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

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OVERVIEW

The results of this study show that the Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS system is a robust analytical tool for the quantitative analysis of pesticide residues in complex matrices to regulatory requirements. Routine mass resolution of 60,000 FWHM and consistent sub-ppm mass accuracy ensures selective and confident compound detection and identification. Repeated injections demonstrate that the system is suitable for routine analysis.

INTRODUCTION

For the complete coverage of the hundreds of pesticides in use, routine residue testing typically requires both liquid and gas chromatographic techniques coupled with triple quadrupole mass spectrometry (MS). These techniques can provide the required sensitivity and selectivity to ensure that residue limits are not exceeded and the regulations enforced. However, targeted MS methods such as these are limited to only detecting pesticides that are measured at the time of acquisition and require careful method optimisation and management to ensure selected ion monitoring windows remain viable. The alternative technique of high resolution Orbitrap mass spectrometry provides distinct advantages over low resolution MS/MS techniques and significantly increases the scope of the analysis. In this work, we evaluated GC Orbitrap MS for the analysis of rarely encountered pesticides in cereals (wheat, barley, oat, rye and rice) to demonstrate an almost unlimited scope in the analysis through full-scan acquisition.

METHODS

Sample Preparation

Cereal flour samples (barley, oat, rice, rye and wheat) were extracted following a citrate buffered QuEChERS procedure. Each cereal type was spiked with 105 pesticides 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. 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 it generated a total of 525 pesticide/matrix combinations. The pesticides chosen are not usually found as part of routine screening. See GC and MS details in Tables 1 and 2.

Tables 1 & 2. GC and MS parameters

TRACE 1310 GC Parameters	
Injection Volume (µL):	1
Liner:	Siltek six baffle PTV liner
Inlet (°C):	70
Transfer rate (°C)	2.5 (final 300 °C)
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 3 (°C):	300
Rate (°C/min):	10
Hold Time (min):	5

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 Acquisition and Analysis

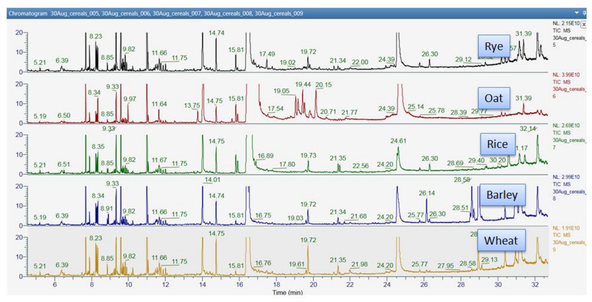
In all experiments a Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer was used. Chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m x 0.25 mm I.D. x 0.25 µm film capillary column with a 5 m integrated guard (P/N:26096-1425). Data were acquired using Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. For target analysis a customised compound database containing the 105 pesticides was created. The database contained the following information; compound name, quantification ion and confirming ion accurate masses, retention times and elemental compositions of parent and fragment masses. For the generation of extracted ion chromatograms an extraction mass window of ±5 ppm was used.

RESULTS

Chromatography

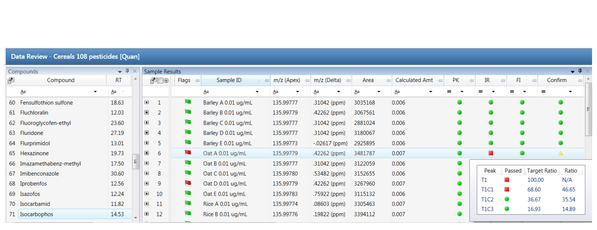
Good chromatographic separation was obtained using the GC conditions (Figure 1) and sample complexity is demonstrated by the varying TIC profiles for the five sample types.

Figure 1. Full scan TIC for five cereal samples.



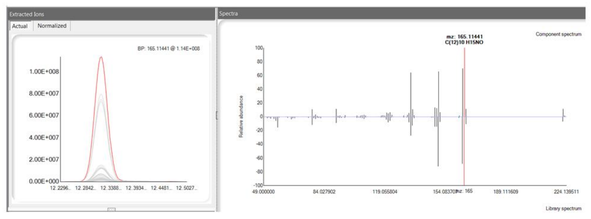
The primary aim of the analysis was to determine how many of the fortified pesticides could be detected at each of the concentration levels (10, 20 and 100 µg/kg). For a positive detection the following criteria based on SANTE guidelines [1] had to be satisfied. TraceFinder software provides automated data acquisition and processing that quickly extracts and displays the identification information for all of the 105 spiked pesticides. The software enables the analyst to rapidly review the data and to confidently confirm the presence of a pesticide (Figure 2).

Figure 2. TraceFinder browser enables fast data review and confirmation. The software quickly points the analyst to the data that supports a positive identification.



As Figure 2 shows, the analyst is presented with a traffic light system alongside raw data to show which identification criteria has been satisfied. More importantly, it will also flag when a particular parameter is outside of expected tolerance and allow the analyst to make the final decision to confirm a positive identification.

Figure 3. TraceFinder deconvoluted peaks (left). Acquired spectrum and library spectrum (right) for mexacarbate with search index score of 905.



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 3. Following the criteria above the lowest concentration level that each pesticide was detected and confirmed in each of the five matrices is summarised in Figure 4. 90% of the 525 pesticide/matrix combinations were confirmed at ≤10 µg/kg and 96% at ≤20 µg/kg.

Figure 4. The lowest concentration confirmed (2 ions within 5 ppm, ion ratios within ±30%) for each pesticide in each of the five sample matrices.

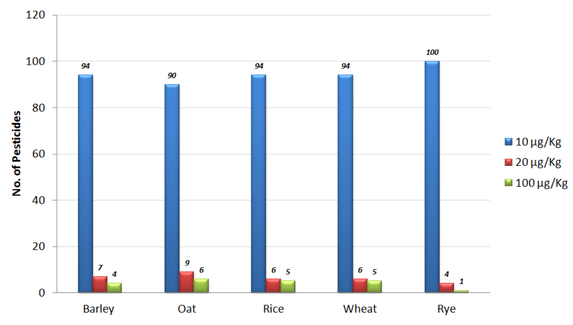
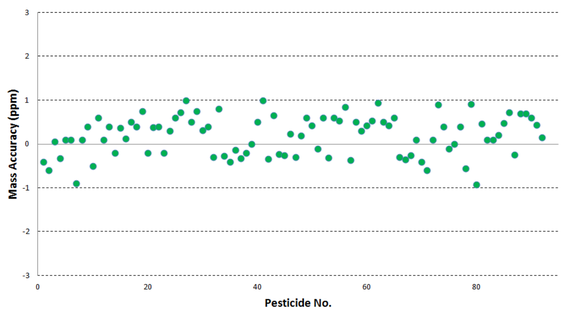


Figure 5. Mass difference measurements at 10 µg/kg for each pesticide in wheat.



Robust Mass Accuracy

The low mass errors (ppm) observed with the Exactive GC are achieved through the high mass resolving power that is able to 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.

When the mass resolution is insufficient, it can result in the mass accuracy of target ions that are outside of the required identification criteria. This is demonstrated in Figure 6 where the oat 20 µg/kg matrix sample was analysed 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 higher mass accuracy 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 <5 ppm and would have been reported as not detected.

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.

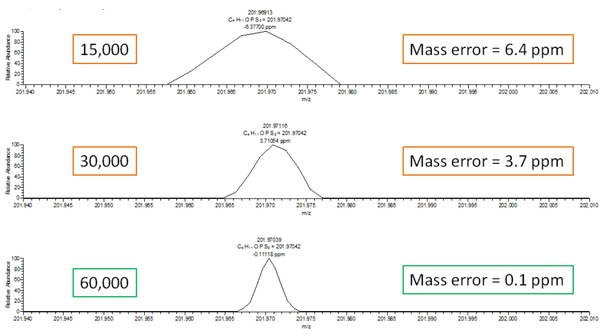
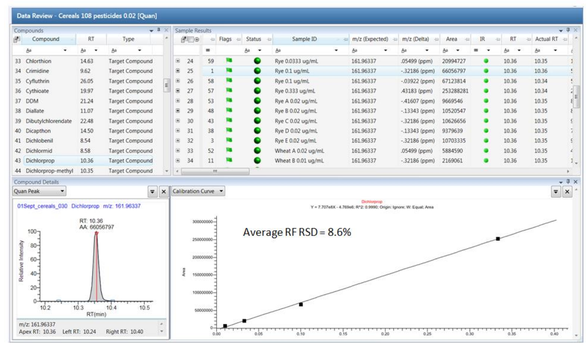


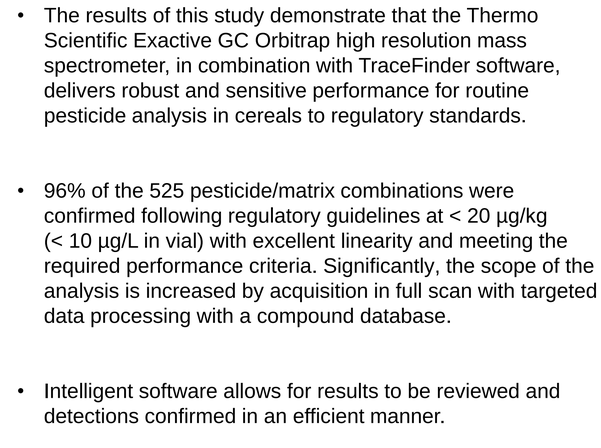
Figure 7. TraceFinder browser showing positively identified pesticides, extracted ion chromatogram and calibration graph (Dichlorprop as an example).



Accurate Quantitation

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 quantification results browser showing dichlorprop is given in Figure 7.

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



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%, significantly lower than the 20% typically accepted limit (Figure 8). This shows that the Exactive GC operated in full scan at 60k resolution has the selectivity and sensitivity required to analyse pesticides in a robust manner at the MRL.

CONCLUSION

- The results of this study demonstrate that the Thermo Scientific 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.
- 96% of the 525 pesticide/matrix combinations were confirmed following regulatory guidelines at < 20 µg/kg (< 10 µg/L in vial) with excellent linearity and meeting the required performance criteria. Significantly, the scope of the analysis is increased by acquisition in full scan with targeted data processing with a compound database.
- 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 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

1. SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Supersedes SANCO/12571/2013. Implemented by 01/01/2016.

TRADEMARKS/LICENSING