

Mass resolving power of 240,000: for confident compound identification

Authors: Jane Cooper, Dominic Roberts, and Cristian Cojocariu

Thermo Fisher Scientific, Runcorn, UK

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Goal

To demonstrate that the ultra-high mass resolution of the Thermo Scientific™ Orbitrap Exploris™ GC 240 delivers the very highest data quality in complex samples to make robust scientific discoveries and answer the most challenging analytical questions.

Introduction

Scientific research laboratories need to deliver fast 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



Figure 1. The Orbitrap Exploris GC 240 benchtop mass spectrometer system configured with a Thermo Scientific™ TriPlus™ RSH™ autosampler

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 proven to be a highly valuable analytical technique for both analytical science and scientific research applications.¹⁻³ 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. This benchtop hybrid quadrupole-Orbitrap mass spectrometer opens new possibilities for increased mass accuracy, sensitivity, and selectivity for GC-amenable compounds. The detailed examples described in this application 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.

The impact of mass resolution on selectivity for targeted analysis

The default acquisition mode for high-resolution, accurate-mass (HRAM) GC-MS experiments is full scan covering a mass range of 50–1000 Da. For targeted compound analysis, the mass of the diagnostic ions is extracted with a narrow mass extraction window (usually ± 5 ppm on Orbitrap systems). This narrow mass window is possible only when the instrument provides sufficient mass accuracy, for which high mass resolving power is essential. However, when there is not sufficient selectivity, mass profiles overlap. This overlap (poor selectivity) can result in the incorrect assignment of the mass of the target compound. The problem is demonstrated in Figure 2, 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).

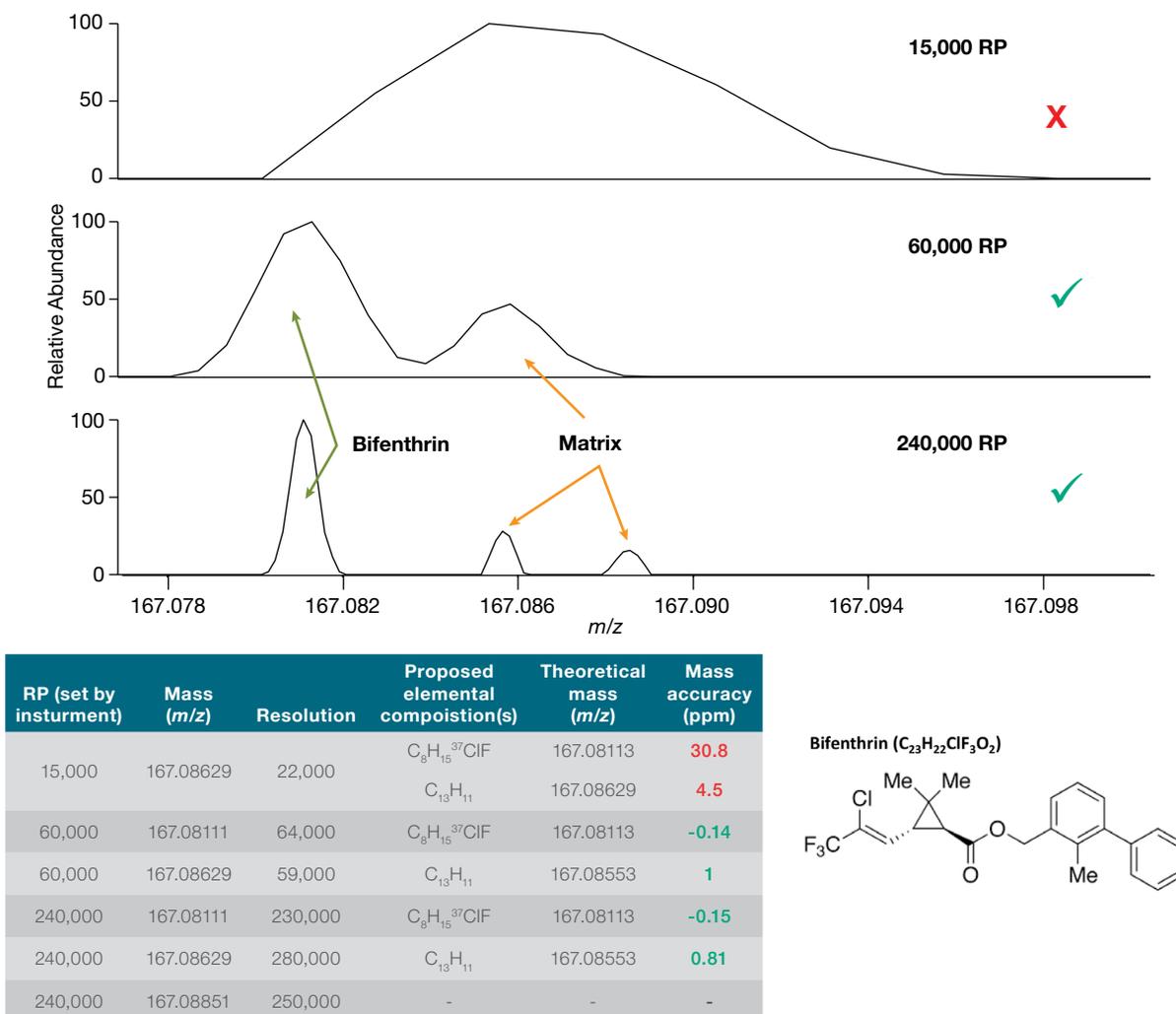


Figure 2. Effect of resolving power (RP) on mass accuracy of an analyte in matrix. Mass spectra of bifenthrin 10 ng/mL in soil acquired at 15,000, 60,000, and 240,000 RP (at FWHM m/z 200). Matrix interference at 15,000 RP prevents separation of this pesticide from the matrix interference, resulting in 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. Under normal screening criteria this pesticide would have been missed (false negative).

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 a 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 <5 ppm, 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 Orbitrap system using a 240,000 RP provided the required selectivity to resolve bifenthrin from other compounds or from matrix ions of similar mass.

Maintaining sensitivity at high resolution

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 3 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.

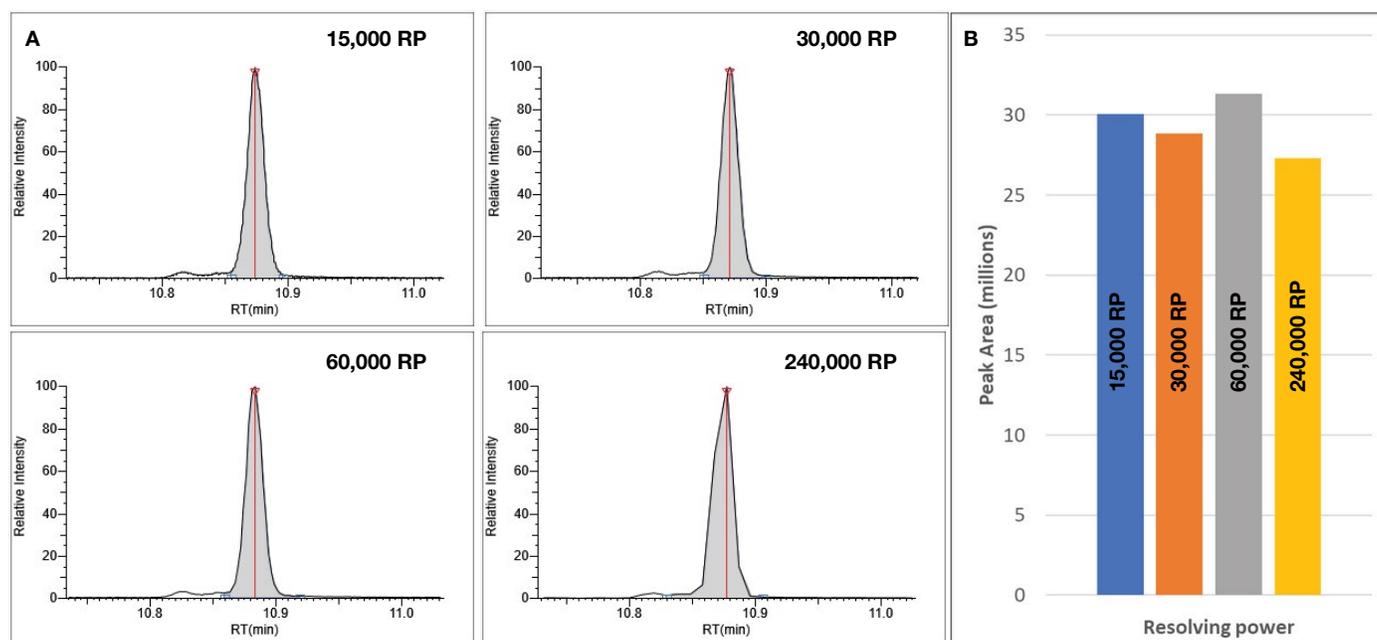


Figure 3. Effect of resolving power (RP) on sensitivity (as absolute peak area response) on pyriproxyfen, in a QuEChERS soil extract at a concentration of 100 ng/mL, showing extracted ion chromatogram of m/z fragment ion (m/z 136.0757) [A], as well as corresponding peak area responses obtained at 15,000, 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.

Compound identification in unknown workflows

For screening of unknown compounds, experiments using the Orbitrap Exploris GC 240 system typically start with a full-scan data acquisition using EI, followed by spectral deconvolution with library matching for putative compound identification. For additional confidence in the identification of unknowns, a confirmation step using positive or negative chemical ionization (PCI and NCI) or MS/MS analysis is also carried out. The typical workflow used for unknown compound identification is summarized in Figure 4.

Typically, when screening for unknown compounds the bottleneck is due to the co-elution of matrix, which can result in a large, unmanageable number of compound assignments, reducing confidence and possibly resulting in potential false positives or false negative assignments.

One of the advantages of having full scan, accurate mass capabilities is that data can be mined retrospectively, and data processed to answer different questions, for example, unassigned peaks can potentially be identified. The mass accuracy of an ion allows elemental compositions to be proposed based on the measured accurate mass and isotopic pattern. The number of possible chemical formulae proposed is based on the elements used in the formula generator and the quality of the spectral data. High-resolution measurements that consistently provide

sub-1 ppm mass accuracy accelerate the identification process by reducing the number of proposed formulae for quick and confident identification. This process is illustrated in Figure 5, where using Thermo Scientific™ Mass Frontier™ structural elucidation software, the ion at m/z 424.33375 from the mass spectra of an unknown peak was submitted to the elemental formula generator. Hits were reported using the following elements Carbon 0-50, Hydrogen 0-100, Oxygen 0-10, Nitrogen 0-10, and Chlorine 0-10. Different ppm mass tolerances from 1.0 to 50 ppm were used to suggest possible formulae, with the number of hits reported in Figure 5C. As expected, the wider the tolerance, the greater the number of suggestions, at 10 ppm, 14 possible hits are proposed. However, with the sub-ppm mass accuracy expected from the Orbitrap Exploris GC 240 system, the number is limited to three formulae at 1.0 ppm.

To further discriminate between these three chemical formulae, comparisons can be made between the theoretical isotopic pattern of each proposed chemical formula and the measured isotopic pattern, as shown in Figure 6, which would suggest the predicted chemical formula to be $C_{29}H_{44}O_2$.

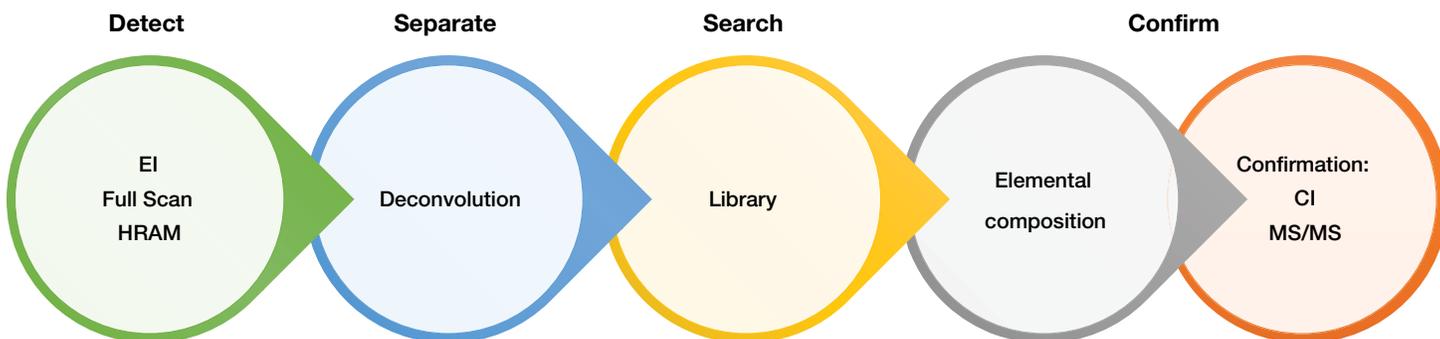


Figure 4. Workflow for screening for unknown compound identification using the Orbitrap Exploris GC 240 system: full scan data acquired using EI full scan HRAM; spectral deconvolution with library search for putative compound identification; confirmation using chemical ionization (CI) data, and if required for additional confirmation, MS/MS for added specificity and selectivity

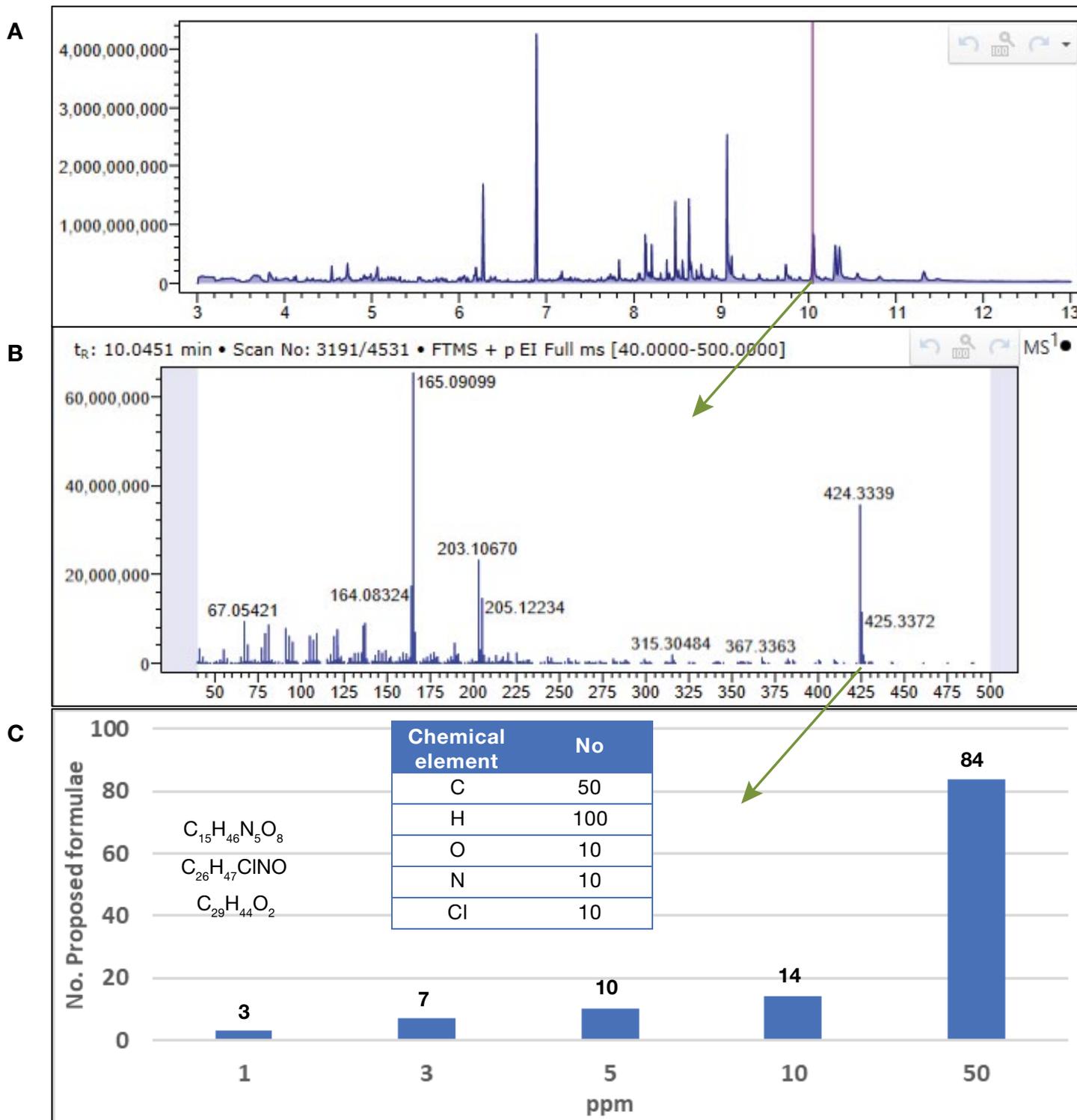


Figure 5. Using Mass Frontier structural elucidation software to aid identification for the elemental compositions for the ion m/z 424.33375 from the mass spectra of an unknown peak (measured with 60,000 RP at FWHM 200 m/z): [A] TIC for a whole flour extract containing an unknown peak at retention time 10.05 min; [B] mass spectrum for the unknown peak; [C] number of proposed formulae (annotated on top of the bars) with different mass tolerances applied, inset table detailing the selected chemical elements used, and proposed formula for a mass tolerance of 1 ppm

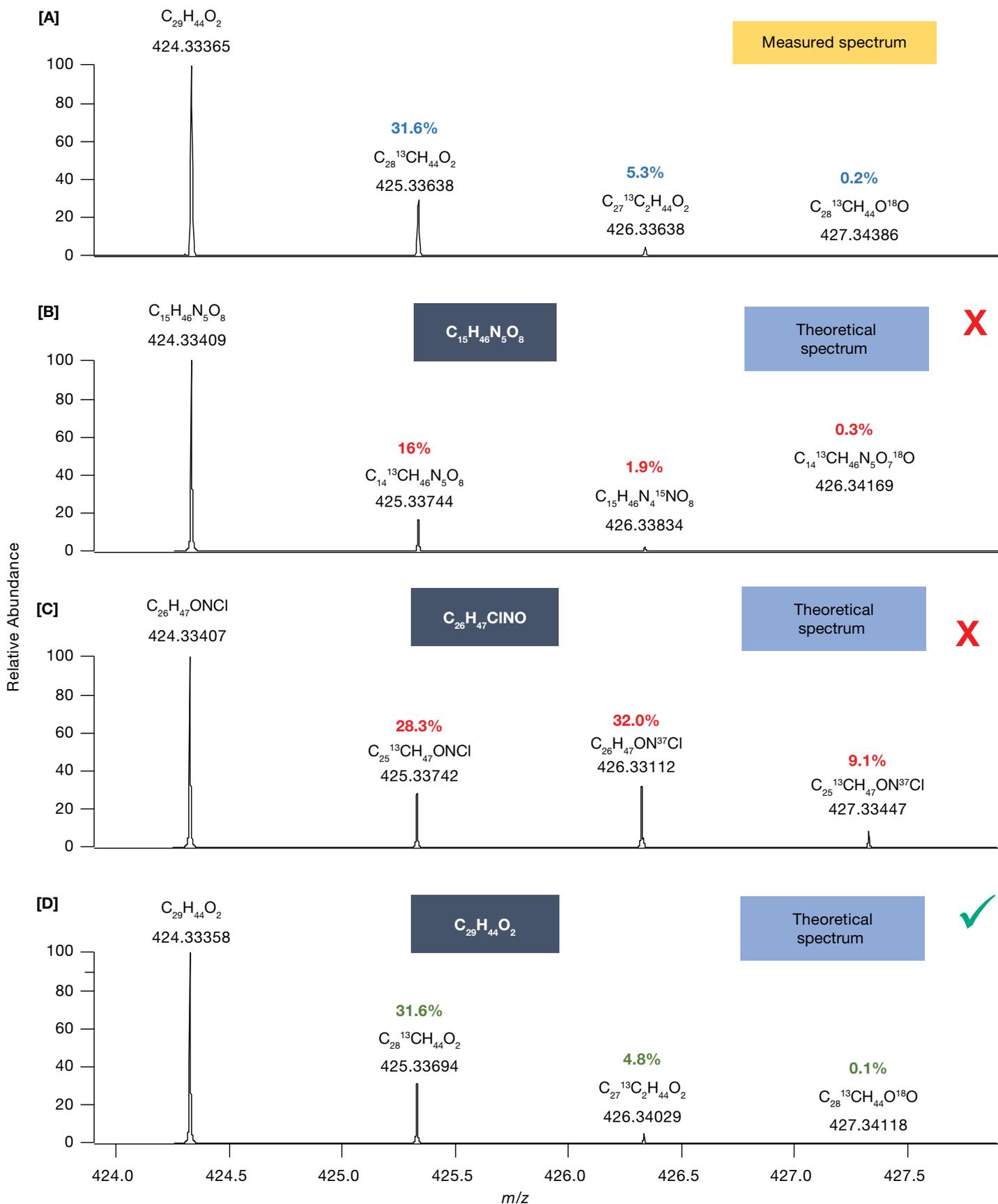


Figure 6. Considering the three formulae suggested with a mass tolerance of 1 ppm for a measured mass ion of m/z 424.33375, the isotopic pattern comparison (measured versus theoretical), used to eliminate possible chemical formulae and confirm the most likely chemical composition. [A] measured isotopic pattern for the measured ion of m/z 424.33375, theoretical spectra for the suggested elemental formula [B] $C_{15}H_{46}N_5O_8$, [C] $C_{26}H_{47}ClNO$, and [D] $C_{29}H_{44}O_2$. Each is annotated with mass, elemental composition, and percentage against relative abundance.

When submitted to the ChemSpider online database, no top hits were returned for the proposed formulae $C_{15}H_{46}N_5O_8$ or $C_{26}H_{47}ClNO$, with the liposoluble phenol α -tocotrienol ($C_{29}H_{44}O_2$) returned as the top hit, with forward and reverse match scores of 841 and 878, respectively.

It has also been observed at $\geq 60,000$ RP, mass differences of several pesticides are less than 1 ppm. In contrast, for $< 60,000$ RP higher mass errors are measured. These higher mass errors are due to inference of chemical background matrix ions with the target ions (Figure 7), which in untargeted workflows can reduce confidence

in compound identification and result in false positive or negative compound identifications.

Spectral fidelity to support identification

Spectral fidelity at high resolving power in matrix is critical to maintain confidence in compound identification when screening for unknown compounds, illustrated in Figure 8, for pentachlorobenzonitrile in a QuEChERS soil extract at a concentration of 100 ng/mL. The mass accuracy for every ion in the isotopic cluster, illustrated for 15,000 RP and 240,000 RP is < 1 ppm giving high confidence in the identification.

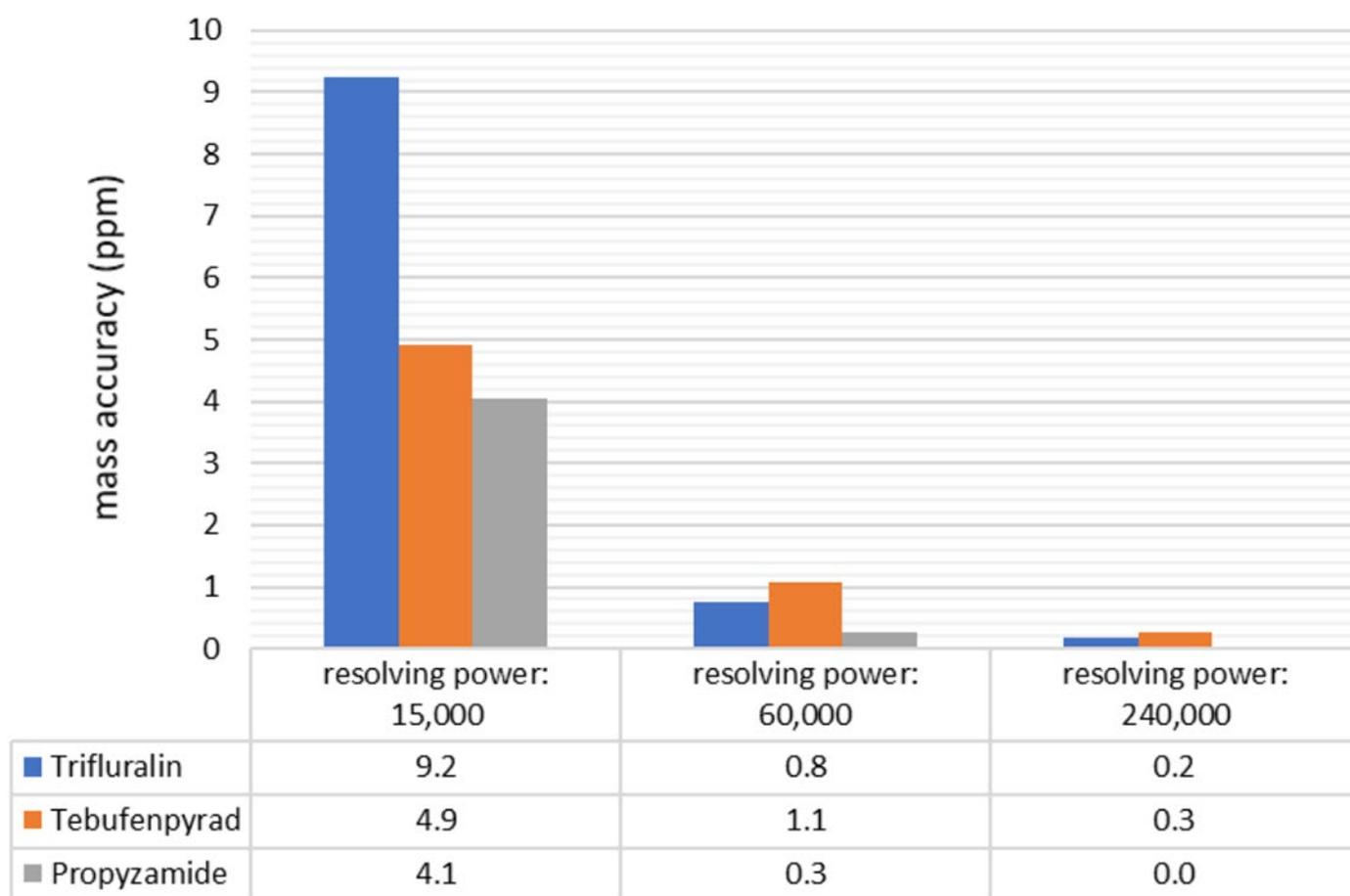


Figure 7. The effect of resolving power (RP) (reported at m/z 200) on mass accuracy measurements of trifluralin, tebufenpyrad, and propyzamide in a QuEChERS soil extract at a concentration of 10 ng/mL (measured over the mass range m/z 50–500). A $\geq 60,000$ RP is required to completely separate these pesticides from the interfering matrix ions and to deliver < 1 ppm mass accuracy.

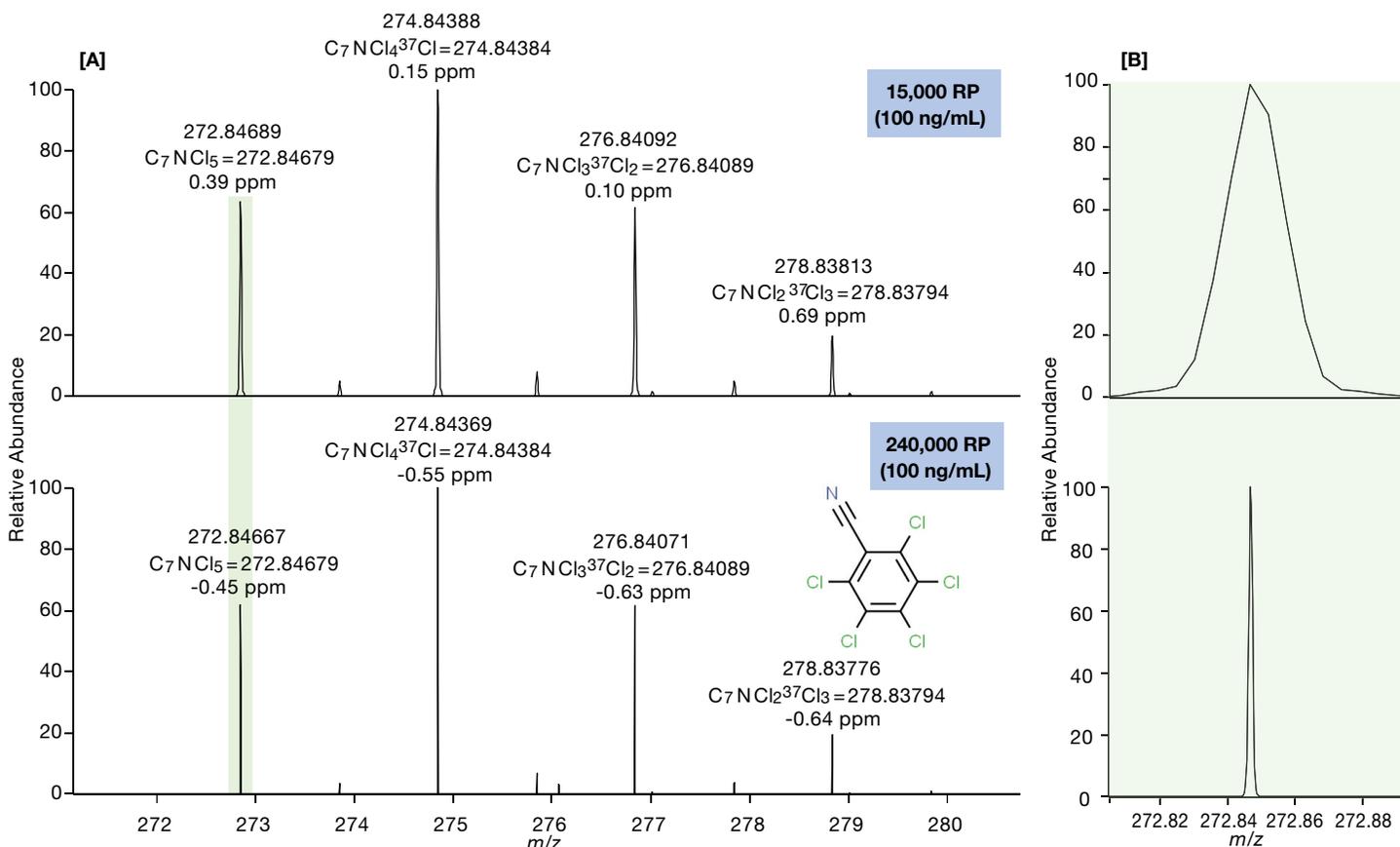


Figure 8. Spectral fidelity illustrated for pentachlorobenzonitrile (C₇Cl₅N) for 15,000 RP and 240,000 RP in a QuEChERS soil extract using the Orbitrap Exploris GC 240 system. [A] EI mass spectra at the molecular ion cluster at each level, annotated with measured mass, elemental composition, theoretical mass and mass accuracy (ppm), [B] EI mass spectra zoomed in the molecular ions ($m/z = 272.84679$) in the ion cluster at 15,000 RP (top) and 240,000 RP (bottom).

Molecular ion confirmation with chemical ionization

When the spectral library match from the EI spectrum is inconclusive, or additional confirmation is required in unknown workflows, 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 (10% ammonia) as the reagent gas mixture, using methane three adducts are typically observed: $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$, and using an additional small amount of ammonia in the reagent gas provides an increased yield of the quasimolecular $[M+1]^+$ ion.

As an example, EI and PCI spectra in a QuEChERS soil extract are shown in Figure 9A and 9B, where the molecular ion for triadimefon corresponding to C₁₄H₁₆CIN₃O₂ (exact mass $m/z = 293.093105$) is not present

in the EI spectrum. The triadimefon quasimolecular $[M+H]^+$ ion in the PCI spectrum with sub-1 ppm mass accuracy can be used to confirm the presence of triadimefon in the soil extract.

To further support the proposed compound identifications PCI MS/MS experiments can also be performed. Figure 9C shows when the triadimefon quasimolecular $[M+H]^+$ ion ($m/z 294.10038$) is isolated in the quadrupole and fragmented in the HCD cell using 20 V energy to generate fragments. Using Mass Frontier software, the assignment of chemical structures to the measured fragment ions in the MS/MS spectrum can be automated to provide detailed information and support compound identification and structural elucidation, as shown in Figure 9C for the PCI MS/MS spectra for triadimefon.

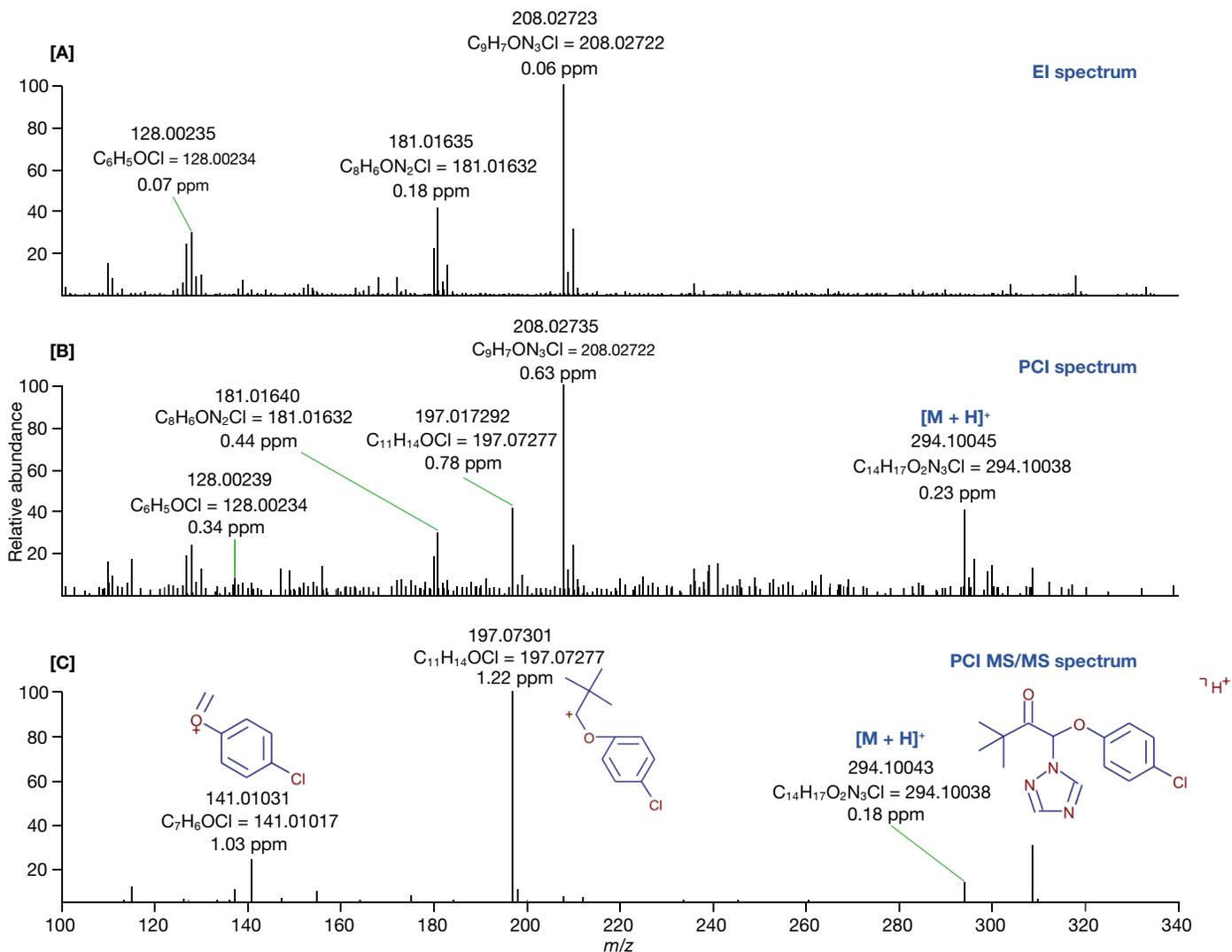


Figure 9. Comparison between the [A] EI, [B] PCI, and [C] PCI MS/MS spectra, illustrating that using PCI and PCI MS/MS data can be used to provide additional compound confirmation, shown for the confirmation of triadimefon in the a QuEChERS soil extract. The molecular ion for triadimefon corresponding to $C_{14}H_{16}ClN_3O_2$ (exact mass $m/z = 293.093105$) is not present in the EI spectrum [A]. In the PCI spectrum the triadimefon quasimolecular $[M+H]^+$ ion is observed with sub-1 ppm mass accuracy when methane (10% ammonia) is used as the reagent gas [B]. In the PCI MS/MS spectrum following m/z 294.10038 fragmentation in the HCD collision cell, showing the product ions produced [C]. Each is annotated with measured mass, theoretical mass, mass accuracy (ppm) and for the PCI MS/MS spectrum the proposed fragment chemical structures.

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 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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