

GC Orbitrap MS delivers increased productivity
to analytical testing



Thermo Scientific™ Exploris GC

“To ensure our food is safe there is a continual demand to detect more compounds and to do so with the very highest confidence. GC triple quadrupole instruments have served us well for many years, but to meet the need for increased scope and to accurately quantify over a wide range, then we have turned to GC high resolution mass spectrometry for the solution”

—Dr Jim Garvey,
Department of Agriculture, Food and the Marine

Food safety and environmental analytical testing laboratories are under ever-increasing pressure to screen samples for more pesticides in a single injection, with a fast turnaround time and at a competitive cost. These challenges are ones faced by Jim Garvey, the Head of Food Chemistry at the Department of Agriculture, Food and the Marine, in Celbridge, Ireland. They are a European Union National reference Laboratory accredited for the measurement of pesticide residues in a wide range of food commodities. One of their primary functions is the implementation of European Union (EU) law within Ireland. This means that each year they face an increasing list of pesticides and metabolites to be included in their scope of analysis and they must also have the ability to respond to emerging food safety matters.

Most laboratories rely on targeted analytical approaches using triple quadrupole mass spectrometry (MS) instrumentation. This technology covers a wide range of chemical classes and provides the required level of sensitivity and selectivity. However, they are limited to only detect those compounds in the method acquisition list. When new compounds are to be added they require careful optimization of acquisition parameters and the daily monitoring of acquisition time windows to ensure detection of the target analyte. An alternative approach to address these demands is to develop methods that use high-resolution accurate mass (HRAM) mass spectrometry (MS). This technology brings distinct advantages, to meet the ever-changing demands in pesticide analysis.

The laboratory has been developing pesticide residue analysis using the Thermo Scientific Orbitrap Exploris GC

system. The team value the high specificity, sensitivity and flexibility of this system to increase the scope of analysis without the need for compound optimization. According to Dr Jim Garvey, “The aim is to cover more than 90% of the regulated pesticides and metabolites using multi-residue methods. This means that our current methods are constantly expanding; for example, we have a plan to add another 45 pesticides to our existing methods by the end of the year. To cope with this, we have had to look at alternatives to our existing triple-quadrupole MS systems and at different workflows such as the introduction of screening methods. For this reason, we have started to develop and validate methods using high-resolution accurate mass (HRAM)-MS systems”.

The priority of the team was to develop a multi-residue method for the analysis of pesticides and polychlorinated biphenyls (PCBs) in fruit and vegetables using GC-HRAM-MS. They had used triple-quadrupole instruments for almost 15 years, but the demand for an increase in scope means that they will have to anticipate methods with scopes of up to 1000 pesticides and metabolites in the near future. There is also the desire to consolidate separate methods onto a single system. Jim explained, “I think methods using triple-quadrupole instruments will struggle to cope with this demand for a number of reasons. We need to find transitions for every new compound added to a method, and these transitions need to be optimized for the instrument being used, which is a considerable amount of work. Once we have done this, the scanning speed of the instruments then limits the number of transitions that we can fit into the method, and this in turn puts a limit on how far we can develop triple-quadrupole methods. These limitations

“Compared to existing triple-quadrupole methods, the selectivity of GC Orbitrap is much higher, the sensitivity is at least comparable and the repeatability and reproducibility are better”

— Dr Jim Garvey

don't exist with high-resolution MS systems operating in full scan, and these systems also give the advantage of high resolution and mass accuracy, which gives us greater confidence in our results".

To develop the protocol, the laboratory built a HRAM database that includes the retention times, exact masses, and confirmatory ions for target compounds of interest. Using the database, the laboratory can quickly set up a method for a confirmatory analysis. The confirmatory method uses an average of four ions—one for quantification and three for confirmation to compare at least two ion ratios—exceeding the SANTE guidelines that require two ions measured by HR MS and one ion ratio.

Following this the method was validated following criteria in the SANTE/2019/12682 guidance document used in the EU. The method was validated for fruit and vegetables - a high water content matrix (cucumber), a high acid content matrix (lemon), and a high chlorophyll content matrix (broccoli). Recovery experiments were carried out across the linear range of the method (5–250 µg/l) using two different analysts, and the repeatability was calculated and the within-laboratory reproducibility. Mass accuracy and the confirmatory ion ratios were also evaluated, and finally they looked at matrix effects. The method contains 167 pesticides, PCBs, and metabolites, and was successfully validated for 94% of these with recoveries within 60–140% with a repeatability and within-laboratory reproducibility of <20%.

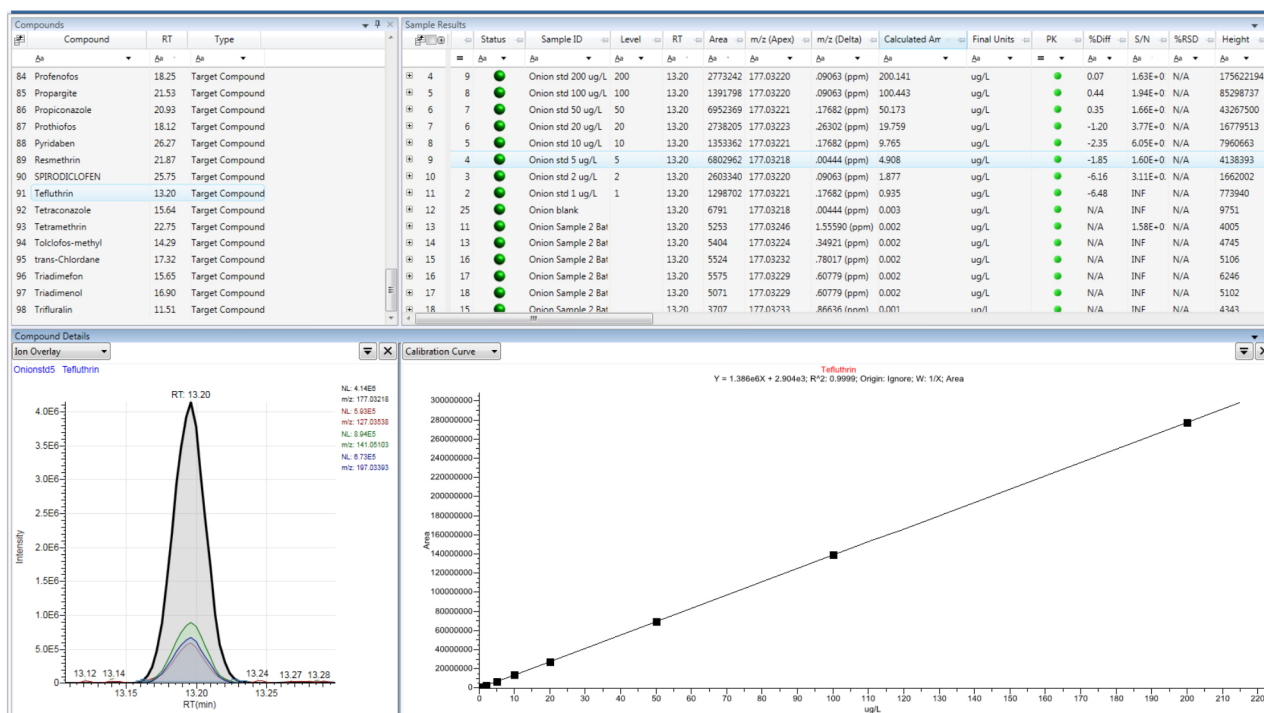


Figure 1. TraceFinder data review. Example showing 5 µg/L Tefluthrin in onion with extracted ion overlay of quan ion and three confirming ions (±5ppm window) and matrix matched calibration series

“For laboratories working in high-throughput analysis of food samples, using HRAM is a good choice and enables the lab to adapt to changing demands”

— Dr Jim Garvey

The sensitivity in full scan mode easily meets the default MRLs of 10 µg/l for 90% of the analytes. The mass accuracy for the target ion and confirmatory ions is less than 2 parts per million (ppm) for most of the analytes. Although it's only an indicative criterion, we evaluated the ratios of confirmatory ion to target ion, and again the majority of these meet the 30% level set in the SANTE document.

In addition to increasing the scope of analysis there are some clear advantages to using HRAM in a high throughput environment. One of those is that the system is very quick to set up with tuning and calibration taking minutes to perform and with data acquired in full scan, there is no need to spend time verifying that SRM windows are correct. It is the data processing options that provide the most significant advantages. With GC-QQQ-MS the analyst is limited to using only 2 or 3 SRM transitions to detect and confirm positive results. With data acquired at 60,000 mass resolution in full scan the user has additional points of identification and therefore the bottle neck of data processing and

peak verification is faster. Full scan allows any ion to be used for confirmation and can be changed according to sample matrix. Spectral matching and isotope pattern score matching can also be used to confirm detections and therefore reduce false positives and negatives. The final benefit is that the retrospective analysis can be performed, enabling compounds to be measured post acquisition or the samples profiled and compared to samples from different time points. This capability can be very useful during method validation where methods can be tweaked without the need to re-analyse samples.

Confirming suspect positives

A recent case highlighted the benefits of having full scan high resolution data, where a suspect positive for molinate from a GC-MS/MS system was proved false by high resolution data. The two molinate targeted SRM transitions produced a peak at the correct retention time and even had acceptable ion ratios. The suspect sample was re-analysed on the GC Orbitrap where the selectivity and flexibility to look at a full spectrum and multiple identification points ruled out the presence of molinate.

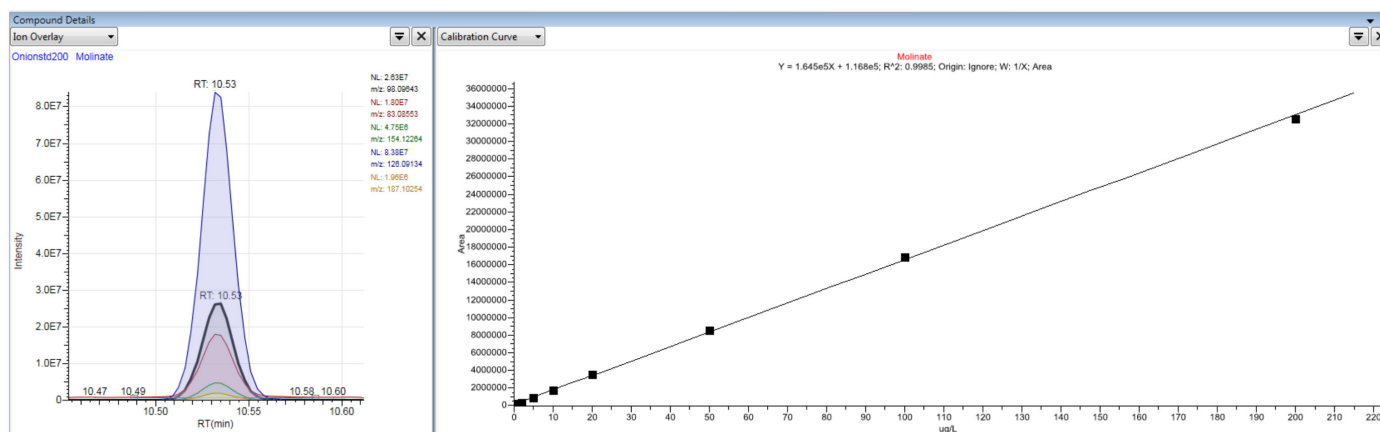


Figure 2. TraceFinder extracted ion overlay of quan ion and four confirming ions for molinate (±5ppm window) and matrix matched calibration series

“The sensitivity in full scan mode easily meets the default MRLs of 10 ppb for 90% of the analytes. The mass accuracy for the target ion and confirmatory ions is less than 2 ppm for most of the analytes”

— Dr Jim Garvey



About Dr Jim Garvey

Dr Jim Garvey graduated from The National University of Ireland (Galway) with a Ph.D. in organic chemistry. He developed his career in the pharmaceutical industry as a Research Chemist, before becoming a process development manager in industry. Since 1999, he has been at the Pesticide Control Laboratory where he is currently managing the introduction of HRAM technology to the residues laboratory. He was recently appointed Head of Food Chemistry at the department of Agriculture, Food and the Marine. As well as pesticides the Food Chemistry division also covers veterinary drugs, compositional analysis of dairy products, contaminants and elemental analysis.

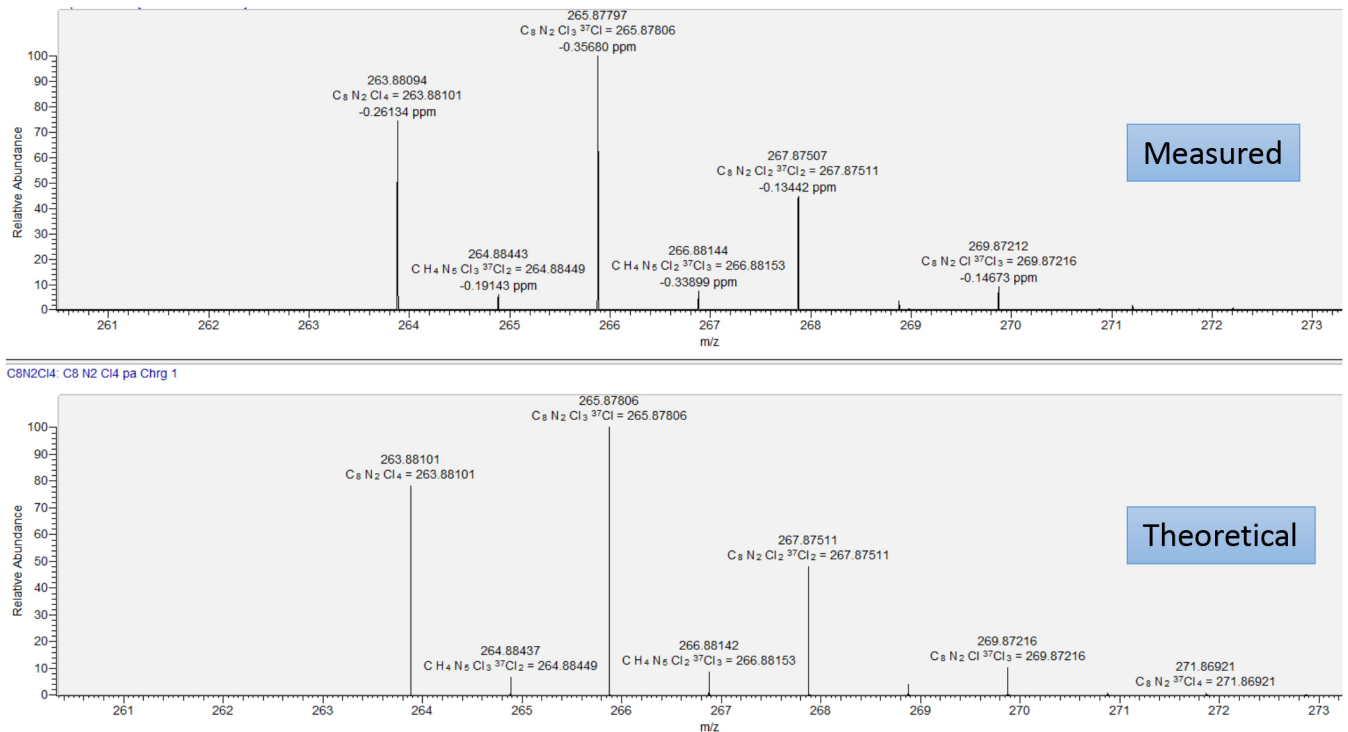


Figure 3. Comparison of measured and theoretical isotope pattern of chlordalonil molecular ion C₈N₂Cl₄. All isotopes measured with correct abundance and excellent mass accuracy

Conclusion

Confidence in the results of food safety and environmental testing is of utmost importance. The Exploris GC system brings the power of HRAM Orbitrap MS to provide the required specificity, sensitivity for target compound analyses in high throughput analytical testing. HRAM brings significant advantages compared with targeted methods including:

- Increased confidence in positive detections.
- Easily increases the scope of analysis.
- Method consolidation options.
- Faster and more confident data processing.
- Retrospective analysis.

All of these enable analytical testing and scientific research laboratories to adapt to ever changing demands.

About Pesticide Control Laboratory at DAFM

The Pesticide Control Laboratory (PCL) at DAFM is the national monitoring laboratory for the analysis of pesticide residues in food and animal feed and for the analysis of formulated products. PCL is also the designated National Reference Laboratory for the analysis of pesticide residues. The laboratory implements the monitoring program for pesticide residues in food, in compliance with EU legislation. The PCL is a fully accredited laboratory, which means that the EU Commission and our customers can have complete confidence in our laboratory's ability to analyse samples for pesticide residues.

The Pesticide Residue laboratory analyses samples of fruit, vegetables, cereals, food of animal origin including animal fat, milk and other dairy products, honey, infant and follow on formula and processed foods (including wine, juices, tinned foods and oils). Sampling of fruit and vegetables is biased in favour of food commodities that are of greater dietary importance. In excess of 800 samples of domestic and imported fruit and vegetables are taken annually. Baby food, fruit and vegetable juices as well as wine fall under this category. Samples are analysed annually for ~460 pesticides and their metabolites.



References

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2. European Commission Directorate General for Health and Food Safety, Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/12682/2019 (Brussels, Belgium, 2019). https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf

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