An Executive Summary

# Implementing GC-HRAM MS for More Efficient and Effective Routine Pesticide Residues Analysis

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The Exactive GC Orbitrap MS system can offer the specificity and sensitivity missing from traditional methods in pesticide residue analysis.

## Overview: Pesticide Residue Analysis and GC-HRAM-MS

Pesticide residue analysis in food and feed products is a challenging endeavor. With 1,630 entries in *The Pesticide Manual*[1], there are a multitude of different compounds to analyze for within hundreds of different food and feed matrices that vary in composition and complexity. This analysis must be able to detect a large number pesticides at low levels, typically 10 ng/g and occasionally at high levels up to 50 µg/g.

Pesticides are basically small molecules that are analyzed by mass spectrometry (MS) coupled with liquid chromatography (LC) or gas chromatography (GC). Some compounds can only be analyzed with LC-MS, while others only by GC-MS. Meanwhile, numerous pesticide compounds can be analyzed by both techniques and in these cases, we can have confirmatory analysis of the GC results by the LC, and vice versa.

Then the question for the analyst is whether to use targeted or non-targeted measurement. There are a number of options for targeted analysis including selected ion monitoring (SIM), and MS/MS. Both options require the analyst to specify, in advance, which pesticides are to be acquired by the instrument. It is not an easy task to set-up the instrument for hundreds of different compounds in one run, especially in the case of MS/MS, which requires the optimization of each precursor to product ion transition. While these techniques provide the highest sensitivity, the number of compounds that can be included in a single analytical run is limited. For example, around 150 pesticides are typically included in a GC-MS/MS targeted analysis.

An emerging approach is to perform non-targeted measurement using full-scan continuous acquisition of all ions during an entire chromatographic run. This more straightforward analysis has an almost unlimited scope; any compound present in the extract that elutes from the column and gets ionized will be detected. In addition, this technique allows analysts to retrospectively analyze the raw data for compounds that were not the focus of the initial analysis.

GC-MS systems have two main types of mass analyzers: nominal mass and high resolution accurate mass (HRAM). In general, single-quadrupole mass spectrometry with nominal mass does not provide the selectivity that is required for pesticide residues analysis, especially if analyzing generic type QuEChERS extracts. For this reason, pesticides analysis is often conducted with nominal mass triple-quadrupole MS/MS because of the higher selectivity compared to single-quadrupole systems.

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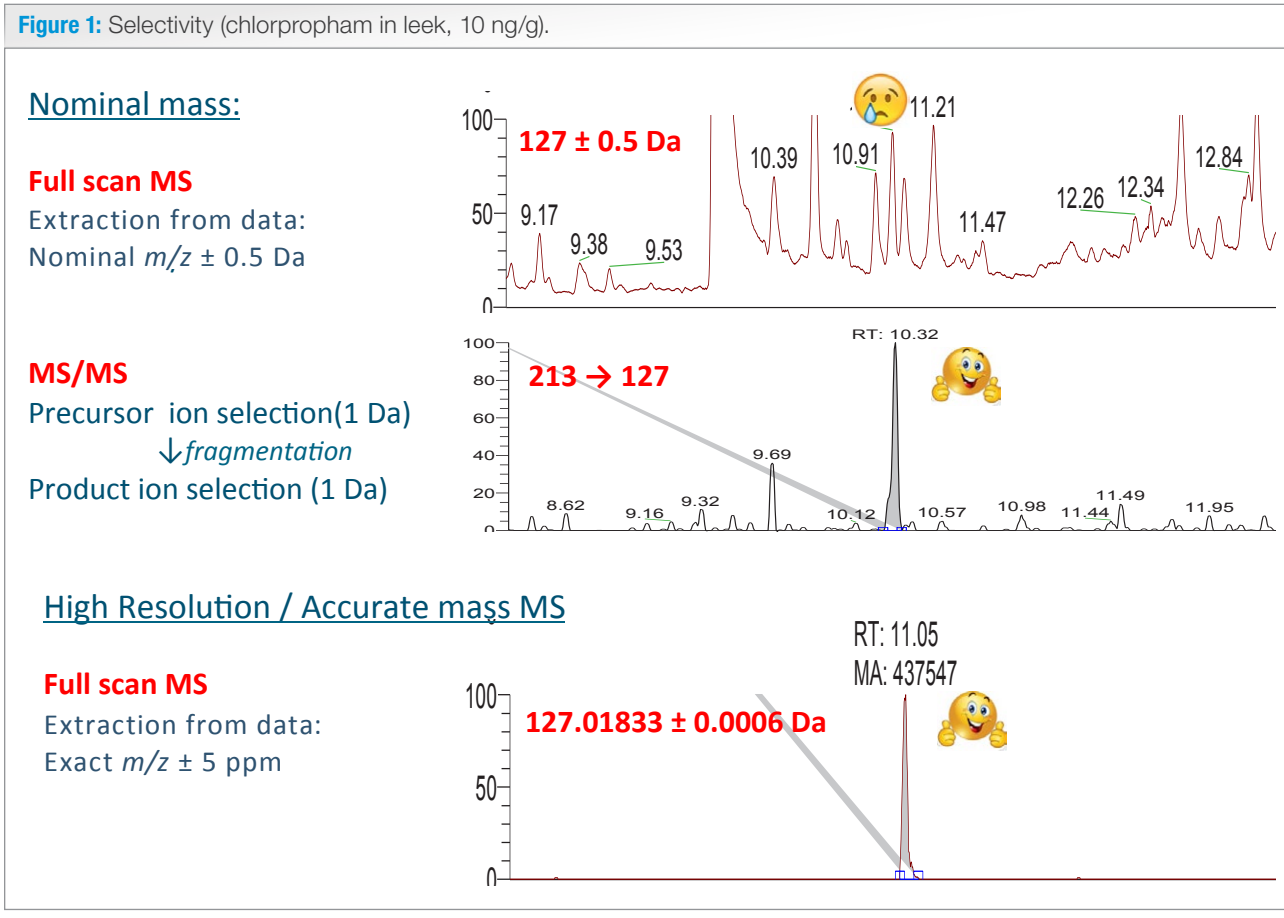
**Figure 1:** Selectivity (chlorpropham in leek, 10 ng/g).




The latest possibility is to use HRAM mass spectrometry using either Time-of-Flight (ToF) or Orbitrap technology. Full-scan acquisition using a GC-Orbitrap system can provide at least equal, if not better selectivity than GC-MS/MS as shown in **Figure 1**.

The top extracted ion chromatogram (XIC) in **Figure 1** illustrates the poor selectivity of mass spectrometry with nominal mass (either full scan or SIM) in a leek sample spiked with chlorpropham at 10 ng/g.

The middle chromatogram shows that nominal mass MS/MS with a triple quadrupole increases selectivity in this targeted measurement.

In the bottom chromatogram, one can see how extraction of an ion with accurate mass and using a narrow mass extraction window (*m/z* ± 5 ppm) from the full-scan data provides the same degree of selectivity as triple-quadrupole MS/MS. The GC-HRAM-MS instrument that collected this data was the Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS system.

## Exactive GC-Orbitrap MS System

A photo of the Exactive GC Orbitrap GC-MS system is shown in **Figure 2**. Two types of GC Orbitrap MS instruments are available: the Exactive GC Orbitrap MS system and the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system.

Both systems have an electron ionization (EI) source or a chemical ionization source (positive or negative), a quadrupole, a C-trap, and the Orbitrap mass analyzer. The Q Exactive system also has a collision cell, allowing for MS/MS analysis and a resolving power of 120,000 (FWHM @ *m/z* 200); the Exactive GC Orbitrap MS system can achieve resolving powers as high as 60,000 (FWHM @ *m/z* 200).

**Resolving power.** With both systems, analysts can choose from several resolving powers. Choices for the Exactive GC Orbitrap MS system are 15,000, 30,000, and 60,000 (FWHM @ *m/z* 200); the Q Exactive GC offers these in addition to 120,000, as noted.

With increased resolving power, scan speed decreases. For instance, if the Exactive GC Orbitrap MS system was set at a resolving power of 15,000, the scan speed would be around 18 Hz. At 60,000, the scan speed decreases to around 7 Hz, and at 120,000 (a resolving power obtainable with the Q Exactive GC), scan speed drops to 3–4 Hz. Selectivity is improved and sensitivity is not affected by increasing resolving power of the Exactive GC Orbitrap MS systems. All of the scan speeds are compatible with capillary gas chromatographic separations with no trade-off with respect to sensitivity.

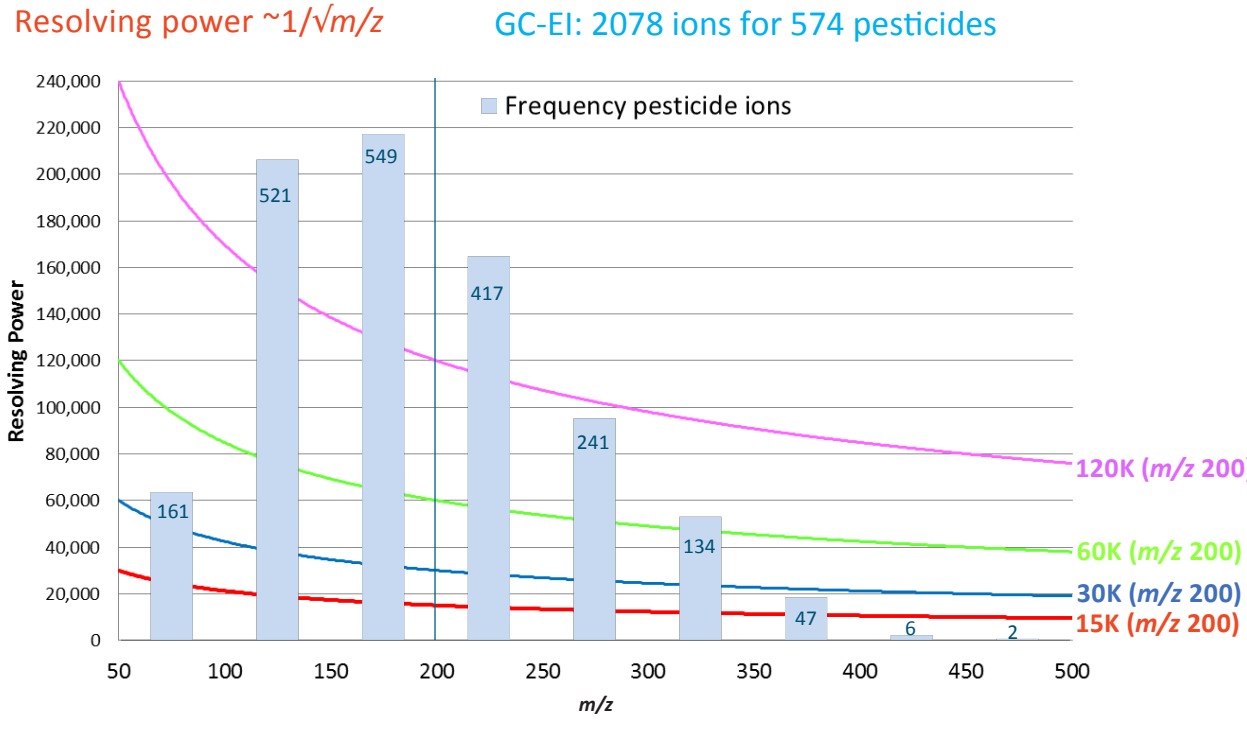
In addition, the resolving power of Orbitrap systems is higher at lower *m/z* values, which is beneficial for small molecules such as pesticides. **Figure 3** shows that the majority of *m/z* values for the quantifier and qualifier ions for 574 pesticides are below 200 *m/z*.

**Comparison to NIST library.** EI Orbitrap spectra were compared with spectra generated with quadrupole instruments. In most cases, the spectra from the Orbitrap system are highly similar to the spectra in the NIST Library. Some differences were found at *m/z* values lower than 90. This did not prove to be much of a problem for library matching because lower *m/z* values usually have a lower weighting in the match value.

**Spectral quality of EI spectra versus amount injected.** Another important aspect to consider is the spectral quality of EI spectra versus amount injected. Maximum residue levels (MRLs) vary widely by pesticide. It is possible that a 1 µL injection of a QuEChERS extract (1 g crop/mL) may have a range of absolute picograms on-column ranging from 5 picogram (if fipronil was present at the MRL) to 30,000 picogram (if boscalid was present at the MRL). Ensuring good-quality spectra and

**Figure 2:** GC-Orbitrap-HRMS: Exactive GC Orbitrap GC-MS system.

**Figure 3:** Most *m/z* values for the quantifier and qualifier ions for 574 pesticides are below 200 *m/z*.



consistent ion ratios across this broad range of concentrations is a concern.

To investigate this matter, hexachlorobenzene at a range of concentrations (0.1 pg–250 pg on column) in solvent standard was studied. Mass spectra were obtained using the Orbitrap mass spectrometer; the results showed that mass accuracy was consistent for all ions across all concentrations, as well as the spectral profile when compared with each other, and with a nominal mass NIST spectrum. The response factors and ions ratios were also consistent across the entire range of concentrations.

## System Set-Up

**Mass extraction window.** Starting with the setting up of the Orbitrap MS: analysts can set up various parameters for full-scan measurement including the ionization mode (typically EI), mass transfer line and ion source temperatures, the mass range and parameters for the C-trap. The automatic gain control was set at the maximum 5e6 and the maximum inject time (MIT) set at 25 ms. It is important to re-emphasize the importance of resolving power selection. The higher the resolving power chosen, the better the mass separation of isobaric compounds, the narrower one can set the mass extraction window to filter the exact mass from the raw data, and the higher the selectivity.

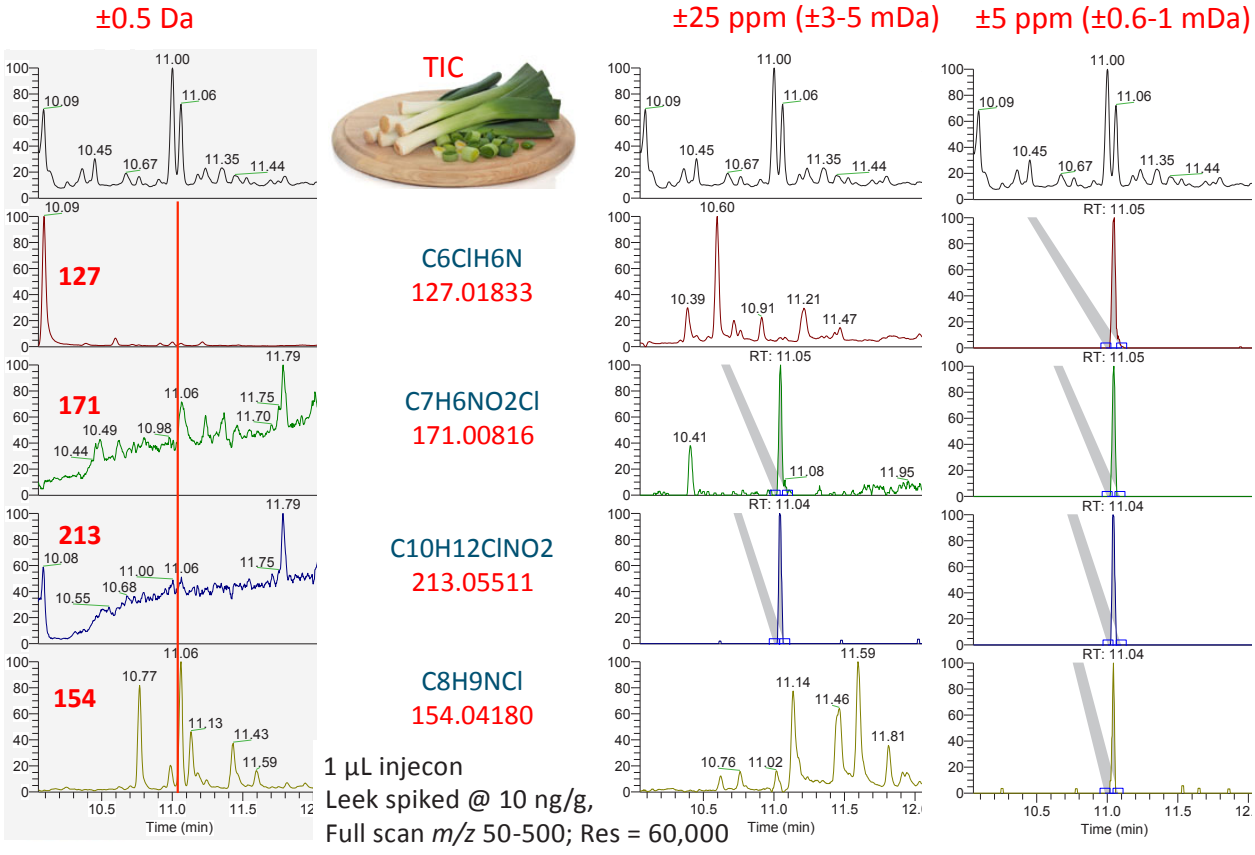
**Figure 4** illustrates the importance of the effect of the mass extraction window on selectivity. Chlorpropham was spiked into leek extract at 10 ng/g and injected onto the Exactive GC Orbitrap MS system. The far-left chromatogram simulates the results that would be generated with a nominal mass quadrupole-only system and a mass extraction window of ±0.5 Da. Selectivity is insufficient.

The central column shows extracted ion chromatograms processed with a mass extraction window of ±25 ppm (±3–5 mDa). Two ions—*m/z* 171.00816 and *m/z* 213.05511—have clean extracted ion chromatograms, but a lot of interference appears for *m/z* 127.01833 and *m/z* 154.04180.

The effect of narrowing the MEW further is illustrated in the far-right column: with a mass extraction window of ±5 ppm (±0.6–1 mDa) all four ions show clean extracted ion chromatograms because of improved selectivity.

This suggests one must have a mass accuracy of 5 ppm or better for the analysis of real, and especially, complex samples. For different pesticide matrix combinations, what type of mass resolving power will achieve this 5-ppm mass accuracy?

**Figure 4:** MEW ↔ selectivity: chlorpropham in leek.



Based on a data set that included 54 compounds in seven matrices, resolving powers of 15,000 and 30,000 are not sufficient. A resolving power of 60,000 and above were found to achieve a mass accuracy of 5 ppm or better, allowing for the use of narrow mass extraction windows (see **Figure 5**). The high selectivity obtained is equal or better than that achieved with triple-quadrupole EI-MS/MS in most cases. However, the selectivity of selected ions varies, even with accurate mass, as shown by the example of cypermethrin in oranges (see **Figure 6**). In this case, it is not a problem because a sufficient number of ions with clean spectra are available. The situation with tetramethrin, a compound composed of C/H/N/O and hence a relative low mass defect, is different. Only *m/z* 164.07061 gives a clean spectra with *m/z* 107.04914, 123.11683, and 135.04406 suffering from interference. Higher resolving does not help because the ions of the matrix coextrative compounds have the identical exact mass as the ions of the analytes of interest. One way to obtain a sufficiently clean qualifier ion in this case is to use the more selective C13 isotope (*m/z* 165.07396) of *m/z* 164.

**GC column selection.** Because, in some cases, interferences can occur with the same exact mass and cannot be resolved mass spectrometrically, then chromatographic separation remains an important component of the analysis. Examples include p,p-DDD/o,p-DDT, which coelute partially, and chlorpropylate/chlorbenzilate, which co-elute perfectly using 5% phenyl dimethyl polysiloxane phases typically used for pesticides analysis. The use of a more dedicated column the TG-OCP I (a functionalized polysiloxane) produced base separation of these critical pairs (see **Figure 7**).

**Injection.** Injection is a critical component of the GC system. For volumes up to 1 µL, hot splitless injection can be used. With a relatively large liner and a CarboFrit insert, this injection mode offers the best performance for more polar solvents such as acetonitrile in QuEChERS-type extracts.

For higher volumes (e.g., 5 µL), a programmed temperature vaporizing (PTV) injector in solvent mode can be used. Best results for extracts in acetonitrile were obtained using a liner with a sintered inner wall, in combination with a syringe that sprays the liquid sideways onto the wall of the liner. This helps to prevent droplets from reaching the bottom of the liner and potentially flooding the column. This injection mode gave a very consistent robust performance with RSDs less than 10% for the majority of 93 pesticides across a series of 60 successive injections.

## Software Database and Processing

Several commercial compound databases or libraries are

**Figure 5:** Resolving power required for ±5 ppm MEW.

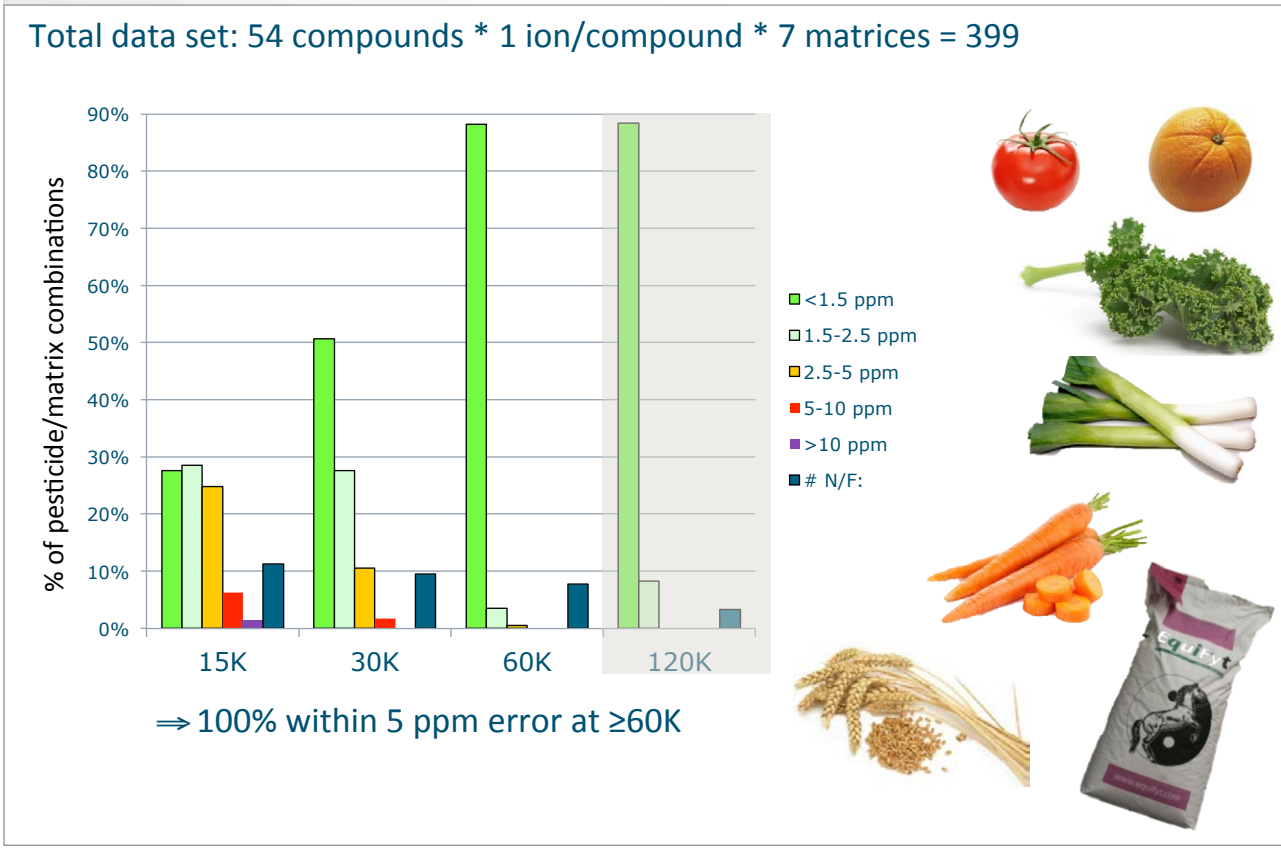
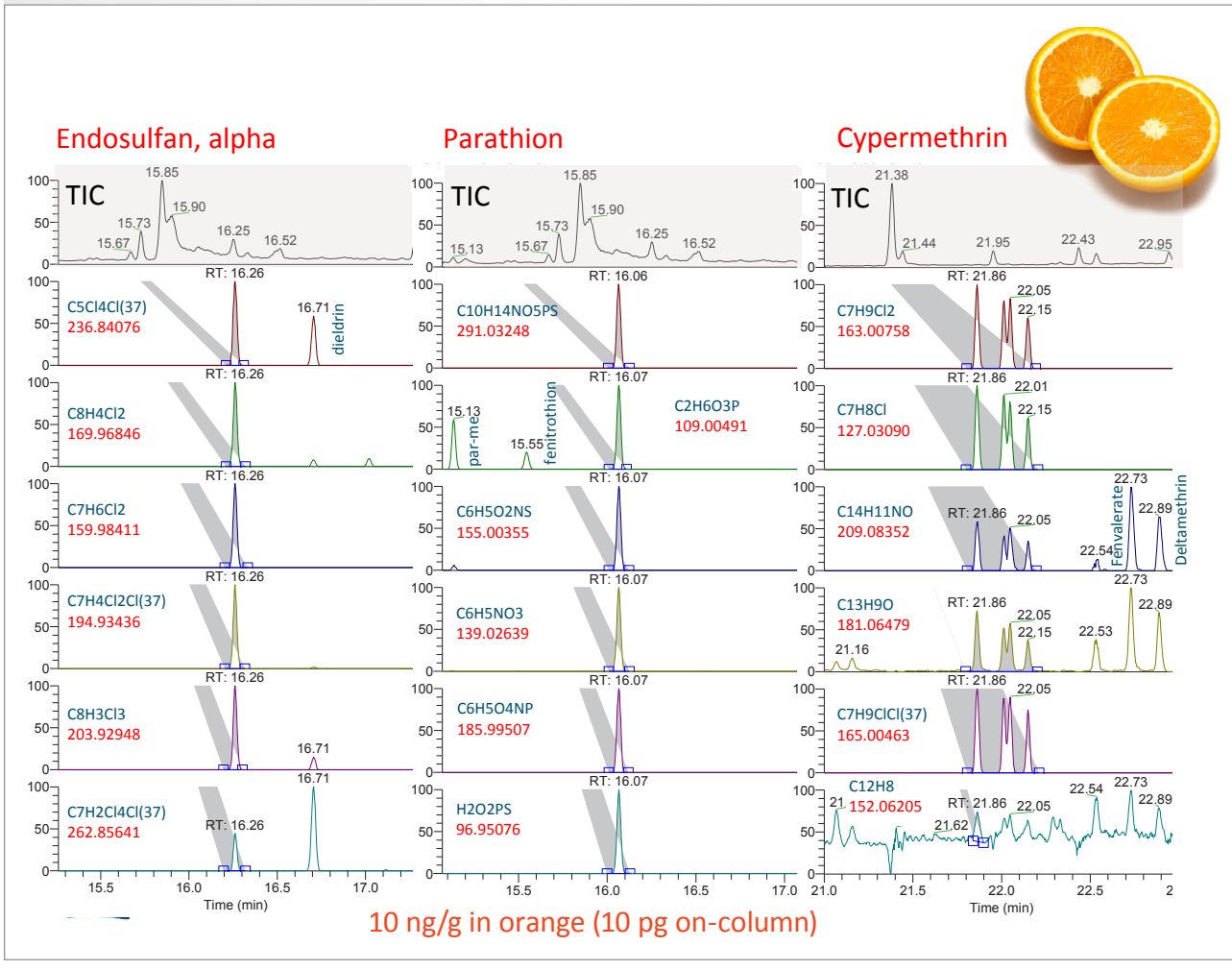


**Figure 6:** Selectivity MEW ±5 ppm.




available for GC-EI MS analysis, but most of these are nominal mass, not accurate mass.

For pesticide applications, Thermo Fisher Scientific has a Thermo Scientific™ TraceFinder™ software library with accurate mass spectra that includes approximately 700 entries, and the number is growing. These accurate mass spectra can be used for quantitative data processing, but ideally it would be better to use exact mass libraries.

To obtain exact masses, one must derive the elemental compositions from the spectra, which requires ions to be annotated. TraceFinder software incorporates a workflow to assist with this task. Solvent standard is injected at the highest resolving power and the peak is found. The annotation aid from the TraceFinder software is used to assign the elemental composition. From there, one can calculate the exact mass for the ion fragments and then perform verification. We can then verify further by creating extracted ion chromatograms using the exact masses calculated in the above process. Then, the most suitable quantifier and qualifier ions can be selected and the data then entered into a user database. The database should contain the name of the compound, the CAS number, the molecular formula of the pesticide, exact mass of the molecular ion, the retention time of the pesticides, the molecular formula, and the exact mass of the quantifier and qualifier ions.

All of this information is then used to create a quantitative data processing method in TraceFinder software. The screen views can be adjusted, for example to display one compound in multiple samples for faster review.

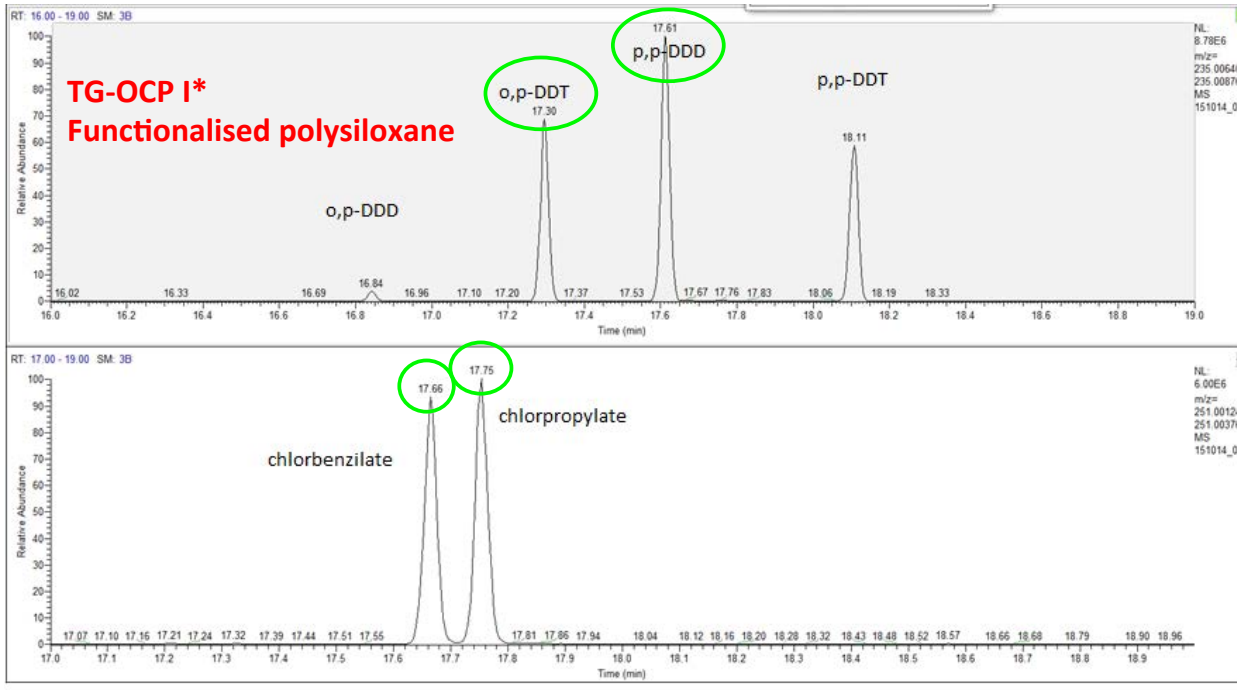
## Quantitative Performance

Firstly, the quantitative performance of Exactive GC Orbitrap MS system for pesticide residue analysis in fruits and vegetables is considered.

The calibration curves in three different matrices (leek, orange, and tomatoes) are typically linear with deviations in back calculated concentrations less than 20% for nearly all compounds.

In a study of 54 pesticides in leek, orange, and tomato,

**Figure 7:** GC separation: critical compounds separated.



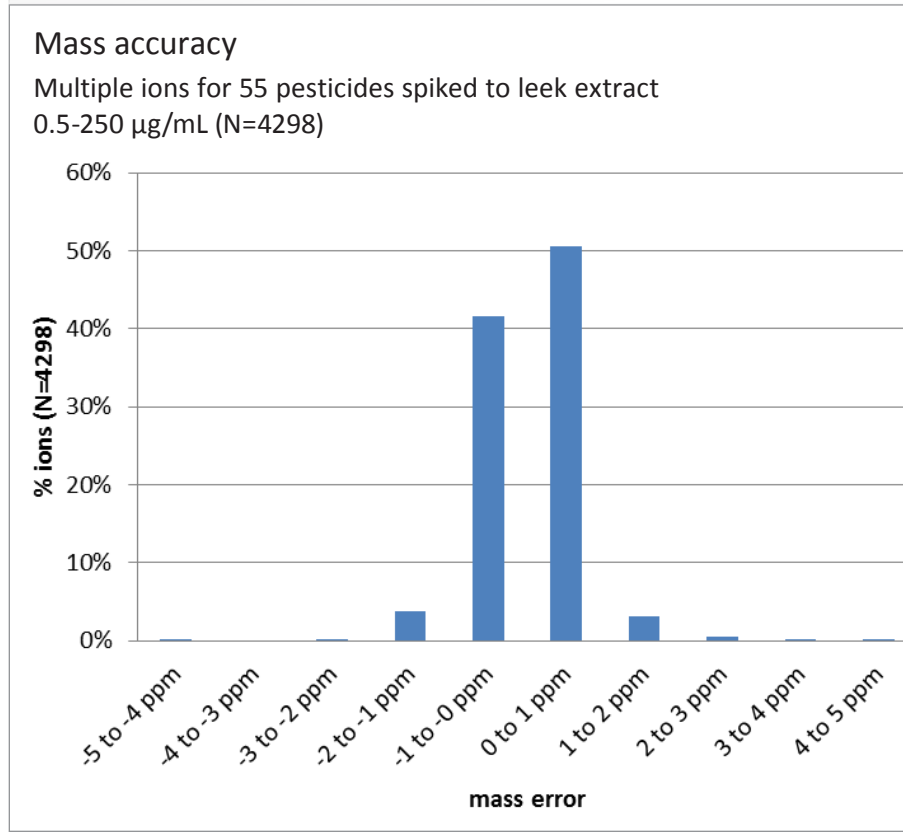

*Similar to: Rtx-CLPesticides

the limit of detection (LOD) and limit of quantification (LOQ) values were 0.5 ng/g or lower in most cases, and all below the default 10 ng/g concentration most laboratories are trying to achieve.

In terms of meeting identification criteria (SANTE/11945/2015[2]), the mass accuracy complies with the guideline 5 ppm and was better than 1 ppm in most cases (see **Figure 8**). Retention time was highly stable (within ±0.1 minute from the reference values acquired in the same chromatographic sequence) in all cases. The limit of identification, fully compliant with the SANTE ion ratios guidelines, was 0.5 ng/g for the majority of compounds and with the exception of 2–3 compounds always less than 10 ng/g.

The full-scan Exactive GC Orbitrap MS approach was also applied to more complex

**Figure 8:** Meeting the identification criteria.



sample types of cereals and feed commodities. This study was carried out using a modified extraction method (0.25 g/mL), but essentially the same instrumental parameters. QuEChERS extracts spiked with 85 pesticides and 6 PCBs were injected (5 µL) directly without solvent exchange. The method validation was done using matrix-matched standards for wheat and standard addition for feed and feed ingredients.

Five different blank samples of varying complexity were evaluated for selectivity. In all cases, no signals were detected above 30% of the reporting limit (the SANTE guideline) demonstrating the excellent selectivity of the Exactive GC Orbitrap MS system.

The average recovery and repeatability for wheat, based on matrix-matched calibration, were excellent and except for two compounds (bromopropylate and endosulfan) were fully compliant with the SANTE guideline criteria (see **Figure 9**). In terms of the validation results for a mixed set of feed samples, analyzed using the standard addition approach, the average recovery was good (71–120%) and repeatability was nearly as good. This slightly higher repeatability compared to wheat was attributed to more lipophilic compounds and PCBs, which were lost into fat that is present in these types of samples. The amount of fat in the different commodities varied as did recovery.

Overall, five outliers were found in the 455 pesticide/matrix combinations. Two wheat compounds—bromopropylate and endosulfan sulfate—co-eluted exactly with a very intense matrix peak at 17.10 minutes. This is a limitation of the C-trap, which restricts the number of ions it samples and may affect analyte detectability. One can overcome this issue by cleaning out the matrix ion via SIM, using the quadrupole to select only the mass of the analyte (e.g. bromopropylate) to restore sensitivity. As we do not want to change the method from a non-targeted to targeted method, this can be done by maintaining the full-scan acquisition but adding a SIM event to enable combined full-scan and SIM in the same run.

This work demonstrates that it is possible for quantification and identification of the usual suspects (pesticides found regularly) in the samples in compliance to official guidelines, but we can also add automated qualitative screening of unexpected pesticide residues as well. The qualitative screening is performed on the same existing raw data but with a reduced analytical quality control burden.

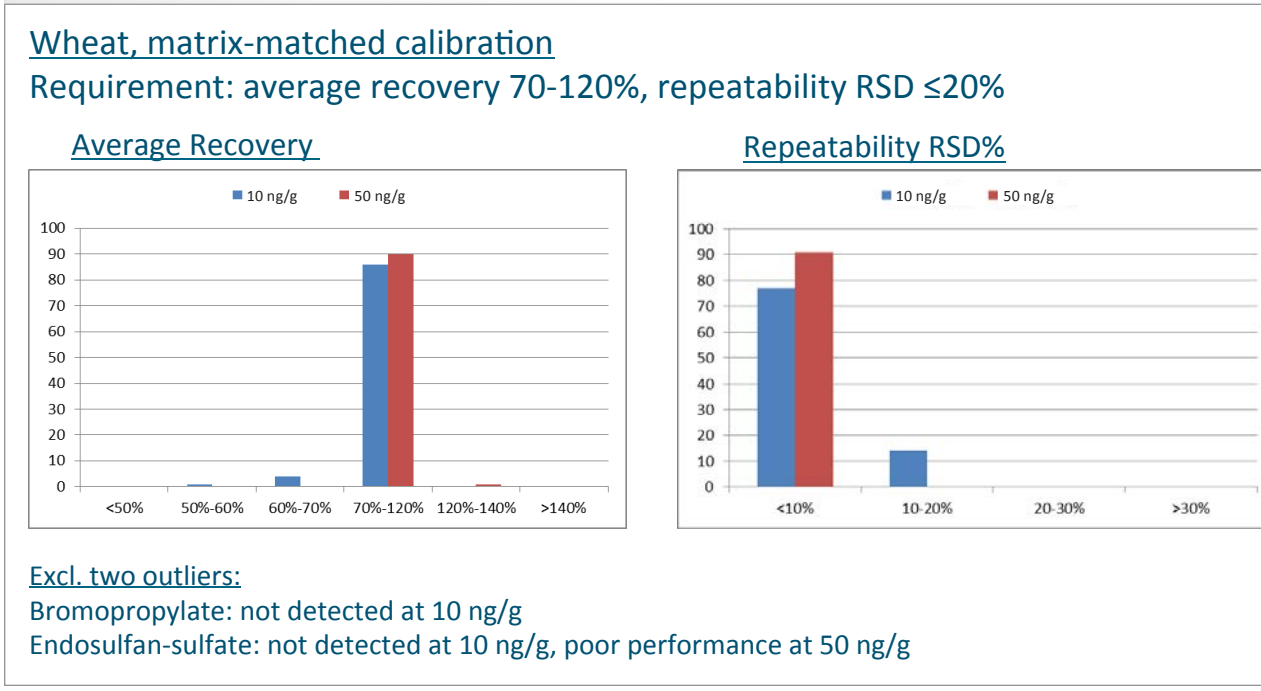
## Qualitative Screening

In terms of qualitative screening, a possible workflow is outlined in **Figure 10**. The same acquired data set is evaluated using different data processing options. One of two approaches can be used:

- spectral matching of the clean deconvoluted spectrum against the library (preferably accurate mass, but nominal mass can also be used), but without retention time information
- screening using two exact masses from the database within a certain retention time window and then have the software report it as a hit.

TraceFinder software has several modules that can be used for this purpose. Within TraceFinder software, there

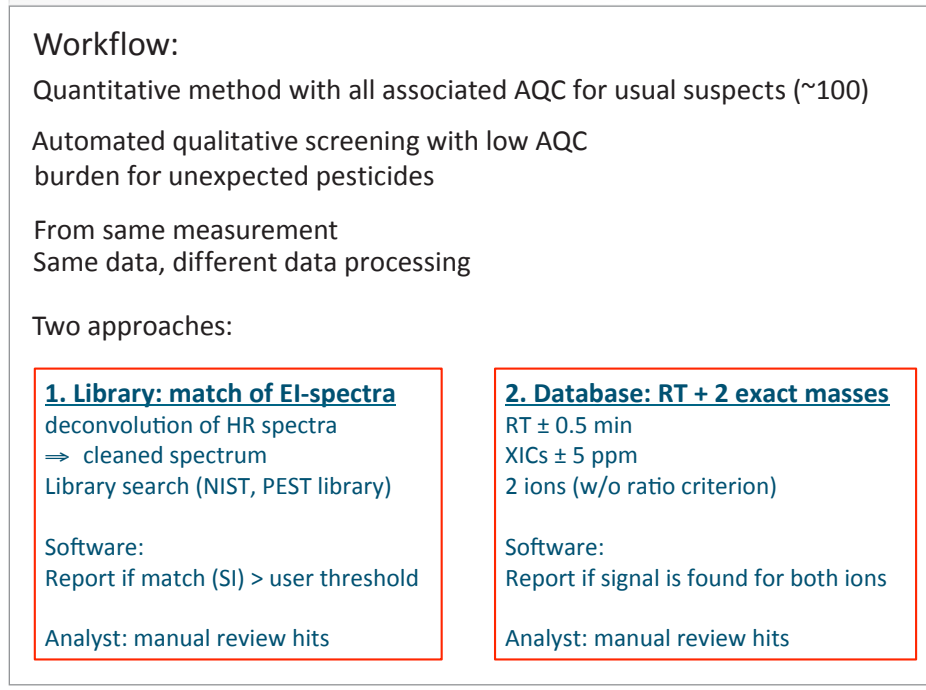
**Figure 9:** Validation results.



is a deconvolution Plug-In tool to assist in the case of option 1. When this approach was applied to the detection of 50 pesticides at 10 ng/g then 94%, 88%, and 86% were automatically detected in tomato, orange, and leek, respectively. Without retention time, the results are promising and further development is ongoing to enhance the performance of this option.

For the second option, the quantitative module was used with automated processing without any manual intervention. At the present time, the second approach out-performs the first option because the data processing is faster, the detection rates are better with a lower number of false detects to investigate. However, the total number of compounds is limited by the number of compounds in the database.

**Figure 10:** Qualitative screening.




## Conclusion

The Exactive GC Orbitrap MS system is highly suited for pesticide residues analysis enabling quantitative and qualitative analysis and identification. The full-scan capability allows for more comprehensive data collection with the flexibility of retrospective data analysis.

The Exactive GC Orbitrap MS system facilitates non-targeted mass analysis while maintaining high levels of specificity and sensitivity. Overall, the performance of this instrument is at least as good as for triple-quadrupole EI-MS/MS while being easier to use and more comprehensive. Data processing for quantitative analysis is similar to that of triple-quadrupole data. In addition, if one faces any problems with selectivity for one ion, another ion can be selected from the raw data retrospectively. Moreover, additional comprehensive qualitative screening for unexpected pesticides can be completed in a more time-efficient fashion.

## References

1. *The Pesticide Manual, 17th Edition*, J.A. Turner, Ed. (British Crop Production Council BCPC, Hampshire, UK, 2015).
2. 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 Apr 16, 2017).

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