An Executive Summary

Multi-residue analysis of pesticides by GC-HRMS

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Introduction

Regulatory authorities throughout the world set and enforce strict limits on the maximum residue levels (MRLs or tolerances) of pesticides permitted on food and feeds. It falls to analytical laboratories to test these products and ensure they meet the relevant standards. The analytical challenge is to look for over 1,500 potential pesticide analytes, at low concentration, in a wide variety of samples of different matrix complexity; it's a big job. The MRLs tend to be low; In the EU, for example, the default MRL is set at 0.01 mg/kg. Finally, laboratories often need to process large numbers of samples each day. Such high throughput analyses call for a flexible, sensitive, and robust platform.

In this paper, we will discuss the use of Thermo Scientific™ GC Orbitrap™ system for multi-residue analysis of pesticides. We'll begin with a brief introduction to the challenge, followed by a description of the operation and then we will highlight the advantages and limitations of the Orbitrap system.

GC Orbitrap for pesticide residues

The standard method for pesticide residue analysis is chromatography with mass spectrometric detection. All of the pesticides we need to detect are amenable to either liquid chromatography (LC) and/or gas chromatography (GC) coupled to mass spectrometric (MS) detectors. Today we'll focus on GC-MS.

GC with quadrupole MS is currently the most popular tool for the analysis of GC amenable pesticides. Single Quadrupole MS operated in full scan mode covers a wide variety of pesticides, but it lacks sensitivity and selectivity. GC with triple quadrupole MS/MS, in contrast, offers high sensitivity and selectivity, but a much more limited scope, limiting the analysis to typically 100-200 target pesticides. In principle, GC with full scan high-resolution accurate mass (HRAM) MS should offer good sensitivity and selectivity, and much wider scope (see **Figure 1**). Thermo's recently introduced GC Orbitrap is just such a system.

There are various options for configuring the GC Orbitrap, but we can demonstrate its capabilities for pesticide analysis using an electron ionization and non-targeted full scan acquisition setup.

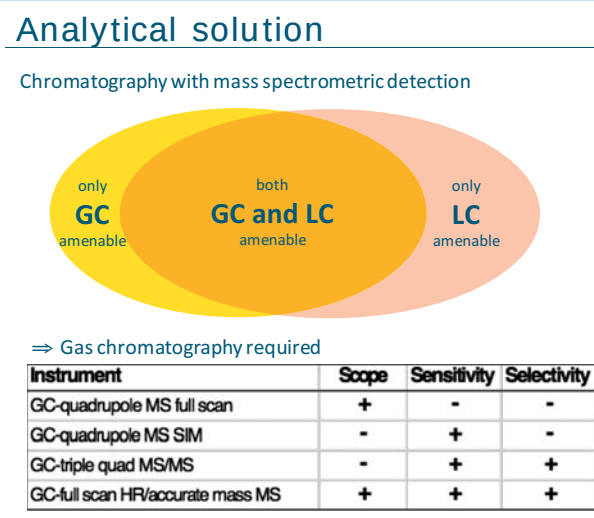
The most abundant ions of almost 600 pesticides are in the range of 100-250 *m/z* which fall exactly within range of the optimal resolving power (RP) of the instrument. There is however, a trade off between resolving power and scan speed. Scanning at 60,000 RP at 5 Hz provides sufficient sample data points (15-20) across a typical GC peak width of three to four seconds wide. When operating at 120,000 RP the scan speed is reduced to 3 Hz, but is still compatible with GC.

Electron ionization (EI) at 70 eV is a well-established, generic ionization technique that

essentially ionizes and fragments every compound of interest in a sample. A single EI acquisition event therefore provides all of the ions we need for analysis. EI-MS spectra are largely instrument-independent, allowing the use of existing libraries of spectra to identify specific pesticides. However, the existing libraries such as NIST and Wiley, are based on nominal mass. It was therefore decided to evaluate the quality of the spectra from the GC Orbitrap to see if the HRAM spectra were suitable for searching the NIST library.

Tests on several pesticides at realistic residue levels show that despite observed deviations at low *m/z* values for a few pesticides, the GC Orbitrap system can use a commercial library (NIST) to produce accurate identifications. The examples of disulfoton and chlorpropham are shown in **Figures 2** and **3** respectively. In every case, the first hit the system identified, using the NIST library, containing more than 200,000 spectra, was correct. Crucially, the spectra remain consistent with excellent mass accuracy (1 ppm) at very low pesticide concentrations, and across a wide range of concentrations (1- 250,000 pg), extending well below and above established regulatory limits for pesticide residues.

Figure 1: GC-HRMS instrumentation maximizes scope, sensitivity, and selectivity for pesticide analysis.

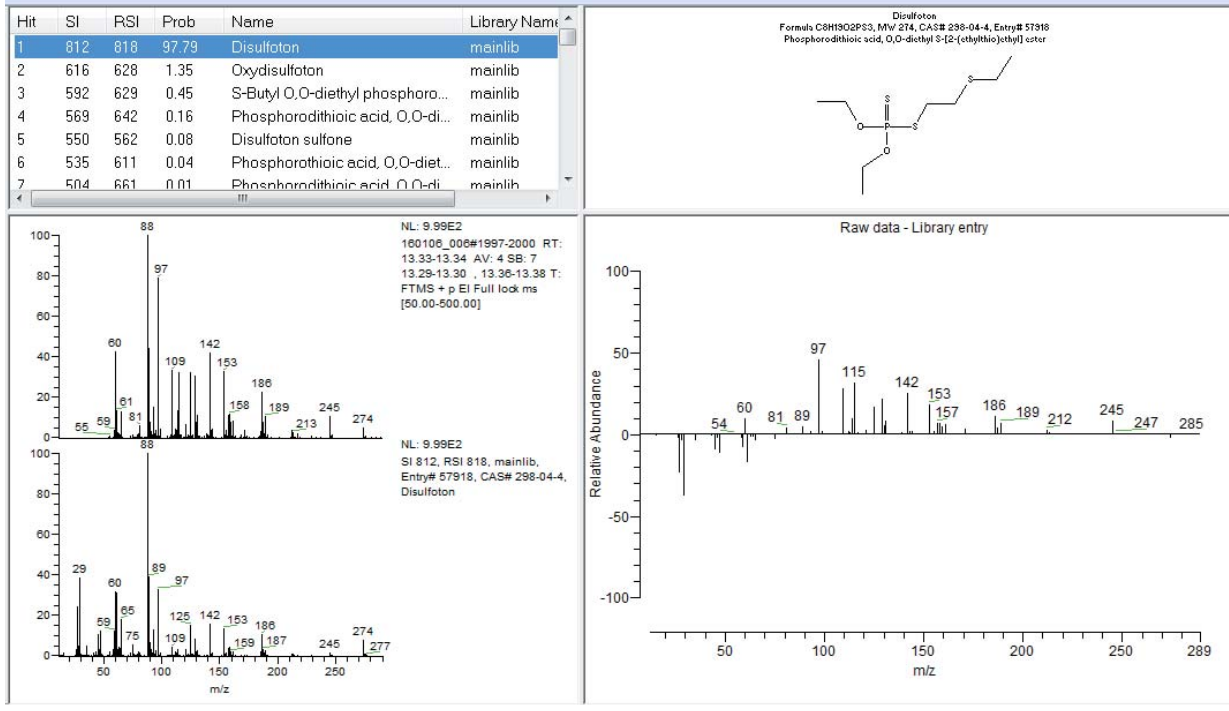


Instrument	Scope	Sensitivity	Selectivity
GC-quadrupole MS full scan	+	-	-
GC-quadrupole MS SIM	-	+	-
GC-triple quad MS/MS	-	+	+
GC-full scan HR/accurate mass MS	+	+	+

Selectivity, method development, system performance

Besides sensitivity, we also need to consider selectivity. Pesticide residues occur in matrices with varying complexity;

Figure 2: Matching spectra against standard libraries aids in the identification of pesticides.



tomatoes are simpler to analyze than leeks, for example. To analyze selectivity, we looked at ion extracted chromatograms using exact mass plus or minus a narrow Mass Extraction Window (MEW), but the question is what MEW is required? Because the width of the MEW required to detect pesticides correctly is an indication of the system's selectivity.

Using chlorpropham in leek extract as an example, we found that we had to narrow the extraction window to ±5 ppm to be able to see three characteristic ions. To be able to use a window that narrow, we need high instrument mass accuracy, and also need to separate the analytes from isobaric compounds. There are three ways to achieve this; sample preparation, chromatographic separation, and mass separation. Only mass separation will work for us in this context and to use mass separation we need sufficient mass resolving power.

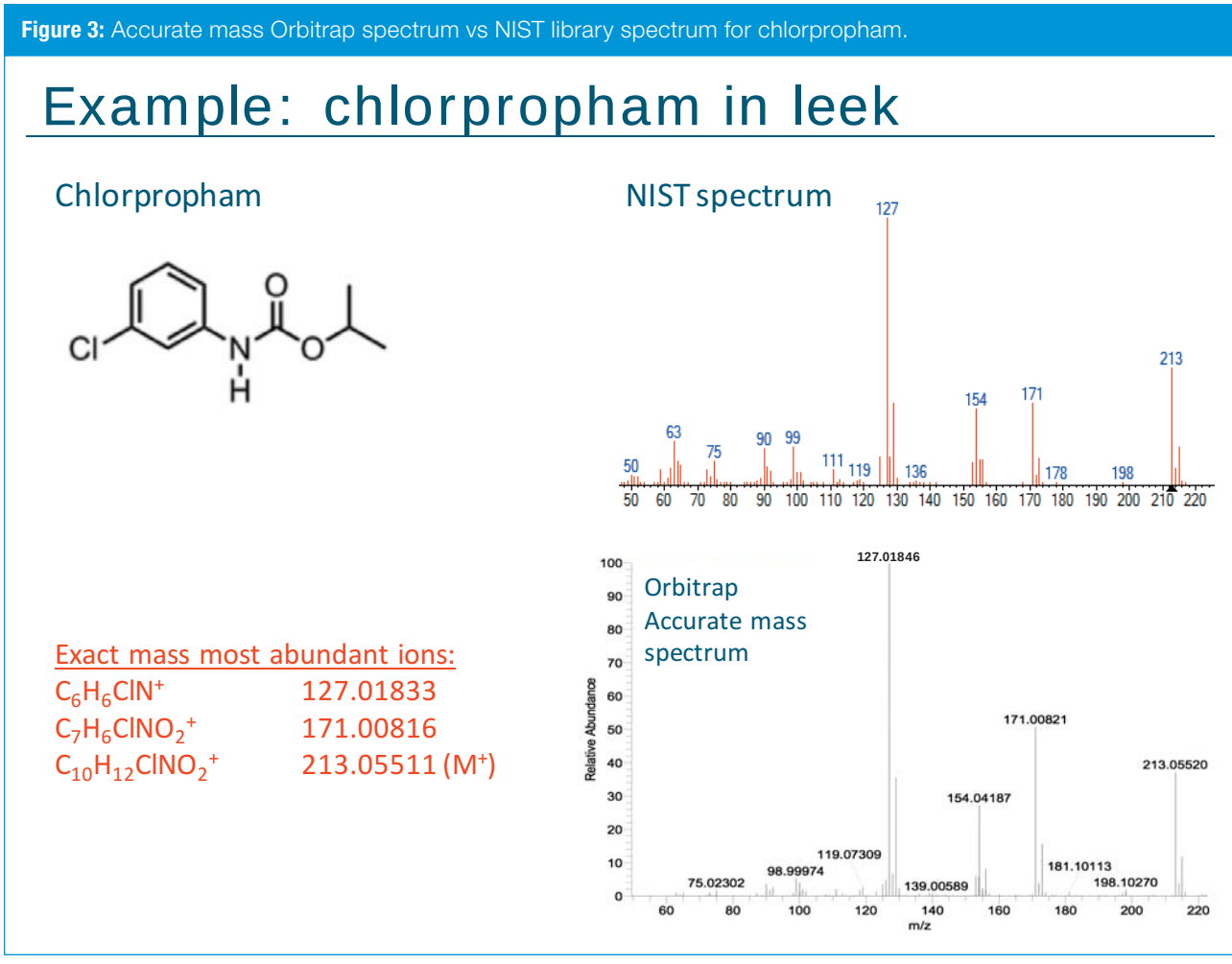
Through analyzing a large number of compounds in different matrices, we determined that operating the GC Orbitrap at a resolving power of 60,000 or higher with a MEW at ±5 ppm provided sufficient selectivity for routine analysis of pesticides at 0.01 mg/kg.

Our sample preparation followed the standard QuECheRS procedure which is familiar to many investigators. The only difference is a solvent switch from acetonitrile to iso-octane, which is better suited to hot splitless injection. After the measurement of our samples, we used TraceFinder software for data analysis. We also need databases of spectra, which can either be libraries such as the NIST library or dedicated databases containing retention time and exact mass information. Currently, Thermo's exact mass pesticide database contains approximately 600 compounds.

Quantitative and qualitative analysis

There are a number of important performance parameters to assess for each pesticide. The instrument limit of detection (LOD) was either based on the normal signal to noise approach (S/N ≥3) if there was continuous background noise to measure, or on a minimum of 5 scans within one peak if there was no background. The instrument limit of quantification (LOQ) was the lowest concentration from a series of matrix-matched calibration standards that could still be accurately quantified (residuals less than 20%).

Figure 3: Accurate mass Orbitrap spectrum vs NIST library spectrum for chlorpropham.



For limit of identification the EU SANTE[1] criteria were used: retention time within 0.1 min of the reference retention time, 2 ions with a mass accuracy within ±5 ppm, and the ion ratio within ±30% of the reference ion ratio.

The developed method was evaluated using 55 different pesticides spiked into samples of leek, orange, and tomato. The system LOD was 0.5 pg on-column for the majority of pesticides as shown in **Figure 4**. The method Limit of Quantification (LOQ) was 0.5 – 5pg and the Limit of Identification (LOI) were 0.5 pg for most of the pesticides and up to 10 pg for some.

Mass accuracy for the vast majority of pesticides at all concentrations was within 1 ppm. Good linearity was obtained based on the deviation of response from the average response factor being less than 20%.

To examine the robustness of the GC Orbitrap, the same extract was repeatedly injected 50 times. Good reproducibility was observed across all 50 runs. Even relatively critical compounds such as iprodione and deltamethrin show results that vary within acceptable limits.

The method was validated using the procedure for a quantitative method described in the SANTE[1] guidelines.

For the majority of the 55 pesticides, the recovery rates were within the acceptable range (70-120%) in all three matrices with associated repeatability for replicates lower than 20% as shown in **Figure 5**. A few pesticides gave low recovery rates, but this was related to the solvent switch to iso-octane, and not to the GC Orbitrap. Despite the low recoveries in those cases, good repeatability and quantitative results that were comparable to those typically seen on triple quadrupole instruments were obtained.

The GC Orbitrap also allows the additional possibility for qualitative screening to detect potential residues in a sample. There are two approaches for this. Firstly, we can match our acquired EI spectra against a published library of electron ionization spectra. To do that we need to deconvolute the high-resolution spectrum, then perform the search of the clean spectra against a database such as the NIST library. The system software handles this process, highlighting results outside a given threshold for manual analysis by the user.

Figure 4: Quantifying the performance of GC Orbitrap for pesticide analysis.

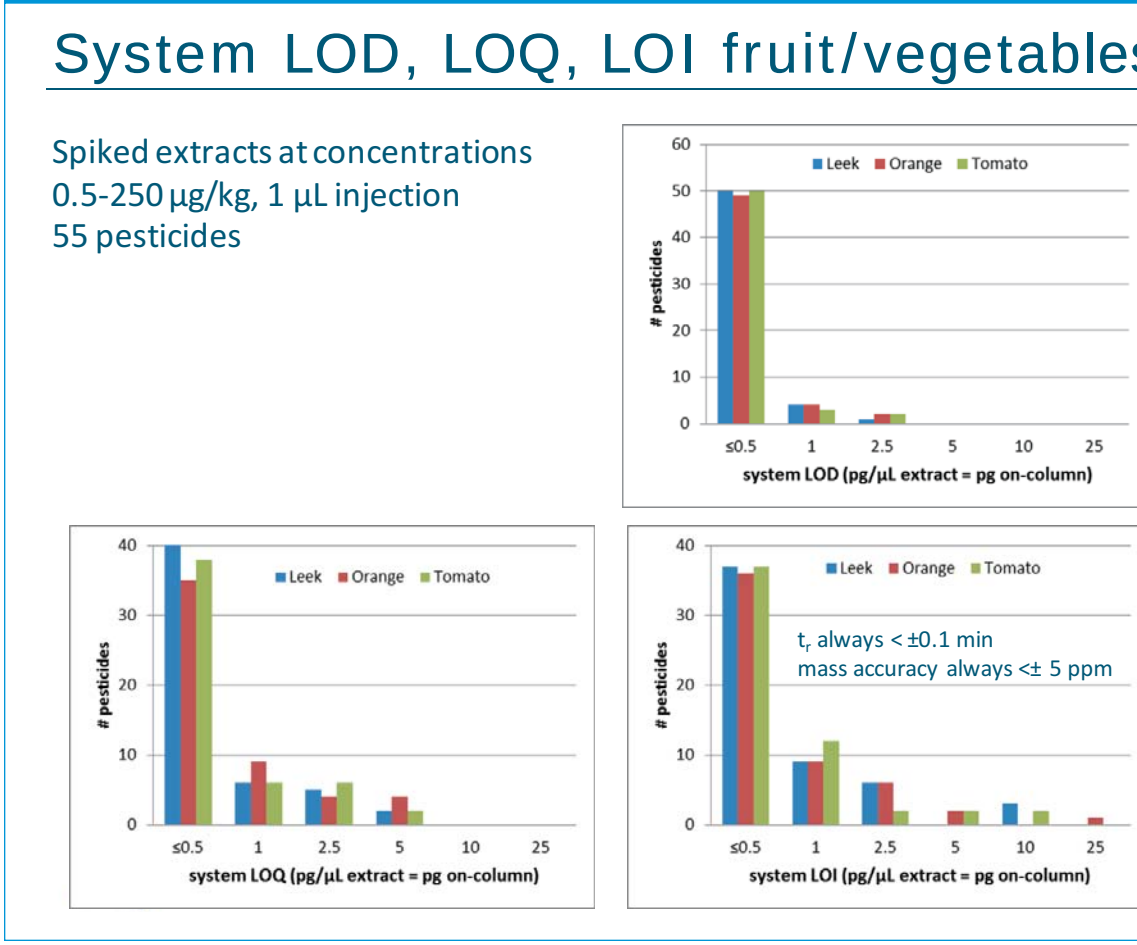
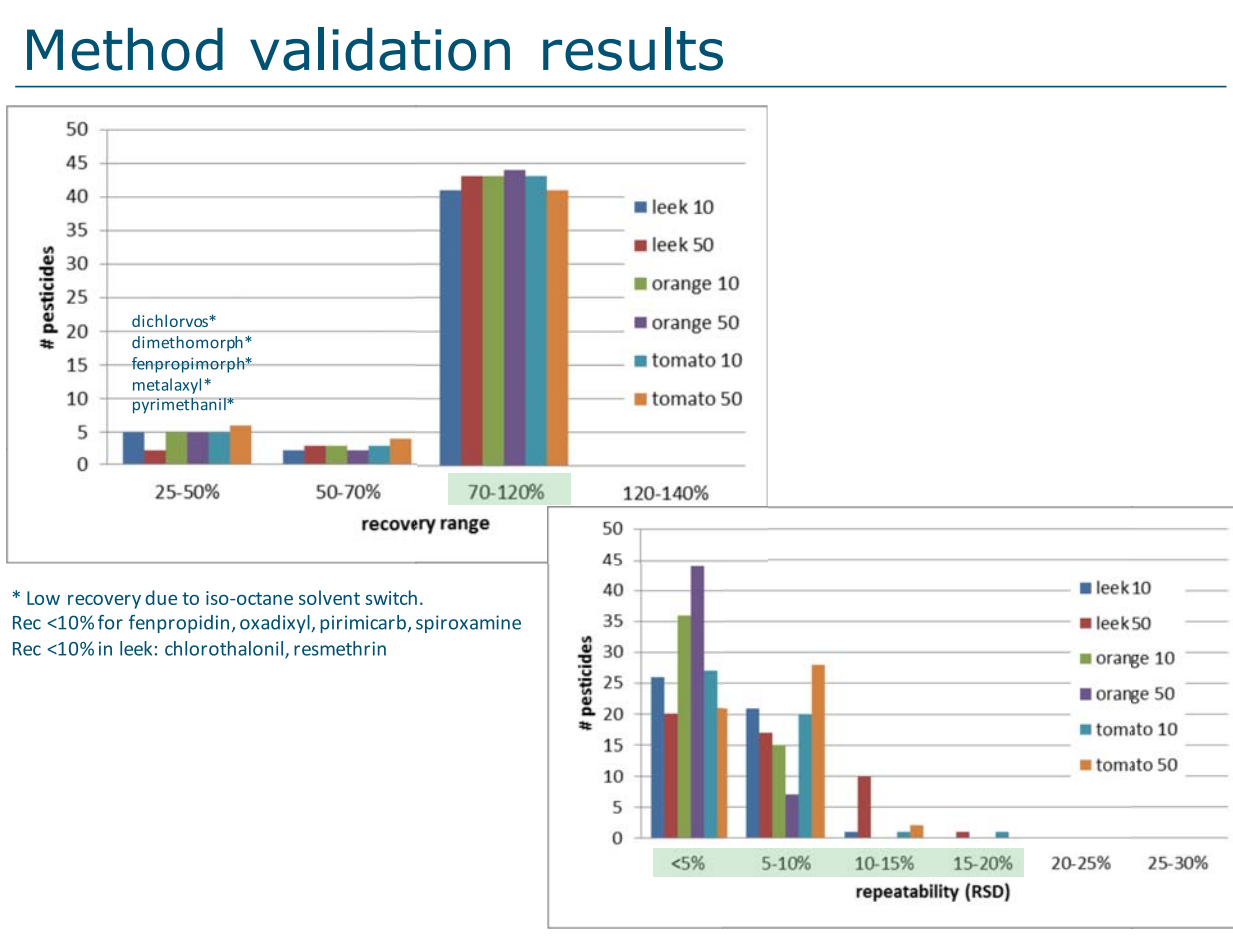


Figure 5: Recovery and repeatability obtained for method validation at 10 and 50 µg/kg.



The second approach is to use a user compound database containing retention times and two exact masses for each pesticide. In this case we compare the information extracted from the sample with the information in the library.

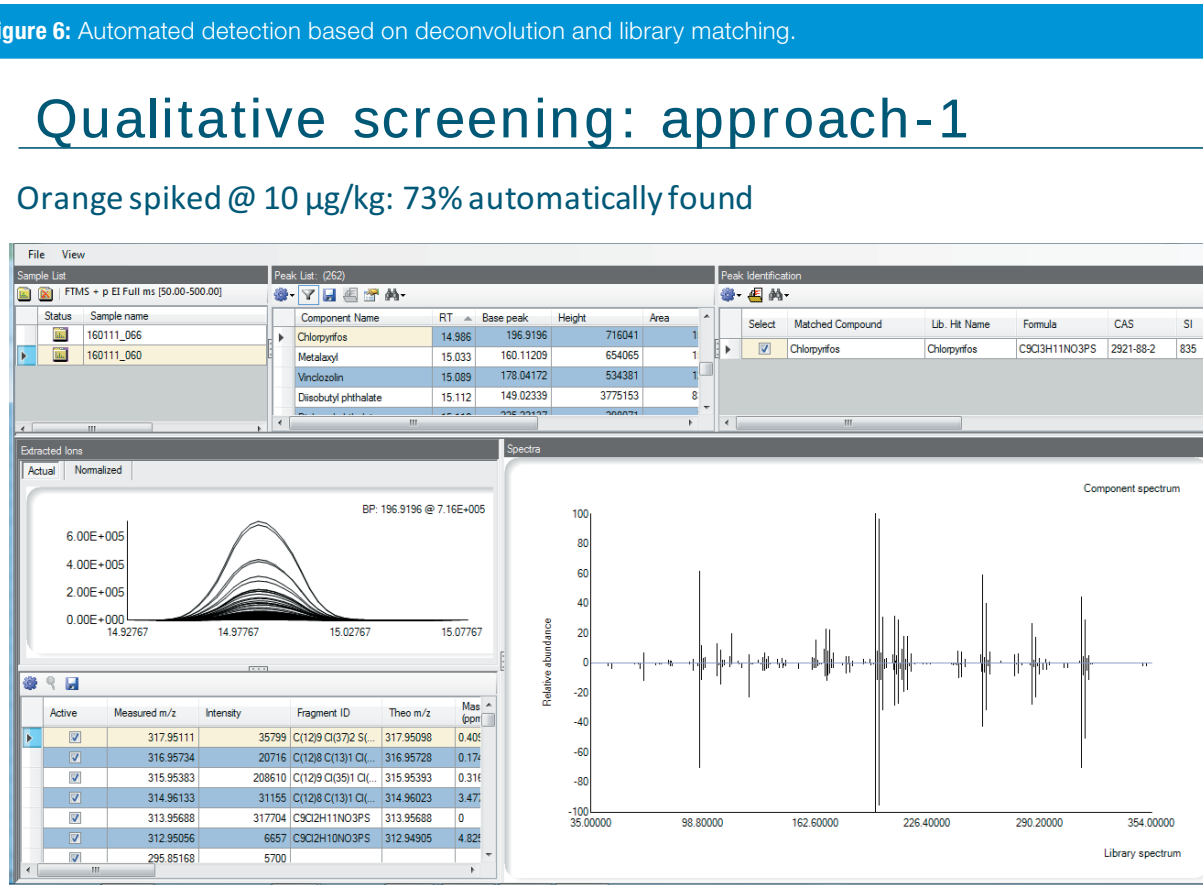
Using the first approach the software automatically found 73% of the pesticides added to the orange matrix at 10 ppb as shown in **Figure 6**. The spectrum for chlorpyrifos at 1.4 ppb could be recognized manually, but was not matched automatically. Interfering peaks from the matrix in this case produces a more complex spectrum which in turn makes it more challenging for the software to automatically match the spectrum of the pesticide at such low concentration in the sample with the corresponding clean spectrum in the library.

Based on these results, we see that we can screen for all pesticides using two techniques with complementary strengths and weaknesses. Using a library match allows screening for analytes that are not yet included in a dedicated high resolution accurate mass database, but it can be challenging for the system to automatically match the spectra at the lower levels. Using a high resolution accurate mass database provides better screening sensitivity, but only for compounds that are in the database.

Summary

For pesticide residue analysis, the GC Orbitrap system matches the wide scope of quadrupole MS operated in full scan, and the high sensitivity and selectivity of selected reaction monitoring in a triple quadrupole MS/MS. The scan speed is sufficiently fast enough for routine GC-MS analysis, and the spectral quality is consistent over wide concentration range and thus NIST searchable. Resolving power: 30/60K ensures reliable high mass accuracy at lower levels in simple/complex matrices and enables use of MEW of ±5 ppm resulting in high selectivity. The system limit of detection is in the low picogram range, and its quantitative performance is fit for routine pesticide residue testing. Compound identification meets the EU SANTE[1] criteria for all of the pesticides tested at 10 ppb or lower. Qualitative screening based on 2-specific ions and retention time proved more sensitive than an approach based on matching

Figure 6: Automated detection based on deconvolution and library matching.



against spectra in a library. The overall situation is expected to improve with the further development of databases and deconvolution software.

Of course, some pesticide residues are not amenable to GC-MS at all, in which case we would use LC-MS to complement GC-MS. Orbitrap MS can already be coupled to LC systems, so the new GC Orbitrap with electron ionization completes the picture.

Reference

1. Method Validation & Quality Control Procedures for Pesticide Residues Analysis in Food & Feed; EC Document No. SANTE 11945/2015