thermo scientific

eBook

Thermo Scientific Orbitrap Exploris GC 240 Mass Spectrometer

ThermoFisher SCIENTIFIC

Contents

Foreword

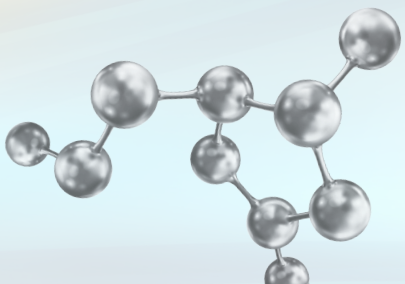
Although the technological principles that underlay its conception date back to 1923, when Kingdon first described orbital trapping using an enclosed metal can and charged wire, the first Orbitrap-based mass spectrometer entered the market more than 80 years later. In 2005, we introduced our first Orbitrap mass spectrometer.

This initial commercial Orbitrap system combined high-resolution, accurate mass (HRAM) detection with the benefits of a linear ion trap. The research community, and especially proteomics as a discipline, enthusiastically welcomed an instrument that met the then-current standard for resolution, mass accuracy, and speed while eliminating the issues that came with FT-ICR machines (maintenance and space concerns) or time-of-flight (TOF) instruments (lower sensitivity, dynamic range and resolution). HRAM capability allowed researchers to detect multiply charged species in complex mixtures and improved database searching with accurate mass detection. For discovery, scientists were able to harness this instrument's full-scan mode to compile a list of precursors suitable for MS/MS by collision-induced dissociation (CID) within a single run. And even though the technology appears to be more complex, the high resolution and accurate mass actually make analyses simpler, reducing the need for tedious method development.

Further accessibility to Orbitrap mass analyzer technology came in the form of the Thermo Scientific™ Orbitrap Exactive™ and Q Exactive™ mass spectrometers. By 2011, the speed of the Orbitrap was further improved by a factor of four, by combining enhanced Fourier transform algorithms that doubled resolving power, and the high-field "compact Orbitrap." The elimination of the ion trap mass analyzer reduced cost and complexity, producing a bench-top instrument that could perform full-scan detection and higher energy collisional dissociation (HCD) without precursor selection. This led to the development of protocols for Orbitrap-only detection, and enabled HRAM screening of known and unknown analytes with very high selectivity (< 5 ppm). It also facilitated retrospective analysis of full scan data. Finally, there was an Orbitrap analyzer that was completely compatible with gas chromatography (GC) separations. In 2015, exactly 10 years after the introduction of the first Orbitrap system, the Q Exactive GC and later Exactive GC were launched.

The Orbitrap Q Exactive GC was an important milestone in GC-MS history but that was just the beginning. The next-generation Thermo Scientific Orbitrap Exploris GC 240 mass spectrometer delivers revolutionary analytical MS performance, versatility, and workflow intelligence to GC-MS within a compact and easy-to-use platform to accelerate scientific understanding. It allows omics, central academic facility and industry research analytical laboratories who require the very highest data quality to make robust scientific decisions and discoveries.

This eBook provides an overview of this exciting and powerful system as well as specifications, applications, case studies, and more. Learn how it can take your laboratory to unprecedented levels of performance and productivity.



Lead discovery with breakthrough performance

Take research capability to a new, unprecedented level of performance with the Thermo Scientific™ Orbitrap Exploris™ GC 240 mass spectrometer and accelerate your scientific discovery. With the unique ability to deliver the very highest-quality, information-rich data, your most complex analytical challenges are simplified.

The Thermo Scientific Orbitrap Exploris GC 240 mass spectrometer with the Thermo Scientific™ TriPlus™ RSH autosampler.

Performance | Discovery | Versatility

- **Ultimate performance and accuracy**

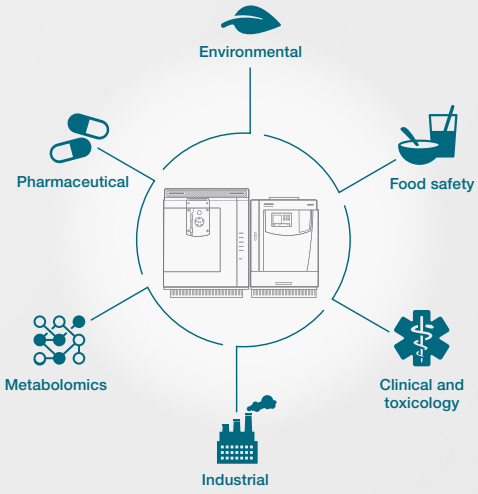
  Get the right answer first time and the flexibility to adapt to evolving research demands. The Orbitrap Exploris GC 240 mass spectrometer brings breakthrough 240,000 mass resolving power, MS/MS capability, and leading sensitivity to researchers across all applications. Lead discovery and be confident that you have the very highest data quality and comprehensive quantitative information to advance your research.

- **Power discovery with ease**

  Focus on results, not instrument setup. Intuitive instrument control and method templates ensure that exceptional data are accessible to all members of your scientific team. Integrated informatics solutions for quantitation and compound discovery facilitate detailed characterization of your most challenging samples to turn data into scientific understanding. The compact Orbitrap Exploris GC 240 mass spectrometer provides definitive gains in resolution, consistent mass accuracy, and robustness to enhance laboratory capability.

- **Versatility for diverse analytical challenges**

  Address diverse analytical challenges today and into the future with ultimate GC-MS performance and flexibility. Expand analytical possibilities with the Thermo Scientific™ ExtractaBrite™ electron ionization/chemical ionization (EI/CI), direct analysis probes, MS/MS capability, and GC modularity. Switching between techniques, ion sources, probes, and columns is fast and easy.

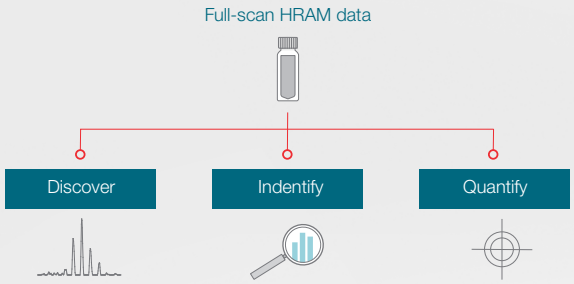


Accelerate exploration with outstanding data quality

Gain a broader and deeper understanding of every sample you analyze with the unprecedented performance that the Orbitrap Exploris GC 240 mass spectrometer delivers. Discover features in your data that would go unnoticed using other technology and gain the power to make confident identifications with uncompromising sensitivity and mass resolving power.

Work smarter to discover more

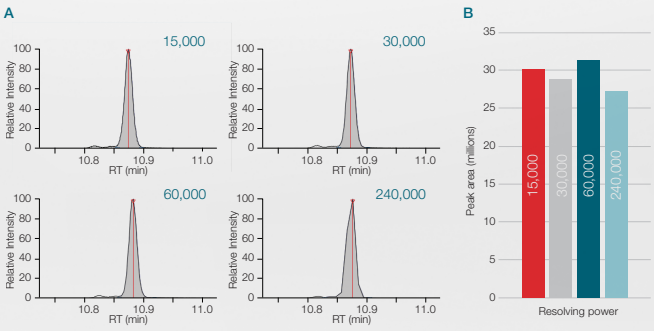
Undetected compounds can have significant implications for your research. The Orbitrap Exploris GC 240 mass spectrometer provides the mass resolving power to cut through the most difficult sample matrices, ensuring you know which compounds are present and at what concentrations. Easily interrogate and re-interrogate full-scan high resolution accurate mass (HRAM) data to answer questions not possible using targeted acquisition. Decide post-acquisition which compounds to analyze and never again be limited to only the ions measured at the time of data acquisition.



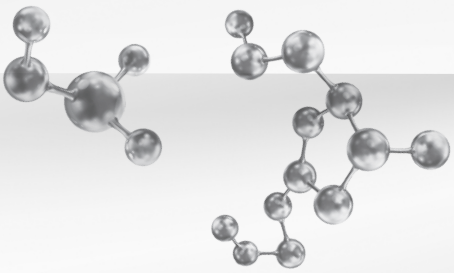
Research flexibility. A single full-scan accurate mass data file answers multiple questions with confidence. Discover emerging compounds, make fast and confident identifications, and determine reliable quantitative information.

Uncompromising sensitivity delivers deeper insights

Full-scan HRAM data with part-per-trillion-level sensitivity, sub-1 ppm mass accuracy, and 240,000 mass resolution (at *m/z* 200) delivers high-capacity component detection in complex samples. Critical to every application, there is never a need to compromise between resolution and sensitivity.



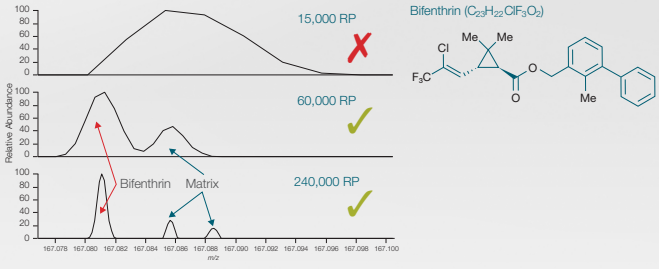
Access the highest resolving power (RP) and sensitivity without compromising data quality. [A] Effect of resolving power on sensitivity (as absolute peak area response) for pyriproxyfen, in a QuEChERS soil extract at a concentration of 100 ng/mL, showing the extracted ion chromatograms of the *m/z* fragment ions, and [B] the corresponding peak area responses obtained at 15,000, 30,000, 60,000, and 240,000 RP.



> For metabolomics experiments, the capability to achieve such high selectivity and maintain sensitivity is revolutionary for our research; having easy access to this data certainty and such wide coverage opens up new research avenues for us."
>
> Dr John Bowden, University of Florida

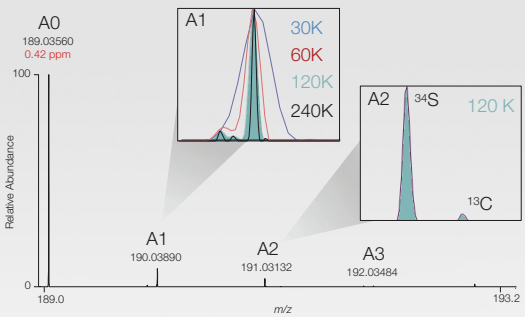
Outstanding mass resolving power provides data certainty

High-resolving power enables you to differentiate between chemical background ions and target ions of interest. Baseline resolution and confident identification of compounds in high matrix samples requires high mass resolving power. Excellent mass accuracy and resolution greater than 120,000 provide access to isotopic pattern determination and reduces the number of potential elemental compositions, providing more assurance in compound assignments.



RP (set by instrument)	Mass (*m/z*)	Proposed elemental composition(s)	Theoretical mass (*m/z*)	Mass accuracy (ppm)
15,000	167.08629	$C_9H_{15}{}^{37}ClF$	167.08113	30.8
		$C_{13}H_{11}$	167.08629	4.5
60,000	167.08111	$C_9H_{15}{}^{37}ClF$	167.08113	-0.14
60,000	167.08629	$C_{13}H_{11}$	167.08553	1
240,000	167.08111	$C_9H_{15}{}^{37}ClF$	167.08113	-0.15
240,000	167.08629	$C_{13}H_{11}$	167.08553	0.81
240,000	167.08851	–	–	–

Mass spectra of bifenthrin 10 ng/mL in soil acquired at 15,000, 60,000, and 240,000 RP. Matrix interference at 15,000 RP prevented separation of the pesticide from the matrix, resulting in a higher than expected mass difference. The bifenthrin fragment ion *m/z* 167 was partially resolved at 60,000 RP and fully resolved at 240,000 RP.

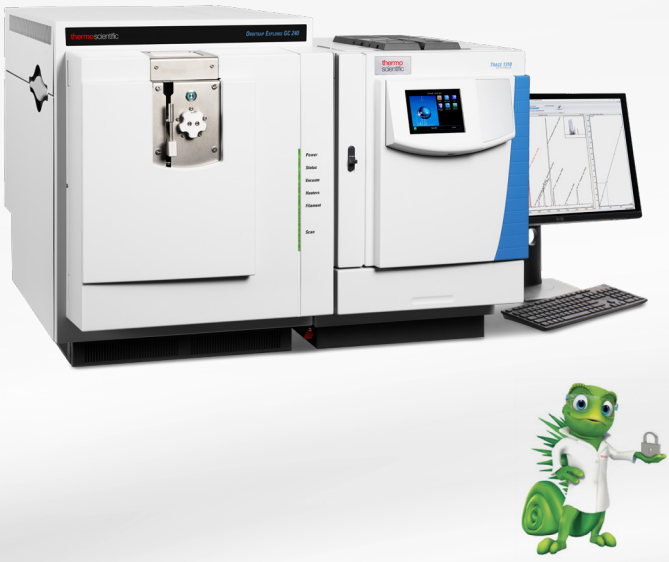


Analysis of tricyclazole demonstrating excellent mass accuracy, and at resolutions greater than 120,000, access to isotopic pattern determination as shown for the A1 and A2 isotopic clusters. Combining these capabilities reduces the number of potential elemental compositions and provides assurance in assignments.

Power targeted workflows with Thermo Scientific software solutions

Research requires powerfully flexible and intelligent identification and quantitation workflows that are accessible to users with different MS expertise levels. Both Thermo Scientific™ Chromeleon™ chromatography data system (CDS) software and TraceFinder software are workflow solutions that increase productivity from method setup to acquiring and processing data, and reporting.

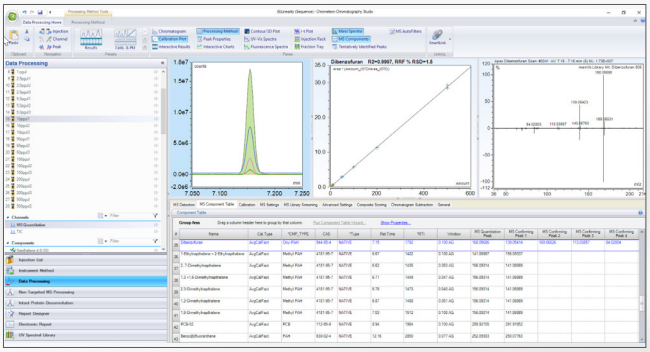
Providing reportable results in a timely manner requires access to a truly connected data processing ecosystem. Regardless of application, Thermo Scientific small-molecule data analysis solutions streamline unknown identification, screening, and quantitation using a powerful suite of software tools.



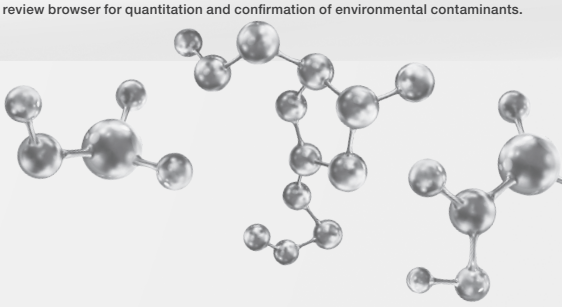
Chromeleon CDS software

Enterprise-ready, regulatory-compliant quantitation

- Streamline chromatography and MS software training using the first CDS with quantitative MS analysis control
- Prepare for audits confidently with support for GLP, GMP, and 21 CFR Part 11 regulations
- Connect multiple sites and locations to a central data center with network failure protection
- Easily connect to third-party software applications and multi-vendor LC and GC chromatography instruments



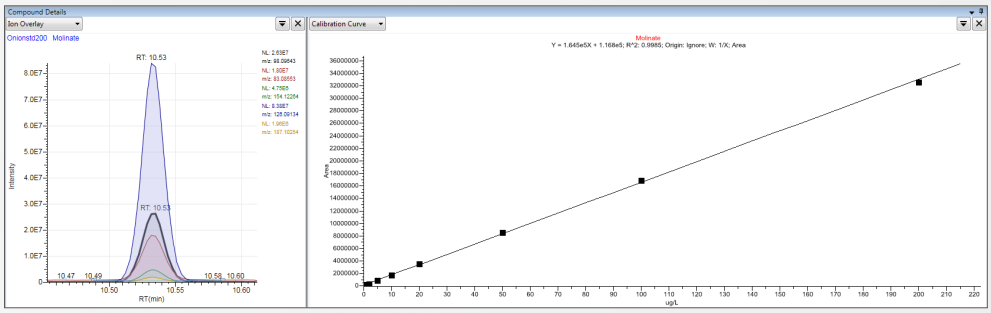
Chromeleon CDS data review browser for quantitation and confirmation of environmental contaminants.



TraceFinder software

High-throughput screening and quantitation

- Save time training staff using a single platform for both screening and quantitation
- View only desired data parameters with a customizable user interface
- Efficiently analyze and report data with customizable flagging and report templates

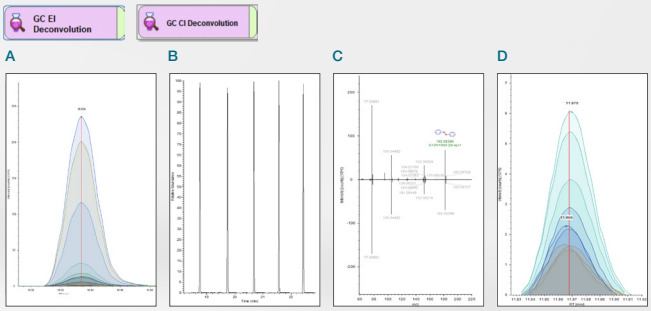


TraceFinder software browser showing extracted ion overlay of the quantitation ion plus four confirming ions for molinate (± 5 ppm extraction window) and matrixed matched calibration series [right].

Compound Discoverer software

Small-molecule unknown identification

- Streamline and customize HRAM data analysis to simplify and gain insights fast. Node based workflows include GC EI and CI deconvolution with statistical analysis tools.
- Confidently identify unknowns using nominal mass and high resolution mass spectral libraries
- Specify desired data flows using drag-and-drop workflow nodes
- Review only data you choose with customizable data visualization



Compound Discoverer software EI and CI node functionality. [A] Peak deconvolution. [B] Retention indexing. [C] Library search. [D] Cross sample peak grouping.

Fourth-generation quadrupole-Orbitrap mass spectrometer

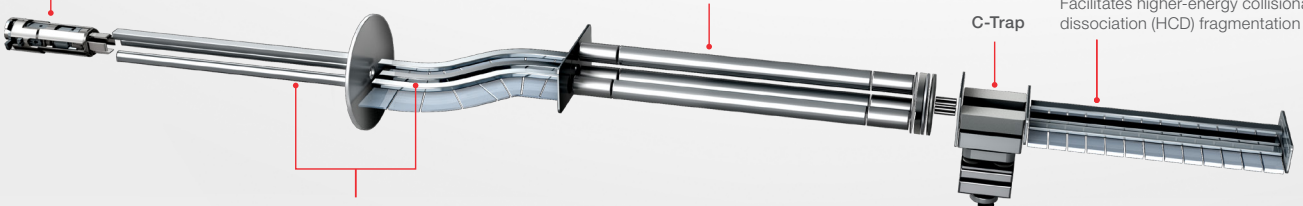
The Orbitrap Exploris GC 240 mass spectrometer combines proven technology refined over more than 20 years with advanced performance and speed capabilities, day-to-day reliability, and a compact footprint. Now both novice and expert high-resolution MS users can efficiently obtain highly reliable and accurate results.

Thermo Scientific ExtractaBrite EI/CI ion source
Robust electron impact (EI) and chemical ionization (CI) performance. Fully removable for maintenance or column changes without breaking vacuum

Advanced Quadrupole Technology (AQT)
Improves sensitivity with 0.4 Da FWHM precursor isolation widths

Ion-routing multipole (IRM)
Facilitates higher-energy collisional dissociation (HCD) fragmentation

C-Trap



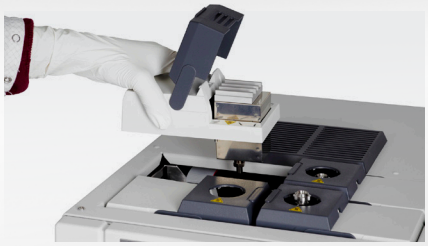
Advanced Active Beam Guide (AABG) with axial gradient
Reduces noise and increases robustness by preventing neutrals from entering the quadrupole mass filter, and by eliminating effects of local charging

High-field Orbitrap mass analyzer
Resolution up to 240,000 (FWHM) at *m/z* 200 and acquisition rates up to 40 Hz (at 7500 resolving power)

Quickly change column, source or switch to CI with the vacuum probe interlock.

PCI MS/MS analysis of an unknown chemical with *m/z* 137 following *m/z* 137.13252 fragmentation in the HCD collision cell showing product ions annotated with measured mass, elemental composition, theoretical mass, mass accuracy (ppm), and proposed structure.

Step into modern gas chromatography



Access unprecedented flexibility. Switch instant-connect injectors and detectors in minutes without tools.

Modularity increases uptime

The unique modular design of the Thermo Scientific™ TRACE™ 1300 Series GC empowers users with new time-saving capabilities and unmatched flexibility. Swapping modules is easy by removing and replacing just three screws, accessible from the top of the GC system. The entire process takes less than five minutes without requiring specialized service assistance. This modularity provides maximum uptime with offline cleaning and servicing of the GC inlet when a spare module is purchased.

Also, rapid response to different application needs or sudden workload requirements is possible with a limited investment in modules.

Take advantage of a comprehensive range of the The Thermo Scientific™ Instant Connect injectors and detectors interchangeable modules, right available at fingertip at any time for any need:

- Instant Connect Helium Saver split/splitless (SSL) injector
- Instant Connect Cold On-Column (COC) injector
- Instant Connect Flame Ionization Detector (FID)
- Instant Connect micro-volume Thermal Conductivity Detector (TCD)
- Instant Connect Electron Capture Detector (ECD)
- Instant Connect Nitrogen Phosphorous Detector (NPD)
- Instant Connect Flame Photometric Detector (FPD)
- Instant Connect Pulsed Discharge Detector (PDD)

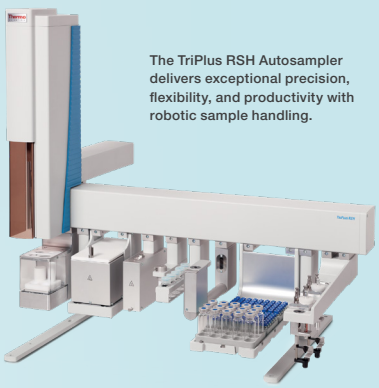
Add productivity with automated sample handling

The TriPlus RSH autosampler offers advanced robotic sample handling to extend automation beyond liquid injection, headspace, and solid-phase microextraction (SPME). Your results will benefit from improved precision and reproducibility, while your laboratory will increase productivity with sample handling flexibility. Several tools are available to reliably automate the most common sample preparation procedures, such as dilution, internal standard addition, and complex derivatization methods, including online microSPE cleanup of QuEChERs extracts.

Ready-to-use prep cycles are available or it is possible to easily create custom workflows using the Thermo Scientific™ Sampling Workflow Editor software, with intuitive drag-and-drop visual programming.

Key benefits

- Improved data repeatability
- Increased automation and laboratory efficiency
- Reduced cost per sample



The TriPlus RSH Autosampler delivers exceptional precision, flexibility, and productivity with robotic sample handling.

Lead discovery

Orbitrap Exploris GC 240 Mass Spectrometer

Benefits

- Premium quantitative and qualitative performance with the fast-scanning High-Field Thermo Scientific™ Orbitrap™ mass analyzer
- Best-in-class resolving power, mass accuracy, sensitivity, and dynamic range
- Compatible Thermo Scientific Compound Discoverer software for chemometric analysis
- EI/CI Thermo Scientific™ ExtractaBrite™ ion source removable under vacuum through vacuum interlock
- Vent-free column exchange with source plug
- VeV low electron energy acquisition for increased molecular ion production

The Thermo Scientific™ Orbitrap Exploris™ GC 240 hybrid quadrupole Orbitrap mass spectrometer is a Thermo Scientific™ quadrupole-Orbitrap™ mass spectrometer based on proven hardware and instrument control software designs of the next-generation Thermo Scientific mass spectrometers and incorporates extensive customer and service engineer feedback.

The Orbitrap Exploris GC 240 takes research capability to a new and unprecedented level of performance to accelerate scientific discovery. With a unique ability to deliver the very highest data quality, the most complex analytical challenges are simplified. By delivering uncompromising qualitative and quantitative performance, the system provides the flexibility to address the demands of scientific research laboratories for compound identification and quantitation. From discovery profiling, metabolomics and applied quantification, scientists gain unparalleled access to information rich data and results with ease.

ThermoFisher SCIENTIFIC

Hardware features

Ion source

- 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)
- Remove entire ion source or change to 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 need for source interchange.

Ion optics

Advanced active beam guide (AABG)

Axial field reduces noise by preventing neutrals and high-velocity clusters from entering the quadrupole mass filter using double bent design geometry.

Advanced quadrupole technology (AQT)

- Segmented quadrupole mass filter for precursor ion selection with variable precursor isolation width from 0.4 to 1,200 Da
- MS/MS and SIM precursor ion selection with high transmission from *m/z* 30 to 2,000

Ion-routing multipole (IRM)

- Robust ion trapping for higher energy collisional dissociation (HCD)
- Nitrogen collision gas

Automatic gain control (AGC)

Reliable AGC measurements for controlled injection of the number of ions.

Thermo Scientific Orbitrap mass analyzer

- High-Field Orbitrap mass analyzer
- Low noise detection pre-amplifier
- 4 kV central electrode voltage

Vacuum system

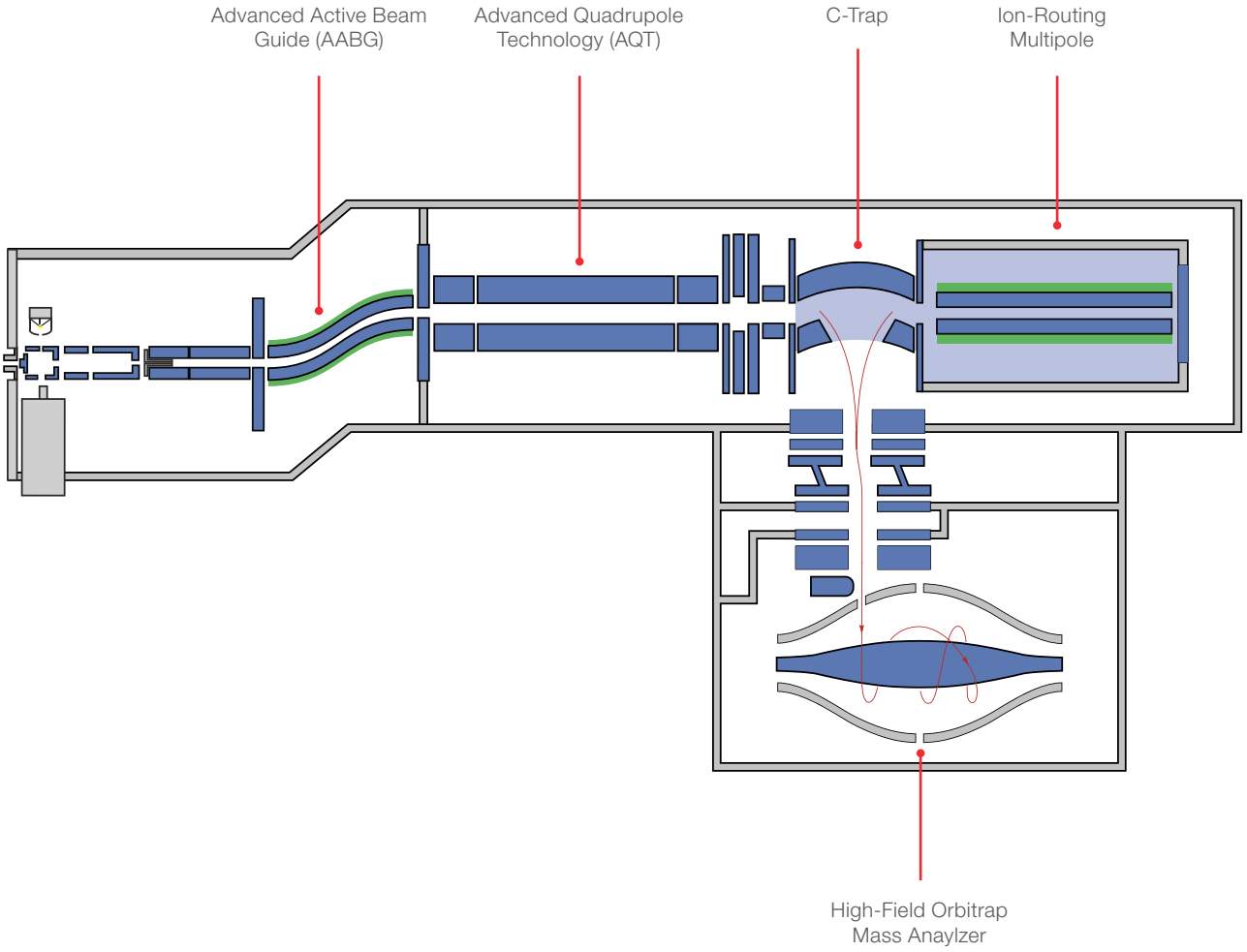
- A compact single turbo pump design providing the adequate vacuum in five stages for the aluminum high-vacuum analyzer chambers
- Advanced vacuum technology reduces pressure in the ultra-high vacuum regions, enhancing transmission of ions to the Orbitrap mass analyzer

Optional hardware

Direct sample probe system option

- Switch to probe <3 min with GC undisturbed
- Available in two styles: rapid heating filament Direct-Exposure Probe (DEP, capable of flash vaporization or pyrolysis at up to 1,600 ˚C) or slower volatilization Direct-Insertion Probe (DIP, capable of accommodating powders and solid samples in a quartz or aluminum crucible) up to 450 ˚C

Orbitrap Exploris GC 240 mass spectrometer ion path



Performance specifications	
Mass range	*m/z* 30–3,000
Orbitrap mass analyzer resolution	Up to 240,000 at *m/z* 200
Scan rate*	Up to 40 Hz at resolution setting 7,500 at *m/z* 200
Mass accuracy*	External calibration achieves <3 ppm RMS drift over 24 hours Internal lock mass calibration achieves <1 ppm RMS drift over 24 hours
Sensitivity	EI: 100 fg octafluoronaphthalene on column, scanning *m/z* 50–300, S/N 10,000:1 EI IDL**: 6 fg octafluoronaphthalene derived at the 99% confidence level PCI: 10 pg benzophenone on column, scanning *m/z* 80–230, S/N 150:1
Dynamic range	$>10^6$ analytical dynamic range* >5,000 within a single Orbitrap mass analyzer spectrum
Polarity switching	One Full Scan cycle*** <700 ms equals >1.4 Hz One tSIM Scan cycle*** <600 ms equals >1.6 Hz
Multiplexing	Up to 20 precursors per scan

* Under defined conditions

** Demonstrated at installation with purchase of Thermo Scientific™ TriPlus™ RSH Autosampler and Exploris GC system IQ/OQ

*** One cycle consists of acquiring one Full scan in positive mode and one Full scan in negative mode at resolution setting 60,000
One tSIM scan in positive mode and one tSIM scan in negative mode at resolution setting 60,000

Data acquisition system

Data system

- High-performance PC with Intel® microprocessor
- High-resolution LED color monitor
- Microsoft® Windows® 10 Enterprise (Long Term Service version) operating system
- High-speed real-time data acquisition and instrument control
- Automatic calibration of all ion transfer and analysis parameters via instrument control software

Thermo Scientific Orbitrap Exploris instrument control software

- Tune application for instrument mass and system calibrations and checks, diagnostics, and manual data acquisition
- Method Editor with a comprehensive application- specific template library, method setup supported by tooltips, and a drag-and-drop user interface to facilitate method development
- Consistent instrument control software whether using Thermo Scientific™ Xcalibur™ or Thermo Scientific™ Chromeleon™ Chromatography Data Systems (CDS) for data acquisition

Included software

Thermo Scientific Xcalibur software

- Xcalibur software is the control software for the next-generation Thermo Scientific mass spectrometer portfolio
- Accelerates familiarization and reduces training needs

Thermo Scientific™ Orbitrap™ GC-MS contaminants library

Allows fast start-up for environmental and food safety screening and quantitation applications. Contains over 800 food and environmental contaminants, including pesticides, PAHs, PCBs, dioxins, and furans. User guide included detailing how to install and make custom enhancements to library.

Optional software

Thermo Scientific Chromeleon Chromatography Data System (CDS)

Streamlined chromatographic and MS screening and quantitative workflows within an enterprise and compliance-ready single software application.

Thermo Scientific™ Compound Discoverer™ software

Streamlines small molecule unknown identification, determination of real differences between samples, and elucidation of biological pathways with an integrated suite of data analysis tools.

Thermo Scientific™ Orbitrap™ GC-MS metabolomics library

Pair with Compound Discoverer to quickly implement metabolite profiling and identification of biomarkers. Contains over 1,000 GC-Orbitrap spectra covering multiple metabolite classes. The majority of metabolite reference spectra have been derivatized with standard MSTFA and methoxyamine protocol.

Thermo Scientific™ TraceFinder™ software

Acquire and process your high-throughput screening and quantitation with built-in intelligence, driving productivity gains from data acquisition and processing to reporting.

Operation modes

Resolution settings

Ranging from 7,500 to 240,000 at *m/z* 200

Scan functions

Full scan

High sensitivity, high selectivity full scan for targeted and untargeted analyses.

tSIM

- Targeted SIM with Mass List Table
- With Targeted Mass Filter for ddMS^2
- Isolation Width, Resolution, Polarity, Microscans set values are definable compound- dependent (w/o msx)
- Isolation Width: 0.4 u to 50 u
- Multiplexing for up to 20 compounds
- MSX ID, multiplexing groups definable
- Isolation Width set values are definable compound-dependent (w/ msx)

tMS^2

- Targeted MS^2 with Mass List Table
- Isolation Width, HCD Collision Energy, Polarity, Microscans set values are definable compound-dependent (w/o msx)
- Isolation Width: 0.4 u to 50 u
- Multiplexing for up to 20 compounds
- Isolation Width set values can be defined compound-dependent (w/ msx)

SIM by Data-Dependent Acquisition (DDA) following a master scan (i.e., a Full MS scan or tSIM scan):

- With up to Top 100 for ddSIM
- With Targeted Mass Filter
- 'Number of Scans' (TopN) and 'Cycle Time' (Top Speed) option
- Isolation Width: 0.4 u to 50 u
- By performing a dependent scan on the most intense ion, if no target mass is found (optional)

MS^2 by Data-Dependent Acquisition (DDA) following a master scan (i.e., a Full MS scan or a tSIM scan):

- With up to Top 100 ddMS^2
- With Targeted Mass Filter
- 'Number of Scans' (TopN) and 'Cycle Time' (Top Speed) option
- Isolation Width: 0.4 u to 50 u
- HCD Collision Energy set value is definable per compound
- By performing a dependent scan on the most intense ion, if no target mass is found (optional)

General

- Multiple experiments can be set up within one method
- One experiment can contain combinations of scans
- Collision Energy Mode' can be selected: 'Fixed' and 'Stepped'

Filters

Filters guide data-dependent (discovery and conformational) decisions on the fly and in real time. To achieve optimum results when applying application- and sample-dependent filter settings, the user is guided with appropriate application-dependent parameter settings and tool tips with tailored recommendations and detailed 'learn more' sections.

Filters can be selected as follows:

- Dynamic Exclusion
- Intensity
- Precursor Fit
- Targeted Inclusion
- Targeted Exclusion
- Apex Detection
- Precursor Selection Range

System templates

System templates provide predefined parameters in each template for users to fast load in Method Editor for data acquisition. To achieve optimum results when applying a template, the user is guided with more detailed information in help files.

System templates categories:

- Food Safety and Environmental
- POPs
- Impurity Testing
- Metabolomics
- Anti-Doping Control
- Flavor and Fragrances
- PCI Data Dependent MSMS

thermo scientific

Installation requirements

Power

- 2 × 208–240 Vac single phase, 15 A, 50/60 Hz, with earth ground for instrument and source vacuum pump
- 208–240 Vac single phase, 15 A, 50/60 Hz, with earth ground for the data system

Gas

Helium

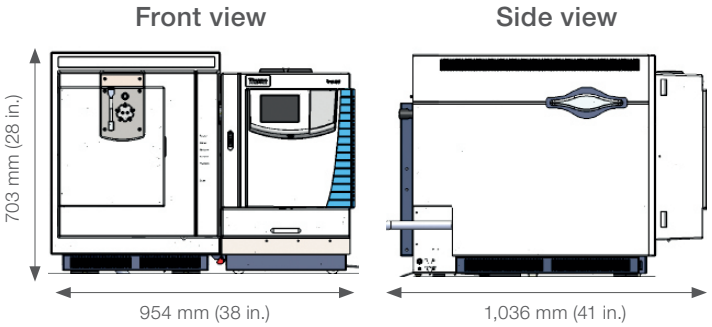
- High-purity helium gas supply (99.999% pure)
- Regulator output pressure adjustable from 300 to 1,000 kPa (3 to 10 bar, 45 to 145 psi)

Methane (required for CI installation)

- High-purity methane gas supply (99.999% ultra high purity)
- Regulator output pressure adjustable from 35 to 240 kPa (0.3 to 2.4 bar, 5 to 35 psi)

Nitrogen

- High-purity nitrogen gas supply (99.999% ultra-high purity)
- Regulator output pressure at 800 ± 30 kPa (8.0 ± 0.3 bar, 166 ± 4 psi)



Dimensions (w, d, h)

- 954 × 1,036 × 703 mm (38 × 41 × 28 in)

Weight

- 156 kg (344 lb) including GC and one injector, without data system, vacuum rough pumps, and optional items

Environment

- System averages 3,440 W (11,730 Btu/h) output when considering air conditioning needs
- Operating environment must be 18–27 ˚C (64–81 ˚F). Relative humidity must be 20–80% with no condensation
- Designed for indoor use at an altitude of up to 3,000 m (10,000 ft) above sea level

Find out more at **thermofisher.com/OrbitrapExplorisGC240**

ThermoFisher SCIENTIFIC

APPLICATION NOTE 10724

Breakthrough performance of the Orbitrap Exploris GC for analytical testing and scientific research applications

Authors: Jane Cooper, Dominic Roberts, and Cristian Cojocariu
Thermo Fisher Scientific, Runcorn, UK

Keywords: Orbitrap Exploris GC, Orbitrap technology, high resolution, mass accuracy, sensitivity, gas chromatography, analytical testing, scientific research, unknown identification, structural elucidation

Goal

To demonstrate the Thermo Scientific™ Orbitrap Exploris™ GC mass spectrometers deliver exceptional analytical performance for both analytical science and scientific research applications.

Introduction

The Orbitrap Exploris GC mass spectrometers have been designed for analytical testing and scientific research applications. These highly selective high-resolution Orbitrap-based analytical platforms aim to deliver unprecedented and flexible performance with up to 240,000 mass resolving power.

The objective of this study was to further explore the power of high resolution and accurate mass using Orbitrap-based GC-MS[1] 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.

Experimental

In all experiments, a Thermo Scientific Orbitrap Exploris GC was used. Some additional experiments were performed on the Thermo Scientific Orbitrap Exploris GC 240, which has a maximum resolving power of 240,000 (at *m/z* 200 FWHM). Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation of the gas-phase chemical components was achieved using a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph equipped with various capillary columns. The Orbitrap Exploris GC was tuned and calibrated using PFTBA to achieve mass accuracy of <1.0 ppm.

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.

Standard and sample preparation

Standard preparation

To assess key analytical parameters, standards were prepared from stock standards in solvent and in matrix:

A) 8270 MegaMix (Restek, catalogue number 31850) was diluted in hexane to produce 12 calibration level standards (ranging from 0.1 ppb to 10,000 ppb)

B) Mixed pesticide calibration standards were prepared from stock standards (Restek, catalogue number 32562), and diluted in matrix to generate 11 calibrations standards (ranging from 0.1 to 500 ppb)

C) Mixed standard, containing octafluoronaphtalene (OFN) (0.1 pg/µL) and hexachlorobenzene (HCB) (10 pg/µL) in iso-octane.

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 ($MgSO_4$), 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 for 10 min. After centrifugation, 5 mL of the supernatant was transferred into a polypropylene tube with 250 mg C18 sorbent, 750 mg $MgSO_4$, 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.

For unknown identification and structural elucidation experiments, commercially available oregano samples were purchased locally. Samples were weighed (150 mg) and transferred into 10 mL crimp top headspace vials (vials P/N 10-CV, caps P/N 20-MCBC-ST3) for analysis.

Results and discussion

Scan speed

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.

Compound identification using spectral libraries

The Exploris Orbitrap 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 Exploris Orbitrap GC for the analysis of aldrin in a mixed pesticide standard, with both forward and reverse library match scores of >890 achieved.

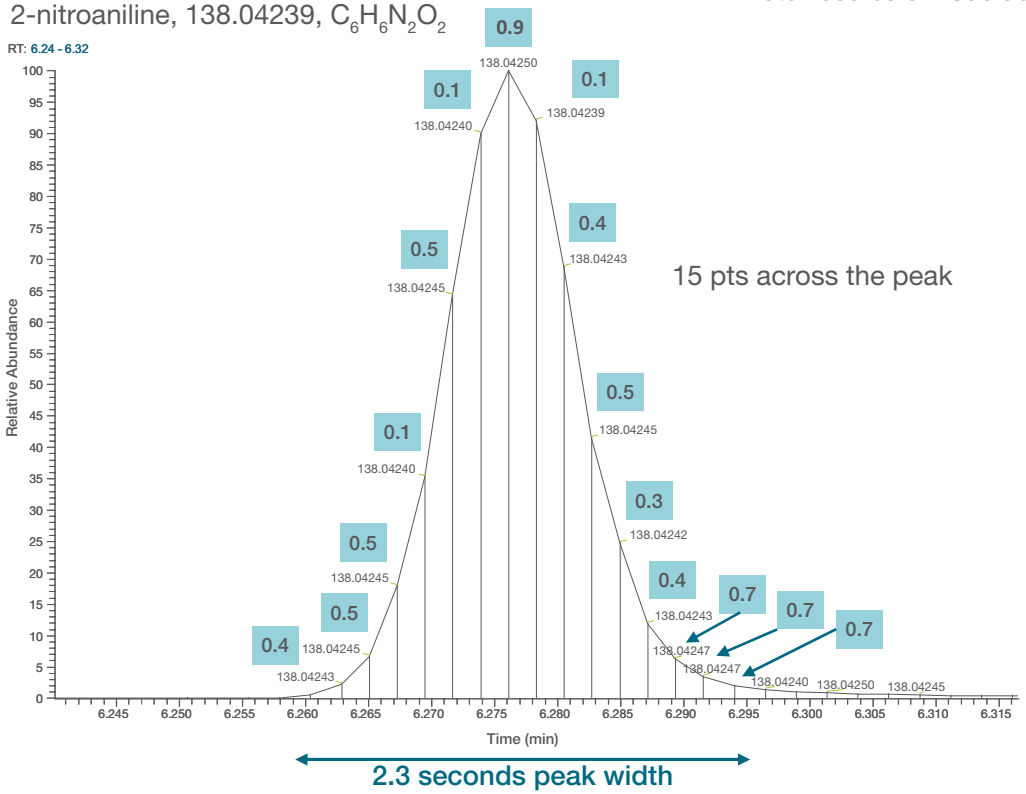


Figure 1. Extracted ion chromatogram (XIC) of 2-nitroaniline (*m/z* 138.04238 ± 5 ppm window) in a 1,000 pg/µL mixed solvent standard. Data acquired in full scan at 60k resolution (FWHM at *m/z* 200). Excellent mass accuracy is shown for each individual scan as well as mass difference (in ppm). An average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

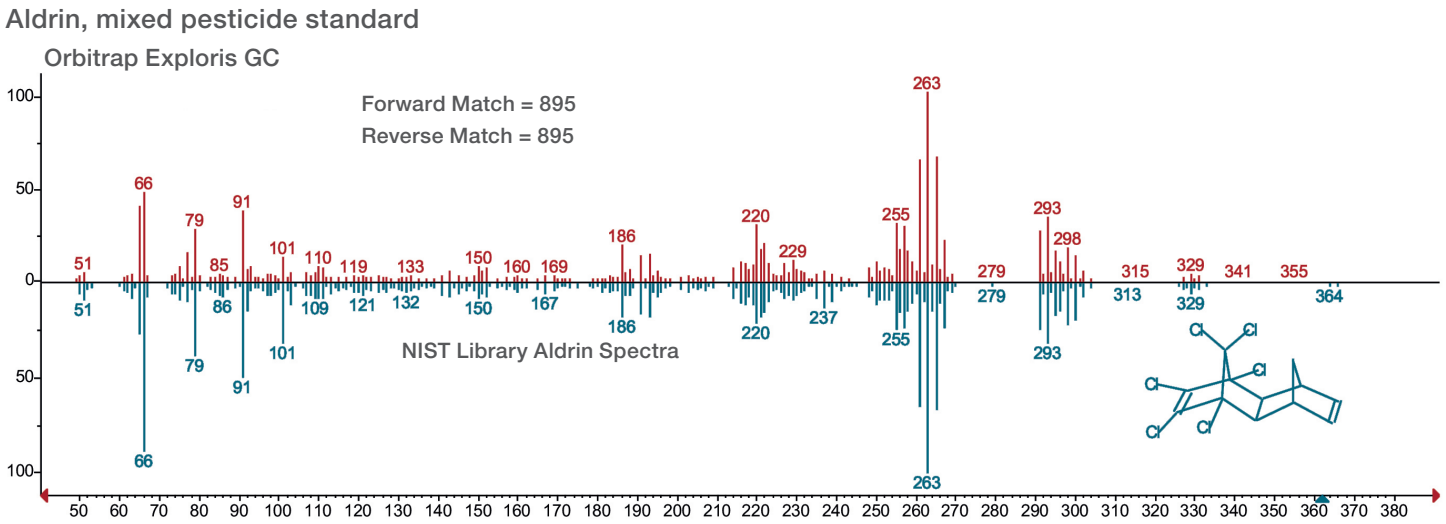


Figure 2. NIST library search mass spectra and match results achieved for the analysis of aldrin in a mixed pesticide standard using an Orbitrap Exploris GC operated in full scan at 60k resolution (FWHM at *m/z* 200)

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.

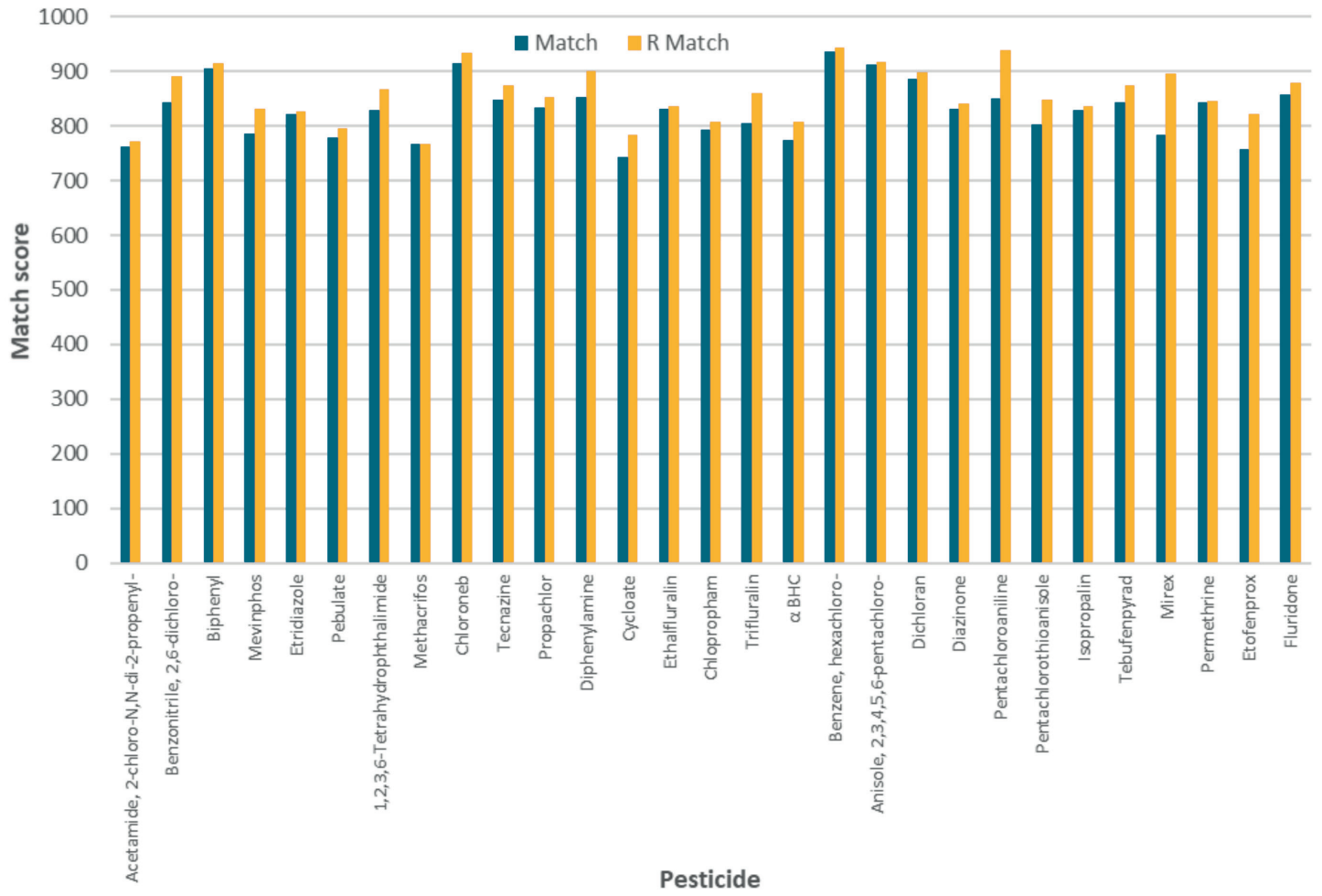


Figure 3. Library search scores achieved using an Orbitrap Exploris GC for a selection of pesticides in a mixed pesticide standard in a whole flour matrix (a score of 1,000 equals a perfect match). Forward search scores (Match) and reverse search scores (R Match) given for each pesticide, when searched against the NIST library. The Orbitrap Exploris GC was operated in full scan at 60k resolution (FWHM at *m/z* 200).

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 4 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.

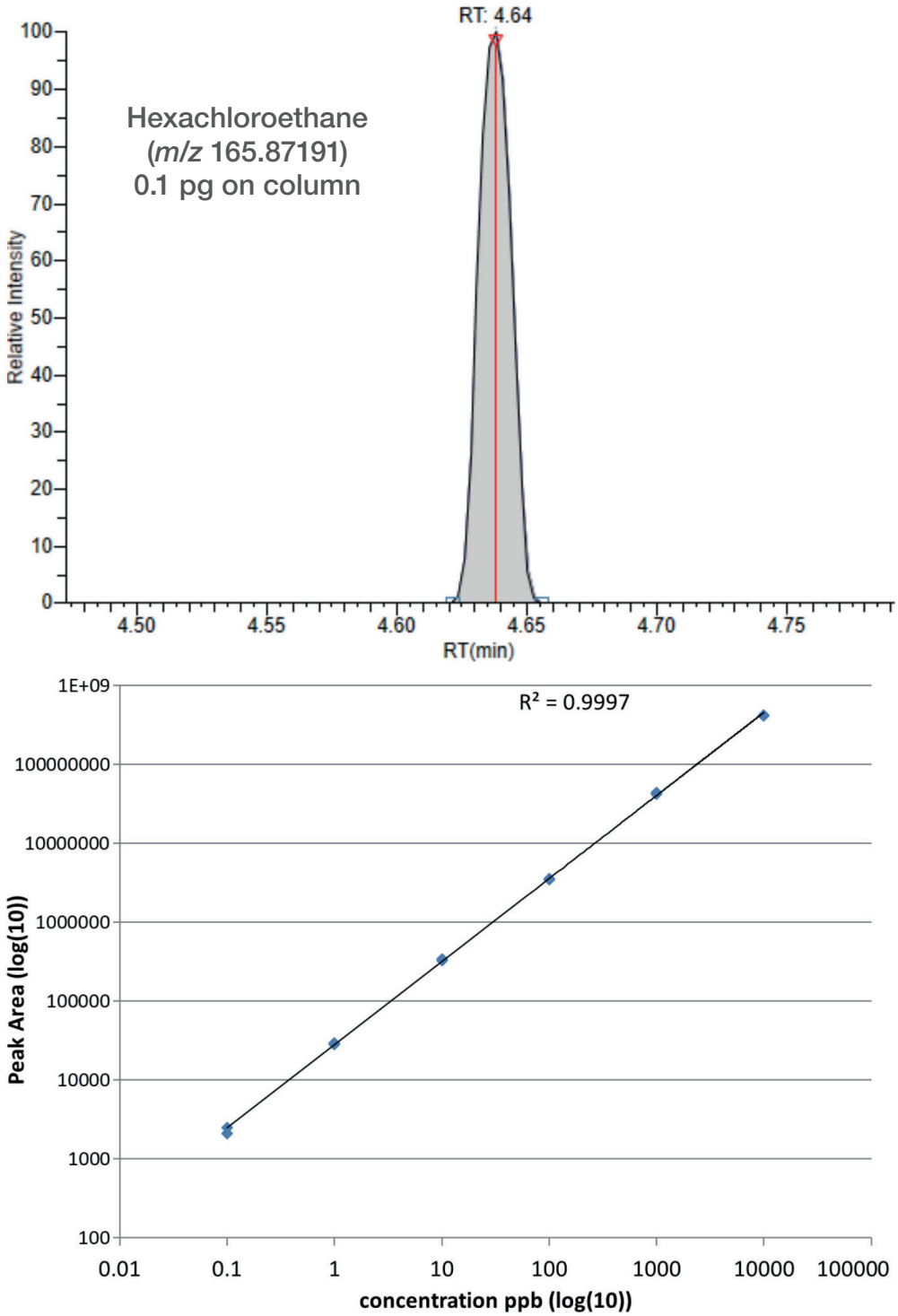


Figure 4. Linear dynamic range of the Orbitrap Exploris GC is demonstrated using hexachloroethane solvent standards injected over six orders of magnitude. The extracted ion chromatogram (*m/z* 165.87191) corresponding to hexachloroethane at 0.1 pg on column is shown together with the coefficient of determination (R^2) values determined over a concentration range of 0.1–10,000 pg on column.

Moreover, excellent peak area repeatability (n=3 injections) was obtained at each concentration level as demonstrated for hexachloroethane in Table 1 which shows %RSD ranged from 1.0 to 4.9 across the six orders of magnitude.

Table 1. Calculated %RSD from n=3 repeat injections of hexachloroethane solvent standard at various on column concentrations. Data from the Orbitrap Exploris GC shown.

Hexachloroethane concentration (pg on column)	Orbitrap Exploris GC %RSD (n=3)
10000	1.7
1000	1.5
100	1.0
10	2.4
1	2.4
0.1	4.9

Sensitivity

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.

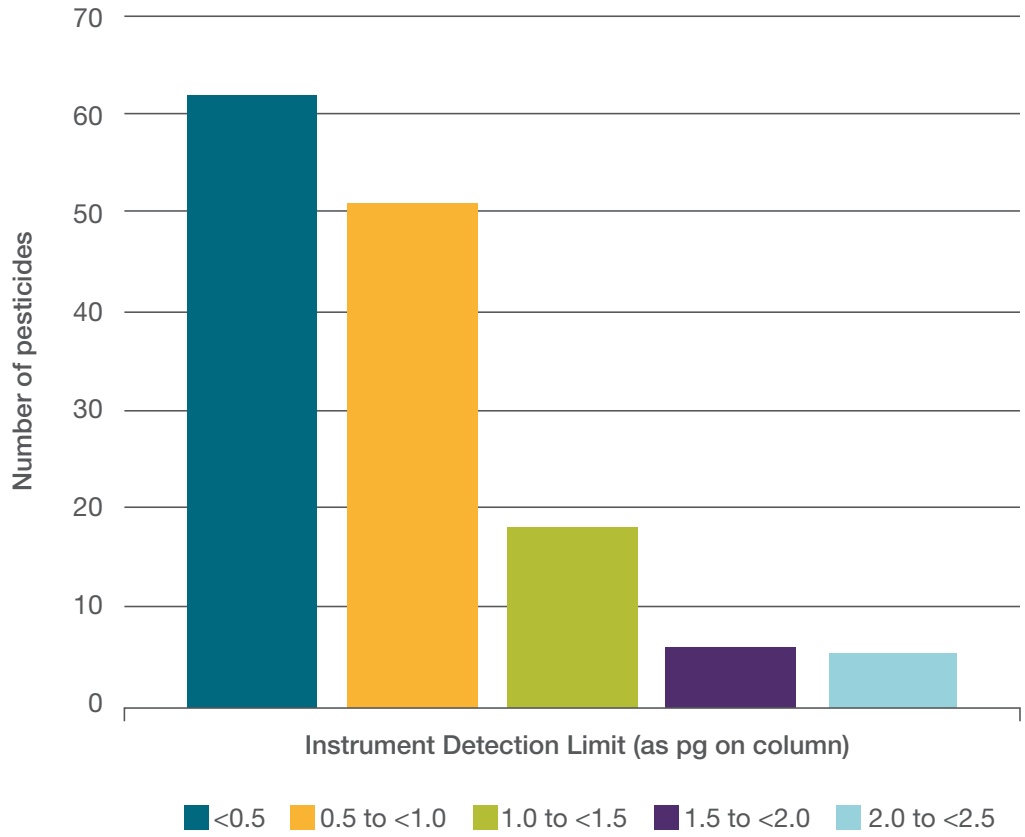
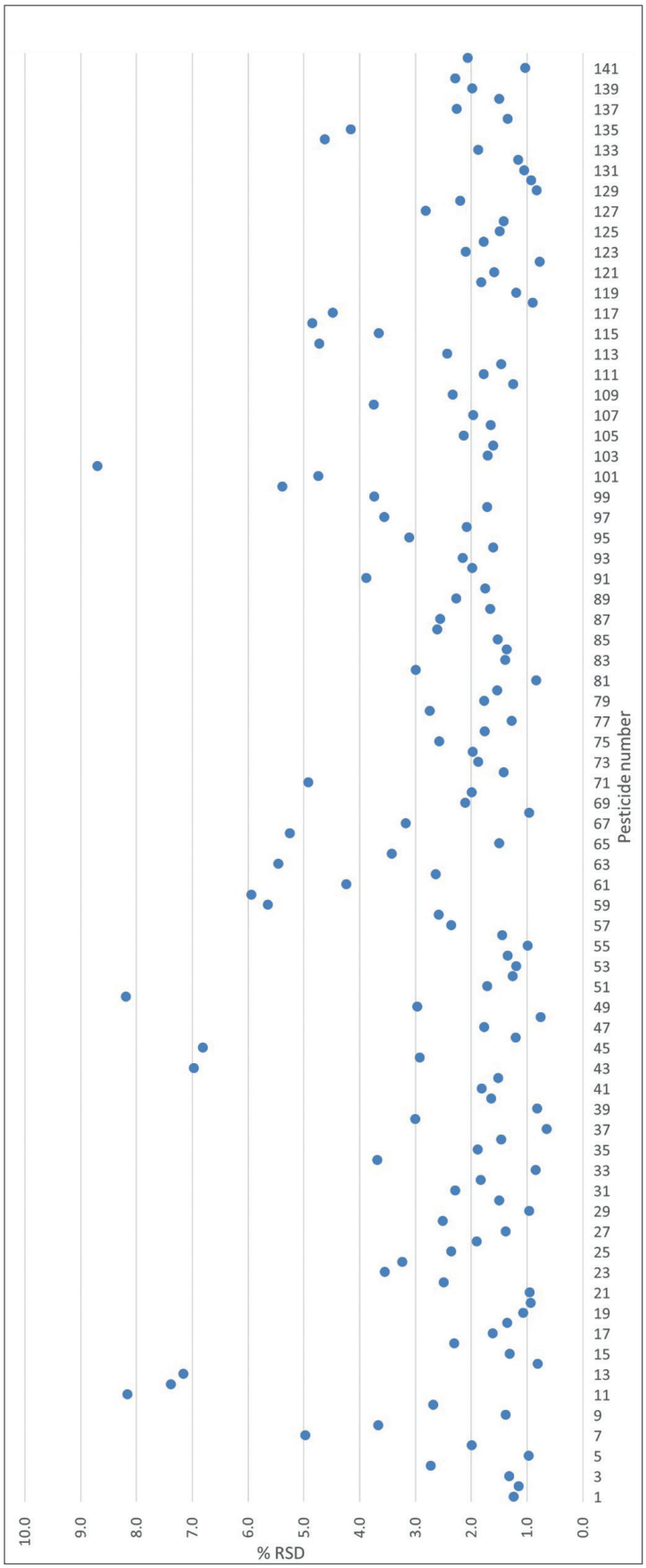


Figure 5. Sensitivity instrument detection limit (IDL as pg on column) determined for 142 pesticides in whole flour using an Orbitrap Exploris GC. Data was obtained by taking into account the amount on column, the Student's-*t* value corresponding degrees of freedom for repeat injections (n=10) of a 10 pg/µL whole flour matrix matched pesticide standard.

Sensitivity expressed as instrument detection limits (IDL) for all the pesticides analyzed was calculated and is reported in Figure 5, and the associated %RSD values are shown in Figure 6. 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.

Orbitrap Exploris GC pesticide %RSD in whole flour



No	Compound	No	Compound
1	2-phenylphenol	72	Fenthion
2	3,4-Dichloroaniline	73	Fluquinconazole
3	4,4'-Dichlorobenzophenone	74	Fluridone
4	9,10-Anthracenedione	75	Flutolanil
5	Acetamide, 2-chloro-N-(2,6-dimethylphenyl)-N-(2-methoxyethyl)-	76	Heptachlor epoxide
6	Acetamide, 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)-	77	Hexazinone
7	Acetamide, 2-chloro-N,N-di-2-propenyl-	78	Iodofenphos
8	Acrinathrin	79	Isazofos
9	Alachlor	80	Isodrin
10	Aldrin/Aldrin-r	81	Isopropalin
11	Azinphos-ethyl	82	Lenacil
12	Azinphos-methyl	83	Leptophos
13	Benfluralin	84	Metazachlor
14	Benzenamine, 2,3,5,6-tetrachloro-	85	Methacrifos
15	Benzene, hexachloro-	86	Methyl parathion
16	Benzonitrile, 2,6-dichloro-	87	Mevinphos
17	Benzonitrile, pentachloro-	88	Mirex
18	BHC, alpha	89	Mitotane
19	BHC, beta	90	N-(2-Ethylhexyl)-5-norbornene-2,3-dicarboximide
20	BHC, delta	91	Nitralin
21	BHC, gamma	92	Nitrofen
22	Bifenthrin	93	Norflurazon
23	Bioallethrin	94	o,p'-DDE
24	Biphenyl	95	o,p'-Methoxychlor
25	Bromfenvinphos-methyl	96	Oxadiazone
26	Bromophos	97	Oxyfluorfen
27	Bromophos-ethyl	98	p,p'-DDE
28	Bromopropylate	99	p,p'-DDT
29	Bupirimate	100	Parathion
30	Carbamothioic acid, bis(1-methylethyl)-, S-(2,3-dichloro-2-propenyl) ester	101	Pebulate
31	Carbophenothion	102	Penconazole
32	Carfentrazone-ethyl	103	Pendimethalin
33	Chlorbenside	104	Pentachloroaniline
34	Chlorfenapyr	105	Pentachlorothioanisole
35	Chlorfenson /Ovex	106	Permethrin
36	Chlorfenvinphos	107	Phosalone
37	Chlorobenzilate	108	Phosmet
38	Chloroneb	109	Phosphonothioic acid, phenyl-, O-ethyl O-(4-nitrophenyl) ester
39	Chlorpropham	110	Piperonyl butoxide
40	Chlorpyrifos(-ethyl)	111	Pirimiphos methyl
41	Chlorpyriphos-methyl	112	Pirimiphos-ethyl
42	Chlorthiophos	113	Pretilachlor
43	Chlorthiophos II	114	Prochloraz
44	Chlozolinate	115	Prodiamine
45	Cis-1,2,3,6-Tetrahydrophthalimide	116	Profenofos
46	cis-Chlordane	117	Profluralin
47	cis-Nonachlor	118	Propanil
48	Clomazone/Dimethazone	119	Propyzamide
49	Coumaphos	120	Pyrazophos
50	Cycloate	121	Pyridaphenthion
51	Cyprodinil	122	Pyrimethanil
52	Diazinone	123	Pyriproxyfen
53	Dichloran	124	Quintozene
54	Diphenamid	125	Sulfotep
55	Diphenylamine	126	Sulprofos
56	Disulfoton	127	Tebuconazole
57	Endosulfan (beta)	128	Tebufenpyrad
58	Endosulfan ether	129	Tecnazene
59	Endosulfan sulfate	130	Tefluthrin
60	Endosulfan, (alpha)	131	Terbacil
61	Endrin	132	Terbufos
62	Endrin ketone	133	Terbuthylazine
63	Endrin-aldehyde	134	Tetrachlorvinphos
64	Ethalfluralin	135	Tetramethrin
65	Ethion	136	Tolclofos-methyl
66	Etofenprox	137	trans-Chlordane
67	Fenamiphos	138	Transfluthrin
68	Fenarimol	139	trans-Nonachlor
69	Fenchlorphos/Ronnel	140	Triadimefon
70	Fenitrothion	141	Triallate
71	Fenson	142	Vinclozolin

Figure 6. IDL repeatability expressed as % RSD achieved for the analysis of 142 pesticides using an Orbitrap Exploris GC, for repeat injections (n=10) of a 10 pg/µL whole flour matrix matched pesticide standard

Spectral fidelity irrespective of concentration

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 7 for pentachloroaniline in whole flour matrix. The mass accuracy for every ion in the isotopic cluster is <1 ppm giving high confidence in the identification.

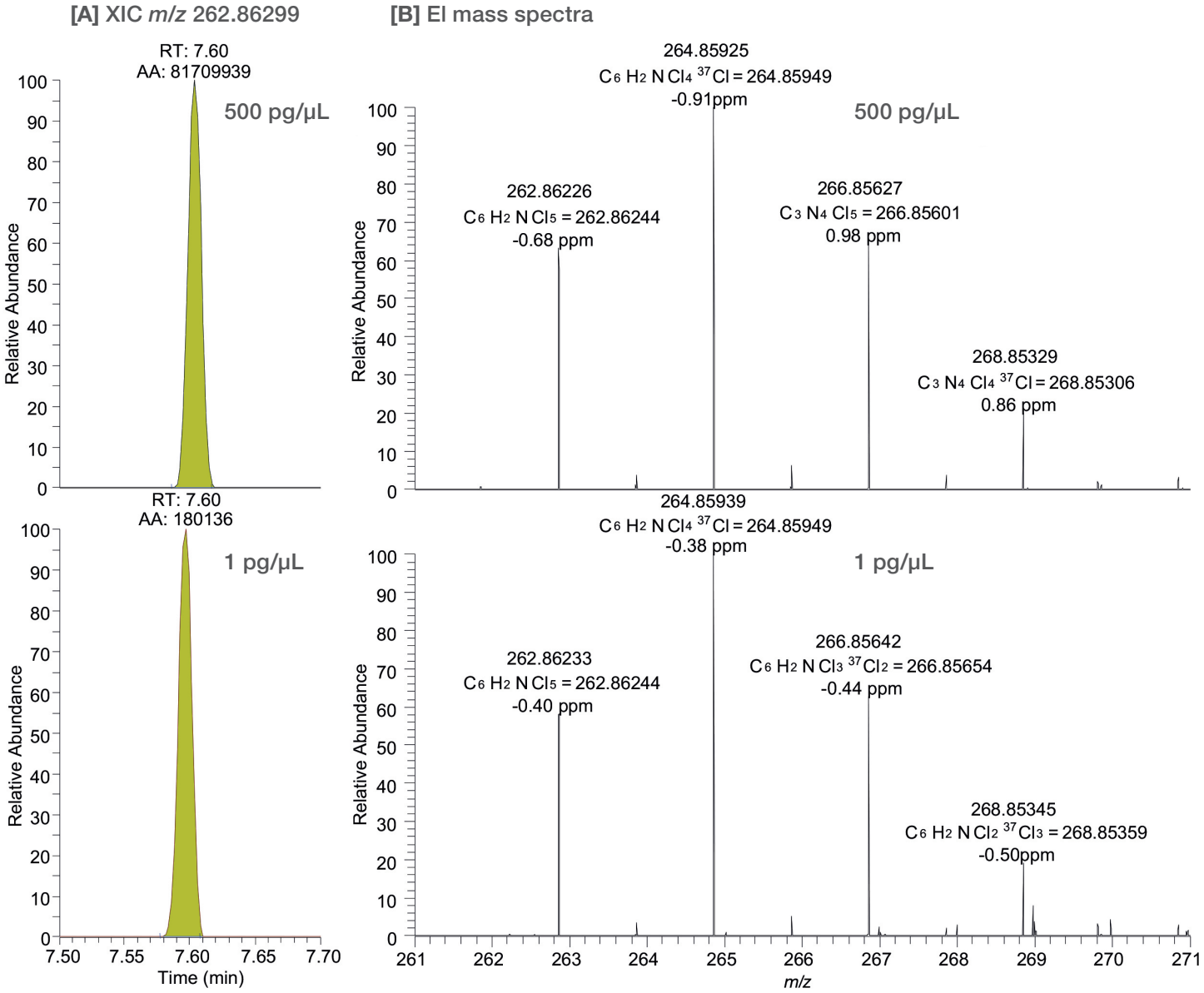
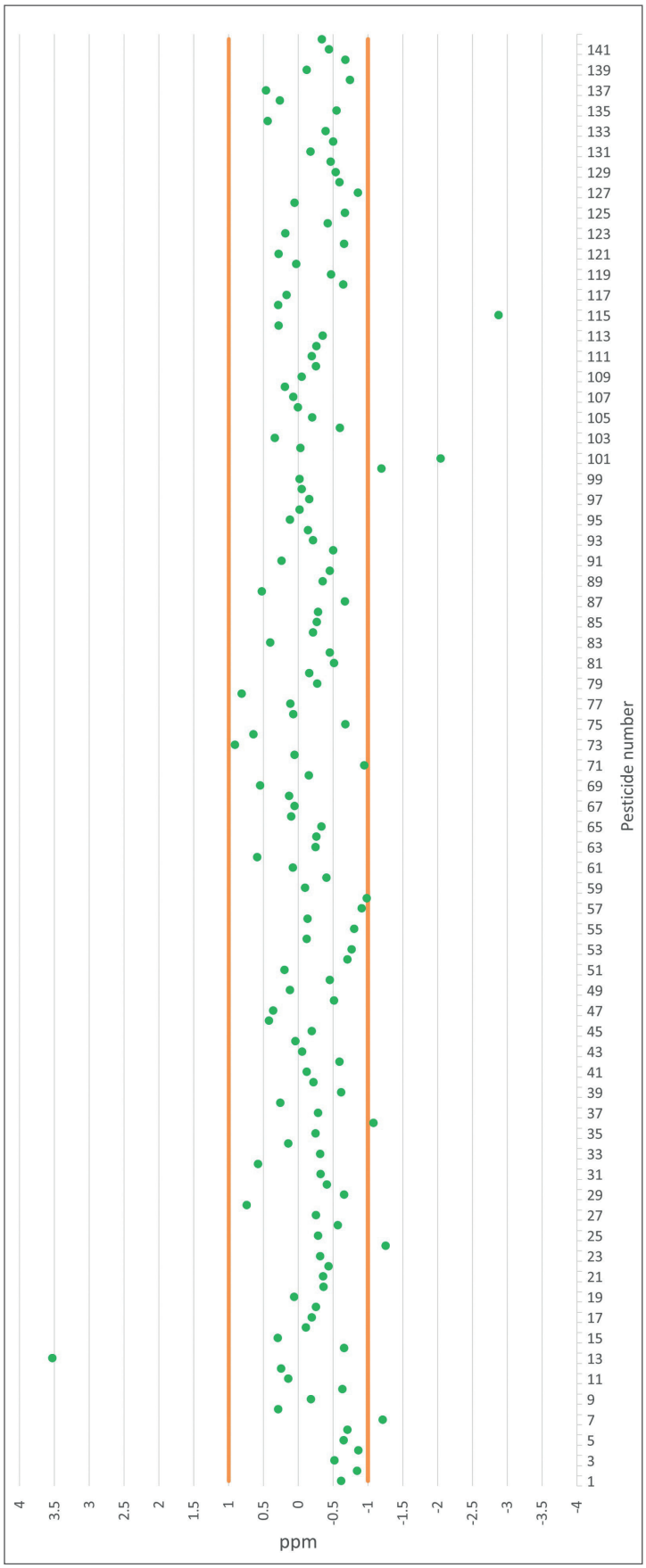


Figure 7. Spectral fidelity illustrated for pentachloroaniline for two levels (1 and 500 pg/µL) in whole flour matrix using an Orbitrap Exploris GC. [A]: Extracted ion chromatograms (XIC) for pentachloroaniline at each level annotated with peak retention time (RT) and peak area (AA); [B]: EI mass spectra 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, low (<1 ppm) mass accuracy is critical. To test the mass accuracy that can be achieved using the Orbitrap Exploris GC, repeat injections (n=10) of mixed pesticides standards (10 pg/µL) were carried out. Examples are reported in Figure 8, with an average mass accuracy of -0.2 ppm.



No	Compound	No	Compound
1	2-phenylphenol	72	Fenthion
2	3,4-Dichloroaniline	73	Fluquinconazole
3	4,4'-Dichlorobenzophenone	74	Fluridone
4	9,10-Anthracenedione	75	Flutolanil
5	Acetamide, 2-chloro-N-(2,6-dimethylphenyl)-N-(2-methoxyethyl)-	76	Heptachlor epoxide
6	Acetamide, 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)-	77	Hexazinone
7	Acetamide, 2-chloro-N,N-di-2-propenyl-	78	Iodofenphos
8	Acrinathrin	79	Isazofos
9	Alachlor	80	Isodrin
10	Aldrin/Aldrin-r	81	Isopropalin
11	Azinphos-ethyl	82	Lenacil
12	Azinphos-methyl	83	Leptophos
13	Benfluralin	84	Metazachlor
14	Benzenamine, 2,3,5,6-tetrachloro-	85	Methacrifos
15	Benzene, hexachloro-	86	Methyl parathion
16	Benzonitrile, 2,6-dichloro-	87	Mevinphos
17	Benzonitrile, pentachloro-	88	Mirex
18	BHC, alpha	89	Mitotane
19	BHC, beta	90	N-(2-Ethylhexyl)-5-norbornene-2,3-dicarboximide
20	BHC, delta	91	Nitralin
21	BHC, gamma	92	Nitrofen
22	Bifenthrin	93	Norflurazon
23	Bioallethrin	94	o,p'-DDE
24	Biphenyl	95	o,p'-Methoxychlor
25	Bromfenvinphos-methyl	96	Oxadiazone
26	Bromophos	97	Oxyfluorfen
27	Bromophos-ethyl	98	p,p'-DDE
28	Bromopropylate	99	p,p'-DDT
29	Bupirimate	100	Parathion
30	Carbamothioic acid, bis(1-methylethyl)-, S-(2,3-dichloro-2-propenyl) ester	101	Pebulate
31	Carbophenothion	102	Penconazole
32	Carfentrazone-ethyl	103	Pendimethalin
33	Chlorbenside	104	Pentachloroaniline
34	Chlorfenapyr	105	Pentachlorothioanisole
35	Chlorfenson /Ovex	106	Permethrin
36	Chlorfenvinphos	107	Phosalone
37	Chlorobenzilate	108	Phosmet
38	Chloroneb	109	Phosphonothioic acid, phenyl-, O-ethyl O-(4-nitrophenyl) ester
39	Chlorpropham	110	Piperonyl butoxide
40	Chlorpyrifos(-ethyl)	111	Pirimiphos methyl
41	Chlorpyriphos-methyl	112	Pirimiphos-ethyl
42	Chlorthiophos	113	Pretilachlor
43	Chlorthiophos II	114	Prochloraz
44	Chlozolinate	115	Prodiamine
45	Cis-1,2,3,6-Tetrahydrophthalimide	116	Profenofos
46	cis-Chlordane	117	Profluralin
47	cis-Nonachlor	118	Propanil
48	Clomazone/Dimethazone	119	Propyzamide
49	Coumaphos	120	Pyrazophos
50	Cycloate	121	Pyridaphenthion
51	Cyprodinil	122	Pyrimethanil
52	Diazinone	123	Pyriproxyfen
53	Dichloran	124	Quintozene
54	Diphenamid	125	Sulfotep
55	Diphenylamine	126	Sulprofos
56	Disulfoton	127	Tebuconazole
57	Endosulfan (beta)	128	Tebufenpyrad
58	Endosulfan ether	129	Tecnazene
59	Endosulfan sulfate	130	Tefluthrin
60	Endosulfan, (alpha)	131	Terbacil
61	Endrin	132	Terbufos
62	Endrin ketone	133	Terbuthylazine
63	Endrin-aldehyde	134	Tetrachlorvinphos
64	Ethalfluralin	135	Tetramethrin
65	Ethion	136	Tolclofos-methyl
66	Etofenprox	137	trans-Chlordane
67	Fenamiphos	138	Transfluthrin
68	Fenarimol	139	trans-Nonachlor
69	Fenchlorphos/Ronnel	140	Triadimefon
70	Fenitrothion	141	Triallate
71	Fenson	142	Vinclozolin

Figure 8. Mass accuracy (ppm) in matrix is illustrated for repeat injections (n=10) of mixed pesticides standards (10 pg/µL)

Maintaining sub-ppm mass accuracy irrespective of compound concentration

Sub-ppm mass accuracy was maintained across compound concentrations using an Orbitrap Exploris GC, as exemplified for hexachloroethane (Table 2). 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.

Mass accuracy repeatability

To evaluate repeatability of mass accuracy over long analysis runs at low concentrations when using the Orbitrap Exploris GC, 140 injections of mixed solvent standards containing octafluoronaphtalene (OFN) (0.1 pg oc) and hexachlorobenzene (HCB) (10 pg oc) were performed, as reported in Figure 9. The results confirmed that by using the Orbitrap Exploris GC, high levels of mass accuracy (<1.1 ppm) over 2 days unattended analysis can confidently be achieved, without instrument maintenance, calibration, or tuning.

Table 2. 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 Δppm	**0.4**	**0.5**	**0.5**	**0.4**	**0.2**

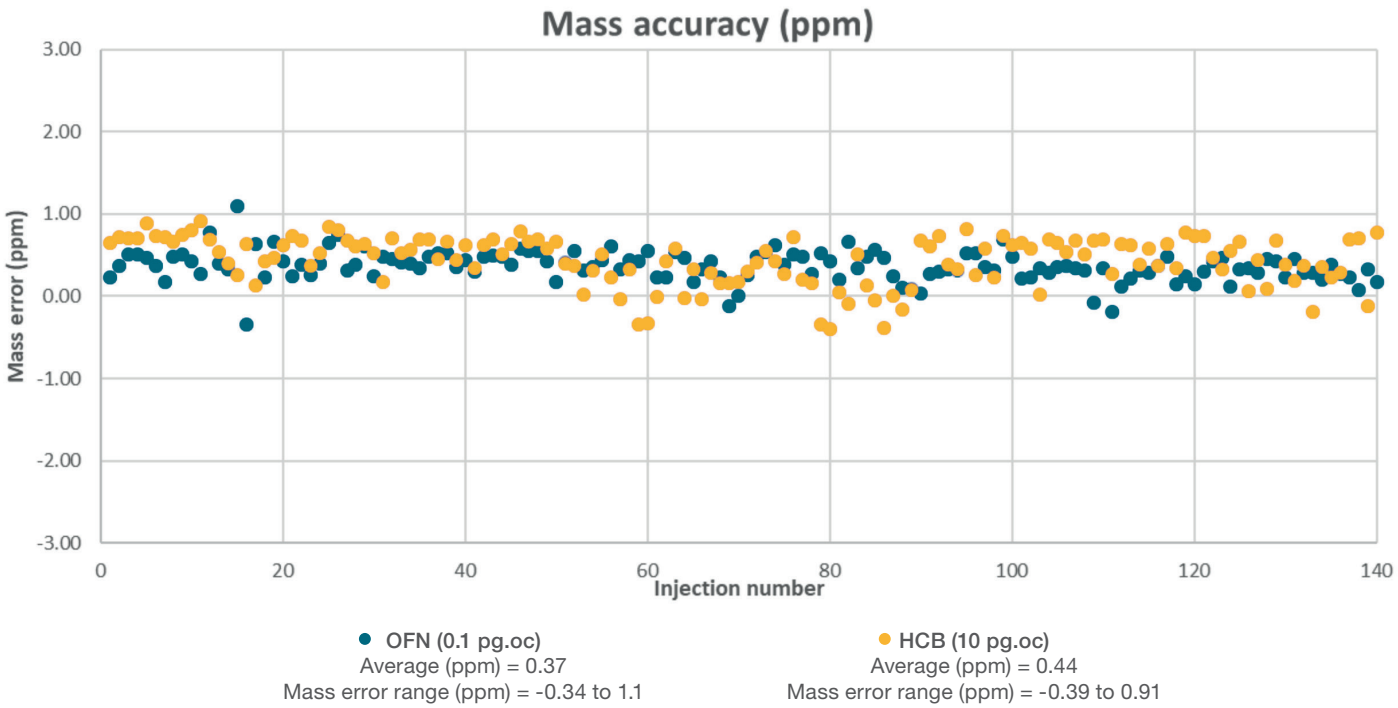


Figure 9. Repeatability of mass accuracy (ppm), illustrated with 140 injections of a solvent standard, containing OFN (0.1 pg on-column) and HCB (10 pg on-column), annotated with average mass accuracy and range achieved using an Orbitrap Exploris GC

Compound confirmation using PCI

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 $[M+C_3H_5]^+$. As an example, EI and PCI spectra of camphene in a sample of thyme are reported in Figure 10. 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.

Resolving power

Acquiring reliable accurate mass measurements is critical when detecting low level pesticides in complex matrices. Through the high-mass resolving power of the Orbitrap Exploris GC 240, discrimination between matrix interferences and target analyte ions can be confidently achieved. When the resolution is insufficient, the mass profile of ions overlaps, which could result in high mass error and incorrect assignment of the mass of the target ion. This is demonstrated in Figure 11 where an oat matrix standard (500 pg/µL) was analyzed at resolving powers of 30K, 60K, 120K, and 240K using the Orbitrap Exploris GC 240. The zoomed in mass spectra show the quantifier ion and a matrix ion of a similar mass causing interference. The diphenylamine ion at 30K and 60K is not resolved resulting in poor mass accuracies of -6.38 and -6.18 ppm, respectively, and is resolved at 120K and even further resolved at 240K with mass accuracies of 1.10 and 0.56 ppm, respectively.

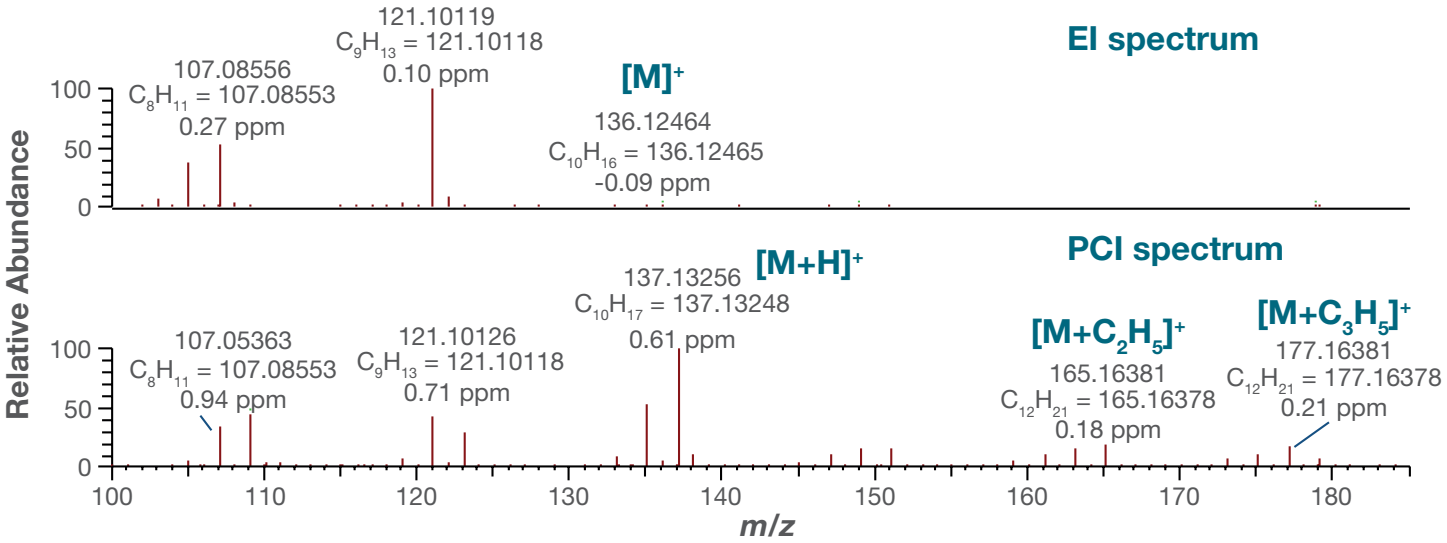


Figure 10. Comparison between EI and PCI spectrum for camphene in a sample of thyme (RT=9.04 min). The molecular ion (*m/z* 136.12468) is visible in the EI spectrum with sub ppm mass accuracy annotated. In the PCI spectrum the typical adducts observed when methane gas is used are clearly visible confirming the molecular ion and the proposed molecular formula for camphene.

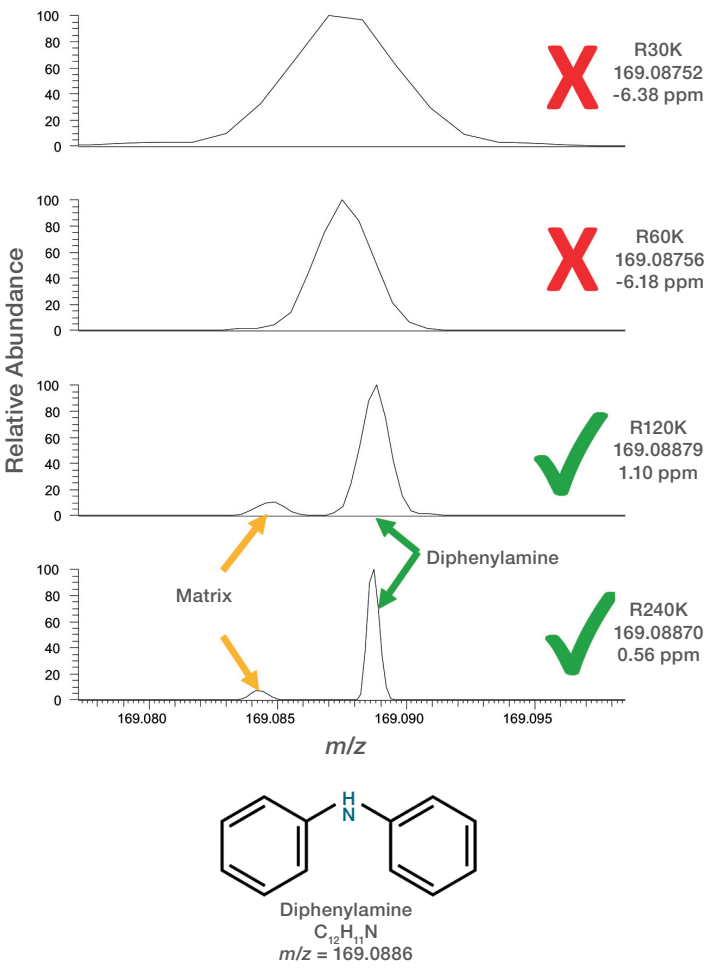


Figure 11. Effects of resolving power on the mass accuracy using the Orbitrap Exploris GC 240 for the detection of diphenylamine in an oat matrix standard (500 pg/µL) acquired at 30K, 60K, 120K, and 240K. The diphenylamine ion at 30K and 60K is not resolved; however, at 120K and 240K the quantifier ion for diphenylamine and a matrix interference ion are sufficiently resolved. Zoomed in mass spectra are annotated with the resolving power, the measured mass, as well as the mass accuracy (ppm).

Conclusions

The data shown here demonstrate that the Orbitrap Exploris GC mass spectrometers deliver exceptional high-quality analytical performance using full scan acquisition for both analytical testing and scientific research applications.

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/µL) make the Orbitrap Exploris GC ideal for analytical testing.

NIST library searchable accurate mass spectra are achievable, enabling confident identification of unknown compounds and confirmation of knowns. Spectral fidelity irrespective of concentration can also be achieved, which is critical to maintain confidence in compound identification.

When the spectral library match from the EI spectrum is inconclusive, or additional confirmation is required, the Orbitrap Exploris GC positive chemical ionization (PCI) data can be used to confirm the elemental composition of the parent molecule using accurate mass information.

Sub-ppm mass accuracy is achievable for the analysis of pesticides in whole flour spiked with pesticides. In addition, the high mass resolving power of the Orbitrap Exploris GC 240 enables superior discrimination between matrix interferences and target analyte ions.

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-Accurate-Mass-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-Orbitrap-Pesticides-Baby-Food-AN10449-EN.pdf

Find out more at **thermofisher.com/HRAMGCMS**

ThermoFisher SCIENTIFIC

TECHNICAL NOTE 10730

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

Authors: Jane Cooper, Dominic Roberts, and Cristian Cojocariu

Thermo Fisher Scientific, Runcorn, UK

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

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 parameters for each compound. High-resolution, full scan mass spectrometry using Orbitrap technology provides a solution to:

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

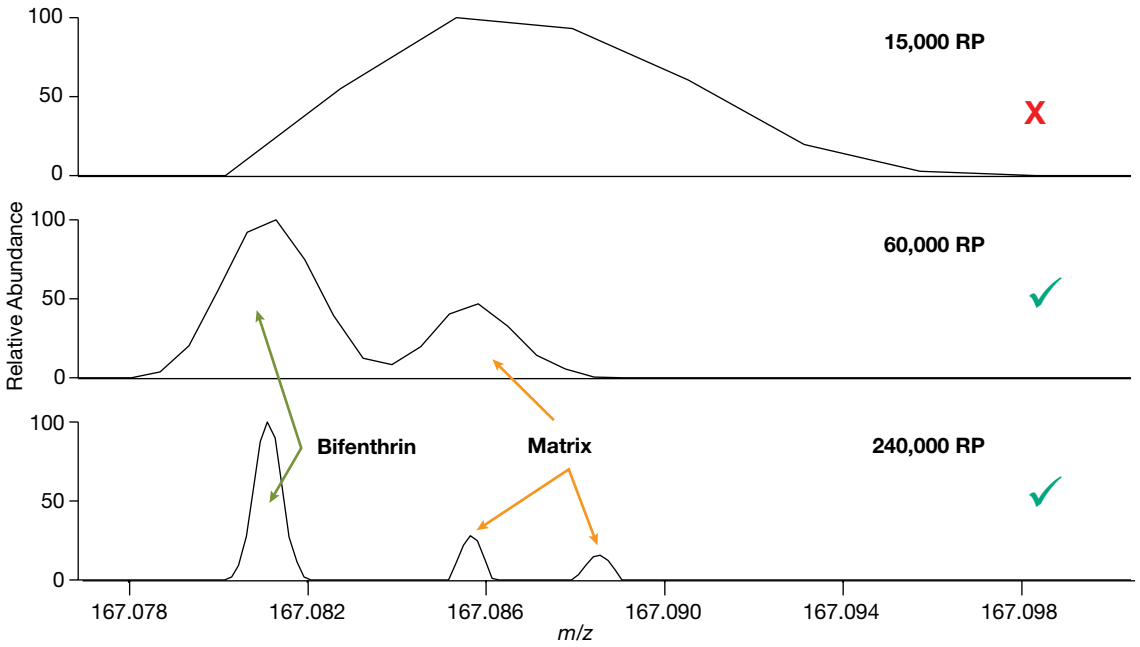
- Detection and quantification of an increasing number of compounds.
- Identification and elucidation of the chemical composition and structure of unknown compounds.
- Retrospective analysis of samples long after data acquisition.

ThermoFisher SCIENTIFIC

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).



RP (set by insturment)	Mass (*m/z*)	Resolution	Proposed elemental compoistion(s)	Theoretical mass (*m/z*)	Mass accuracy (ppm)
15,000	167.08629	22,000	$C_8H_{15}{}^{37}ClF$	167.08113	30.8
			$C_{13}H_{11}$	167.08629	4.5
60,000	167.08111	64,000	$C_8H_{15}{}^{37}ClF$	167.08113	-0.14
60,000	167.08629	59,000	$C_{13}H_{11}$	167.08553	1
240,000	167.08111	230,000	$C_8H_{15}{}^{37}ClF$	167.08113	-0.15
240,000	167.08629	280,000	$C_{13}H_{11}$	167.08553	0.81
240,000	167.08851	250,000	-	-	-

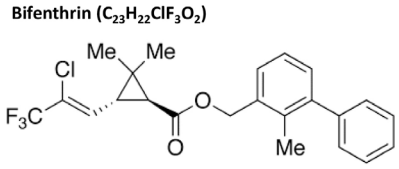


Figure 2. Effect of resolving power (RP) on mass accuracy of an analyte in matrix. Mass spectra of bifenthrin 10 ng/mL in soil acquired at 15,000, 60,000, and 240,000 RP (at FWHM *m/z* 200). Matrix interference at 15,000 RP prevents separation of this pesticide from the matrix interference, resulting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

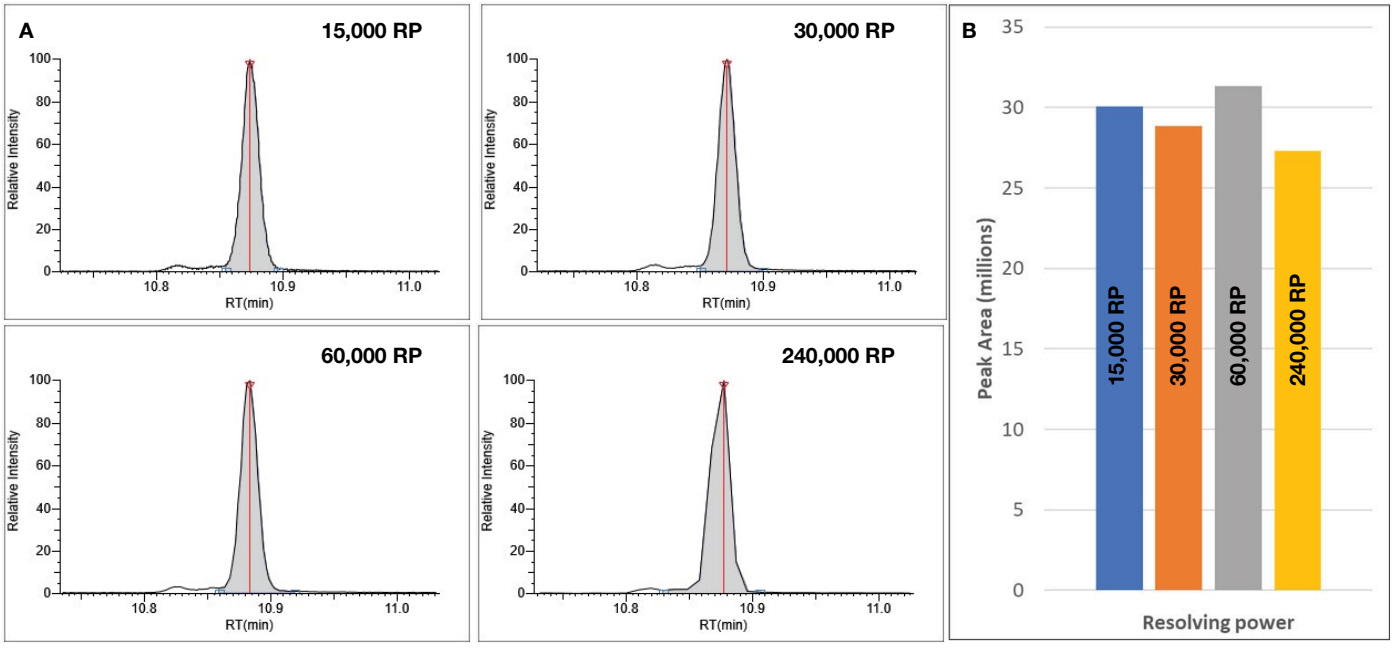


Figure 3. Effect of resolving power (RP) on sensitivity (as absolute peak area response) on pyriproxyfen, in a QuEChERS soil extract at a concentration of 100 ng/mL, showing extracted ion chromatogram of *m/z* fragment ion (*m/z* 136.0757) [A], as well as corresponding peak area responses obtained at 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 $C_{29}H_{44}O_2$.

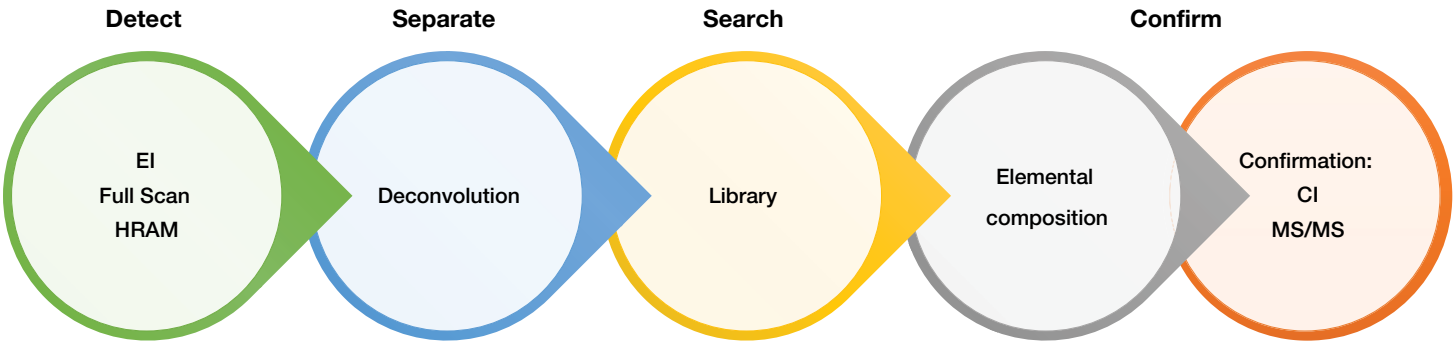


Figure 4. Workflow for screening for unknown compound identification using the Orbitrap Exploris GC 240 system: full scan data acquired using EI full scan HRAM; spectral deconvolution with library search for putative compound identification; confirmation using chemical ionization (CI) data, and if required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

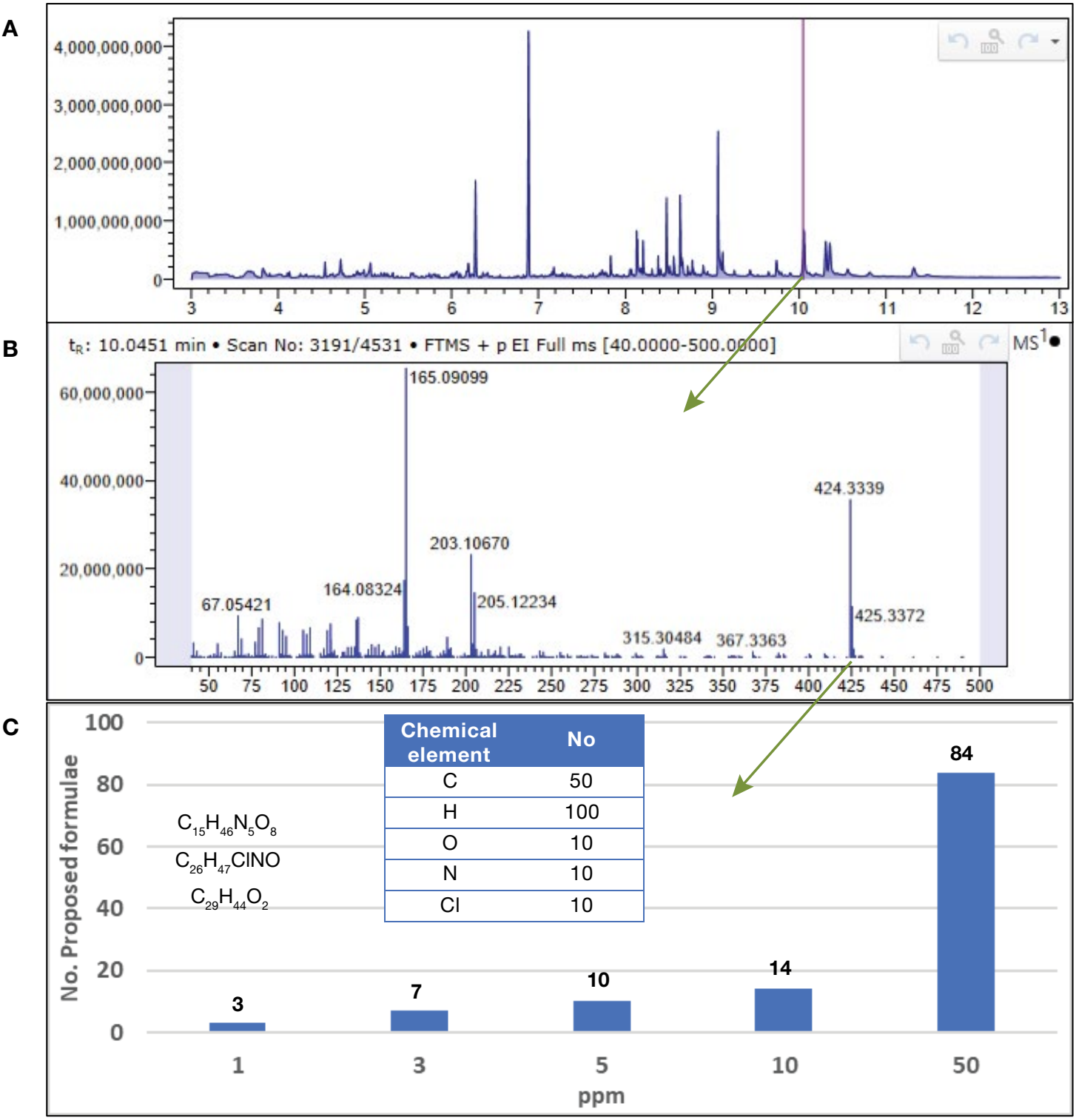


Figure 5. Using Mass Frontier structural elucidation software to aid identification for the elemental compositions for the ion *m/z* 424.33375 from the mass spectra of an unknown peak (measured with 60,000 RP at FWHM 200 *m/z*): [A] TIC for a whole flour extract containing an unknown peak at retention 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

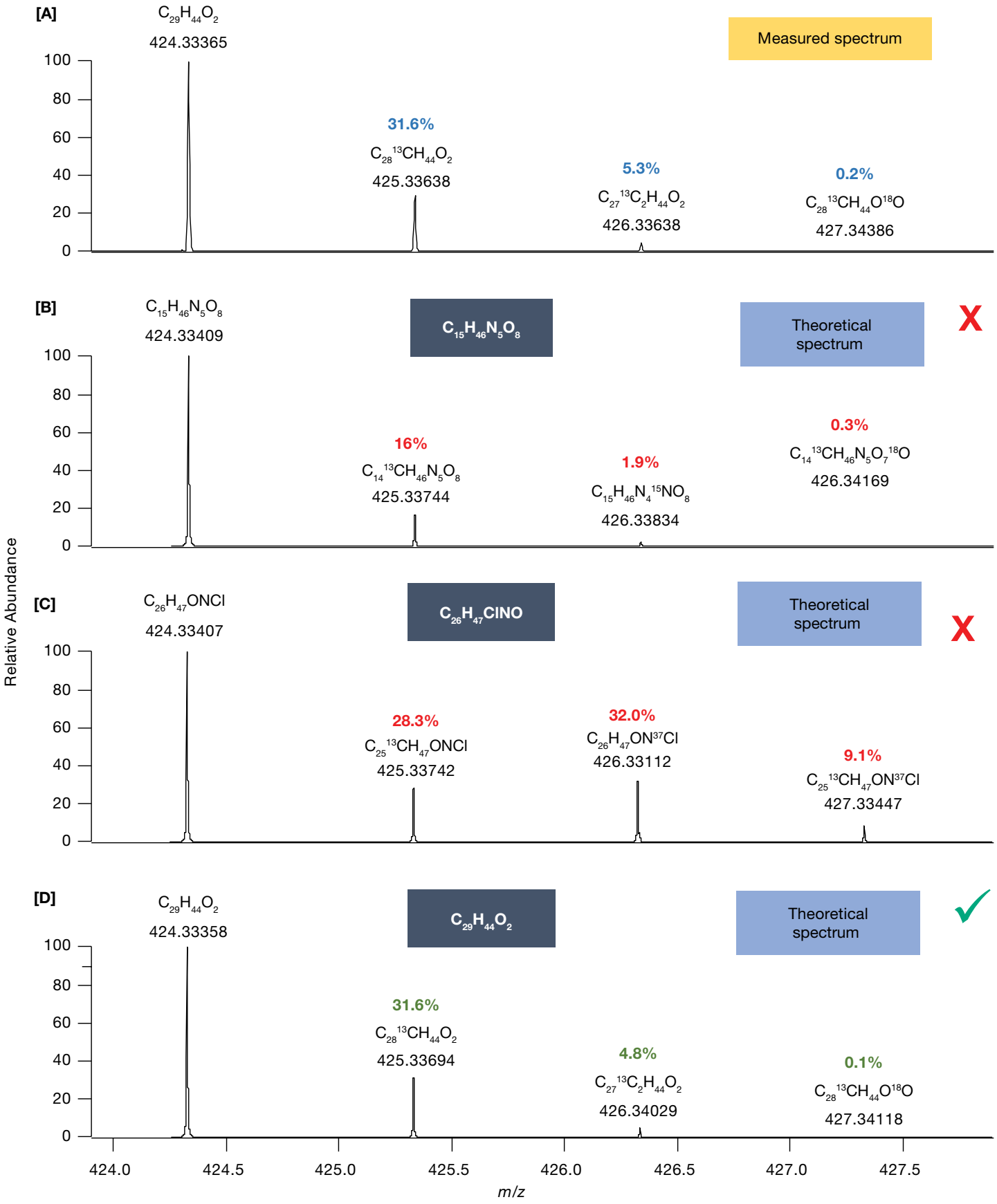


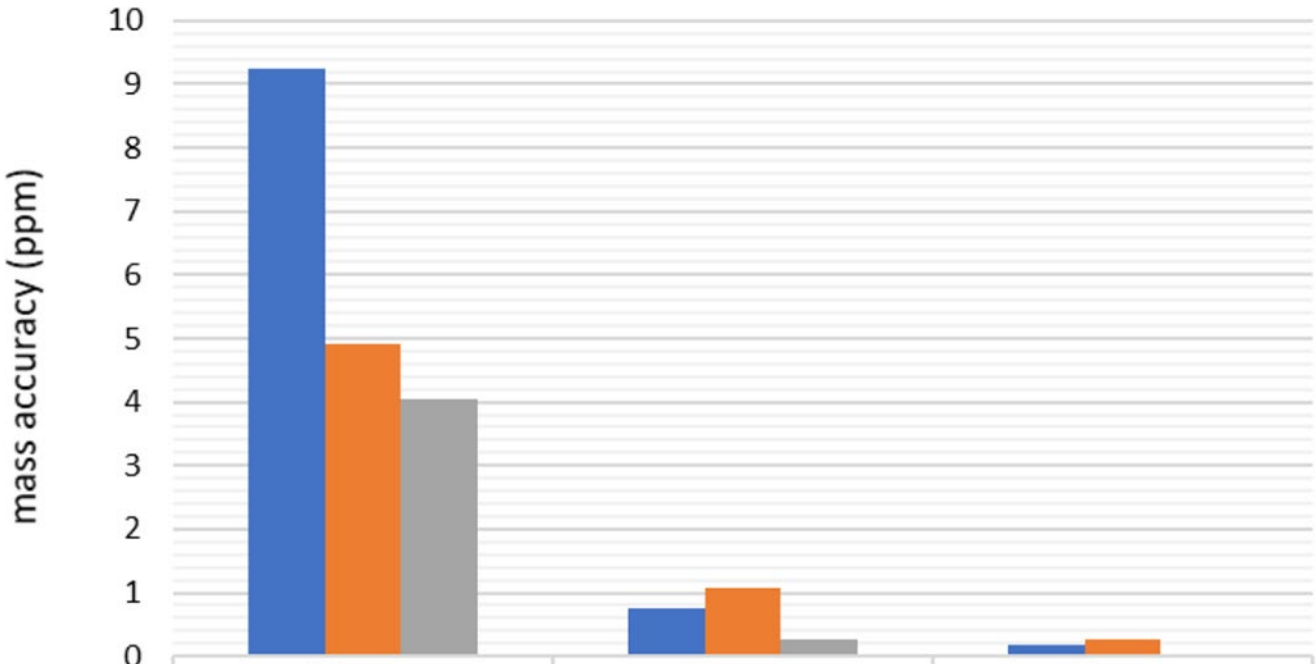
Figure 6. Considering the three formulae suggested with a mass tolerance of 1 ppm for a measured mass ion of *m/z* 424.33375, the isotopic pattern comparison (measured versus theoretical), used to eliminate possible chemical formulae and confirm the most likely chemical composition. [A] measured isotopic 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 ≥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.



	resolving power: 15,000	resolving power: 60,000	resolving power: 240,000
Trifluralin	9.2	0.8	0.2
Tebufenpyrad	4.9	1.1	0.3
Propyzamide	4.1	0.3	0.0

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 ≥60,000 RP is required to completely separate these pesticides from the interfering matrix ions and to deliver <1 ppm mass accuracy.

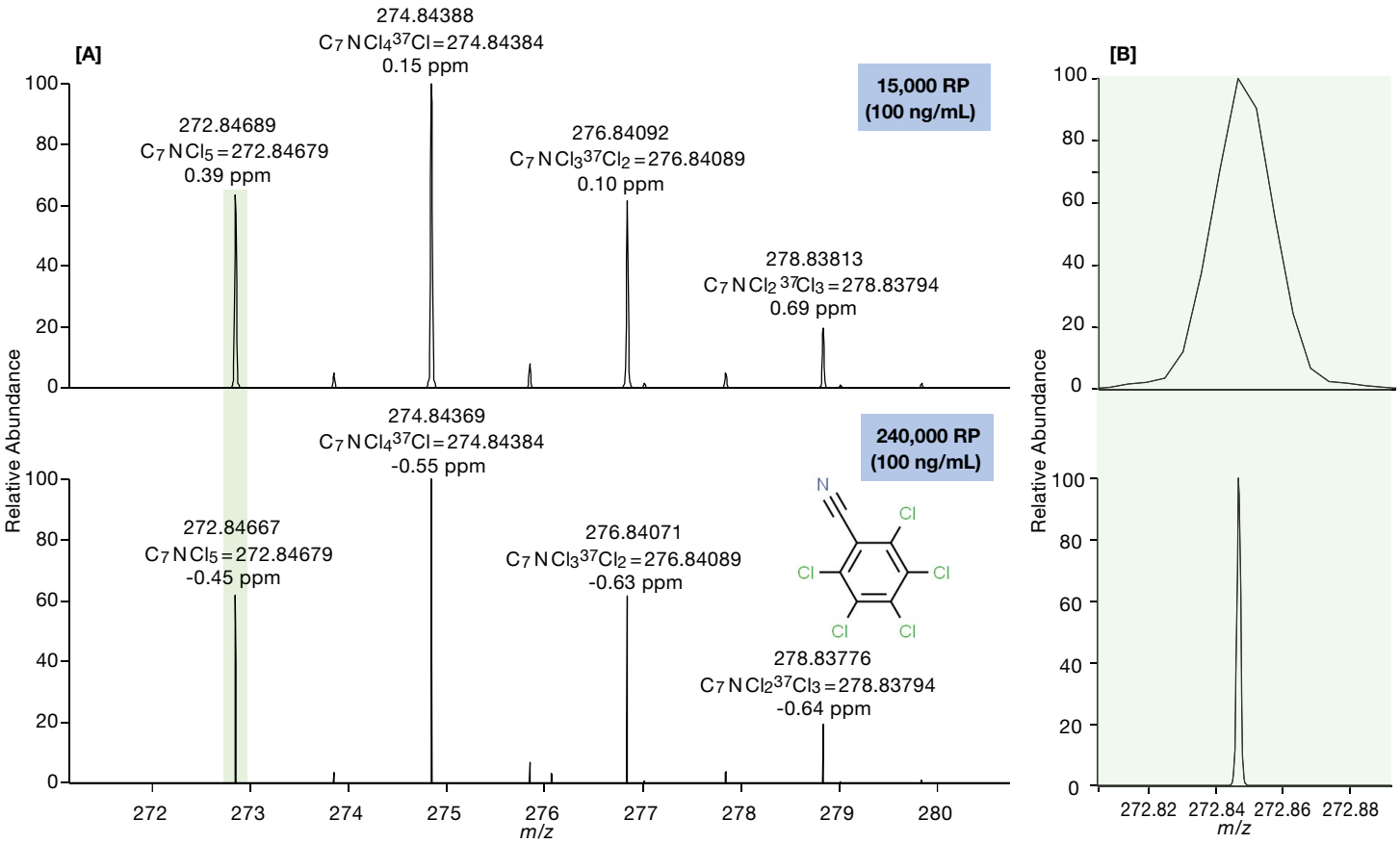


Figure 8. Spectral fidelity illustrated for pentachlorobenzonitrile (C_7Cl_5N) for 15,000 RP and 240,000 RP in a QuEChERS soil extract using the Orbitrap Exploris GC 240 system. [A] EI mass spectra at the molecular ion cluster at each level, annotated with measured mass, elemental composition, theoretical 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 $C_{14}H_{16}ClN_3O_2$ (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.

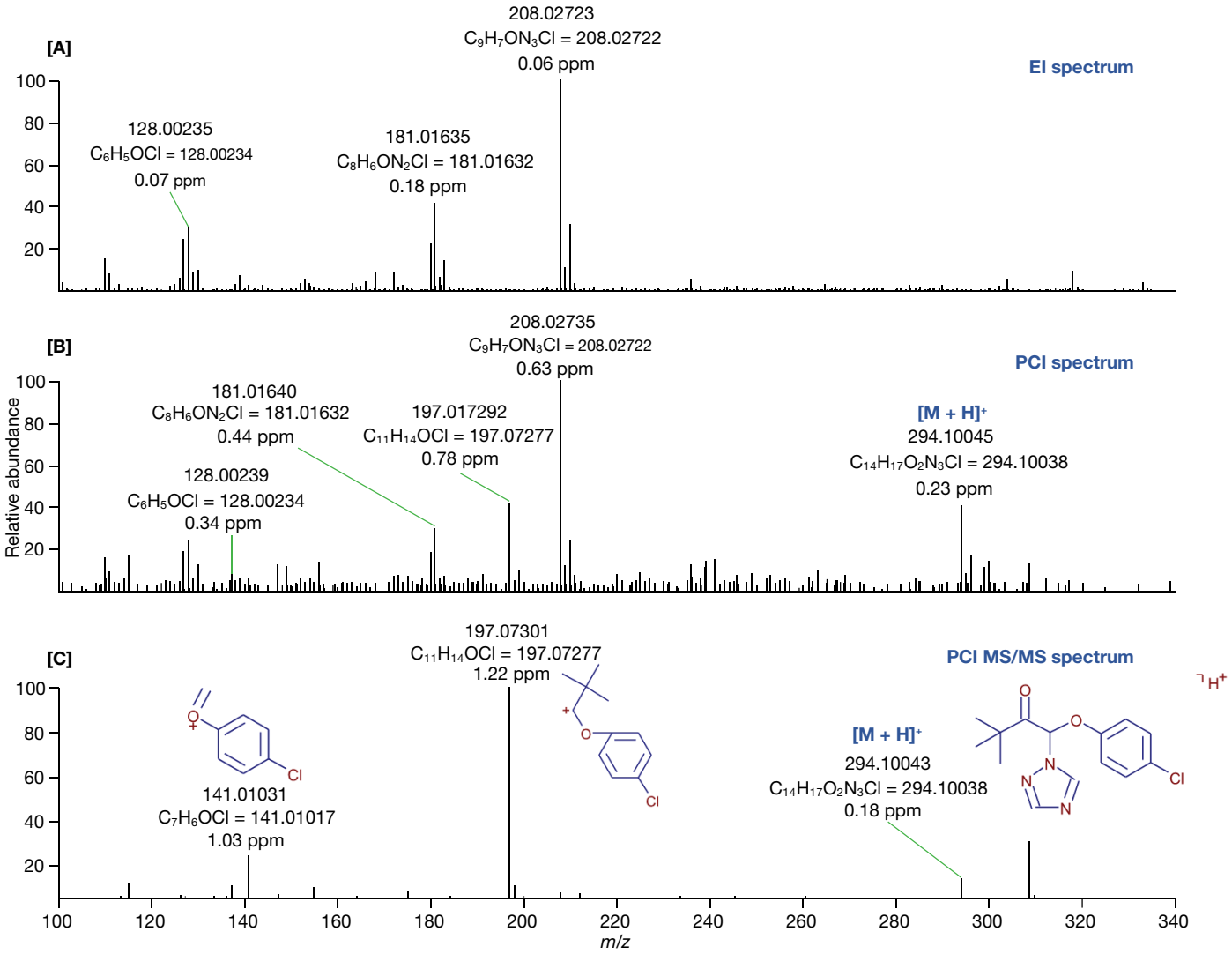


Figure 9. Comparison between the [A] EI, [B] PCI, and [C] PCI MS/MS spectra, illustrating that using PCI and PCI MS/MS data can be used to provide additional compound confirmation, shown for the confirmation of triadimefon in the a QuEChERS soil extract. The molecular ion for triadimefon corresponding 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.

thermo scientific

References

1. Thermo Scientific Application Note 617: Quantitative and Qualitative Confirmation of Pesticides in Beet Extract Using a Hybrid Quadrupole-Orbitrap Mass Spectrometer. San Jose, California, USA and Bremen, Germany. [Online] https://www.thermofisher.com/content/dam/tfs/ATG/CMD/cmd-documents/sci-res/app/ms/lc-ms/Orbitraps/AN-617-LC-MSMS-Pesticides-Beets-AN64284-EN.pdf (accessed March 10, 2020).
2. Thermo Scientific Application Note 592: Metabolomics of Hermaphroditic *C. elegans* via Isotopic Ratio Outlier Analysis (IROA) Using High-Resolution, Accurate-Mass LC-MS/MS. Thermo Fisher Scientific, NJ, USA, University of Florida, FL, USA and IROA Technologies, MI, USA. [Online] https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FCMD%2FApplication-Notes%2FAN-592-LC-MS-Hermaphroditic-Caenorhabditis-Elegans-AN63914-EN.
3. Thermo Scientific Application Note 502: Simple and Rapid Screening of Melamine in Milk Products with High Resolution Accurate Mass Benchtop Orbitrap LC-MS. San Jose, California, USA. [Online] https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FCMD%2FApplication-Notes%2FAN502_63314_ASMS09_Th668_MelamineMilk_11-10.

Find out more at **thermofisher.com/OrbitrapExplorisGC240**

ThermoFisher SCIENTIFIC

CASE STUDY 10750

Versatile, highly sensitive and reproducible: How Orbitrap Exploris GC 240 strengthens metabolomics

Introduction

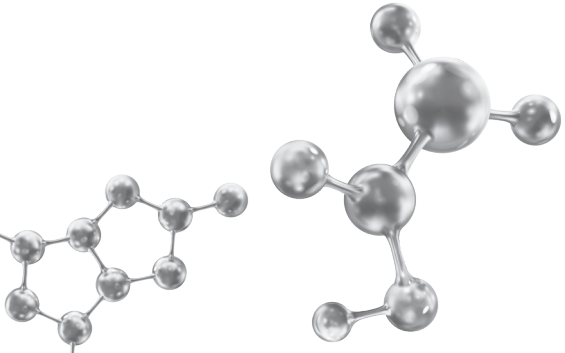
Over the last decade, metabolomics has demonstrated immense potential in accelerating biomarker research in human health and disease. The metabolome, influenced by both genetic and environmental factors, closely represents the underlying molecular phenotype, having direct applications towards both basic and translational research. Dr. John Bowden's work at the University of Florida takes the 'omics' approach to study biomarkers associated with pollutant exposure. His laboratory focuses on two main research questions: (1) what effect does the environment have on human and wildlife health, and (2) what effect does human activity have on environmental health. To tackle these research questions, the laboratory develops and employs mass spectrometric workflows to measure the presence of environmental pollutants as well as metabolomic workflows to identify biomarkers related to exposure. As industry experts in mass spectrometry (MS) techniques, the Bowden laboratory is prolific in its diverse MS-based metabolomics applications.

Recently, the team added a high-resolution accurate mass (HRAM) gas chromatography-mass spectrometry (GC-MS) system to take advantage of its higher separation power and unparalleled sensitivity for identifying and quantifying metabolites. Here, we discuss how the Bowden laboratory successfully expanded its capability with GC-MS and highlight key factors to consider for setting up a robust metabolomics platform.

A versatile, multi-platform approach to metabolomics

The expansive portfolio in the Bowden laboratory includes translational applications, such as human and wildlife exposure projects, pollutant measurements and the development of novel analytical methods. Running multiple parallel projects with a wide variety of applications is only made possible with instrument versatility. Centered around mass spectrometry, Dr. Bowden's laboratory is equipped with an orthogonal assortment of instruments to tackle these studies from varying perspectives. "In analyzing any sample, I want the capability to interrogate the widest chemical landscape possible," says Dr. Bowden.

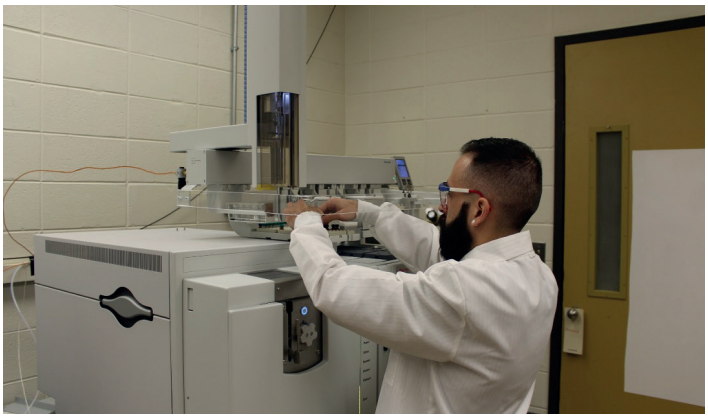
The instrumentation suite for metabolomics is diverse and includes the Thermo Scientific Orbitrap Exploris GC 240. This technology has provided the research group with the necessary workflow to develop robust methods, perform compound identification, analyze structures and obtain (semi-) quantitation across a variety of chemical classes within a diverse array of matrices. "It has made us a one-stop-shop to perform exposure-related studies," says Dr. Bowden. "We can analyze both the external measures of exposure (the pollutants) as well as internal measures of exposure (biomarkers) using the available instrumentation."



ThermoFisher SCIENTIFIC

"For metabolomics experiments, the capability to achieve such high selectivity and maintain sensitivity is revolutionary for our research; having easy access to this data certainty and such wide coverage opens up new research avenues for us"

– Dr. Bowden



The Thermo Scientific Orbitrap Exploris GC 240 system in the Bowden laboratory

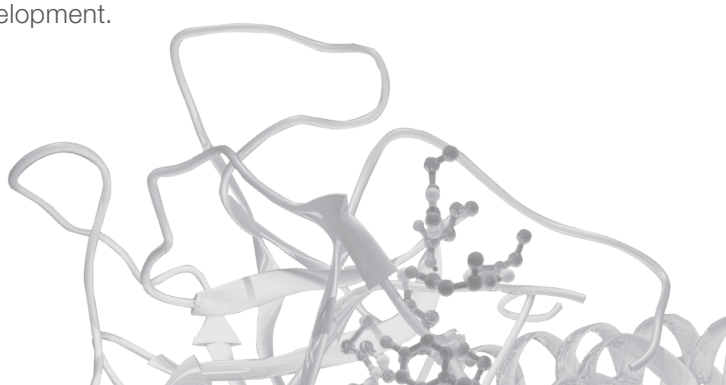
Prior to acquiring the Orbitrap Exploris GC 240, the team performed metabolomics studies solely using LC-MS/MS workflows on the Q-Exactive system. "The utilization of GC for metabolomics is gradually starting to receive the same level of attention as LC-MS-based approaches," says Dr. Bowden. "Incorporating both LC and GC into our metabolomics workflow has immensely broadened our compound identification capability. We can now identify a multitude of compounds ranging from volatile to nonvolatile, stable to thermally unstable and polar to nonpolar, providing us with a more comprehensive profile to interrogate."

With limitless potential on metabolite analyses, the research team put the technology suite to work to help execute multiple 'exposomics' and metabolomics projects.

How the comprehensive 'omics' setup serves multiple projects

The Bowden laboratory extends its metabolomics approach towards a wide variety of projects, breaking new ground with novel research questions and the use of unusual samples. Below are a handful of ongoing projects:

- **Measuring pollutants within biological systems:** Products of anthropogenic pollution, volatile per- and polyfluoroalkyl substances (PFAS) are known to infiltrate and persist in biological systems due to their high bioaccumulation potential. Using the Orbitrap Exploris GC 240, this study investigates volatile variants of PFAS present in both environmental and biological matrices of a wide variety of animal species, such as manatees. These studies will set new frontiers for PFAS research and trigger future high-profile studies involving other emerging areas of concern, such as pollutants in landfill emissions and indoor air.
- **The heterogeneity of lupus:** A part of a large longitudinal study, this project aims to understand the genetic and environmental mechanisms behind lupus. Metabolomics and lipidomics strategies are applied towards plasma and urine samples collected from a cohort of Gullah African American patients, a subpopulation with a high risk for lupus. To better understand the heterogeneity of lupus pathophysiology, the study will examine associations between lupus subtypes, environmental contaminants, diet, genetic and auto-immune risks, and the generated metabolomic and lipidomic profiles.
- **Wildlife exposure studies:** The goal of this project is to determine the extent to which wildlife populations are affected by environmental stressors, providing potential clues towards assessing human health risks. By adapting omics-based strategies typically employed in human health research to measure adverse health consequences in marine mammals, fish, crocodilians and apex predators, these projects challenge the status quo of metabolomics and venture into a vastly unexplored area in both biomarker discovery and method development.



Using GC-MS to expand metabolomic readouts

Choosing the right GC-MS system largely depends on the types of samples being measured, the level of expertise in the laboratory, and the availability of other complementary instruments to aid downstream or upstream experiments. As such, success with GC-MS is dictated by producing high-quality data which includes the number of compounds the research team can confidently identify, while ensuring all quality assurance and quality control requirements have been met.

"When a laboratory decides to expand its metabolomics workflow with GC-MS, there are a few key characteristics that need to be considered," notes Dr. Bowden. "At the top of the list are versatility, sensitivity, ease of use and rigorous ID capabilities." With rare and scarce samples at the heart of numerous projects, the Bowden laboratory needs to reliably extract the most information they can within each sample in one run. Dr. Bowden continues: "For laboratories like ours, where it's important to maximize the amount of information obtained from a single injection, acquiring systems that exceed the above characteristics is crucial."

With the installation of the Orbitrap Exploris GC 240 system, the Bowden laboratory is now equipped with a set of technologies to examine any metabolite. One of the distinguishing benefits of the Orbitrap Exploris GC 240 system is its superior resolution that allows compounds to be identified against a complex chemical background. Moreover, the flexibility of tandem MS enables structural investigations and the identification of unknowns, a helpful tool when investigating profiles generated with non-model species. Unlike time-of-flight technology, that calls for a compromise between resolving power and sensitivity, the Orbitrap Exploris GC 240 system provides an unprecedented depth of analysis by offering both high selectivity and sensitivity at the same time.

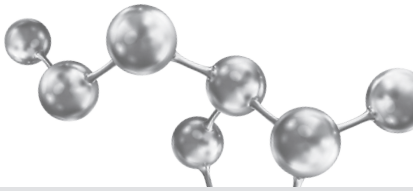
Additionally, the system itself is designed to facilitate easy and routine use by all members of the analytical team, irrespective of their level of expertise or training. Straightforward and uncomplicated workflows eliminate repeated user adjustments, thereby providing consistent and reproducible results, even across multiple users. If desired, laboratories can further minimize analyst variability by incorporating automated sample prep. Once raw data is obtained, the user-friendly software simplifies and speeds-up data processing and analyses.

Members of the Bowden laboratory generally use non-targeted approaches for initial pilots, exploratory studies or to generate new hypotheses. The high resolving power of the Orbitrap Exploris GC 240 is particularly well-suited for non-targeted studies. Its mass accuracy of <1 ppm boosts confidence in metabolite identification, even during exploration. Targeted methods, on the other hand, are applied towards established hypotheses or routine analyses that require higher specificity and reproducibility. More often than not, broad nontargeted studies in the Bowden laboratory feed into specific, targeted studies. "Targeted or not, either way, we can use mass spectrometry to interrogate both pollutants and biological molecules with a simple change in sample preparation, chromatography and scanning strategies," says Dr. Bowden.

Key factors for setting up a robust metabolomics platform

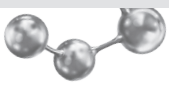
Optimizing a reproducible workflow

From sample prep to data analysis, each step of the workflow needs to be optimized for individual matrices or sample types to deliver accurate, reproducible results. "The optimization of the entire metabolomics workflow, irrespective of LC- or GC-based separation, needs to be accomplished to provide a level of confidence in the method and ease with the process," advises Dr. Bowden. Every stage of the method warrants meticulous optimization, starting from the initial experimental design and sample preparatory steps, such as extraction and derivatization, and extending to mass spectrometric scanning strategies, data analysis and interpretation.



"For laboratories like ours, where it's important to maximize the amount of information obtained from a single injection, acquiring systems that exceed the above characteristics is crucial."

– Dr. Bowden



Method-based studies or application notes outlining optimized workflows are helpful but need to be repeated from scratch in each laboratory and for every matrix type to ensure optimal translation. To better support the community, the Bowden laboratory is currently working towards building and disseminating a more practical approach to optimize metabolomics workflows. The team utilizes commercially available Standard Reference Materials (SRM), such as plasma, urine, stool and so on, to evaluate their in-house methods, and assess their robustness and reproducibility over a long period of continuous operation. The homogenous nature of the SRM overrides any variability arising from the starting sample, while the intricacies of the method itself get undivided focus for optimization. "Our ultimate goal would be to produce 'sample-method-data' products, where laboratories new to metabolomics can optimize their own methods by purchasing the same SRM we used, testing them with our methods that are available online and finally, validating their results with our published data," informs Dr. Bowden.

Standardizing analytical measurements

Even with an optimized method, external or inherent variables, such as the standards used or sample stability, can result in differential outputs with the same protocol. Inconsistencies stemming from these variables can inadvertently generate false reports in this emerging field and negatively impact the metabolomics community rather than advancing it. A community-wide standardization of analytical measurements is, therefore, essential to foster collaboration and data sharing in the long run. One way to achieve this is to incorporate modern instruments, such as the Orbitrap Exploris GC 240 system, that are fitted with 'intelligent run controls,' and other smart functionality tools to help minimize errors and achieve reproducible, high-quality data.

Having access to expansive databases

Working with non-model species and unique matrices requires having comprehensive databases to identify compounds. "We often encounter peaks that we're unable to identify due to the lack of available databases. This is especially common when moving away from mammalian species, and is further compounded by the lack of standards and variable derivatization methods," notes Dr. Bowden.

To eliminate database-related limitations from novel exploratory studies, the Orbitrap Exploris GC 240 uses an extensive breadth of libraries to generate candidate compounds. Researchers can tap into both commercially available nominal mass libraries, such as NIST or Wiley, as well as the Orbitrap GC-MS HRAM metabolomics library (Figure 1). In the case of cutting-edge studies, it's also possible to construct one's own library. A faster and more confident route to compound identification is supported by the Compound Discoverer software built into the Orbitrap Exploris GC 240. Its deconvolution and library searching capabilities extract meaningful information from large datasets.

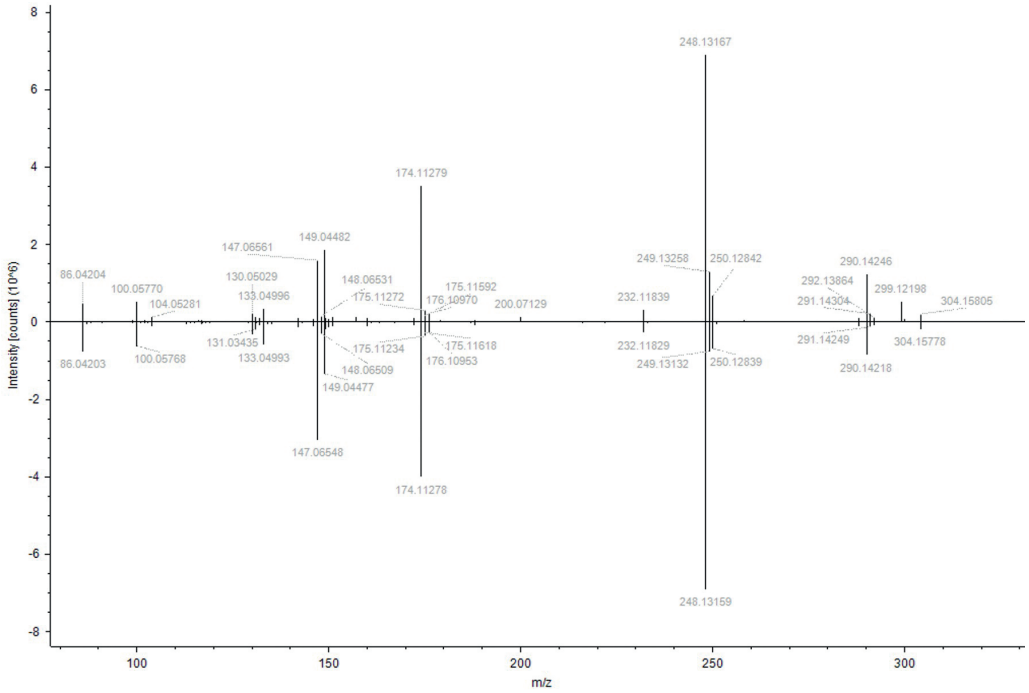


Figure 1. The deconvoluted spectrum of L-Alanine 3TMS mirrored with the library spectrum from the Thermo Scientific Orbitrap GC-MS HRAM metabolomics library. Search index score of 909 forward fit and 934 reverse search index.

> "We extensively communicated with the Thermo Fisher team before the instrument was delivered to make sure on-site requirements and specifications, such as electrical supply, gas inlet and ventilation, were met, they provided us with clear and precise instructions, which we were then able to pass on to our operations personnel."
>
> – Dr. Bowden

Reliable technical support

Incorporating new technology into current workflows isn't without technical and logistical challenges. Reliable technical support provided by manufacturers can preempt and alleviate some of the issues associated with installation and troubleshooting. Even before the Orbitrap Exploris GC 240 was installed, members of the Bowden laboratory worked in close collaboration with the Thermo Fisher technical support staff to ensure the new equipment seamlessly assimilated into the existing facility. "We extensively communicated with the Thermo Fisher team before the instrument was delivered to make sure on-site requirements and specifications, such as electrical supply, gas inlet and ventilation, were met," informs Dr. Bowden. "They provided us with clear and precise instructions, which we were then able to pass on to our operations personnel."

After delivery, the Bowden laboratory has continued collaborations with the Thermo Fisher staff to roll out the installation process and get the system running. Dr. Bowden continues: "The engineers had clear plans and defined goals for what they needed to accomplish on each day of the installation. They were good at thoroughly explaining every step to us and answering our questions. As a part of the training, they provided multiple resources, including manuals and videos, that clearly explained how to set up the instrument, perform calibrations, clean the filament and switch out the column, giving us the necessary foundation to start using the system."

Once installation was complete, seeking technical support to validate and troubleshoot workflows on the new system can eliminate hours of trial and error. "During the demo stage and throughout method development on our own system, we have worked closely with engineers, application scientists and other support staff at Thermo Fisher. The team has provided us with exceptional support, including recommendations for sample preparation, useful guidance for instrument maintenance, and tips for troubleshooting and working with software, such as Compound Discoverer," says Dr. Bowden. Collaborating with well-informed technical support staff before, during and after set-up undoubtedly shortens the learning curve for laboratory members and yields results faster.

Conclusion

Choosing the most appropriate technology and leveraging its capabilities to answer new research questions in metabolomics has catapulted the potential of the Bowden laboratory. The recent addition of the Orbitrap Exploris GC 240 to the laboratory's orthogonal assortment now offers technical flexibility to work on a range of metabolomics projects to answer both current and future research questions. This comprehensive metabolomics suite that is capable of tackling samples from mammalian and rare species alike, with both nontargeted and targeted approaches, all while consistently providing reliable data, has attracted fruitful collaborations and earned the laboratory additional grant funding to further advance the field of metabolomics.

> "We have worked closely with engineers, application scientists and other support staff at Thermo Fisher. The team has provided us with exceptional support, including recommendations for sample preparation, useful guidance for instrument maintenance, and tips for troubleshooting and working with software."
>
> – Dr. Bowden

thermo scientific

Dr. John A. Bowden

Dr. John A. Bowden is an Assistant Professor in the Department of Physiological Sciences in the College of Veterinary Medicine at the University of Florida (UF). He also has a joint appointment in the Department of Chemistry and has affiliations with the Center for Human and Environmental Toxicology and the Engineering School of Sustainable Infrastructure & Environment. He received his Ph.D. in Analytical Chemistry from UF under the guidance of Dr. Richard A. Yost in 2009. After a two-year postdoctoral fellowship at Saint Louis University, he joined the National Institute of Standards and Technology (NIST) as an NIST/NRC postdoctoral fellow in 2011, working at the Hollings Marine Laboratory in Charleston, SC, where he would later serve as a Research Chemist until 2018. In his current position, his research is focused on employing novel mass spectrometric methods at the chem/bio interface, with an expanding interest in the fields of metabolomics, lipidomics and exposomics.

Find out more at **thermofisher.com/OrbitrapExplorisGC240**

ThermoFisher SCIENTIFIC

APPLICATION NOTE 10723

Untargeted analysis with GC-Orbitrap: a powerful tool for the authentication of spices and herbs

Authors: Giulia Riccardino[1], Dominic Roberts[1], Cristian Cojocariu[1], and Michele Suman[2]; [1]Thermo Fisher Scientific, Runcorn, UK; [2]Barilla SpA – Advanced Research Laboratories, Parma, IT

Keywords: Oregano, spices, herbs, fraud, adulteration, volatile organic compounds (VOCs), Orbitrap, gas chromatography, GC, high resolution mass spectrometry, HRMS, full-scan, ExtractaBrite, electron ionization, EI, positive chemical ionization, PCI, unknowns

Introduction

Oregano is widely used as an ingredient in food and beverage products and as a flavoring ingredient for culinary purposes due to its organoleptic properties. Adulteration of oregano can be accidental or intentional with the latter driven by price and demand. Leaves from other plants (e.g., olive, thyme, marjoram, sumac, myrtle, and hazelnut) are frequently used as adulterants as they are difficult to detect by visual inspection.[1] As a consequence, food manufacturers must check regularly for the quality and purity of oregano outsourced from various suppliers to ensure the quality and consistency of the end product.

Oregano is a complex matrix containing essential oils, phytosterols, and pigments; its aroma derives from a complex mixture of volatiles, mainly monoterpenes and sesquiterpenes, which can be easily extracted and concentrated in one single step using the headspace solid-phase microextraction (HS-SPME) technique.

This allows for minimal sample preparation, a critical point in non-targeted analysis since every manipulation could alter the sample composition.[2] The fingerprint of the oregano aroma constituents can be investigated with a multiplatform approach using isotope ratio, liquid or gas chromatography coupled with mass spectrometry (LC or GC–MS), or high resolution mass spectrometry (LC or GC-HRMS), and in combination with software tools for data reprocessing and statistical analysis. The high-resolution GC-MS approach has become very popular as it offers the advantage of full-scan data acquisition combined with high sensitivity, high resolving power (up to 240,000 FWHM), and accurate mass (< 5 ppm). Moreover full-scan data acquisition allows for targeted, non-targeted, and retrospective data analysis.[2]

In this study GC-Orbitrap technology coupled with solid-phase micro-extraction (SPME) with Arrow technology was used to assess the volatile profile of intentionally adulterated and native oregano samples. Data were acquired in full-scan electron ionization (EI) mode and analyzed with Thermo Scientific™ Compound Discoverer™ software. Positive chemical ionization (PCI) was used to confirm the elemental composition of the molecular ions using accurate mass information, isotopic match (measured versus theoretical), and presence of specific adducts. Additional MS/MS data were acquired and used to explain the proposed chemical structure of the compounds identified via mass spectrum matching.

Experimental

In all experiments, a Thermo Scientific™ Orbitrap™ Exploris™ GC 240 system equipped with two Thermo Scientific™ Instant Connect Split/Splitless (SSL) Injectors—one used for SPME Arrow fiber conditioning and the second used for GC sample introduction, both equipped with SPME Arrow liner 1.7 mm i.d. (P/N 453A0415)—was coupled with a Thermo Scientific™ TriPlus RSH™ autosampler with SPME Arrow configuration. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-1MS capillary column, 30 m × 0.32 mm × 1.0 µm (P/N 26099-2970). Additional HS-SPME Arrow and Orbitrap Exploris GC parameters are detailed in Table 1. The triple coating phase of the DVB/CWR/PDMS fiber (P/N 36SA11T3) allowed for effective extraction of a wide range of volatiles including alcohols, aldehydes, ketones and esters.

Table 1 (part 1). TriPlus RSH-SPME Arrow and Orbitrap Exploris GC experimental parameters used for the assessment of the volatile fraction of oregano

TriPlus RSH – HS-SPME Arrow parameters	
Fiber	SPME Arrow DVB/CWR/PDMS (P/N 36SA11T3)
Coating phase thickness (µm)	110
Coating phase length (mm)	20
Incubation temperature (˚C)	60
Incubation time (min)	15
Agitation speed (rpm)	500
Extraction temperature (˚C)	60
Extraction time (min)	15
Stirring speed (rpm)	1,500
Fiber depth in vial (mm)	25
Fiber depth in injector (mm)	70
Desorption time (min)	2
Analysis time (min)	40
Inlet for fiber conditioning temperature (˚C)	270
Inlet module and mode	SSL, splitless
Fiber pre-conditioning time (min)	0
Fiber post-conditioning time (min)	15
Septum purge flow (mL/min)	5, constant
Purge carrier gas, flow (mL/min)	He, 6.0
Fiber depth in injector (mm)	70
Trace 1310 GC parameters	
Inlet temperature (˚C)	220
Liner	Thermo Scientific™ SPME Arrow liner 1.7 mm i.d. (P/N 453A0415)
Inlet module and injection mode	SSL, split
Split ratio	30:1
Septum purge flow (mL/min)	5, constant
Carrier gas, flow (mL/min)	He, 1.8
Oven temperature program	
Temperature (˚C)	40
Hold time (min)	2
Rate (˚C/min)	10
Temperature 2 (˚C)	150
Rate (˚C/min)	5
Temperature 3 (˚C)	260
Rate (˚C/min)	25
Temperature 4 (˚C)	300
Hold time (min)	3

Table 1 (part 2). TriPlus RSH-SPME Arrow and Orbitrap Exploris GC experimental parameters used for the assessment of the volatile fraction of oregano

Column	
Thermo Scientific™ TraceGOLD™ TG-1MS	30 m, 0.32 µm, 1.0 µm film (P/N 26099-2970)
Vials and caps	
Vials	Thermo Scientific™ 10 mL crimp top HS vials (P/N 10-CV)
Caps	Thermo Scientific™ 20 mm magnetic crimp caps (P/N 20-MCBC-ST3)

Exploris GC mass spectrometer parameters for EI	
Transfer line temperature (˚C)	280
Ionization type	EI
Ion source lemperature (˚C)	280
Electron energy (eV)	70
Aquisition mode	Full Scan
Mass range (Da)	40–450
Resolving power (FWHM)	60,000 @ *m/z* 200
Lockmass (*m/z*), column bleed	207.03235

Exploris GC mass spectrometer parameters for PCI	
Transfer line temperature (˚C)	280
Ionization type	CI
Ionization gas	Methane
Ionization gas flow (mL/min)	1.3
Ion source temperature (˚C)	190
Electron energy (eV)	90
Emission current (µA)	100
Aquisition mode	Full Scan
Mass range (Da)	80–450
Resolving power (FWHM)	60,000 @ *m/z* 200

Data acquisition, processing, and reporting

Data were acquired using Thermo Scientific™ Xcalibur™ software. This single platform integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. Compound Discoverer software, version 3.2, was used for spectral deconvolution, compound identification, and multivariate statistical analysis. Thermo Scientific™ Mass Frontier™ Spectral Interpretation software, version 8.0 was used to elucidate the chemical structure of putatively identified compounds via NIST mass spectra library matching.

Sample preparation

Three commercially available oregano samples were purchased from different retailers. Each oregano jar was well mixed to homogenize the matrix. Herb samples were weighed (150 mg) and transferred into 10 mL crimp top headspace vials for analysis. Samples were prepared in triplicate. In order to simulate an oregano fraud occurrence, thyme, marjoram, and olive leaves were purchased from local and online retailers and used to adulterate the oregano samples. One of the native oregano samples was randomly chosen and added with the three herbs to obtain a “fit for purpose” adulterated sample. Three bulks of fraudulent oregano samples were obtained by weighing 600 mg of native oregano and transferring into three glass containers. These containers were then adulterated by adding 10% of native marjoram, thyme, and ground olive leaves, respectively. Samples for analysis were prepared in triplicate as described above. A blend was obtained by pooling together the native and the adulterated oregano samples. In order to reduce the bias in the results, the sample vials were analyzed in a randomized order. A retention index mix (Sigma-Aldrich, C7-C30 saturated alkanes, P/N 49451-U) was injected at the beginning of the sequence and used to derive the RI of chemical components putatively identified by NIST17 library search following spectral deconvolution.

Results and discussion

Workflow to assess the volatile profile in oregano samples and to identify adulterated samples

Full-scan (FS) data for native and adulterated samples were analyzed with Compound Discoverer 3.2 software for chemometric assessment and putative identification of peaks. A complete workflow was used to isolate unique components, identify compounds with a high degree of confidence, and detect the variations suggesting a possible fraud. The workflow used is reported in Figure 1. FS data was acquired using EI mode at 60,000 FWHM resolution and then imported in Compound Discoverer 3.2 software. The software was used to deconvolute, align, and filter the peaks to putatively identify the compounds using mass spectral library match (NIST 17). Multivariate statistical analysis (principal component analysis (PCA), loading and volcano plots) was used to select the significant features, defined by their *m/z* and retention time, contributing to the group differences. Full-scan data were acquired using PCI mode at 60,000 FWHM resolution to confirm the molecular ion and propose a chemical formula. Additional MS/MS experiments were performed to confirm that the fragments were from the molecular ion. This streamlined workflow allowed for a comprehensive characterization of the aroma components in oregano samples.

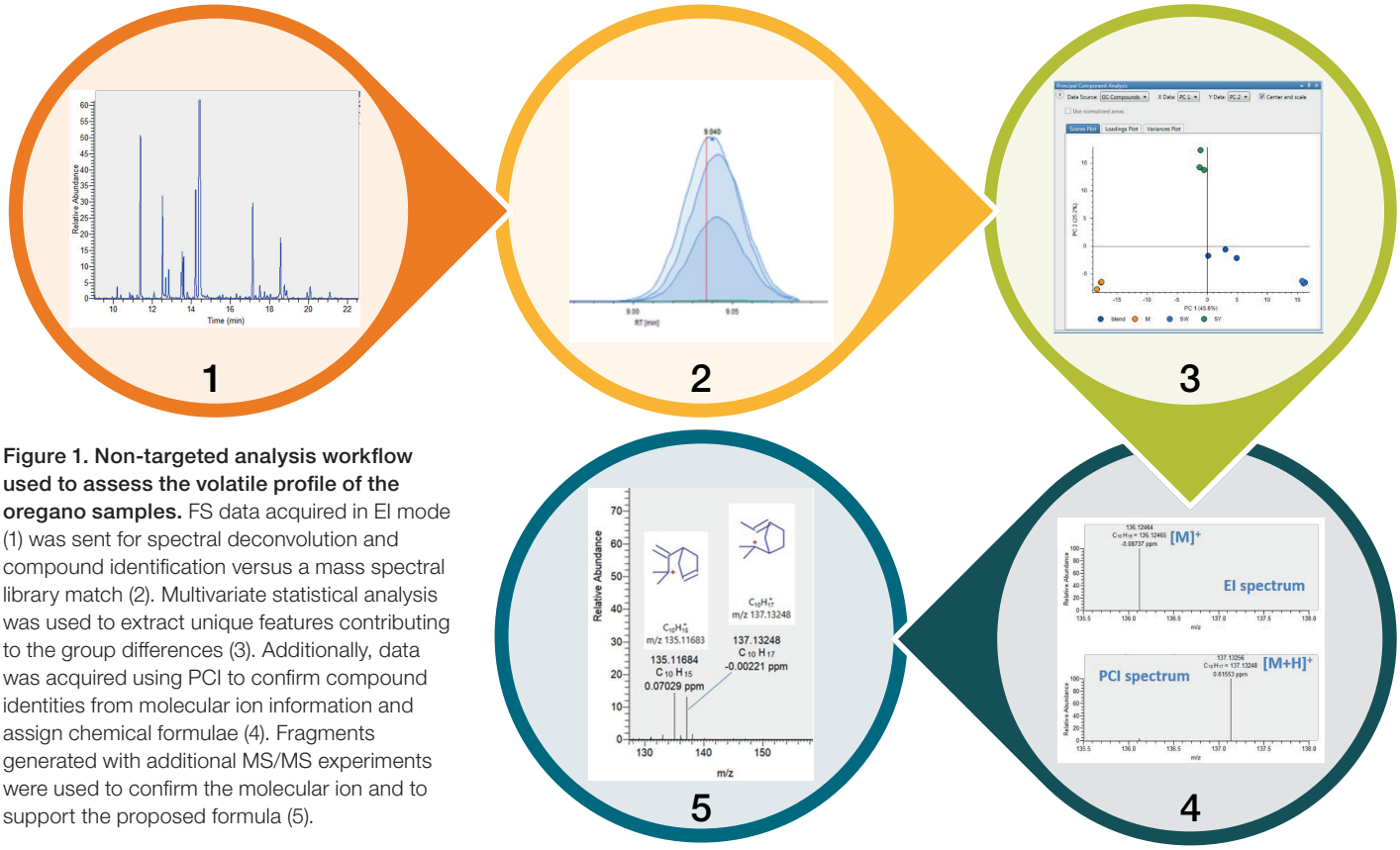


Figure 1. Non-targeted analysis workflow used to assess the volatile profile of the oregano samples. FS data acquired in EI mode (1) was sent for spectral deconvolution and compound identification versus a mass spectral library match (2). Multivariate statistical analysis was used to extract unique features contributing to the group differences (3). Additionally, data was acquired using PCI to confirm compound identities from molecular ion information and assign chemical formulae (4). Fragments generated with additional MS/MS experiments were used to confirm the molecular ion and to support the proposed formula (5).

Aroma profile in adulterated samples

Differences in chromatographic profiles of the native herbs (oregano, marjoram, thyme, and olive leaves) and the fraudulent samples were visible when comparing the full-scan total ion chromatograms (TICs). Differences related to the prevalence of the common aroma components, as well as typical components of both native oregano and native adulterants, could be found in the simulated fraudulent samples. As an example, the comparison between native oregano, native thymol, and adulterated sample (oregano/10% thymol) is reported in Figure 2. Some components, putatively identified based on the spectral library (NIST 2017) SI score as α-pinene (RT=8.79 min) and camphene (RT=9.04 min), originated from thyme exclusively. Thymol (RT=14.23 min) and carvacrol (RT=14.41 min) are present in both native thyme and oregano but with different abundances; thymol is predominant in thyme while carvacrol is the main constituent of oregano aroma. Although differences can be observed in the TIC comparison, all features were extracted from the data and analyzed statistically.

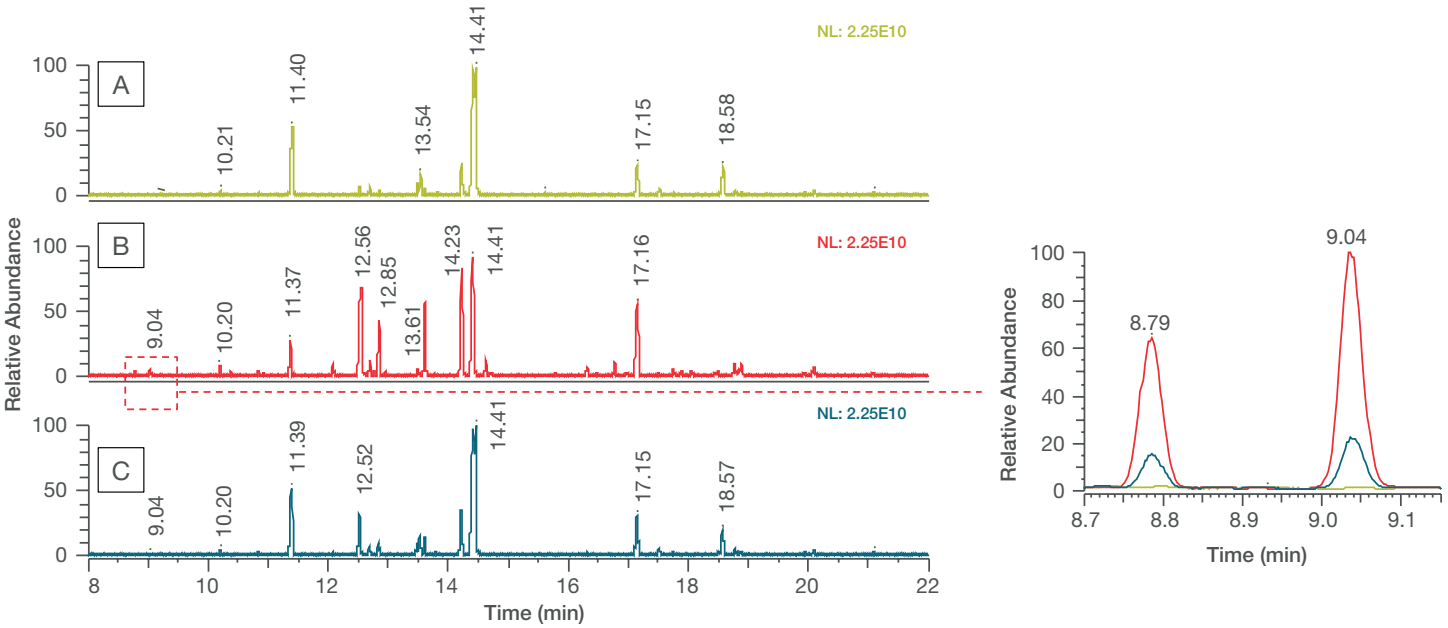


Figure 2. FS TIC obtained for native oregano (A), thyme (B), and adulterated oregano sample (C). Typical components of native thyme (e.g., α-pinene, RT=8.79 min and camphene, RT=9.04) as well as differences in the amounts of the common aroma components (e.g., thymol, RT=14.23 min and carvacrol, RT=14.41 min) or oregano unique components (e.g., aromadendrene, RT=18.58 min) could be found in the simulated fraudulent sample.

Compound identification in fraudulent samples

The Compound Discoverer platform includes a streamlined workflow for GC EI data allowing for extraction, deconvolution, and putative identification of the unknowns based on mass spectral library (NIST 2017).

The software first performed non-targeted peak detection within 5 ppm extraction windows. Accurate mass chromatographic deconvolution was then performed by grouping together all extracted ion peaks above a customizable signal to noise (S/N) threshold that maximize at the same retention time. The deconvoluted spectra were then searched against NIST 2017 nominal mass spectral library, and the hits were scored based on the total score derived from a combination of library search index (RSI), high-resolution filtering (HRF) value, and presence/absence of the molecular ions as well as elemental percentage. The use of a retention index acquired under the same conditions used for sample analysis helped to increase the confidence in compound identification. An example of this workflow is reported in Figure 3 with the Compound Discoverer browser showing the overlaid XIC (extracted ion chromatogram) of the peak eluting at 9.04 min (*m/z* 93.06982), the result table with the top hit, and the EI spectrum – deconvoluted versus NIST library. The peak was putatively identified as camphene with a total score=94.9, RSI=801, and HRF=97.53. This approach allowed putative identification of most of the detected peaks; however, for some compounds the EI spectral library match was inconclusive. In this case PCI data and accurate mass become essential to discriminate the chemical formula and provide confidence in identification.

Multivariate statistical analysis: PCA and differential analysis

Multivariate statistical analysis was carried out using Compound Discoverer 3.2 software. PCA is a well-known statistical approach that highlights variation between sample groups and allows visualization of strong patterns in complex datasets. An example of a PCA plot is reported in Figure 4. The generated PCA plot shows clear separation between the native oregano sample and the adulterated ones.

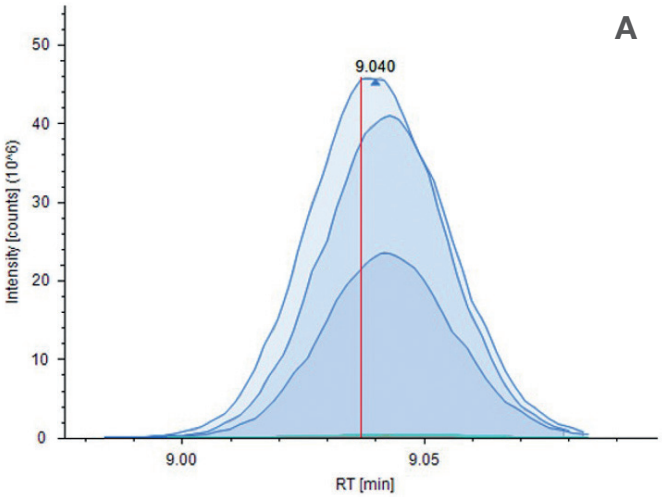
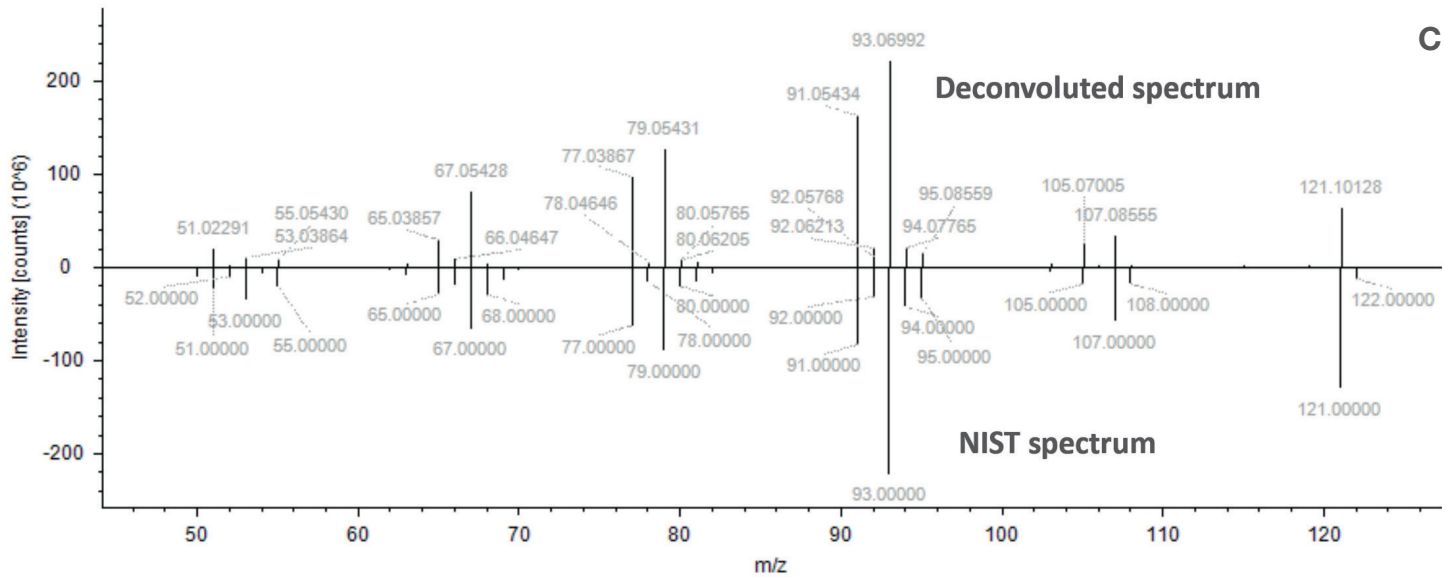


Figure 3. Compound Discoverer results browser showing an example of compound identification for the peak eluting at RT=9.04 min, putatively (RSI 801) identified as camphene. Overlaid XIC (base peak *m/z* 93.06982) for camphene (A); results table with the matched compound identified based on library search and retention index (B); EI spectrum of camphene – measured vs. NIST library (C).

B

Matched Compound	Formula	Score	HRF Score	SI	RSI	RI Delta	Elements Found[%]	Theo Mol Mass	Observed Mol. Mass	RI Column type	RI Diff[%]
Camphene	C10H16	95.0	97.7356	794	801	4	100.0	136.12465	136.12457	StandardNonPolar	0.4



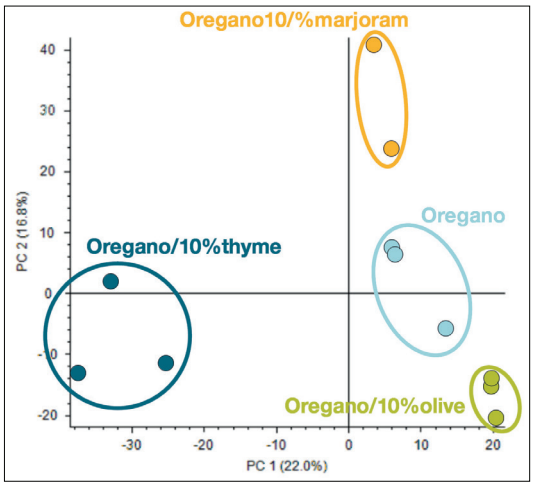


Figure 4. PCA score plot of the volatile compounds that differentiate the native oregano from the adulterated samples. A complete separation between the sample groups was observed.

Differential analysis was carried out using the volcano plots (V-plot), useful to quickly identify changes in large data sets composed of replicate data. The V-plot obtained comparing oregano adulterated with thyme and native oregano is reported in Figure 5. The main compounds responsible for differences in the analyzed groups are highlighted with light blue dots. This approach allowed the identification of suspected adulteration of native oregano, by both highlighting the changes in the amount (increase or decrease) of some compounds and the presence of compounds that are not usually found in native oregano. For oregano adulterated with 10% thyme, for example, it was possible to identify two main components such as camphene and α-pinene with a content increase by a 7-fold factor and a 3-fold factor, respectively. Allyl furoate increased by 3.5-fold in oregano adulterated with olive leaves, while methyl cinnamate had a log2-fold change of 2.5 in oregano adulterated with marjoram as reported in Table 2.

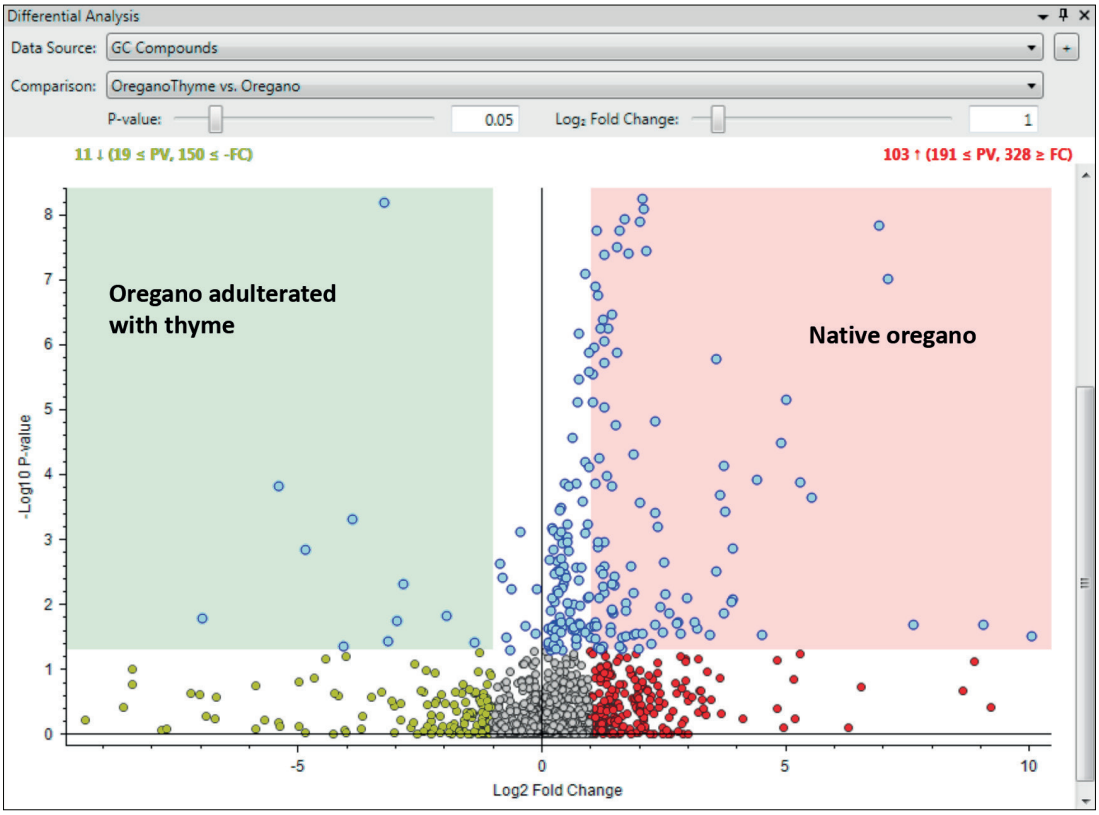


Figure 5. V-plot scatterplot showing the statistical significance (P value) versus magnitude of change (fold change) when comparing the oregano adulterated samples (with thyme) versus the native oregano. The main chemical components that are responsible for sample diversity between two sample groups are selected (light blue dots).

Table 2. Table of fold change of main compounds that could suggest an adulteration of the native oregano. In particular, camphene and α-pinene content increased by a 7-fold and 3-fold factor, respectively, when oregano was adulterated with thyme; allyl furoate increased by 3.5-fold in oregano adulterated with olive leaves; and methyl cinnamate had a log2-fold change of 2.5 in oregano adulterated with marjoram.

Putative ID	RT [min]	Reference *m/z*	Total score	Log2 fold change
Oregano adulterated with 10% thyme				
α-Thujene	8.63	77.03867	92.9	1.9
α-Pinene	8.79	91.05434	95.9	3.6
Camphene	9.04	93.06992	95.1	6.9
trans-ß-Ocimene	10.60	91.05424	93.6	-0.3
Camphor	12.08	95.08559	95.1	1.1
Pinocamphone	12.34	95.08554	94.0	1.4
cis-Ocimenol	12.85	93.06992	95.3	1.6
cis-Dihydrocarvone	12.95	67.05428	94.4	1.6
Duroquinone	13.54	117.06986	92.3	1.2
Carvacrol	14.41	135.08051	96.2	-0.1
Germacrene	20.31	133.10118	91.1	-0.3
Levomenthol	24.97	109.10131	92.8	1.5
Oregano adulterated with 10% olive leaves				
Furfuryl disulfide	9.41	81.03341	97.1	1.2
Allyl furoate	10.69	95.04906	92.8	3.5
Thymol	14.22	135.08049	96.0	-0.3
α-Ocimene	11.40	93.06971	94.1	-0.2
Sabinene	10.84	91.05434	95.3	-0.4
4-Thujanol	10.92	93.06988	95.6	-0.2
Oregano adulterated with 10% marjorm				
α-Thujene	8.63	77.03867	92.9	1.4
Sabinene	9.42	91.05424	92.6	1.3
Thujone	11.50	110.1089	92.7	-0.2
Carvacrol	14.41	135.08051	96.2	-0.1
4-Terpinenyl acetate	14.64	94.07311	94.3	1.6
Methyl cinnamate	15.75	161.0597	93.7	2.5

Compound confirmation using PCI

Further confirmation in the identification of compounds was achieved by assessing the PCI spectra to identify the elemental composition of the parent ion by looking at common adducts. In PCI experiments using methane as the reagent gas three adducts are typically observed: $[M+H]^+$, $[M+C_2H_5]^+$, $[M+C_3H_5]^+$. As an example, EI and PCI spectra of camphene are reported in Figure 6. The observed molecular ion corresponding to *m/z* 136.12464 is present in the EI spectrum with a mass difference of -0.09 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* 136.12464 as the molecular ion for camphene (RT=9.04 min) and supported the elemental composition of the proposed molecule.

Molecular ion fragment annotation using PCI MS/MS data

Additional PCI MS/MS experiments were assessed to support the proposed formula and to derive structural information. An elemental composition calculator included in the Thermo Scientific™ FreeStyle™ application was used to propose a formula for $[M+H]^+$ ion as shown in Figure 7. The ion *m/z* 137.13252 was isolated in the quadrupole and fragmented in the HCD cell using 10 V energy to generate fragments as shown in Figure 8. Mass Frontier software was used for assignment of fragment structures to measured ions in the MS/MS spectrum. The measured fragments provided detailed information with respect to the proposed chemical formula with <1 ppm mass accuracy.

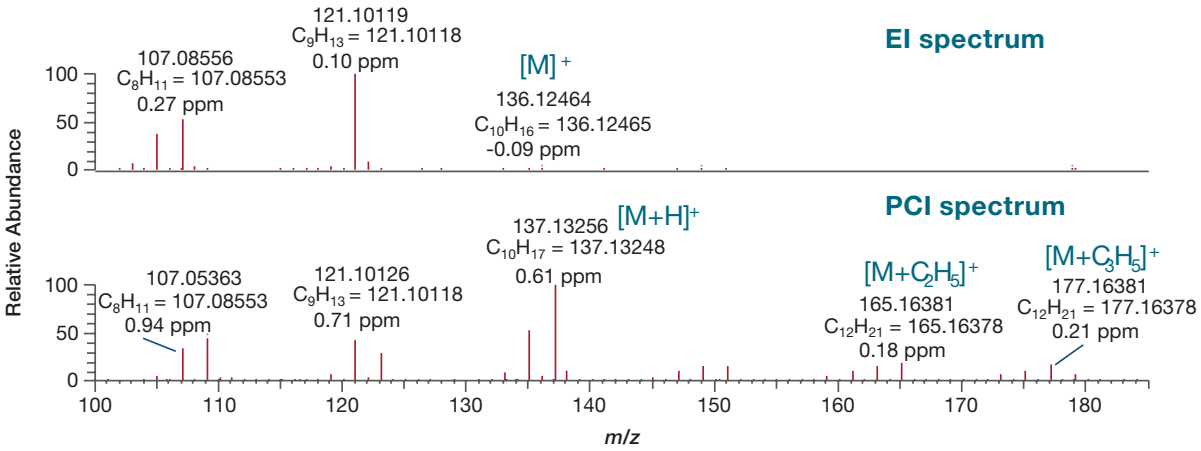


Figure 6. Comparison between EI and PCI spectrum for camphene (RT=9.04 min). The molecular ion (*m/z* 136.12464) is visible in the EI spectrum with <1 ppm mass accuracy annotated. In the PCI spectrum the typical adducts observed when methane gas is used are clearly visible confirming the molecular ion and the proposed molecular formula for camphene.

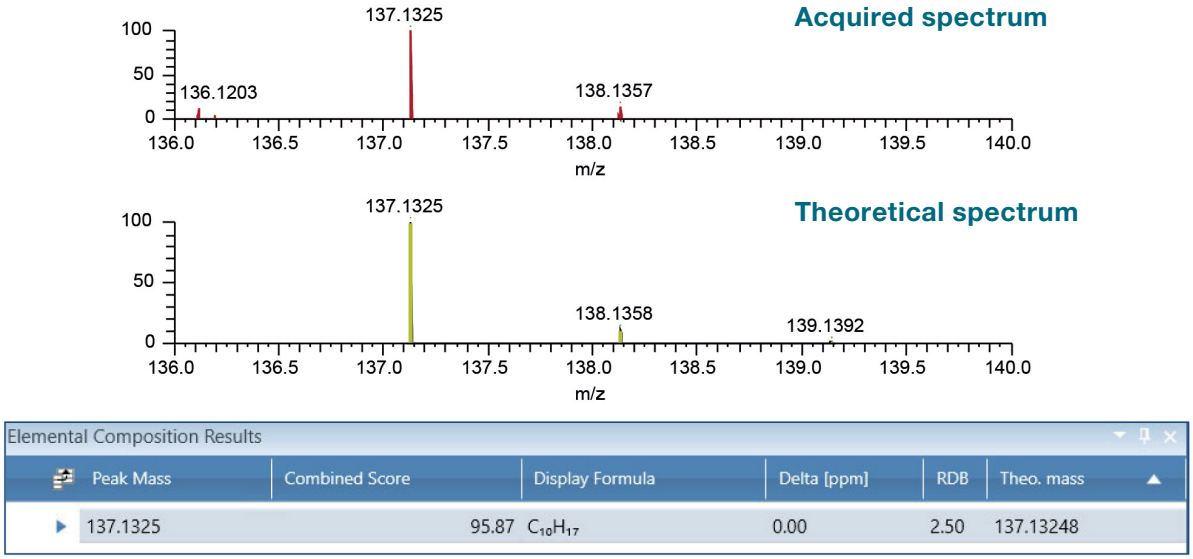


Figure 7. Elemental composition calculator in the FreeStyle application proposing the chemical formula $C_{10}H_{17}$ for the $[M+H]^+$ ion based on accurate mass and isotope pattern. One candidate is proposed with 0.00 ppm mass error and 96% isotopic match with the theoretical pattern.

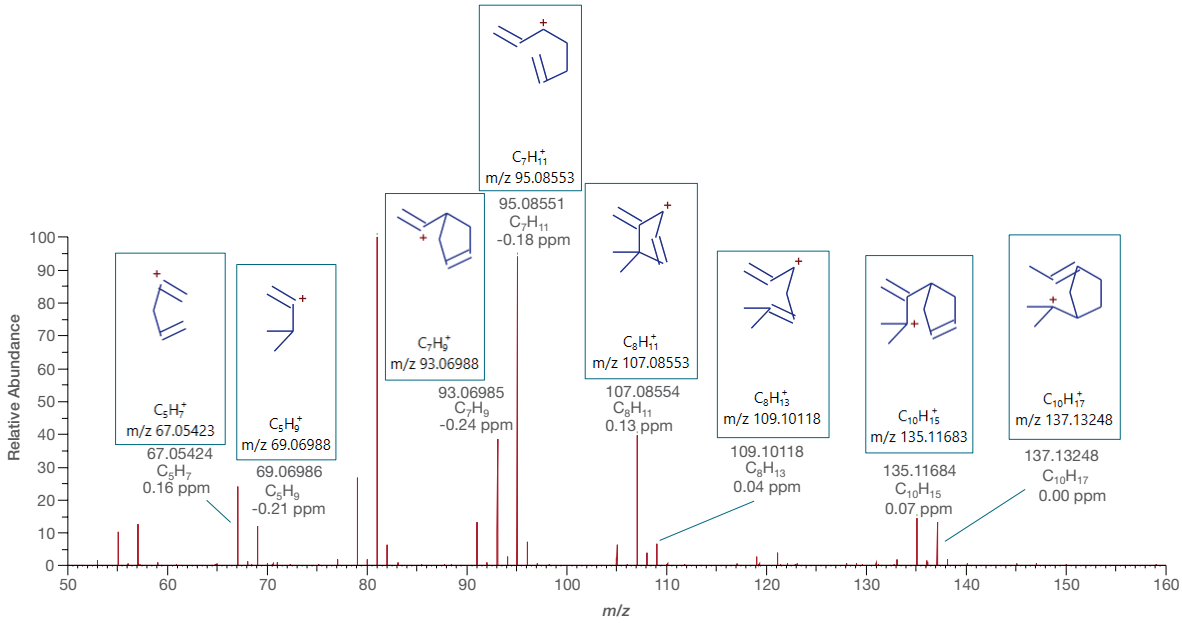


Figure 8. PCI MS/MS following *m/z* 137.13252 fragmentation in the HCD collision cell showing the product ions annotated with measured mass, elemental composition, theoretical mass, mass accuracy (ppm), and the proposed chemical structures.

thermo scientific

Conclusions

The results obtained in this study demonstrate that the Orbitrap Exploris GC system represents a powerful tool for assessing food authenticity, providing an integrated omics approach for profiling complex samples and identifying unknown compounds intentionally added with fraudulent purposes.

- Automated headspace sampling with the SPME Arrow removes the need for sample preparation and speeds up the analysis.
- The high resolving power (60,000 at *m/z* 200) and consistent sub-1 ppm mass accuracy and the wide dynamic range allow for fast and confident characterization of a large number of compounds regardless of their concentration or matrix complexity.
- The streamlined GC-EI data processing workflow in Compound Discoverer software allows for extraction, deconvolution, identification of unknown compounds, and multivariate statistical analysis.
- Principal components and differential analysis allow for the detection of differences in the composition of the aroma profile, which can identify unique compounds present in adulterated oregano samples.
- Rapid change-over from EI (for spectral library search) to softer ionization such as PCI (for molecular ion confirmation using adduct information) and the ability to perform accurate mass MS/MS experiments (for structural elucidation), combined with the use of the FreeStyle elemental composition calculator and Mass Frontier software for predictive fragmentation and structural elucidation, enable confident compound identification with unprecedented ease.

References

1. Dubrabova, L.; Alvarez-Rivera, G.; Suchanova, M.; Schusterova, D.; Pulkrabova, J.; Tomaniova, M.; Kocourek, V.; Chevallier, O.; Elliot, C.; Hajslova, J. Food fraud in oregano: pesticide residues as adulteration markers, *Food Chemistry,* **2019**, *276,* 726-734.
2. Cavanna, D.; Righetti, L.; Elliot, C.; Suman, M. The scientific challenges in moving from targeted to non-targeted mass spectrometric methods for food fraud analysis: A proposed validation workflow to bring about a harmonized approach, *Trends in Food Science & Technology,* **2018**, *80,* 223-241.
3. Díaz-Maroto, M.C.; Pérez-Coello, M.S.; Cabezudo, M.D. Headspace solid-phase microextraction analysis of volatile components of species, *Chromatographia,* **2002**, *55,* 729-735.

Find out more at **thermofisher.com/HRAMGCMS**

ThermoFisher SCIENTIFIC

APPLICATION NOTE 65914

Intelligent omics workflow using an Orbitrap Exploris GC 240 mass spectrometer for food characterization

Authors: Giulia Riccardino, Dominic Roberts, and Cristian Cojocariu

Thermo Fisher Scientific, Runcorn, UK

Keywords: *Origanum vulgare,* characterization, volatile organic compounds (VOCs), Orbitrap, Orbitrap Exploris GC 240, gas chromatography, GC, high resolution accurate mass mass spectrometry, HRMS, full scan, electron ionization, EI, positive chemical ionization, PCI

Goal

The aim of this application note is to demonstrate the performance of the Thermo Scientific™ Orbitrap Exploris™ GC 240 coupled to SPME Arrow technology for the assessment of the aroma profile in *Origanum vulgare* samples grown in different geographical areas.

Introduction

Origanum vulgare is widely used as an ingredient and flavoring for culinary purposes because of its organoleptic properties and enjoyable taste.[1]

Oregano is a complex matrix with an extremely variable composition of phytosterols, pigments, and essential oils. The differences in the amounts of its major constituents can be used to discriminate between individual plants. For example, the monoterpene content is strongly affected by the climatic conditions playing an important role in up/downregulating the cymyl-, sabinyl-, and linalool/linalyl acetate pathways.[2] Plants originating from the Mediterranean climate usually exhibit active cymyl- and/or linalool pathways, with the first being characterized by a higher content of carvacrol, thymol, and their biosynthetic precursors (*γ*-terpinene and *p*-cymene) and the second one characterized by higher concentration of linalool and linalyl acetate. Plants originating from continental climate are usually poorer in monoterpenes, showing a higher content of sesquiterpenes (mainly sabinene and *trans/cis*–sabinene hydrate and their acetates) generated by the sabinyl-pathway.[2]

Flavor analysis presents some challenges as the number of compounds that must be extracted from the matrix and identified is significant. Moreover, these chemicals usually have different chemical properties (structure, reactivity, polarity, boiling point). Aroma compounds can be present at very low concentrations; therefore, their extraction, identification, and quantitation become critical to obtain reliable results. Oregano aroma compounds can be extracted using different techniques such as distillation, Soxhlet extraction, static-headspace sampling (SHS), and purge and trap (P&T), although they have some limitations. Monoterpenes can undergo chemical changes under the conditions applied for distillation, while volatile compounds can be lost with solvent extraction. Static headspace and purge and trap can result in low sensitivity and risk of cross-contamination. Headspace solid phase micro-extraction (HS-SPME) has become common in aroma analysis as it is rapid, simple, and allows for the extraction of volatile and semi-volatile compounds with minimal sample preparation, which is a critical point in non-targeted analysis since every manipulation could alter the sample composition.[3]

Flavor analysis can be carried out using either liquid or gas chromatography coupled to mass spectrometry (LC or GC–MS) or high resolution accurate mass mass spectrometry (LC or GC-HRMS) and the use of effective software tools for data reprocessing and statistical analysis. The high resolution GC approach with the Orbitrap system offers the advantage of full-scan data acquisition combined with high sensitivity, high resolving power (up to 240,000 FWHM at *m/z* 200), and accurate mass (<1 ppm), allowing for targeted, non-targeted, and retrospective data analysis.[3]

In this study, the Orbitrap Exploris GC 240 system coupled to SPME Arrow technology was used to assess the aroma profile of several *Origanum vulgare* samples grown in different geographical areas with either Mediterranean or continental climate. Data was acquired in full-scan (FS) electron ionization (EI) and positive chemical ionization (PCI) modes, and reprocessed using the streamlined workflows integrated in the Thermo Scientific™ Compound Discoverer™ 3.2 software platform.

Experimental

In all experiments, an Orbitrap Exploris GC 240 system with a Thermo Scientific™ Instant Connect split/splitless SSL (equipped with SPME Arrow liner 1.7 mm ID (P/N 453A0415)) was coupled to a Thermo Scientific™ TriPlus™ RSH autosampler with SPME Arrow configuration. In place of the standard SPME Arrow Conditioning Station, a second IC-SSL injector (equipped with SPME Arrow liner 1.7 mm ID (P/N 453A0415)) was used for fiber conditioning. Chromatographic separation was achieved using a Thermo Scientific™ TraceGOLD™ TG-1MS capillary column, 30 m × 0.32 mm × 1.0 µm (P/N 26099-2970). Additional HS-SPME Arrow and Orbitrap Exploris GC 240 system parameters are detailed in Tables 1a and 1b, respectively. The triple coating phase of the DVB/CWR/PDMS fiber (P/N 36SA11T3) allowed for effective extraction of monoterpenes, cyclic and acyclic terpenes, sesquisterpenes, and bornane compounds.

Table 1a. TriPlus RSH-SPME Arrow experimental parameters used for the assessment of the volatile fraction of oregano

TriPlus RSH – HS - SPME Arrow parameters	
Fiber	SPME Arrow DVB/CWR/PDMS (P/N 36SA11T3)
Coating phase thickness (µm)	110
Coating phase length (mm)	20
Incubation temperature (°C)	60
Incubation time (min)	15
Incubation speed (rpm)	500
Extraction temperature (°C)	60
Extraction time (min)	15
Stirring speed (rpm)	1500
Fiber depth in vial (mm)	25
Fiber depth in injector (mm)	70
Desorption time (min)	2
Analysis time (min)	40
Fiber conditioning	
Inlet temperature (°C)	270
Liner	SPME Arrow liner 1.7 mm I.D. (P/N 453A0415)
Inlet module and mode	SSL, splitless
Fiber pre-conditioning time (min)	0
Fiber post-conditioning time (min)	15
Septum purge flow (mL/min)	5, constant
Carrier gas (mL/min)	He, 6.0
Fiber depth in injector (mm)	70

Table 1b. Trace 1310 GC and Orbitrap Exploris GC 240 mass spectrometer experimental parameters used for the assessment of the volatile fraction of oregano

Trace 1310 GC parameters	
Inlet (°C)	220
Liner	Arrow liner 1.7 mm I.D. (P/N 453A0415)
Inlet module and mode	SSL, split
Split ratio	30:1
Septum purge flow (mL/min), mode	5, constant
Carrier gas (mL/min)	He, 1.8
Oven temperature program	
Temperature (°C)	40
Hold time (min)	2
Rate (°C/min)	10
Temperature 2 (°C)	150
Rate (°C/min)	5
Temperature 3 (°C)	260
Rate (°C/min)	25
Temperature 4 (°C)	300
Hold time (min)	3
Column	
TraceGOLD TG-1MS	30 m, 0.32 µm, 1.0 mm (P/N 26099-2970)
Vials and caps	
Vials	10 mL crimp top HS vials (P/N 10-CV)
Caps	20 mm magnetic crimp caps (P/N 20-MCBC-ST3)
Orbitrap Exploris GC 240 mass spectrometer parameters	
Parameters for EI	
Transfer line temperature (°C):	280
Ionization type:	EI
Ion source temperature (°C):	280
Electron energy (eV):	70
Aquisition mode:	Full Scan
Mass range (Da):	40–450
Resolving power (FWHM):	60,000 @ *m/z* 200
Lockmass, column bleed:	207.03235
Parameters for PCI	
Transfer line temperature (°C):	280
Ionization type:	CI
Ionization gas:	Methane
Ionization gas flow (mL/min):	1.3
Ion source temperature (°C):	190
Electron energy (eV):	90
Aquisition mode:	Full Scan
Mass range (Da):	80–450
Max resolving power (FWHM):	120,000 @ *m/z* 200

Data acquisition, processing, and reporting

Data were acquired using Thermo Scientific™ Chromeleon™ 7.3 CDS software and imported in Compound Discoverer 3.2 software for chemometric assessment. Chromeleon CDS integrates instrument control, method development functionality, and quantitation-focused workflows in compliance with Title 21 of the Code of Federal Regulations. Compound Discoverer 3.2 software was used to reprocess EI data (spectral deconvolution, compound identification, and multivariate statistical analysis) as well as CI data (elemental composition of the molecular ions and presence of specific adducts confirmation).

Sample preparation

Three commercially available *O. vulgare* samples were purchased at different retailers. Each oregano jar was well mixed to homogenize the matrix. Herb samples were prepared in triplicate by weighing (150 mg) and transferring into 10 mL crimp top headspace vials (vials P/N 10-CV, caps P/N 20-MCBC-ST3) for analysis. A blend was obtained by pooling together the oregano samples and was used for confirmatory purposes. To reduce the bias in the results, the sample vials were analyzed in a randomized order. A retention index (RI) mix (Sigma-Aldrich, C7-C30 saturated alkanes, P/N 49451-U) was injected at the beginning of the sequence and used to derive the RI of chemical components putatively identified by the NIST™ Mass Spectral Library (NIST20) and the Thermo Scientific™ Orbitrap™ GC-MS HRAM Metabolomics Library (P/N 1R120400-0080) following spectral deconvolution.

Results and discussion

Workflows to assess the volatile profile in oregano samples

Full scan EI and CI data were processed using Compound Discoverer 3.2 software for chemometric assessment and putative identification of peaks as reported in Figure 1. Multivariate statistical analysis (principal component analysis, PCA and volcano plot, v-plot) was used to select the significant features, defined by their *m/z* and retention time, contributing to the group differences. Chromatographic peaks were then deconvoluted, aligned, filtered, and putatively identified using mass spectral library match (NIST20 nominal mass library and Orbitrap GC-MS HRAM Metabolomics Library). The PCI workflow in Compound Discoverer software enabled confirmation of the presence of the molecular ion and the adducts and proposal of a chemical formula. These streamlined workflows allowed for a comprehensive characterization of the aroma components in oregano samples.

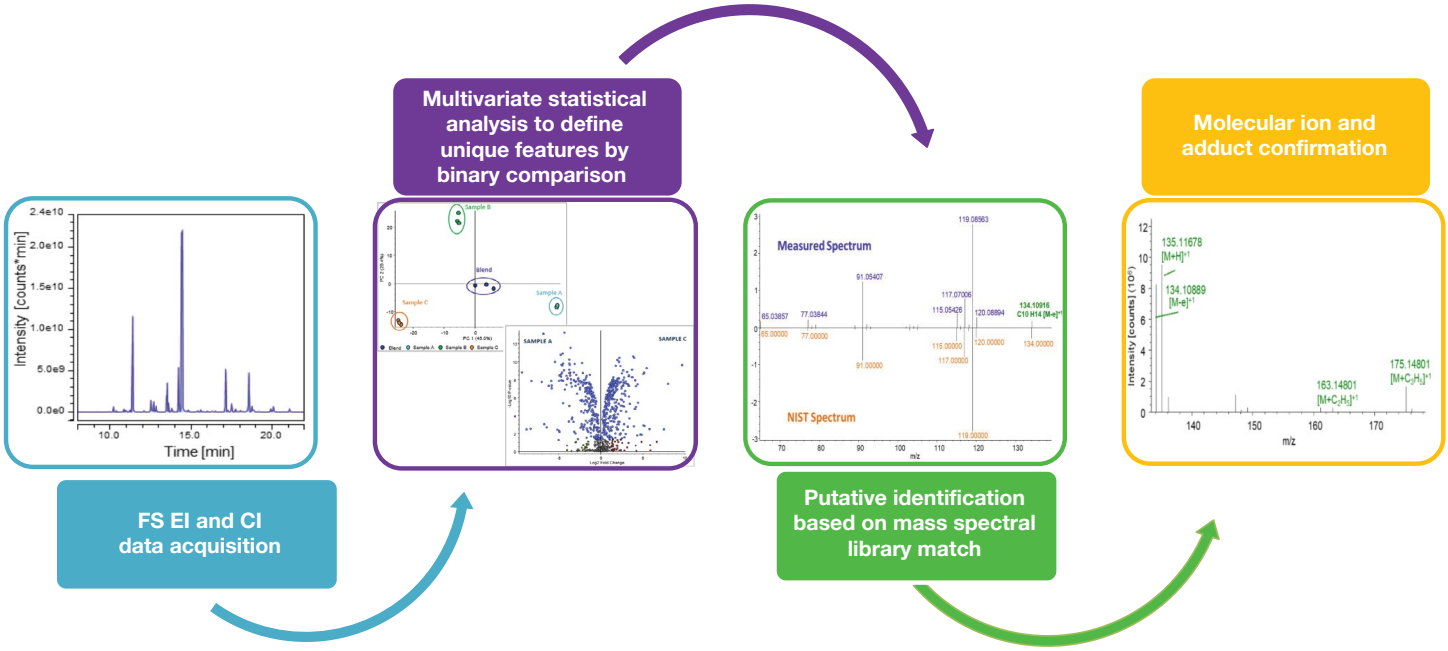


Figure 1. Workflow used to assess the volatile profile of the oregano samples. FS data was acquired in EI and PCI modes: multivariate statistical analysis was performed to identify unique features contributing to the group differences in EI data; peak putative identification was made using mass spectral library match (NIST20 and Orbitrap GC-MS HRAM Metabolomics Library); compound identification was confirmed using soft ionization PCI data and the presence of quasimolecular and/or adduct ions.

Multivariate statistical analysis: PCA and V-Plot

Full scan EI data were imported in Compound Discoverer software and a multivariate statistical analysis step was carried out to assess the sample differences and to isolate the main features responsible for such variances. PCA is a well-known statistical approach that highlights variation between sample groups and allows visualization of strong patterns in complex datasets. By employing PCA analysis in Compound Discoverer software, significant differences were observed between the volatile profiles of the analyzed oregano samples. The generated PCA plot is reported in Figure 2, highlighting a clear separation between the oregano samples with the blend (pooled samples) centered in-between the groups. To isolate the chemical components responsible for these variances, differential analyses were carried out using the volcano-plots, useful to quickly identify changes in large data sets composed of replicate data. A V-plot obtained by comparing sample A and sample C is shown as an example in Figure 3.

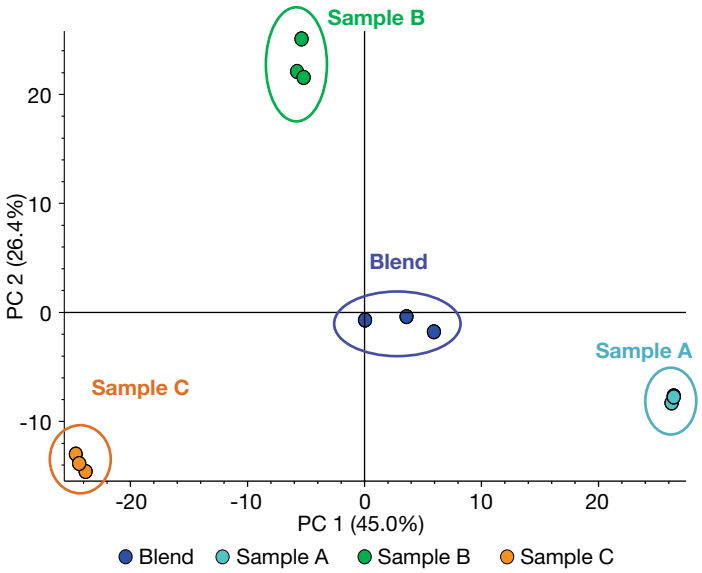


Figure 2. Centered PCA score plot obtained for oregano samples. The PCA plot shows a complete separation between the sample classes with the blend (pooled) samples in the clustered in the center of the plot.

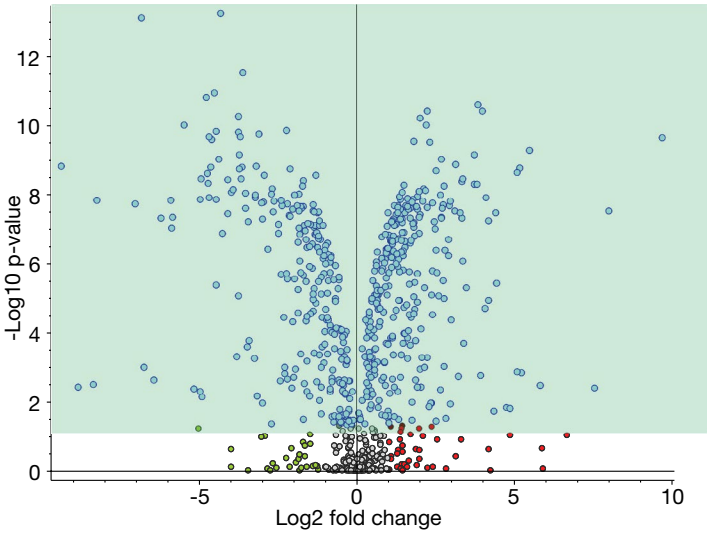


Figure 3. Volcano-plot scatterplot showing the statistical significance (p-value) versus magnitude of change (fold change) when comparing sample A and C. Significant chemical components that are responsible for sample diversity between two groups are selected (as light blue dots).

Compound identification based on NIST20 and Orbitrap GC-MS HRAM Metabolomics Library match

Compound Discoverer 3.2 platform includes a streamlined workflow for GC EI data, which allows for extraction, deconvolution, and putative identification of the unknowns based on mass spectral library matching. The software first performed untargeted peak detection within 5 ppm extraction windows. Accurate mass chromatographic deconvolution was then performed by grouping together all extracted ion peaks above a customizable signal to noise (S/N) threshold that maximize at the same retention time. The deconvoluted spectra were then searched against the NIST20 nominal mass spectral library and Orbitrap GC-MS HRAM Metabolomics Library and the hits were scored based on the total score derived from a combination of library search index (SI), high resolution filtering (HRF) value and presence/absence of the molecular ions as well as elemental percentage match. The use of a retention index acquired under the same conditions used for sample analysis helped to boost the confidence in compound identification. An example of this workflow is reported in Figure 4 with the Compound Discoverer 3.2 browser showing the overlaid extracted ion chromatograms (XIC) of the peak eluting at 10.21 min (*m/z* 119.08563), the result table with the top hit for peak deconvolution and library search (NIST20 and Orbitrap GC-MS HRAM Metabolomics Library), and the EI spectrum – measured versus the NIST20 library. According to the NIST20 library, the peak was putatively identified as *p*-cymene with a total score of 95.2, SI = 796, and HRF = 98.1.

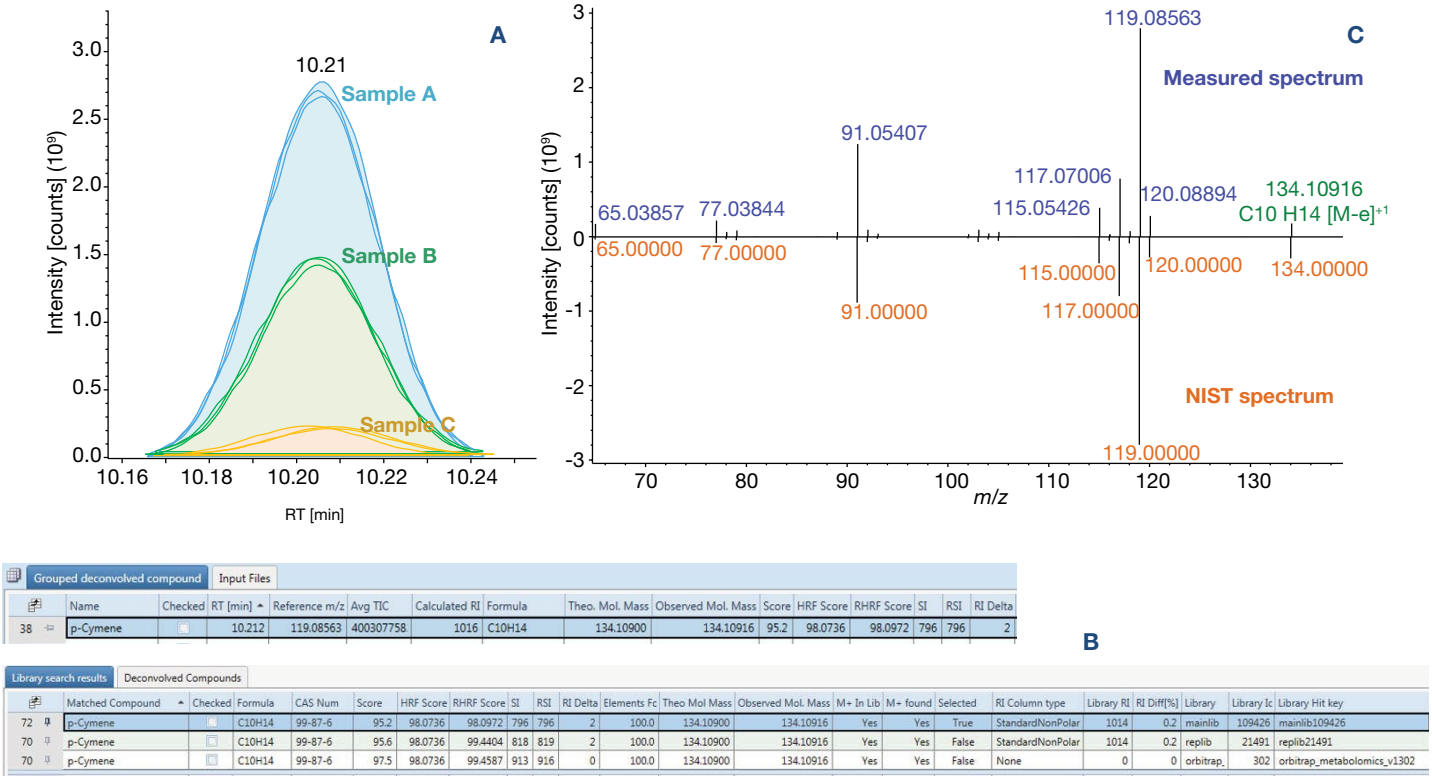


Figure 4. Compound Discoverer software showing peak deconvolution results for the compound eluting at RT = 10.21 min and putatively identified as *p*-cymene (*m/z* 119.08563). XIC for *p*-cymene (A); result table with deconvoluted compound and library search results for NIST20 and Orbitrap GC-MS HRAM Metabolomics Library (B); EI spectrum of *p*-cymene – measured versus NIST20 library (C).

The putative identification was supported by the Orbitrap GC-MS HRAM Metabolomics Library match with a total score of 97.5, SI = 913, and HRF = 99.5. This approach allowed putative identification of most of the detected peaks; however, for some compounds the EI spectral library match resulted inconclusive. In this case, PCI data and accurate mass become essential to discriminate the chemical formula and provide confidence in identification.

Volatile composition of *Origanum vulgare* samples of various geographical origin

The differences in the composition of the aroma profile allowed for the discrimination between samples even with a limited data set as reported in Figure 2. Sample A showed a higher content of cymyl-type compounds such as *p*-cymene (4-fold change), *γ*-terpinene (2-fold change), and thymol (3-fold change). Sample B resulted to be poor in cymyl-type compounds but richer in acyclic compounds such as β-ocimene (4-fold change) with a higher amount of sesquiterpenes such as germacrene D (2-fold change). Sample C resulted to be richer in β-ocimene and sequiterpenes such as germacrene D and β-caryophyllene. The differences in the chemotypes can be representative of the different climate where *O. vulgare* varieties were grown with the predominance of phenolic monoterpenes in plants grown in the Mediterranean area and sesquiterpenes predominant in continental regions, although as reported in literature, oregano characterization is difficult due to the huge diversity in the aroma composition of the existing oregano populations.[2]

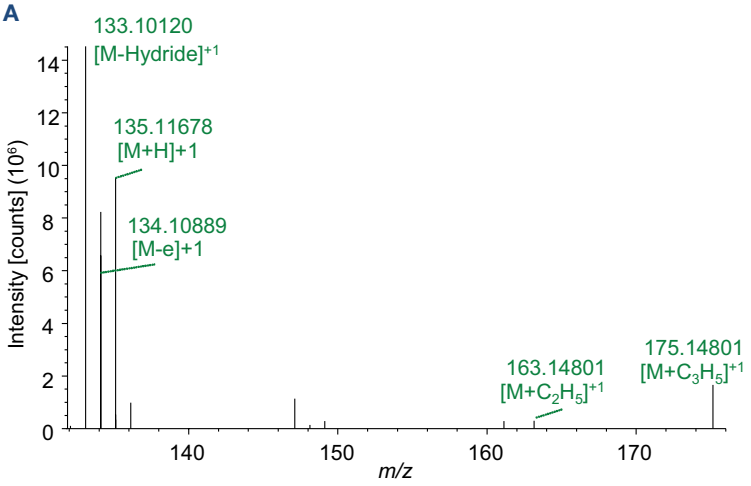
Molecular ion and adduct confirmation using PCI

Further confirmation in the identification of compounds was achieved by assessing the PCI spectra to identify the elemental composition of the parent ion by looking at common adducts. In PCI experiments using methane as the reagent gas, three adducts are typically observed: $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$. PCI data were imported in Compound Discoverer software and reprocessed to detect the characteristic adducts. As an example, the Compound Discoverer software results showing the PCI spectra of *p*-cymene are reported in Figure 5. The PCI workflow embedded in the software allowed the prediction of the chemical formula for the unknown compounds and assignment of the compound annotation based on multiple sources such as mzCloud™ or ChemSpider™. The presence of the methane adducts in the PCI spectrum confirmed *m/z* 134.10889 as the molecular ion for *p*-cymene (RT = 10.20 min) and supported the elemental composition of the proposed molecule.

Table 2. Table of fold change of main volatile compounds constituents of *O. vulgare* samples with different geographical provenances. In particular, sample A showed a significantly higher level of cymyl-type compounds such *p*-cymene (4-fold change), γ-terpinene (2-fold change), and thymol (3-fold change). Sample B had lower levels of cymyl-type compounds but was higher in acyclic compounds such as β-ocimene (4-fold change) and sesquiterpenes (such as germacrene D (2-fold change)). Sample C had significantly higher concentrations of β-ocimene and sequiterpenes (such as germacrene D).

Name	RT [min]	Chemical Formula	Reference m/z	EI			PCI			Total Score	Log2 Fold Change		
				Measured *m/z*	Theoretical m/z	Mass error (±5	$[M+H]^+$	$[M+C_2H_5]^+$	$[M+C_3H_5]^+$		Sample A / Sample C	Sample B / Sample C	Sample B / Sample A
α-Thujene	8.64	C10H16	91.05417	136.12463	136.12465	-0.1	137.13254	165.16379	177.16380	93.4	4.0	3.3	-0.7
α-Pinene	8.79	C10H16	91.05423	136.12469	136.12465	0.3	137.13252	165.16377	177.16377	93.4	3.4	3.0	-0.4
p-Cymene	10.21	C10H14	119.0856	134.10892	134.10900	-0.6	135.11688	163.14818	175.14815	96.2	3.7	2.8	-0.9
γ-Terpinene	10.84	C10H16	91.05424	136.12457	136.12465	-0.6	137.13234	165.13659	177.16353	95.2	2.3	2.6	0.3
β-Ocimene	11.40	C10H16	93.06971	136.12466	136.12465	0.1	137.13266	165.16393	177.16391	90.2	-4.4	-0.6	3.9
Camphor	12.09	C10H16O	95.08548	152.11957	152.11957	0.0	153.12727	181.15852	193.15848	96.6	0.6	1.1	0.5
Thymoquinone	13.54	C10H12O2	149.0596	164.08311	164.08318	-0.4	165.09077	193.12198	205.12195	94.4	0.1	0.4	0.3
Methyl thymyl ether	13.61	C11H16O	149.0962	164.11960	164.11957	0.2	165.12726	193.15863	205.15863	97.4	1.5	2.0	0.4
Thymol	14.23	C10H14O	135.0804	150.10382	150.10392	-0.6	151.11169	179.14310	191.14310	96.1	2.8	-0.3	-3.1
Carvacrol	14.42	C10H14O	135.0805	150.10384	150.10392	-0.5	151.11163	179.14302	191.14294	95.7	0.3	0.2	0.0
Eugenol	15.36	C10H12O2	164.0831	164.08321	164.08318	0.2	165.09100	193.12228	205.12231	96.9	-1.5	0.2	1.7
Methyleugenol	16.02	C11H14O2	178.0989	178.09885	178.09883	0.1	179.10660	207.13788	219.13788	96.2	-2.6	0.2	2.9
γ-Elemene	17.00	C15H24	189.1639	204.18726	204.18725	0.0	205.19524	233.22658	245.22647	92.1	0.5	0.2	-0.3
β-Caryophyllene	17.15	C15H24	91.05417	204.18721	204.18725	-0.2	205.19489	233.22615	245.22627	95.3	0.5	-0.1	-0.6
Humulene	17.76	C15H24	93.06991	204.18729	204.18725	0.2	205.19513	233.22646	245.22649	94.5	1.8	-0.1	-1.9
Isoledene	18.06	C15H24	105.0699	204.18713	204.18725	-0.6	205.19519	233.22652	245.22665	96.2	1.2	0.9	-0.3
Germacrene D	18.22	C15H24	147.1167	204.18727	204.18725	0.1	205.19514	233.22649	245.22647	95.5	-0.9	0.9	1.7
Alloaromadendrene	18.58	C15H24	91.05424	204.18716	204.18725	-0.4	205.19485	233.22614	245.22618	95.8	-0.6	0.7	1.3
γ-Muurolene	18.78	C15H24	161.1327	204.18719	204.18725	-0.3	205.19499	233.22635	245.22636	93.6	-0.3	0.2	0.5
Isospathulenol	19.94	C15H24O	91.05419	220.18230	220.18217	0.6	221.19000	249.22171	261.22125	94.5	-1.3	0.0	1.3
Caryophyllene oxide	20.10	C15H24O	91.05419	220.18199	220.18217	-0.8	221.18999	249.22130	261.22129	94.7	-0.2	-0.3	-0.1

A



B

GC CI Compounds | ChemSpider Results | Mass List Search Results | Input Files | Study Information

	Name	Formula	RT [min]	Annot. Source: Predicted Compositions (ChemSpider Search, MassList Search)	# Adducts	Annot. ΔMass [ppm]	Molecular Weight	Reference m/z
83	p-cymene	C10 H14	10.154		5	-0.38	134.10950	93.06992

Figure 5. Compound Discoverer software results showing the PCI spectrum for *p*-cymene (RT = 10.15 min) (A) and the results table (B). The typical adducts formed when methane gas is used are labeled in the spectrum plot in green. The annotation sources (ChemSpider and Mass List) used to propose the chemical formula for *p*-cymene, as well as the number of adducts found, the mass accuracy (in ppm), the molecular weight and the reference *m/z*, are listed in the table.

Conclusions

The results presented in this study demonstrate that the Thermo Scientific Orbitrap Exploris GC 240 mass spectrometer in combination with SPME Arrow technology and Compound Discoverer 3.2 software represents an integrated omics approach for the characterization of the volatile fraction of food samples.

- Flavor profiling is a challenging analysis as the sample matrices encountered are chemically complex, the compounds are present over a wide dynamic range, and profiling requires sensitive and stable systems.
- Significant differences in the oregano chemotypes were detected and these can be representative of the different climate where *O. vulgare* varieties were grown with the predominance of phenolic monoterpenes in plants grown in the Mediterranean area and sesquiterpenes predominant in continental regions.
- Cymyl-type compounds (*p*-cymene, *γ*-terpinene, and thymol) were predominant in sample A, whereas other samples had high levels of acyclic compounds and sequiterpenes (such as germacrene D and β-caryophyllene).

- The high resolving power and consistent sub-1 ppm mass accuracy as well as the wide linear and dynamic range lead to fast and confident characterization of a large number of compounds regardless of their concentration or matrix complexity.
- Automated headspace sampling with the SPME Arrow eliminates the need of sample preparation and speeds up the analysis.
- The streamlined GC-EI/PCI data processing workflow integrated in Compound Discoverer 3.2 software allows for multivariate statistical analysis, extraction, deconvolution, and putative identification of the unknown compounds. The EI data obtained can be used for candidate compound identification against existing commercial libraries. Importantly, as often the chemicals detected are not included in such libraries, the consistent sub-ppm mass accuracy measurements as well as the retention index information will greatly aid in the determination of the elemental composition and subsequent structural elucidation of unknown chemicals. Moreover, softer ionization such as positive chemical ionization with methane can be used to confirm the elemental composition of the molecular ion of a chemical.

References

1. Black C.; Haughey S.A.; Chevallier O.P.; Galvin-King P.; Elliott C.T. A comprehensive strategy to detect the fraudulent adulteration of herbs: the oregano approach, *Food Chemistry* **2016,** *210,* 551–557.
2. Lukas B.; Schmiderer C.; Novak J. Essential oil diversity of European *Origanum vulgare* L. (Lamiaceae), *Phytochemistry* **2015,** *119,* 32–40.
3. Cavanna D.; Righetti L.; Elliot C.; Suman M. The scientific challenges in moving from targeted to non-targeted mass spectrometric methods for food fraud analysis: A proposed validation workflow to bring about a harmonized approach, *Trends in Food Science & Technology* **2018,** *80,* 223–241.

Find out more at **thermofisher.com/HRAMGCMS**

ThermoFisher SCIENTIFIC