High Efficiency, Broad Scope Screening of Pesticides Using Gas Chromatography High Resolution Orbitrap Mass Spectrometry

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Introduction

Pesticides are used globally to improve the production and yields of agricultural crops and their use is essential to ensure a sufficient global food supply. However, this widespread use of pesticides and the potential for them to remain in the final product is of significant concern to consumers and to governments whose responsibility it is to ensure a safe food supply. Consequently, legislation exists to protect consumers from exposure to contaminated foods. This legislation requires that foods are monitored for both the type and quantity of the pesticide present, with each pesticide given a maximum residue limit (MRL) in a particular sample commodity. The list of compound and sample combinations is extensive, creating a challenge for accurate and reliable routine monitoring.

Laboratories are under ever-increasing pressure to screen samples for pesticides in a single analysis, with a fast turnaround time and at a competitive cost. Most existing laboratories rely on targeted analytical approaches using both gas chromatography and liquid chromatography coupled to mass spectrometry instrumentation. These techniques cover the wide range of chemical classes that need to be monitored and at the required levels of sensitivity and selectivity. However, they are limited to only those compounds in the target list, which are usually selected based on the residue definition and legislation requirements to demonstrate that the food is fit for consumption. These techniques require careful optimization of acquisition parameters for each compound and the monitoring of acquisition time windows to ensure detection of the analyte.

To increase the scope of the analysis, chemical screening methods using high-resolution, full-scan mass spectrometry have received significant attention in recent years. These methods use non-targeted acquisition, in which a generic full scan acquisition is run, followed by targeted data processing of a list of compounds within a database.

Although data interrogation is performed against a list of target compounds, retrospective data analysis is possible in order to identify new compounds that were not screened for at the time of acquisition. For this approach to be used in routine analysis, screening data processing software needs to be fast and accurate enough to detect residues at low concentrations with an acceptably low level of false negative results, as described in the European Union guidelines.[1] There is no recommendation for the number of false positives, but it is necessary for routine laboratories to keep this number as low as possible to minimize the time required for additional investigation. The majority of samples that pass through a laboratory are compliant with the legislation. Therefore, it is efficient to quickly screen compliant samples from those that are suspected to be contaminated. Following an initial screen, the suspect positive samples are reanalyzed using a second confirmatory method (e.g., GC-MS/MS) to confirm suspect positives and to accurately determine the concentration of the pesticide present. The confirmatory analysis contains a complete calibration series in an appropriate matrix that is not included in the screening analysis.

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In this study, we evaluate the performance of the Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer (MS) for the accurate screening of GC-amenable pesticides. The Q Exactive GC Orbitrap MS provides high mass resolving power up to 120,000 (*m/z* 200) full width half maxima (FWHM) to facilitate highly accurate mass measurements and to enable confident discrimination of co-eluting and isobaric compounds in complex samples. Fast scan speeds and a high intrascan dynamic range (>5000) facilitate the detection of trace compounds in the presence of high matrix components.

Experimental Conditions

Sample Preparation

Food and feed samples were extracted following an acetate buffered QuEChERS-based approach. Briefly, 10 mL of acidified (1% acetic acid) acetonitrile was added to 5 g (cereals/feed) or 10 g (fruit/vegetables) of homogenized sample. A mixture of salts was added and the centrifuge tube shaken and spun. The final acetonitrile extracts (0.5 or 1 g/mL in acetonitrile) were fortified with a mixture of 55 pesticides at concentrations corresponding to 0.5–100 ng/g (ppb). A variety of difficult sample matrices were studied including wheat, leek, and horse feed.

Instrument and Method Setup

In all experiments, a Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler and chromatographic separation was obtained using a Thermo Scientific™ TRACE™ 1310 gas chromatograph (GC) and a Thermo Scientific™ TraceGOLD TG-5SilMS™ 15 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N: 26096-1301).

Additional details of instrument parameters are displayed.

GC and Injector Conditions	
TRACE 1310 GC Parameters	
Injection Volume (µL):	1
Liner:	Asymmetric baffled (P/N: 45352062)
Inlet (°C):	75
Inlet Module and Mode:	PTV, cold splitless
PTV Transfer delay (min):	1
Injection time (min):	0.1
Transfer rate (°C/sec):	2.5
Transfer temperature (°C):	300
PTV Transfer time (min)	3
Cleaning rate (°C/sec):	330
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Temperature 2 (°C):	180
Rate (°C/min)	25
Temperature 3 (°C):	300
Rate (°C/min)	100
Hold Time (min):	3

Mass Spectrometer Conditions	
Q Exactive Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source(°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (Da):	50–500
Resolving power (FWHM):	60,000
Lockmass (*m/z*):	207.03235

The Q Exactive GC system was operated in EI full scan mode using 60,000 (FWHM *m/z* 200) resolving power. Additional experiments were run at different resolution modes of 15K, 30K, and 120K. Chromatographic data was acquired with a minimum of 11 points/peak to ensure consistent peak integration.

Data Processing

Data was acquired and processed using the Thermo Scientific™ TraceFinder™ software. This single software package integrates instrument control, method development functionality, and qualitative-screening and quantitation-focused workflows.

Results and Discussion

The objective of this study was to screen for a wide range of pesticides in different sample matrices with the highest level of confidence. The aim of the analysis was to determine if a pesticide is present in a sample above the lowest MRL, which is typically 10 ppb. This assessment was made by screening fortified wheat, horse feed, and leek extracts spiked at different concentrations to determine their limits of detection for screening under the conditions described. These matrices were selected because they are known to be highly complex and challenging matrices for pesticide analysis, as is shown in the total ion chromatograms in Figure 1.

The sample extraction techniques used in routine pesticide analysis are very generic (e.g., QuEChERS) and produce highly complex and variable solutions. The lack of selectivity in sample preparation stages has to be made up for by selectivity in the instrumental analysis. This selectivity can be achieved using high mass resolving power and high mass accuracy. As sample types increase in complexity, the resolving power of the mass spectrometer becomes a key factor in reliable pesticide detection. This resolving power has already been demonstrated for the analysis of LC-amenable pesticides.[2] Furthermore, high-resolution, full-scan analysis increases the scope of the analysis without the need for optimization of the acquisition parameters.

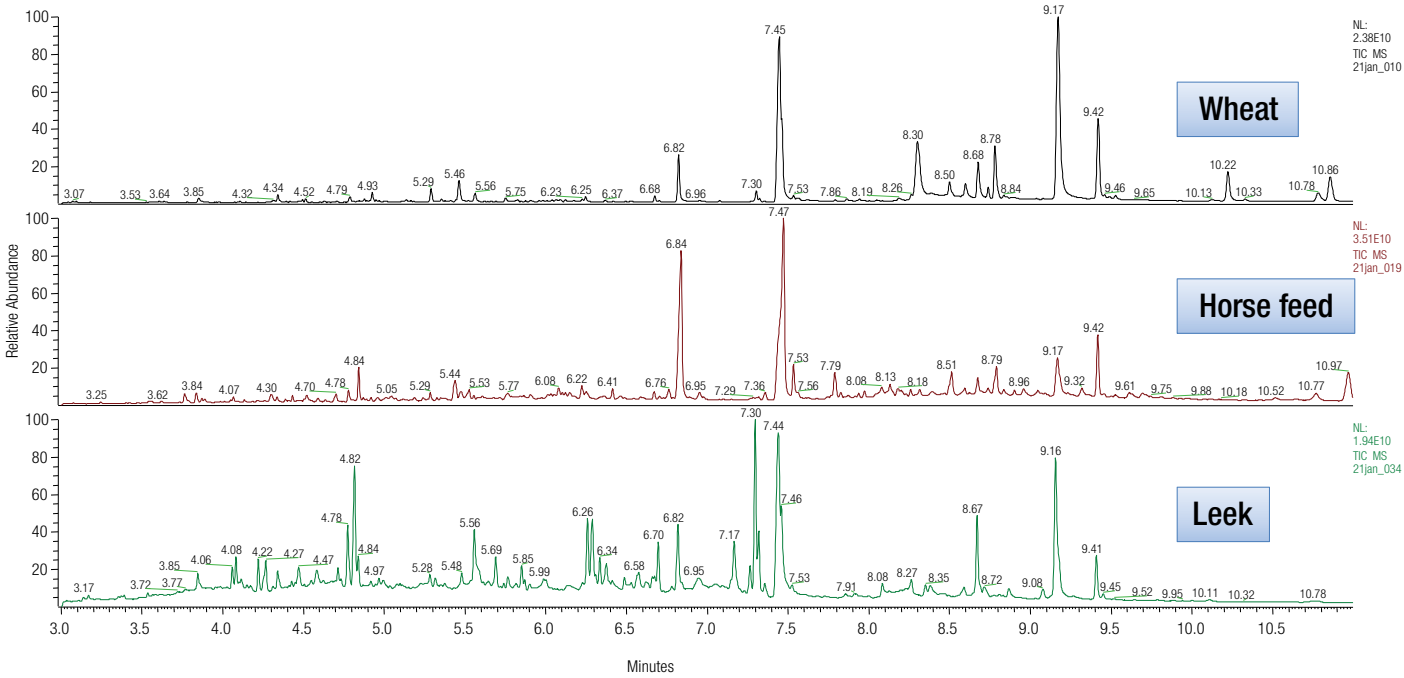

Figure 1. Full scan total ion chromatogram (TIC) for 55 pesticides spiked into wheat, horse feed, and leek extracts showing the complexity of the samples.

Sample Throughput

Sample throughput is a key consideration in pesticide analysis. As such, a fast chromatographic method was used to test the system under typical conditions. This method resulted in a complete analysis within 17 minutes (injection to injection), enabling up to 84 analyses to be performed within a 24 hour period. Although this is a fast GC method, the scan speed of the mass spectrometer provided a minimum of at least 11 points/peak. Figure 2 shows the peak for diazinon with 11 points across the 1.8 second peak.

Screening

Following full scan analysis at a mass resolution of 60,000, TraceFinder software was used to process the data. An in-house database of 183 pesticides, containing information for formula, accurate mass, retention time, isotopic pattern (via formula of diagnostic ion), and fragments was used to screen the samples. Although all parameters can be used for identification, the criterion used by the software for a positive identification was that a peak must be observed in the extracted ion chromatogram (XIC) of the main diagnostic ion at the expected retention time within ± 20 seconds, and the exact mass of the ion should be within ± 2 ppm of the theoretical value.

Pesticide detection can be confirmed by assessing the retention time and mass accuracy of the fragment ions as well as the isotopic pattern fit. The inclusion of these parameters increases the confidence in the detection and reduces the number of false positives.

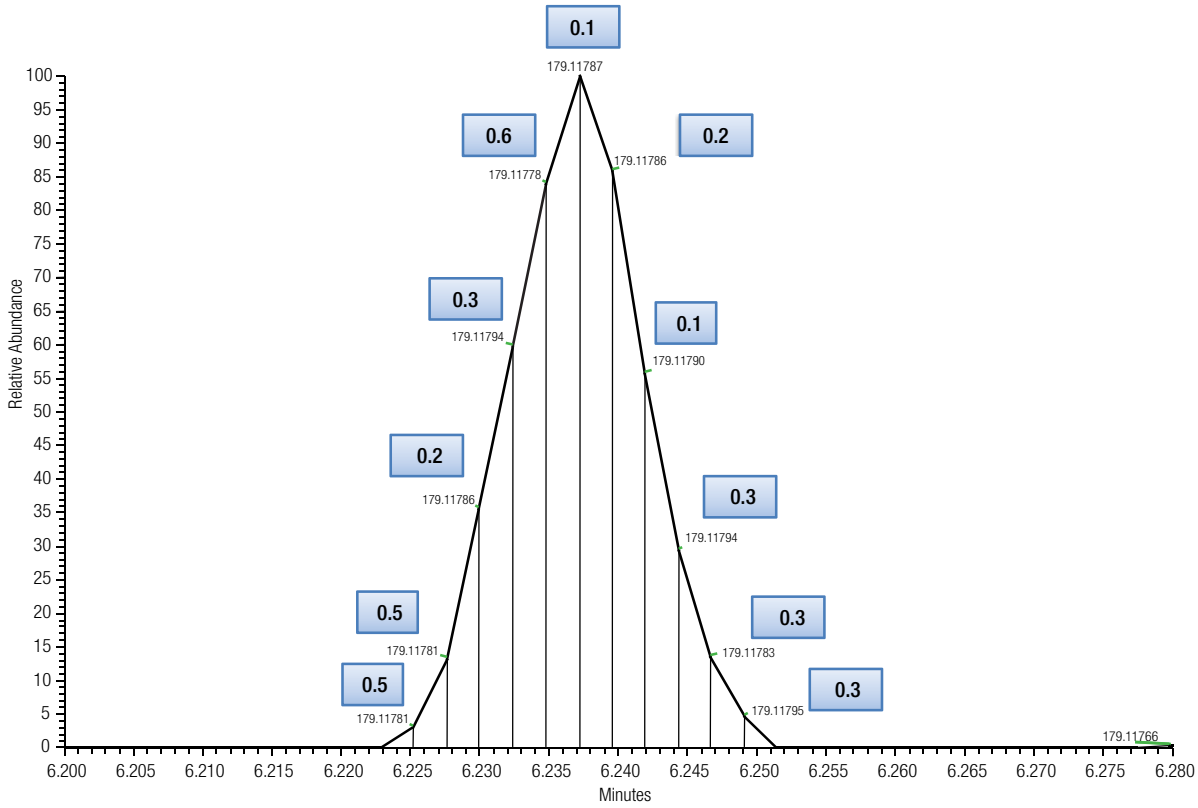

Figure 2. Extracted ion chromatogram (XIC) of diazinon (*m/z* 179.11789 ± 5 ppm mass window) in wheat spiked at 10 ng/mL showing ~11 scans/peak (peak width 1.8 sec). Data acquired in full scan at 60,000 FWHM at *m/z* 200 resolving power. Excellent accurate mass is shown for each individual scan as well as mass difference (in ppm). Average mass difference of 0.3 ppm across the peak.

Screening Software

The processing software is critical to the successful implementation of routine screening. TraceFinder software was used to quickly screen the data for the presence of the target pesticides. A target compound database was used to detect and report the pesticides found and to indicate which criteria were satisfied. Figure 3 shows an example TraceFinder browser window for some of the detected pesticides in wheat spiked with 10 ng/mL. The pesticide p,p'-DDT, which has been detected and confirmed based on retention time, accurate mass (0.21 ppm), fragment, and isotopic match is highlighted in the summary window. The data is displayed to the user in a traffic light system that enables quick review of the data. More detailed information is available in the summary columns and in the window panes, showing in this example the XIC and the measured and theoretical isotopic pattern for p,p'-DDT. The exceptional accurate mass provided by this system, even in complex matrices, enables compounds to be detected with a high degree of confidence. All pesticides are screened at < 2ppm and, as shown in Figure 3, the accurate mass is typically sub ppm. This specificity of accurate mass for both the main diagnostic ions and fragments enables the false detects to be screened out automatically or quickly assessed by the user.

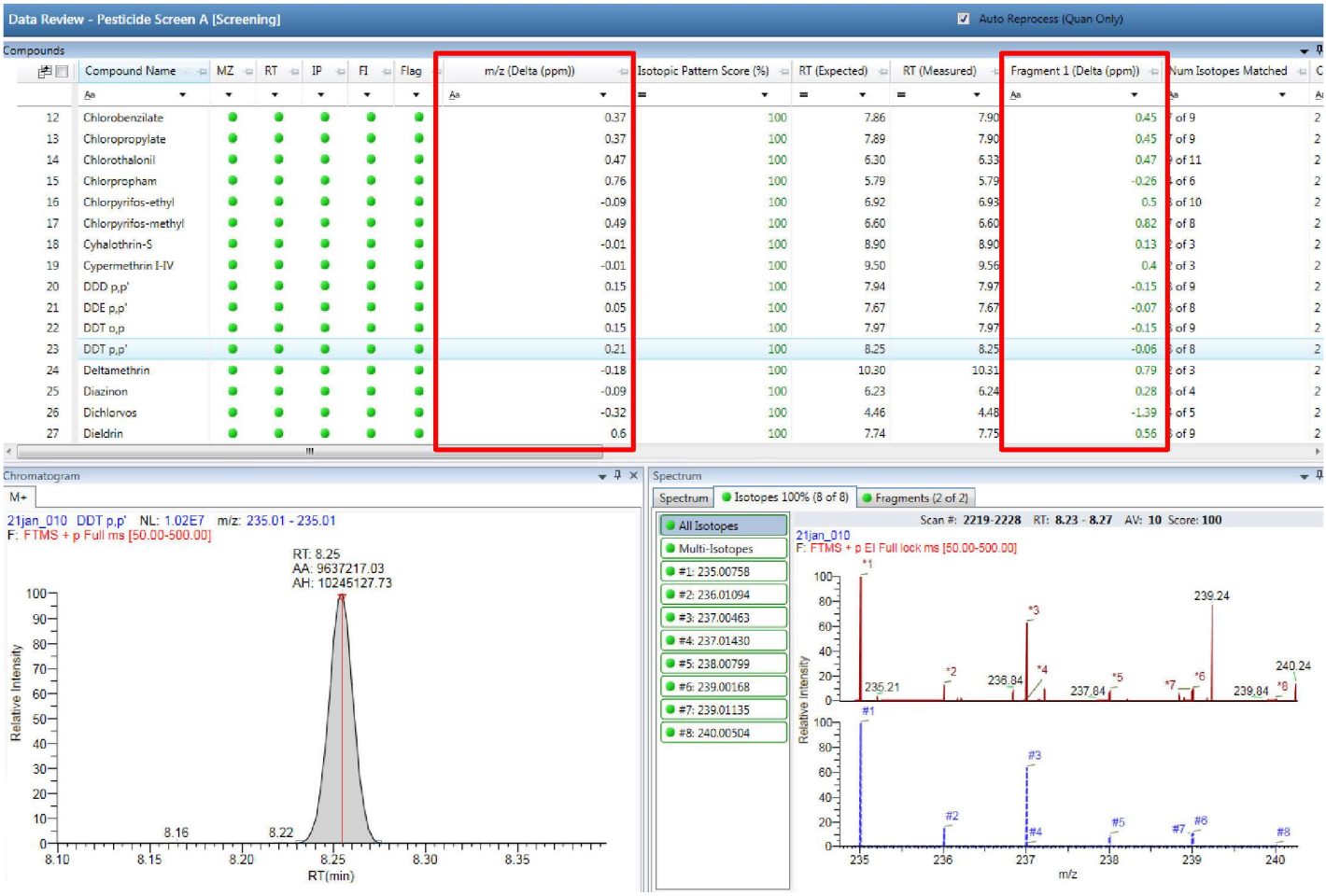

Figure 3. TraceFinder screening browser showing positively identified pesticides (p,p'-DDT as an example) in wheat spiked at 10 ng/mL, based on accurate mass confirmation (± 2 ppm mass window), retention time (RT), isotopic pattern (IP), fragment ions (FI). Sub-ppm mass accuracy for both main and confirmatory ions is highlighted in red boxes.

Screening Below MRL

In this study, all 55 pesticides were detected in the wheat, horse feed, and leek samples when spiked with 10 ng/mL. The majority of pesticides were detected at much lower concentrations. As shown in Figures 4 and 5, 53 pesticides were detected at a concentration of < 2.5 ng/mL in wheat matrix with 47 detected in the 0.5 ng/mL spiked extract. This excellent sensitivity in complex matrices makes confident screening at, or even below, the MRL a unique feature of the Q Exactive GC system.

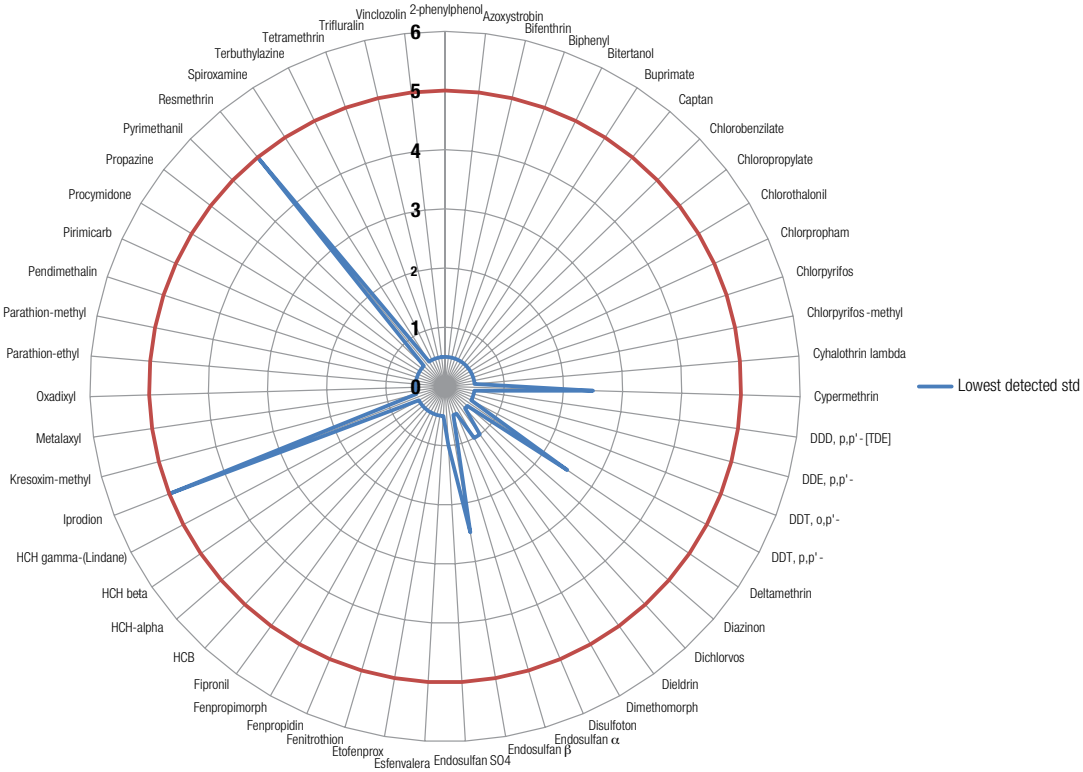

Figure 4. Graph showing the lowest detected standard for 55 pesticides in wheat. Identification based on accurate mass < 2ppm and retention time ± 20 seconds. 5 ng/mL level displayed.

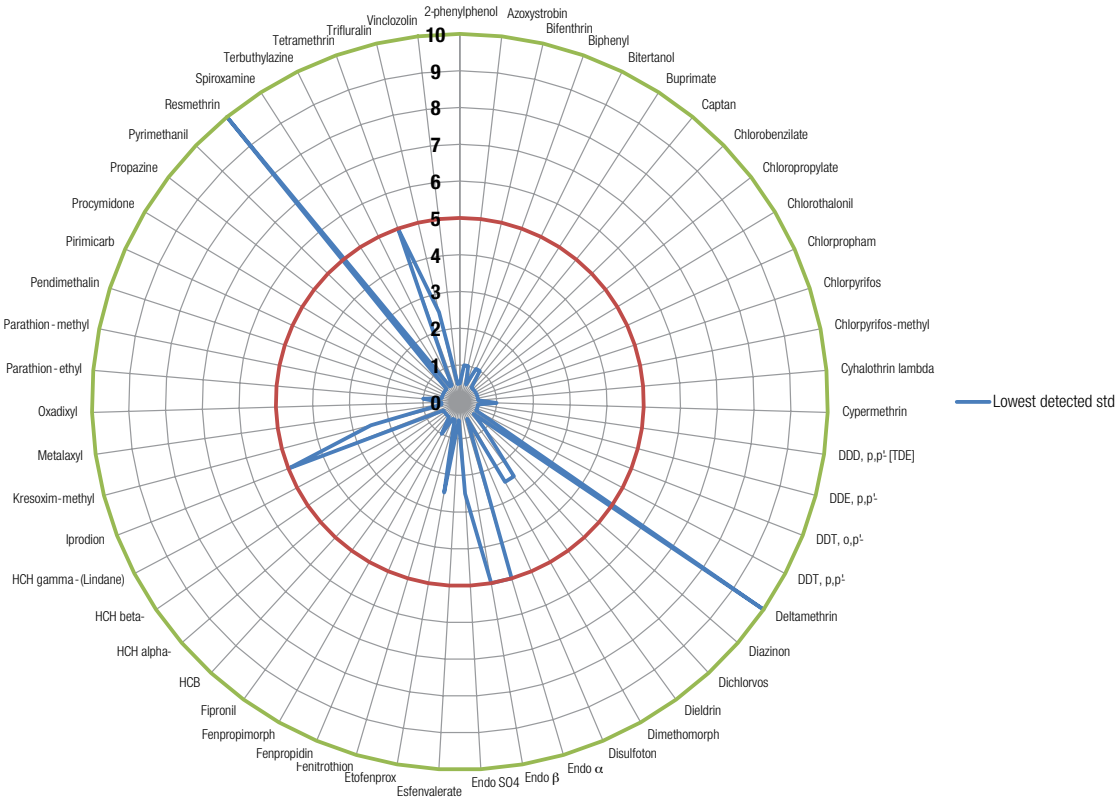

Figure 5. Graph showing the lowest detected standard for 55 pesticides in horse feed. Identification based on accurate mass < 2ppm and retention time ± 20 seconds. 5 ng/mL and 10 ng/mL levels highlighted.

Avoiding False Negatives Using Resolving Power

The use of a narrow mass accuracy tolerance is possible only when the resolving power is sufficient to isolate target compounds from matrix interferences or other target compounds. When two mass profiles overlap, the measured mass profile is the sum of the two individual profiles. This summed profile results in the incorrect assignment of the mass of the target compound. This phenomenon is demonstrated in Figure 6, where the leek sample was analyzed four times at resolving powers of 15K, 30K, 60K, and 120K. The mass spectra show a diagnostic ion of chlorpropham and a background matrix ion at a similar mass, resulting in interference. The expected mass accuracy was achieved at 60K and 120K with near baseline resolution. However, at 15K and 30K, chlorpropham was not resolved from the interference, resulting in poorer mass accuracy. At 15K, the mass accuracy is significantly affected with a value of 18.4 ppm mass difference. Under the screening criteria used in this study, and even under a wider tolerance of 10 ppm, this peak would have resulted in a false negative for chlorpropham. This result shows that a minimum resolving power is needed. The required minimum resolving power depends on the complexity of the sample being analyzed and the concentration of both target analytes and interferences.

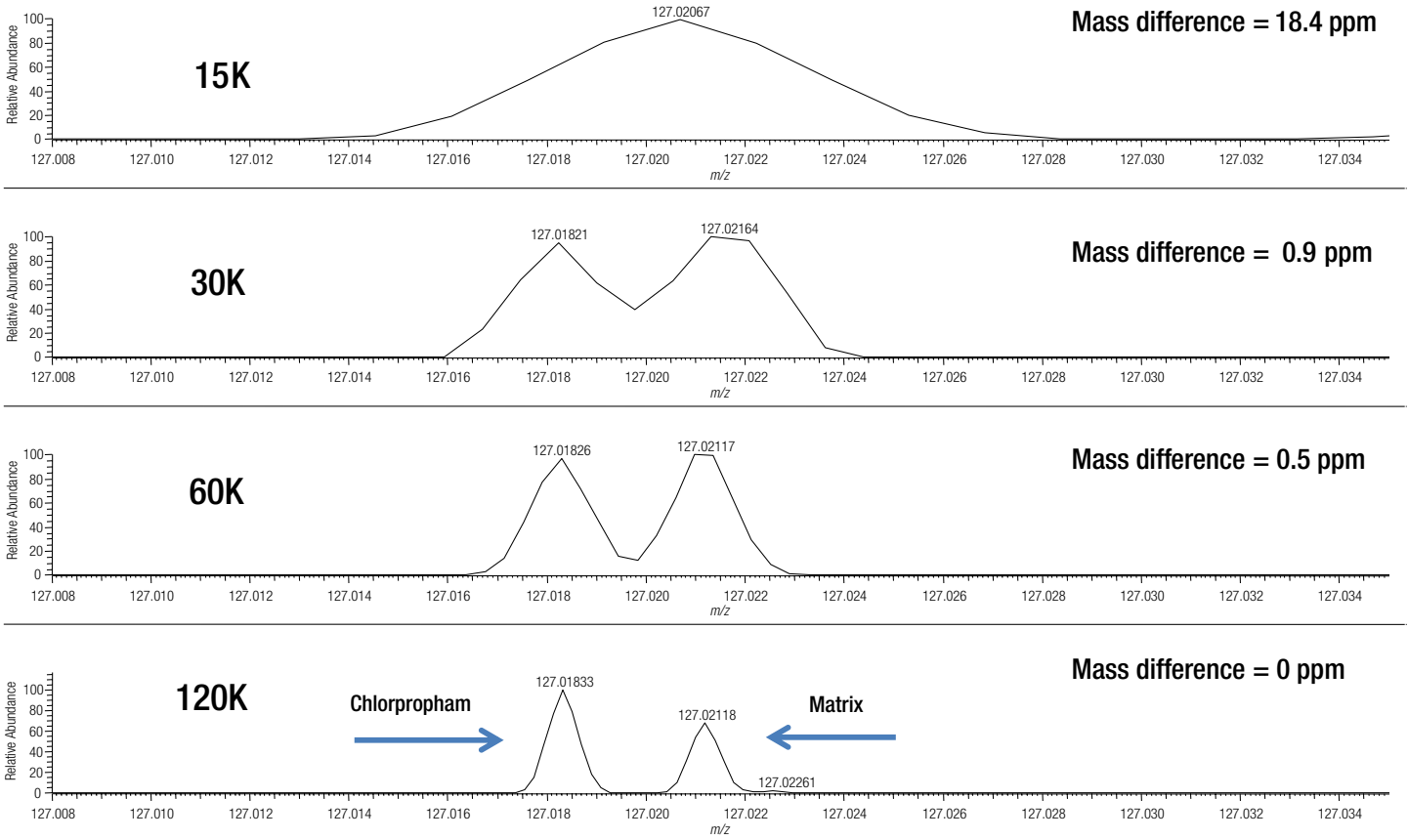

Figure 6. Effect of resolving power on mass accuracy of an analyte in matrix. Mass profiles of a diagnostic ion of chlorpropham at 10 ng/mL in leek, acquired at different resolutions of 15K, 30K, 60K, and 120K. At 15K and 30K the chlorpropham ion is not resolved from matrix interference resulting in poorer mass accuracy. At 15K, under screening criteria applied in this study, this pesticide would have been missed (false negative).

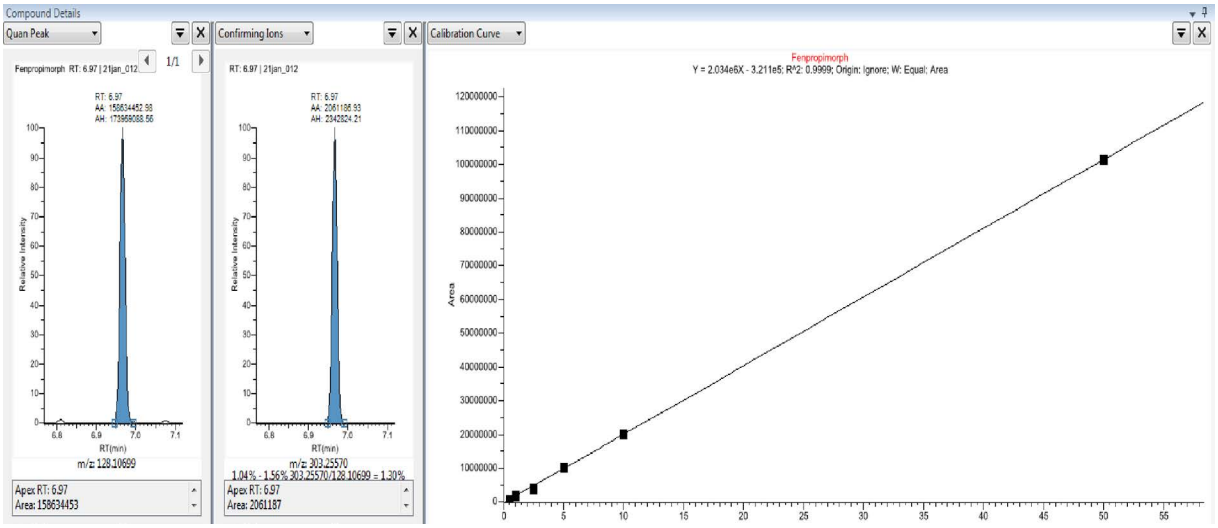

Figure 7. TraceFinder software view of the extracted ion chromatograms and calibration curve for fenpropimorph in leek. Triplicate injections of the calibration series were performed with good linearity.

Quantitative Pesticide Performance

The next step in routine analysis is to determine the concentration of the pesticide detected in the sample. Pesticide linearity was assessed across a concentration range of 0.5–50 ng/mL using matrix-matched standards and using triplicate injections of each calibration standard. In all cases, the coefficient of determination (R^2) was >0.99 with an average value of $R^2 = 0.997$ and with residual values from the regression line of <25%. An example of compound linearity for fenpropimorph is shown in Figure 7. Full quantitation of detected compounds was not in the scope of this study, but is reported in more detail for pesticides in Thermo Scientific Application Note 10449.[3]

Conclusions

The results of this evaluation demonstrate that the Thermo Scientific Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer, in combination with TraceFinder software, is an extremely effective tool for the routine screening of pesticides in food and feed samples. The Orbitrap mass spectrometer delivers excellent resolving power, mass accuracy, and sensitivity.

- Screening using full-scan, high-resolution mass spectrometry is an effective way to increase the scope of an analysis. This technology allows for more compounds to be analyzed from a single injection without prior optimization of the acquisition parameters.
- Fast GC analysis and acquisition speeds allow for increased laboratory productivity and sample throughput. The outstanding mass accuracy, in combination with excellent sensitivity, makes confident routine pesticide screening possible.
- Routine resolving power of 60,000 FWHM eliminates matrix interferences, increasing confidence in results when screening pesticides in complex matrices. Consistent sub-ppm mass accuracy achieved for all compounds ensures confident compound identification.

References

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3. Thermo Scientific Application Note 10449: Fast screening, identification, and quantification of pesticide residues in baby food using GC Orbitrap MS technology. Runcorn, UK.

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