APPLICATION NOTE 65722

A multiresidue method for pesticide profiling using an Orbitrap Tribrid mass spectrometer

Authors: Ed George, Seema Sharma, Scott Peterman, and Richard Fussell

Thermo Fisher Scientific, San Jose, CA

Keywords: Orbitrap ID-X mass spectrometer, Tribrid, pesticide residues screening, non-targeted, high-resolution accurate-mass, Vanquish UHPLC, identification points, part per billion (ppb), Compound Discoverer, TraceFinder, quantitation of pesticide residue, intelligent MS, data dependent acquisition, dynamic exclusion, AcquireX

Goal

Develop a method based on full scan acquisition that enables the detection and quantitation of multiple targeted pesticide residues and simultaneous post-acquisition screening analysis of unknown/unexpected pesticide residues. The resulting method must be able to acquire compound-specific precursor and product ions with sufficient sensitivity, mass accuracy, and resolution to selectively extract ion intensity attributed to compounds of interest. Meeting these requirements will enable accurate quantitation and help to maintain a high degree of reproducibility and robustness for the duration of the study. In addition, the overall LC-MS^n workflow must be easy to use, applicable to most sample types and matrices, and extremely efficient.

Introduction

Pesticides are routinely applied to crops for preventing, destroying, or controlling pest activity. In order to protect consumers and ensure they are not being exposed to pesticide levels harmful to their health, pesticides are regulated in many food sources and several countries have established maximum residue levels (MRLs) or tolerances. Given the large number of pesticides used and the globalization of the food supply, multiresidue methods offer a great advantage, allowing analysis of hundreds of pesticides in a single experiment.

A maximum residue level (MRL) is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly in accordance with Good Agricultural Practice. When a pesticide has been registered on a particular crop, the MRL for the pesticide in/on the crop is usually set at a valued determined from “supervised field residue trials”. However, if a pesticide has not been approved for use on a crop, the MRL can be set at the limit of detection (LOD). In the EU, the default LOD MRL is 0.01 mg/kg.

Liquid chromatography coupled to mass spectrometry (LC-MS) is the preferred method for performing multiresidue analysis of LC-amenable pesticides.[1-4] Traditionally, panels of SRM transitions associated for each pesticide are monitored using triple quadrupole mass spectrometers for rapid and accurate quantitation of targeted pesticides.[2-5] Each method contains a specific set of precursor/product ion mass pairs per pesticide for data acquisition and uses known product ion ratios, internal standards, and/or known retention times for identification and quantitation. While these methods meet the required analytical performance metrics, expanding target panels with additional SRM transitions must be individually evaluated to ensure target selectivity in new matrices. Also, targeted methods cannot detect or identify the presence of unknown/unexpected pesticides, their metabolites, or degradation products.

Recently, high-resolution accurate-mass (HRAM) MS data was used to demonstrate effectiveness for non-targeted pesticide detection and identification in the presence of complex matrices.[1,4,6-8] Instrument methods using full scan HRAM MS acquisition facilitated post-acquisition extracted ion chromatographic (XICs) analysis to detect pesticides with high mass accuracy (±5 ppm). Furthermore, automated tandem mass spectral acquisition routines complemented HRAM MS data by acquiring full scan HRAM product ion spectra, resulting in confident pesticide identification based on measuring at least one compound-specific product ion.[4] (See the EU SANTE Guidelines 12682/2019 or the FDA Guidelines describing the acceptance criteria for residue detection and identification.) The benefit of acquiring full scan HRAM MS^n data enabled retrospective data mining for not only known target compounds, but also suspected compounds.

With the recent introduction of the Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer, a new paradigm of multiresidue characterization and screening has become available.[7] The Orbitrap ID-X mass spectrometer has redefined intelligent MS workflows by implementing a new approach (Thermo Scientific™ AcquireX™ method)[4] to automate the generation of a comprehensive background exclusion list to enhance the detection and identification of pesticide residues at low levels. The AcquireX workflow effectively manages HRAM MS and data-dependent MS^n data acquisition by performing real-time identification of precursor features associated with the matrix and those associated with compounds of interest. This directs the mass spectrometer to acquire full scan HRAM MS/MS (or higher order MS^n spectra) on only the latter. By automating the generation of the exclusion list, the AcquireX workflow maximizes the instrument cycle time to be spent acquiring higher quality precursor and product ion spectra for enhanced confidence in detection, identification, and quantitation.

An AcquireX workflow implemented on the Orbitrap ID-X Tribrid mass spectrometer was evaluated for the analysis of 250 pesticides spiked into strawberry matrix to demonstrate the efficiency of the workflow for identifying the pesticides spiked at different levels. Detection and identification were determined by matching experimentally acquired MS and MS/MS data to validated spectral libraries. The efficiency of the AcquireX workflow was evaluated against a standard data-dependent acquisition and dynamic exclusion method that did not utilize an exclusion list.

Experimental

Sample preparation

Strawberry samples were obtained from a local retail store. Following homogenization using an IKA ULTRA-TURRAX homogenizer (Sigma-Aldrich, St. Louis, MO), strawberry sub-samples were extracted using a QuEChERS approach.[2,7] Briefly, 10 g of sample was weighed into the ready-to-use QuEChERS extraction tubes with 4 g of $MgSO_4$, 1 g of trisodium citrate dehydrate and 0.5 g sodium citrate for buffered extraction. A total of 10 mL of acetonitrile was added and the sample was then mixed using a vortex mixer. Samples were shaken and centrifuged and an aliquot of the supernatant retained for analysis. Matrix-matched standards were prepared by spiking the 250 pesticide standards into the extracted matrices at concentration levels ranging from 0.05 to 200 ng/mL (equivalent to ng/g in the samples). The spiked levels are lower than the reported MRLs for many pesticides on fruit as listed within the CODEX database.

Liquid chromatography

Chromatographic separation was performed on a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system using a Thermo Scientific™ Accucore™ aQ C18 column with dimensions of 100 × 2.1 mm and 2.6 µm particles. Mobile phase A consisted of 98:2 water/MeOH containing 5 mM ammonium formate and 0.1% formic acid. Mobile phase B consisted of 98:2 MeOH/water containing 5 mM ammonium formate and 0.1% formic acid. The flow rate was 300 µL/min and the column temperature was set to 25 °C. Analysis time was 15 min including 3 min equilibration time, and each experiment was performed using a 1 µL injection volume.

Mass spectrometry

All experiments were performed on an Orbitrap ID-X Tribrid mass spectrometer using the AcquireX workflow for automated generation of the background exclusion list and management of data-dependent acquisition (DDA) and dynamic exclusion (DE). The exclusion list was automatically generated from the matrix blank and exported to the data acquisition methods for subsequent analyses. The automatic gain control (AGC) target value was set at 2e5 for the full MS and 5e4 for the MS/MS spectral acquisition. The mass resolution was set to 60,000 (@*m/z* 200) for full scan MS and 15,000 for MS/MS events. All full scan MS/MS spectra were acquired using a Top 7 DDA method with dynamic exclusion implemented. High-energy collision dissociation (HCD) was performed with a stepped collision energy of 20, 40, and 70%. All samples were analyzed using a second mass spectral method using the same DDA/DE acquisition parameters but without the AcquireX generated exclusion list.

Data processing

Full scan data processing was performed with Thermo Scientific™ TraceFinder™ software. Quantitation and reproducibility analysis were performed on the precursor ion with mass extraction tolerance settings of ±5 ppm. The limits of detection were determined based on reproducibly measuring precursor response and the S/N ≥ 3. The limit of quantitation was based on S/N ≥ 10 and the coefficient of variance (%CV) and relative standard deviation (%RSD) were less than 20%. Qualitative data analysis and spectral library matching were performed using Thermo Scientific™ Compound Discoverer™ 3.1 software in which the experimentally acquired product ion spectra were matched against Thermo Scientific™ mzCloud library spectra, and successful matching was based on dot-product correlation coefficients.

Results and discussion

The role of HRAM MS^n analysis in efficient, confident, comprehensive non-targeted pesticide screening

Comprehensive, non-targeted pesticide screening requires data acquisition strategies to generate compound-specific mass spectral data, enabling post-acquisition detection and identification. For non-targeted full scan acquistion LC-MS methods, identification consists of detecting two ions, preferably the precursor and at least one product ion, with high mass accuracy (≤5 ppm). Full scan MS data acquired at resolving powers of 50,000 or greater can eliminate interference from background ions in the matrix, enabling selective extraction of precursor ions for qualitative and quantitative analysis. High resolving power also enables confident isotope detection and more accurate intensity measurements to help discern chemical formula of unknowns. High mass measurement accuracy maximizes confidence in precursor/product ion assignments based on spectral matching routines for known pesticides as well as significantly reducing false positive detections.

The Orbitrap ID-X mass spectrometer is capable of acquiring high resolution MS, MS/MS, and higher order MS^n data while maintaining high mass measurement accuracies (±1 ppm when using internal standard) across a wide dynamic range and for all MS^n spectra. This performance characteristic is critical for implementation of narrow mass extraction tolerance settings to enhance automated processing routines at MRLs or lower levels for unauthorized or unknown toxic compounds. For example, the EU apply a default MRL of 10 ppb (0.01 mg/kg) to pesticide-sample type combinations for which substantive MRLs (those based on field trial data) do not exist.

The AcquireX workflow uniquely facilitates non-targeted pesticide screening studies by automating intelligent MS^n data acquisition. The AcquireX workflow uses two different data acquisition methods, one for LC-MS precursor mapping and the second for LC-MS^n acquisition based on DDA/DE. The workflow automatically creates the acquisition sequence for blanks, matrix background, and samples as well as manages data storage. The workflow first acquires the precursor map of a representative blank matrix, processes the data, and creates the exhaustive exclusion list consisting of precursors and corresponding retention times. The resulting exclusion list is automatically imported into the LC-MS^n method enabling more selective acquisition of tandem MS spectra by real-time data

analysis to bypass matrix peaks. The workflow is easy to set up, amenable to any matrix (provided there are matrix blanks), and can effectively profile large numbers of pesticides across a wide dynamic range.

A stock solution mixture for the 250 pesticides was used to perform spiking levels to mimic different residue concentrations on strawberries. The lowest spiking level used in this is 100-fold lower than the EU default MRL. Figure 1 shows the post-acquisition data analysis for the pesticide ametryn. The resulting quantitation curve is used to evaluate the Orbitrap ID-X mass spectrometer performance and stability over the course of the study using a narrow mass tolerance for precursor extraction and integration. A 1/× weighting scheme was applied to the curve, resulting in a linear regression of 0.99. The inset shows the expanded curve covering the spiking levels of 0.5 to 5 ppb. The coefficient of variance calculated for the three replicate injections at 0.5 ppb was 4.5% and the relative standard deviation was 4.4%.

The parameters used for post-acquisition data processing were ideal for both known and unknown organic compounds due to the unbiased HRAM MS^n data acquired. Evaluating the data showed average chromatographic peak widths around 6 s wide. Despite the narrow peak widths, an average of seven DDA cycles were acquired enabling reproducible area under the curve measurements and acquisition of full scan MS/MS. The resulting data generated a high number of identification points (IPs) measured per compound per spiking level.[9-10] Identification points are achieved based on variance between empirical and reference measurements for chromatographic retention times, precursors, and product ion detection and relative abundance values. For example, mass errors higher than 10 mDa for a precursor scored 1.0 IPs and 1.5 for product ions as compared to mass errors below 2 mDa scoring 2.0 and 2.5 for precursors and product ions, respectively. This criteria has been updated by the EU SANTE guidelines (SANTE/12682/2019) requiring a minimum of a precursor and product ion measured with a mass error ≤5 ppm. Increased confidence is achieved with each additional precursor and product ion. Confident acquisition of product-ion rich tandem mass spectra facilitates spectral matching.

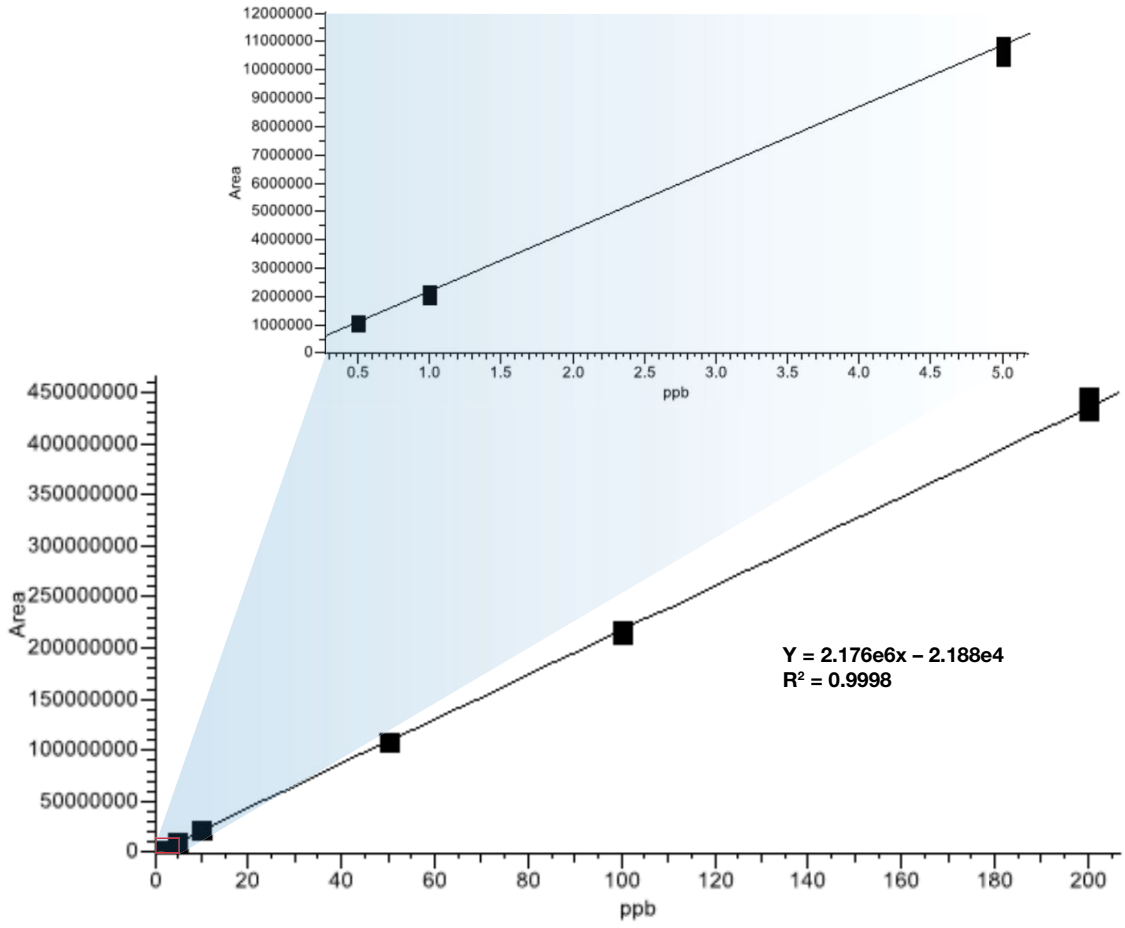

Figure 1. Quantitation curve for the pesticide ametryn in strawberry matrix across the spiking range of 0.5 to 200 ppb. The inset shows the expanded curve at the low end of the spiking range. A mass tolerance of ±5 ppm was used for post-acquisition data extraction, integration, and analysis using an automated processing software routine.

For the current work, a precursor *m/z* extraction tolerance of ±5 ppm was used for all post-acquisition data processing and analyses. For the ametryn example, the mass measurement error was less than 2 mDa consistently measured for all spiked levels. Since a single data processing routine was used for the analysis of all spiking levels, a stable chromatographic and mass measurement accuracy is achieved across the entire study. In addition, full scan product ion spectra were acquired and matched against spectral library entries (examples shown below). Product ion spectral matching utilized ±5 ppm tolerance achieving the highest IP scores per pesticide studied.

Non-targeted pesticide analysis

The detection efficency and quantitative ranges for all spiked pesticides were evaluated using the same automated, post-acquisition data processing workflows defined above. To maintain the quantitative accuracy at the low spiking levels for all 250 pesticides, the full scan detection capabilities of the Orbitrap ID-X mass spectrometer must have a high dynamic detection range to measure the ion signal attributed to the spiked pesticides in the presence of the background matrix. In addition, the full scan mass spectra must be acquried with high resolution to ensure selectivity for target extraction, and the mass measurement accuracy must remain stable across the entire study. Table 1 lists the reproducibility measurements for the three technical replicates at the LOQ for the selected pesticides. The representative 18 pesticides had LOQ levels between 0.5 and 1 ppb and measured %RSDs and %CVs were less than 8%. To provide context, the LOQ for both carbaryl and dinotefuran was 1.0 ppb compared to published Codex MRLs of 800 and 500 ppb, respectively.

Evaluation of the measured LODs and LOQs for all pesticides was performed using the same post-acquisition data processing described above. Automated data processing was performed on all pesticides across all levels and replicates to first determine the linear regression and %CV/RSD per level. The LOD/LOQ levels per pesticide were determined and manually evaluated for S/N determination. Figure 2 shows the pesticide distribution for the respective LOD and LOQ levels as a function of the spiking level. The presented workflow successfully detected and quantified all spiked pesticides at 10 ppb (ng/g) and 96% were detected 100-fold lower. Almost 94% of the pesticides analyzed had LOQ values at the 0.5 ppb (ng/g) level, demonstrating excellent detection and quantitation using HRAM MS data.

Table 1. List of representative pesticides, respective LOQ levels, and reproducible measurements across the three replicates

Pesticide residue	LOQ (ppb)	% Difference injection 1	% Difference injection 2	% Difference injection 3	%RSD	%CV
Ametryn	0.5	-0.07	-6.27	-8.01	4.38	4.48
Carbaryl	1.0	4.14	-6.79	3.43	6.10	5.10
Chloridazon	1.0	-0.13	-3.33	8.17	5.84	5.86
Clomazone	0.5	0.54	4.46	-9.50	7.31	7.24
Cyanazine	0.5	-4.15	-1.42	-1.71	1.54	1.53
Cyazofamid	0.5	-4.48	-4.40	-7.92	2.13	2.01
Dicrotophos	0.5	3.66	2.87	9.13	3.24	3.62
Dinotefuran	1.0	2.14	8.90	1.67	3.88	4.88
Fensulfothion	0.5	1.08	-4.20	5.61	4.87	4.74
Fuberidazole	0.5	-1.24	7.81	1.97	4.46	4.47
Hexazinone	0.5	-5.17	-0.35	-2.01	2.51	2.55
Heptonophos	1.0	-0.37	3.72	-0.37	2.34	2.40
Methabezthiazuron	0.5	3.38	6.83	2.54	2.18	2.13
Metosulam	1.0	-1.21	-1.81	-2.98	0.92	0.72
Ofurace	0.5	6.41	-1.51	-0.11	4.16	4.14
Tebufebpyrad	1.0	1.39	-5.94	6.06	6.02	5.60
Thiabendasole	0.5	1.49	-2.71	-5.99	3.84	3.81
Tricyclazole	0.5	-1.04	-4.31	-2.49	1.68	1.73

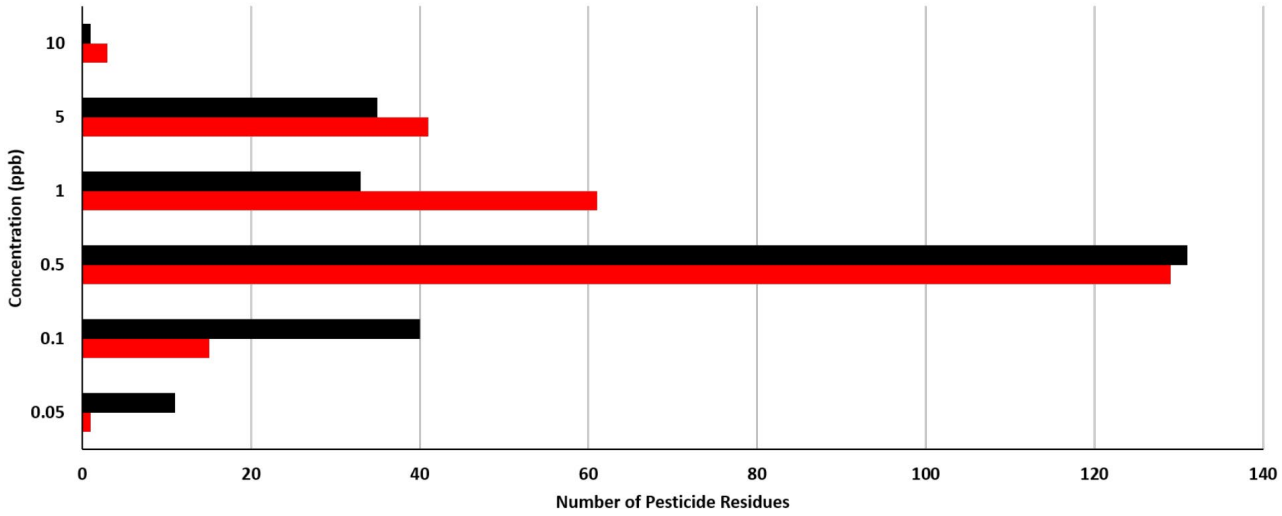

Figure 2. Comparative histogram for the measured LOD and LOQ levels for the pesticides tested. The distribution of pesticides for LOD are shown in black and LOQ in red.

The variance threshold was used as the first criteria for establishing LOD/LOQ for the measured pesticide response with the cutoff set to 20%. Figure 3 shows that all pesticides were measured with acceptable %CVs down to 10 ppb, which meets the EU requirements for pesticides. As further demonstration of the precursor detection capabilities in full scan MS data performance of the Orbitrap ID-X mass spectrometer, reproducible measurements were maintained for 205 pesticides down to 1 ppb.

Increasing unknown pesticide characterization using the AcquireX workflow and automated spectral library matching

The second challenge to effectively screen known and unknown pesticides is to acquire product ion spectra for structural confirmation or characterization. Data-dependent acquisition with dynamic exclusion (DDA/DE) has been routinely implemented to handle unknown targets in the presence of background matrix features. Thus a level of intelligent data acquisition must be performed by the

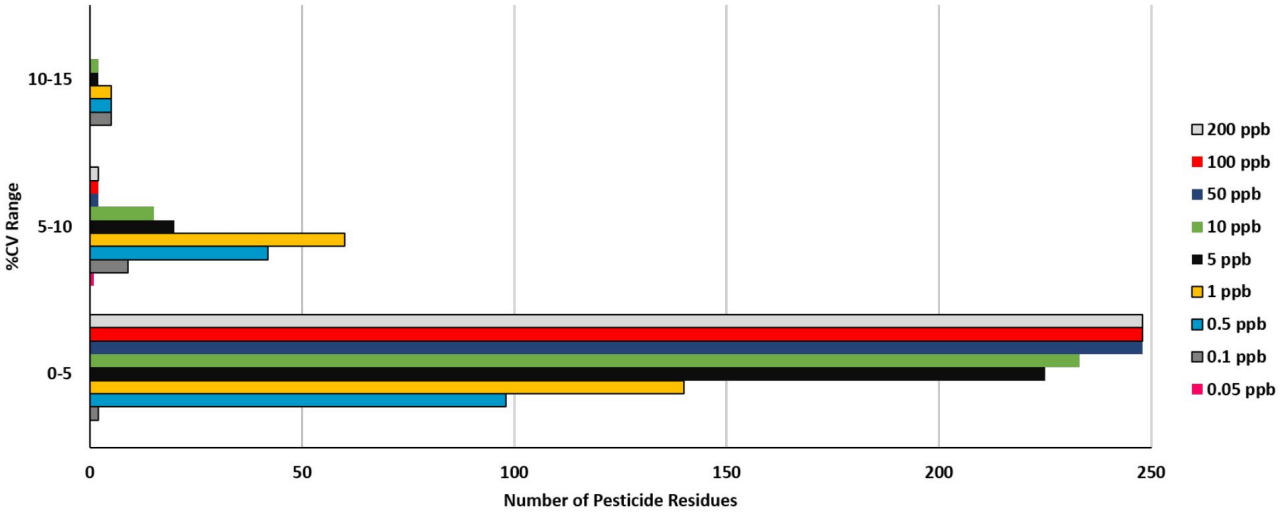

Figure 3. Distribution profile for all pesticides as a function of spiking level based on measured %CV

mass spectrometer to determine which features should be targeted for tandem mass spectral analysis and which ones should be avoided due to the precursor *m/z* value being previously interrogated within the user-defined time. Generally, the DDA/DE method prioritizes the more intense precursor *m/z* values, requiring a greater number of MS^2 spectral acquisitions to overcome matrix precursors to target potential pesticides. This could result in using longer cycle times, reducing the number of HRAM MS data points across the chromatographic peak and potentially compromising relative quantitative accuracy. Another option used to maintain a sufficient number of DDA/DA acquisition cycles utilizes lower precursor resolution settings (30,000) to devote more of the cycle time for DDA MS/MS spectra acquisition. Both options may still be insufficient for comprehensive non-targeted pesticide sampling.

Automating the exclusion list using the AcquireX workflow enhances the intelligent MS^n acquisition, resulting in a greater potential for interrogating primarily features associated with the sample, even at very low spiking levels. Coupling the AcquireX methods with traditional DDA/DE substantially increases DDA/DE efficiency by significantly reducing the number of precursors considered for tandem mass spectral analysis due to the extensive exclusion list. Therefore, implementing a static cycle time for the DDA/DE method can ensure enough full scan MS data points for post-acquisition quantitation as well as manage tandem mass spectral acquisiton through the setting of the AGC target value and maximum ion fill times. The resulting HRAM MS and MS^2 spectra are used to confirm the pesticide structure through spectral library matching or identify putative matches through chemical database matching.

The non-targeted pesticide selection efficiency was evaluated across all spiking levels. The same exclusion list generated at the onset of the study by the AquireX routine was used for all levels. Precursor *m/z* values not on the exclusion list but measured with sufficient intensities were then selected based on DDA/DE routines. Using this approach provides three advantages:

- Bypassing the background matrix features enables lower precursor ion intensity thresholds used to target compounds of interest.
- Increasing the maximum ion fill times enhances product ion spectral quality.
- Maintaining adequate cycle times allows for precursor quantitation.

All of these extend the dynamic range for non-targeted screening and relative quantitative analysis.

The resulting product ion spectra were searched against the mzCloud spectral library using Compound Discoverer 3.1. software. Figure 4 shows four pesticides selected for tandem mass spectral acquistion and the resulting mzCloud spectral library match. The product ion spectra were acquired at the LOQ values. Of particular interest is the quality of the spectra acquired at 0.5 and 1 ppb. The measured product ions contained in the spectral libraries are accounted for as well as the product ion distribution, resulting in a Pearson dot product correlation coefficient of almost 1. In addition, the product ion spectra were measured in the Orbitrap mass analyzer with a resolving power of 15,000 and mass accuracies less than 3 ppm for maximum confidence.

The mzCloud library has the world's largest HRAM LC-MS spectral library and continues to grow weekly. Over 16,000 compounds covering wide chemical diversity support over 17 different small molecule markets. A total of 745 pesticides and herbicides compounds are validated with 1289 ion trees and almost 144,000 spectra. Each compound has product ion spectra acquired at multiple collision energies and at MS^3 and MS^4 stages for increased matching and sub-structural matching routines. In addition, the mzCloud library is used in a unique unknown search strategy to help identify putative unknown structures not in the spectral library.

The spectral library matching shown in Figure 4 underscores the fragmentation and product ion detection efficiency of the Orbitrap ID-X Tribrid mass spectrometer. The empirical data was compared against validated spectral libraries for scoring. As stated above, the greater the number of measured product ions, the greater the IP score. Despite the low spiking level, each product ion spectrum showed at least four product ions with mass errors of ca. 0.4 mDa, each of which contributes 2 IPs per pesticide. In addition, the relative product ion distribution ratios fit additional requirements for increased IPs.

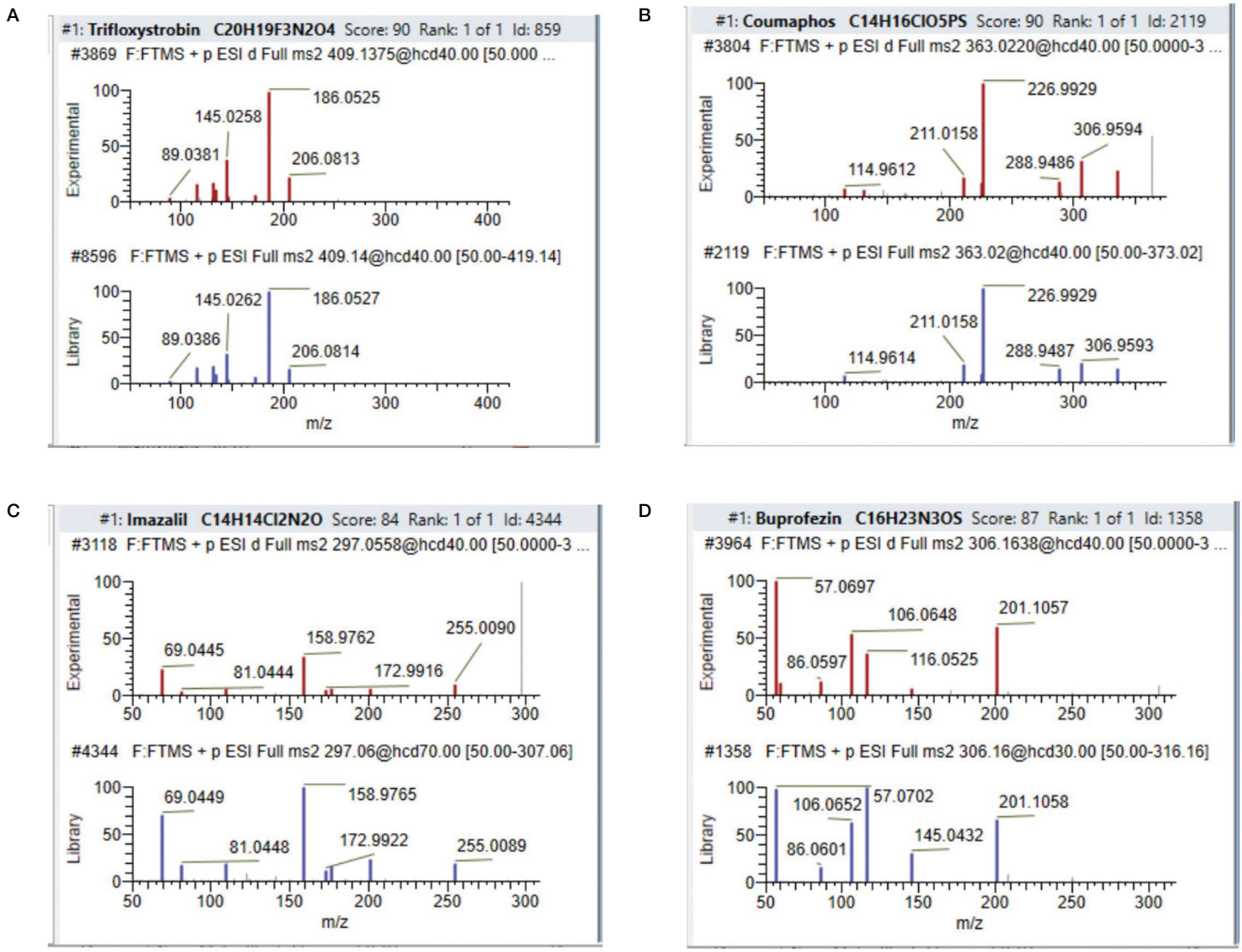

Figure 4. Evaluation of the MS² product ion spectra for four different pesticides and comparative spectral library match. The product ion spectra were acquired at 0.5 ppb for (A) trifloxystrobin and (b) coumaphos and 1 ppb for (c) imazalil and (d) buprofeszin.

Figure 5 shows the success of the intelligent tandem mass spectral acquisition at each spiking level as well as the reproducibility between two replicate studies. As demonstrated above, the resulting product ion spectra acquired per spiked pesticide resulted in a high-quality tandem mass spectrum that could be successfully matched against the reference spectrum for pesticide confirmation. About 92% of the spiked pesticides were interrogated and matched at 10 ppb or less, which is approximately an order of magnitude below the reported MRLs for the pesticides involved in the study. The success of the precursor selection method remains almost 90% down to the 5 ppb level and almost 60% at the 0.5 ppb level. In addition, the second study shows excellent reproducibility at all levels differing only by 1 pesticide at any level without manually creating a targeted precursor inclusion list. Note the success of the automated data acquisition scheme to reproducibly quantify and interrogate 12 and 13 pesticides at the 0.1 ppb level, which is 100-fold lower than the default thresholds established in the EU for pesticides.

The same samples were re-evaluated using the standard DDA/DE methods to assess automated pesticide selection and spectral matching. The loop count and cycle times were identical to that used for the AcquireX workflow, ensuring similar performance on post-acquisition HRAM MS quantitation. The difference is the lack of a comprehensive, automated exclusion list to enhance data-dependent selection of the compounds of interest. Figure 6 shows the comparative results for the two methods. The AcquireX method demonstrated better pesticide interrogation and spectral matching success at every level. The difference in the number of matched pesticides was much greater at the 5 ppb level and lower using the AcquireX workflow, and the standard DDA/DE method did not interrogate any of the spiked pesticides at the lowest spiking level. This was expected as the number of possible precursors surpassing the user-defined triggering thresholds was too great to enable automated selection of the low-intensity precursors associated with the spiked pesticides.

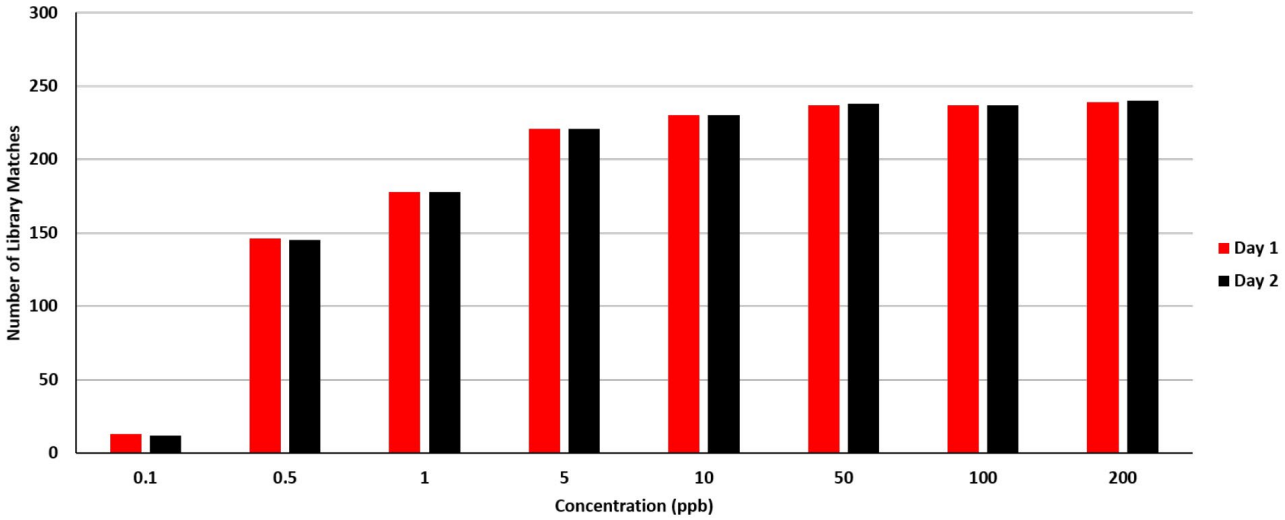

Figure 5. Comparative analysis of the number of pesticides that were confidently matched through mzCloud library searching for the same study on successive days. The entire AcquireX routine was evaluated on each day for the same samples.

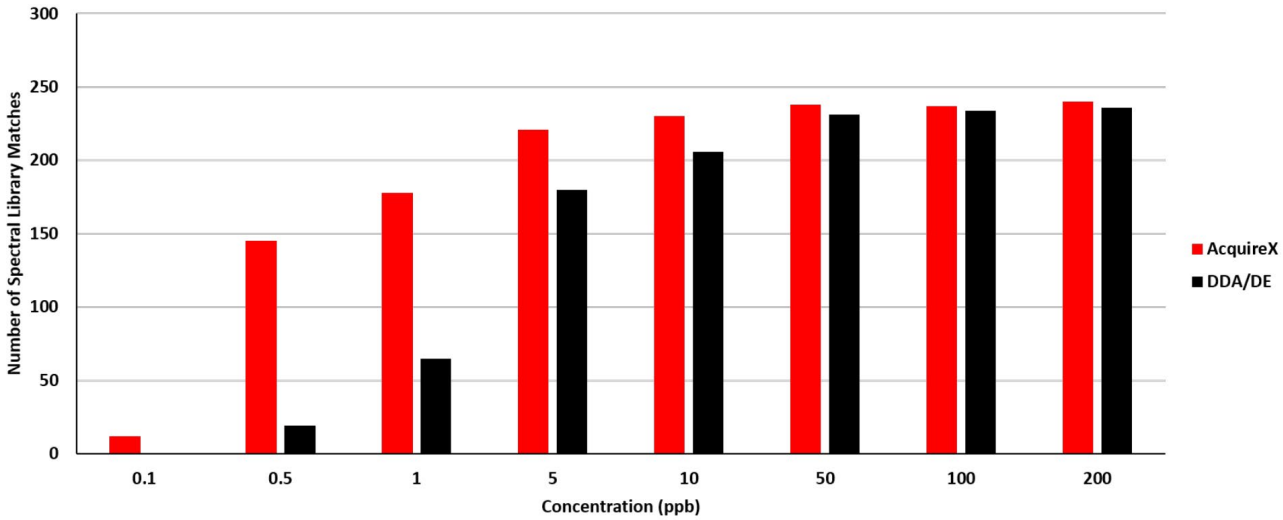

Figure 6. Comparative results for the two methods

thermo scientific

Conclusions

The AcquireX workflow operated on the Orbitrap ID-X Tribrid mass spectrometer presents a new paradigm for non-targeted, multiresidue LC-MS pesticide profiling. The incorporation of intelligent MS routines managed by the AcquireX workflow removes the burden of manually creating inclusion/exclusion lists previously needed to ensure pesticide detection and confirmation at LOD/LOQ levels. The results presented demonstrate the enhanced profiling capability for large numbers of pesticides across a wide range of residue levels substantially lower than existing MRLs. In addition, the HRAM MS^n data analysis in the Orbitrap mass analyzer results in high resolution and mass measurement accuracy for both MS and MS^2, enabling selective and sensitive automated post-acquisition data identification and extraction and processing routines to further enhance the workflow efficiency without sacrificing confidence. By automating the exclusion list generation and implementation, the AcquireX workflow is easy-to-use, amenable to any matrix, and ideal for post-acquisition analysis of known and unknown pesticides or sample-specific compounds.

References

1. del Mar Gomez-Ramos, M. R.-A. Liquid chromatography Orbitrap mass spectrometry with simultaneous full scan and tadem MS/MS for highly selective presticide residue analysis. *Anal. Bioanal. Chem.* **2015,** *407*(21), 6317–6326.
2. Wong, J. Development and interlaboratory validation of a QuEChERS-based liquid chromatography-tandem mass spectrometry method for multiresideue pesticide analysis. *J. Agric. Food Chem.* **2010,** *58,* 5897–5903.
3. Niessen, W. M. Matrix effects in quantitative pesticide analysis using liquid chromatography-mass spectrometry. *Mass Spectrom. Reviews*, **2006,** *25,* 881–899.
4. Ferrer, I. G.-R.-A. Multi-residue pesticide analysis in fruits and vegtables by liquid chromatography-time-of-flight mass spectrometry. *J. Chrom. A* **2005,** *1082,* 81–90.
5. Barbieri, M. V.-A.-A. Analysis of 52 pesticides in fresh fish muscle by QuEChERS extraction followed by LC-MS/MS determination. Sci. of the Total Environment, 653, 958-967.Hernandez, F. I. (2004). Comparison of different mass spectrometric techniques combined with liquid chromatography for confirmation of pesticides in environmental water based on the use of identification points. Anal. *Chem.* **2019,** *76,* 4349–4357.
6. Martinez-Dominguez, G. R.-G. Multi-class methodology to determine pesticides and mycotoxins in green tea and royal jelly supplements by liquid chromatography coupled to Orbitrap high resolution mass spectrometry. *Food Chemistry* **2016,** *197,* 907–915.
7. Kalli, A. S. A multiresidue method for pesticide analysis using an Orbitrap Tribrid mass spectrometer and automatic background exclusion. American Society of Mass Spectrometry. Atlanta, GA: *J. Am. Soc. Mass Spectrom.* **2019.**
8. Shi, F. G. Application of a high resolution benchtop quadrupole-Orbitrap mass spectrometer for the rapid screening, confirmation, and quantification of illegal adulterated phosphoidesterase-5 inhibitors in herbal medicines and dietary supplements. *J. Chromatography A* **2014,** *1344,* 91–98.
9. Koplin, D. W. Response to comment on “pharamceuticals, hormones, and other organic wastewater contaminants in U. S. streams, 1999-2000: a national reconnaissance”. *Environ. Sci. Technol.* **2002,** *36*(18), 4007–4008.
10. Hernandez, F. I. Comparison of different mass spectrometric techniques combined with liquid chromatography for confirmation of pesticides in environmental water based on the use of identification points. *Anal. Chem.* **2004,** *76,* 4349–4357.

Find out more at **thermofisher.com**

ThermoFisher SCIENTIFIC