



GC-MS

## Grant application resource: Using the Orbitrap Exploris GC 240 mass spectrometer to accelerate research

### Keywords

Orbitrap Exploris GC 240 mass spectrometer, Orbitrap technology, accurate mass, complex matrices, high resolution, screening, gas chromatography, unknown identification, structural elucidation

### Goal

This document highlights why the Thermo Scientific™ Orbitrap Exploris™ GC 240 mass spectrometer is the premium quantitative high-resolution, accurate-mass (HRAM) Orbitrap system that delivers unprecedented depth of analysis. With technology innovations and curated ready-to-use, application-specific method templates, this system delivers complete quantitative strategies for scientific research, such as untargeted analysis to quantitative targeted experiments without compromising accuracy, coverage, or depth of analysis. With industry-leading sensitivity, the Orbitrap Exploris GC 240 MS is designed for scientists in research and core laboratories looking to expand into new research areas with the highest confidence, leading to impactful publications and results. The information provided here highlights technologies that are different from previous Thermo Scientific™ Q Exactive™ instrumentation as well as quadrupole time-of-flight (QTOF) analyzers.

### Introduction

Scientific research laboratories need to obtain confident results while maintaining the highest levels of accuracy and confidence. For many researchers, it is critical to have the flexibility and analytical power to tackle a diverse range of analytical challenges to gain a comprehensive understanding of their samples. Most of these laboratories rely on both targeted and untargeted analytical approaches, using both gas chromatography and liquid chromatography coupled to single quadrupole or triple quadrupole mass

spectrometry (MS) instrumentation. These systems cover the wide range of chemical classes to be detected but provide only limited information for discovery workflows. For targeted applications, they are limited to detect only those compounds in the target list, and they require careful optimization of acquisition parameters for each compound. High-resolution, full scan mass spectrometry using Orbitrap technology provides a solution to:

- Detection and quantification of an increasing number of compounds
- Identification and elucidation of the chemical composition and structure of unknown compounds
- Retrospective analysis of samples long after data acquisition

High-resolution Orbitrap mass spectrometry has been available with both liquid and gas chromatography for many years and has proven to be a highly valuable analytical technique. More recently, the technology in gas chromatography moved to join the Thermo Scientific Orbitrap Exploris Mass Spectrometer series. This new platform of a benchtop hybrid quadrupole-Orbitrap mass spectrometer opens up new research opportunities in a system with significantly reduced footprint, saving both energy and raw materials in their manufacture.

High-resolution Orbitrap mass spectrometry has proven to be a highly valuable analytical technique for both analytical science and scientific research applications.<sup>1-3</sup> Orbitrap mass spectrometry technology coupled to gas chromatography (GC) has evolved with the Orbitrap Exploris GC 240 mass spectrometer system (Figure 1), which delivers a maximum resolving power of 240,000 (FWHM at  $m/z$  200), in a compact design and with intelligent informatic solutions. Researchers gain the ability to have the right answers the first time and the flexibility to adapt to ever changing needs from superior mass accuracy, dynamic range, and robustness.



Figure 1. The Thermo Scientific Orbitrap Exploris GC 240 mass spectrometer system

This quadrupole Orbitrap mass spectrometer opens new possibilities for increased mass accuracy, sensitivity, and selectivity for GC-amenable compounds. The detailed examples described in this white paper highlight the benefits of high-resolution MS coupled to GC for the confident detection of both known and unknown compounds in targeted and untargeted workflows. Figure 2 shows the different analytical approaches to increase scope of analysis through full scan high-resolution, accurate-mass data from a targeted list of analytes to screening and unknowns compound identification.

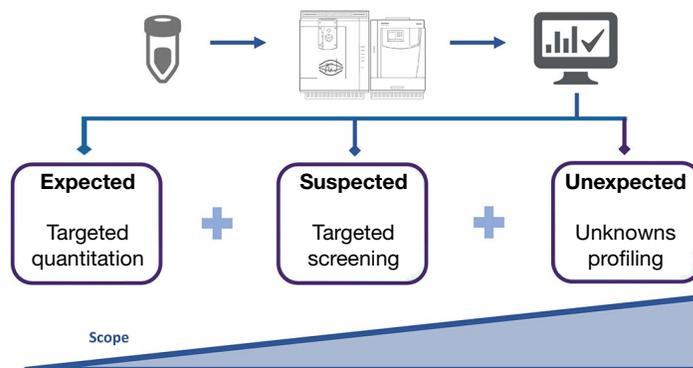


Figure 2. Analytical approaches to increase scope of analysis through full scan high-resolution, accurate-mass data

Here, we highlight how next generation technologies in mass spectrometry hardware and software innovations detailed in Figure 3 and Table 1 achieve increased sensitivity and selectivity on the Orbitrap Exploris GC 240 system.

#### Additional features for research applications:

- Thermo Scientific™ ExtractaBrite™ Electron Ionization (EI) source
- Ion source includes ion volume, repeller, source lenses, RF lens, and dual filaments in all ionization modes, programmable from 50 °C to 350 °C
- VeV tuning allows optimized low electron energy acquisition down to 8 eV
- Chemical ionization (CI) source for acquisition with positive ion chemical ionization (PCI) and negative ion chemical ionization (NCI)
- Entire ion source can be removed or changed to a CI source in under 2 minutes without venting
- Vent-free column exchange with patented source plug combination EI/PCI/NCI ion volume can be used without the need for source interchange

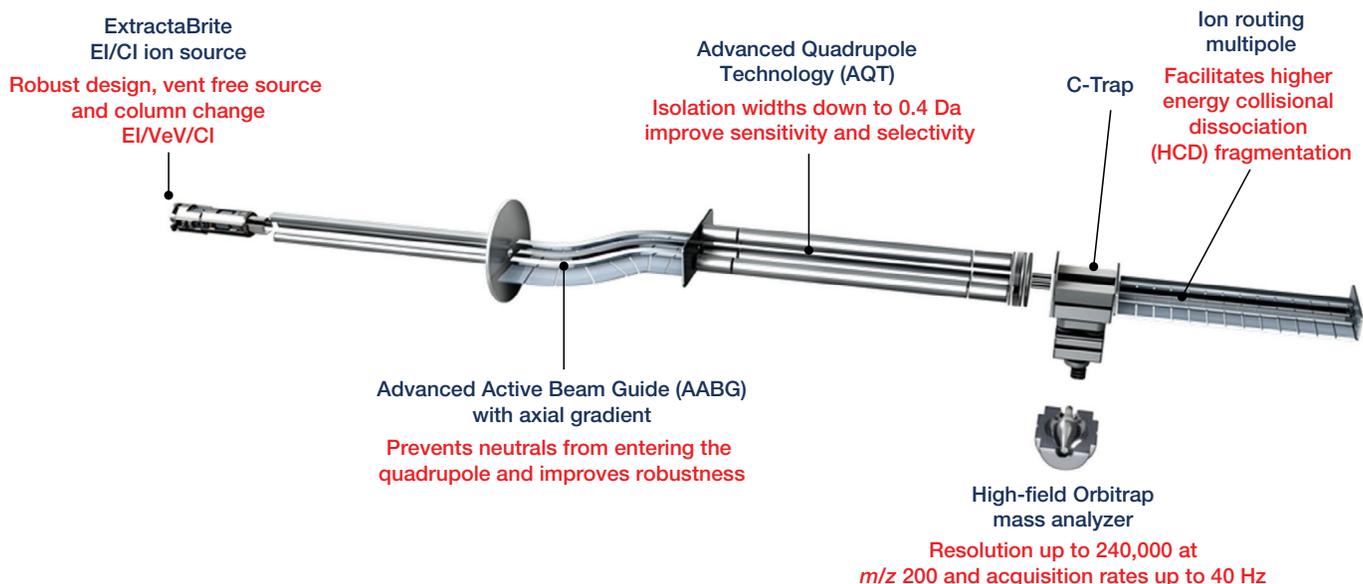


Figure 3. Schematic diagram of the internal components used in the Orbitrap Exploris GC 240 MS

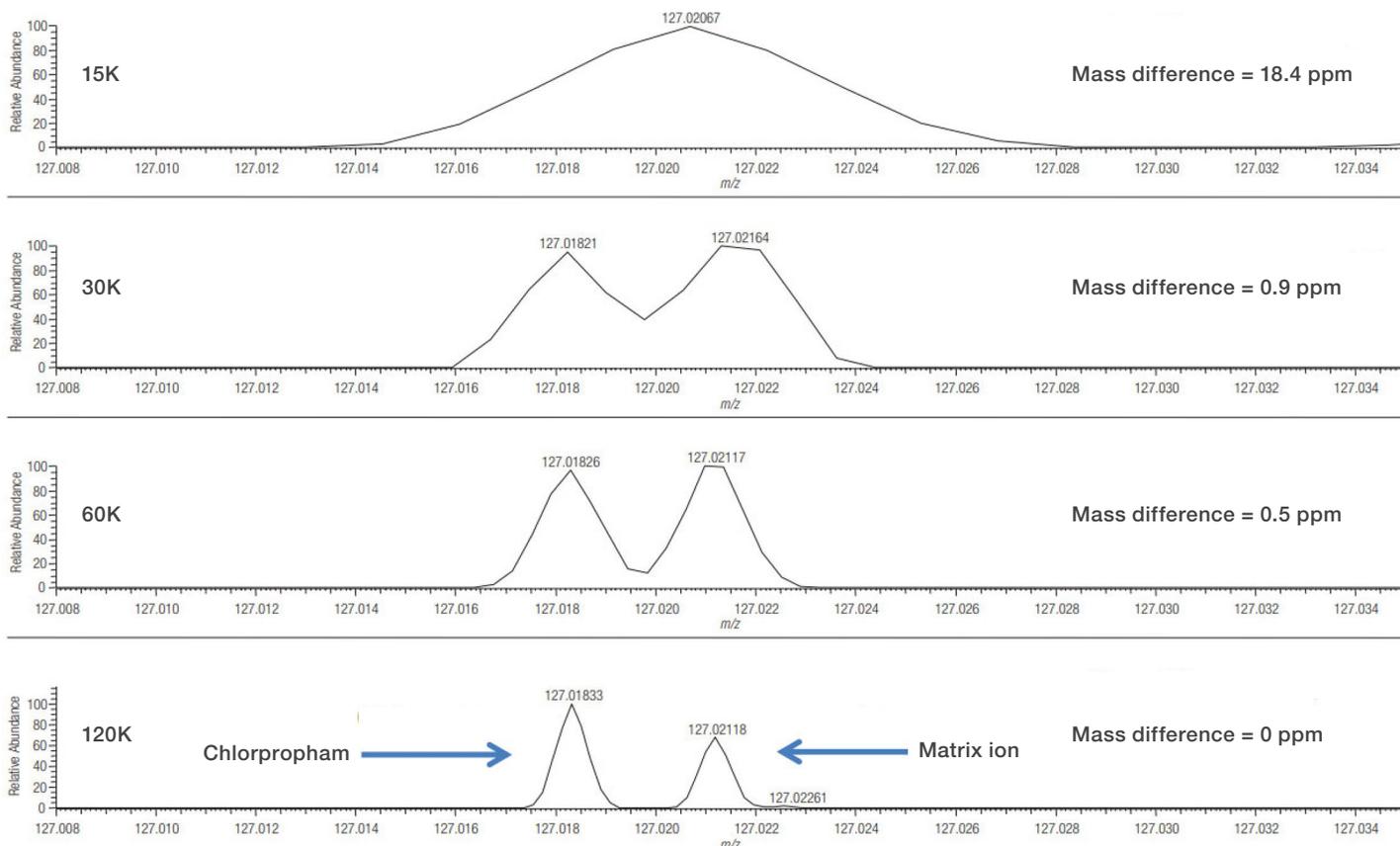
Table 1. Technology specifications for fundamental benefits of Orbitrap Exploris GC 240 MS instrumentation

Benefit	Detail	Technology
<b>Unprecedented performance</b>	Delivers breakthrough performance with unrivalled 240,000 mass resolving power and the sensitivity to get the right answer first time	Orbitrap mass spectrometer Up to 240,000 (FWHM) at $m/z$ 200
<b>Versatility for research</b>	Flexibility to adapt to changing research demands and address diverse challenges through maximum hardware capability	NeverVent™ enabling source exchange, EI to CI and column change in minutes without breaking vacuum
<b>Highest data quality</b>	Fast, simple, and standardized instrument setup to be assured that all users obtain the very highest data quality	Six orders of linear dynamic range and sub 1 ppm mass accuracy
<b>Speed</b>	Fast scan rates for improving deconvolution and quantitation workflows	Up to 40 Hz at resolution setting 7500 (FWHM) at $m/z$ 200
<b>Robustness</b>	Robust and reliable performance with a compact footprint to minimize instrument downtime with easy-to-maintain/clean and simple calibration procedures.	Proven Orbitrap technology delivering reliable performance; ExtractaBrite ion source for robust and consistent ionization
<b>Informatics</b>	Spectral libraries including dedicated application specific GC Orbitrap HRAM libraries, that are fully integrated into Thermo Scientific™ Compound Discoverer™ software	Discover new insights with Compound Discoverer software and HRAM spectral libraries

### Impact of mass resolution on selectivity for targeted analysis

High-resolution, accurate-mass experiments typically provide a full scan analysis of a sample, and for small molecule analyses, the scan range is typically 30–1,000 Da. Orbitrap technology provides the required selectivity to resolve the target compound from other compounds or from matrix ions of similar mass. For targeted compound analysis, the accurate mass of the diagnostic ion is extracted with a narrow mass extraction window

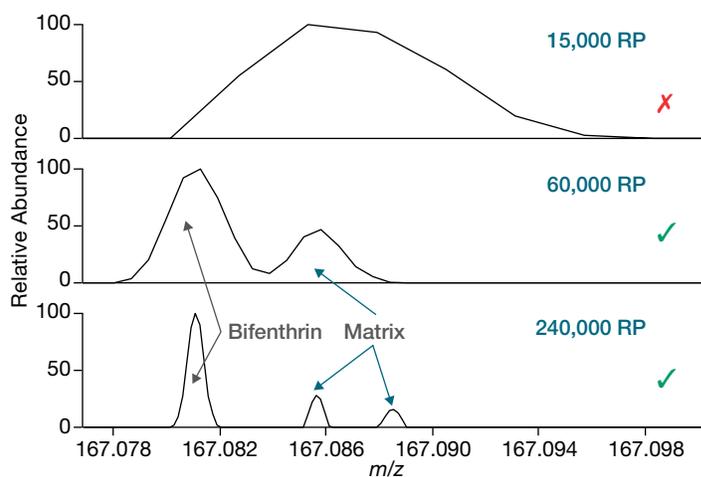
(typically < 5 ppm). This narrow window is possible only when the instrument provides sufficient mass accuracy, for which high mass resolving power is essential. However, when two mass profiles overlap, the measured mass profile is the sum of the two individual profiles. This overlap results in the incorrect assignment of the mass of the target compound. The problem is demonstrated in Figure 4, where a QuEChERS leek extract in acetonitrile was analyzed four times at resolving powers of 15K, 30K, 60K, and 120K ( $m/z$  200).



**Figure 4. Effect of resolving power on mass accuracy of an analyte in matrix.** Mass profiles of chlorpropham 10 ng/g in leek acquired at resolutions of 15K, 30K, 60K, and 120K. Matrix interference at 15K and 30K prevents separation of the pesticide from the interference and higher-than-expected mass difference. Chlorpropham is resolved at 60K and 120K with improvements in mass accuracy. Under normal screening criteria this pesticide would have been missed (false negative).

The Orbitrap Exploris GC 240 mass spectrometer has been designed for complex scientific research applications. The highly selective high-resolution Orbitrap MS platforms aim to deliver unprecedented and flexible performance with up to 240,000 mass resolving power. An additional example of the importance of high resolution is demonstrated in Figure 5, where a QuEChERS soil extract in *n*-hexane was analyzed at resolving powers of 15,000, 60,000, and 240,000 (at FWHM  $m/z$  200). The mass spectra show the pesticide bifenthrin (fragment ion  $m/z$  167.08113 used often as confirmatory ion) and a background matrix ion at a similar mass creating interference. Excellent mass accuracy was achieved for bifenthrin using a resolving power (RP) of 240,000, with near baseline resolution. However, at 15,000 RP, bifenthrin was not sufficiently resolved from the matrix interference, resulting in a poorer mass accuracy assignment. At 15,000 RP, the mass accuracy was significantly affected with a value of 30.8 ppm mass difference. Under typical targeted screening criteria of < 5ppm, and even under a wider tolerance of 10 ppm, this mass difference would have resulted in a false negative (non-detection) for this pesticide. This example clearly shows that a minimum 60,000 RP is needed. The required resolving power needed depends on the complexity of the sample being analyzed, the mass of the analyte, and the abundance of both target analytes and interferences.

In this example, the Orbitrap Exploris GC system using 240,000 RP provided the required selectivity to resolve bifenthrin from other compounds or from matrix ions of similar mass.



**Figure 5. Effect of resolving power (RP) on mass accuracy of an analyte in matrix.** Mass spectra of bifenthrin 10 ng/mL in soil acquired at 15,000, 60,000, and 240,000 RP (at FWHM  $m/z$  200). Matrix interference at 15,000 RP prevents separation of this pesticide from the matrix interference, resulting in a higher than expected mass difference. The bifenthrin fragment ion  $m/z$  167 is partially resolved at 60,000 RP and fully resolved at 240,000 RP.

Being able to separate two compounds that are close in mass is one of the significant advantages of high-resolution, accurate-mass instruments. This is demonstrated in Figures 6–8 where the compounds flurenol methyl ester  $C_{15}H_{12}O_3$  ( $m/z = 240.0781$ ) and dimetilan  $C_{10}H_{16}N_4O_3$  ( $m/z = 240.1217$ ) were analyzed at equal intensity at three different resolving power levels of 30,000, 60,000, and 240,000. The zoomed spectra show excellent

separation at all resolution levels with improvements at 60K and 240K showing clear benefits. When analyzed at 10:1 ratio (Figure 9), there is still good separation and mass accuracy. The latter reflects the real world where compounds will be at varying intensities, and it is essential that mass accuracy is maintained to make confident identifications.

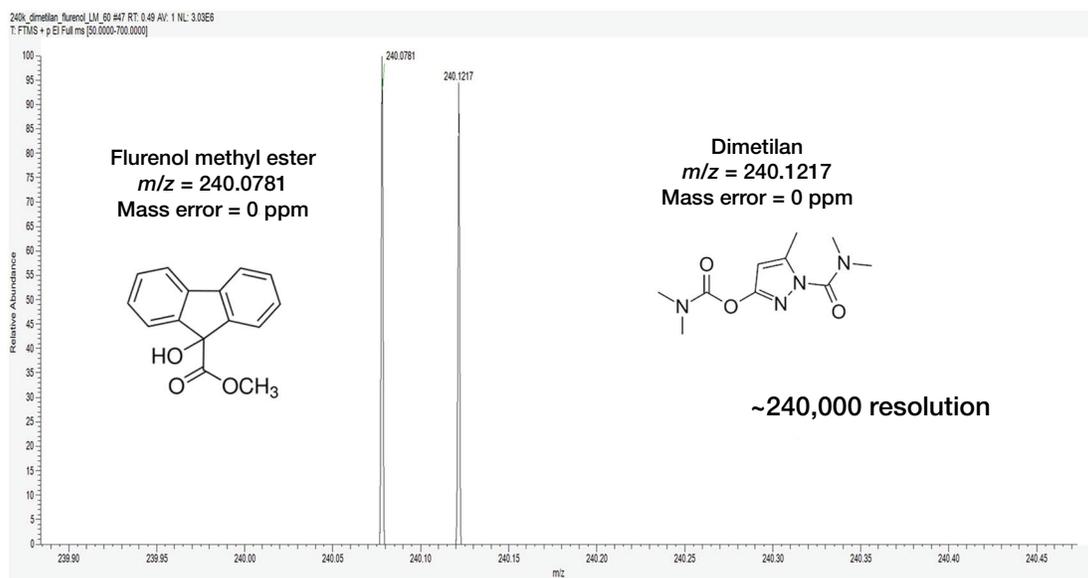


Figure 6. Zoomed spectrum of two similar accurate mass compounds flurenol methyl ester  $C_{15}H_{12}O_3$  ( $m/z = 240.0781$ ) and dimetilan  $C_{10}H_{16}N_4O_3$  ( $m/z = 240.1217$ ) acquired at 240,000 resolving power

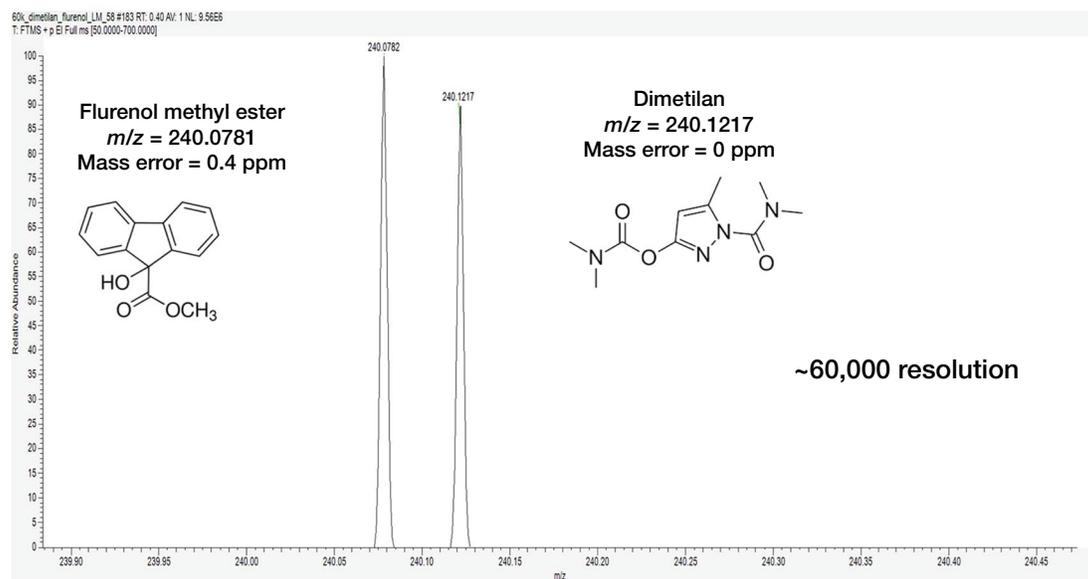


Figure 7. Zoomed spectrum of flurenol methyl ester  $C_{15}H_{12}O_3$  ( $m/z = 240.0781$ ) and dimetilan  $C_{10}H_{16}N_4O_3$  acquired at 60,000 resolving power

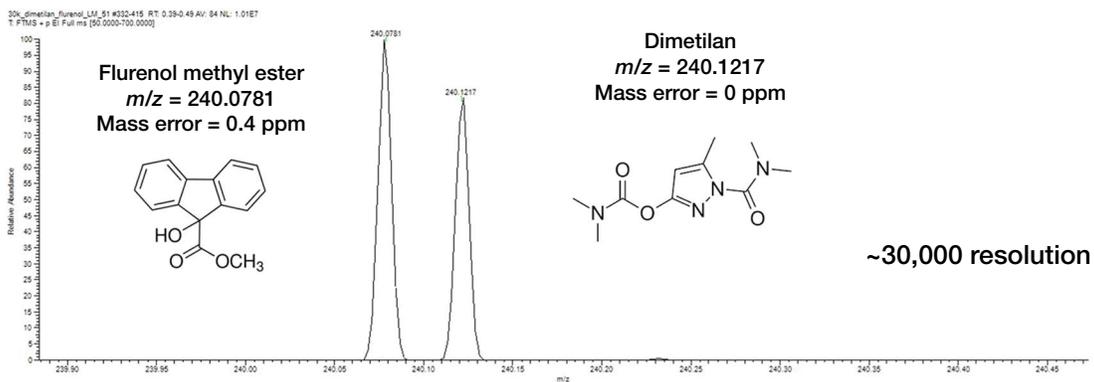


Figure 8. Zoomed spectrum of flurenol methyl ester  $C_{15}H_{12}O_3$  ( $m/z = 240.0781$ ) and dimetilan  $C_{10}H_{16}N_4O_3$  ( $m/z = 240.1217$ ) acquired at 30,000 resolving power

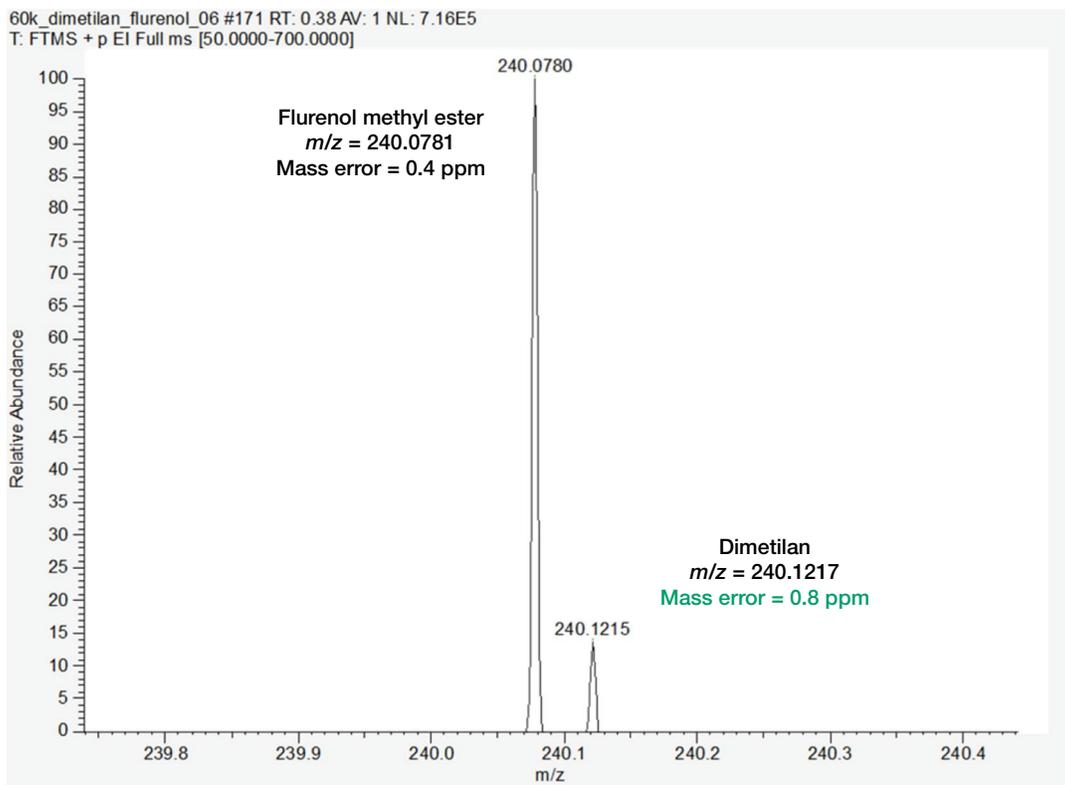


Figure 9. Zoomed spectrum at 10:1 intensity ratio of flurenol methyl ester  $C_{15}H_{12}O_3$  ( $m/z = 240.0781$ ) and dimetilan  $C_{10}H_{16}N_4O_3$  ( $m/z = 240.1217$ ) acquired at 60,000 resolving power

## High sensitivity and high-resolution mass spectrometry

With other types of high-resolution GC-MS technology, increasing the instrument resolving power results in decreased ion transmission (sensitivity). Consequently, the precision of the measurement and the achievable range of detection can be affected. For low-level targeted screening, quantification, and profiling in complex matrices, it is essential to maintain instrument sensitivity while operating at high resolving power. While resolution is extremely important, it is also essential to maintain sensitivity at the higher resolution modes of 240,000. The Orbitrap Exploris GC 240 system does not lose signal intensity as significantly with increasing resolution as other types of high-resolution mass spectrometers. Figure 10 shows an example of the pesticide pyriproxyfen and the corresponding peak area responses at a concentration of 100 ng/mL in soil QuEChERS acetonitrile extracts. These extracts were analyzed at 15,000, 30,000, 60,000 RP, and 240,000 RP in full scan. Increasing resolution is shown to have no significant effect of sensitivity (as absolute peak area response) of pyriproxyfen. This consistency

provides access to both the very highest resolving power and sensitivity that is required across all applications from target quantitation through to discovery omics studies.

## Chemical ionization for compound confirmation

When the spectral library match from the EI spectrum is inconclusive, or additional confirmation is required, positive chemical ionization (PCI) data can be used to confirm the elemental composition of the parent molecule using accurate mass information. In PCI experiments using methane as the reagent gas, three adducts are typically observed:  $[M+H]^+$ ,  $[M+C_2H_5]^+$ , and  $[M+C_3H_9]^+$ . As an example, EI (70 eV and 12 eV) and PCI spectra of ethoprophos are reported in Figure 11. The observed molecular ion corresponding to  $m/z$  136.12468 is present in the EI spectrum with a mass difference of 0.2 ppm from the theoretical  $m/z$  136.12465 for the formula  $C_{10}H_{16}$ . The presence of the methane adducts in the PCI spectrum with sub-1 ppm mass accuracy confirmed  $m/z$  136.12468 as the molecular ion for camphene (RT = 9.04 min) and supported the elemental composition of the proposed molecule.

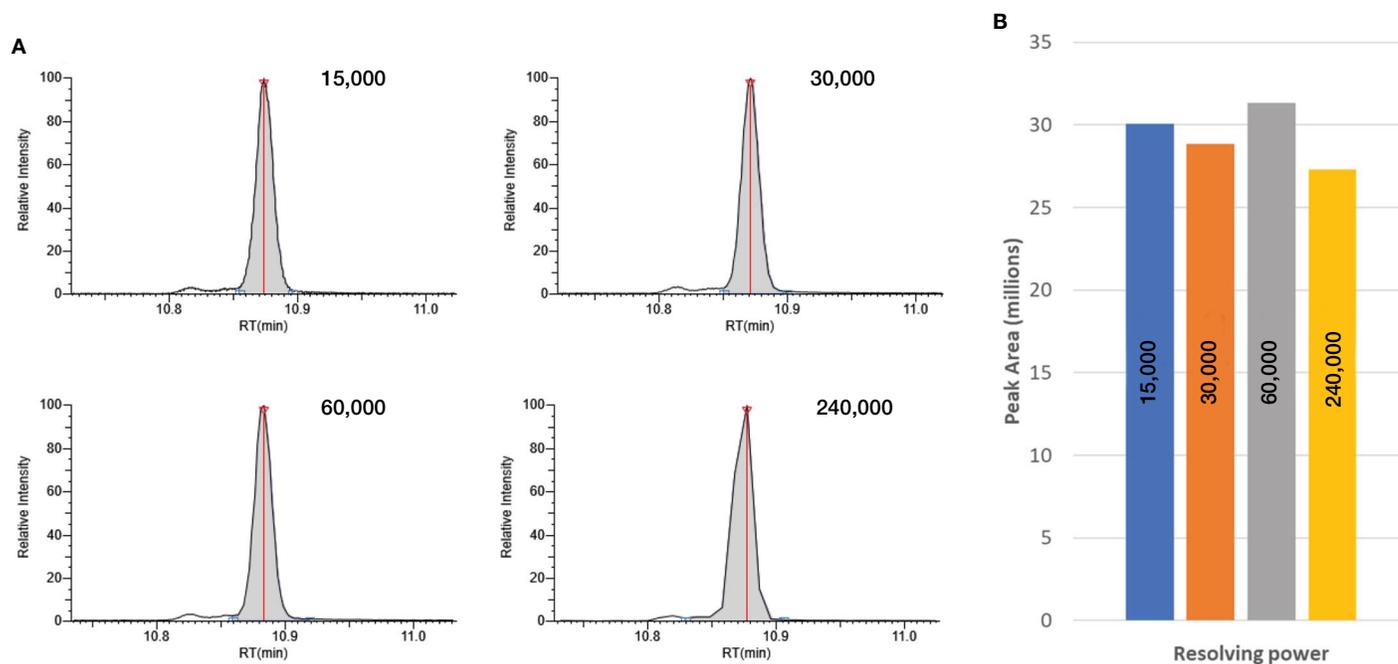


Figure 10. Effect of resolving power (RP) on sensitivity (as absolute peak area response) on pyriproxyfen, in a QuEChERS soil extract at a concentration of 100 ng/mL, showing the extracted ion chromatogram of  $m/z$  fragment ion ( $m/z$  136.0757) [A], as well as corresponding peak area responses obtained at 15,000, 30,000, 60,000 RP and 240,000 RP (at FWHM at  $m/z$  200) [B]. Data was acquired in EI full scan. Sensitivity (as absolute peak area response) is maintained across the resolution modes.

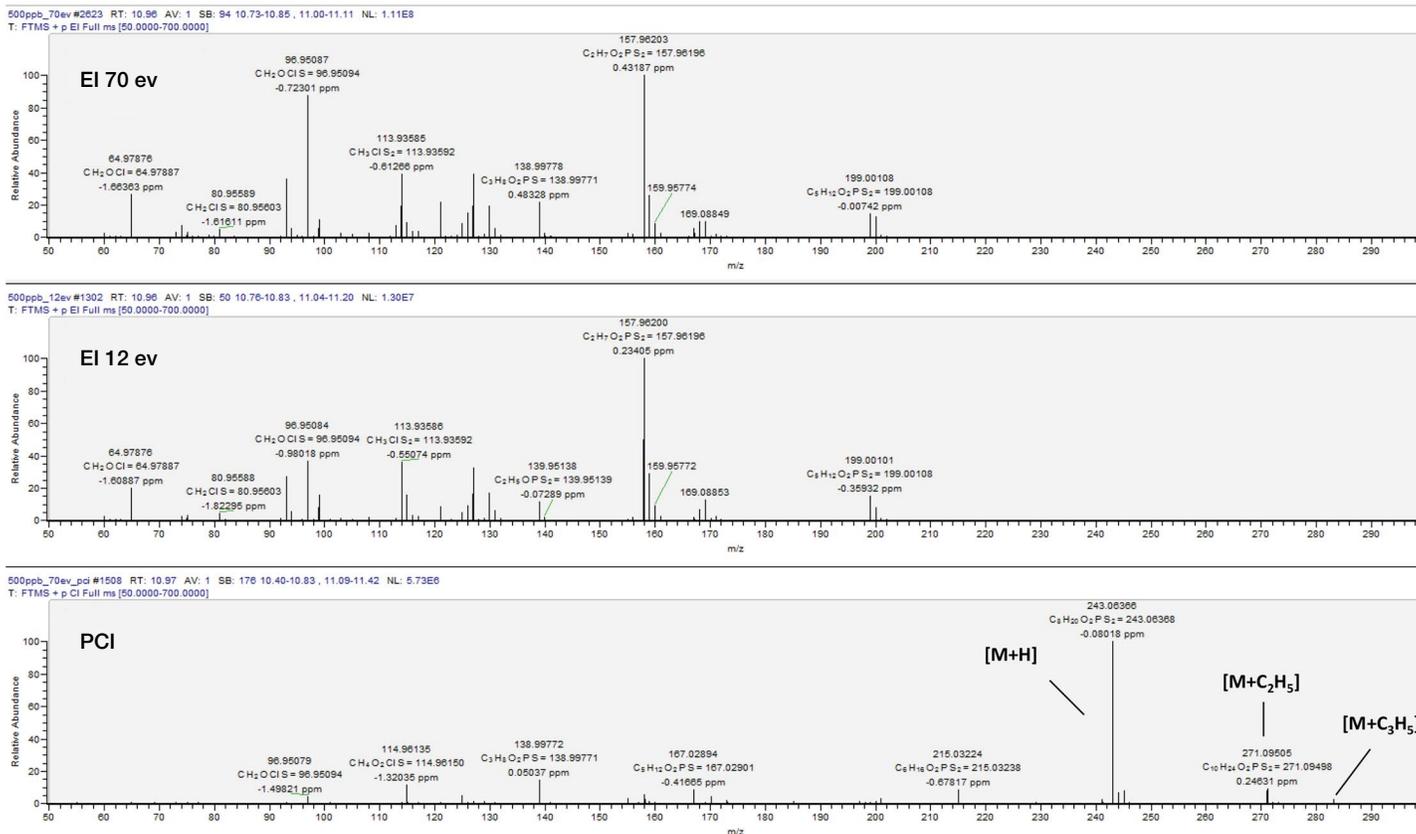


Figure 11. Spectra of compound acquired with EI 70 eV (upper), low EI at 12 eV (middle), and positive chemical ionization (lower). The molecular ion can only be determined in the PCI spectrum from the mass adducts. In this case EI at either 12 or 70 eV does not yield molecular ion.

## Conclusions

- With unprecedented resolving power of 240,000 and consistent sub-ppm mass accuracy, the Orbitrap Exploris GC 240 mass spectrometer is a unique laboratory tool for targeted and discovery workflows, where screening, quantitation, compound identification, and structural elucidation applications are required.
- The Orbitrap Exploris GC 240 mass spectrometer provides selectivity to resolve target compounds from other interfering compounds and/or from matrix ions of similar mass, which is essential for the compound confirmation in targeted or untargeted experiments. As an example, a mass resolving power of 240,000 (corresponding to a mass resolution of 230,000 at  $m/z$  167.08113) is needed to separate bifenthrin from the background interfering ions in a soil sample extract.
- High sensitivity is maintained across all resolving power settings, ensuring unmatched analytical performance irrespective of matrix complexity and providing limits of detection of ppt levels.
- Excellent sub-ppm mass accuracy accelerates the identification of elemental composition and compound identification in unknown workflows by allowing the use of narrow mass tolerances.
- Availability of soft chemical ionization, such as PCI coupled with MS/MS, allows for structural elucidation and confirmation of parent molecules using accurate mass information.



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