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)

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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

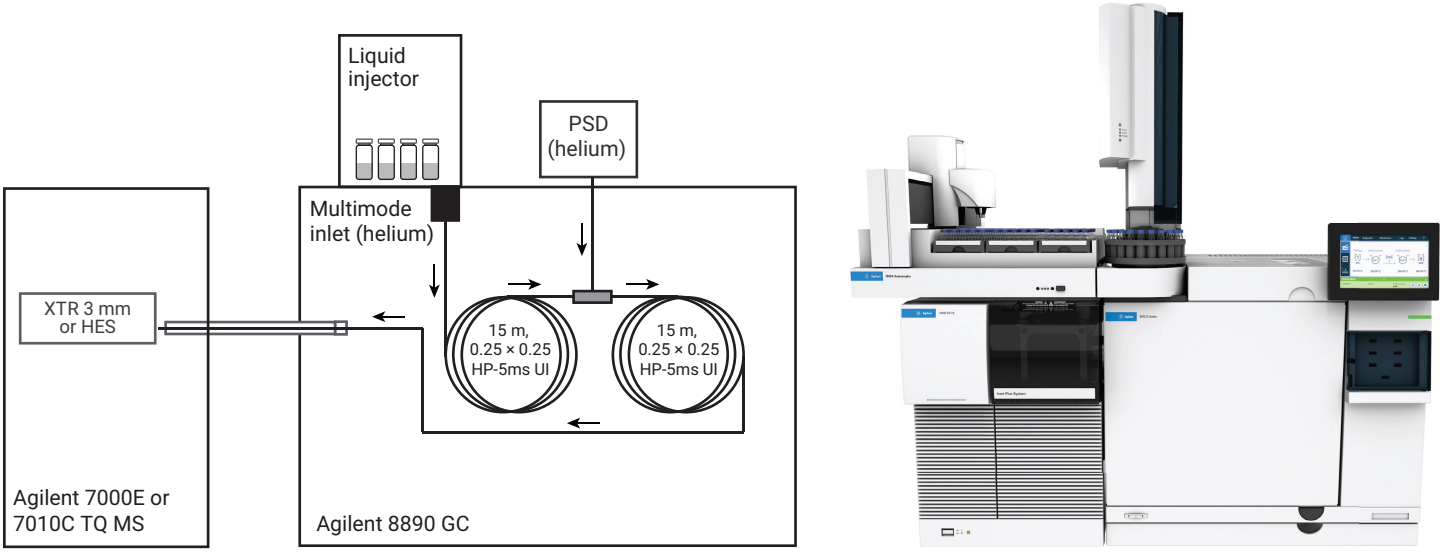

Figure 1. The Agilent 8890/7000E and 8890/7010C GC/TQ system (A) and system configuration (B).

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

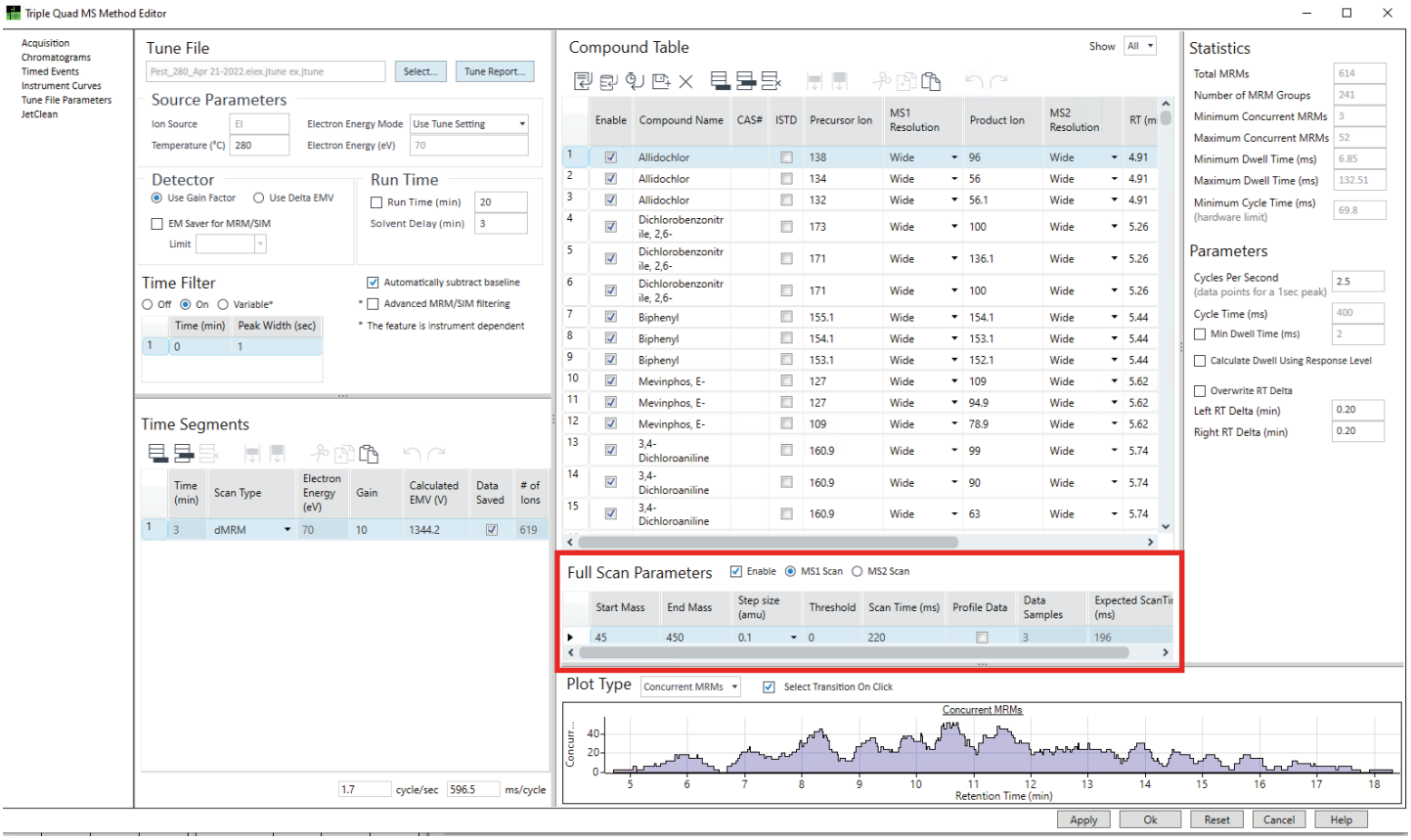

Figure 2. Triple quadrupole MS Method Editor showing the full scan acquisition parameters used for simultaneous dMRM/scan in this work.

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.

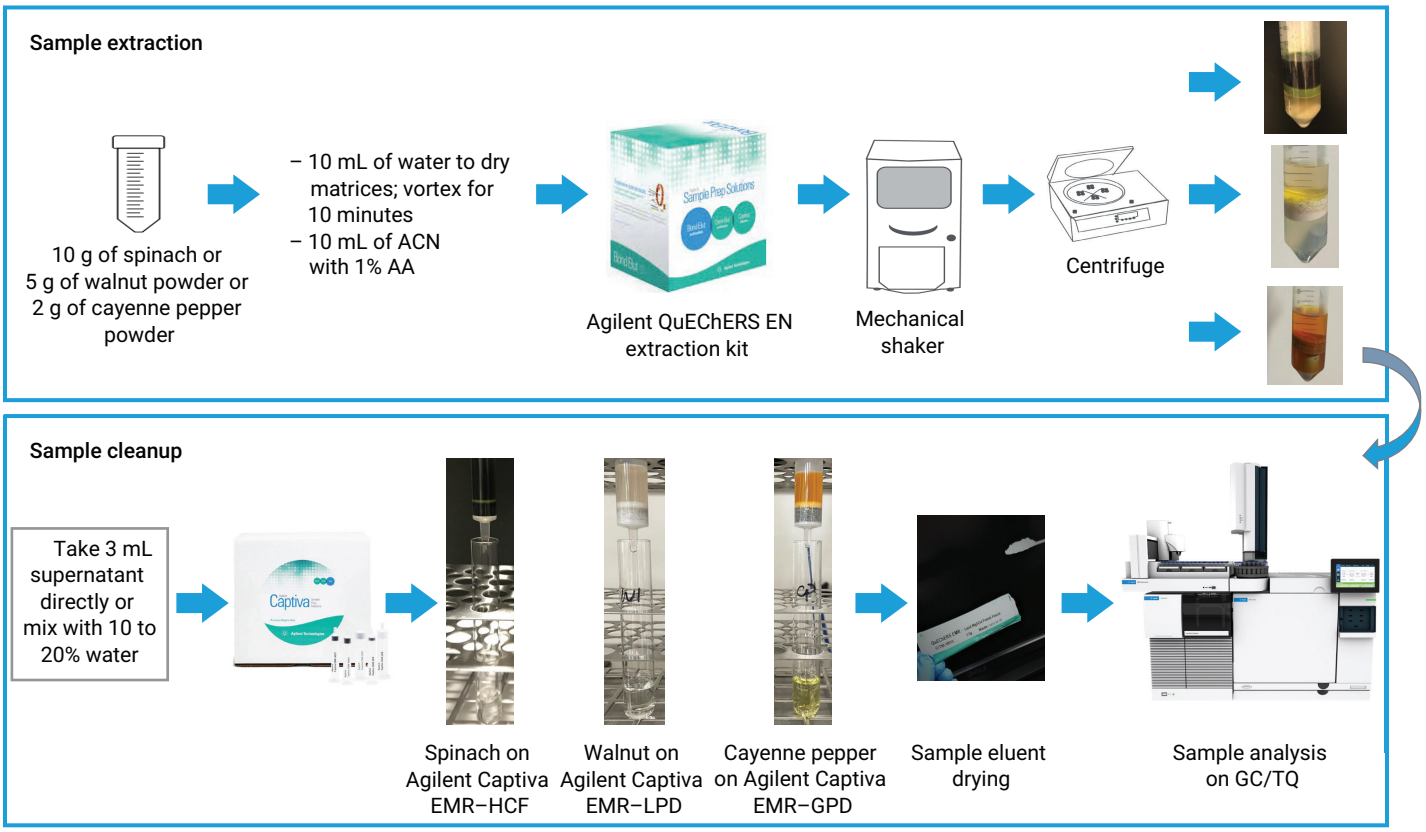

Figure 3. Sample preparation flowchart including traditional QuEChERS extraction, followed with Captiva EMR pass-through clean up.

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.

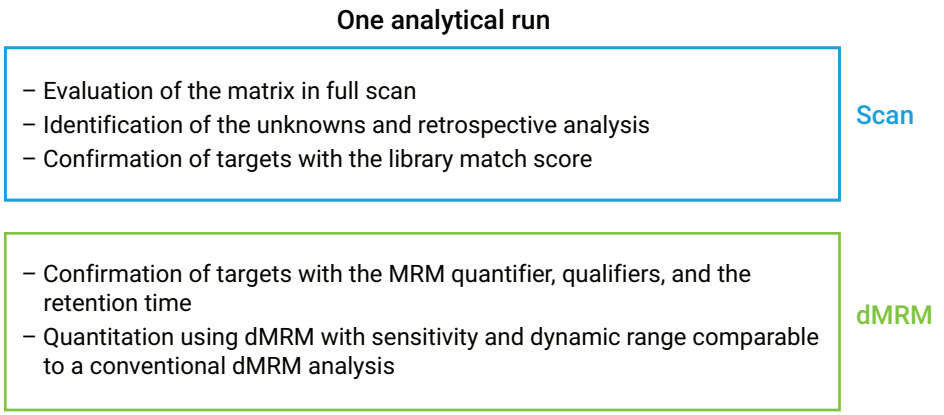

Figure 4. Functionality enabled with simultaneous dMRM/scan data acquisition mode within one analytical run.

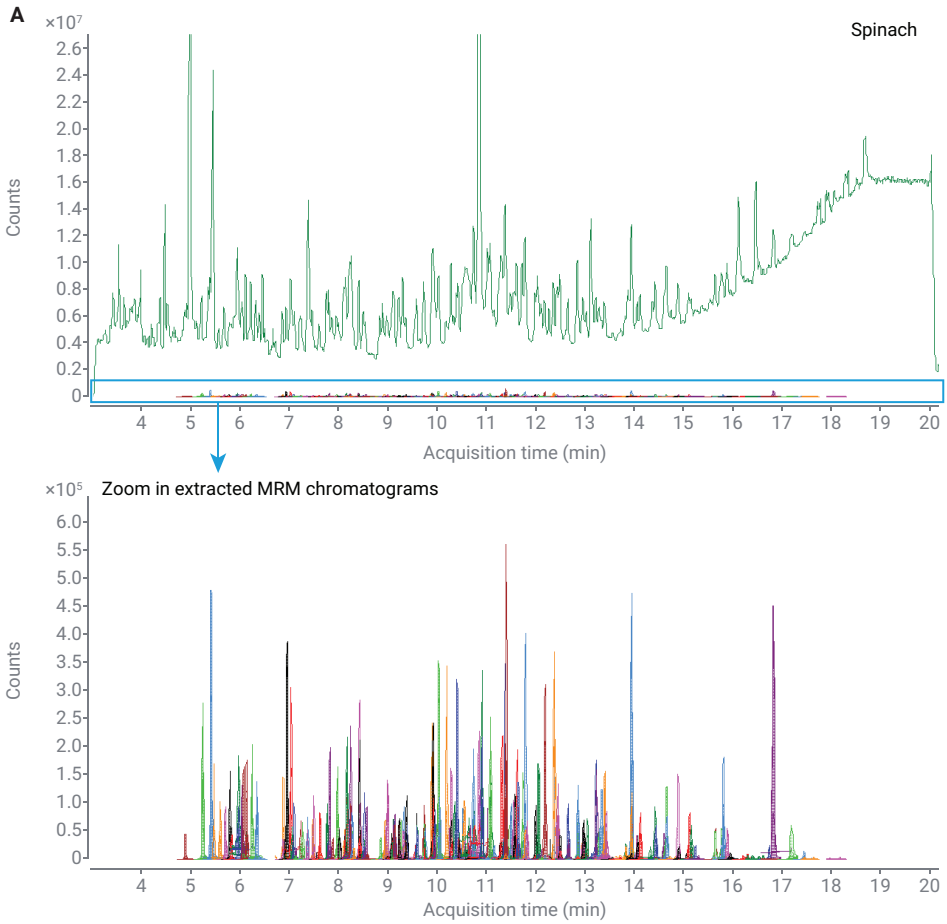

Figure 5A. Scan (on top) and dMRM (magnified on the bottom) TIC acquired in simultaneous dMRM/scan data acquisition mode for spinach extract.

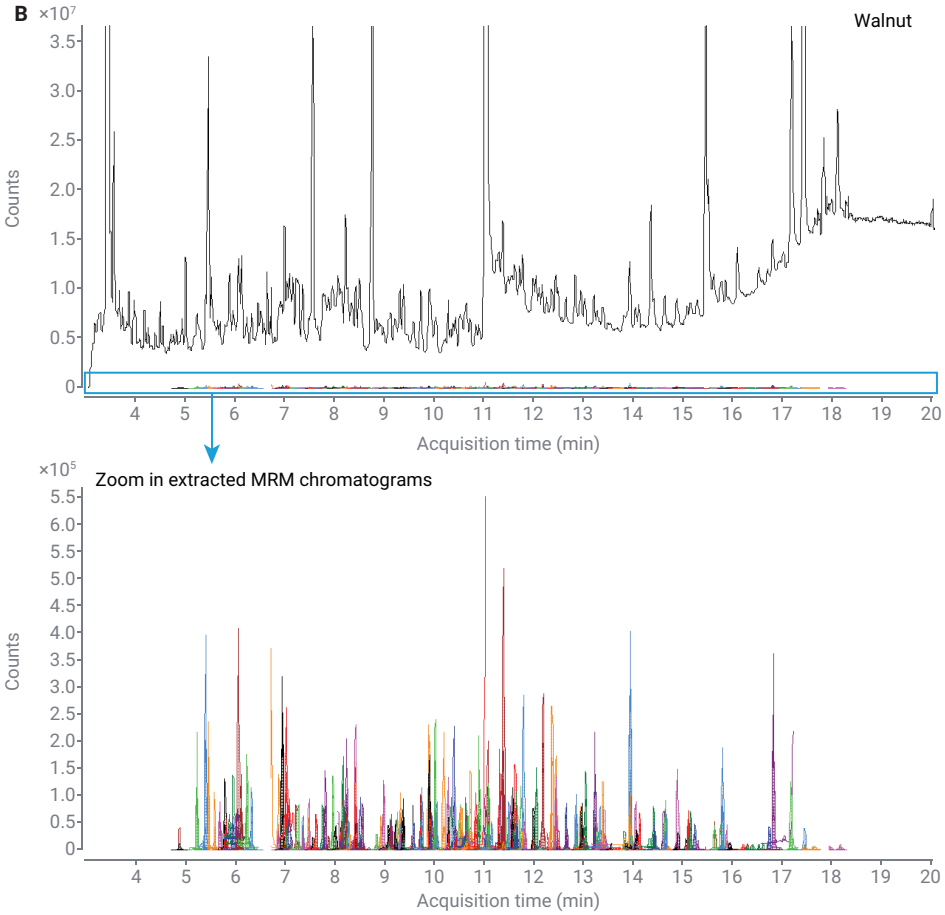

Figure 5B. Scan (on top) and dMRM (magnified on the bottom) TIC acquired in simultaneous dMRM/scan data acquisition mode for walnut extract.

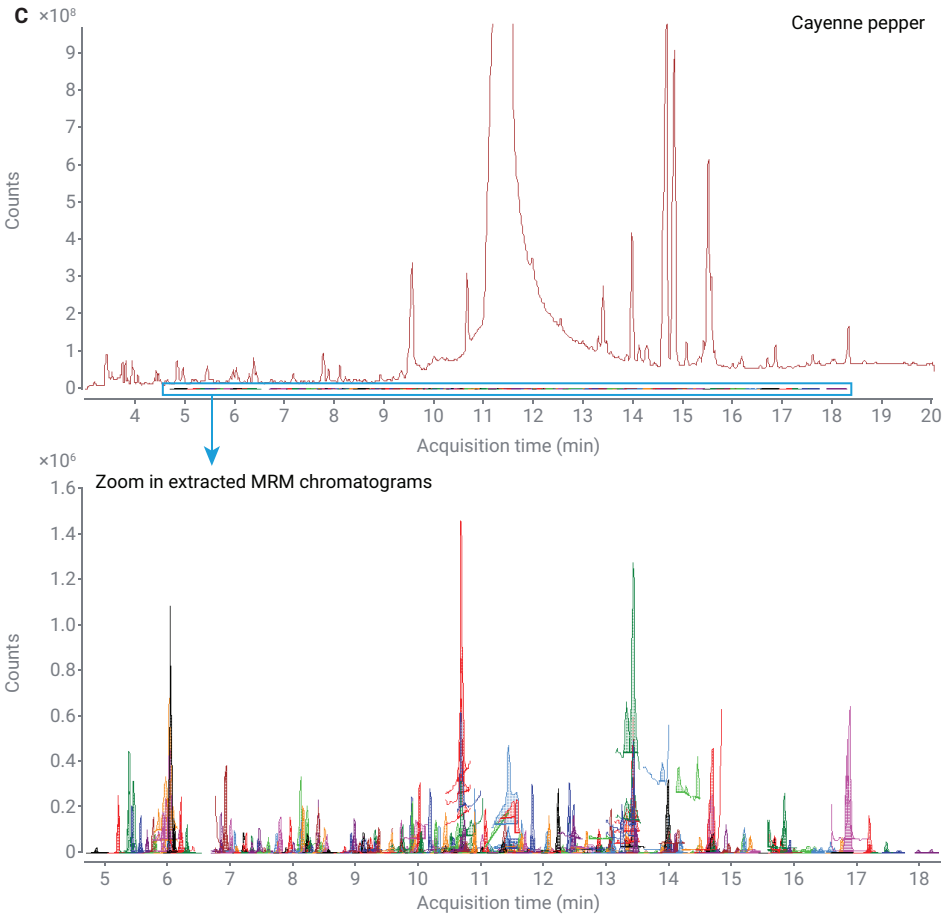

Figure 5C. Scan (on top) and dMRM (magnified on the bottom) TIC acquired in simultaneous dMRM/scan data acquisition mode for cayenne pepper extract.

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.

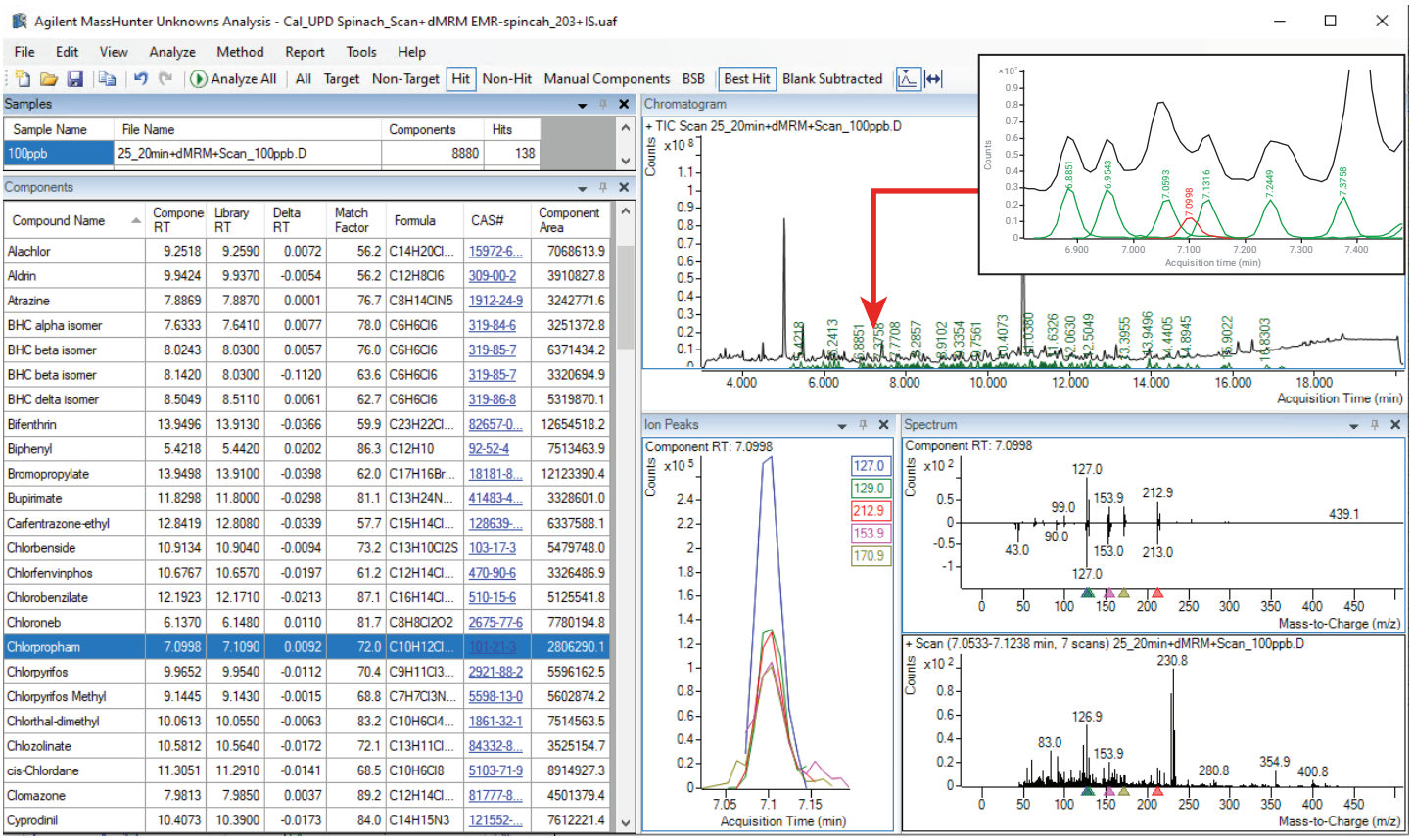

Figure 6. A partial list of search results for spinach extract spiked with a pesticide mixture at 100 ppb against a retention time-locked spectral library. Chlorpropham is selected in the components table and its extracted ion chromatograms and corresponding spectral information are shown on the lower 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

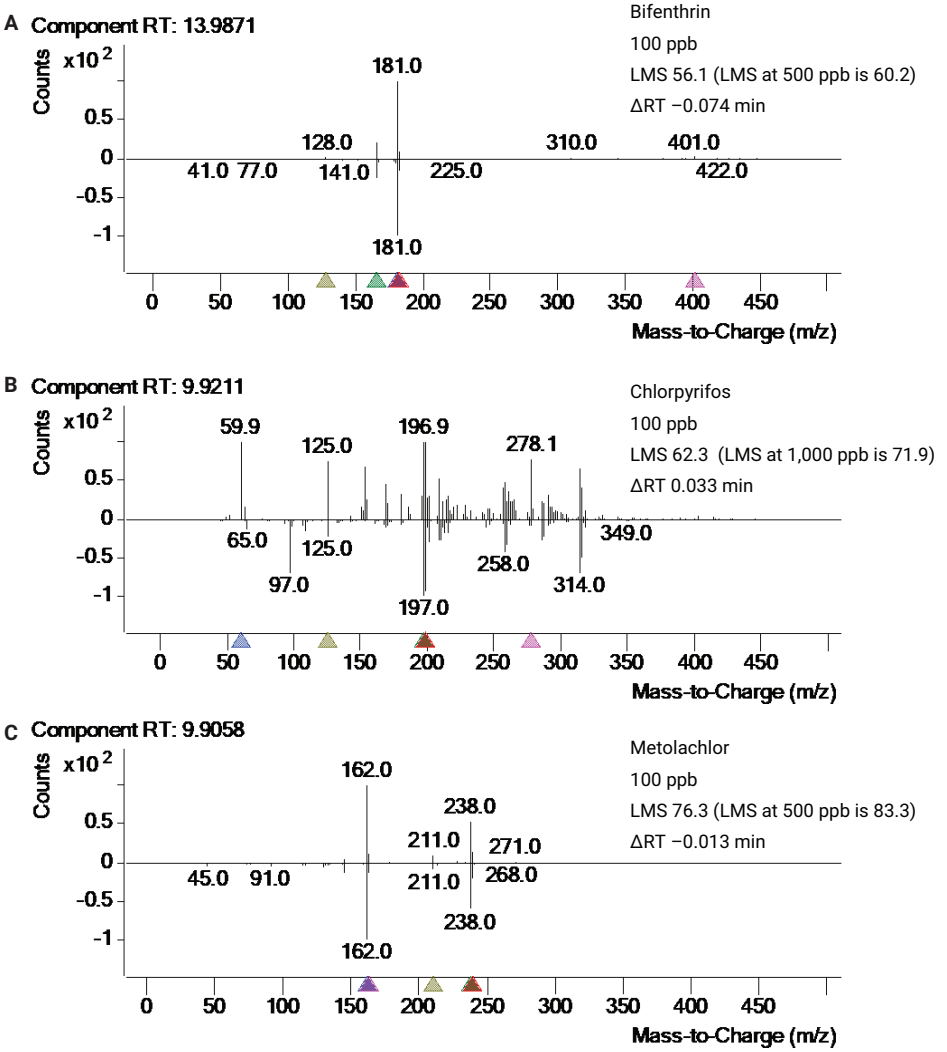

Figure 7. Spectral confirmation with library match score for bifenthrin (A), chlorpyrifos (B), and metolachlor (C) spiked at 100 ppb in cayenne pepper with the Agilent 7000E GC/TQ in simultaneous dMRM/scan data acquisition mode.

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.

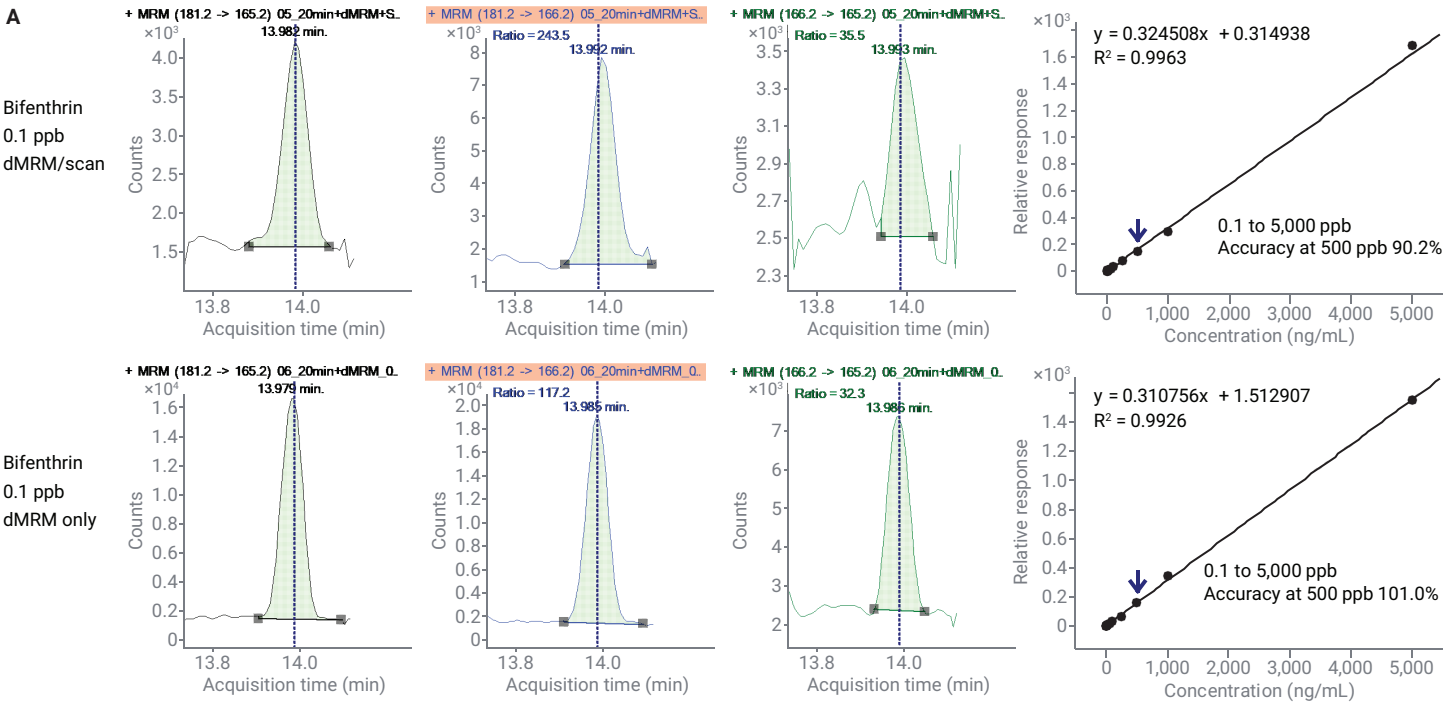

Figure 8A. Quantifier and qualifier ion profiles and matrix-matched calibration curves over 0.1 to 5,000 ppb for bifenthrin spiked at 100 ppb in cayenne pepper with the Agilent 7000E GC/TQ in simultaneous dMRM/scan and dMRM only data acquisition modes.

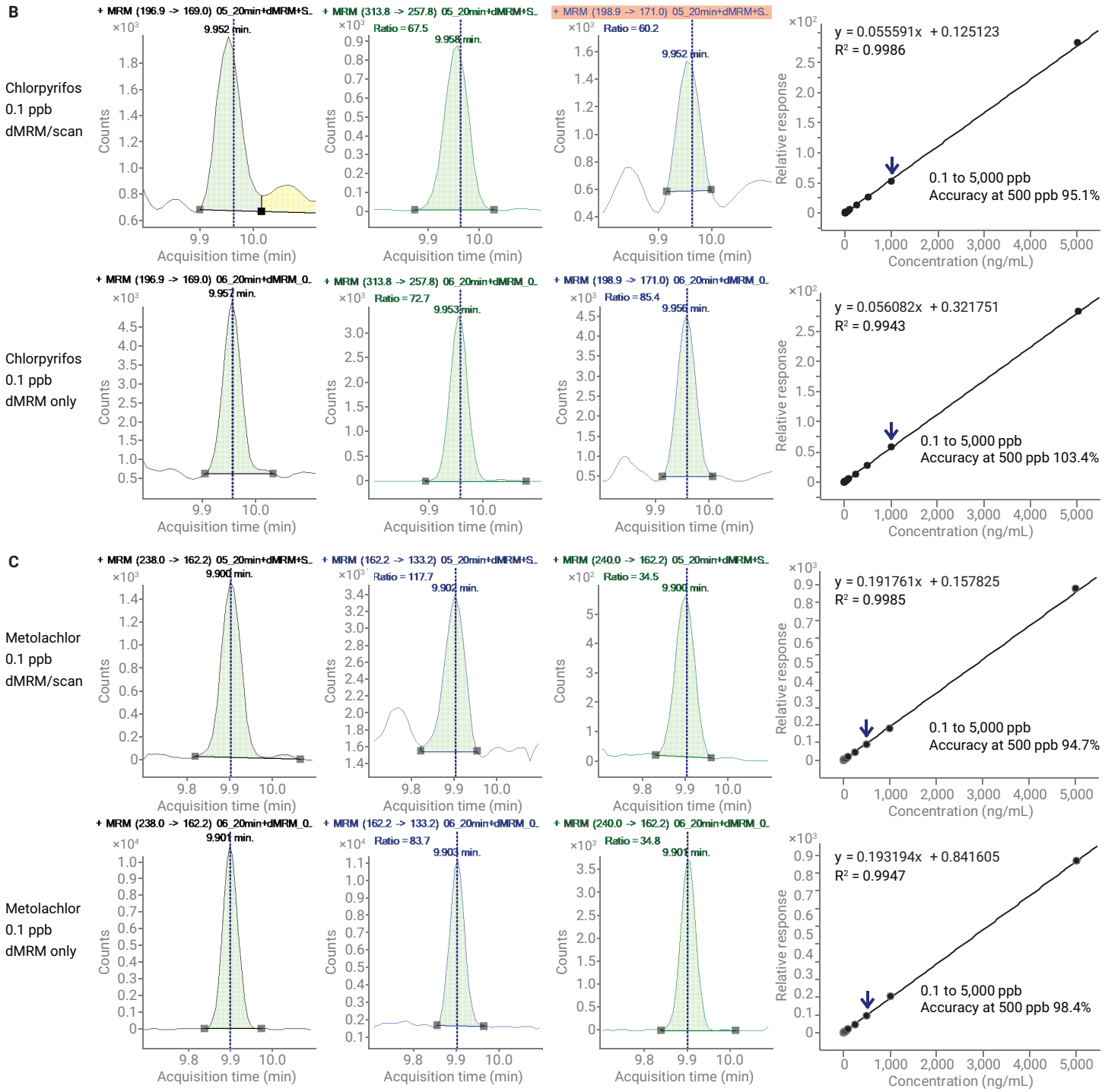

Figure 8B,C. Quantifier and qualifier ion profiles and matrix-matched calibration curves over 0.1 to 5,000 ppb for chlorpyrifos (B) and metolachlor (C) spiked at 100 ppb in cayenne pepper with the Agilent 7000E GC/TQ in simultaneous dMRM/scan and dMRM only data acquisition modes.

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.

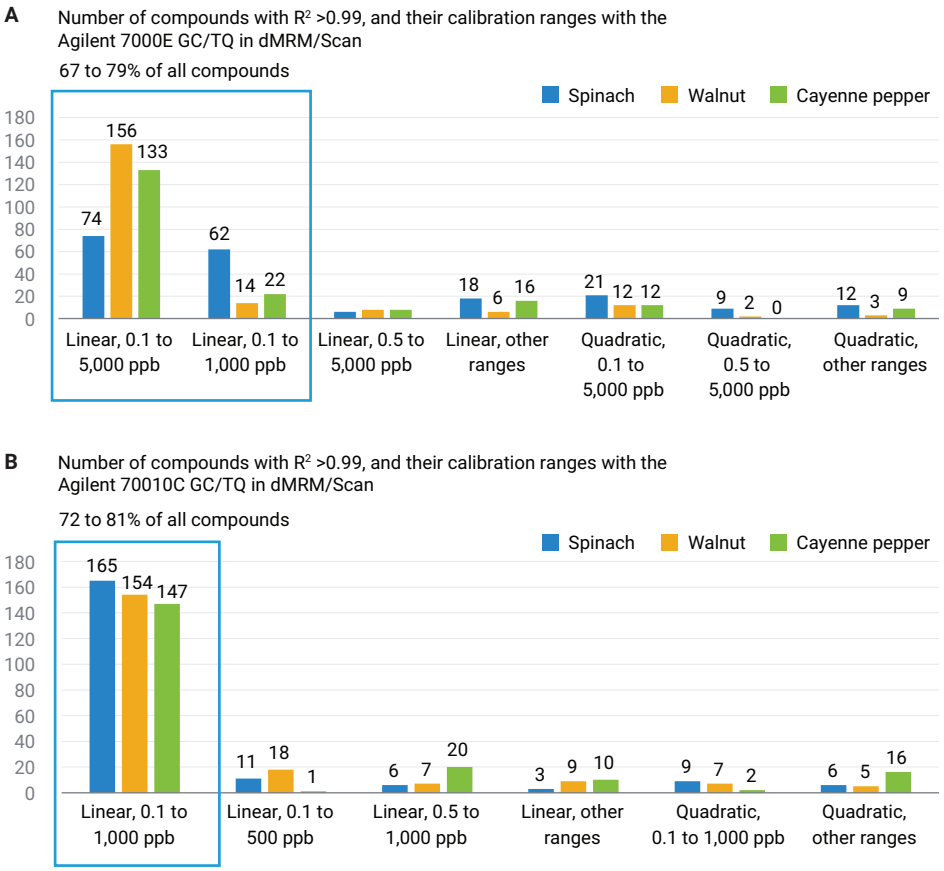

Figure 9. Calibration performance for the 203 pesticides with an Agilent 7000E (A) and Agilent 7010C (B) GC/TQ in spinach, walnut, and cayenne pepper QuEChERS extracts. The graph shows the number of compounds and their calibration ranges.

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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