An Assessment of GC Orbitrap MS Technology for the Routine Screening and Quantification of Pesticide Residues in Food

Paul Silcock, Dominic Roberts, and Cristian Cojocariu; Thermo Fisher Scientific, Tudor Road, Runcorn, Cheshire, UK, WA7 1TA

ABSTRACT

The results of this study show that the Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS system is a robust analytical tool for the analysis of pesticide residues in complex matrices to regulatory requirements. The scope of the analysis is increased by offering high performance full-scan analysis. 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

Pesticides are measured by liquid chromatography (LC) and gas chromatography (GC) analytical methodologies. GC offers good separation efficiency and a choice of MS detectors such as single or triple quadrupoles. However, targeting specific compounds during acquisition limits the scope of analysis. This limitation has led to increased interest in the development of methods using MS analyzers that can operate in full-scan with a high-mass resolving power, but provide similar levels of selectivity and quantitative performance. In this work, we demonstrate the use of GC Orbitrap technology in the context of the SANTE guidelines1 for high throughput pesticide residues analysis in fruits and vegetable samples with an almost unlimited scope in the analysis through full-scan acquisition.

MATERIALS AND METHODS

Sample Preparation

Tomato, leek and orange samples were extracted using the acetate buffered QuEChERS protocol. A mixture of salts was added and the centrifuge tube shaken for 4 minutes and centrifuged for 5 minutes at 3700 rpm. 5 mL was transferred to a 15 mL PTFE centrifuge tube containing magnesium sulphate and 125 mg of PSA. The extract was shaken in a vortex mixer and centrifuged as above. The final acetonitrile extracts (1g/mL) were used as blank matrix. The calibration series was prepared by taking 100 µl of acetonitrile blank matrix and drying under a stream of nitrogen. The sample was reconstituted in 100 µl ethyl acetate containing the appropriate concentration of 51 pesticides. See GC and MS details in Tables 1 and 2.

Table 1. GC and MS Parameters

TRACE 1310 GC Parameters	
Injection Volume (mL):	1
Liner:	LinerGOLD™ single taper
Inlet (° C):	280
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

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):	50–550
Resolving power (FWHM at *m/z* 200):	60,000
Lockmass, column bleed (*m/z*):	207.03235

Data Acquisition and Analysis

A ten point matrix matched calibration series was run from 0.5 – 500 µg/Kg. In addition, 10 replicates were analyzed of each sample at 10 µg/Kg. A final repeatability test involved 100 repeat injections of tomato at 10 µg/Kg.

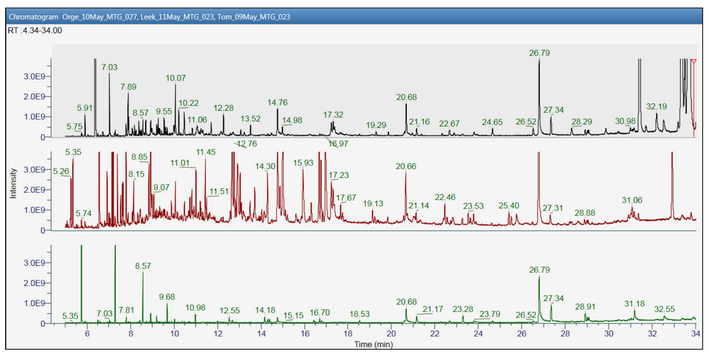
Data was acquired and processed using the Thermo Scientific™ TraceFinder™ software. TraceFinder allows easy data acquisition, reviewing and data reporting.

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 three sample types.

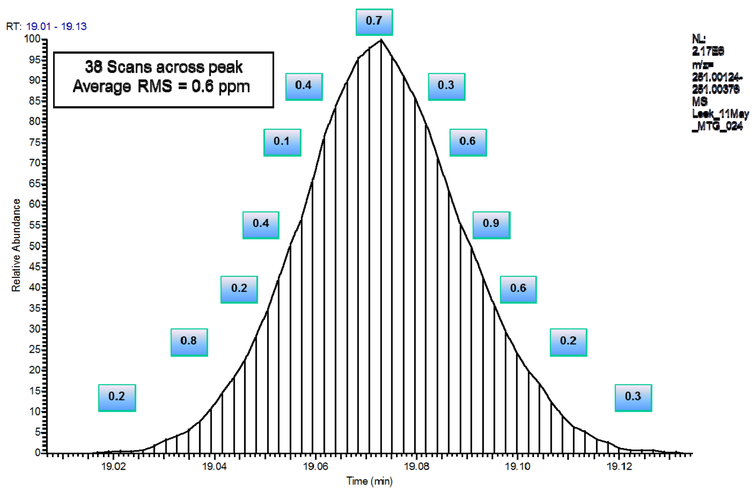
Figure 1. Full scan TIC for orange, leek and tomato.



MS Acquisition Speed

Using GC run times requires fast MS acquisition rates in order to obtain sufficient scans/peak. An example of typical number of scans acquired using the Exactive GC system operated at 60,000 resolution is shown below (Figure 2). Noticeably, beside the adequate number of scans/peak, excellent mass accuracy for every single scan across the peak was obtained (<0.6 ppm RMS).

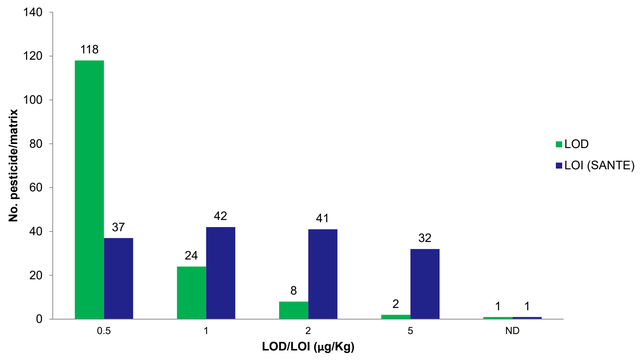
Figure 2. XIC of chlorobenzilate (*m/z* 251.0025) showing 38 scans/peak. Data acquired in full-scan at 60,000 FWHM resolution Mass accuracy/scan shown as ppm.



Sensitivity

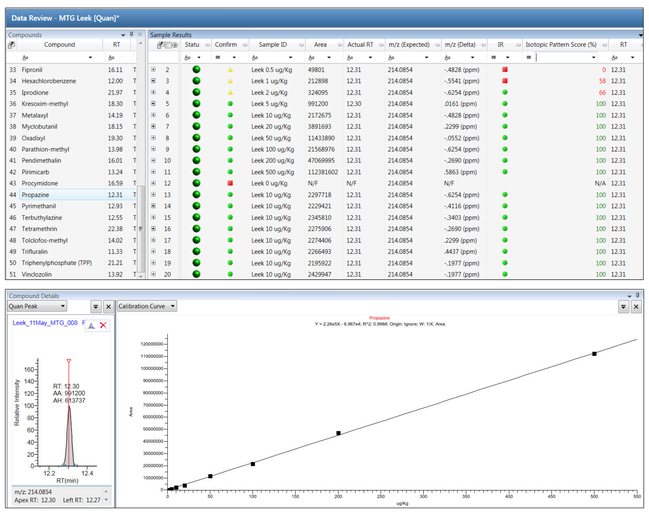
All pesticides were detected and confirmed following SANTE guidelines at ≤5 µg/Kg (Figure 3) with the exception of chlorothalonil in leek, which is known to degrade in this matrix.[2] The LOI required 2 ions to be detected with <5 ppm mass accuracy, retention times within 0.1 minute and ion ratios of <30%. LODs were much lower with 93% of pesticides detected at ≤1 µg/Kg.

Figure 3. The limit of detection (LOD) and limit of identification (LOI) to SANTE guidelines for pesticide matrix combinations.



For HR-MS to be used in routine it is essential that the processing software is fast and accurate. TraceFinder software is used to process the data and present the results to the analyst. Flags are used to indicate when criteria are met or fail (Figure 4). All detected pesticides were linear R^2 >0.99 and an example is shown below.

Figure 4. TraceFinder browser showing positively identified pesticides, extracted ion chromatogram and calibration graph (propazine as an example).



Mass Accuracy

When the mass resolution is inadequate, the mass profile of two ions overlap, which results in the incorrect assignment of the mass of the target compound. This is demonstrated in Figure 5 where the leek 10 µg/Kg matrix standard was analyzed at resolving powers of 15K, 30K and 60K. An interference is observed that adversely impacts on the mass accuracy at 15K and 30K. Obtaining accurate mass information in a consistent manner is critical for determining the identity of a pesticide. The mass accuracy for all 51 pesticides was assessed at the pesticides LOI level and is summarized in Figure 6.

Figure 5. Effect of resolving power on mass accuracy of the diagnostic ion of pyrimethanil at 10 µg/Kg in leek acquired at different resolutions of 15K, 30K and 60K.

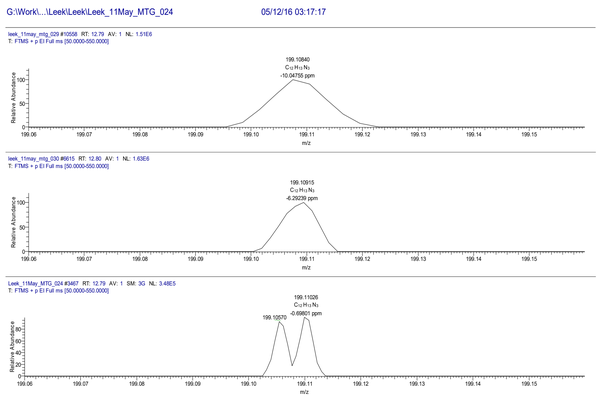
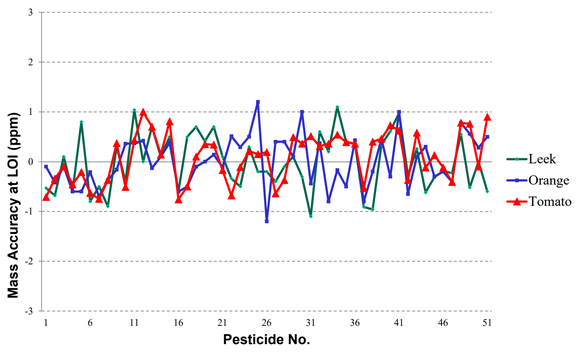
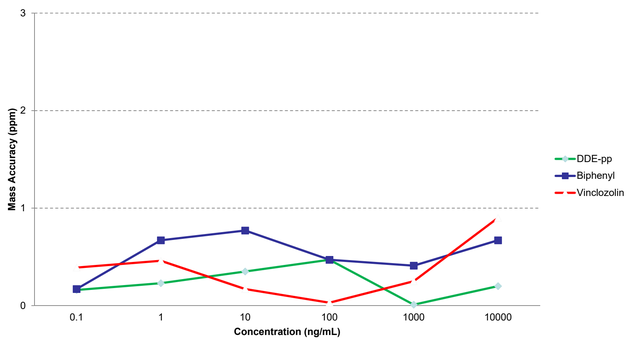


Figure 6. Mass difference measurements at the LOI level for each pesticide across the three matrices.



In pesticide analysis it is also essential that the instrument is able to maintain mass accuracy across the complete range of possible analyte concentrations encountered. It would not be acceptable if a high concentration pesticide violation was missed due to detector saturation. On the Exactive GC system, the Orbitrap is protected from saturation by the C-Trap which regulates the ions being injected. This ensures that no matter what concentration is encountered the mass accuracy performance is preserved. This is demonstrated in Figure 7 which shows the mass accuracy for three pesticides at concentrations from 0.1 to 10,000 ng/mL (six orders of magnitude) is always <1 ppm.

Figure 7. Mass accuracy measurements across 6 orders of concentration (0.1–10,000 ng/mL) for DDE-p,p, biphenyl and vinclozolin in ethyl acetate.



Repeatability

To evaluate the repeatability of the Exactive GC system over a longer period, a tomato extract at 10 µg/Kg was repeatedly injected 100 times from a single vial. Prior to commencing analysis, a new liner was installed, the source tuned and the MS calibrated. No further interventions were made during the 66 hours of continual analysis. Peak areas showed good RSD% and mass accuracy maintained at ≤1 ppm (Figures 8 and 9).

Figure 8. Repeat injections (n=100) of a tomato extract spiked at 10 µg/Kg showing that the sensitivity is maintained over the 66 hours of continual operation.

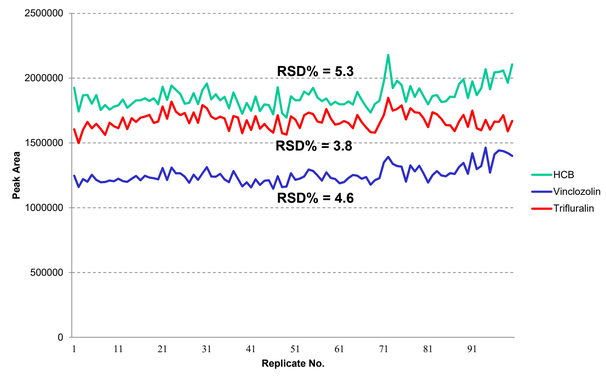


Figure 9. Mass accuracy (ppm) over 100 injections for hexachlorobenzene, vinclozolin and trifluralin in tomato extract at 10 µg/Kg.

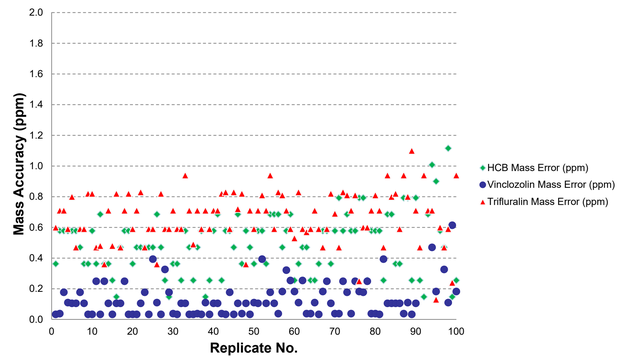
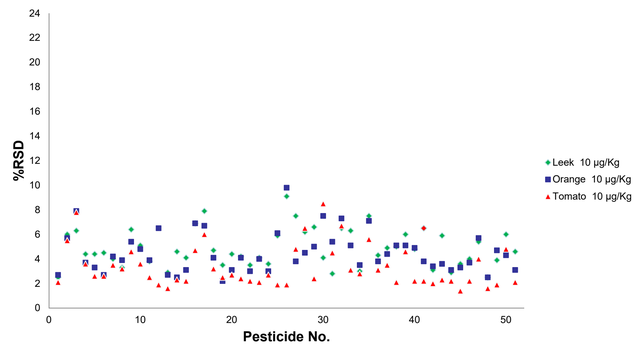


Figure 10. Repeatability (%RSD) for 10 µg/Kg (n=10) for each pesticide in the three matrices studied. SANTE guideline of 20% threshold shown in green.



The results of the 10 replicate injections at 10 µg/Kg in all three matrices are presented in Figure 10. All of the detectable pesticides had RSD% less than 10%, well below the 20% threshold requirement in the SANTE guidance document. This shows that the system 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 Mass Spectrometer, in combination with TraceFinder software, is a robust and sensitive instrument for routine pesticide analysis in fruits and vegetables following the SANTE guidance document.
- 99.3% of the pesticide/matrix combinations were detected below the MRL with excellent linearity and meeting the required performance criteria.
- Mass accuracy was sub 1 ppm increasing confidence in identifications.
- Repeated injections of a tomato 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.
2. Belmonte Valles N., Retamal M., Martinez-Uroz M.A., Mezcua, M., Fernandez-Alba AR, de Kok, A. (2012) Determination of chlorothalonil in difficult-to-analyse vegetable matrices using various multiresidue methods. Analyst, 137, 10, p 2513–2520.

ACKNOWLEDGEMENTS

The authors would like to thank Professor Amadeo Fernández-Alba and Samanta Uclés Duque at the European Reference Laboratory for Fruits and Vegetables, Almeria, Spain for providing samples, standards and discussion.

TRADEMARKS/LICENSING