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Multi-residue pesticide screening in cereals using GC-Orbitrap mass spectrometry

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Keywords

Pesticides, QuEChERS, Cereals, GC Orbitrap Mass Spectrometry, Screening, Quantitation, Accurate Mass, High Resolution, TraceFinder

Goal

To demonstrate the performance of the Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer for the routine analysis of GC-amenable pesticides in cereals (wheat, barley, oat, rye and rice).

Introduction

Pesticides are used to improve cereal crop yields and to minimize degradation during storage and processing. However, the widespread use of pesticides and the potential for residues to remain on the final product is of concern to consumers and to governments whose responsibility it is to ensure a safe food supply. Consequently, legislation has been introduced to protect consumers from exposure to contaminated foods.[1] Pesticide application to cereal crops is regulated by international organizations, and maximum residue levels (MRLs) are set for each pesticide/commodity combination. In the EU, if no substantive MRL has been set, a default MRL value of 0.01 mg/kg is usually applied.

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For complete coverage of the hundreds of pesticides in use, routine residue testing requires both liquid and gas chromatographic (GC) techniques coupled with mass spectrometers. Triple quadrupole mass spectrometers can provide the required sensitivity and selectivity to ensure that residue limits are not exceeded and the regulations are enforced. However, such targeted MS methods are limited to only detecting pesticides that are measured at the time of data acquisition and require careful method optimization and management to ensure selected reaction monitoring (SRM) windows remain viable. The alternative technique of high-resolution Orbitrap mass spectrometry provides distinct advantages over low-resolution MS/MS techniques and can substantially increase the scope of the analysis. With high-resolution mass spectrometry (HRMS), the default acquisition mode is untargeted (full-scan), making it simple to manage methods and allowing for a potentially unlimited number of pesticides to be monitored in a single injection. Unlike SRM acquisition on a triple quadrupole MS, high-resolution, full-scan data acquisition provides increased selectivity and enables retrospective interrogation of samples to search for emerging pesticides or other contaminants that were not screened for at the time of acquisition.[2, 3]

In this study, the performance of the Thermo Scientific Exactive GC Orbitrap mass spectrometer was evaluated for the routine analysis of GC-amenable pesticides in cereals (wheat, barley, oat, rye, and rice). The Exactive GC-MS system is routinely operated at a resolving power of 60,000 (measured at *m/z* 200 as full width at half maximum) for the detection of trace compounds against a complex chemical background as encountered in cereal sample extracts.

Experimental conditions

Sample preparation

Cereal samples (barley, oat, rice, rye, and wheat) were ground (or milled) to flour and then extracted using a citrate buffered QuEChERS procedure. The final acetonitrile extracts were acidified with 5% formic acid and diluted 1:1 with acetonitrile so that the standards and samples had the same level of matrix.

Each cereal type was spiked with 105 pesticides prior to extraction at a concentration of 100 µg/kg with five replicate extractions performed. Further dilutions of this extract were made to 10 and 20 µg/kg. These concentrations were equivalent to 5, 10, and 50 µg/L in the vial after the 1:1 dilution. For the assessment of compound linearity, a calibration series in rye matrix was prepared over the range from 10 to 300 µg/kg. The 105 pesticides included in the study cover a wide range of chemical classes and, with the five matrices, a total of 525 pesticide/matrix combinations were generated. The pesticides chosen in this study are not usually found as part of routine screening, therefore, their performance on the system was tested. The performance of more routine pesticides has been studied previously.[2,3]

Instrument and method setup

In all experiments, an Exactive GC Orbitrap mass spectrometer was used. Automatic sample injection was performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column with a 5 m integrated guard (P/N 26096-1425). Additional details of instrument parameters are displayed in Table 1 and Table 2.

Table 1. GC and injector conditions.

TRACE 1310 GC system parameters	
Injection Volume (µL):	1 splitless
Liner:	Siltek 1, splitless six baffle PTV liner (P/N: 453T2120)
Inlet (°C):	70
Split Flow (mL/min):	50
Transfer Rate (°C):	2.5
Final Temperature (°C):	300
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Temperature 2 (°C):	90
Rate (°C/min):	25
Hold Time (min):	1.5
Temperature 3 (°C):	280
Rate (°C/min):	5
Hold Time (min):	0
Temperature 4 (°C):	300
Rate (°C/min):	10
Hold Time (min):	5

Table 2. Mass spectrometer conditions.

Exactive GC mass spectrometer parameters	
Transfer Line (°C):	280
Ionization type:	EI
Ion Source (°C):	250
Electron Energy (eV):	70
Acquisition Mode:	Full-scan
Mass Range (Da):	50–600
Resolving Power (FWHM at *m/z* 200):	60,000
Lockmass, Column Bleed (*m/z*):	207.03235

Data processing

Data were acquired using the Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. For targeted analysis, a customised compound database contained the 105 compound names, accurate masses for quantification and identification ions, retention times, and elemental compositions of fragment masses. For the generation of extracted ion chromatograms, an extraction mass window of ±5 ppm was used.

Results and discussion

The objective of this study was to screen for 105 pesticides in five replicate extractions of different cereal matrices with a high degree of confidence. The lowest concentration at which each pesticide could be detected was to be determined. Further assessments of mass accuracy, linearity in matrix, and repeatability are also reported.

The five sample types chosen provided both typical and difficult matrices that are encountered in routine cereals testing. The full-scan total ion chromatograms shown in Figure 1 illustrate the high complexity and diversity of the different cereal samples. This is one reason why high-resolution, accurate-mass mass spectrometry is required to selectively extract target analytes from background chemical noise. In comparison to most fruit and vegetable samples, cereals have a high fat content that results in heterogeneous extracts when generic extraction techniques are used. The low selectivity of the QuEChERS sample extraction approach needs to be compensated for by selective instrumental analysis. On the Exactive GC, this is achieved using high mass resolving power. This capability, in combination with a full-scan acquisition, increases the scope of the analysis without the need for optimization of acquisition parameters, as is the case with targeted analyses.

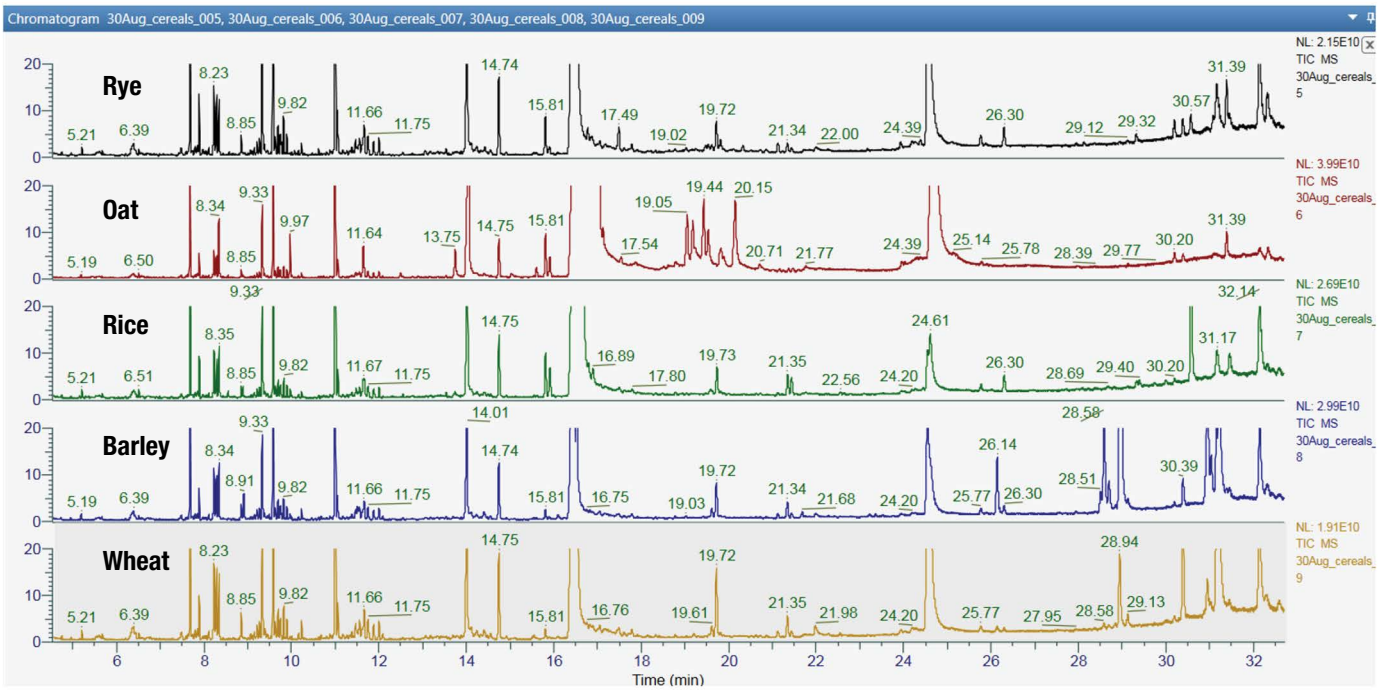

Figure 1. Full-scan total ion chromatogram (TIC) with zoomed Y axis of cereal extracts showing the complexity of the sample matrices used in this study.

The primary aim of the analysis was to determine how many of the fortified pesticides could be detected at each of the concentration levels (10, 20, and 100 µg/kg). For a positive detection, the following criteria based on SANTE guidelines[4] had to be satisfied:

1. Two ions detected for each pesticide with mass accuracy < 5 ppm and peak S/N > 3.
2. Retention time tolerance of ± 0.1 minutes compared with standards in the same sequence.
3. Ion ratio within ±30% of the average of calibration standards from the same sequence.

Intelligent data processing

TraceFinder software provides automated data acquisition and processing that quickly extracts and displays the identification information for all 105 spiked pesticides in approximately 20 seconds per sample file (0.75 GB). The software enables the analyst to rapidly review the data and to confidently confirm the presence of a pesticide. As Figure 2 shows, the analyst is presented with a traffic light system alongside raw data to show which identification criteria have been satisfied. More importantly, it will also flag when a parameter is outside of expected tolerance and alert the analyst to carefully review all of the available information before making the final decision to confirm a positive identification. In the example in Figure 2, the ion ratio of one of the fragment ions of isocarbophos in oat sample A (46.7%) is just outside the allowable ratio window of 48–89% due to peak integration. This is flagged to the analyst by a red square in the ion ratio (IR) column. By hovering over this square, further details are displayed. In this case, isocarbophos can be confirmed despite this flag as the other criteria are met and alternative fragment ion ratios are within the 30% tolerance. The multiple identification points provided by full-scan analysis along with user friendly software enables a faster time to result, which is vital in routine pesticide analysis.

Following the criteria listed previously, the lowest concentration level at which each pesticide was detected and confirmed in each of the five matrices is summarized in Figure 3. Of the 525 pesticide/matrix combinations, 90% were confirmed at ≤ 10 µg/kg and 96% at ≤ 20 µg/kg. Having multiple identification points and

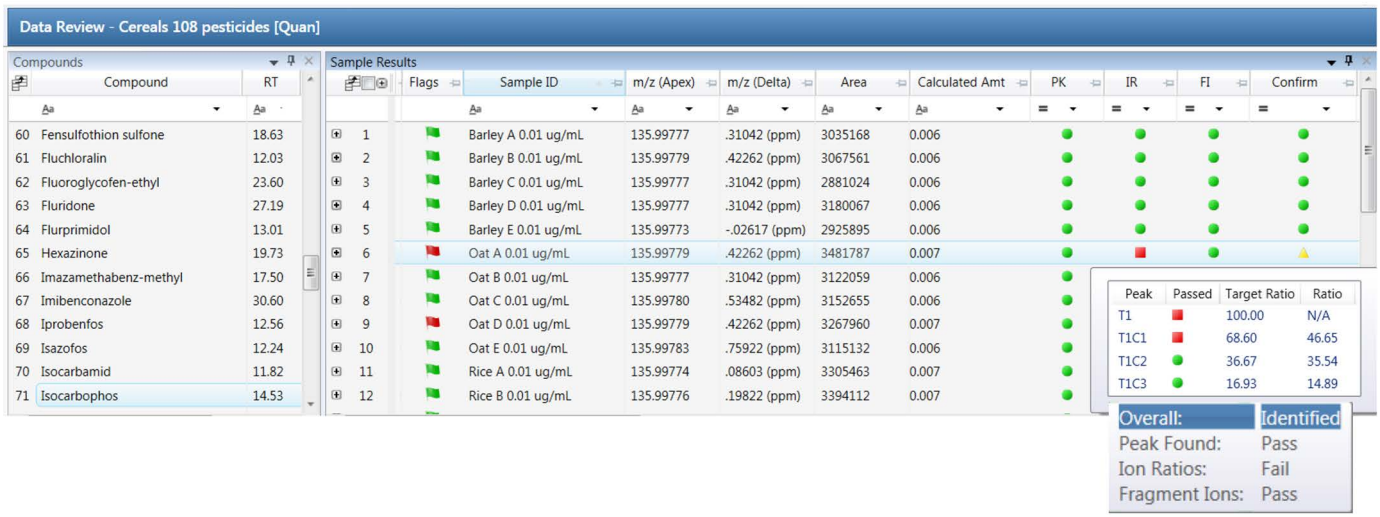

Figure 2. TraceFinder software browser enables fast data review and confirmation. The software quickly points the analyst to the data that supports a positive identification using a traffic light system along with real data values. More importantly, it will flag when a parameter is outside of tolerance, and by what value, and allow the analyst to make the final decision to confirm an identification. Hovering above the red square (below) brings up further details.

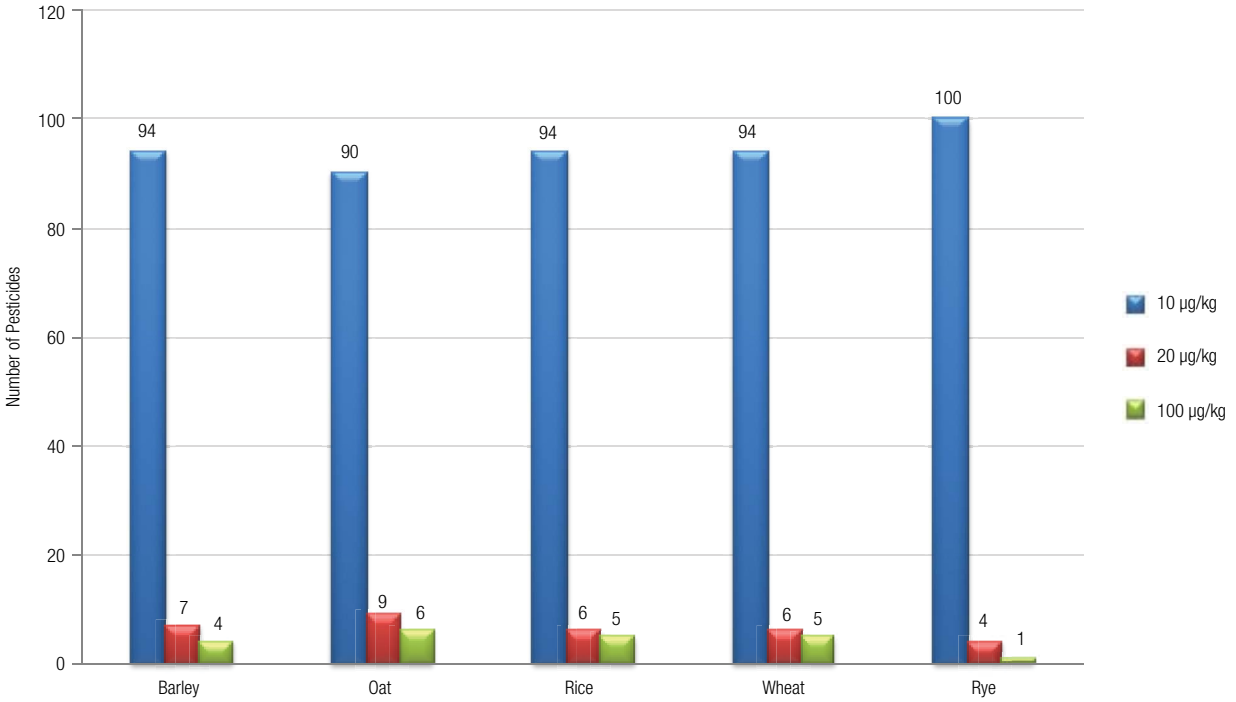

Figure 3. The lowest concentration confirmed (two ions within 5 ppm, ion ratios within ±30%) for each pesticide in each of the five sample matrices. The total number of pesticides is 105.

limits of detection below the MRL increases the confidence in positive detections. This also minimizes the risk of false negative results and ensures that the limits of false positive detects are at a manageable level within a routine environment. All 105 pesticides were detected at concentrations lower than 10 µg/kg (5 µg/L in vial) if screened based on retention time and the main quantifier ion. The limiting factor for confirmed identification in the case of a few analytes was the sensitivity of additional ions that were much lower in intensity compared to the main ion. As the criteria applied here has shown, using electron ionization (EI) in combination with full-scan acquisition provides the opportunity to use multiple diagnostic ions for the identification of pesticides. In addition to individual ions, compound spectra can be used to confirm identifications. The Exactive GC generates standard EI spectra that are highly reproducible and library searchable (using nominal- or high-resolution MS libraries commercially available or custom made). An example of spectral matching with NIST 2014 for the pesticide mexacarbate (SI 905) is shown in Figure 4.

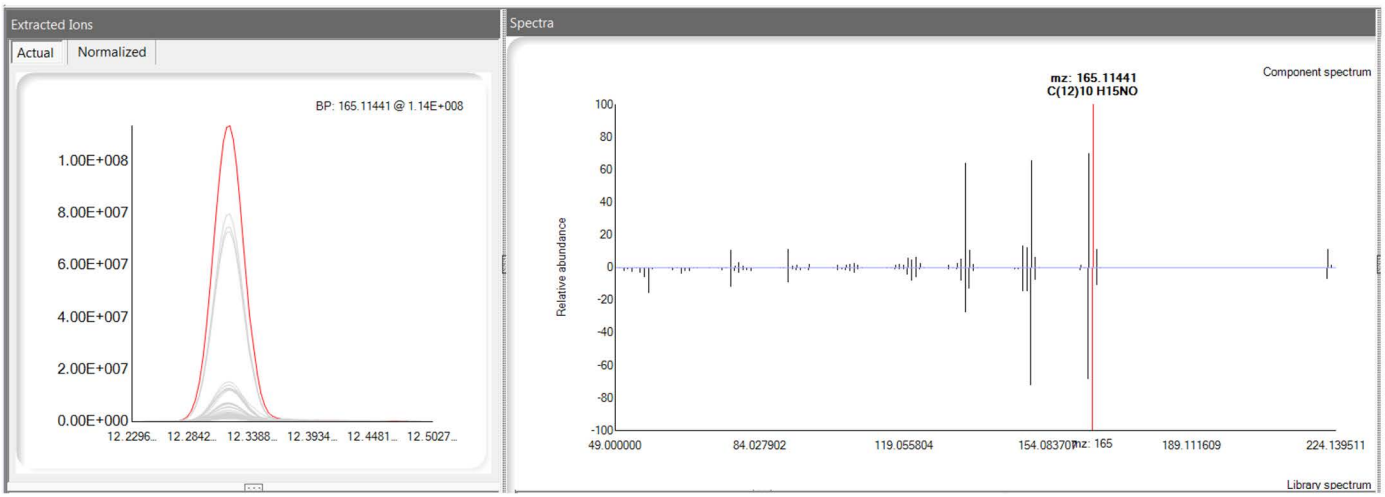

Figure 4. TraceFinder software deconvoluted peaks (left). Acquired spectrum and library spectrum (right) for mexacarbate with search index score of 905.

True mass accuracy

Acquiring reliable accurate mass measurements is critical when detecting pesticide residues at low concentrations in complex sample matrices. Low mass errors ensure that compound selectivity is high and that detection and indentification are robust. The low mass errors (ppm) observed with the Exactive GC are achieved through the high mass resolving power that can discriminate between matrix interferences and target analyte ions. Internal mass correction enables mass accuracies of ≤ 1 ppm to be consistently achieved regardless of analyte concentration or matrix complexity. As an example, the mass accuracy of all detected pesticides in wheat at 10 µg/kg is shown in Figure 5. All pesticides are detected with sub-1 ppm mass accuracy, well below the guideline limit of 5 ppm (< 1 mDa for *m/z* < 200), delivering the highest confidence in accurate and selective detection. The low mass accuracy also allows for tighter tolerances to be applied for extracted ion chromatograms, which will result in fewer false positive detects thus increasing efficiency by reducing the need for manual review.

When the mass resolution is insufficient, it can result in target ions that have a mass accuracy outside of the required identification criteria. This is demonstrated in Figure 6 where the oat 20 µg/kg matrix sample was analyzed at resolving powers of 15K, 30K, and 60K. The zoomed mass spectra show the quantifier ion for tribufos. At 15K and 30K, the *m/z* 201.97042 ion demonstrates poor mass resolution resulting in mass accuracies of 6.4 and 3.7 ppm, respectively. However, the ion is well resolved at 60K resulting in the expected sub-1 ppm mass accuracy. At 15K this pesticide would have failed the identification criteria of < 5ppm and would have been reported as not detected.

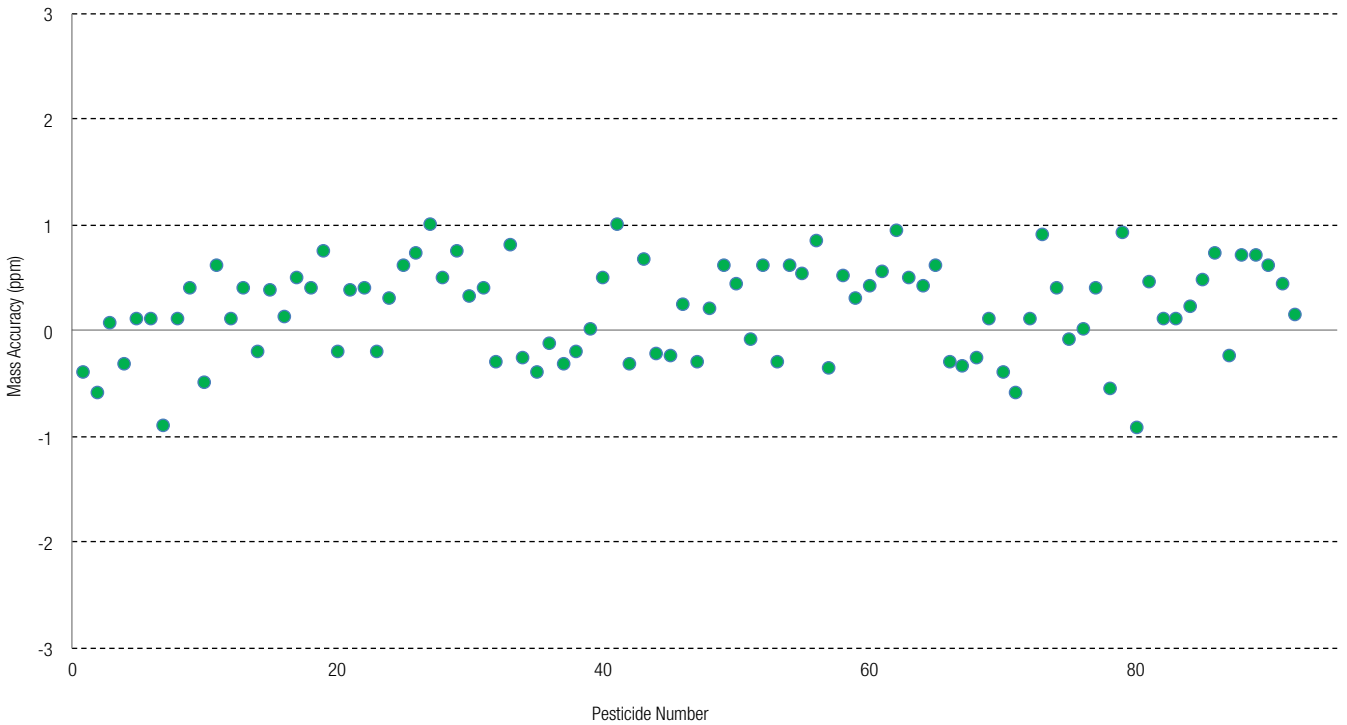

Figure 5. Mass difference measurements at 10 µg/kg for each pesticide in wheat.

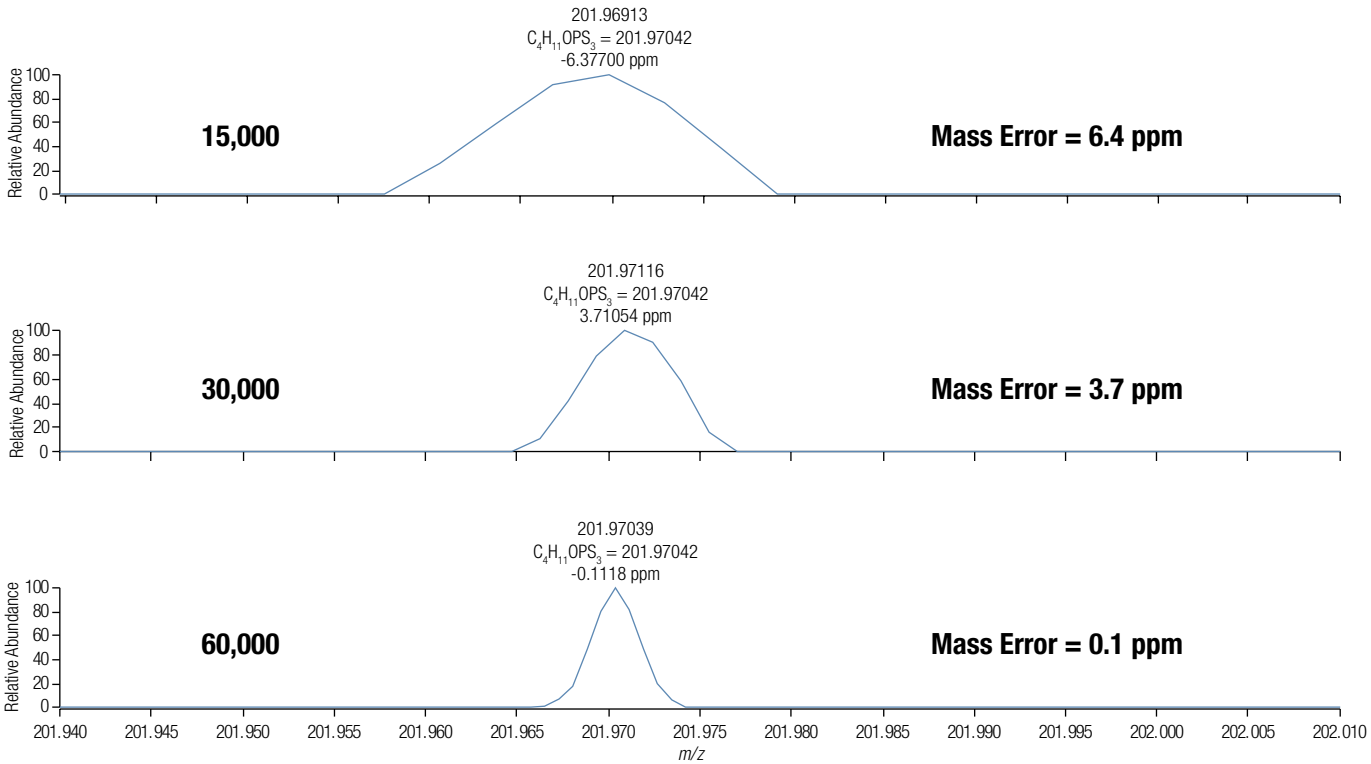

Figure 6. Effect of resolving power on mass accuracy of diagnostic ion (*m/z* 201.97042) tribufos at 20 µg/kg in oat acquired at different resolutions of 15K, 30K, and 60K.

Robust quantitative performance

Having reliably identified a pesticide in a sample, the final stage is to determine its concentration. The Exactive GC quantitative linearity was assessed using matrix matched standards in rye across a concentration of 10–300 µg/kg. In all cases, the coefficient of determination (R^2) was > 0.99 for each pesticide from its LOD value to 300 µg/kg. An example of the TraceFinder software quantification results browser showing dichlorprop methyl ester is given in Figure 7.

A final assessment was made of the peak area repeatability at low analyte level by running n = 20 replicate injections at 10 µg/kg in wheat. All detected pesticides had RSD% of less than 13%, (Figure 8). This shows that the Exactive GC operated in full-scan at 60k resolution has the selectivity and sensitivity required for robust and reliable routine anlysis of pesticides residues at or below the MRLs in a range of different types of cereal samples.

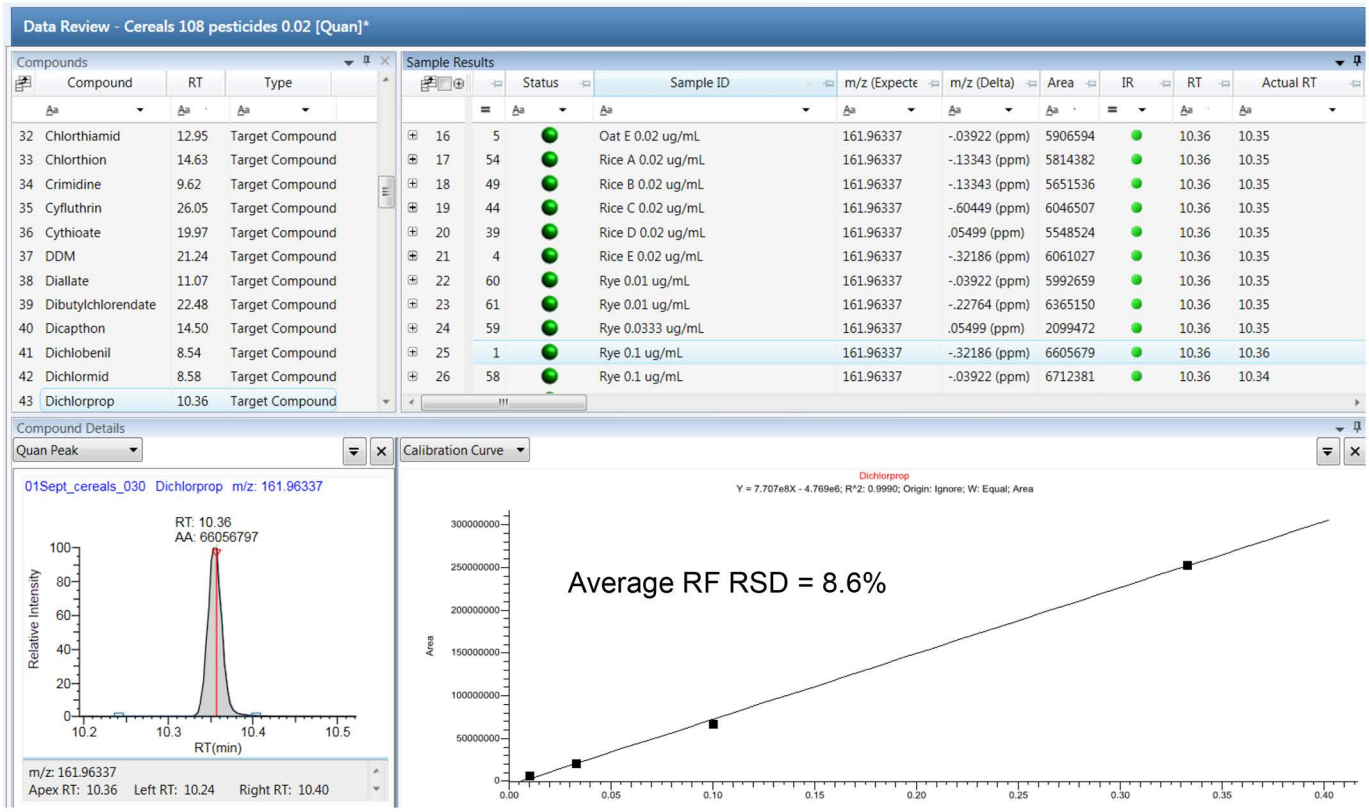

Figure 7. TraceFinder software browser showing positively identified pesticides, extracted ion chromatogram, and calibration graph (dichlorprop methyl ester as an example). Sub-ppm mass accuracy for dichlorprop across the calibration range and in replicates of 20 mg/kg. Identification criteria information is available and flagged when out of tolerance for quick data review.

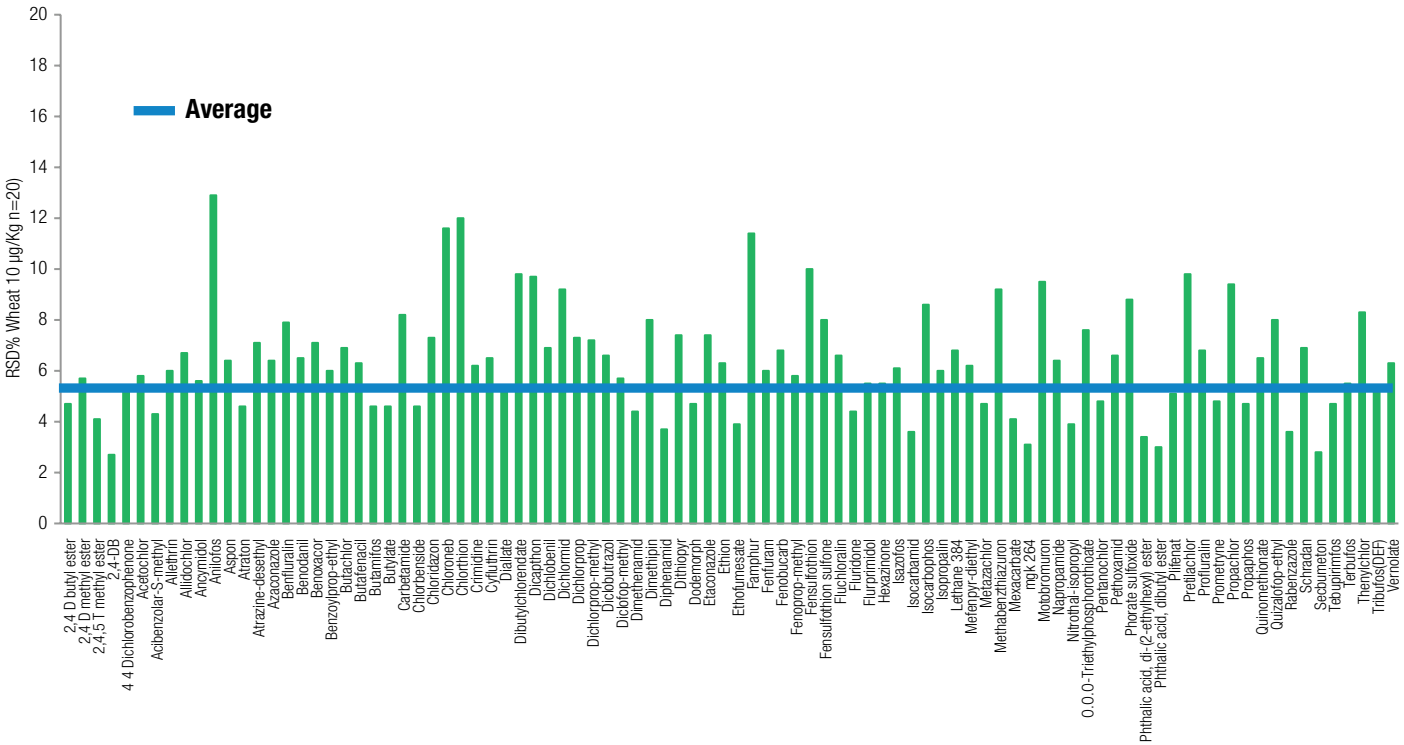

Figure 8. Repeatability (%RSD) for 10 µg/kg (n=20) for each pesticide in wheat.

Conclusions

The results of this study demonstrate that the Exactive GC Orbitrap high-resolution mass spectrometer, in combination with TraceFinder software, delivers robust and sensitive performance for routine pesticide analysis in cereals to regulatory standards.

- All 105 pesticides were detected at 10 µg/kg (5 µg/L in vial). 96% of the 525 pesticide/matrix combinations were confirmed at < 20 µg/kg (< 10 µg/L in vial) with excellent linearity, and in full compliance with the EU SANTE method performance criteria.
- The full scan acquisition permits efficient targeted data processing by use of a compound database and has the capability to easily add further analytes into the method scope.
- Intelligent software allows for results to be reviewed and detections confirmed in an efficient manner.
- Consistent sub-ppm mass accuracy was achieved for all compounds over a wide concentration range, ensuring that compounds are detected with high confidence at low and high concentration levels.
- Repeated injections of a wheat matrix at 10 µg/kg showed that the system is able to maintain a consistent level of performance over an extended period of time as is demanded by a routine testing laboratory.

References

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