

Applying High-Resolution GC-Orbitrap Mass Spectrometry for Quantitation of Pesticides and PCB's in food samples



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ABSTRACT

Purpose: To evaluate high resolution accurate mass performance for the quantitative analysis of pesticides and PCBs in food matrices.

Methods: In all experiments a bench top GC Orbitrap MS with 60,000 mass resolution was used. It was tuned and calibrated using PFTBA to achieve mass accuracy of <1.0 ppm prior to use. The method used a TG-SIMS, 0.25 mm x 0.25 µm column (Thermo Fisher Scientific) with a 5-m safeguard. A programmed temperature vaporizing (PTV) injector with a temperature ramp and a 1-µL injection volume. The default mode of operation was full scan at various resolving powers. Data acquired was lock-mass corrected using GC column bleed siloxane masses. All data were acquired and processed using Thermo Scientific™ TraceFinder™ software. One diagnostic ion was used for quantitation and three confirmatory ions were used to confirm detections.

Results: The first objective of the study was to assess the linearity of response over a calibration range of 5-200 ppb (µg/kg); the linear response as indicated by the coefficient of determination R² was >0.994 and residual average response factor RSD% was < 20% for all detected compounds. Secondly, the system sensitivity was tested by repeat injections of serially diluted matrix-matched fruit and vegetable samples spiked with up to 25 compound at various levels and establishing the limit of detection (LOD). Experiments to assess the stability of mass accuracy and stability of the response were repeated in two different days for each matrix. The method contains 167 pesticides, PCBs, and metabolites, and was successfully validated for 94% of these analytes. The sensitivity in full scan mode easily meets the default MRLs of 10 ppb for 90% of the analytes¹. The mass accuracy for the target ion and confirmatory ions is less than 2 parts per million (ppm) for most of the analytes. The ion ratios of confirmatory ion to target ion meet the 30% level set in the SANTE document².

INTRODUCTION

The objective of this work was to assess the performance and 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.

MATERIALS AND METHODS

Sample Preparation

Fruit and vegetable samples were obtained from the market and extracted using the mini-Luke procedure. Acetone (30 mL) was added to 15 g of cryogenically homogenized sample in a PTFE centrifuge tube. The sample was blended using an ULTRA-TURRAX®. Dichloromethane (30 mL) and petroleum ether, 40–60 °C, and sodium sulfate were added and the sample re-blended using the ULTRA-TURRAX blender. The sample was centrifuged at 3500 rpm for 5 min and 60 mL of the supernatant taken (equivalent to 1 g/mL sample). The sample volume was reduced by rotary evaporation and a solvent exchange into ethyl acetate (EA) was performed. The sample was transferred to a 10 mL volumetric flask and made up to volume with EA. A series of matrix-matched calibration standards containing 167 pesticides and 7 PCBs, equivalent to 1, 2, 5, 10, 20, 50, 100, and 200 µg/kg, were prepared by spiking apple and carrot extracts.

MS Method

The routine ionization mode was electron ionization (EI) and the mass spectrometers were operated using full scan with default 60,000 mass resolution (FWHM, measured at *m/z* 200). Data acquired was lock-mass corrected using GC column bleed siloxane masses. Table 1 and 2 details the instrument parameters used in the study.

Data Analysis

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.

Method

Method details

Table 1. GC parameters used for data acquisition

TRACE 1310 GC Parameters	
Injection Volume (µL):	1
Liner:	Siltek six baffle PTV liner (PN: 453T2120)
Inlet (°C):	70
Transfer rate(°C):	5
Final temperature (°C)	300
Transfer time (min)	2
Inlet Module and Mode:	PTV, splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Temperature 2 (°C):	90
Rate (°C/min):	25
Hold Time (min):	1.5
Temperature 3 (°C):	280
Rate (°C/min):	5
Hold Time (min):	0
Temperature 3 (°C):	300
Rate (°C/min):	10
Hold Time (min):	5

Table 2. Orbitrap Exploris GC method parameters used.

Orbitrap Exploris GC Mass Spectrometer Parameters	
Transfer line (°C):	250
Ionization type:	EI
Ion source(°C):	250
Electron energy (eV):	70
Acquisition Mode:	full scan
Mass range (Da):	50-700
Resolving power (FWHM at <i>m/z</i> 200):	60,000
Lockmass, column bleed (<i>m/z</i>):	207.03235

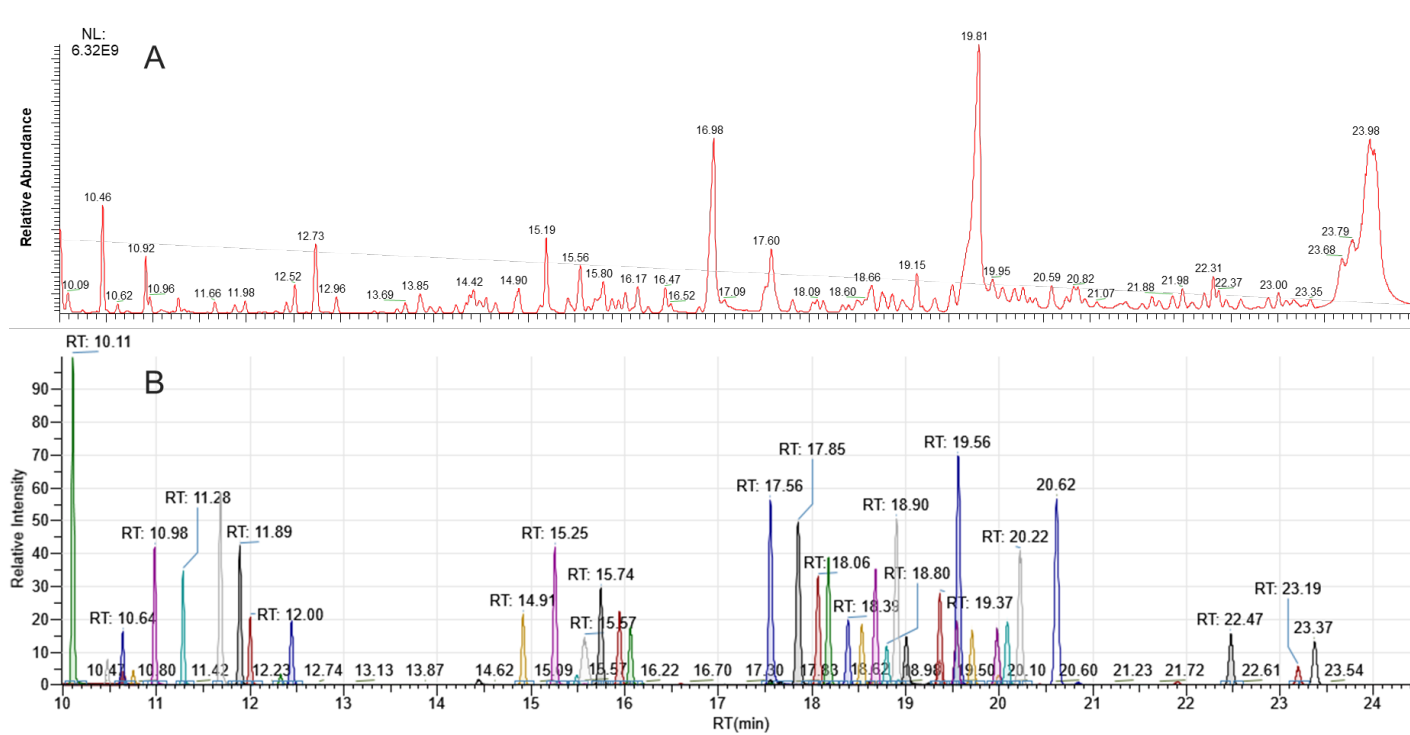
RESULTS

Limits of quantitation in matrix

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 utilizes modular injectors that allow for either 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.

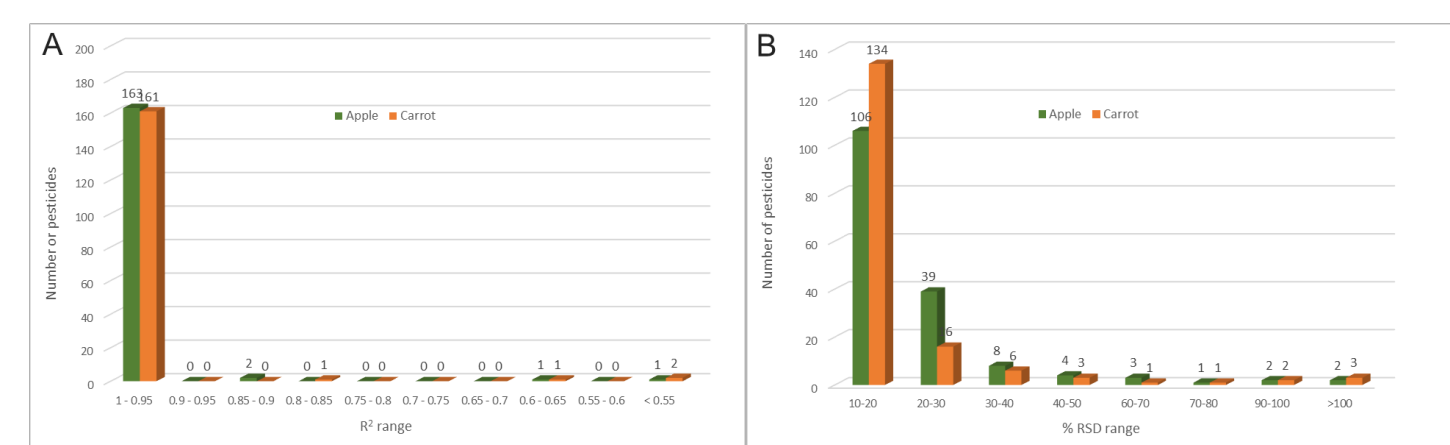
A wide linear dynamic range is essential, especially when dealing with applications where the samples analyzed contain a complex chemical background that could potentially interfere with the analytes of interest. The sensitivity of target compounds in matrix is a key parameter when assessing the suitability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

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



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/x, and with curves not forced through the origin. The residual values variation (as %RSD) and coefficient of determination (R²) were used to assess linearity. R² values of ≥0.95 and residuals ≤30% are considered acceptable. Figure 2 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. Therefore, when operating at a resolving power of 60,000, the established RL exceed the detection requirements for pesticide residue monitoring.

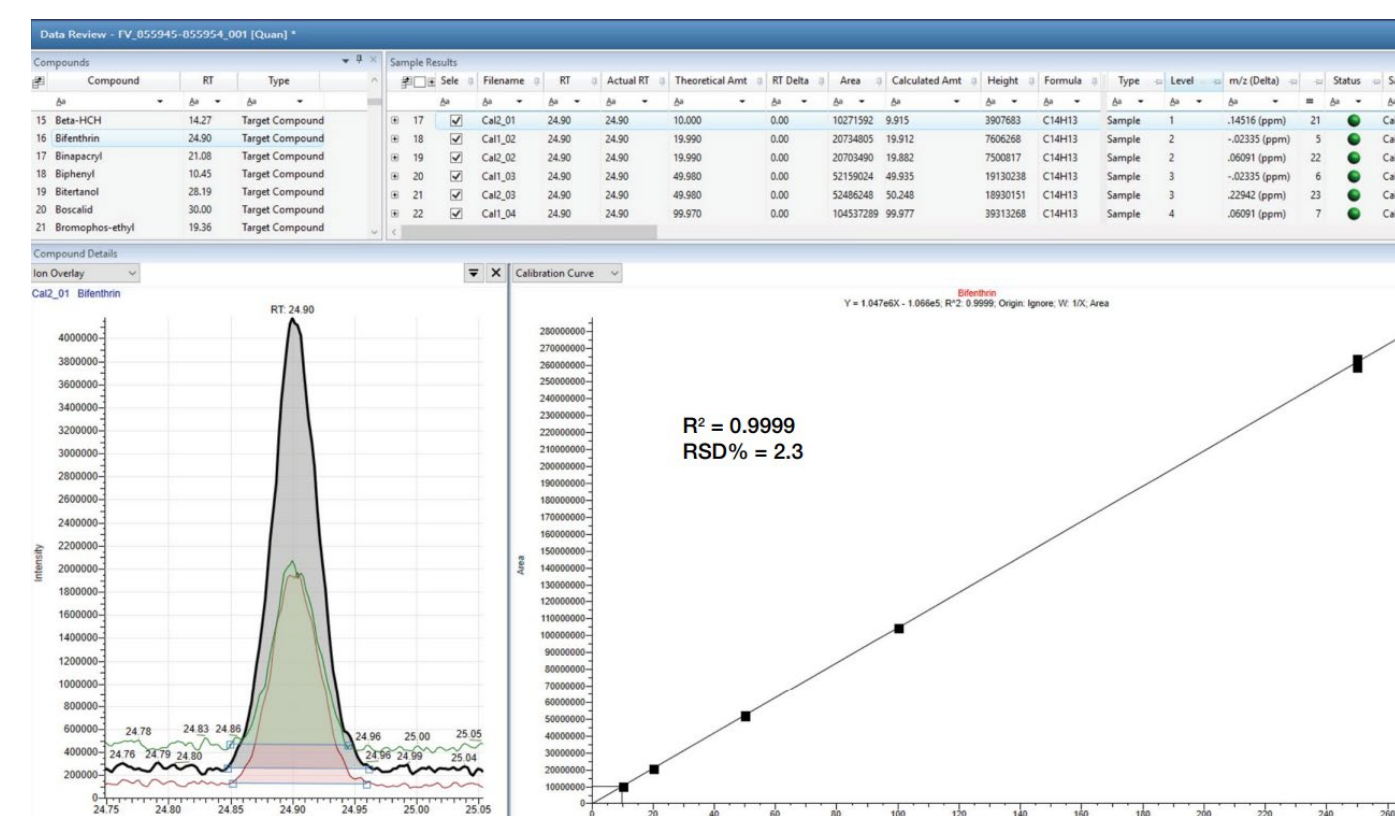
Figure 2. Summary of linearity achieved for pesticides in apple and carrot matrices, expressed as (A) R² and (B) % RSD



Linear dynamic range

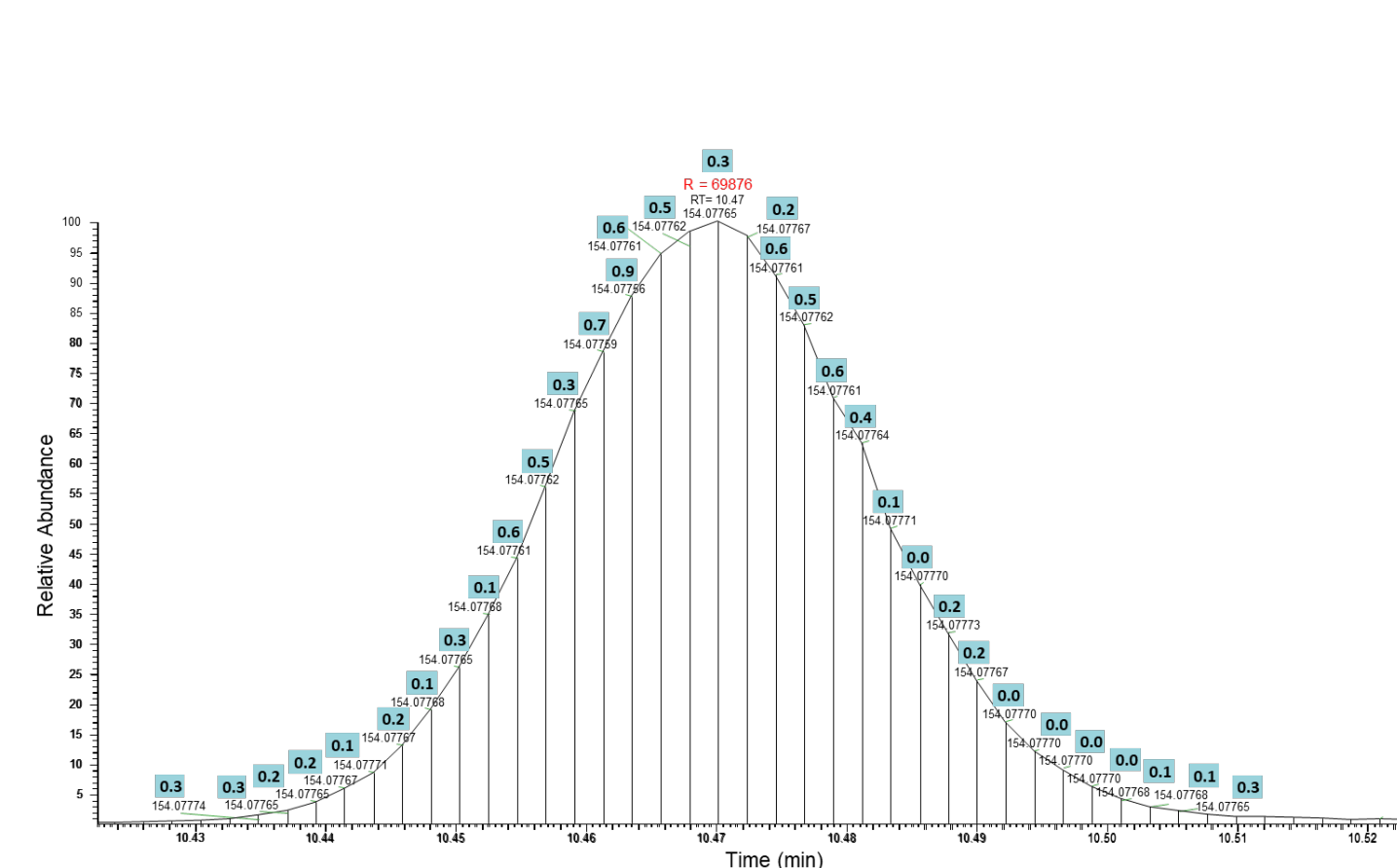
An example of compound sensitivity is shown in Figure 3 for bifenthrin in carrot. Overlay of the diagnostic ions at 10 µg/kg and the linear response for this compound are shown in the customizable views in TraceFinder software, which allows the user to quickly review the key detection criteria and any parameters outside of specified tolerances can be flagged automatically

Figure 3. 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 R² = 0.9999 and residual value as RSD% = 2.3 are shown.



For accurate compound identification and quantitation, at least 10 data points (scans) across a chromatographic peak are usually considered necessary. At 60,000 mass resolving power setting the data acquisition speed is approximately 7 per second. Mass accuracy for each scan is <1ppm. Figure 4 shows biphenyl peak in apple at 10 pg on column with ~33 scans across a 4.8 s wide peak.

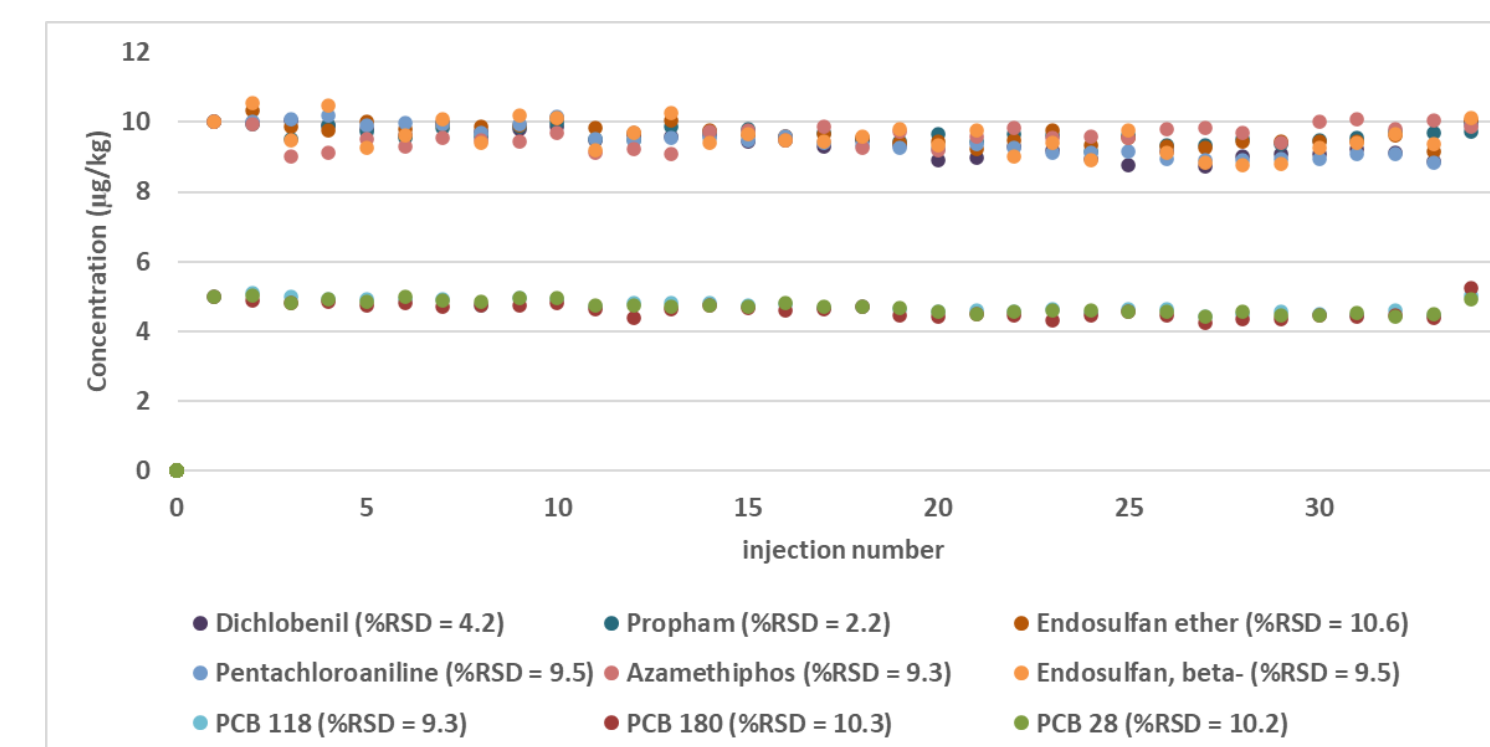
Figure 4. Biphenyl acquired in full scan at 60k resolution in an apple sample at 10 pg on column (corresponding to 10 µg/kg level), showing ~33 scans/peak (4.8 s peak width). Consistent sub 1-ppm accuracy was obtained for each individual scan.



Reproducible results for high throughput analysis

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 5, for a selection of pesticide and PCBs with % RSDs .

Figure 5. Robustness data achieved for the analysis of a selection of pesticides and PCBs in apples and carrots using the Exploris Orbitrap GC, with %RSD



CONCLUSIONS

- Sensitivity below the MRL; the majority of mass accuracy 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.
- Full scan high resolution data enables the scope of analysis to be increased without reanalysis of the samples or different ions used for confirmation of compound presence. Consistent results were demonstrated across two days of continual analysis.

REFERENCES

- The Power of High Resolution Accurate Mass Using Orbitrap Based GC-MS. Thermo Scientific White Paper 10456, May 2015. [Online] <https://tools.thermofisher.com/content/sfs/brochures/WP-10456-GC-MS-Orbitrap-High-Resolution-AccurateMass-WP10456-EN.pdf>
- SANTE/12682/2019. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.

TRADEMARKS/LICENSING

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