Application Note

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High-Throughput Pesticide Residue Analysis Using an Agilent Ultivo Triple Quadrupole LC/MS and the MassHunter Productivity App

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Abstract

There are >1,000 pesticides used globally, and food safety laboratories are under pressure to widen the scope of their analytical methods for routine pesticide monitoring. As a result, method development and data analysis have become a time-consuming and labor-intensive task for analytical laboratories. To address this growing issue, Agilent has developed the MassHunter Productivity App, which streamlines routine, high-throughput quantitation workflows in food testing laboratories. This Application Note demonstrates the capabilities of the software for multiresidue screening of 254 pesticides in two matrices, using the Agilent Ultivo triple quadrupole LC/MS.

Introduction

To ensure good agricultural practice and, ultimately, the safety of the food produced, screening and quantitation of pesticide residues is essential. Governmental agencies around the world set maximum residue limits (MRLs), in the parts-per-billion (ppb) range[1], for hundreds of pesticides and their metabolites in food. For practical reasons, not all of these pesticides are screened in products. Risk assessment is used to determine the best scope.

There is increasing concern within governments and industry, that more of these listed compounds should be screened by testing labs. This has prompted efforts to perform pesticide monitoring using faster and more sensitive analytical techniques with broader scope. The increased quantity of data means data analysis has now become the major bottleneck for most control laboratories. The development of integrated and automatic data processing and reporting tools can allow labs to make faster and more accurate decisions when reviewing their data, and speed up delivery of analytical results.

The MassHunter Productivity App has been designed to simplify data collection and data processing for targeted screening and quantitation. Its goal is to save time and increase productivity in routine MS testing laboratories. The software offers features to automate routine and tedious tasks and provides a simple, unified procedure for a laboratory technician to submit a sequence of samples, review the results, and create a report.

This Application Note illustrates the ease-of-use of the MassHunter Productivity App for targeted quantitation of a broad range of pesticide residues in broccoli and strawberry, using data from the Ultivo triple quadrupole LC/MS.

Experimental

Reagents

All reagents and solvents used in this study were HPLC grade. Ammonium formate solution was obtained from Agilent (p/n G1946-85021). Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA). Ammonium fluoride was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was produced with a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid was purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Pesticide standards were obtained from Agilent (LC/MS pesticide comprehensive test mix (p/n 5190-0551)), AccuStandard (New Haven, CT, USA), and Chem Service. Inc (West Chester, PA, USA). The stock solution of pesticides including 254 compounds was prepared at 10 µg/mL concentration, and used for spiking the QuEChERS extracts. The calibration standards ranged from 1 to 100 ng/mL.

Sample preparation

Organic and nonorganic broccoli and strawberry samples were obtained from local grocery stores. The same sample preparation procedure was used for both organic and nonorganic samples. Ten grams of homogenized fruit and vegetable were weighed into 50-mL polypropylene tubes. The sample matrices were then extracted using 10 mL of acetonitrile according to the EN 15662 QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-5650). Broccoli and strawberry extracts were cleaned using an Agilent dispersive SPE (dSPE) kit (p/n 5982-5256).

Equipment

An Agilent 1290 Infinity HPLC coupled to an Ultivo triple quadruple LC/MS system was used for sample analyses. The Ultivo was equipped with an Agilent Jet Stream (AJS) ESI source, and operated in dynamic MRM (dMRM) and polarity switching. Tables 1 and 2 show the HPLC and MS source parameters.

Software

Data acquisition and analysis was performed using the MassHunter Productivity App. To streamline the analysis workflow (Figure 1), this software provides a unified user experience from run setup to data review and reporting.

The MassHunter Productivity app is distributed with a comprehensive pesticide database and acquisition method with 700 compounds (>2,000 MRMs), allowing a novice user to select a desired set of target compounds and perform data acquisition and analysis quickly without any method optimization and development. This database was used to acquire and analyze the data presented in this Application Note.

The acquired data were automatically analyzed by the app using a built-in data analysis function to select correct chromatographic peaks. This option modified target retention times and qualifier ratios in the analysis method based on the calibration sample results in the current run:

- Target retention times (RTs) were automatically determined based on the RT of the largest peak in the top-level calibration sample.
- Target qualifier ratios were automatically determined based on the average of the qualifier ratios across all calibration samples.

Table 1. Agilent 1290 Infinity HPLC.

Parameter	Value
Guard Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 5 mm, 1.8 µm (p/n: 821725-901)
Column	Agilent ZORBAX Eclipse Plus C18 3.0 × 150 mm, 1.8 µm (p/n: 959759-302)
Injection volume	2 µL
Column temperature	45 °C
Flow rate	0.45 mL/min
Mobile phase	A) Water + 4.5 mM ammonium formate + 0.5 mM ammonium fluoride + 0.1 % formic acid B) Methanol + 4.5 mM ammonium formate + 0.5 mM ammonium fluoride + 0.1 % formic acid
Gradient	Time (min) %B 0.00 2 0.50 2 1.00 50 4.00 65 16.0 100 18.0 100 18.1 2 20.0 2
Post run time	4 minutes

Table 2. AJS ESI source parameters.

Parameter	Value
Ion mode	Positive and negative
Scan type	Dynamic MRM
Drying gas temperature	250 °C
Drying gas flow	11 L/min
Nebulizer	40 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Capillary voltage	(±ESI) 3,500 V
Nozzle voltage	(+ESI) 300 V (-ESI) 1,000 V

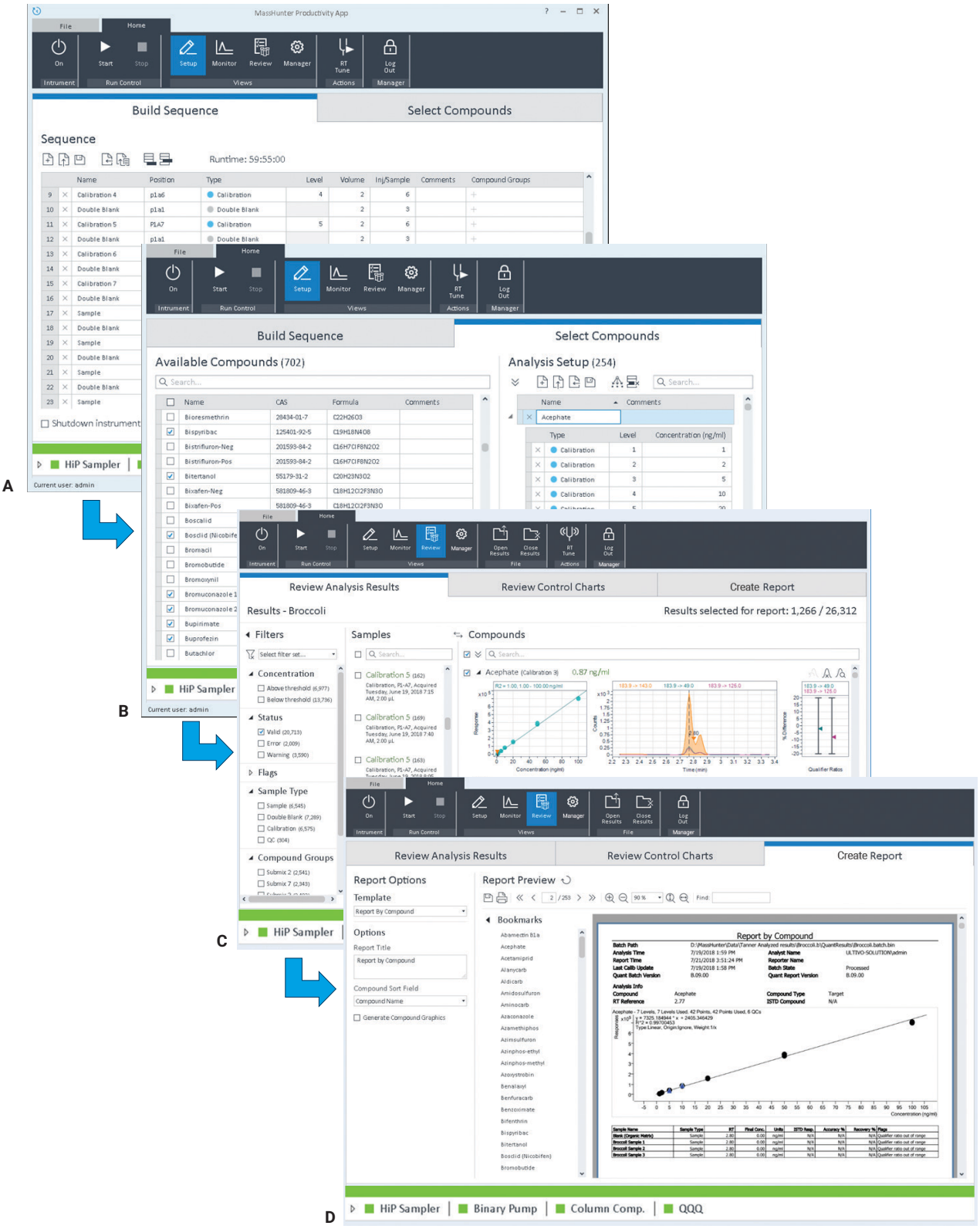

Figure 1. The MassHunter Productivity App streamlines the pesticide quantitation workflow from start to finish. Data acquisition, analysis, and reporting are performed in four steps: (A) setting up sample sequence, (B) setting up target pesticides and analysis parameters, (C) reviewing analysis results, and (D) creating reports.

Results and discussion

Four samples of broccoli, including three nonorganic and one organic, and three samples of strawberry, including two nonorganic and one organic, were examined in this study.

A broad range of 254 pesticides covering several classes were analyzed in the method, and at least two MRM transitions were analyzed per compound. The total run time was 20 minutes.

Matrix-matched calibration standards were prepared at concentration levels of 1, 2, 5, 10, 20, 50, and 100 ng/g, and yielded correlation coefficient values (R^2) >0.99 for most of the pesticides in the broccoli and strawberry matrices. All pesticides were detected at 1/5 MRL concentration. In these cases, accuracy was in the range of 80–120 % for at least four out of six replicates with relative standard deviations of less than 10 %. These values are within parameters of method validation guidelines from the European Union[2]. Figure 2 shows a representative chromatogram of pesticides spiked at 1 ng/g in organic strawberry matrix.

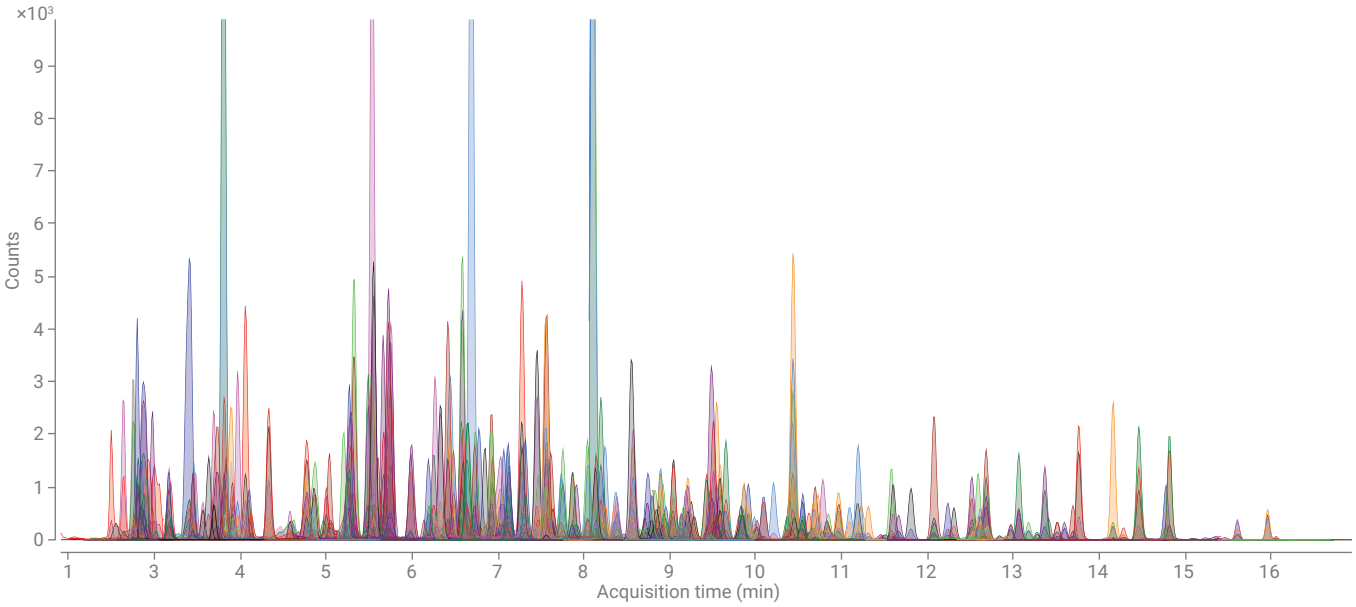

Figure 2. LC/MS/MS chromatogram of more than 250 pesticides in strawberry extract matrix at 1 ng/g.

Data review

Analysis of nonorganic broccoli and strawberry matrices revealed the presence of pesticides, some above their MRLs. A few pesticides were detected in organic matrices of broccoli and strawberry, though never above the 1/5 MRL level (2 ng/g). Table 3 shows the list of pesticides detected in nonorganic samples including results well over MRL values (and beyond calibration range).

Table 3. Pesticides detected in nonorganic broccoli and strawberry samples in positive ion polarity.

Compound	Concentration (ng/g)	Number of replicates (n)	R^2 value	RSD %
Nonorganic broccoli sample1				
Thiamethoxam	1.33	6	>0.998	3.53
Nonorganic broccoli sample 2				
Imidacloprid	4.90	6	>0.992	1.2
Nonorganic broccoli sample 3				
Azoxystrobin	>100	6	>0.998	1.21
Dimethomorph-isomer 1	>100	6	>0.998	0.933
Dimethomorph-isomer 2	>100	6	>0.995	1.06
Mandipropamid	>100	6	>0.998	1.03
Cyazofamid	45.1	6	>0.995	2.23
Bosclid (Nicobifen)	39.8	6	>0.997	1.02
Imidacloprid	15.9	6	>0.992	0.826
Novaluron	7.11	6	>0.991	8.91
Pyraclostrobin	6.82	6	>0.998	1.72
Fludioxonil	3.07	6	>0.994	2.98
Metalaxy	1.58	6	>0.996	2.41
Nonorganic strawberry sample 1				
Abamectin B1	23.4	5	>0.995	3.52
Bifenazate	18.2	5	>0.989	2.25
tetraconazole	7.16	5	>0.981	1.49
Nonorganic strawberry sample 2				
Chlorantraniliprole	27.9	5	>0.996	2.32
Cyprodinil	13.3	5	>0.996	1.02
Trifloxystrobin	13.1	5	>0.996	2.37
Fludioxonil	9.86	5	>0.993	1.42
Spinosyn A	2.44	5	>0.996	4.72
Fenhexamid	2.08	5	>0.961	3.04

The quantitation results were reviewed using the MassHunter Productivity App, which allows filtering results based on concentration, flags, sample types, compound groups, and error status. Each filtered result contains an image of the calibration curve, an extracted ion chromatogram plot, and a qualifier ion ratio plot, facilitating quick review. Figure 3 illustrates an example of using filters for reviewing detected pesticides in a broccoli sample. Applied filters in Figure 3 allowed quick and targeted review of analytes, instead of labor-intensive and time-consuming manual review of the results for all 254 compounds in the sample.

The Productivity App automatically calculates the quantifier to qualifier ion ratio for each compound and flags a compound result if it is not within specified ion ratio tolerance (set to 20 % in this study). Figure 4 shows detected pesticides in a nonorganic strawberry sample. The qualifier fragment ions and their ratios to the quantifier ion peak area were reviewed to verify the identification of Spinosyn D and Tetraconazol.
The qualifier ratio for the compound identified as Tetraconazol fell within the targeted range. However, the qualifier ratio for Spinosyn D was outside of the targeted range, revealing a false positive identification for this pesticide. The user can review chromatograms in detail, and assess potential incorrect identification quickly, in a single interface.

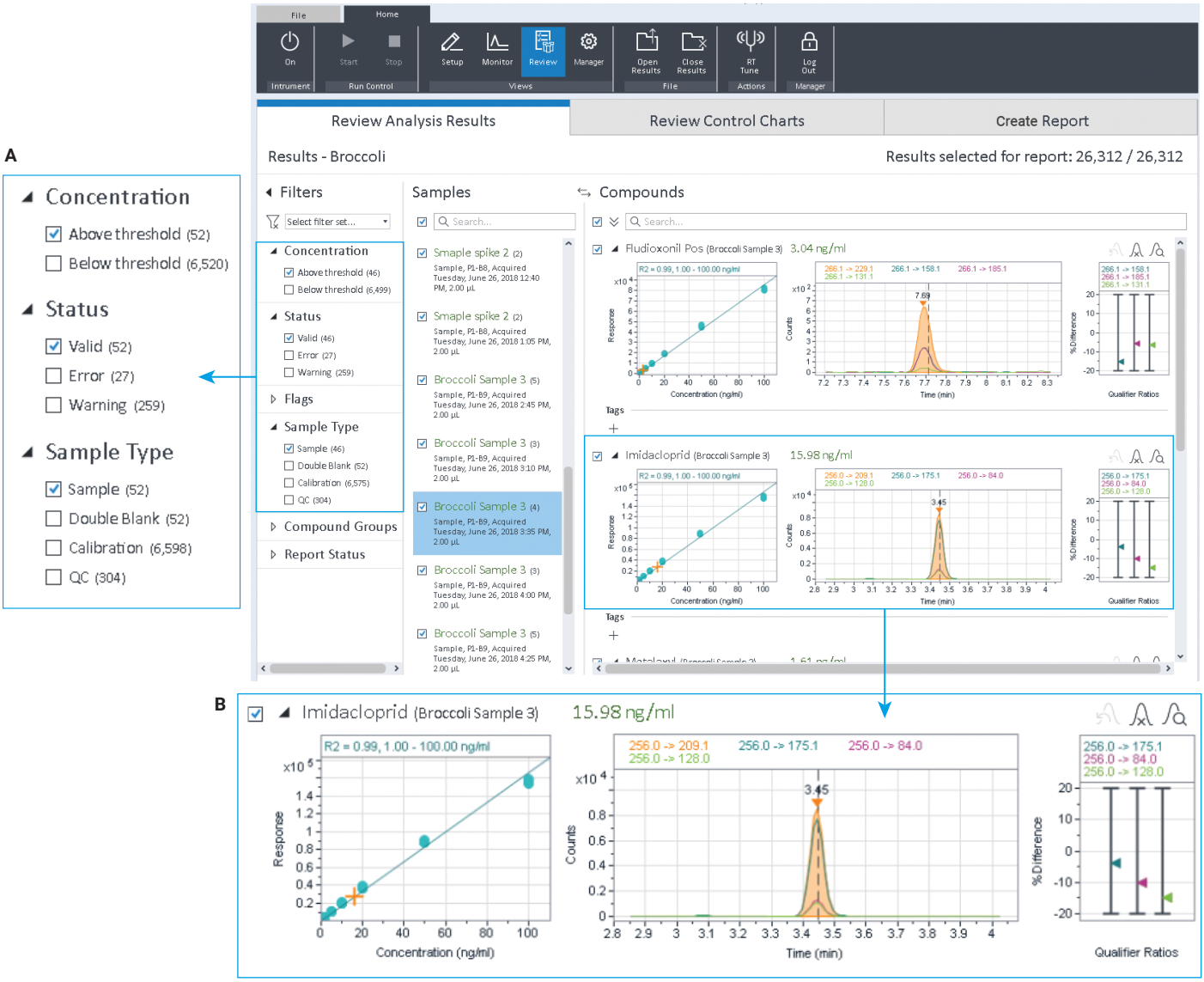

Figure 3. The MassHunter productivity App, showing detected pesticides in a nonorganic broccoli sample. (A) Type of filter used for reviewing results. (B) Calibration curve, extracted ion chromatogram, and qualifier ratio for detected Imidacloprid.

Figure 4. (A) Identified Spinosyn D and Tetraconazol in a nonorganic strawberry sample. (B, C) Overlaid plots of quantifier ion of identified pesticides in nonorganic strawberry and two calibration samples. (B) shows the incorrect RT for the Spinosyn D quantifier ion in nonorganic strawberry compared to two calibration samples (false positive identification). (C) shows the correct RT for standard Tetraconazol in calibration and nonorganic strawberry samples (true identification).

Spinosyn D results were further evaluated by overlaying the quantifier ion chromatograms of the compounds in nonorganic strawberry sample with the calibration samples. For example, a quantifier ion chromatogram of Tetraconazol in nonorganic broccoli sample was overlaid onto two calibration samples with similar RT (Figure 4C). Conversely, the overlaid quantifier ion chromatogram for Spinosyn D in calibration samples and nonorganic broccoli sample shows incorrect identification, and confirmed the false positive identification of Spinosyn D (Figure 4B).

The MassHunter Productivity App can flag analysis results for items that require attention such as accuracy, RT, ion ratio, and R^2 when values are outside of the user-defined limits in the analysis method. This flagging feature helps the user to quickly filter and review data and identify compounds with issues. Another benefit of the flagging feature in MassHunter Productivity App is the capability of quick identification of pesticides above MRL and calibration range. Figure 5 shows results filtered based on concentration above a threshold of 0.5 ng/g, sample type, and compound with a flag of *Above calibration range*. Using these filters, we were able to quickly identify the compounds Dimethomorph and Mandipropamid as compounds with concentrations >100 ng/g in the nonorganic broccoli sample.

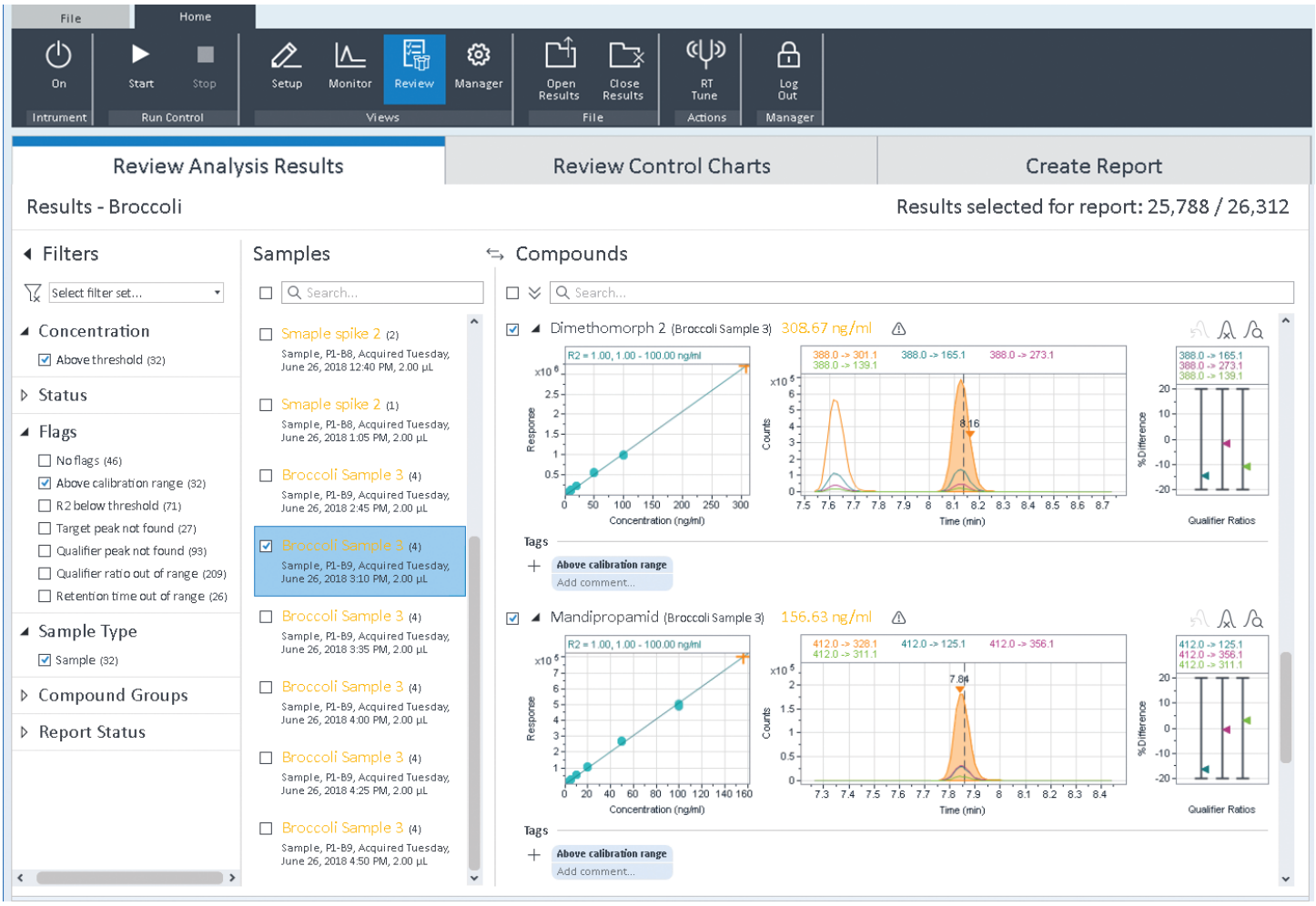

Figure 5. The MassHunter Productivity App enables fast and targeted reviewing of analysis results using filters. Analysis results are shown for detected Dimethomorph and Mandipropamid in nonorganic broccoli sample based on *Above threshold*, *Sample Type*, and *Above calibration range* filters. These two compounds were detected at concentrations above the calibration range (>100 ng/g or ppb).

MassHunter Productivity App enables analyst to have a comparative view for reviewing the analysis results. For example, the user can switch between Samples and Compounds views, and display compound results across all the samples. Figure 6 shows an example of a comparative view for Imidacloprid pesticide in different nonorganic broccoli samples. This feature allows quick comparison of a compound response across multiple samples.

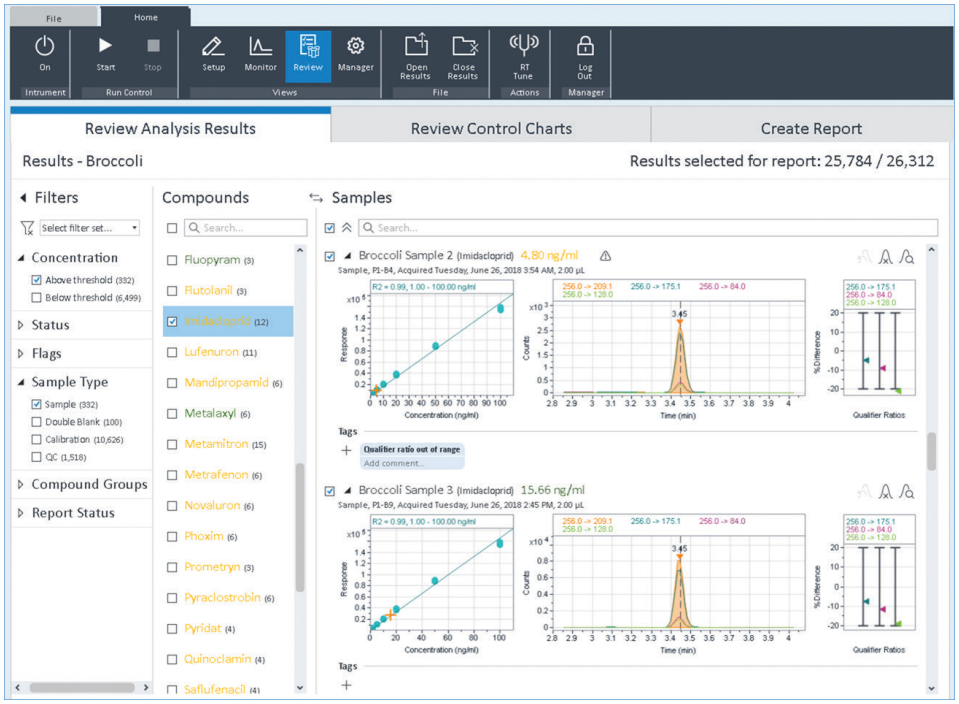

Figure 6. The MassHunter Productivity App provides a customizable user interface for reviewing quantitation results by switching between Samples and Compounds view. Here, results for detected Imidacloprid can be viewed across two different nonorganic broccoli samples in the batch.

Additionally, the MassHunter Productivity App offers a metric plot for compound RT, quality control (QC) concentration, and internal standard (ISTD) response across different samples for easy, quick assessment of data quality and instrument performance. Figure 7 shows an example of a metric plot for QC samples.

Generating reports

Once the data have been reviewed, the MassHunter Productivity App allows users to generate a customized report for the entire batch or for selected results. The default report also displays all tags and associated comments used for data analysis and review. Figure 8 depicts an example of a report generated by the App, showing the pesticides that were detected in nonorganic broccoli samples.

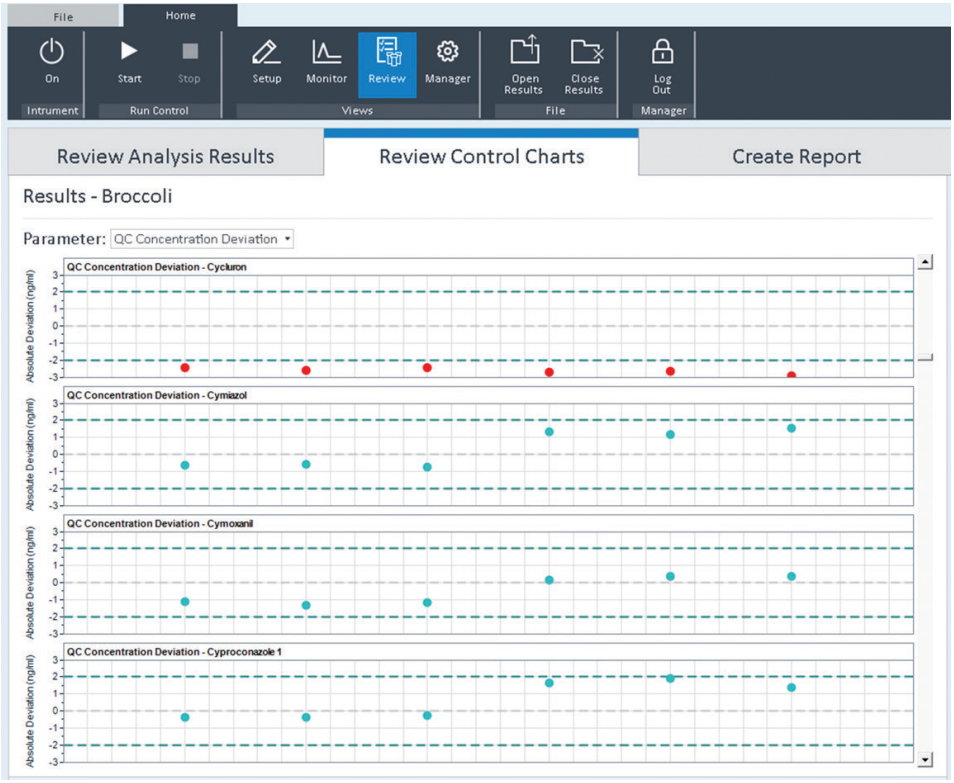

Figure 7. QC concentration deviation chart for each compound in QC samples. Each filled circle represents a QC sample. Blue circles are QC samples with concentration within the predefined limit, and red circles are QC samples with concentration outside of the predefined limit. The dashed vertical lines identify a predefined concentration deviation of ±2 ng/g.

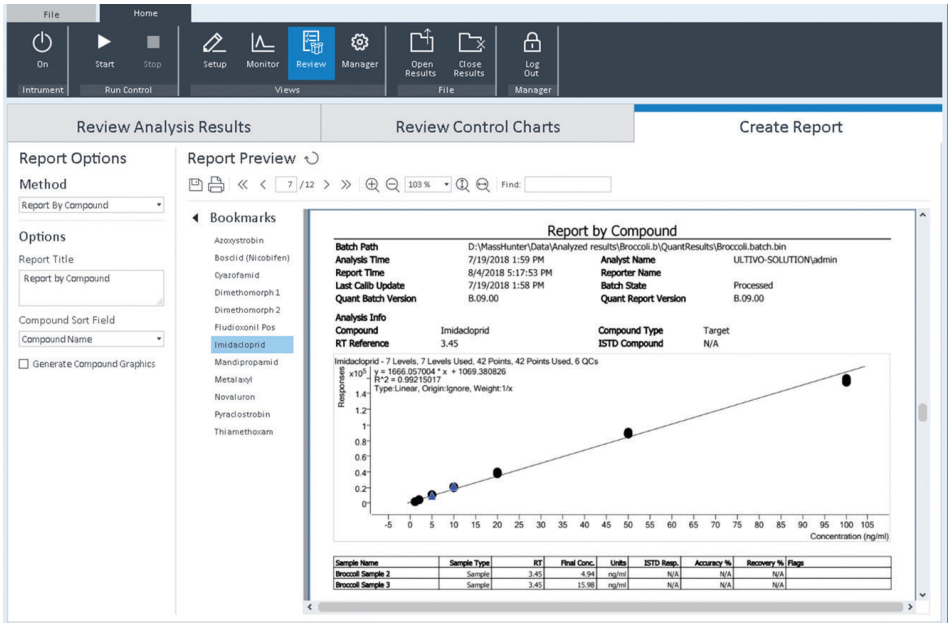

Figure 8. Report preview generated by the MassHunter productivity App based on compound results across different nonorganic broccoli samples. This preview shows a summary of analysis results for Imidacloprid across different samples.

Conclusion

The Ultivo triple quadrupole LC/MS and MassHunter Productivity App enabled high-throughput quantitative analysis of pesticides in complex food matrices. Most of the pesticides were detected in the spiked matrices at concentrations lower than the MRLs. The MassHunter Productivity App enabled faster run setup and data review because it has been purposefully designed for routine analysis in high-throughput laboratories. The Productivity App improves analysis throughput by:

- Streamlining the analytical laboratory workflow from beginning to end using a single, intuitive user interface
- Reducing method development time using the built-in acquisition and analysis methods
- Providing real-time monitoring of the run progress and instrument status
- Automating batch creation and data analysis
- Automating flagging of analysis results
- Offering faster data review using grouping, filtering, and tagging features
- Offering flexible analysis report generation

References

1. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin (including amendments as of 18 March 2008) and complying with regulation (EC) 1107/2009.
2. Guidance document on analytical quality control and method validation procedures for pesticides residues and analysis in food and feed, SANTE/11813/2017, 21-22 November 2017. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf

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