Breakthrough Analytical Performance of a Novel Gas Chromatography – Orbitrap Mass Spectrometry for Routine and Research Applications

ABSTRACT

Purpose: To demonstrate the Thermo Scientific[™] Orbitrap Exploris[™] GC mass spectrometer's exceptional analytical performance for both analytical science and scientific research applications.

Methods: In all experiments a benchtop Orbitrap Exploris GC 240 with a maximum resolving power of 240,000 was used, tuned and calibrated using PFTBA to achieve mass accuracy of <1.0 ppm prior to use. Methods were developed, using liquid split/splitless sample injection, on a TG-5SilMS GC capillary column, and using both EI and CI (with methane as reagent gas) ionization modes. The default mode of operation was full scan at various resolving powers (30,000, 60,000, 120,000 and 240,000 measured as FWHM, at *m*/z 200). Data acquired was lock-mass corrected using GC column bleed siloxane masses. All data were acquired and processed using Thermo Scientific™ TraceFinder™ software.

Results: Fast scan speed with typically 15 points across a 2sec wide peak were demonstrated for 2nitroaniline (m/z = 138.04239) while maintaining sub-ppm mass accuracy for each individual scan. NIST library searchable accurate mass spectra were achieved, with greater than 750 match and reverse match NIST library search scores achieved for the identification of mixed pesticides in a whole flour matrix. Linear dynamic range extending to six orders of magnitude (0.1 -10,000 pg oc), with excellent peak area repeatability was demonstrated for hexachlorethane. Sensitivity, expressed as instrument detection limit (IDL), was demonstrated for >140 pesticides in a whole flour matrix, with IDL values of between 0.2 and 2.5 pg/µL, with an average value of 0.7 pg/µL. Spectral fidelity, which is critical to maintain confidence in compound identification, was illustrated for pentachloroaniline in a whole flour matrix, with the mass accuracy for every ion in the isotopic cluster <1 ppm at both low level (1 $pg/\mu L$) and high level (500 $pg/\mu L$) and an isotope match score of 100%.

Sub-ppm level mass accuracy was achieved for the analysis of pesticides in a spiked whole flour matrix ($10pg/\mu L$, n=10), with an average mass accuracy of 0.2 ppm.

INTRODUCTION

The objective of this study was to further explore the power of high resolution and accurate mass using Orbitrap-based GC-MS^{1,2} by evaluating key analytical parameters that are essential for analytical testing and scientific research applications. These include linear dynamic range, sensitivity, NIST library search matching, spectral fidelity, scan speed, mass accuracy, robustness, compound confirmation using positive chemical ionization, and resolving power.

MATERIALS AND METHODS

Sample Preparation

Locally purchased organic wheat flour or oats (10 g) were weighed into a 50 mL centrifuge tube. Acetonitrile (10 mL), containing 1% (v/v) of acetic acid, was then added to the sample, which was then vortexed for 1 min. To this, 3 g of magnesium sulfate (MgSO4), 1.7 g of sodium acetate, and 0.5 g of disodium hydrogen citrate were added, and the tube was vortexed for 1 min and centrifuged at 4,000 rpm. Figure 1 depicts an extracted ion chromatogram (XIC) of 2-nitroaniline (m/z 138.04238 \pm 5 ppm window) in a 1,000 pg/µL mixed solvent standard. Data were acquired in full scan at 60k resolution (FWHM at m/z 200). Excellent mass accuracy is shown for each individual scan as well as mass difference (in ppm). An average mass difference of 0.4 ppm was measured across the peak. Compound identification using spectral libraries The Orbitrap Exploris GC instrument, with full scan range mass accuracy and sensitivity, enables accurate and reliable commercial library (e.g. NIST/Wiley) matching. Figure 2 shows the NIST library search results achieved using the Orbitrap Explors GC mass spectrometer for the analysis of aldrin in a mixed pesticide standard, with both forward and reverse library match scores of >890 achieved. for 10 min. After centrifugation, 5 mL of the supernatant was transferred into a polypropylene tube with 250 mg C18 sorbent, 750 mg MgSO4 and 750 mg primary/secondary amine (PSA). The tube was vortexed for 1 min and centrifuged for 10 min at 4,000 rpm and supernatant used for GC-MS analyses

MS Method

Electron ionization (EI) mode was employed, and the mass spectrometers were operated using full scan with default 60,000 mass resolution (FWHM, measured at m/z 200). Data acquired was lockmass corrected using GC column bleed siloxane masses.

Data Analysis

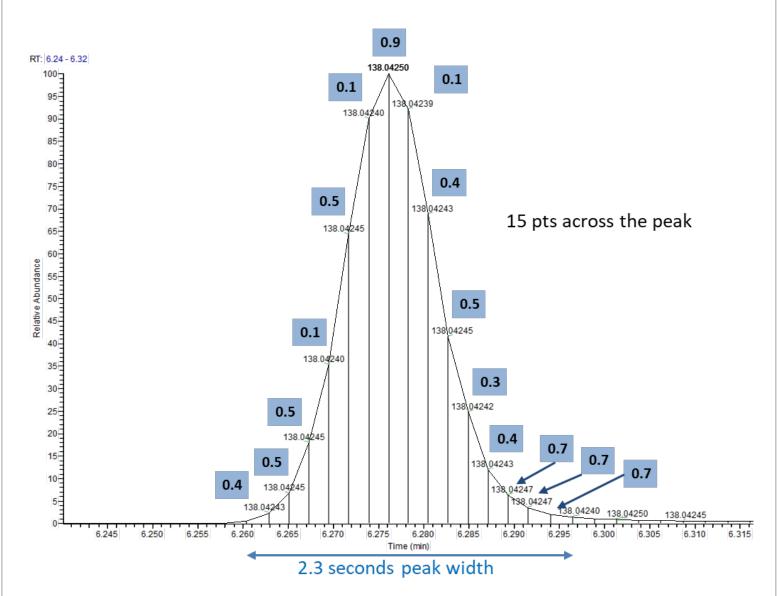
Data analysis was performed using both TraceFinder and Thermo Scientific[™] Compound Discoverer[™] software.

RESULTS

Data acquisition rate

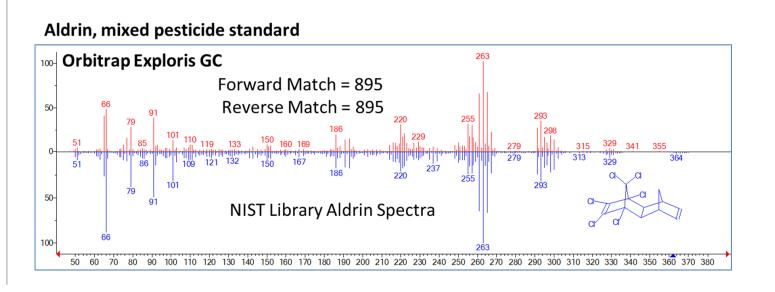
Fast data acquisition, to allow sufficient data points across narrow GC peaks, is critical in order to achieve accurate and precise compound identification. An example using the Orbitrap Exploris GC is shown in Figure 1, where 15 data points across the 2.3 s wide peak for 2-nitroaniline (extracted ion chromatogram of m/z 138.04238) were obtained.

Figure 1. Extracted ion chromatogram (XIC) of 2-nitroaniline (m/z 138.04238 \pm 5 ppm window) in a 1,000 pg/µL mixed solvent standard. Data acquired in full scan at 60k resolution (FWHM at m/z 200). Excellent mass accuracy is shown for each individual scan as well as mass difference (in ppm). An average mass difference of 0.4 ppm was measured across the peak.



The Orbitrap Exploris GC, with full scan range mass accuracy and sensitivity, enables accurate and reliable commercial library (e.g. NIST/Wiley) matching. Figure 2 shows the NIST library search results achieved using the Orbitrap Exploris GC for the analysis of aldrin in a mixed pesticide standard, with both forward and reverse library match scores of >890 achieved.

60k resolution (FWHM at m/z 200)...



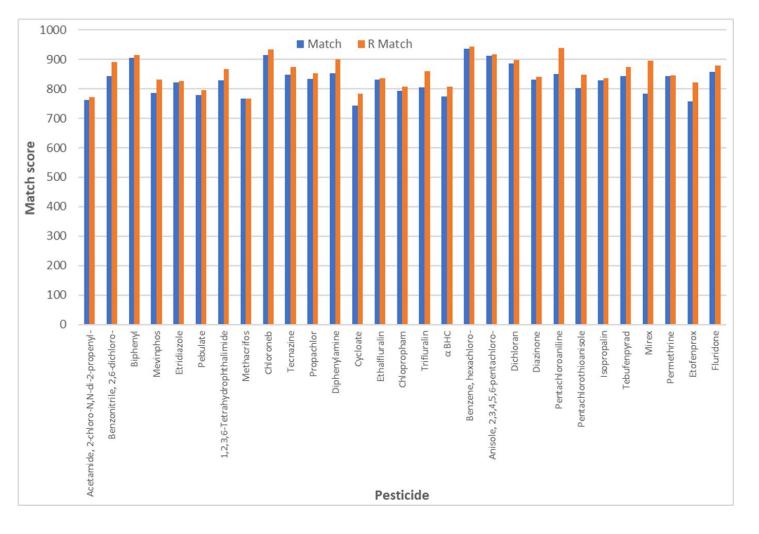
Xin Zheng, Jason Cole¹, Lori Dolata, Dominic Roberts², and Cristian I. Cojocariu². Thermo Fisher Scientific, Austin, USA. Thermo Fisher Scientific, Hemel Hempstead, United Kingdom

Figure 2. NIST library search mass spectra and match results achieved for the analysis of aldrin in a mixed pesticide standard using an Orbitrap Exploris GC operated in full scan at

Spectral library matching

Additional results achieved using the Orbitrap Exploris GC are shown in Figure 3 for a selection of pesticides in a mixed pesticide standard in whole flour matrix, where similar NIST library results were obtained considering both reverse and forward search results.t

Figure 3 Library search scores achieved using an Orbitrap Exploris GC for a selection of pesticides in a mixed pesticide standard in a whole flour matrix (a score of 1,000 equals a perfect match). Forward search scores (Match) and reverse search scores (R Match) given for each pesticide, when searched against the NIST library.



The sensitivity achievable with the Orbitrap Exploris GC was evaluated for the analysis of whole flour spiked with pesticides. For this a whole flour sample extract was spiked with pesticides at 10 pg/µL level (equivalent to the European Union (EU) default maximum residue level (MRL) set at 10 µg/kg) and repeat injections (n=10) were performed. Sensitivity expressed as instrument detection limits (IDL) for all the pesticides analyzed was calculated and is reported in Figure 4. The IDL was calculated taking into account the Student's-t critical values for the corresponding degrees of freedom (99% confidence). Excellent sensitivity with IDL values ranging from 0.18 to 2.45 pg/µL was achieved, with an average value of 0.7 pg/µL. The results confirmed that the Orbitrap Exploris GC has the sensitivity levels to meet the regulatory analysis of pesticides in matrix matched standards.

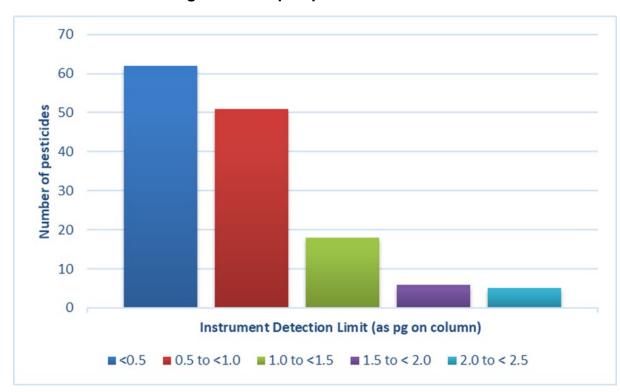
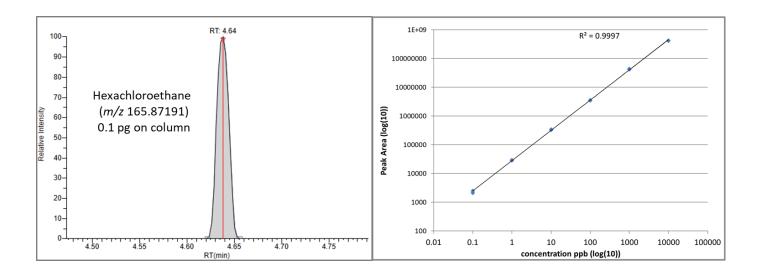


Figure 4. Sensitivity instrument detection limit (IDL as pg on column) determined for 142 pesticides in whole flour using an Orbitrap Exploris GC.

Linear dynamic range

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 (e.g., pesticide screening and quantification, metabolomics). To test the linear dynamic range using the Orbitrap Exploris GC, repeat injections (n=3) of increasing concentration levels (0.1 pg to 10,000 pg on column) of mixed solvent standards were performed. An example of compound linearity obtained using log(10) is shown in Figure 5 for hexachloroethane, the results demonstrating linear dynamic range extending to six orders of magnitude (0.1–10,000 pg on-column) making the Orbitrap Exploris GC an ideal platform for quantitative analysis.

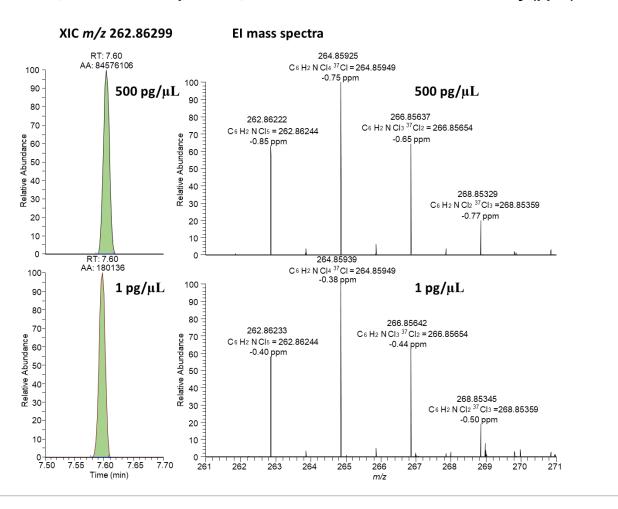
Figure 5. Linear dynamic range of the Orbitrap Exploris GC is demonstrated using hexachloroethane solvent standards injected over six orders of magnitude. The extracted ion chromatogram (m/z 165.87191) corresponding to hexachloroethane at 0.1 pg on column is shown together with the coefficient of determination (R2) values determined over a concentration range of 0.1–10,000 pg on column



Spectral fidelity

Maintaining spectral fidelity over the full analytical concentration range in matrix is critical to maintain confidence in compound identification, even at low levels, as illustrated in Figure 6 for pentachloroaniline in whole flour matrix. The mass accuracy for every ion in the isotopic cluster is <1 ppm giving confidence in the identification.

Figure 6. Spectral fidelity illustrated for pentachloroaniline for two levels (1 and 500 pg/µL) in whole flour matrix using an Orbitrap Exploris GC. [A]: Extracted ion chromatograms (XIC) for pentachloroaniline at each level annotated with peak retention time (RT) and peak area (AA); [B]: El mass spectra zoomed at the molecular ion cluster at each level, annotated with measured mass, elemental composition, theoretical mass and mass accuracy (ppm).



Mass accuracy

To have a high degree of confidence in compound identification, good mass accuracy is critical. Subppm mass accuracy was maintained across compound concentrations using an Orbitrap Exploris GC, as exemplified for hexachloroethane (Table 1). In all cases, irrespective of the *m*/*z* and concentration level, <1 ppm values were observed. This is essential as any compromise in accuracy of mass measurements can result in false identification and non-detection of toxic chemicals such as pesticides in a screening experiment.2 It is also necessary to maintain this performance at all concentration levels as any level can be encountered in real world samples.

Table 1. Mass accuracy (ppm) over six orders of magnitude for five selected ions of hexachloroethane measured using the Orbitrap Exploris GC

Level	Orbitrap Exploris GC				
ppb on column	m/z 118.90306	m/z 116.90601	m/z 120.90011	m/z 165.87191	m/z 202.83781
0.1	0.1	0.4	0.7	0.1	-0.3
1	0.0	0.0	0.2	0.1	-0.1
10	0.4	0.6	0.5	0.6	0.3
100	0.4	0.5	0.4	0.5	0.3
1000	0.7	0.8	0.7	0.8	0.5
10000	0.5	0.6	0.5	0.6	0.3
average ∆ppm	0.4	0.5	0.5	0.4	0.2

CONCLUSIONS

- The data shown here demonstrate that the Orbitrap Exploris GC mass spectrometers deliver exceptionally high guality analytical performance using full scan acquisition for both analytical testing and scientific research applications.
- NIST library searchable accurate mass spectra are achievable, enabling confident identification of unknown compounds and confirmation of knowns.
- The fast scan speeds available allow sufficient data points across narrow chromatographic peaks to accurately describe the peak area and to ensure signal and spectra reproducibility. The linear dynamic range extending to six orders of magnitude and the sensitivity demonstrated for >140 pesticides in whole flour (IDL values of between 0.18 and 2.45 $pg/\mu L$).

REFERENCES

- 1. 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
- 2. Fast Screening, Identification, and Quantification of Pesticide Residues in Baby Food Using GC Orbitrap MS Technology. Thermo Scientific Application Note 10449. [Online] https://tools.thermofisher.com/content/sfs/brochures/AN-10449-GC-MS-OrbitrapPesticides-Babv-Food-AN10449-EN.

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