Enhanced quantitative performance for analytical testing laboratories with Orbitrap Exploris GC

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Goal

To demonstrate the benefits of the Thermo Scientific™ Orbitrap Exploris™ GC mass spectrometer for the analysis of pesticide residues and polychlorinated biphenyls (PCBs) at trace levels in food, in compliance with SANTE method performance criteria.

Introduction

In high-throughput analytical testing laboratories, robust streamlined analytical and data processing workflows are key requirements for the accurate and reliable determination of trace level residues and contaminants (such as pesticides and PCBs) in food. These methods must overcome the challenges of an ever-growing list of compounds and diversity of sample matrices, in addition to ever-demanding sensitivity and identification requirements. Typically, gas chromatography coupled to low resolution, nominal mass triple quadruple mass spectrometers

(GC-MS/MS) has been the system of choice for the sensitive and selective detection of a wide range of target compounds. A GC-MS/MS acquisition method requires at least two precursor ions for product selected reaction monitoring (SRM) transitions to be optimized for selectivity and sensitivity for each analyte. Furthermore, the retention time for each analyte has to be pre-programmed into the acquistion method. This initial method development can be a time-consuming process.

The development of additional hyphenated GC-MS analytical systems such as high-resolution accurate mass (HRAM) Orbitrap mass spectrometry coupled to GC has proved to be a valuable alternative to triple quadrupole GC-MS.[1-5] With HRAM mass spectrometry, the default acquisition mode is untargeted (full-scan) meaning that all the ions are acquired with high selectivity at the same time

across a specified mass range, making the method setup and data acquisition simple to manage and giving the analyst the flexibility to decide which compounds to focus on. This can extend into retrospective analysis of data to evaluate for the presence/absence of other contaminants not necessarily of interest at the time of acquisition.

In the European Union (EU), the default maximum pesticide residues level (MRL) is regulated at 10 μg/kg.[6,7] The guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed is SANTE/12682/2019,[7] which includes quantitative and identification criteria for HRAM MS data:

- At least two ions per target compound, measured with mass accuracy of ≤5 ppm (≤5 mDa for masses below 200 Da)

- Retention time tolerance of ±0.1 min

- Precision for bracketed (beginning and end of the run) matrix-matched calibration standards % RSD ≤20% for each calibration level is required

- The lowest standard ≤ the reporting limit

- Recoveries within the range 60% to 140%, or ± 2× RSD (determined from validation data or ongoing QC results)

- For HRAM compared to nominal mass analysis, ion ratio guidelines state matching ion ratios are not necessary, but could be used for additional confirmation

In the experiments described below, the analytical performance and suitability of a benchtop HRAM Orbitrap GC-MS for analytical testing laboratories was assessed. System setup simplicity and method setup as well as typical method performance parameters were tested including sensitivity, linearity, quantitation, recovery, accurate mass, and ion ratios.

Experimental
Sample and standard preparation
Homogenized test-portions of fruit and vegetable samples were extracted using the mini-Luke procedure,[8] and prepared as detailed in a previously published Application Note.[9] A series of matrix-matched calibration standards containing pesticides and PCBs, over varying concentration ranges, were prepared by spiking apple and carrot extracts.

Instrument and method setup
Automatic sample injection was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was performed using a Thermo Scientific™ TRACE™ 1310 GC system equipped with a PTV injector and fitted with a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 μm film capillary column with a 5 m integrated guard (P/N 26096-1425). Finally, a Thermo Scientific Orbitrap Exploris GC mass spectrometer was used for accurate mass measurements in full-scan mode at 60,000 mass resolution (FWHM *m/z* 200). Ease of use maintenance features, such as changing columns or removing the source that can be carried out without venting the MS, can save valuable time and add flexibility with increased efficiency. Additional details of instrument parameters are as detailed in previously published Application Note.[9]

Data processing
Data were acquired and quickly processed using Thermo Scientific™ TraceFinder™ software, which allows intuitive instrument control, method development, and data processing capabilities. Ready-to-go templates for instrument and processing method setup allowing walk up and use capability. For targeted analysis, a compound database was prepared containing compound name, accurate masses for the quantification and qualifying ions, retention times, and the elemental compositions of the molecular ion. To generate the extracted ion chromatograms (EIC), a mass window of ±5 ppm was used, meaning that only ions with a mass accuracy ≤5 ppm were extracted.

Results and discussion
The objective of this study was to evaluate the quantitative, ease of use, and performance of the Exploris GC system for the analysis of pesticides and PCBs in fruit and vegetable matrices with varying complexity considering the performance requirements detailed in the SANTE guidelines.[3]

Pesticides were assessed in typical food matrices such as apples and carrots. Chromatographic separation was achieved in under 33 min with the TRACE GC system, which comes with modular injectors that allow for PTV and SSL selection. A typical TIC chromatogram in an apple matrix is shown in Figure 1, versus overlaid EICs for a selection of pesticides. The results achieved demonstrate excellent selectivity for the analysis of pesticides and PCBs even in complex samples.

The Orbitrap Exploris GC-MS, with markedly reduced footprint, can be tuned and calibrated very quickly (~1.5 min) and efficiently, using a next generation tune software designed for ease of use, while offering maximum functionality, see Figure 2.
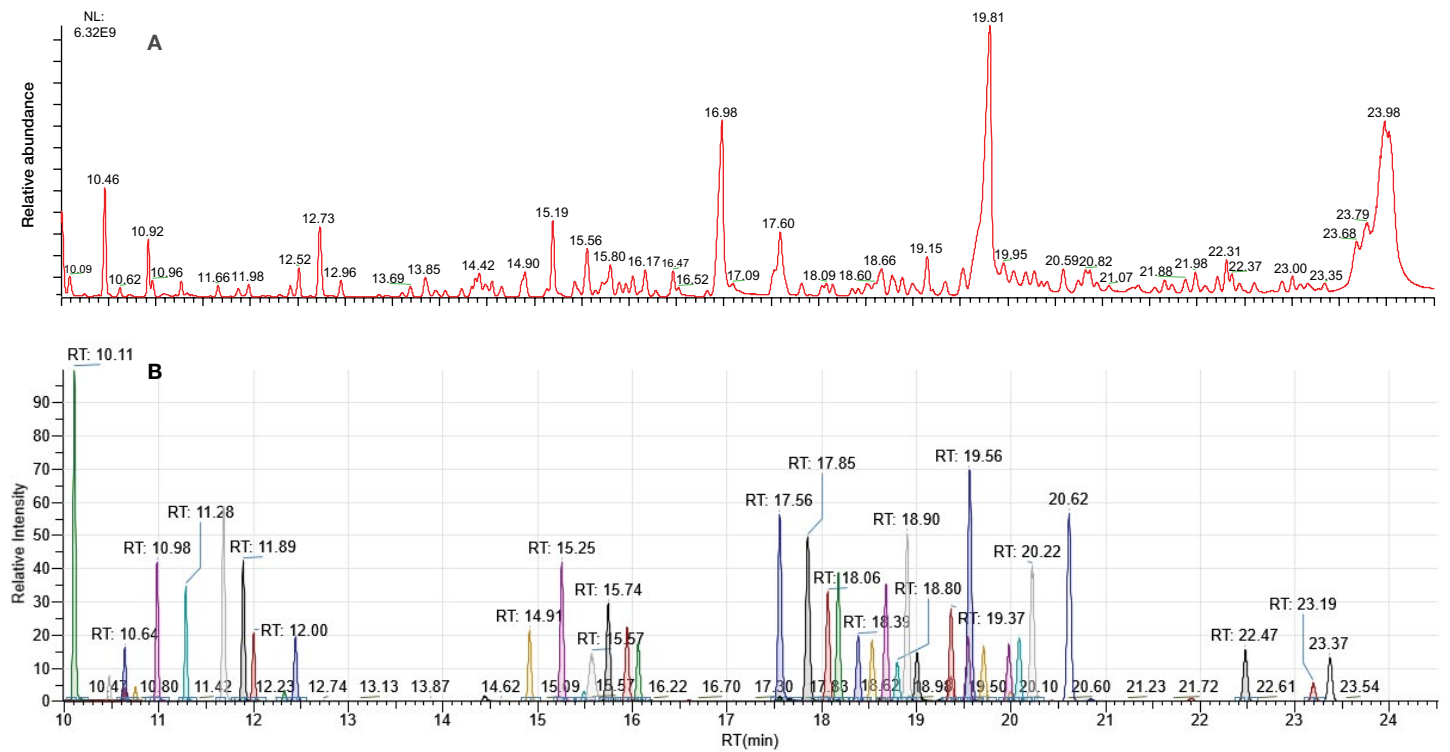

Figure 1. Apple sample chromatogram (spiked 100 µg/kg): [A] TIC full scan; [B] EICs for a selection of pesticides
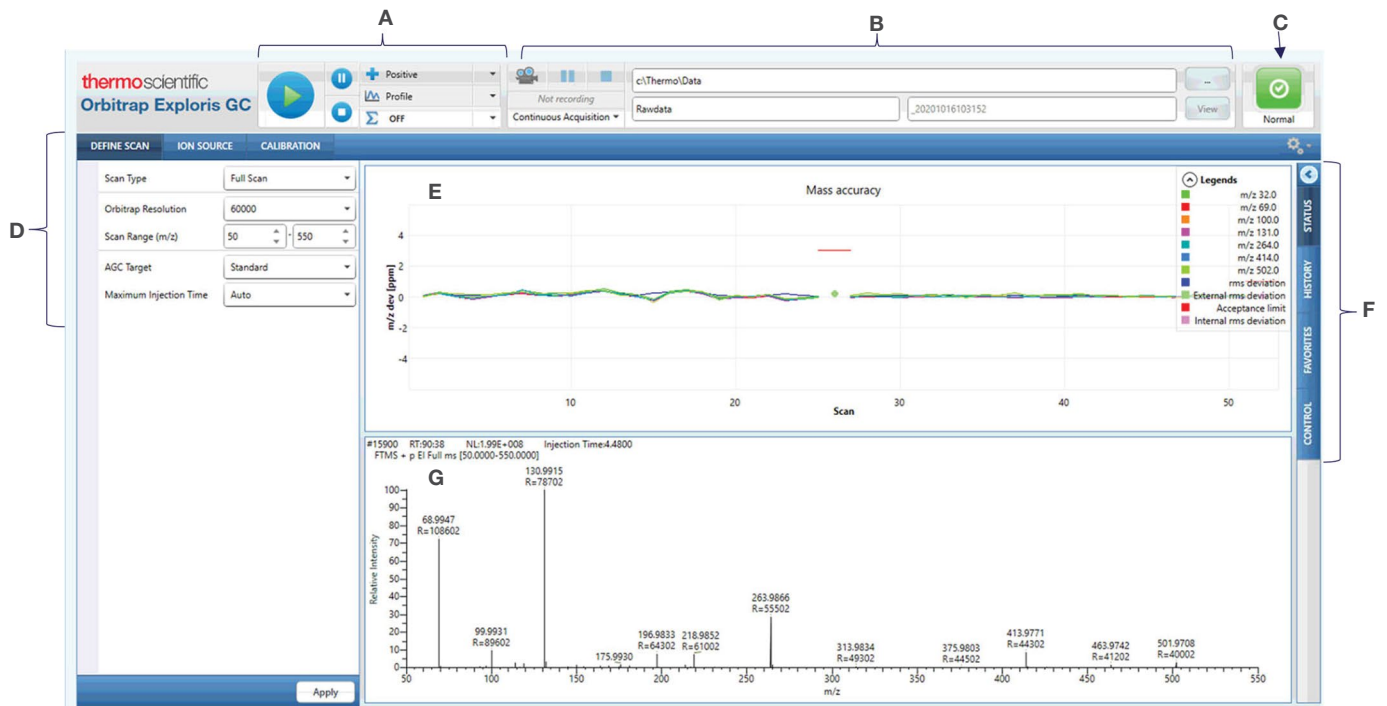

Figure 2. Orbitrap Exploris GC-MS tune page user interface developed for simplicity of use while offering maximum functionality to enable fast and efficient system setup. System calibration and tuning take ~1.5 minutes and are stable for a week or longer. The tune page includes: [A] power and instrument icons; [B] data acquisition buttons; [C] instrument status icon; [D] scan, ion source, and calibration panes; [E] plot view; [F] status panes, and [G] spectrum view.

The method editor is simple and intuitive and features ready to use, pre-optimized method templates for a wide range of typical application, including food safety (Figure 3).

Linearity and sensitivity

A wide linear dynamic range is essential, especially when dealing with applications where the samples analyzed contain a complex chemical background that could potentially interfere with the analytes of interest.[10] The sensitivity of target compounds in matrix is a key parameter when assessing the suitability of a quantitative analytical technique.

The SANTE guidelines[7] specify that precision and sensitivity for bracketed (beginning and end of the run) matrix-matched calibration standards % RSD ≤20% for each calibration level is required, with the lowest standard less than or equal to the reporting limit (RL).

An external matrix-matched five-point calibration covering a 5 to 250 ppb range was used, with a linear curve fit, a standard weighting of 1/×, and with curves not forced through the origin. The residual values variation (as %RSD) and coefficient of determination (R^2) were used to assess linearity. R^2 values of ≥0.95 and residuals ≤30% are considered acceptable. Figure 4 summarizes the linearity achieved for pesticides in apple and carrot matrices.

Achieving sufficient sensitivity when analyzing food contaminants is critical. With increasing resolution, the Orbitrap Exploris GC signal intensity is not affected unlike other types of high-resolution mass spectrometers where doubling the resolution will result in a significant drop in sensitivity.[12] Therefore when operating at a resolving power of 60,000, the established RL exceed the detection requirements for pesticide residue monitoring.
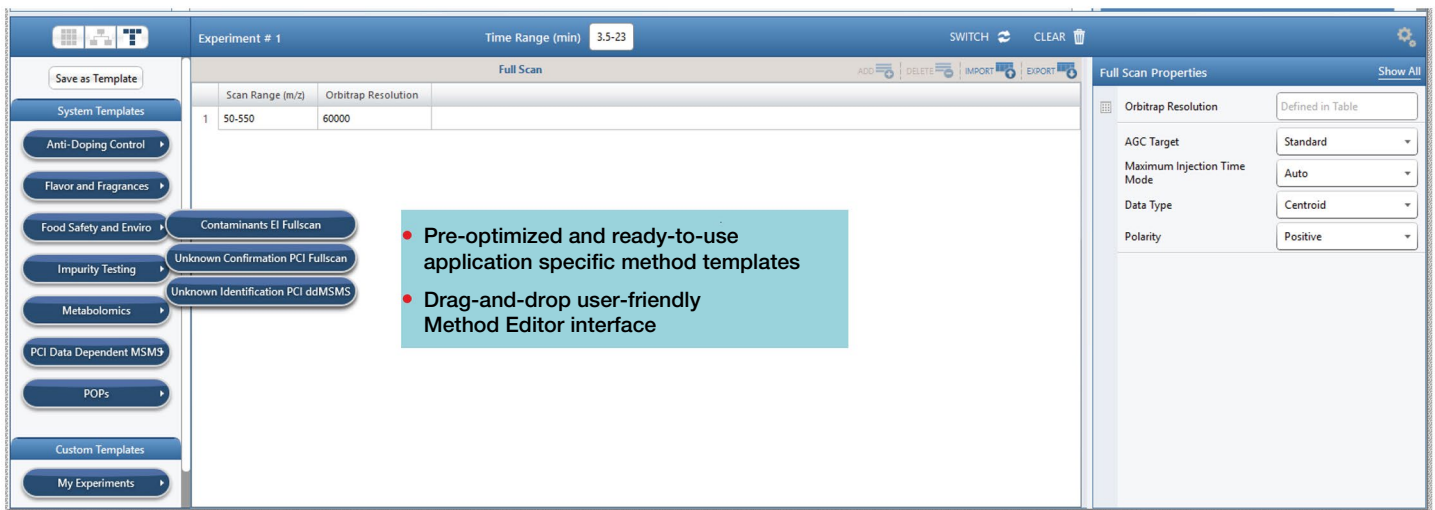

Figure 3. Orbitrap Exploris GC method editor system templates, enabling user friendly method setup using pre-optimized application specific method templates
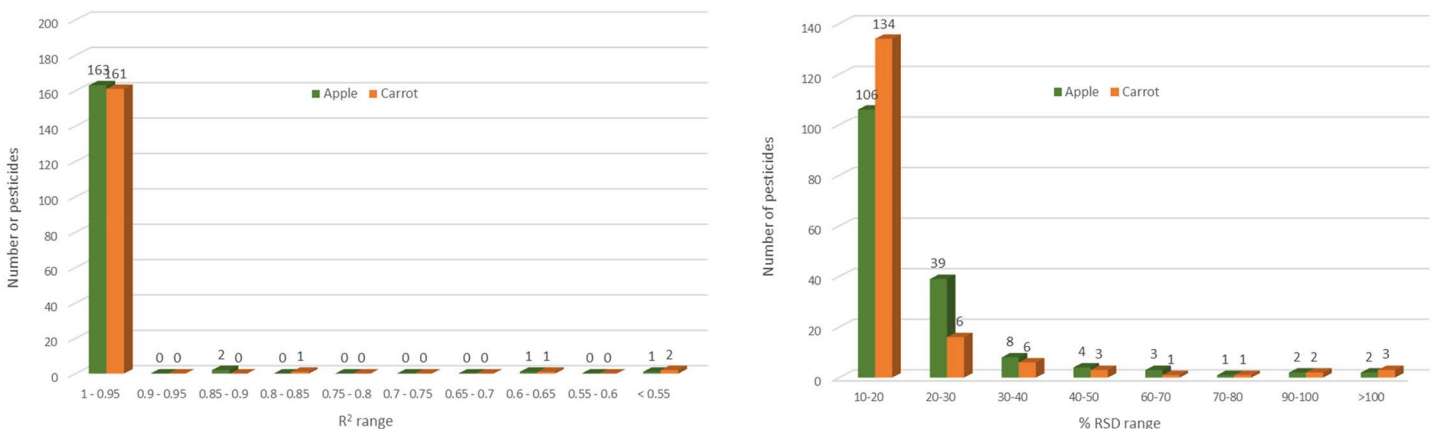

Figure 4. Summary of linearity achieved for pesticides in apple and carrot matrices, expressed as (A) R^2 and (B) % RSD

An example of compound sensitivity is shown in Figure 5 for bifenthrin in carrot. Overlay of the diagnostic ions at 10 µg/kg and the linear response for this compound are shown in the customizable views in TraceFinder software, which allows the user to quickly review the key detection criteria and any parameters outside of specified tolerances can be flagged automatically.

Accurate quantitation

For accurate compound identification and quantitation, at least 10 data points (scans) across a chromatographic peak are usually considered necessary. Figure 6 shows biphenyl peak in apple at 10 pg on column with ~33 scans across a 4.8 s wide peak.

To assess the detectability and accuracy of quantitation, spiked apple and carrot samples were analyzed. Figure 7 summarizes these recovery results for 167 pesticides, which show good agreement between the spiked and calculated concentrations, with 97% of

pesticides within the SANTE guidelines[8] (60–140% for routine recoveries) for compounds outside this range quoted include pesticides that are a challenge for GC-MS and typically are also analyzed using LC-MS.

High accurate mass for confident compound identification

With the Orbitrap Exploris GC system operated routinely at 60,000 resolving power, consistent high mass accuracy information is always obtained, which is essential to increase the confidence in compound identification and avoid reporting false positive results. The SANTE guideline[8] recommends a criterion of <5 ppm for mass accuracy for identification of target pesticides in food and feed samples. As shown in Figure 8, this was achived for all pesticides analyzed, with ~90% of compounds showing <2 ppm mass accuracy for both the quantification and qualifier ions and 100% of compounds <5 ppm. This was achieved by performing a mass calibration at the start of the anlytical run, with no additional calibrations performed during the analytical sequence.
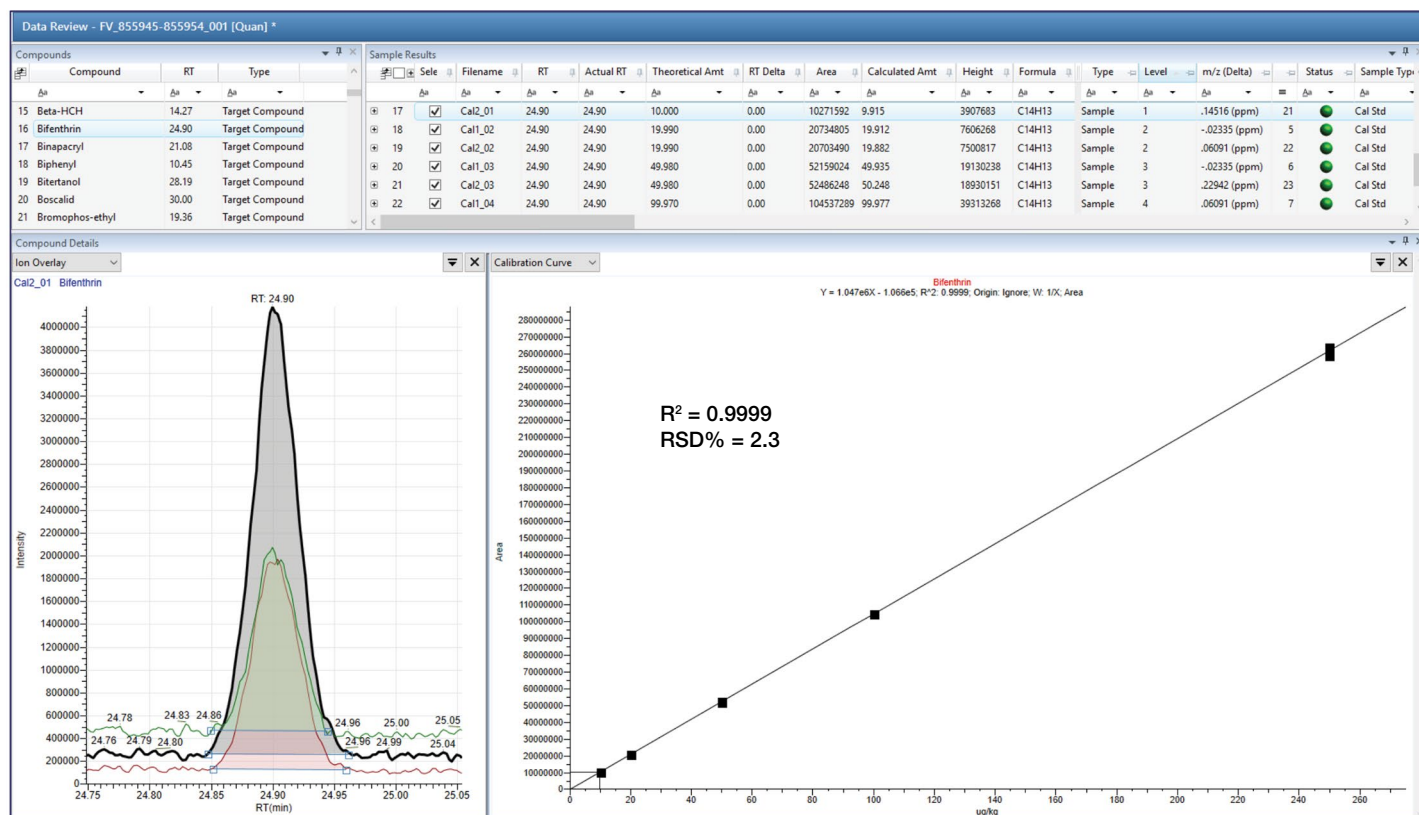

Figure 5. TraceFinder quantification results browser showing bifenthrin as an example. The overlay of extracted ion chromatograms for the quantification and two confirmatory ions as well as the linear response for bifenthrin over a concentration range of 10 to 250 ppb (equivalent to 10–250 µg/kg in matrix) with R^2 = 0.9999 and residual value as RSD% = 2.3 are shown.
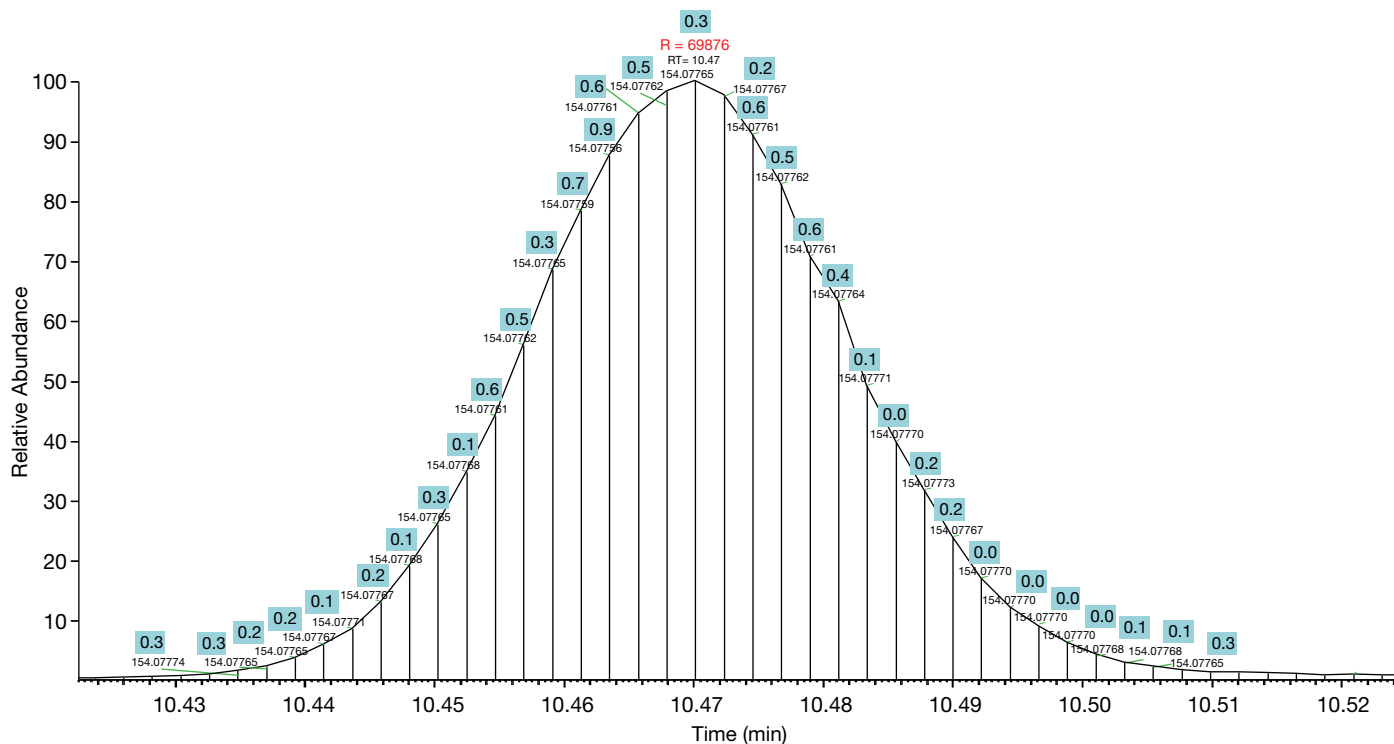
Figure 6. Biphenyl acquired in full scan at 60k resolution in an apple sample at 10 pg on column (corresponding to 10 µg/kg level), showing ~33 scans/peak (4.8 s peak width). Consistent sub 1-ppm accuracy was obtained for each individual scan.
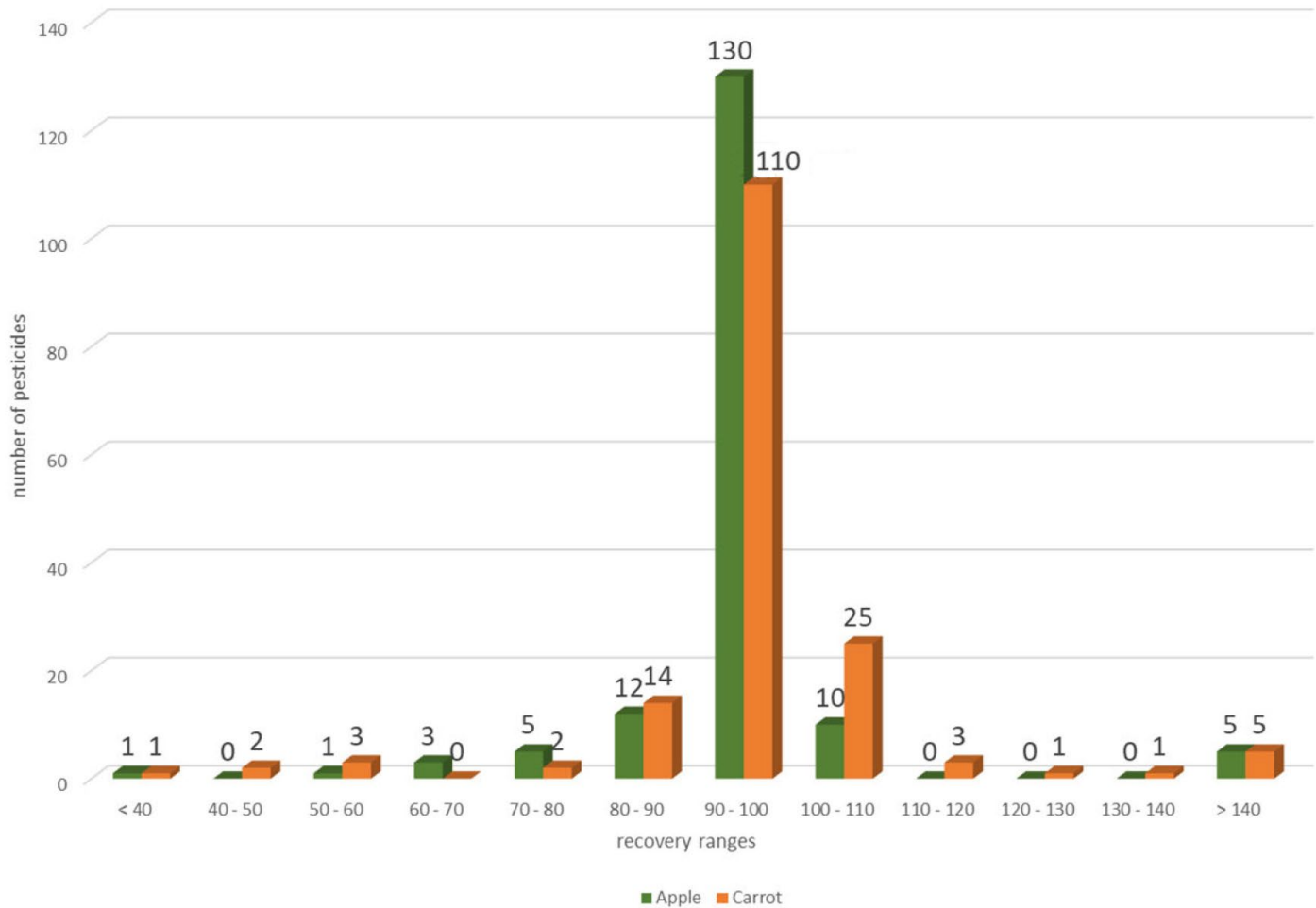

Figure 7. Summary of the % recoveries for 167 pesticides spiked at 100 ppb level (equivalent to 100 µg/kg) in apple and carrot matrices
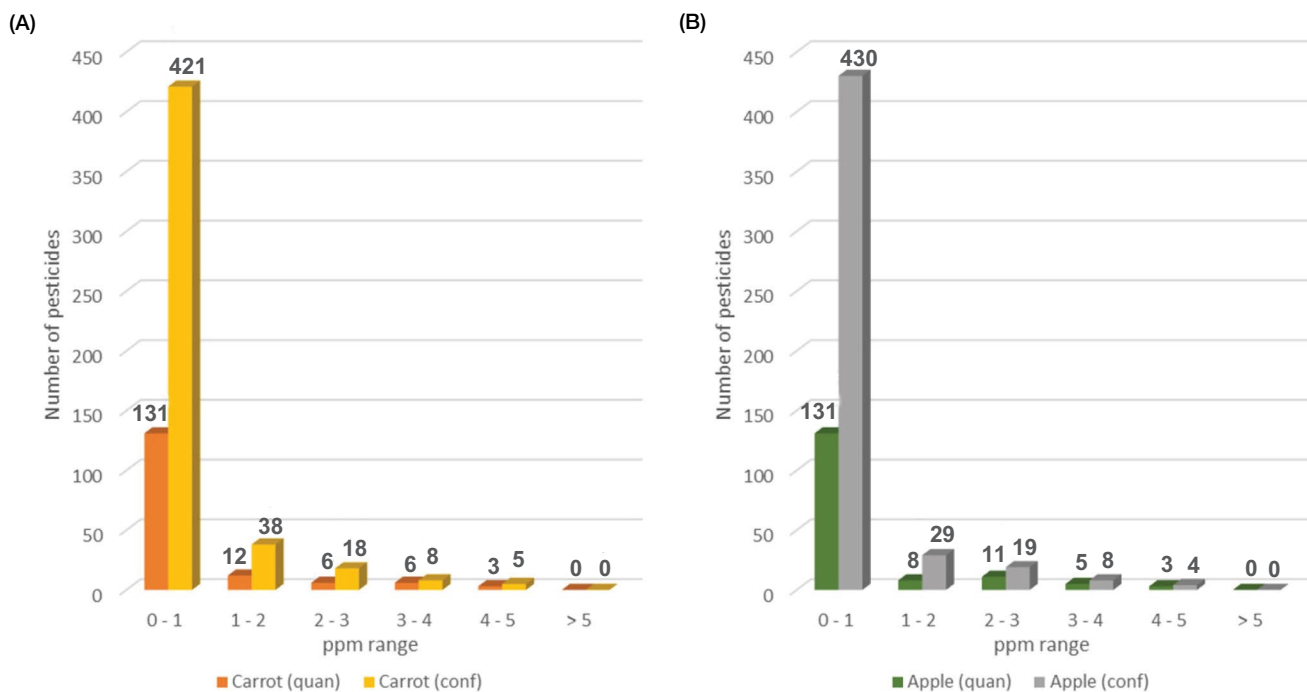
Figure 8. Summary of mass accuracy (as ppm) results for the quantification and qualifier ions in pesticides and PCBS spiked in (A) carrot and (B) apple

Ion ratios for additional compound identification

When evaluating mass spectrometry data, considering ion ratios achieved can provide additional verification. The SANTE guideline document allows a variation of 30% between ion ratios of the standards and the sample, but it also documents when using HRAM ion ratios are of secondary importance. A summary of the ion ratios results achieved is shown in Figure 9, which shows that for apple and carrots 93% of ion ratios differences are ≤30%.
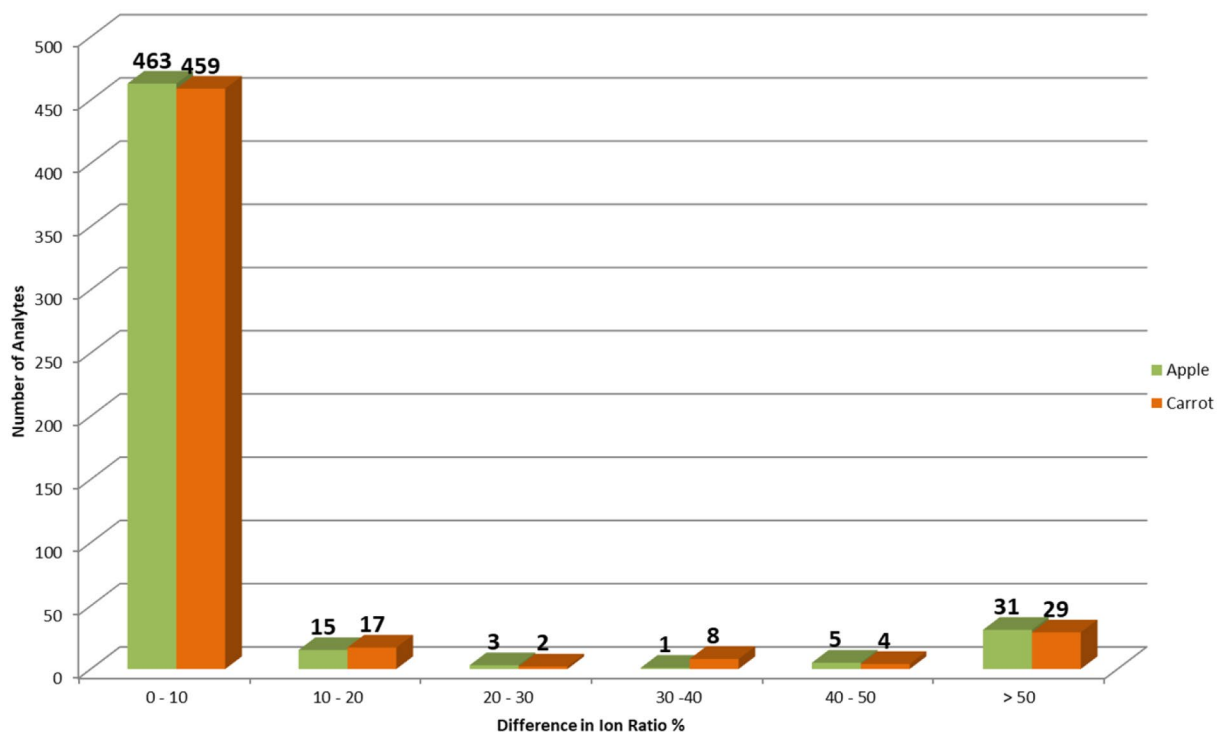

Figure 9. Summary of the difference in ion ratios between standards and recovery spikes by matrix

Compound identification against spectral NIST libraries

Where additional confirmation is required, the Exploris Orbitrap GC with full scan range mass accuracy and sensitivity enables accurate and reliable commercial library (e.g., NIST/Wiley) matching. Figure 10 shows the NIST library search results achieved using the Orbitrap Exploris GC for the analysis of biphenyl and hexachlorobenzene in a mixed pesticide standard, with both forward and reverse library match scores of >900 achieved.

Ensuring consistency of results across daily sample batches

Consistent instrument performance is key to ensure quality of results. This was evaluated for the analysis of pesticides and PCBs in food using n=35 apple matrix injections over two days of continuous operation by repeatedly injecting an apple extract (10 µg/kg). Response of pesticides and PCBs were consistently stable, illustrated in Figure 11, for a selection of pesticide and PCBS with % RSDs <11, demonstrating a robust system performance critical for analytical testing.

(A) Biphenyl

Forward match = 935
Reverse match = 936

NIST library biphenyl spectra

(B) Hexachlorobenzene

Forward match = 919
Reverse match = 951
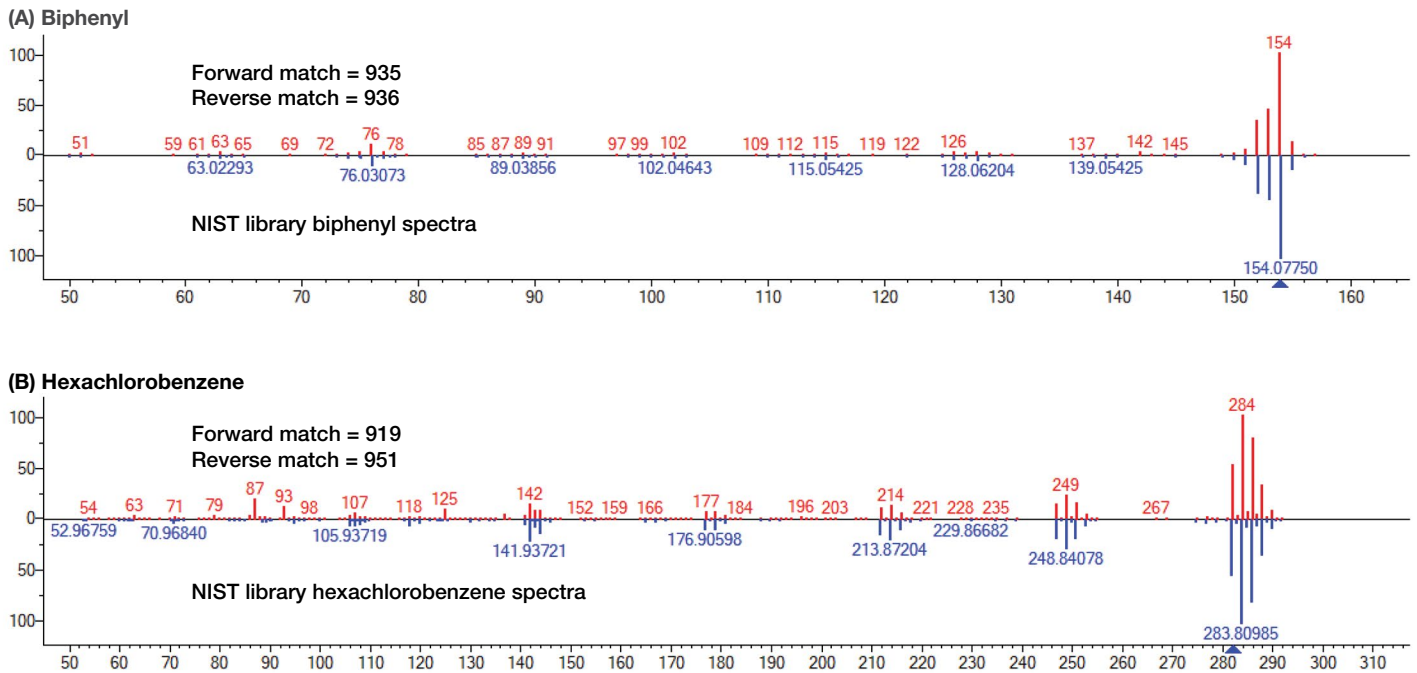
NIST library hexachlorobenzene spectra

Figure 10. NIST library search results achieved using the Exploris Orbitrap GC for the analysis of biphenyl and hexachlorobenzene in a mixed pesticide standard (10 µg/kg), with both forward and reverse library match scores of >900 achieved

- Dichlobenil (%RSD = 4.2)
- Propham (%RSD = 2.2)
- Endosulfan ether (%RSD = 10.6)
- Pentachloroaniline (%RSD = 9.5)
- Azamethiphos (%RSD = 9.3)
- Endosulfan, beta- (%RSD = 9.5)
- PCB 118 (%RSD = 9.3)
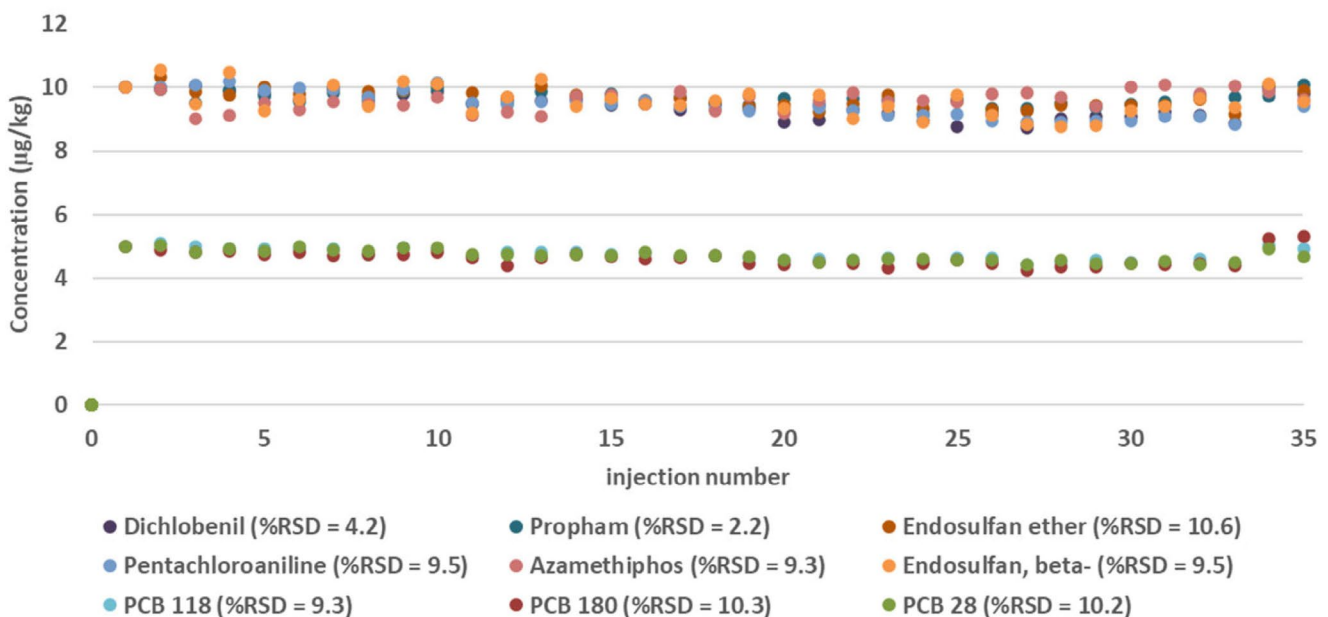- PCB 180 (%RSD = 10.3)
- PCB 28 (%RSD = 10.2)

Figure 11. Robustness data achieved for the analysis of a selection of pesticides and PCBs in apples and carrots using the Exploris Orbitrap GC, with %RSD <11 (n=35), demonstrating consistent system performance, essential for analytical testing application

Summary

The results summarized in this application note demonstrate that the Orbitrap Exploris GC mass spectrometer coupled to the TRACE 1310 GC represents a suitable alternative to traditional GC-MS/MS approaches for the analysis of trace level contaminants in food. The smaller instrument footprint and simplified system setup enables operational use while offering powerful gains in quantitative performance, ease of use, simplicity, and productivity in line with regulatory requirements such as SANTE/12682/2019.

Sensitive and robust full-scan analysis allows for easy and flexible method setup, data acquisition, and processing, meeting SANTE guidelines for the analysis of pesticides and PCBs:

- Sensitivity below the MRL; the majority of mass accuracy <2 ppm; 93% of ion ratios with differences of ≤30% between ion ratios of standards and samples achieved; excellent linearity with $R^2 > 0.95$; average response factors RSD% < 20 across the 5-point (5–1250 µg/kg) matrix-matched calibration series; recoveries for pesticides spiked in apple and carrot samples showed reliable detection and accurate quantitation of spiked compounds, with >92% of pesticides tested within 70–120% recovery obtained.

- A compact footprint offering regulated testing with robust quantitative performance with results confidently reportable with fast turnarounds, all at a competitive cost

- Ease of use features enabling maintenance such as changing columns or removing the source to be carried out without venting the MS, saving valuable time, adding flexibility with increased efficiency

- Quick and efficient tuning and calibration using Orbitrap Exploris tune software designed for ease of use, while offering maximum functionality

- Options for retrospective addition of compounds enhance productivity.

- Method editor incorporated with System Templates, enabling user friendly method setup using pre-optimized application specific method templates

- The TRACE 1310 GC brings the power to the user through its instant connect modular injectors and analogue detectors, which can be interchanged within minutes, and fast cooling capability, reducing the time between injections.

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