoranthene	3.29	4.16	4.89	4.89	3.75	7.44
Benzo[e]pyrene	2.52	4.32	3.33	3.67	2.79	5.75
Benzo[a]pyrene	4.53	4.62	4.60	4.44	4.46	4.92
Perylene	1.55	1.81	2.49	3.03	1.94	2.65
Dibenz[a,c]anthracene	4.61	5.89	4.86	6.91	5.08	6.36
Dibenz[a,h]anthracene	5.03	7.49	9.34	7.86	8.80	8.27
Indeno[1,2,3-cd]pyrene	5.26	7.23	9.31	8.99	6.86	8.38
Benzo[ghi]perylene	6.40	7.74	9.01	11.13	8.08	9.89

After each set of 100 injections, the liner and septa were replaced, which resulted in the concentration for dibenz[a,c] anthracene, dibenz[a,h]anthracene, indeno[1,2,3-cd]pyrene, and benzo[ghi] perylene to recover back to starting concentrations. The UI mid-frit liner performed well at trapping complex matrix, similar to previous studies.[9,10] The observation of a decline in concentration at approximately injection 70 for the four late-eluting compounds demonstrates that the liner was becoming saturated with matrix. As the liner saturates, the transfer of late-eluting compounds becomes inhibited. Table 7 shows the RSDs for only the first 70 injections of each set of 100, and the RSD for the total set of injections that comprise of just the first 70 injections. RSDs for each set of injections and total injections are improved when considering only the first 70 injections for dibenz[a,c]anthracene, dibenz[a,h]anthracene, indeno[1,2,3-cd] pyrene, and benzo[ghi]perylene. Also, at 300 injections the gold seal was changed, which resulted in tighter RSDs for 17 of 27 PAHs (Table 6).

Table 7. Calculated concentration RSD% of the first 70 injections of every 100 injections and total injections (using only the first 70 from each set) of extracted soil matrix spiked with 100 pg of PAH standard and 500 pg of ISTD.

Analyte	Injection RSD (%)					
	1 to 70	101 to 170	201 to 270	301 to 370	401 to 470	All (1 to 500)
Naphthalene	2.19	2.56	3.00	1.18	2.91	2.70
1-Methylnaphthalene	1.87	2.80	4.19	2.17	2.38	5.22
2-Methylnaphthalene	1.82	2.90	3.38	2.14	3.89	5.31
Biphenyl	1.73	2.35	3.99	2.34	1.78	3.53
2,6-Dimethylnaphthalene	1.89	3.09	3.98	2.15	1.55	4.43
Acenaphthylene	2.48	2.63	2.22	4.72	4.92	5.73
Acenaphthene	1.50	2.05	2.30	1.83	1.29	2.97
2,3,5-Trimethylnaphthalene	1.11	2.03	3.21	1.17	1.16	4.11
Fluorene	1.23	1.95	2.75	2.52	1.76	2.55
Dibenzothiophene	1.77	2.37	2.26	1.45	1.11	2.70
Phenanthrene	2.13	2.42	3.40	1.40	2.58	3.08
Anthracene	3.88	3.24	3.26	4.55	3.77	5.42
1-Methylphenanthrene	1.82	2.00	2.57	1.47	1.12	3.13
Fluoranthene	1.94	3.25	3.74	1.73	0.93	4.59
Pyrene	2.49	2.56	3.43	3.59	2.25	7.48
Benz[a]anthracene	2.89	2.80	2.70	4.25	1.91	3.88
Chrysene	1.95	2.43	2.28	1.10	0.87	2.93
Benzo[b]fluoranthene	2.99	3.53	3.41	3.00	4.76	5.53
Benzo[k]fluoranthene	2.36	4.31	4.13	2.34	1.69	3.85
Benzo[j]fluoranthene	3.27	4.44	3.64	4.85	2.07	6.52
Benzo[e]pyrene	2.55	4.45	2.94	3.01	2.37	5.45
Benzo[a]pyrene	3.96	4.09	2.72	4.19	4.16	4.25
Perylene	1.45	1.65	2.14	2.85	1.99	2.63
Dibenz[a,c]anthracene	4.10	5.00	3.43	4.52	4.48	5.64
Dibenz[a,h]anthracene	3.10	5.79	6.06	3.98	5.98	5.61
Indeno[1,2,3-cd]pyrene	3.24	4.26	5.51	5.34	4.49	5.82
Benzo[ghi]perylene	4.38	5.82	5.37	7.23	6.06	7.83

Conclusion

The triple quadrupole GC/MS method for analyzing PAHs using hydrogen carrier gas, the Agilent HydroInert source, and backflush described here demonstrated several improvements over previous hydrogen[8] and helium[5] methods:

- Excellent chromatographic peak shape with little or no tailing
- MDL and linearity comparable to or better than obtained with helium
- Better chromatographic resolution with a shorter run time
- ISTD response stability across four orders of calibration
- Excellent linearity over 0.1 to 1,000 pg for 26 out of 27 analytes
- Average MDL of 0.09 pg for 27 analytes
- Reliable and accurate quantitation over 500 injections of a challenging soil extract with routine maintenance
- Excellent performance of the Agilent universal Ultra Inert mid-frit inlet liner when analyzing challenging soil matrix

For those laboratories looking to change their PAH analysis to the more sustainable hydrogen carrier gas, the HydroInert source with the 9 mm extractor lens enables the transition with equivalent or better performance.

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Application Note

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Analysis of Semivolatile Organic Compounds with US EPA 8270E Using the Agilent 7000E Triple Quadrupole GC/MS

Authors

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Abstract

This application note illustrates a sensitive method used to analyze semivolatile organic compounds (SVOCs) on an Agilent 7000E triple quadrupole GC/MS system (GC/TQ). The use of GC/TQ instrumentation for analysis of SVOCs offers significant advantages. High selectivity afforded by multiple reaction monitoring (MRM) mode results in faster batch review and increased confidence due to the elimination of matrix interferences. These interferences are often present when using selective ion monitoring (SIM) or scan acquisition modes. Increased sensitivity can facilitate smaller extraction volumes that improve sustainability, reduce waste, and decrease costs associated with sample preparation, solvent usage, and waste disposal. A primary objective of this work was to demonstrate the ability of a GC/TQ to detect SVOCs at low levels to meet these laboratory needs while maintaining an excellent dynamic range.

Introduction

The analysis of SVOCs can be challenging as there is a wide variety of target analytes that include bases, neutrals, and acids. These analytes span a wide range of molecular weights and boiling points. The United States Environmental Protection Agency (US EPA) has issued regulations and guidelines in Method 8270E for the analysis of these analytes by GC/TQ. Typical samples that are analyzed for SVOCs include surface or ground water as well as solid samples. These samples are then extracted before analysis. If method sensitivity can be improved, there is an opportunity to reduce sample and extract volumes that can result in decreased costs and increased lab sustainability. A preferable analytical method can also demonstrate a wide dynamic range to reduce the need for sample dilution and reanalysis.

Experimental

Sample preparation

A 2,000 µg/mL stock standard of SVOCs was sourced from Agilent (part number US201-1). Initial calibration curve standards were prepared by dilution of the stock and working standards into dichloromethane. Eleven calibration levels were prepared at the following concentrations: 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 µg/mL. A 2,000 µg/mL internal standard (ISTD) solution was also sourced from Agilent (part number ISM-560-1). This solution contained six internal standards: 1,4-dichlorobenzene-d4, acenaphthene-d10, chrysene-d12, naphthalene-d8, phenanthrene-d10, and perylene-d12. This ISTD solution was diluted and added to the calibration vials at a concentration of 4 µg/mL.

Instrumental method

An Agilent 8890 GC system and 7693A automatic liquid sampler (ALS) were used for sample introduction. The 8890 GC was configured with a split/splitless (SSL) inlet. An Agilent 7000E triple quadrupole mass spectrometer (TQ/MS) was used as the detector.

Initial method parameters were obtained from two Agilent application notes.[1,2] GC and MS method settings are shown in in the following tables.

The key techniques below were employed which increased method success:

- Using a GC/TQ provided greater sensitivity for low level analysis and simplified data reduction due to increased selectivity.
- A pulsed split injection with a 5:1 split ratio offered excellent sensitivity while preserving the advantages of a split injection.
- The 9 mm extractor lens enhanced linearity and improved overall performance for challenging analytes.
- Retention time locking protected against losing peaks, which may have otherwise drifted out of an MRM analysis window after column trimming.
- Dynamic MRM (dMRM) analysis mode reduced the number of simultaneous transitions that were monitored and simplified the process of adding and removing analytes.

GC Settings	
Analytical Column	Agilent J&W DB-8270D UI, 30 m × 0.25 mm, 0.25 µm (p/n 122-9732)
Injection Volume	1 µL
Inlet Temperature	Isothermal 280 °C
Injection Mode	Pulsed split
Split Ratio	5:1
Injection Pulse Pressure	30 psi until 0.6 min
Liner	Ultra Inert split, low pressure drop glass wool (p/n 5190-2295)
Oven Temperature Program	40 °C, hold for 0.5 min Ramp at 25 °C /min to 260 °C, hold 0 min Ramp at 5 °C /min to 280 °C, hold 0 min Ramp at 25 °C /min to 320 °C, hold 2 min
Run Time	16.9 min
Equilibration Time	1 min
Carrier Gas	Helium, constant flow at 1.55 mL/min (adjusted by RT locking)
Transfer Line Temperature	320 °C

MS Settings	
Ion Source	Extractor with 9 mm lens
Ion Source Temperature	300 °C
Quadrupole Temperature	150 °C
Collision Gas	Nitrogen at 1.5 mL/min
Quench Gas	Helium at 2.25 mL/min
Ionization Mode	EI
Solvent Delay	1.7 min
EMV mode	Gain factor
Gain Factor	3
Scan Type	Dynamic MRM

Several injection techniques were evaluated including split and splitless modes, with and without pulsed injections. A pulsed split injection with a 5:1 split ratio was selected as it offered excellent sensitivity while preserving the advantages of a split injection. Split injections allow for faster sample transfer from the inlet to the column. This faster transfer can improve performance for thermally sensitive analytes as they spend less time at high temperature in the GC inlet. Split injections also diminish the deposition of nonvolatile matter at the head of the GC column.

This method also used a 9 mm diameter extractor lens (part number G3870-20449) in the MS source. The 9 mm lens has been shown to significantly enhance method performance for polycyclic aromatic hydrocarbons and for many other challenging analytes such as 2,4-dinitrophenol by Anderson *et al.*[3]

The implementation of retention time locking (RTL) was critical to ensure exact retention time fidelity even after repeated inlet maintenance and column trimming. After trimming the column during maintenance, a single injection was made that allowed the Agilent MassHunter acquisition software for GC/MS systems to make a slight adjustment to the GC flow. This adjustment realigned all the analyte retention times. The method was retention time locked to acenaphthene-d10 at 7.08 minutes. This technique protects against losing peaks that may otherwise drift out of a dMRM analysis window after column maintenance.

The method also used dMRM acquisition mode. This approach addresses the limitations of time segment methods for a large batch of compounds by replacing the group segmentation with individual time windows for every analyte transition. It also dramatically reduces, the number of individual MRM transitions that are monitored during each MS scan.[4] Dynamic MRM mode simplifies the addition and removal of analytes of interest. The dMRM mode overcomes many challenges associated with time segmented methods targeting an abundance of analytes in a short elution window.

Early method experiments used a 25 °C oven ramp from 40 to 320 °C. The oven ramp was modified such that the oven ramp rate from 260 to 280 °C was decreased to 5 °C per minute. By optimizing the oven ramp, improved chromatographic resolution was achieved for benzo[b]fluoranthene and benzo[k]fluoranthene. Isomers are considered resolved if the height of the valley between two isomer peaks is less than 50% of the average of the two peak heights.[5] As shown in Figure 1, 88.6% resolution was achieved at a concentration of 2.0 µg/mL. Indeno[1,2,3-cd]pyrene and dibenz[a,h] anthracene were also acceptably separated at 62.6% resolution, as shown in Figure 2.

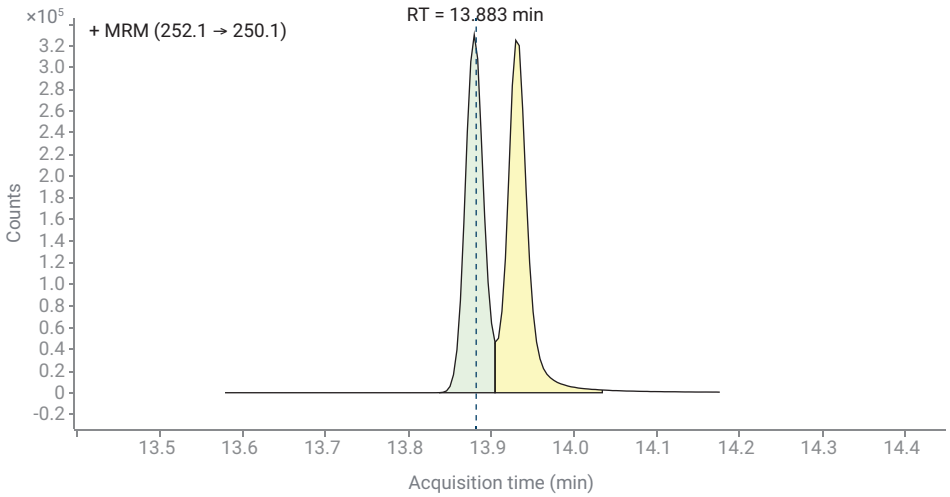

Figure 1. Benzo(b)fluoranthene and benzo(k)fluoranthene at 2.0 µg/mL (88.6% resolution).

Results and discussion

Manufacturer recommended tune

On a single quadrupole MS, the instrument would be challenged with a DFTPP (decafluorotriphenylphosphine) solution to verify mass accuracy and resolution. DFTPP tune checks are not appropriate for tandem MS analysis using MRM. However, the laboratory must demonstrate, prior to the initial calibration, that the MS system achieves mass accuracy and mass resolution criteria specified by the instrument manufacturer for the perfluorotributylamine (PFTBA) internal calibrant or another appropriate chemical.[5] The MS tune was verified using the Agilent manufacturer recommended tune protocol for the GC/TQ. Figure 4 shows an example check tune report from the Agilent manufacturer recommended tune. This procedure assists the analyst in using the GC/TQ by generating tune evaluation tests and reports to quickly evaluate and document the operability of the MS system.

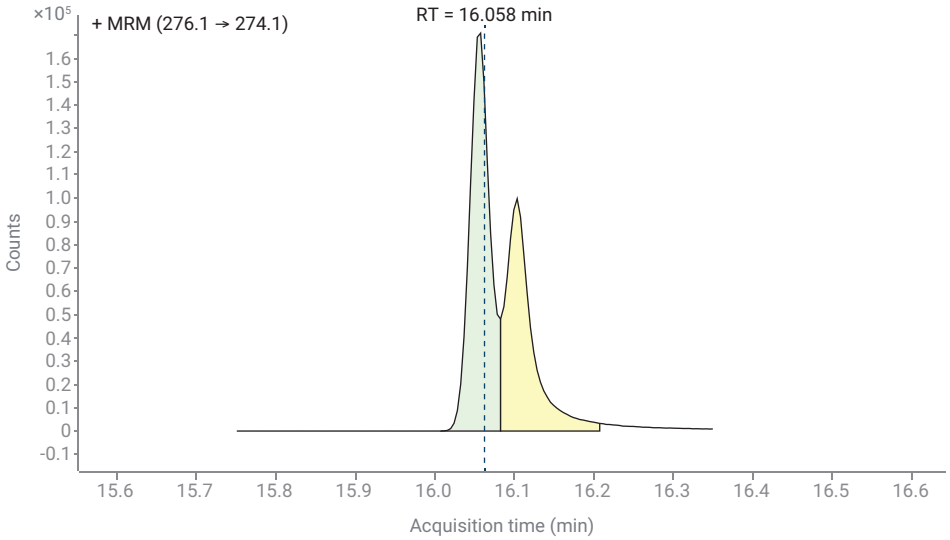

Figure 2. Indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene at 2.0 µg/mL (62.6% resolution).

Calibration

The initial calibration included 74 analytes. The 3- and 4-methyl phenol isomers were not separated and were reported as a combined result. The initial calibration was performed by introducing 11 different calibration solutions across more than three orders of magnitude in the range of 0.005 to 10 µg/mL. Each analyte was monitored using at least two MRM transitions, one of which was selected to quantify the results while the second was used as a qualifier. Some calibration curve ranges were trimmed at the top and/or bottom of the working range to meet method criteria.

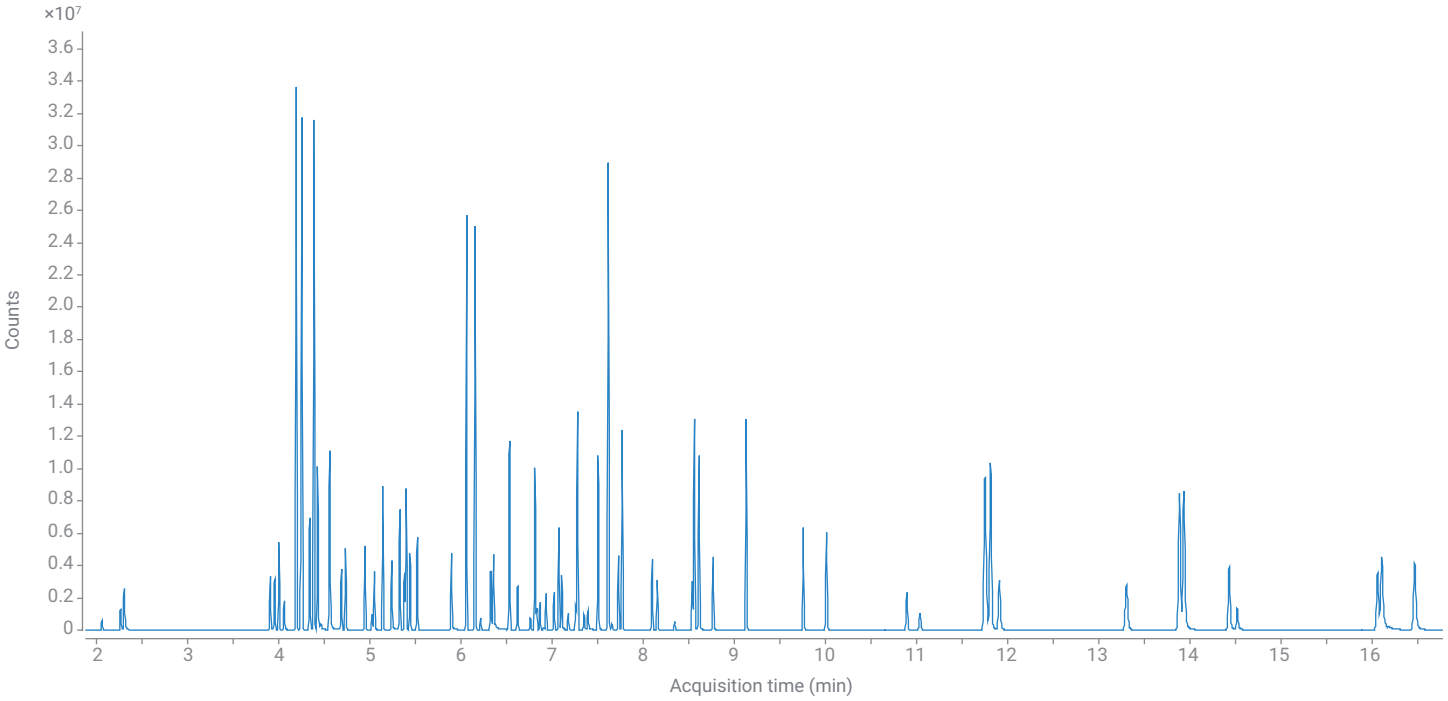

Figure 3. Total ion chromatogram from composite of all dMRM transitions showing separation in 16.9 minutes.

Triple Quadrupole GC/MS Checktune Report

Agilent | Trusted Answers

Instrument Information **EI with Extractor Ion Source – High Sensitivity Tune**

MS Model	G7000E	Tune Timestamp	2022-03-30 11:30:51-04:00
Instrument Name		Save Timestamp	2022-03-30 11:30:56-04:00
SW/FW Version		Tune File	first.eiex
		Tune Level	Full Autotune

Instrument Actuals

Emission (µA)	35.1	Rough Vac (mTorr)	1.04E+2	Column 1 (mL/min)	1.550
Source Temp. (°C)	300	High Vac (Torr)	7.64E-5	Column 2 (mL/min)	0.000
MS1 Quad Temp. (°C)	150	Turbo 1 Speed (%)	100.0	Collision Cell (mL/min)	1.500
MS2 Quad Temp. (°C)	150	Turbo 1 Power (W)	0.0	Quench Flow (mL/min)	2.250
Transfer Line (°C)	320				

MS1/MS2 Quadrupole Checktune Results

Target Mass	Actual Mass (m/z)		MS1 Abundance			MS2 Abundance		
(m/z)	MS1	MS2	Abundance	Ratio %	Acceptable %	Abundance	Ratio %	Acceptable %
69.0	69.0	69.0	11,924,296	100.00	50.0 - 110.0	39,580,079	100.00	50.0 - 110.0
219.0	219.0	219.0	10,837,233	90.88	70.0 - 110.0	15,324,358	38.72	10.0 - 40.0
264.0	264.0	264.0	3,749,068	31.44	10.0 - 80.0	12,500,412	31.58	10.0 - 60.0
414.0	414.0	414.0	952,894	7.99	0.1 - 40.0	3,333,806	8.42	0.1 - 20.0
502.0	502.0	502.0	560,982	4.70	0.1 - 40.0	964,475	2.44	0.1 - 12.0

Isotope M+1	MS1 Abundance			MS2 Abundance		
(m/z)	Iso M+1 Abund	Iso M+1 Ratio %	Acceptable %	Iso M+1 Abund	Iso M+1 Ratio %	Acceptable %
70.0	137,009	1.15	0.63 - 1.72	545,237	1.38	0.63 - 1.72
220.0	471,869	4.35	2.94 - 6.42	687,613	4.49	2.94 - 6.42
265.0	213,584	5.70	4.09 - 8.37	731,141	5.85	4.09 - 8.37
415.0	84,401	8.86	7.29 - 12.08	294,690	8.84	7.29 - 12.08
503.0	55,587	9.91	8.75 - 12.88	94,539	9.80	8.75 - 12.88

Detector Checktune Results

Detector Checktune Results	Value	Recommended Limit
EMV (V)	1158	≤ 2,900
Maximum Gain Factor	100	≥ 100

Air and Water Checktune Results

Air / Water	Absolute Abundance	Relative Abundance (%)	Recommended Limit
PFTBA(69)	11,357,567	100	---
Water	21,511	0.19	≤ 20
Oxygen	22,816	0.20	≤ 2.5
Nitrogen*	85,036	0.75	≤ 10

* Nitrogen values are calculated from oxygen abundance

Page 1 of 1

Report Generated: 2022-03-30 11:31:05-04:00

Figure 4. Example check tune report for manufacturer recommended tune.

Some analytes in the 8270 list are prone to difficulty in calibration. These analytes may be labile or active in the GC inlet, particularly at lower concentrations. This may manifest as variation in response factor relative to analyte concentration. Section 1.4.7 of the 8270 method[5] lists several such analytes and notes that they may be subject to erratic chromatographic behavior. 2,4-Dinitrophenol is one of the most difficult from this list and the calibration is shown in Figure 5. The response factor moderately increases with concentration, but method requirements were met as the average response factor (avg RF) relative standard deviation was 18.07%, which is less than the requirement of 20%. Method 8270 allows curve fitting for some analytes to alleviate this difficulty, provided that the coefficient of determination (R^2) is greater than 0.99. An alternate quadradic curve fit for 2-4-dinitrophenol is shown in Figure 6 with a R^2 of 0.9979. Pentachlorophenol is another of these listed potentially difficult analytes and the calibration curve is shown in Figure 8. In this case, a quadradic curve fit was selected with a R^2 value of 0.9966. These calibration curves demonstrate that calibration criteria may be met even with difficult analytes at low concentrations. An example of a more ideal calibration curve is shown for NDMA in Figure 9. NDMA itself can be a difficult analyte if chromatographic conditions are not optimized due to early elution and potential difficulty in complete resolution from the solvent. In this example, NDMA has an avg RF relative standard deviation of 5.71% and demonstrates exemplary linearity across the calibrated range.

Table 1. Calibration results.

Compound	Curve Fit	% RSE	R^2	Low Std (ppm) (default is 0.005 to 10 ppm)	High Std (ppm) (default is 0.005 to 10 ppm)
1,2,4-Trichlorobenzene	Avg RF	5.7			
1,2-Dichlorobenzene	Avg RF	5.3			
1,3-Dichlorobenzene	Avg RF	4.5			
1,3-Dinitrobenzene	Avg RF	16.4		0.025	5
1,4-Dichlorobenzene	Avg RF	7.8			
1,4-Dinitrobenzene	Avg RF	11.8		0.025	
1-Methylnaphthalene	Avg RF	6.8			
2,2'-oxybis[1-chloropropane]	Avg RF	4.3		0.050	
2,3,4,6-Tetrachlorophenol	Avg RF	14.1			
2,3,5,6-Tetrachlorophenol	Avg RF	9.6		0.025	
2,4,5-Trichlorophenol	Avg RF	8.2			
2,4,6-Trichlorophenol	Avg RF	5.2			
2,4-Dichlorophenol	Avg RF	4.2			
2,4-Dimethylphenol	Avg RF	3.4		0.010	
2,4-Dinitrophenol	Avg RF	18.1		0.050	5
2,4-Dinitrotoluene	Quadratic	5.4	0.9967	0.025	
2,6-Dinitrotoluene	Quadratic	8.3	0.9937	0.010	
2-Chloronaphthalene	Avg RF	3.5			
2-Chlorophenol	Avg RF	6.5			
2-methyl-4,6-dinitrophenol	Avg RF	13.0		0.025	5
2-Methylnaphthalene	Avg RF	4.1			
2-Methylphenol	Avg RF	6.7		0.010	
2-Nitroaniline	Avg RF	10.4			
2-Nitrophenol	Avg RF	7.8			
3+4-Methylphenol	Avg RF	3.5			
3-Nitroaniline	Avg RF	14.7			5
4-bromophenyl phenyl ether	Avg RF	3.9			
4-chloro-3-methylphenol	Avg RF	4.9			
4-Chloroaniline	Avg RF	3.0			
4-Chlorophenyl phenyl ether	Avg RF	2.1			
4-Nitroaniline	Quadratic	7.0	0.9954		
4-Nitrophenol	Avg RF	11.9			5
Acenaphthene	Avg RF	9.8		0.010	
Acenaphthylene	Avg RF	4.3		0.010	
Aniline	Avg RF	7.6		0.010	
Anthracene	Avg RF	5.2			
Azobenzene	Avg RF	3.9			
Benz[a]anthracene	Avg RF	6.7			
Benzo[a]pyrene	Avg RF	7.9			
Benzo[b]fluoranthene	Avg RF	7.2			
Benzo[g,h,i]perylene	Avg RF	8.0			
Benzo[k]fluoranthene	Avg RF	8.7			
Benzyl alcohol	Avg RF	2.7		0.010	
bis(2-Chloroethoxy)methane	Avg RF	3.2			
bis(2-Chloroethyl)ether	Avg RF	7.1			

Compound	Curve Fit	% RSE	R^2	Low Std (ppm) (default is 0.005 to 10 ppm)	High Std (ppm)
Bis(2-ethylhexyl) phthalate	Avg RF	14.3		0.025	
Butyl benzyl phthalate	Avg RF	10.3			
Carbazole	Avg RF	5.0			
Chrysene	Avg RF	5.7			
Dibenz[a,h]anthracene	Avg RF	14.4			5
Dibenzofuran	Avg RF	5.0			
Diethyl phthalate	Avg RF	7.6		0.100	
Dimethyl phthalate	Avg RF	4.1			
Di-*n*-butyl phthalate	Avg RF	3.2		0.025	
Di-*n*-octyl phthalate	Quadratic	6.2	0.9960		
Diphenylamine	Avg RF	4.9		0.025	
Fluoranthene	Avg RF	3.9			
Fluorene	Avg RF	3.0			
Hexachlorobenzene	Avg RF	7.1			
Hexachlorobutadiene	Avg RF	3.7			
Hexachlorocyclopentadiene	Avg RF	14.4		0.010	
Hexachloroethane	Avg RF	2.6		0.010	
Indeno[1,2,3-cd]pyrene	Avg RF	7.9			5
Isophorone	Avg RF	5.6			
Naphthalene	Avg RF	6.8			
NDMA	Avg RF	5.7		0.010	
Nitrobenzene	Avg RF	10.9		0.010	
N-Nitrosodi-*n*-propylamine	Avg RF	3.4		0.050	
Pentachlorophenol	Quadratic	6.7	0.9966	0.010	
Phenanthrene	Avg RF	5.7			
Phenol	Avg RF	5.7			
Pyrene	Avg RF	3.6			
Pyridine	Avg RF	5.2		0.025	
	Average = 7.0				

In this data set, 69 of the 74 analytes were calibrated using an avg RF fit with a relative standard deviation of less than or equal to 20%. The remaining five analytes (2,4-dinitrotoluene, 2,6-dinitrotoluene, 4-nitroaniline, di-*n*-octyl phthalate, and pentachlorophenol) were calibrated using weighted least squares regression with quadratic fits having R^2 values above 0.99. The relative standard error was calculated for each analyte and found to be less than or equal to 20% for each calibration curve. The mean relative standard error across all analytes was 6.96%. Also, the accuracy for all calibration points used was within ±30% of the theoretical value for each concentration. At least six data points were used for each calibration curve.

If a calibration working range is desired which covers higher concentrations, it is recommended to either dilute the samples or increase the ratio of the pulsed split injection. This modification would have the additional benefit of reducing matrix that reaches the column and detector and would likely reduce maintenance frequency.

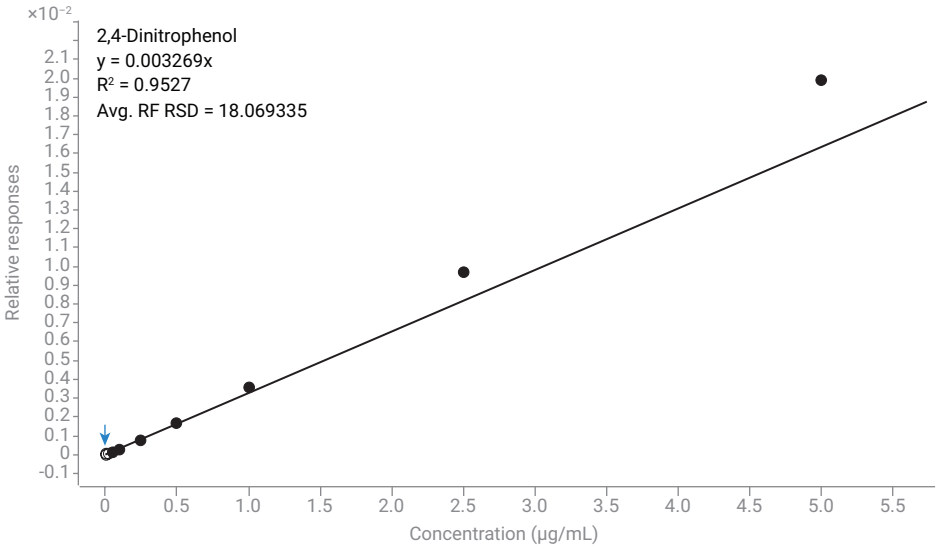

Figure 5. Avg RF calibration curve for challenging analyte 2,4-dinitrophenol 0.05 to 5 µg/mL. Avg. RF RSD = 18.07. Calibration points 1, 2, 3, and 11 are excluded.

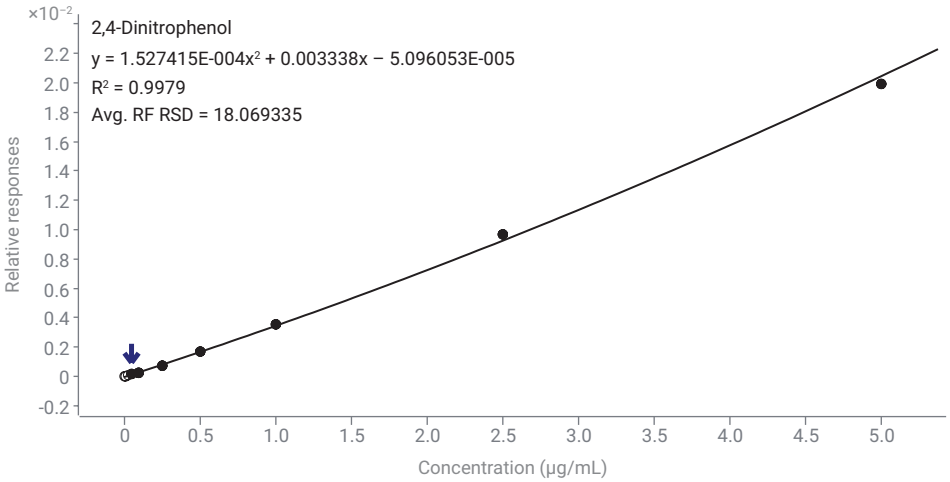

Figure 6. Alternate calibration curve for 2,4-dinitrophenol with a quadradic curve fit 0.05 to 5 µg/mL. R^2 = 0.9979. Calibration points 1, 2, 3, and 11 are excluded.

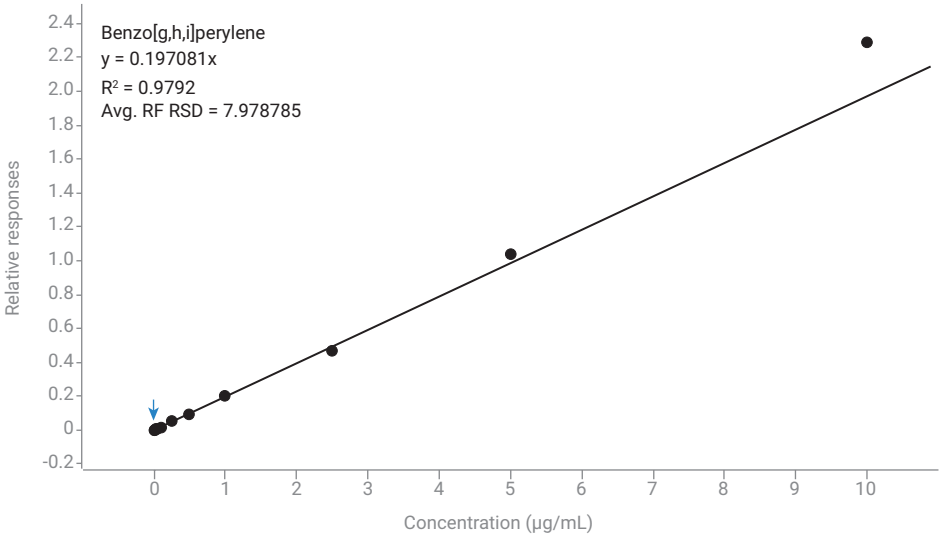

Figure 7. Avg RF calibration curve for benzo[g,h,i]perylene 0.005 to 10 µg/mL. Avg RF RSD = 7.98.

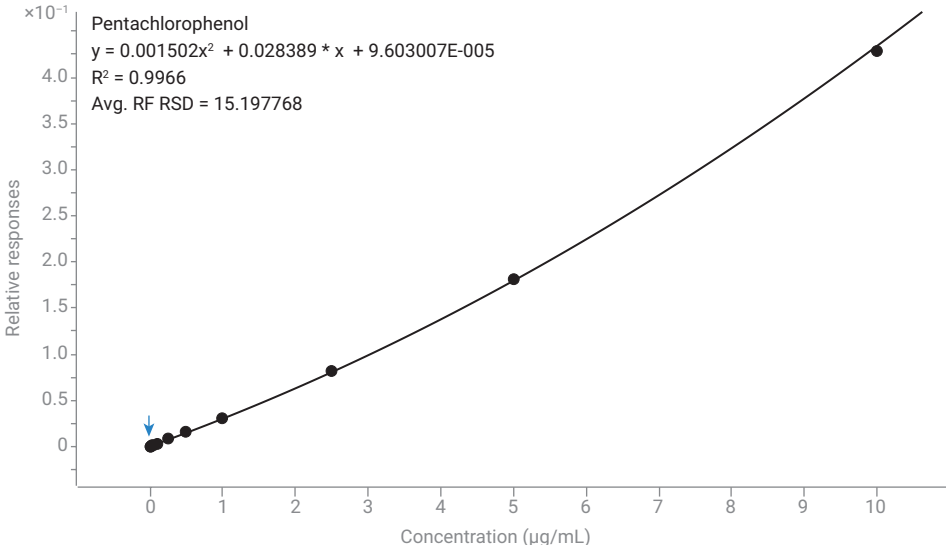

Figure 8. Calibration curve for pentachlorophenol 0.01 to 10 µg/mL. R^2 = 0.9966. Calibration point 1 excluded.

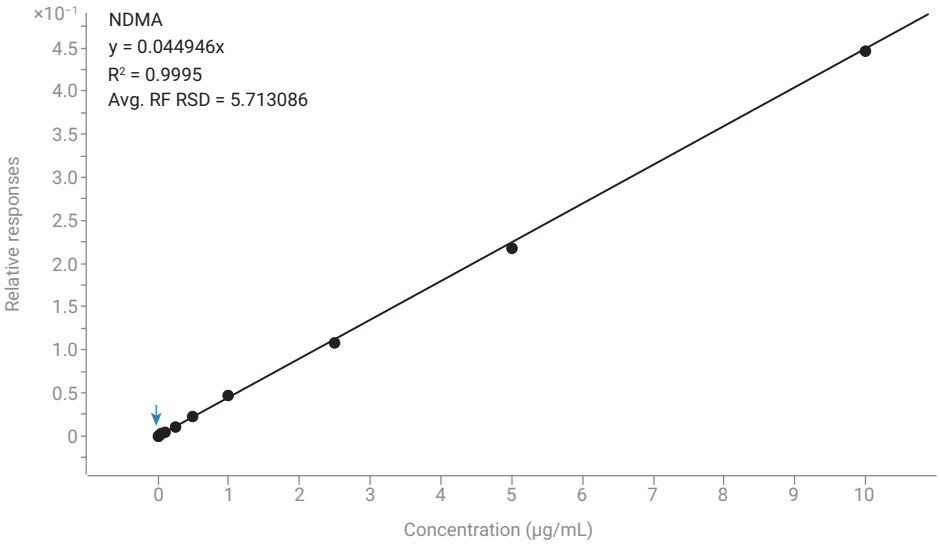

Figure 9. Calibration curve for NDMA. 0.01 to 10 µg/mL. Avg. RF RSD = 5.71. Calibration point 1 excluded.

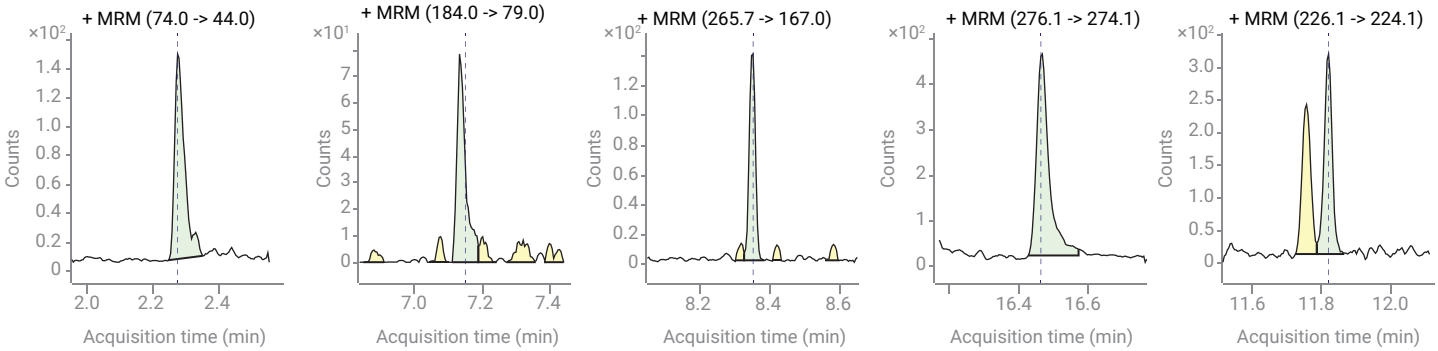

Figure 10. NDMA 0.01 µg/mL, 2,4-dinitrophenol 0.05 µg/mL, PCP 0.01 µg/mL, benzo[g,h,i]perylene 0.005 µg/mL, and chrysene 0.005 µg/mL.

Conclusion

A sensitive method for analysis of SVOCs has been developed that also demonstrates an extended dynamic range. Many analytes were shown to have a wide working calibration range over more than three orders of magnitude from 0.005 to 10 µg/mL. The collected data were evaluated with the quality criteria outlined in EPA 8270E.

GC/TQ offers significant advantages over the single quadrupole GC/MSD system in the analysis of SVOCs:

- High selectivity results in faster batch review by reducing the complexity of the data due to elimination of matrix interferences.
- Increased sensitivity opens the door for reduced sample sizes and smaller extraction volumes, which may:
  - Reduce waste while improving sustainability
  - Decrease costs associated with sample transport, solvent usage, and waste disposal
- Dynamic MRM mode generally reduces the number of individual MRM transitions during each MS scan. This improves instrument performance and makes adding and removing analytes from the method easy.
- The manufacturer recommended tune protocol simplifies tuning verification on the GC/TQ.

Key techniques for SVOC analysis by GC/MS which can improve results are

- Retention time locking ensures exact retention time fidelity even after column trimming which:
  - Eliminates the need to manually adjust retention times after maintenance
  - Makes data interchangeable across multiple instruments and multiple laboratories
- A pulsed split injection can enhance sensitivity over a standard split injection while maintaining a wide dynamic range.
- A 9 mm extractor lens gives outstanding linearity for all compounds while affording excellent sensitivity for many difficult analytes.

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Disclaimer

Although reference is made to EPA documents for review of the data, the contents of this publication have not been subjected to EPA review and the opinions of the authors do not reflect EPA policy.

Appendix

A List of calibrated compounds and transitions is shown in the following table.

Compound Name	CAS No.	Retention Time (min)	Precursor Ion	Product Ion	Left RT Delta	Right RT Delta	CE
NDMA	62-75-9	2.25	74	44	0.3	0.3	6
NDMA	62-75-9	2.25	74	42	0.3	0.3	14
Pyridine	110-86-1	2.4	79	52	0.3	0.5	25
Pyridine	110-86-1	2.4	79	51	0.3	0.5	25
Phenol	108-95-2	3.92	94	66.1	0.3	0.3	15
Phenol	108-95-2	3.92	94	65.1	0.3	0.3	20
Aniline	62-53-3	3.96	93	66	0.3	0.3	10
Aniline	62-53-3	3.96	92	65	0.3	0.3	10
bis(2-Chloroethyl)ether	111-44-4	4.01	95.1	65	0.3	0.3	5
bis(2-Chloroethyl)ether	111-44-4	4.01	93.1	63	0.3	0.3	0
2-Chlorophenol	95-57-8	4.06	128	64	0.3	0.3	30
2-Chlorophenol	95-57-8	4.06	128	63	0.3	0.3	15
1,3-Dichlorobenzene	541-73-1	4.2	146	111	0.3	0.3	15
1,3-Dichlorobenzene	541-73-1	4.2	146	75	0.3	0.3	30
1,4-Dichlorobenzene-d4	3855-82-1	4.25	150	115	0.2	0.2	15
1,4-Dichlorobenzene-d4	3855-82-1	4.25	150	78	0.2	0.2	30
1,4-Dichlorobenzene	106-46-7	4.27	146	111	0.3	0.3	15
1,4-Dichlorobenzene	106-46-7	4.27	146	75	0.3	0.3	30
Benzyl alcohol	100-51-6	4.35	108	79	0.3	0.3	15
Benzyl alcohol	100-51-6	4.35	107	79	0.3	0.3	5
1,2-Dichlorobenzene	95-50-1	4.39	146	111	0.3	0.3	15
1,2-Dichlorobenzene	95-50-1	4.39	146	75	0.3	0.3	30
2-Methylphenol	95-48-7	4.44	108	107	0.3	0.3	15
2-Methylphenol	95-48-7	4.44	107	77	0.3	0.3	15
2,2'-oxybis[1-chloropropane]	108-60-1	4.47	121	77	0.3	0.3	5
2,2'-oxybis[1-chloropropane]	108-60-1	4.47	121	49	0.3	0.3	30
3+4-Methylphenol	108-39-4	4.57	108	107.1	0.3	0.3	15
3+4-Methylphenol	108-39-4	4.57	108	80	0.3	0.3	0
N-Nitrosodi-*n*-propylamine	621-64-7	4.58	113.1	71	0.3	0.3	10
N-Nitrosodi-*n*-propylamine	621-64-7	4.58	101	70	0.3	0.3	0
Hexachloroethane	67-72-1	4.69	200.9	165.9	0.3	0.3	15
Hexachloroethane	67-72-1	4.69	118.9	83.9	0.3	0.3	35
Nitrobenzene	98-95-3	4.74	123	77	0.3	0.3	10
Nitrobenzene	98-95-3	4.74	77	51	0.3	0.3	15
Isophorone	78-59-1	4.96	138	82	0.3	0.3	5
Isophorone	78-59-1	4.96	82	54	0.3	0.3	5
2-Nitrophenol	88-75-5	5.03	138.9	81	0.3	0.3	15
2-Nitrophenol	88-75-5	5.03	109	81	0.3	0.3	10
2,4-Dimethylphenol	105-67-9	5.06	121	107	0.3	0.3	10
2,4-Dimethylphenol	105-67-9	5.06	107.1	77.1	0.3	0.3	15
bis(2-Chloroethoxy)methane	111-91-1	5.15	95	65	0.3	0.3	5

Compound Name	CAS No.	Retention Time (min)	Precursor Ion	Product Ion	Left RT Delta	Right RT Delta	CE
bis(2-Chloroethoxy)methane	111-91-1	5.15	93	63	0.3	0.3	5
2,4-Dichlorophenol	120-83-2	5.25	163.9	63	0.3	0.3	30
2,4-Dichlorophenol	120-83-2	5.25	162	63	0.3	0.3	30
1,2,4-Trichlorobenzene	120-82-1	5.34	179.9	145	0.3	0.3	15
1,2,4-Trichlorobenzene	120-82-1	5.34	179.9	109	0.3	0.3	30
Naphthalene-d8	1146-65-2	5.39	136.1	108.1	0.2	0.2	20
Naphthalene-d8	1146-65-2	5.39	136.1	84.1	0.2	0.2	25
Naphthalene	91-20-3	5.41	128.1	102.1	0.3	0.3	20
Naphthalene	91-20-3	5.41	128.1	78.1	0.3	0.3	20
4-Chloroaniline	106-47-8	5.46	127	92	0.3	0.3	15
4-Chloroaniline	106-47-8	5.46	127	65	0.3	0.3	20
Hexachloro-1,3-butadiene	87-68-3	5.53	226.8	191.9	0.3	0.3	15
Hexachloro-1,3-butadiene	87-68-3	5.53	224.7	189.9	0.3	0.3	15
4-chloro-3-methylphenol	59-50-7	5.91	142	107	0.3	0.3	15
4-chloro-3-methylphenol	59-50-7	5.91	107	77	0.3	0.3	15
2-Methylnaphthalene	91-57-6	6.07	142	141	0.3	0.3	15
2-Methylnaphthalene	91-57-6	6.07	141	114.9	0.3	0.3	15
1-Methylnaphthalene	90-12-0	6.16	142	114.9	0.3	0.3	30
1-Methylnaphthalene	90-12-0	6.16	114.9	89	0.3	0.3	20
Hexachlorocyclopentadiene	77-47-4	6.22	236.7	143	0.3	0.3	20
Hexachlorocyclopentadiene	77-47-4	6.22	236.7	119	0.3	0.3	20
2,4,6-Trichlorophenol	88-06-2	6.34	197.8	97	0.3	0.3	25
2,4,6-Trichlorophenol	88-06-2	6.34	195.8	97	0.3	0.3	25
2,4,5-Trichlorophenol	95-95-4	6.37	197.8	97	0.3	0.3	30
2,4,5-Trichlorophenol	95-95-4	6.37	195.8	97	0.3	0.3	25
2-Chloronaphthalene	91-58-7	6.54	162	126.9	0.3	0.3	20
2-Chloronaphthalene	91-58-7	6.54	162	77	0.3	0.3	35
2-Nitroaniline	88-74-4	6.63	138	92	0.3	0.3	15
2-Nitroaniline	88-74-4	6.63	138	65	0.3	0.3	25
1,4-Dinitrobenzene	100-25-4	6.77	168	75	0.2	0.2	20
1,4-Dinitrobenzene	100-25-4	6.77	122	92	0.2	0.2	5
Dimethyl phthalate	131-11-3	6.82	163	92	0.3	0.3	30
Dimethyl phthalate	131-11-3	6.82	163	77	0.3	0.3	20
1,3-Dinitrobenzene	99-65-0	6.84	168	75	0.3	0.3	20
1,3-Dinitrobenzene	99-65-0	6.84	122	92	0.3	0.3	5
2,6-Dinitrotoluene	606-20-2	6.87	165	90.1	0.3	0.3	15
2,6-Dinitrotoluene	606-20-2	6.87	165	63	0.3	0.3	25
Acenaphthylene	208-96-8	6.94	151.9	102	0.3	0.3	30
Acenaphthylene	208-96-8	6.94	150.9	77	0.3	0.3	25
1,2-Dinitrobenzene	528-29-0	6.95	168	78	0.3	0.3	5
1,2-Dinitrobenzene	528-29-0	6.95	168	63	0.3	0.3	35
3-Nitroaniline	99-09-2	7.03	138	92	0.3	0.3	15
3-Nitroaniline	99-09-2	7.03	138	80	0.3	0.3	5
Acenaphthene-d10	15067-26-2	7.08	164.1	162.1	0.5	0.5	15
Acenaphthene-d10	15067-26-2	7.08	162.1	160.1	0.5	0.5	20
Acenaphthene	83-32-9	7.11	153.9	127	0.3	0.3	40
Acenaphthene	83-32-9	7.11	152.9	77	0.3	0.3	45

Compound Name	CAS No.	Retention Time (min)	Precursor Ion	Product Ion	Left RT Delta	Right RT Delta	CE
2,4-Dinitrophenol	51-28-5	7.14	184	107	0.3	0.3	25
2,4-Dinitrophenol	51-28-5	7.14	184	79	0.3	0.3	25
4-Nitrophenol	100-02-7	7.19	138.9	109	0.3	0.3	5
4-Nitrophenol	100-02-7	7.19	109	81	0.3	0.3	10
2,4-Dinitrotoluene	121-14-2	7.27	165	119	0.3	0.3	5
2,4-Dinitrotoluene	121-14-2	7.27	165	63	0.3	0.3	45
Dibenzofuran	132-64-9	7.29	167.9	139.1	0.3	0.3	25
Dibenzofuran	132-64-9	7.29	138.9	63	0.3	0.3	35
2,3,5,6-Tetrachlorophenol	935-95-5	7.36	232	167.9	0.2	0.2	15
2,3,5,6-Tetrachlorophenol	935-95-5	7.36	230	165.9	0.2	0.2	15
2,3,4,6-Tetrachlorophenol	58-90-2	7.4	231.9	167.9	0.3	0.3	15
2,3,4,6-Tetrachlorophenol	58-90-2	7.4	230	165.9	0.3	0.3	15
Diethyl phthalate	84-66-2	7.51	149	93	0.3	0.3	15
Diethyl phthalate	84-66-2	7.51	149	65	0.3	0.3	20
4-Chlorodiphenyl ether	7005-72-3	7.62	204	77	0.3	0.3	30
4-Chlorodiphenyl ether	7005-72-3	7.62	141.1	115.1	0.3	0.3	20
Fluorene	86-73-7	7.62	166	165.1	0.3	0.3	15
Fluorene	86-73-7	7.62	164.9	163.1	0.3	0.3	35
4-Nitroaniline	100-01-6	7.64	138	108.1	0.3	0.3	5
4-Nitroaniline	100-01-6	7.64	108	80	0.3	0.3	15
4,6-dinitro-o-cresol	534-52-1	7.66	198	167.9	0.3	0.3	5
4,6-dinitro-o-cresol	534-52-1	7.66	198	121	0.3	0.3	10
Diphenylamine	122-39-4	7.75	170	169.2	0.3	0.3	15
Diphenylamine	122-39-4	7.75	167	166.2	0.3	0.3	20
Azobenzene	103-33-3	7.79	105	77.1	0.3	0.3	5
Azobenzene	103-33-3	7.79	77	51	0.3	0.3	15
4-bromophenyl phenyl ether	101-55-3	8.1	250	141	0.3	0.3	20
4-bromophenyl phenyl ether	101-55-3	8.1	248	141	0.3	0.3	20
Hexachlorobenzene	118-74-1	8.16	283.7	213.8	0.3	0.3	30
Hexachlorobenzene	118-74-1	8.16	248.7	214	0.3	0.3	15
Pentachlorophenol	87-86-5	8.35	265.7	167	0.3	0.3	25
Pentachlorophenol	87-86-5	8.35	165	130	0.3	0.3	25
Phenanthrene-d10	1517-22-2	8.54	188.3	160.2	0.2	0.2	20
Phenanthrene-d10	1517-22-2	8.54	188.3	158.2	0.2	0.2	35
Phenanthrene	85-01-8	8.57	177.9	152	0.3	0.3	25
Phenanthrene	85-01-8	8.57	175.9	149.9	0.3	0.3	25
Anthracene	120-12-7	8.62	178.1	151	0.3	0.3	30
Anthracene	120-12-7	8.62	177.9	152	0.3	0.3	25
Carbazole	86-74-8	8.77	167	139	0.3	0.3	45
Carbazole	86-74-8	8.77	167	89	0.3	0.3	60
Di-*n*-butyl phthalate	84-74-2	9.13	149	121	0.3	0.3	15
Di-*n*-butyl phthalate	84-74-2	9.13	149	65	0.3	0.3	25
Fluoranthene	206-44-0	9.76	201.9	151.9	0.3	0.3	30
Fluoranthene	206-44-0	9.76	200.9	199.9	0.3	0.3	15
Pyrene	129-00-0	10.02	202.1	151	0.3	0.3	45
Pyrene	129-00-0	10.02	201.1	200	0.3	0.3	15

Compound Name	CAS No.	Retention Time (min)	Precursor Ion	Product Ion	Left RT Delta	Right RT Delta	CE
Butyl benzyl phthalate	85-68-7	10.9	149	65	0.3	0.3	25
Butyl benzyl phthalate	85-68-7	10.9	91	65	0.3	0.3	15
Benz[a]anthracene	56-55-3	11.75	228.1	226.1	0.3	0.3	30
Benz[a]anthracene	56-55-3	11.75	226.1	224.1	0.3	0.3	35
Chrysene-d12	1719-03-5	11.77	240.2	236.2	0.3	0.3	35
Chrysene-d12	1719-03-5	11.77	236.1	232.1	0.3	0.3	40
Chrysene	218-01-9	11.81	226.1	224.1	0.3	0.3	40
Chrysene	218-01-9	11.81	113.1	112.1	0.3	0.3	10
Bis(2-ethylhexyl) phthalate	117-81-7	11.9	167	149	0.3	0.3	5
Bis(2-ethylhexyl) phthalate	117-81-7	11.9	149	65	0.3	0.3	25
Di-*n*-octyl phthalate	117-84-0	13.29	149	93	0.3	0.3	20
Di-*n*-octyl phthalate	117-84-0	13.29	149	65	0.3	0.3	25
Benzo[b]fluoranthene	205-99-2	13.88	252.1	250.1	0.3	0.3	35
Benzo[b]fluoranthene	205-99-2	13.88	126	113.1	0.3	0.3	10
Benzo[k]fluoranthene	207-08-9	13.93	252.1	250.1	0.3	0.3	30
Benzo[k]fluoranthene	207-08-9	13.93	126.1	113.1	0.3	0.3	10
Benzo[a]pyrene	50-32-8	14.42	252.1	250.1	0.3	0.3	35
Benzo[a]pyrene	50-32-8	14.42	125	124.1	0.3	0.3	10
Perylene-d12	1520-96-3	14.5	264.2	260.1	0.3	0.3	35
Perylene-d12	1520-96-3	14.5	260.1	256.1	0.3	0.3	40
Indeno[1,2,3-cd]pyrene	193-39-5	16.05	276.1	274.1	0.3	0.3	40
Indeno[1,2,3-cd]pyrene	193-39-5	16.05	137	136	0.3	0.3	15
Dibenz[a,h]anthracene	53-70-3	16.1	278.1	276.1	0.3	0.3	35
Dibenz[a,h]anthracene	53-70-3	16.1	125	124	0.3	0.3	10
Benzo[g,h,i]perylene	191-24-2	16.47	276.1	274.1	0.3	0.3	45
Benzo[g,h,i]perylene	191-24-2	16.47	138	137	0.3	0.3	15

Consumables	Part Number
Sample Containment	
Vials, screw top, amber, deactivated, 2 mL, 100/pk	5183-2072
Cap, screw, PTFE/silicone septa, 100/pk	5040-4681
Vial inserts, 250 µL, deactivated, 100/pk	5181-8872
Instrument Supplies	
Syringe, Blue Line, 10 µL, fixed needle, 23-26s/42/cone, 6/pk	G4513-80200
Inlet septa, Advanced Green, nonstick, 11 mm, 50/pk	5183-4759
Inlet liner, Ultra Inert, split, low pressure drop, glass wool	5190-2295
GC inlet seal, gold plated, with washer, Ultra Inert, 10/pk	5190-6145
Lens, extraction, 9 mm	G3870-20449
Separation	
J&W DB-8270D Ultra Inert GC column, 30 m × 0.25 mm, 0.25 µm	122-9732

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Analysis of Semivolatile Organic Compounds with Hydrogen Carrier Gas and HydroInert Source by Gas Chromatography/Triple Quadrupole Mass Spectrometry (GC/MS/MS)

Author

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Abstract

Gas chromatography/mass spectrometry (GC/MS) is integral to the analysis of semivolatile organic compounds (SVOCs) in environmental matrices. Some methods have extended instrumentation to include gas chromatography/triple quadrupole mass spectrometry (GC/MS/MS) as users push towards lower detection limits. Recent pressure on the helium (He) supply has required organizations to actively investigate hydrogen (H_2) carrier gas, but most GC/MS and GC/MS/MS analyses have reduced sensitivity and hydrogenation or dechlorination in the existing mass spectrometry products. New advances in mass spectrometer design have reduced hydrogenation and dechlorination reactions in the source. The Agilent HydroInert source retains the ability to analyze a wide calibration range, for some compounds from 0.02 to 100 µg/mL, and meet the U.S. Environmental Protection Agency (EPA) method 8270 calibration criteria when using H_2 carrier gas.

Introduction

GC/MS/MS has been determined to be suitable for use with the U.S. EPA method 8270 (version 8270E) in solid waste, soil, air, and water extracts.[1,2] Previous application notes have discussed using He carrier gas with GC/MS/MS to extend the calibration range of EPA method 8270 down to 0.02 µg/mL, while retaining the top range of the method at 160 µg/mL.[3]

The availability of He has been a concern for several years, but interest in transitioning to alternative carrier gases has significantly increased in recent years. However, existing mass spectrometry systems have issues with hydrogenation of some functional groups, such as nitro groups, or dechlorination of heavily chlorinated compounds. These issues would alter the mass spectrum of a peak and lead to potential misidentification of compounds, or no identification of compounds if the precursor or product ions are affected by reactions with H_2 in a source. One example is with nitrobenzene, where H_2 carrier gas and nitrobenzene exposed to metal and heat, such as in a mass spectrometer source, will hydrogenate nitrobenzene (molecular weight (MW) 123 *m/z*) to aniline (MW 93 *m/z*). This is observed by the identification of aniline at the retention time of nitrobenzene and increase in 93 *m/z* fragment intensity compared to 123 *m/z*. A newly designed extractor source called the HydroInert source, for Agilent 7000C/D/E Inert Plus triple quadrupole GC/MS systems, addresses these H_2-related issues and helps improve performance with H_2 carrier gas in GC/MS and GC/MS/MS applications, including SVOC analyses. The HydroInert source with H_2 carrier gas retains mass spectral fidelity and can allow users to continue to use existing He-based mass spectral libraries, quantitative methods, and multiple reaction monitoring transitions (MRMs).

This application note demonstrates the ability of the HydroInert source to allow the use of H_2 carrier gas, while retaining critical functional groups, such as nitro groups and halogens. Retention of mass spectral fidelity is a breakthrough for the use of H_2 carrier gas with GC/MS systems, especially for environmental analyses such as EPA method 8270. Additionally, a method for EPA 8270 has been developed that retains similar sensitivity of a He carrier gas analysis, which allows for most compounds to be calibrated between 0.02 to 100 µg/mL with less than 20% of compounds requiring linear or quadratic curve fits.

Experimental

A set of stock standards containing 120 target compounds and surrogates was selected to provide a representative mixture of acids, bases, and neutral compounds, as well as comprising various compound classes, from nitrophenols to PAHs. The nine stock standards of target analytes were at concentrations of 2,000 µg/mL; part numbers for these stock standards are as follows: SVM-160, SVM-121, SVM-122, SVM-123, SVM-124, SVM-125, SVM-126-1, SVM-127, and US-211. Pyridine was diluted from a pure standard to 1,000 µg/mL as a working standard. The surrogate standard (part number ISM-332) contained six compounds at 2,000 µg/mL, indicated in Table 1. An internal standard mixture of six deuterated PAHs was used for recovery and calibration. The stock standards were combined and diluted in dichloromethane to make a working standard at 200 µg/mL. The working standard was then diluted to form the following nominal concentrations for the targets and surrogates for calibration standards: 0.02, 0.05, 0.1, 0.2, 0.5, 0.8, 1, 2, 5, 10, 20, 35, 50, 75, and 100 µg/mL. Internal standards were added to each calibration standard at a concentration level of 40 µg/mL. Table 1 lists the compounds that were used in the study. The compound numbers in Table 1 were assigned based on retention order of the targets and surrogates, with the internal standards listed at the end of the table out of retention order.

The tuning standard (part number GCM-150), containing a mixture of benzidine, pentachlorophenol, 4,4'-dichlorodiphenyltrichloroethane (4,4'-DDT), and decafluorotriphenylphosphine (DFTPP) was diluted to a concentration of 25 µg/mL and used to verify GC flow path inertness.

A composite mixture of soils extracted with dichloromethane was prepared for EPA method 8270 analysis. The mixture is a representative matrix residue that is typically encountered in the lab and was procured from Pace Analytical (Mt. Juliet, TN).

Table 1. Target, surrogates, and internal standards.

No.	Compound	No.	Compound	No.	Compound
1	N-Nitrosodimethylamine (NDMA)	43	4-Chloro-3-methyl phenol	85	Pentachloronitrobenzene
2	Pyridine	44	2-Methylnaphthalene	86	4-Aminobiphenyl
3	2-Picoline	45	1,2,4,5-Tetrachlorobenzene	87	Propyzamide
4	N-Nitroso-N-methylethylamine	46	Hexachlorocyclopentadiene	88	Phenanthrene
5	Methyl methanesulfonate	47	2,4,6-Trichlorophenol	89	Dinoseb
6	2-Fluorophenol (surrogate)	48	2,4,5-Trichlorophenol	90	Disulfoton
7	N-Nitrosodiethylamine	49	2-Fluorobiphenyl (surrogate)	91	Anthracene
8	Ethyl methanesulfonate	50	1-Chloronaphthalene	92	Parathion-methyl
9	Phenol-d_6 (surrogate)	51	2-Chloronaphthalene	93	Di-*n*-butyl phthalate
10	Phenol	52	2-Nitroaniline	94	4-Nitroquinoline-1-oxide
11	Aniline	53	Dimethyl phthalate	95	Parathion
12	Bis(2-chloroethyl)ether	54	Acenaphthylene	96	Fluoranthene
13	2-Chlorophenol	55	2,6-Dinitrotoluene	97	Benzidine
14	1,3-Dichlorobenzene	56	3-Nitroaniline	98	Pyrene
15	1,4-Dichlorobenzene	57	Acenaphthene	99	*p*-Terphenyl-d_{14} (surrogate)
16	Benzyl alcohol	58	2,4-Dinitrophenol	100	Aramite I
17	1,2-Dichlorobenzene	59	Pentachlorobenzene	101	Aramite II
18	2-Methylphenol (*o*-cresol)	60	4-Nitrophenol	102	4-Dimethylaminoazobenzene
19	Bis(2-Chloro-1-methylethyl)ether	61	Dibenzofuran	103	Chlorobenzilate
20	4-Methylphenol (*p*-cresol)	62	2,4-Dinitrotoluene	104	3,3'-Dimethyl benzidine
21	N-Nitrosopyrrolidine	63	1-Naphthylamine	105	Famphur
22	Acetophenone	64	2,3,4,6-Tetrachlorophenol	106	Butyl benzyl phthalate
23	4-Nitrosomorpholine	65	2-Naphthylamine	107	Benz[a]anthracene
24	N-Nitrosodi-n-propylamine	66	Diethyl phthalate	108	3,3'-Dichlorobenzidine
25	*o*-Toluidine	67	Fluorene	109	Chrysene
26	Hexachloroethane	68	Thionazin	110	Bis(2-ethylhexyl) phthalate
27	Nitrobenzene-d_5 (surrogate)	69	5-Nitro-*o*-toluidine	111	Di-*n*-octyl phthalate
28	Nitrobenzene	70	4-Chlorophenyl phenyl ether	112	Benzo[b]fluoranthene
29	N-Nitrosopiperidine	71	4-Nitroaniline	113	7,12-Dimethylbenz[a]anthracene
30	Isophorone	72	2-methyl-4,6-dinitrophenol (DNOC)	114	Benzo[k]fluoranthene
31	2-Nitrophenol	73	N-Nitrosodiphenylamine	115	Benzo[a]pyrene
32	2,4-Dimethylphenol (2,4-xylenol)	74	Diphenylamine	116	3-Methylcholanthrene
33	Benzoic acid	75	Azobenzene	117	Dibenz[a,j]acridine
34	Bis(2-Chloroethoxy)methane	76	2,4,6-Tribromophenol (surrogate)	118	Indeno[1,2,3-cd]pyrene
35	2,4-Dichlorophenol	77	Sulfotep	119	Dibenz[a,h]anthracene
36	1,2,4-Trichlorobenzene	78	Dimethoate	120	Benzo[g,h,i]perylene
37	Naphthalene	79	Diallate I	121	1,4-Dichlorobenzene-d_4 (internal standard)
38	4-Chloroaniline	80	Phorate	122	Naphthalene-d_8 (internal standard)
39	2,6-Dichlorophenol	81	Phenacetin	123	Acenaphthalene-d_{10} (internal standard)
40	Hexachlorobutadiene	82	4-Bromophenyl phenyl ether	124	Phenanthrene-d_{10} (internal standard)
41	*p*-Phenylenediamine	83	Hexachlorobenzene	125	Chrysene-d_{12} (internal standard)
42	N-Nitrosodi-n-butylamine	84	Pentachlorophenol	126	Perylene-d_{12} (internal standard)

Instrumental methods

The Agilent 8890B GC was configured with a multimode inlet (MMI) and an Agilent J&W DB-5ms Ultra Inert GC column (part number 121-5522UI) interfaced with an Agilent 7000E Inert Plus triple quadrupole GC/MS system and an Agilent HydroInert source. Table 2 summarizes the GC/MS instrumentation and consumables used in this study. The GC and MS/MS method parameters (Table 3) have been optimized to provide a 12-minute method, while retaining the required resolution for isomer pairs and following the EPA 8270 guidelines for method parameters. The mass spectrometer was operated in electron ionization mode and was autotuned with the etune algorithm. Check tunes were run periodically to verify that the ion ratios and mass positions of the tune calibrant, perfluorotributylamine (PFTBA), were within tolerances. The analytical method used an Agilent Ultra Inert low pressure drop inlet liner with the 20:1 split injection and an Agilent J&W DB-5ms Ultra Inert GC column, 20 m × 0.18 mm, 0.18 µm; this column choice is preferred with H_2 carrier gas to maintain reasonable inlet pressures, as well as requiring a split injection to avoid overloading the column. Additionally, the split injection is better for the GC/MS/MS, which is commonly used for trace analyses with target analyte concentrations below 1 µg/mL. The 20:1 split drops the 100 µg/mL highest standard down to 5 µg/mL on column. With the ramped temperature of the inlet, H_2 carrier gas, and dichloromethane solvent, it is critical to verify extracted samples do not contain water; extraction steps must include a step to remove residual water to reduce the risk of generating hydrochloric acid in the inlet and causing damage to the instrument and consumables. The acquisition method was retention time locked to the internal standard, acenaphthene-d_{10}, to maintain consistent retention times across column changes and different instruments, which is critical. The final oven temperature hold time was tested at 2 minutes and 2.7 minutes; benzo[g,h,i]perylene eluted at 10.13 minutes and the 2-minute final hold would result in a method run time of 11.3 minutes, if cycle time is a concern. No quench gas is used with H_2 carrier gas; disconnect the He tubing from the back of the electronic pressure control module. Data was collected using dynamic MRM (dMRM) for more efficient use of the GC/MS/MS analytical time.

MRM transitions from previous application notes and methods were leveraged for this work to reduce the development of MRM transitions, but collision energies were reoptimized using Agilent MassHunter Optimizer. Additionally, some compounds were not listed in previous work and MassHunter Optimizer was used to identify the best MRM transitions and collision energies for the following compounds: 2,6-dichlorophenol, N-nitrosomethylethylamine, and N-nitrosomorpholine. For the GC/MS tuning mixture runs, a scan mode acquisition method was used, as DFTPP, DDT, and the breakdown products of DDT were not in the MRM acquisition method.

Instrumentation

Table 2. GC and MSD instrumentation and consumables.

Parameter	Value
GC	Agilent 8890 GC system
MS	Agilent 7000E Inert Plus triple quadrupole GC/MS with the Agilent HydroInert source
Extraction Lens	9 mm HydroInert
Syringe	Agilent Blue Line autosampler syringe, 10 µL, PTFE-tip plunger (p/n G4513-80203)
Column	Agilent J&W DB-5ms Ultra Inert GC column, 20 m × 0.18 mm, 0.18 µm (p/n 121-5522UI)
Inlet Liner	Agilent Ultra Inert inlet liner, low pressure drop, glass wool (p/n 5190-2295)

Instrument conditions

Table 3. GC and MSD instrument conditions.

Parameter	Value
Injection Volume	1 µL
Multimode Inlet	Split 20:1 250 °C (hold 0.3 min) ramp 200 °C/min to 350 °C (hold for run length) Postrun: 350 °C/min with 100 mL/min split flow
Column Temperature Program	40 °C (hold 0 min), 30 °C/min to 320 °C (hold 2 to 2.7 min*) Post run: 320 °C hold for 2 min
Carrier Gas and Flow Rate	H_2 at 1.2 mL/min**, constant flow
Transfer Line Temperature	320 °C
Ion Source Temperature	300 °C
Quadrupole Temperature	150 °C
Collision Gas and Flow Rate	Nitrogen, 1.5 mL/min
Quench Gas	No quench gas is used with H_2 carrier gas
EMV Mode	Gain factor
Gain Factor	1 (optimized for each system)
Scan Type	dMRM

* Oven hold time set to 2 minutes would generate a run time of 11.3 minutes; benzo[g,h,i]perylene eluted at 10.13 minutes.

** RT locking may result in a different flow rate on different instruments.

Results and discussion

GC/MS tuning mix

Even though the GC/MS/MS system can be and was tuned with the manufacturer's recommended tune, which is the etune default for Agilent 7000 series triple quadrupole GC/MS systems, the DFTPP ion ratio criteria from Table 3 of EPA method 8270E were used to test the HydroInert source with H_2 carrier gas.[1,2] Table 4 summarizes the relative abundances of the DFTPP ion ratios at 25 µg/mL, the method criteria, and if the measured relative abundances matched the criteria, where all measured relative abundances pass the 8270E ion ratio criteria.

There is always concern of inlet and column cleanliness for EPA method 8270 to work, no matter the carrier gas; DDT, pentachlorophenol, and benzidine are used to track inlet breakdown and column health. Increased DDT breakdown indicates a need for inlet maintenance, while increasing tailing factors of benzidine and pentachlorophenol inform the user to trim or change the column. With the introduction of H_2 carrier gas, users may be worried about increased reactions of active compounds such as DDT in the inlet; the recommendation is to lower the inlet temperature to 230 to 250 °C and use a temperature-programmable inlet, such as the MMI, to protect the active compounds, while still being able to increase the temperature to 320 or 350 °C and drive out the PAHs. In this note, we have used the MMI.

Reviewing the results of the GC/MS tuning mixture for DDT breakdown and compound tailing factors from a scan mode run, the DDT (%) breakdown was 1.4%, the pentachlorophenol tailing factor was 1.0, and the benzidine tailing factor was 1.4. All values are within the EPA method 8270 criteria of <20% DDT breakdown and tailing factors <2.0.

Initial calibration

Figure 1 displays a total ion chromatogram (TIC) for the separation of 120 target analytes and six internal standards. A multipoint calibration was performed with 15 concentration levels from 0.02 to 100 µg/mL, and the relative response factor (RF) was determined for each compound at each calibration level. The average RF was calculated for the calibration curve of each compound along with the relative standard deviation (%RSD). The preferred passing criteria for EPA method 8270 is an average RF %RSD less than 20%; if not attainable with six or more calibration levels, a linear curve fit requires an R^2 value of 0.990 or greater, as does a quadratic curve fit. Accuracy of the lowest data point must be within 30% of the estimated concentration.

Table 4. DFTPP ions, abundance criteria from EPA method 8270E[2], measured relative abundance and pass/fail of the relative abundance for the Agilent HydroInert source in a GC/MS/MS system with H_2 carrier gas.

Target Mass (*m/z*)	Ion Abundance Criteria	Measured Relative Abundance	Pass/Fail
68	<2% of 69 *m/z*	0 %	Pass
69	Present	36.4 %	Pass
70	<2% of 69 *m/z*	1.1 %	Pass
197	<2% of 198 *m/z*	0 %	Pass
198	Base peak or present	100 % (base peak)	Pass
199	5 to 9% of 198 *m/z*	7.0 %	Pass
365	>1% of Base peak	1.8 %	Pass
441	<150% of 443 *m/z*	51.8 %	Pass
442	Base peak or present	46.7% (base peak)	Pass
443	15 to 24% of 442 *m/z*	21.9 %	Pass

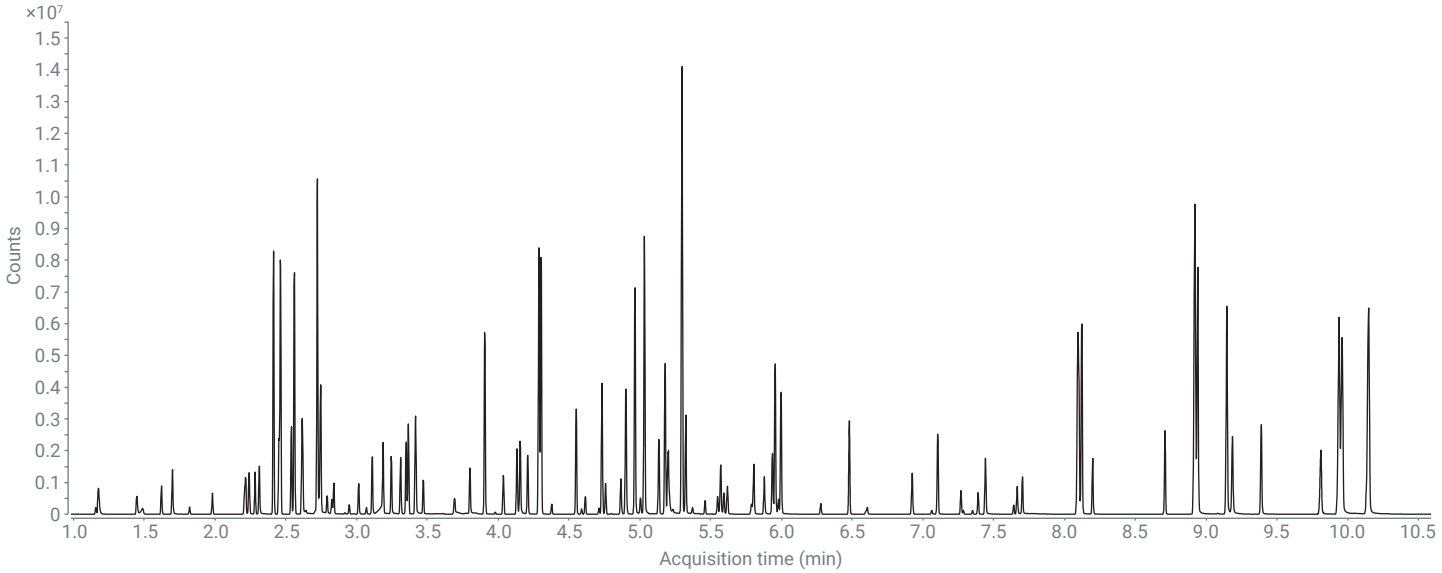

Figure 1. TIC of the 50 µg/mL calibration standard showing separation in under 10 minutes.

Critical pair resolution

With the shorter method time and different column, critical pair resolution above 50% was verified for phenanthrene and anthracene (MRM transition of 178.1 → 152.1 *m/z*), benz[a] anthracene and chrysene (228.1 → 226.1 *m/z*), and benzo(b) fluoranthene and benzo(k)fluoranthene (252.1 → 250.1 *m/z*). All three isomer pairs are shown in Figure 2 at a midlevel concentration of 5 µg/mL; phenanthrene and anthracene (Figure 2A) have baseline resolution, benz[a]anthracene and chrysene (Figure 2B) are nearly baseline resolved, and benzo(b)fluoranthene and benzo(k)fluoranthene (Figure 2C) are ~70% resolved, satisfying the EPA method 8270 criteria.

Mass spectral fidelity

A common concern of using H_2 carrier gas is the reactivity of H_2 at active sites, such as the hot metal inside of a source, which can cause hydrogenation and dechlorination reactions. Compound transformations, such as hydrogenation of nitro functional groups to amine groups could cause low or no response for MRM transitions that have been identified with He carrier gas and result in no identification or misidentification of a compound in a sample. Retention of existing method MRM transitions is preferred to reduce method development work. With the HydroInert source, users can retain the same MRM transitions with H_2 carrier

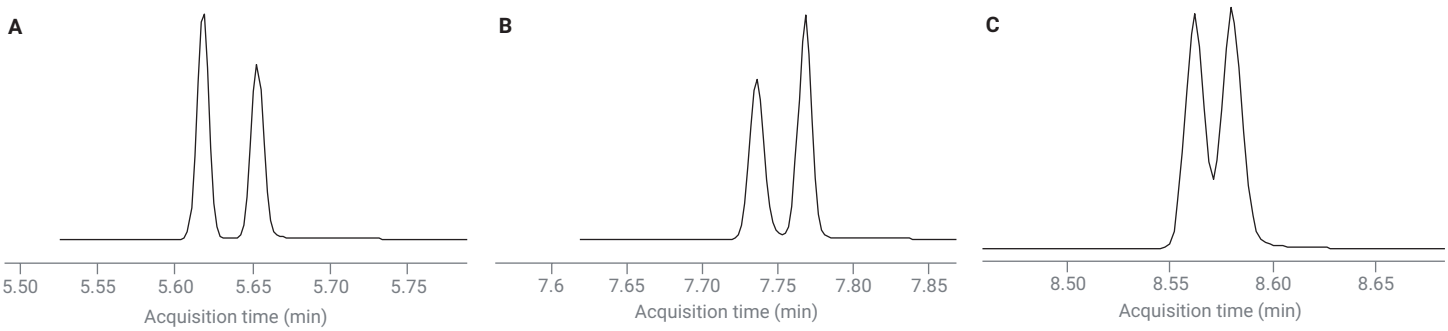

Figure 2. Midlevel standard (5 µg/mL) MRM transition extracted ion chromatograms (EICs) for critical isomer pairs: (A) phenanthrene and anthracene (MRM transition of 178.1 → 152.1 *m/z*); (B) benz[a]anthracene and chrysene (228.1 → 226.1 *m/z*); (C) benzo(b)fluoranthene and benzo(k)fluoranthene (252.1 → 250.1 *m/z*).

gas that they developed with He systems. Retention times and collisions energies must be re-evaluated, especially for retention times if column dimensions and oven temperature ramps are altered. The compound list above has several nitro compounds and heavily chlorinated compounds that would be susceptible to reactions with H_2 in the normal extractor source, including nitrobenzene, pentachlorophenol, hexachlorobenzene, and pentachloronitrobenzene. We can observe retention of functional groups by verifying the MRM transition EICs exist and the expected ratios between the quantifier and qualifier MRM transitions. If the ratios for the qualifier transitions (compared to the quantifier transition) are close to 100%, reactions with H_2 are not occurring. Missing, very low, or very high MRM transition ratios would indicate reaction with H_2. Figure 3 shows a set of overlays of the MRM transitions for parathion (Figure 3A), a compound with a nitro group, and hexachlorobenzene (Figure 3B), a heavily chlorinated compound. Figures 3A and 3B each have the transition ratio percentages listed in the top-left corner. For parathion, if the nitro functional group was hydrogenated to an amine group, the 291 → 109 transition would be lower in abundance and ratio to the quantifier transition, as the MW would be 259 *m/z*, instead of 291 *m/z*. As shown in Figure 3A, the transition ratios were at 100%, indicating retention of the nitro functional group. For hexachlorobenzene, dechlorination would result in higher abundance of the 249 → 214 transition and lower abundance at 284 → 214 transition; however, Figure 3B displays retention of the expected ratio between these two transitions at 100%, and no significant dechlorination occurred.

Calibration data

Of 120 compounds, six compounds required linear fits and 10 quadratic fits were required. Table 5 summarizes the calibration results for the 120 target compounds and surrogates with average response factor (RF) %RSD values, the curve fit and R^2 value, if required, and the lowest and highest concentration level, if the values are different than the extended calibration range, 0.02 to 100 µg/mL. Over 86% of the 120 compounds pass the calibration criteria with an average RF %RSD below 20%. Of the 120 compounds, 13 compounds (<11%) had a calibration range narrower than the normal EPA method 8270 range of 0.1 to 100 µg/mL, but all still passed EPA method 8270E criteria by at least seven calibration levels or more. Looking at the previous work using EPA method 8270E and GC/MS/MS with He carrier gas, eight compounds required curve fits to pass the calibration criteria.[3] An increase in linear and quadratic fits is predictable since H_2 is more reactive than He. Also, the inlet is initially set to a lower temperature to avoid formation of hydrochloric acid in the presence of higher temperatures and water in the inlet, whether from carrier gas or the sample extraction procedure. In both He and the H_2 carrier gas results, bis(2-ethylhexyl)phthalate and di-n-octyl phthalate required quadratic fits to pass the calibration criteria. However, some of the compounds requiring curve fits were different between the two data sets. For example, N-nitrosodipropylamine passed with average RF %RSD of 12.3% for the He data, but required a linear fit for the H_2 carrier gas with the HydroInert source. N-nitrosodimethylamine (NDMA) required a linear fit from 0.2 to 100 µg/mL for the He-generated data, but passed

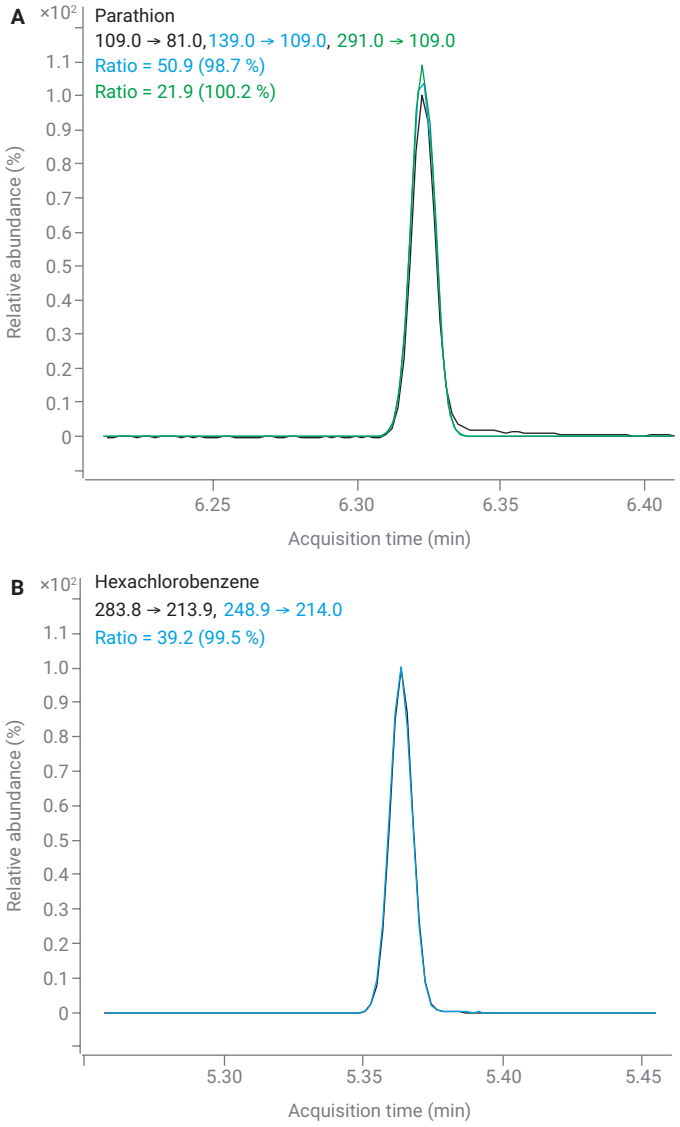

Figure 3. Overlays of MRM transition EICs for (A) parathion and (B) hexachlorobenzene, when using H_2 carrier gas and the Agilent HydroInert source on a GC/MS/MS system, showing retention of key functional groups in the presence of H_2.

Table 5. Initial calibration results for 120 target compounds and surrogates for H_2 carrier gas and the Agilent HydroInert source in GC/MS/MS for EPA method 8270.

Name	RT (min)	Avg. RF	Average RF %RSD	Curve Fit R^2	Curve Fit	Low Standard (µg/mL)	High Standard (µg/mL)
						Default is 0.02 to 100 µg/mL	
NDMA	1.1613	0.074	17.28			0.02	100
Pyridine	1.1832	0.487	16.17			0.05	100
2-Picoline	1.4508	0.154	11.23			0.05	100
N-Nitroso-N-methylethylamine	1.4893	0.101	13.58			0.02	100
Methyl methanesulfonate	1.6215	0.385	6.18			0.02	100
2-Fluorophenol (surrogate)	1.6962	0.515	12.02			0.02	100
N-Nitrosodiethylamine	1.8184	0.069	15.15			0.02	100
Ethyl methanesulfonate	1.9794	0.307	7.28			0.02	100
Phenol-d_6 (surrogate)	2.2064	0.287	9.81			0.02	100
Phenol	2.2135	0.278	12.45			0.05	100
Aniline	2.2394	0.638	11.65			0.02	100
Bis(2-chloroethyl)ether	2.2817	0.538	4.95			0.02	100
2-Chlorophenol	2.3106	0.536	11.28			0.02	100
1,3-Dichlorobenzene	2.413	0.922	2.68			0.02	100
1,4-dichlorobenzidine-d_4 (ISTD)	2.450		3.46			0.02	100
1,4-Dichlorobenzene	2.461	0.917	3.36			0.02	100
Benzyl alcohol	2.5379	0.388	14.57			0.02	100
1,2-Dichlorobenzene	2.5582	0.879	2.65			0.02	100
2-Methylphenol (*o*-cresol)	2.6123	0.524	7.24			0.02	100
Bis(2-chloro-1-methylethyl)ether	2.639	0.031	7.60			0.02	100
N-Nitrosopyrrolidine	2.7006	0.029	14.89			0.05	100
4-Methylphenol (*p*-cresol)	2.7173	0.738	8.05			0.02	100
Acetophenone	2.7202	0.971	7.46			0.05	100
N-Nitrosodi-n-propylamine	2.722	0.027		0.9951	Linear	0.1	100
4-Nitrosomorpholine	2.7331	0.097	16.61			0.02	100
o-Toluidine	2.741	0.735	9.62			0.02	100
Hexachloroethane	2.7897	0.150	6.42			0.02	100
Nitrobenzene-d_5 (surrogate)	2.8228	0.074	11.46			0.02	100
Nitrobenzene	2.837	0.259	12.83			0.05	100
N-Nitrosopiperidine	2.9445	0.049	15.16			0.1	100
Isophorone	3.0114	0.251	9.29			0.02	100
2-Nitrophenol	3.0661	0.067	16.02			0.02	100
2,4-Dimethylphenol (2,4-xylenol)	3.107	0.441	7.45			0.02	100
Benzoic acid	3.1093	0.202		0.9965	Linear	2	100
bis(2-Chloroethoxy)methane	3.186	0.741	6.02			0.02	100
2,4-Dichlorophenol	3.2418	0.420	17.51			0.02	100
1,2,4-Trichlorobenzene	3.3073	0.577	7.97			0.02	100
Naphthalene-d_8 (ISTD)	3.348		3.25			0.02	100
Naphthalene	3.3634	0.902	3.21			0.02	100
4-Chloroaniline	3.4127	0.558	5.69			0.02	100
2,6-Dichlorophenol	3.4162	0.353	15.57			0.02	100
Hexachlorobutadiene	3.4689	0.410	4.92			0.02	100

Name	RT (min)	Avg. RF	Average RF %RSD	Curve Fit R^2	Curve Fit	Low Standard (µg/mL)	High Standard (µg/mL)
						Default is 0.02 to 100 µg/mL	
p-Phenylenediamine	3.6874	0.232	11.54			0.1	100
N-Nitrosodi-*n*-butylamine	3.6903	0.069	8.48			0.02	100
4-Chloro-3-methylphenol	3.7999	0.372	11.05			0.02	100
2-Methylnaphthalene	3.9022	1.689	4.44			0.02	100
Hexachlorocyclopentadiene	4.0322	0.034	18.12			0.02	100
1,2,4,5-Tetrachlorobenzene	4.0348	0.230	6.13			0.02	100
2,4,6-Trichlorophenol	4.1305	0.171	19.08			0.02	100
2,4,5-Trichlorophenol	4.1537	0.255	15.58			0.02	100
2-Fluorobiphenyl (surrogate)	4.2061	0.364	3.16			0.02	100
1-Chloronaphthalene	4.2848	0.810	4.80			0.02	100
2-Chloronaphthalene	4.2998	0.784	4.74			0.02	100
2-Nitroaniline	4.3763	0.060	15.70			0.02	100
Dimethyl phthalate	4.5458	0.799	10.18			0.02	100
2,6-Dinitrotoluene	4.5829	0.034	9.97			0.02	100
Acenaphthylene	4.6136	0.146	7.06			0.02	100
3-Nitroaniline	4.7069	0.034	16.75			0.1	100
Acenaphthene-d_{10} (ISTD)	4.731		3.03			0.02	100
Acenaphthene	4.7548	0.184	2.87			0.02	100
2,4-Dinitrophenol	4.801	0.006		0.9988	Linear	1	100
Pentachlorobenzene	4.8623	0.149	4.46			0.02	100
4-Nitrophenol	4.8639	0.055	15.34			0.1	100
Dibenzofuran	4.8969	1.389	4.27			0.02	100
2,4-Dinitrotoluene	4.9036	0.030	17.05			0.1	100
1-Naphthylamine	4.9616	0.746	10.88			0.02	100
2,3,4,6-Tetrachlorophenol	5.0024	0.066	18.19			0.1	75
2-Naphthylamine	5.0276	0.906	7.70			0.02	100
Diethyl phthalate	5.1254	0.583	12.91			0.1	100
Fluorene	5.1741	1.433	4.42			0.02	100
Thionazin	5.1855	0.037		0.9992	Quadratic	0.05	100
5-Nitro-*o*-toluidine	5.1925	0.052	17.22			0.2	100
4-Chlorophenyl phenyl ether	5.1941	0.363	8.62			0.02	100
4-Nitroaniline	5.1986	0.111	15.16			0.1	100
2-Methyl-4,6-dinitrophenol (DNOC)	5.2271	0.009		0.9992	Linear	0.2	75
N-Nitrosodiphenylamine	5.2922	2.207	5.19			0.02	100
Diphenylamine	5.2923	2.697	5.23			0.02	100
Azobenzene	5.3216	0.966	19.48			0.1	100
2,4,6-Tribromophenol (surrogate)	5.3661	0.048	18.64			0.05	100
Sulfotep	5.4547	0.046		1.0000	Quadratic	0.1	100
Dimethoate	5.4556	0.004		0.9996	Quadratic	0.1	100
Diallate I	5.5446	0.056		0.9995	Quadratic	0.2	100
Phorate	5.5454	0.112	19.23			0.05	50
Phenacetin	5.5584	0.395		0.9926	Linear	0.2	100
4-Bromophenyl phenyl ether	5.591	0.214	4.60			0.02	100
Hexachlorobenzene	5.6139	0.411	3.63			0.02	100

Name	RT (min)	Avg. RF	Average RF %RSD	Curve Fit R^2	Curve Fit	Low Standard (µg/mL)	High Standard (µg/mL)
						Default is 0.02 to 100 µg/mL	
Pentachlorophenol	5.785	0.106		0.9996	Quadratic	0.5	100
Pentachloronitrobenzene	5.7933	0.053	17.34			0.02	100
4-Aminobiphenyl	5.8011	0.415	7.12			0.02	100
Propyzamide	5.8731	0.228	18.96			0.1	75
Phenanthrene-d_{10} (ISTD)	5.936		2.96			0.02	100
Phenanthrene	5.9516	1.117	6.24			0.02	100
Dinoseb	5.9596	0.046	16.84			0.2	100
Disulfoton	5.9761	0.189		0.9999	Quadratic	0.05	100
Anthracene	5.9921	0.857	3.53			0.02	100
Parathion-methyl	6.2746	0.068	18.32			0.02	100
Di-*n*-butyl phthalate	6.4745	0.567	19.97			0.05	100
4-Nitroquinoline-1-oxide	6.5908	0.011	19.12			0.2	75
Parathion	6.6037	0.032	16.40			0.05	100
Fluoranthene	6.9204	0.344	4.85			0.02	100
Benzidine	7.0591	0.029	17.04			0.1	100
Pyrene	7.1006	0.361	4.52			0.02	100
p-Terphenyl-d_{14} (surrogate)	7.2656	0.141	3.33			0.02	100
Aramite I	7.2822	0.014	12.68			0.02	100
Aramite II	7.3467	0.013	11.52			0.02	100
4-Dimethylaminoazobenzene	7.3855	0.053		0.9989	Quadratic	0.05	100
Chlorobenzilate	7.4376	0.171	19.35			0.02	75
Famphur	7.6348	0.061	11.33			0.02	50
3,3'-Dimethyl benzidine	7.6608	0.097	11.45			0.05	100
Butyl benzyl phthalate	7.6991	0.155		0.9986	Quadratic	0.05	100
Benz[a]anthracene	8.0875	1.018	9.47			0.05	100
3,3'-Dichlorobenzidine	8.0933	0.075	16.78			0.1	100
Chrysene-d_{12} (ISTD)	8.100		3.61			0.02	100
Chrysene	8.1151	0.437	6.10			0.02	100
bis(2-Ethylhexyl) phthalate	8.1936	0.250		0.9992	Quadratic	0.05	100
Di-*n*-octyl phthalate	8.7044	0.470		0.9991	Quadratic	0.05	100
Benzo[b]fluoranthene	8.9096	1.258	3.89			0.02	100
7,12-Dimethylbenz[a]anthracene	8.9135	0.603	14.52			0.02	100
Benzo[k]fluoranthene	8.9307	1.258	4.48			0.02	100
Benzo[a]pyrene	9.1396	0.922	11.99			0.02	100
Perylene-d_{12} (ISTD)	9.183		5.97			0.02	100
3-Methylcholanthrene	9.3835	0.455	19.13			0.02	100
Dibenz[a,j]acridine	9.7986	0.375		0.9923	Linear	0.2	100
Indeno[1,2,3-cd]pyrene	9.9277	0.961	12.31			0.02	100
Dibenz[a,h]anthracene	9.9494	0.140	10.41			0.02	100
Benzo[g,h,i]perylene	10.133	1.265	4.92			0.02	100

calibration criteria across the full default range of 0.02 to 100 µg/mL, with an average RF %RSD of 17.3% using the H_2 carrier gas with the HydroInert source.[3] Individual differences in specific compounds are expected since the method was moved from an inert gas to a more reactive gas, and changes were made to the inlet and oven parameters.

During method development, the starting MMI temperature was varied to test for the best results across the entire run time. The best results were generated when the MMI was ramped up from 250 to 350 °C in this method. The inlet was also tested starting at a lower inlet temperature of 230 °C, which had better results for some of the earlier-eluting sensitive compounds, such as benzoic acid, but the later-eluting PAHs did not perform as well with respect to the linear ranges, and there was some risk of carryover. The specific inlet parameters should be optimized by the user for their analysis needs.

Sensitivity loss with H_2 carrier gas and existing mass spectrometer systems has been well reported. Due to this concern, particular attention was paid to the calibration range and verifying that most compounds were able to achieve the same calibration range as previous He analyses. On the topic of sensitivity, 77 compounds were analyzed in a previous application for EPA method 8270 with He carrier gas on GC/MS/MS.[3] Comparing these compounds with the same set using the HydroInert source and H_2 carrier gas (also GC/MS/MS), only 8 more compounds required linear or quadratic fits than the He data. As is normal, benzoic acid required a linear fit with a calibration range of 2 to 100 µg/mL, where the curve fit and calibration range was the same between He and H_2 data. For 2,4-dinitrophenol, both analyses required linear fits but the H_2 data had a narrower range, starting at 1 µg/mL instead of 0.5 µg/mL for He. When starting at 230 °C for the inlet temperature, the 2,4-dinitrophenol calibration range started at 0.5 µg/mL; if 2,4-dinitrophenol detection is most critical, then the method should be built for this sensitive compound. Pentachlorophenol had the same curve fit, quadratic, and a calibration range of 0.5 to 100 µg/mL for both H_2 with HydroInert source and He results. On the other hand, 4-nitrophenol passed calibration criteria with an average RF %RSD of 17.4% with a 0.1 to 100 µg/mL range for the H_2 analysis, while the He results required a linear fit from 5 to 160 µg/mL. Also, benzidine was routinely identifiable in all analyses with H_2 and HydroInert source in the GC/MS/MS; in this specific method, the average RF %RSD was 17.5% for the full extended calibration range from 0.02 to 100 µg/mL, while the benzidine data was not included in the He results. Another pair of examples of extended calibration range with the H_2 and HydroInert data can be shown with bis(2-ethylhexyl) phthalate and di-*n*-octylphthalate. Both phthalate compounds had a wider calibration range of 0.05 to 100 µg/mL with a quadratic fit for the H_2 data, compared to the He quadratic fit from 0.5 to 100 µg/mL. Reviewing the internal standards, the average RF %RSDs are all below 6%, indicating consistent performance for the H_2 carrier gas, HydroInert source, and GC/MS/MS, and no issues with hydrogenation of deuterated compounds. The deuterated surrogate compounds, nitrobenzene-d_5, phenol-d_6, and *p*-terphenyl-d_{14}, further support the retention of deuterium bonds with average RF %RSDs below 12% for the extended calibration curves. Of the 77 comparable compounds between the H_2 and He data, 80% (60 compounds) had similar or wider calibration ranges for H_2 and HydroInert results. H_2 carrier gas with the HydroInert source retains the sensitivity for most compounds when compared to the He data.

Response factor (RF) comparison

There is always concern about sensitivity and maintenance of response factors (RFs) for both single quadrupole and triple quadrupole systems when moving an analysis from He to H_2 carrier gas. Table 6 lists the RFs from EPA method 8270E guidance criteria (Table 4), RFs from a GC/MS analysis with He carrier gas, and RFs for GC/MS/MS analysis with the HydroInert source and H_2 carrier gas. All of these test systems used 9 mm extraction lenses, respective of the source type (e.g. the HydroInert source had a HydroInert 9 mm extraction lens). The RFs from EPA method 8270E Table 4 are guidance criteria and not requirements to pass the method, but ideally the RFs should be similar to these guidance values. For the He GC/MS analysis, two compounds have RFs below the guidance criteria: hexachloroethane and N-nitroso-di-*n*-propylamine. For the H_2 HydroInert GC/MS/MS analysis, there were 14 more compounds with RF values lower than the guidance criteria than the He GC/MS system, but the GC/MS/MS also opens the potential to analyze lower concentration levels, down to 20 ng/mL, when the normal calibration range is 100 ng/mL to 100 µg/mL. Seven of these low RF compounds are within 0.2 counts of the suggested RF value. It is difficult to determine the significance of the difference, since the reference RF values are data generated on single quadrupole GC/MS systems using He carrier gas.

Repeatability in matrix

Table 6. RFs for select compounds (in alphabetical order) from EPA method 8270E (Table 4)[4], GC/MS single quadrupole analysis with He carrier gas and GC/MS/MS triple quadrupole analysis with the Agilent HydroInert source and H_2 carrier gas.

Compound	RF from EPA 8270E[4]	RF He GC/MS	RF H_2 and HydroInert GC/MS/MS
Acenaphthene	0.9	1.3	0.2
Acenaphthylene	0.9	1.9	0.1
Acetophenone	0.01	1.2	1.0
Anthracene	0.7	1.1	0.9
Benzo(a)anthracene	0.8	1.4	1.0
Benzo(a)pyrene	0.7	1.2	1.0
Benzo(b)fluoranthene	0.7	1.4	1.2
Benzo(g,h,i)perylene	0.5	1.1	1.3
Benzo(k)fluoranthene	0.7	1.2	1.3
Bis(2-chloroethoxy)methane	0.3	0.4	0.7
Bis(2-chloroethyl)ether	0.7	0.8	0.5
Bis-(2-ethylhexyl)phthalate	0.01	0.8	0.2
4-Bromophenyl-phenyl ether	0.1	0.3	0.2
Butyl benzyl phthalate	0.01	0.6	0.1
4-Chloroaniline	0.01	0.4	0.6
4-Chloro-3-methylphenol	0.2	0.3	0.4
2-Chloronaphthalene	0.8	2.4	0.7
2-Chlorophenol	0.8	0.8	0.5
4-Chlorophenyl-phenyl ether	0.4	0.7	0.3
Chrysene	0.7	1.2	0.4
Dibenz(a,h)anthracene	0.4	1.1	0.2
Dibenzofuran	0.8	1.7	1.4
Di-*n*-butyl phthalate	0.01	1.3	0.5
3,3'-Dichlorobenzidine	0.01	0.5	0.1
2,4-Dichlorophenol	0.2	0.3	0.4
Diethyl phthalate	0.01	1.4	0.6
Dimethyl phthalate	0.01	1.4	0.8
2,4-Dimethylphenol	0.2	0.3	0.4
4,6-Dinitro-2-methylphenol	0.01	0.2	0.01
2,4-Dinitrophenol	0.01	0.2	0.01
2,4-Dinitrotoluene	0.2	0.4	0.02
2,6-Dinitrotoluene	0.2	0.3	0.03
Di-*n*-octyl phthalate	0.01	1.3	0.4
Fluoranthene	0.6	1.2	0.4
Fluorene	0.9	1.3	1.4
Hexachlorobenzene	0.1	0.3	0.4
Hexachlorobutadiene	0.01	0.2	0.4
Hexachlorocyclopentadiene	0.05	0.3	0.03
Hexachloroethane	0.3	0.2	0.1
Indeno(1,2,3-cd)pyrene	0.5	1.2	1.1
Isophorone	0.4	0.6	0.3
2-Methylnaphthalene	0.4	0.7	1.7
2-Methylphenol	0.7	0.7	0.6
4-Methylphenol	0.6	1.0	0.7
Naphthalene	0.7	1.1	0.9
2-Nitroaniline	0.01	0.4	0.05
3-Nitroaniline	0.01	0.3	0.02
4-Nitroaniline	0.01	0.3	0.1
Nitrobenzene	0.2	0.3	0.3
2-Nitrophenol	0.1	0.2	0.1
4-Nitrophenol	0.01	0.2	0.05
N-Nitroso-di-*n*-propylamine	0.5	0.4	0.03
N-Nitrosodiphenylamine	0.01	2.1	2.9
2,2'-Oxybis-(1-chloropropane)	0.01	0.5	0.03
Pentachlorophenol	0.05	0.2	0.1
Phenanthrene	0.7	1.2	1.1
Phenol	0.8	0.9	0.3
Pyrene	0.6	1.3	0.3
1,2,4,5-Tetrachlorobenzene	0.01	0.4	0.2
2,3,4,6-Tetrachlorophenol	0.01	0.4	0.07
2,4,5-Trichlorophenol	0.2	0.3	0.2
2,4,6-Trichlorophenol	0.2	0.3	0.2

The large EPA method 8270 mixture of compounds was also diluted to a concentration of 0.4 µg/mL to act as a calibration verification standard, since 0.4 µg/mL was not a specific calibration point. To test the repeatability of the HydroInert source in GC/MS/MS with H_2 carrier gas, the standard was sandwich-injected with 1 µL of a composite soil matrix to simulate a spiked matrix sample. This injection was repeated 10 times to understand the robustness of the method and to look for matrix enhancement, suppression, or potential contamination from the soil matrix. Table 7 contains the following data for each compound: calculated concentration of 0.4 µg/mL calibration verification in solvent, average concentration of the 10 replicates of 0.4 µg/mL calibration verification in soil matrix, the %RSD for the 10 replicate injections in soil matrix, and the recovery percentage comparing the soil matrix and solvent concentrations.

Compounds with calibration ranges that did not include 0.2 µg/mL or lower were not included in the table. For the 0.4 µg/mL solvent standard, only five compounds fell outside of the ±20% calibration verification window: sulfotep, dimethoate, diallate I, aramite I, and 7,12-dimethylbenz[a] anthracene. The first three compounds all were calibrated with quadratic fits and this verification concentration is low, which may be the reason for the high values. Normally, the calibration verification standard is closer to the midpoint of the calibration curve, but this study was pushing towards to lower limits with an on-column concentration of 0.02 µg/mL. Aramite I is just above the 20% limit at 0.481 µg/mL, while 7,12-dimethylbenz[a]anthracene is approximately half the expected concentration at 0.22 µg/mL. All other compounds near 7,12-benz[a]anthracene are within the 20% limit, and it is unclear why this result is very low. For the replicate injections in soil, all but two compounds have a %RSD for the replicate injections below 10%, indicating the method is robust, even when running samples in matrix.

For the average concentrations in matrix, 17 compounds are outside the ±20% limit; 5 of these compounds are just above 0.48 µg/mL (less than 0.49 µg/mL), which may be minor signal enhancements from the matrix. Ten of these compounds are within 140% of the expected concentration of 0.4 µg/mL; furthermore, when the recovery percentage is calculated comparing the soil concentration to the solvent concertation, only six compounds fall outside of a ±20% recovery range, which again suggests signal enhancement. Bis(2-ethylhexyl) phthalate has a reported average concentration of 0.89 µg/mL, suggesting that there was bis(2-ethylhexyl) phthalate in the soil matrix. On the other hand, famphur appears to be suppressed by the matrix, as the average concentration in matrix was 0.272 µg/mL, but 0.402 µg/mL in solvent. In summary, for the soil matrix testing, we can easily detect the 0.4 µg/mL calibration verification standard consistently in matrix with over 85% of the compounds reporting inside the ±20% calibration range requirement. Typically, calibration verification is completed in solvent, where more than 95% of the compounds are inside the ±20% calibration range requirement.

Table 7. Comparison of the solvent-calculated concentration of the 0.4 µg/mL calibration verification standard, the average concentration (10 replicate injections) of the 0.4 µg/mL standard in soil matrix, the %RSD of the 10 replicate injections, and recovery percentage of the 0.4 µg/mL standard in matrix compared to solvent.

No.	Name	Calculated Concentration (0.4 µg/mL in Solvent)	Average Concentration in Matrix of 0.4 µg/mL Spike	%RSD of 10 Replicates	Recovery in matrix
1	NDMA	0.45	0.47	1.95%	104%
2	Pyridine	0.46	0.45	2.68%	97%
3	2-Picoline	0.45	0.45	2.54%	100%
4	N-Nitroso-N-methylethylamine	0.44	0.46	1.75%	106%
5	Methyl methanesulfonate	0.47	0.46	0.31%	99%
6	2-Fluorophenol	0.46	0.45	0.94%	99%
7	N-Nitroso-N-diethylamine	0.46	0.46	1.37%	100%
8	Ethyl methanesulfonate	0.45	0.45	0.68%	99%
9	Phenol-d_6	0.46	0.45	0.67%	99%
10	Phenol	0.46	0.44	1.73%	96%
11	Aniline	0.46	0.46	1.51%	100%
12	bis(2-Chloroethyl)ether	0.46	0.45	0.87%	99%
13	2-Chlorophenol	0.44	0.45	1.28%	101%

No.	Name	Calculated Concentration (0.4 µg/mL in Solvent)	Average Concentration in Matrix of 0.4 µg/mL Spike	%RSD of 10 Replicates	Recovery in matrix
14	1,3-Dichlorobenzene	0.46	0.46	0.56%	100%
15	1,4-Dichlorobenzene	0.47	0.46	0.57%	98%
16	Benzyl alcohol	0.42	0.45	2.08%	108%
17	1,2-Dichlorobenzene	0.47	0.46	0.87%	99%
18	2-Methylphenol (*o*-cresol)	0.44	0.44	1.50%	99%
19	bis(2-Chloro-1-methylethyl)ether	0.47	0.46	4.86%	97%
20	N-Nitrosopyrrolidine	0.45	0.47	3.45%	103%
21	4-Methylphenol (*p*-Cresol)	0.40	0.42	1.65%	104%
22	Acetophenone	0.45	0.45	1.71%	100%
23	N-Nitrosodi-*n*-propylamine	0.42	0.43	5.84%	103%
24	4-Nitrosomorpholine	0.42	0.45	3.11%	107%
25	*o*-Toluidine	0.47	0.47	1.44%	99%
26	Hexachloroethane	0.44	0.48	2.32%	109%
27	Nitrobenzene-d_5	0.43	0.49	2.66%	112%
28	Nitrobenzene	0.43	0.48	3.02%	110%
29	N-Nitrosopiperidine,	0.42	0.43	2.72%	104%
30	Isophorone	0.43	0.44	1.53%	103%
31	2-Nitrophenol	0.46	0.49	2.06%	106%
32	2,4-Dimethylphenol	0.43	0.43	1.30%	100%
33	bis(2-Chloroethoxy)methane	0.44	0.44	0.54%	101%
34	2,4-Dichlorophenol	0.40	0.43	0.92%	106%
35	1,2,4-Trichlorobenzene	0.46	0.46	0.56%	100%
37	Naphthalene	0.47	0.46	0.66%	98%
38	4-Chloroaniline	0.45	0.46	1.13%	102%
39	2,6-Dichlorophenol	0.41	0.44	1.32%	106%
40	Hexachlorobutadiene	0.46	0.46	0.52%	100%
41	*p*-Phenylenediamine	0.45	0.44	3.75%	97%
42	N-Nitrosodi-*n*-butylamine	0.42	0.44	1.67%	104%
43	4-Chloro-3-methylphenol	0.43	0.43	1.45%	101%
44	2-Methylnaphthalene	0.47	0.47	0.60%	99%
45	Hexachlorocyclopentadiene	0.41	0.40	3.72%	96%
46	1,2,4,5-Tetrachlorobenzene	0.47	0.47	1.39%	99%
47	2,4,6-Trichlorophenol	0.42	0.43	1.47%	103%
48	2,4,5-Trichlorophenol	0.41	0.39	4.58%	97%
49	2-Fluorobiphenyl	0.47	0.46	0.74%	99%
50	1-Chloronaphthalene	0.47	0.46	0.78%	98%
51	2-Chloronaphthalene	0.47	0.46	1.55%	98%
52	2-Nitroaniline	0.44	0.53	0.90%	120%
53	Dimethyl phthalate	0.42	0.44	0.92%	106%
54	2,6-Dinitrotoluene	0.44	0.47	2.90%	106%
55	Acenaphthylene	0.44	0.43	2.28%	99%
56	*m*-Nitroaniline	0.39	0.43	4.35%	112%
57	Acenaphthene	0.48	0.46	1.14%	95%
59	Pentachlorobenzene	0.46	0.45	1.85%	98%
60	4-Nitrophenol	0.37	0.44	3.35%	120%

No.	Name	Calculated Concentration (0.4 µg/mL in Solvent)	Average Concentration in Matrix of 0.4 µg/mL Spike	%RSD of 10 Replicates	Recovery in matrix
61	Dibenzofuran	0.47	0.46	0.58%	99%
62	2,4-Dinitrotoluene	0.42	0.44	3.98%	105%
63	1-Naphthylamine	0.37	0.47	1.19%	126%
64	2,3,4,6-Tetrachlorophenol	0.40	0.42	1.79%	106%
65	2-Naphthylamine	0.40	0.44	1.66%	110%
66	Diethyl phthalate	0.41	0.45	1.02%	111%
67	Fluorene	0.47	0.47	0.82%	101%
68	Thionazin	0.42	0.46	2.38%	109%
69	5-Nitro-*o*-toluidine	0.40	0.45	8.22%	114%
70	4-Chlorophenyl phenyl ether	0.48	0.46	1.00%	96%
71	4-Nitroaniline	0.43	0.38	7.92%	88%
72	2-Methyl-4,6-dinitrophenol (DNOC)	0.46	0.52	5.22%	112%
73	N-Nitrosodiphenylamine	0.46	0.46	0.97%	101%
74	Diphenylamine	0.45	0.47	0.94%	104%
75	Azobenzene	0.47	0.50	2.62%	107%
76	2,4,6-Tribromophenol	0.42	0.43	3.11%	104%
77	Sulfotep	0.53	0.52	4.03%	97%
78	Dimethoate	0.64	0.52	12.70%	81%
79	Diallate I	2.70	0.53	2.91%	102%
80	Phorate	0.47	0.53	2.47%	111%
81	Phenacetin	0.42	0.44	1.40%	105%
82	4-Bromophenyl phenyl ether	0.45	0.44	2.94%	98%
83	Hexachlorobenzene	0.46	0.46	1.43%	100%
85	Pentachloronitrobenzene	0.41	0.46	3.62%	111%
86	4-Aminobiphenyl	0.44	0.45	1.56%	103%
87	Propyzamide	0.40	0.43	1.92%	107%
88	Phenanthrene	0.48	0.48	0.67%	101%
89	Dinoseb	0.42	0.43	3.59%	103%
90	Disulfoton	0.43	0.48	2.15%	111%
91	Anthracene	0.44	0.46	1.26%	104%
92	Parathion-methyl	0.42	0.40	1.25%	94%
93	Di-*n*-butyl phthalate	0.38	0.41	1.25%	106%
94	4-Nitroquinoline-1-oxide	0.42	0.41	11.49%	97%
95	Parathion	0.41	0.45	2.50%	112%
96	Fluoranthene	0.47	0.47	0.79%	100%
97	Benzidine	0.42	0.45	7.96%	105%
98	Pyrene	0.47	0.48	0.38%	101%
99	*p*-Terphenyl-d_{14}	0.46	0.46	0.82%	101%
100	Aramite I	0.48	0.51	2.28%	106%
101	Aramite II	0.48	0.50	2.85%	105%
102	*p*-(Dimethylamino)azobenzene	0.47	0.51	2.10%	108%
103	Chlorobenzilate	0.41	0.45	1.07%	108%
104	Famphur	0.40	0.27	3.75%	68%
105	3,3'-Dimethylbenzidine	0.46	0.47	2.96%	101%
106	Butyl benzyl phthalate	0.40	0.43	1.32%	109%

No.	Name	Calculated Concentration (0.4 µg/mL in Solvent)	Average Concentration in Matrix of 0.4 µg/mL Spike	%RSD of 10 Replicates	Recovery in matrix
107	Benz[a]anthracene	0.44	0.45	0.31%	101%
108	3,3'-Dichlorobenzidine	0.41	0.43	2.23%	105%
109	Chrysene	0.47	0.47	0.62%	99%
110	bis(2-Ethylhexyl) phthalate	0.44	0.89	1.80%	205%
111	Di-*n*-octyl phthalate	0.43	0.45	1.37%	104%
112	Benzo[b]fluoranthene	0.44	0.46	1.25%	105%
113	7,12-Dimethylbenz[a]anthracene	0.22	0.40	1.83%	182%
114	Benzo[k]fluoranthene	0.46	0.43	2.74%	94%
115	Benzo[a]pyrene	0.41	0.42	2.09%	103%
116	3-Methylcholanthrene	0.40	0.41	1.34%	104%
117	Dibenz[a,j]acridine	0.44	0.46	1.56%	104%
118	Indeno[1,2,3-cd]pyrene	0.41	0.42	1.01%	104%
119	Dibenz[a,h]anthracene	0.43	0.44	3.11%	103%
120	Benzo[g,h,i]perylene	0.43	0.44	1.87%	104%

Conclusion

Due to the high sensitivity achieved with MRM mode and the inertness of the Agilent HydroInert source with H_2 carrier gas, 92.5% of the 120 tested compounds were detected and calibrated in the normal calibration range for EPA method 8270E from 0.1 to 100 µg/mL, and 77 compounds reached the extended calibration range of 0.02 to 100 µg/mL. Additionally, only 16 compounds required curve fits to pass EPA Method 8270E calibration criteria. Method criteria for EPA method 8270E were met for initial calibration over a working range of 0.02 to 100 µg/mL in a single 12-minute run using H_2 carrier gas and the HydroInert source, while retaining mass spectral fidelity and existing MRM transitions for compounds susceptible to H_2 reactivity.

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