

Shifting the Paradigm in GC-MS Pesticide Analysis



Amadeo Fernández-Alba, Ph.D.
*European Reference Laboratory
for Pesticide Residues in
Fruits and Vegetables,
Analytical Chemistry Dept.,
University of Almería, Spain*

Introduction

There is growing interest in the use of gas chromatography high-resolution accurate mass (HRAM) mass spectrometry for the non-targeted screening of pesticides in food, an approach enabled by the improved full-scan performance of the latest GC-HRAM systems. It is now possible to analyze targeted, suspected, and unexpected residues in a single workflow with an easy-to-use GC-MS system that provides an unprecedented level of performance in routine analysis. This manuscript describes the evaluation of the required resolving power, mass accuracy, and compliance with the SANTE guidelines for the quantification and identification of pesticides. The ease of use and maintenance, as well as other potential benefits from the implementation of full-scan GC-MS technology in the routine analysis of pesticide residues in food, are also considered.

Sample Preparation and Instrument Setup

A targeted list of 210 different pesticides, including the pesticides in the European Multiannual Control Programme, was used for the evaluation. Sample extraction was performed using the European version of the QuEChERS approach based on acetonitrile with a citrate buffer. An aliquot, normally 100 μ L, of the acetonitrile extract was evaporated to near dryness and reconstituted in ethyl acetate prior to splitless injection (1 μ L). Typical GC conditions are summarized in **Figure 1**.

To facilitate the analysis, the Thermo Scientific™ TraceFinder™ Software compound database, which has accurate mass information for more than 700 different contaminants, can be used in combination with the classical nominal mass NIST database. Databases can also be generated manually by using the Thermo Scientific™ Xcalibur™ Software. Standards are used to obtain retention times and theoretical masses of the fragments of interest. When constructing databases, it is important to assign exact masses, rather than measured (accurate) masses, to enable more accurate assessment of mass errors. We prefer to use as many fragments as possible, typically 3–5.

During instrument setup, it is important to consider spectral resolution, mass accuracy, sensitivity, linearity, reproducibility, and robustness. In practice, a resolving power of 60,000 provides a good compromise between the number of data points required for good quantitation, and sufficient mass resolution of the ions of interest from coeluting matrix ions, even when analyzing difficult matrices as shown by the analysis of carbofuran in leeks (see **Figure 2**).

To obtain excellent mass accuracy, there are two options: use lock mass or use external calibration. External calibration is preferred for routine analyses since the error differences between the two approaches are relatively small. Also, co-extractives from complex matrices can occasionally interfere with the lock mass, resulting in increased mass errors. The mass accuracy is always stable across the peak and is typically less than 1 mDa. Another important requirement is to optimize the automatic gain control (AGC). In general, an AGC setting of 1e6 will provide a good area response and good peak shape.

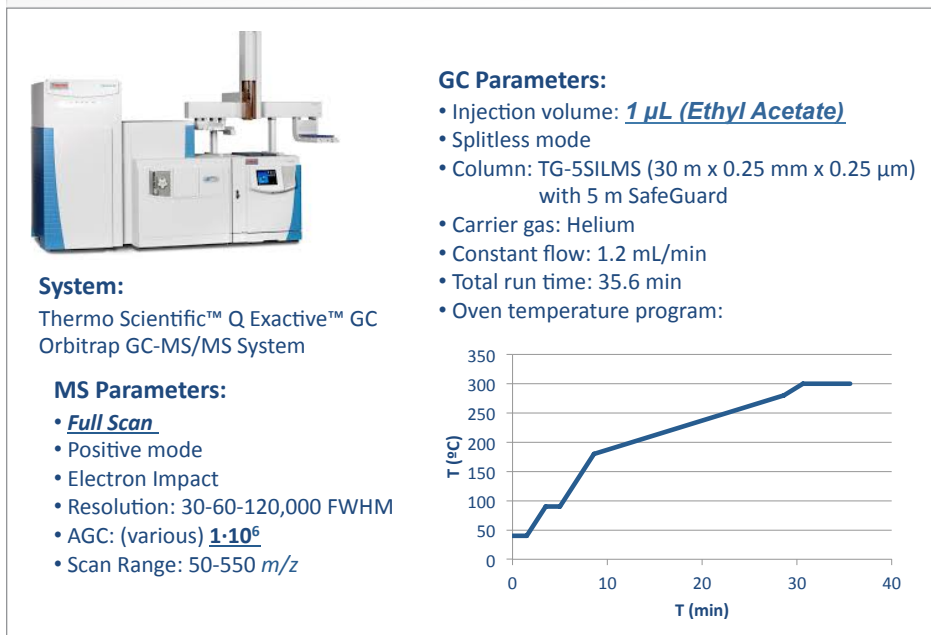
Until now, poor sensitivity has been one of the main limitations of GC-HRMS, particularly when analyzing complex matrices like leek. Fortunately, the latest Thermo Scientific™ Orbitrap™ GC-MS systems are capable of routinely achieving detection limits of 10 ppb over a linear range up to 500 ppb, with precision of 10% RSD or less, for the majority of pesticide-commodity combinations. The precision exceeds 20% in very

SPONSORED BY

ThermoFisher
SCIENTIFIC

LC|GC
north america

Figure 1: GC-MS conditions.



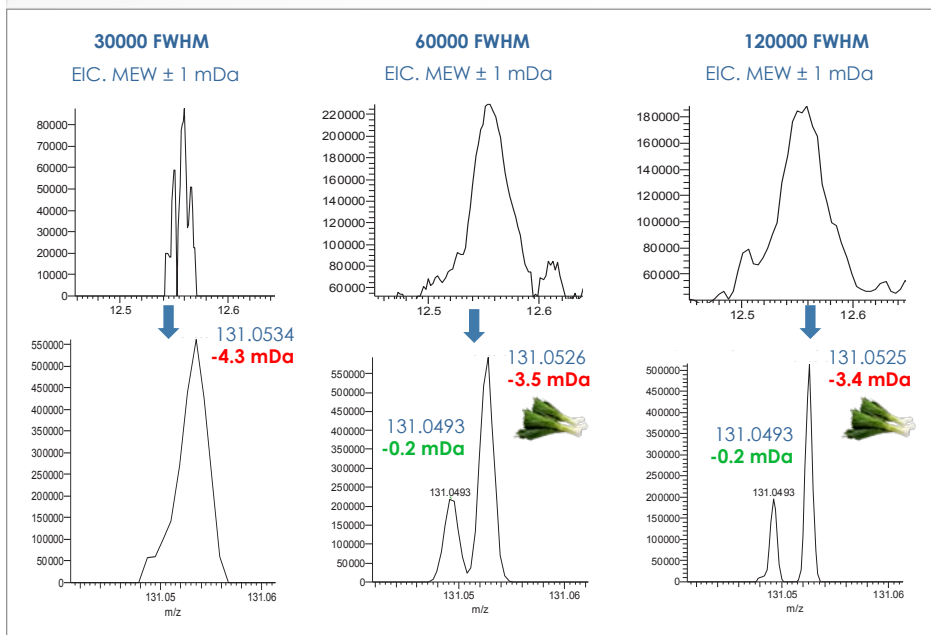
time; typical results for hundreds of samples injected over 12 days are below ± 0.4 mDa.

Benefits of a GC-Full Scan MS Workflow

There are several stages in a typical GC-MS pesticide residue analysis workflow: batch creation, raw data file duplication, file processing, and manual revision and exporting of the results to the final reports. The Thermo Trace Finder 4.0 software automates this workflow, thus allowing the analysis of 18 samples, including two calibration curves by matrix-matched standards (three levels), two recovery checks, and five solvent injections in as little as one hour. Using a full-scan MS like the Orbitrap GC-MS system in combination with Trace Finder Software can provide a significant advantage over the GC-triple quadrupole instruments, as illustrated by the analysis of buprofezin at 10 ppb in the tomato matrix (see **Figure 3**). As seen in **Figure 2** on the right, the second transition on the triple quadrupole instrument is very low. Even the third transition on the Orbitrap GC-MS system (lower trace) shows excellent sensitivity.

Another benefit to using full-scan non-targeted acquisition workflows is that the acquired data can be used for both target and non-target data processing. The decision is made after acquisition and is dependent on the software, libraries, and databases used for data processing. The main difference between the “target” and “non-target” data processing approaches is that the

Figure 2: The benefit of resolving power in the analysis of carbofuran in leek matrix.



few cases, which means that the data are essentially in full compliance with the SANTE guidelines.¹ It is possible to detect almost 100% of the pesticides at 5 ppb with one fragment ion and almost 100% of the pesticides at 10 ppb with two fragment ions. The number of pesticides detected in the leek matrix was lower than in other matrices, but this is not a problem with the instrument; rather, it is a real problem with the leek matrix. The high concentration of sulphur compounds in leek traps chlorinated pesticides, thus reducing their detector response. In our laboratory, the robustness of the Orbitrap GC-MS system was evaluated by injecting isotopically labeled standard over

more targeted approach uses databases that include retention time information, while the non-targeted libraries (e.g., NIST) do not include retention time information.

For targeted analysis, the EU quality control guidelines specify criteria for: retention time ± 0.1 minute, % ppm mass error (or 1 mDa < 200 m/z), and two ions < 5 ppm, with a ratio of within $\pm 30\%$, compared to the same ions in the standard. By contrast, the U.S. FDA acceptance criteria for identity confirmation of chemical residues using exact mass² data considers only the retention time, not the ion ratio.

Analysis of Samples

Test materials of potato, green pepper, broccoli, and spinach from the official European Union Proficiency Testing (EUPT) scheme for screening methods were analyzed using the Orbitrap GC-MS system in combination with databases including retention time information. All 24 pesticides (100%) were detected and identified with no false detects, as summarized in **Figure 4**.

Screening without Retention Time Information

When working without a target list, or without retention times, peaks at all retention times are candidate compounds. Automatic checks against the NIST library or user databases with two, three, or four ions are performed; the more ions used, the lower the number of false negative and/or positive detects obtained. For example, as presented in **Figure 5**, only three ions are reported for picolinafen from the database or NIST library spectrum (lower trace).

While the experimental spectrum also yields three ions (upper spectrum in **Figure 5**), the ion at 256 has a different relative abundance in the library database versus the experimental spectrum. This is a rare occurrence, but when it does happen, without retention information, the identification can be in doubt. Nonetheless, by comparing the two other ion ratios for m/z 238 and m/z 376 against a standard and with the retention time, a positive identification can be made, as illustrated in **Figure 6**. Not every pesticide produces as many as four ions on electron ionization. Ultimately the identification or quantitation of the remaining candidates must be performed *a posteriori* with a second analysis alongside the analysis of the relevant standard reference material.

It is also important to consider the number of ions used, especially to minimize false detects. These peaks, which result from the matrix, not a pesticide, require the data to be re-evaluated, needlessly affecting laboratory efficiency and workflow. A worst case is false negative detects whereby pesticides actually present in the sample go undetected; unlike positive detects, false negative results are not scrutinized.

Figure 3: Comparison of selectivity between GC-QqQ-MS/MS and Orbitrap GC-MS.

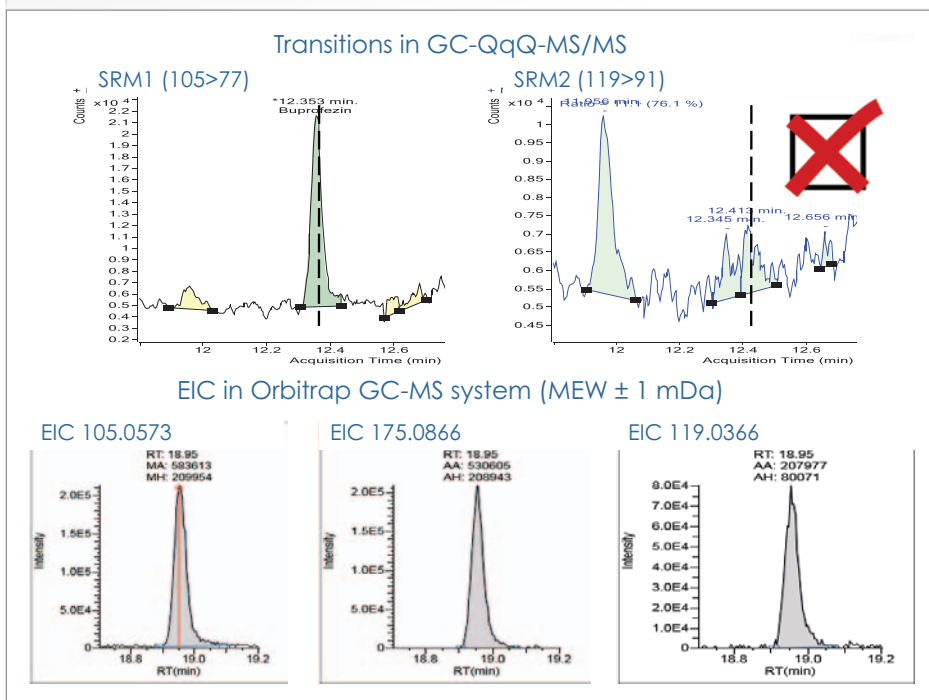


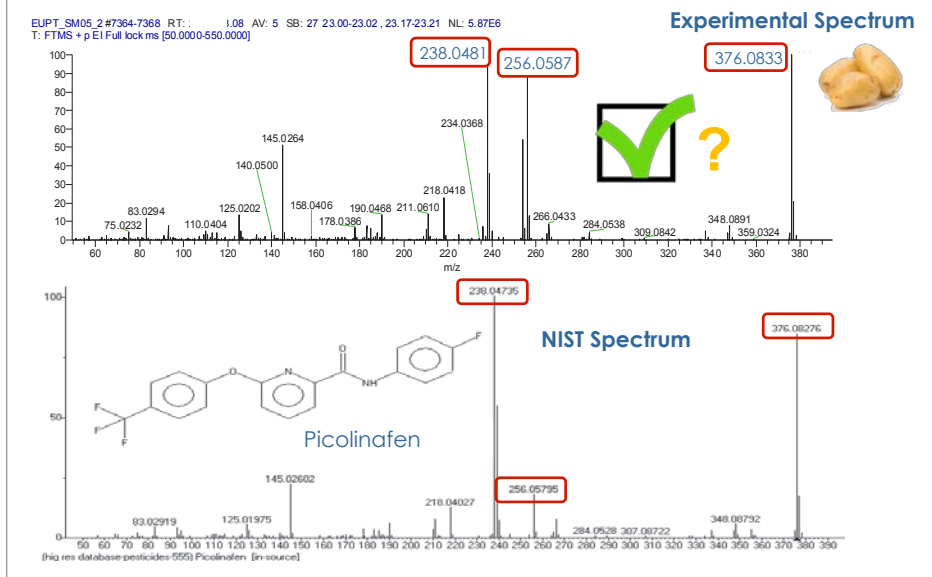
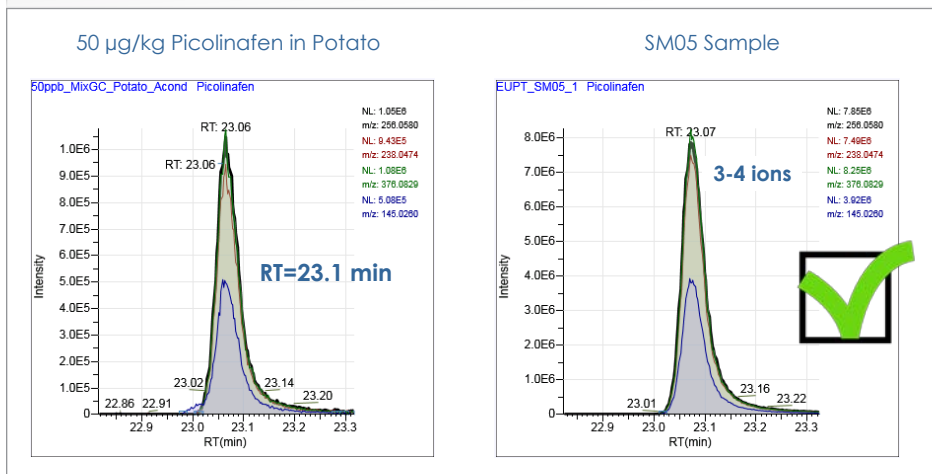
Figure 4: Results obtained using a database with retention time for the targeted analysis of pesticides in EUPT samples.

	Matrix	No. Compounds	Compounds Screened	2 ions < 5 ppm	Ion ratio within 30%
SM05	Potato	5	210	✓	✓
SM06	Green Pepper	11	210	✓	✓
SM07	Broccoli	1	210	✓	✓
SM08	Spinach	7	210	✓	✓

100 % detection/identification rate: No False detects

The advantage of including a higher number of fragment ions is to reduce the number of false detects and additional work, especially when retention time information is not available. An example is summarized in **Figure 7**.

The four different EUPT-test materials analyzed above (see **Figure 4**) were evaluated without using retention time information (see **Figure 7**). Even without retention times, 100% of the pesticides across all samples were detected, but the number of false positive detects varied depending on the number of ions used; 52 false detects were obtained with two ions, 18 with three ions, and 10 with four ions. This further emphasizes that the more ions used, the lower the number of false negative and/or positive detects that are obtained.

Figure 5: Detection of picolinafen using the NIST library (no retention time information).

Figure 6: Detection of picolinafen using a database with retention time information.

Figure 7: Results obtained using a database without retention time for the targeted analysis of pesticides in EUPT samples.

	Matrix	No. of detected Compounds	Compounds Screened
SM05	Potato	5 (100%)	210
SM06	Green Pepper	11 (100%)	210
SM07	Broccoli	1 (100%)	210
SM08	Spinach	7 (100%)	210

No of False Detects (-); 100 % detection rate

Number of False Detects with 2 ions (+52), 3 ions (+18) or 4* ions (+10)

Conclusion

The Thermo Scientific Orbitrap GC-MS system enables new comprehensive workflows for the simultaneous quantitative analysis of targeted compounds and qualitative screening of untargeted pesticides in routine laboratories.

Excellent mass accuracy (<1 mDa) for each scan across a peak in a complex matrix, at a resolving power of 60,000, and the simplicity of one acquisition event to get multiple accurate mass ions that can make use of existing libraries (such as NIST and Wiley) offer significant advantages for pesticide residue analyses. Other benefits include the possibility to increase the scope even without standards.

Non-targeted acquisition allows the screening for obsolete pesticides and pesticides for which standard reference materials are extremely expensive or not commercially available. Even pesticide transformation products that are produced by the analytical method can be detected. Above all, the Exactive™ GC Orbitrap™ GC-MS System, should provide improved efficiency and accuracy of screening workflows in routine laboratories.

References

- European Commission Directorate-General for Health and Food Safety (SANTE)/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. http://ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdoc_11945_en.pdf (accessed 25 Nov 2016)
- FDA Office of Foods and Veterinary Medicine. Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data. <http://www.fda.gov/ScienceResearch/FieldScience/ucm273423.htm> (accessed 25 Nov 2016)