Mass spectrometry

WHITE PAPER

Grant application resource for Orbitrap IQ-X Tribrid mass spectrometer

Reveal complex chemical structures with ultimate confidence, ease, and experimental versatility

Keywords

Metabolite identification, untargeted metabolomics, lipidomics, lipids, oligonucleotides, natural products, compound annotation, unknown analysis, structure elucidation, isotope fine structure, high resolution accurate mass (HRAM), mass spectrometry, Orbitrap IQ-X Tribrid mass spectrometer, MS^n, AcquireX workflows, mzLogic, mzCloud, Real-Time Library Search

Goal

Built specifically for small molecule analysis, the Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ MS utilizes technology that significantly augments unknown analysis capabilities in metabolite identification (Met-ID), untargeted metabolomics, and lipidomics research, but also shows benefits for structural elucidation of oligonucleotides and natural products research. The information provided here describes the technologies that set the Orbitrap IQ-X Tribrid MS apart from other instrumentations including previous generation Thermo Scientific™ Orbitrap ID-X™ Tribrid™ MS, Thermo Scientific™ Orbitrap Exploris™ platform, Thermo Scientific™ Q Exactive™ instruments, and quadrupole time-of-flight (QTOF) mass analyzers.

Summary

Thermo Scientific™ Orbitrap™ Tribrid™ mass spectrometers are an essential tool for high-end life science research. Equipped with a combination of the best of quadrupole, linear ion trap and ultra-high resolution Orbitrap mass analyzer that deliver acquisition of the richest MS^n data. This unique hardware combination provides superior analytical performance that enables multiple modes of analysis due to the parallel isolation and detection of ions, which is not available with on Orbitrap Hybrid mass spectrometer or QTOF instrumentation. The Orbitrap IQ-X Tribrid MS (Figure 1) has been specifically designed to unravel complex chemical structures while delivering the highest versatility and comprehensive workflow support for analysis of small molecule unknowns. This became possible with the recent advances in mass spectrometry technology including real-time spectral library search, multiple fragmentation techniques, higher precursor ion selectivity and a fully automated calibration source.

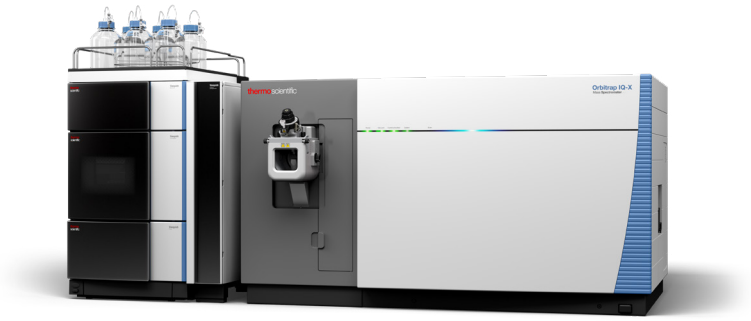

Figure 1. The Orbitrap IQ-X Tribrid mass spectrometer with the Thermo Scientific™ Vanquish™ Duo UHPLC Systems provides superior small-molecule characterization.

Introduction

Identification of unknown small molecule analytes is a demanding application due to multiple challenges, including:

- Small molecules have a wide range of chemical properties that affect their chromatographic and MS behavior.
- They often have many isomeric forms.
- They often exist in a complex chemical matrix.
- Structural elucidation software tools are diverse and can be difficult to use.

One of the main strategies used for identification of unknowns is to reduce sample complexity by applying several different chromatographic methods prior to analysis with a high-resolution accurate mass (HRAM) Orbitrap mass spectrometer.[1] However, knowing the chemical structure of a compound of interest benefits diverse application areas including metabolomics to biomarker discovery, environmental and food safety, natural products, oligonucleotides, pharma metabolite or impurity identification, extractables and leachables to forensic or clinical toxicology. Indeed, identification of hundreds to thousands of small molecules in complex matrices such as human plasma is one of the most difficult challenges faced by scientists.[2] Detection and unambiguous identification of an unknown requires a combination of mass spectrometric tools including high mass accuracy, ultra-high resolution (for isotopic fine structure), multiple dissociation techniques, and an ability to collect a high-resolution mass spectral fingerprint consisting of high-quality MS^n spectral trees.[3] HRAM LC-MS datasets contain many thousands of features where only some of them are related to the biological sample or while the rest belong to chemical background.[4] Data reduction and false positives are a major roadblock in these experiments. The Orbitrap IQ-X Tribrid mass spectrometer was specifically developed with all of these analytical challenges in mind. This versatile LC-MS^n instrument is optimized to obtain the highest quality information from small molecule samples. High resolution with robust mass accuracy ensures that every full MS and MS^n scan is used effectively for obtaining comprehensive small molecule identification. Intelligent acquisition methods allow for real-time decision making when profiling precious samples, making deeper characterization of known and unknown compounds possible.

Identification of low-abundance metabolites in human plasma, isotopomer mixture analysis, characterization of isomeric flavonoid or lipid species in biological extracts, and obtaining high quality MS^n spectral reference libraries can be fully achieved on the versatile Orbitrap IQ-X Tribrid mass spectrometer. The Orbitrap IQ-X Tribrid mass spectrometer is optimized for small molecules and can perform multiple fragmentation techniques: (higher energy collision dissociation (HCD), collisional induced dissociation (CID) and optional Ultraviolet Photodissociation (UVPD) at every level of MS^n analysis. The instrument provides up to 500,000 resolution at *m/z* 200, high sensitivity, and rapid acquisition rates needed for obtaining high quality data for demanding applications such as isotopic tracer studies, with optional 1,000,000 (1M) resolution at *m/z* 200 for fine isotopic structure. The Orbitrap IQ-X Tribrid mass spectrometer system extends the dynamic range for small molecule detection, compound annotation and quantitation needed to achieve a broad range of analyte coverage, by combining the versatility of Tribrid architecture, selectivity of Orbitrap technology and real-time spectral library searching to generate high-quality MS^n spectra. The integration of Thermo Scientific™ AcquireX intelligent data dependent acquisition methods and the use of advanced scan filters to target specific compound classes provide unparalleled flexibility in designing experiments that meet the real world challenges in unknown small molecule characterization today. All of this is made easier with an expansive library of application specific small molecule methods for metabolomics, lipidomics, metabolite ID, impurities, extractables and leachables, and more. The methods in the library which are fully customizable and organized as easy-to-use, one-click method templates.

12 reasons for selecting the Orbitrap IQ-X Tribrid mass spectrometer for small molecule research

- Intelligent acquisition with Real-Time Library Search provides higher confidence small molecule analysis by using on-the-fly MS^n spectral matching against a local spectral library for decision-based triggering of MS^n scan events.
- Confident metabolite annotations with high quality HCD MS^n and CID MS^n data
- Improved fragmentation for lipidomics and other small molecule analysis with optional UVPD analysis for MS^n provides lipid double bond localization, site specific glucuronidation, complementary fragmentation for structure assignment.
- Ultra-high resolution and consistently high mass accuracy allows for in-depth small molecule coverage with high confidence. Optional 1M resolution allows detecting fine isotope structure improving the confidence of elemental composition assignment.
- AcquireX data acquisition workflows make it possible to obtain higher LC-MS^n coverage with annotation of more compounds of interest.
- Spend more time on results, less time on instrument set-up with the new Auto-Ready ion source that simplifies maintenance with weekly pre-scheduled and remote one-click calibration.

What's new on Orbitrap IQ-X Tribrid MS compared to previous instrumentation?

The Orbitrap IQ-X Tribrid mass spectrometer redefines small molecule identification, quantitation, and characterization by leveraging the proven and trusted Tribrid architecture with intelligent MS^n acquisition, based on Real-Time Library Search, to improve annotation and structural characterization of unknown analytes. This instrument delivers unrivaled performance and ease-of-use with an integrated autocalibration source that simplifies maintenance combined with a fit-for-purpose software interface to overcome the traditional bottlenecks in small molecule structural identification and characterization. Key performance characteristics of Orbitrap IQ-X Tribrid MS are summarized in Figure 2 and Table 1.

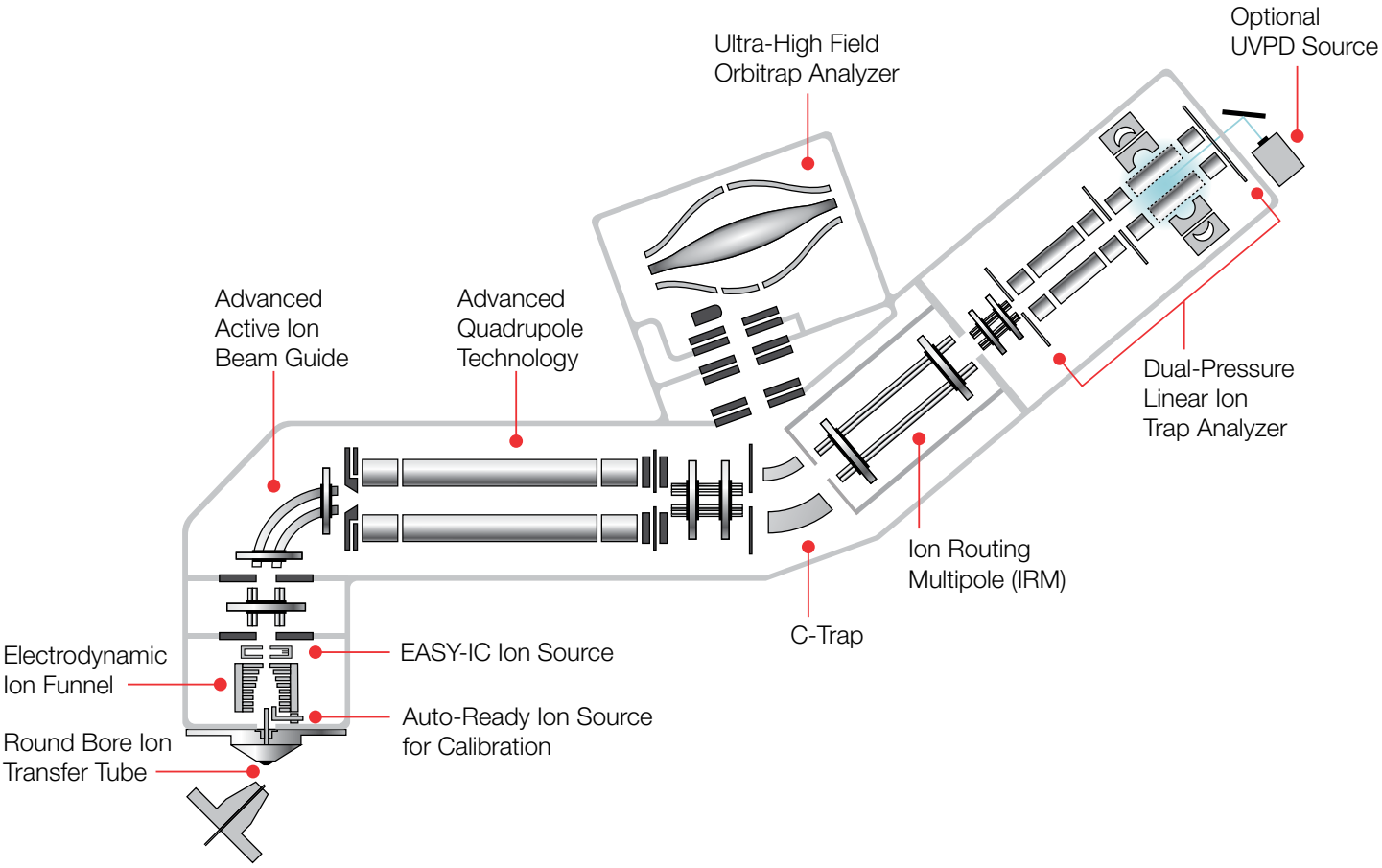

Figure 2. Orbitrap IQ-X Tribrid mass spectrometer schematic.

Table 1. Key technology differentiators by instrument.

Key Technology Features	Q Exactive Plus mass spectrometer	Orbitrap Exploris 240 mass spectrometer	Orbitrap IQ-X Tribrid mass spectrometer	Competitor QTOF
Resolution	Up to 140,000	Up to 240,000	Up to 1,000,000 (1M option)	Less than 50,000
Mass accuracy	<3 ppm	<1 ppm (EASY-IC)	<1 ppm (EASY-IC)	>3 ppm
Ion mobility	None	Differential Ion Mobility (FAIMS Pro interface)	Differential Ion Mobility (FAIMS Pro Duo interface)	Drift Tube or Trapped Ion Mobility
Unified software and sources	Not available	Included	Included	Not available
Real-time library search	Not available	Not available	Spectral library matching in real-time to control instrument acquisition	Not available
AcquireX workflows	Not available	Automated inclusion and exclusion list generation and routines	Automated inclusion and exclusion list generation and routines	Not available
Mass analyzers	Two: Quadrupole and Orbitrap	Two: Quadrupole and Orbitrap	Three: Quadrupole, Linear Ion Trap, and Orbitrap	Two: Quadrupole and TOF
MS^n capabilities	None	None	Multiple fragmentation techniques—CID and HCD with optional UVPD—for any stage of MS^n	None

The following sections details new features and benefits driven by the latest technological advancements in mass spectrometry that are including with the Orbitrap IQ-X Tribrid MS, providing the highest confidence data for small molecule characterization with increased ease-of-use.

Real-Time Library Search

Real-Time Library Search provides higher confidence small molecule analysis with Real-Time spectral matching against a local, customizable mzVault library for decision-based triggering of MS^n acquisition events (Figure 3). Its use includes, but not limited to, library directed MS^n data acquisition to increase confidence in metabolite annotation, including structurally related compounds and improve structured assignment of isomeric species, and search-based unknown characterization.

Electrodynamic Ion Funnel with round bore ion transfer tube

The unique combination of an electrodynamic ion funnel with a round bore ion transfer tube efficiently captures ions as they enter the mass spectrometer, while providing gentle transmission, reducing ion losses and increasing sensitivity.

Segmented quadrupole

The segmented quadrupole mass filter on the Orbitrap IQ-X Tribrid MS enables higher transmission at narrower isolation width down to 0.4 *m/z*. This improved sensitivity and selectivity benefits the identification of co-eluting compounds.

Mild Trapping for labile compounds

Within the small-molecule method templates, a Mild Trapping mode can be optionally applied for particularly labile compounds to provide gentler ion trapping within the mass spectrometer. This mode functions as a global setting that amends the entire analysis.

Extended mass range

Extended mass range down to 40 *m/z* on the Orbitrap IQ-X Tribrid MS provides additional fragment ions to increase specificity and improved structure assignment of isomeric species for metabolomics and lipidomics.

Improved Advanced Precursor Determination (APD)

Small molecule specific improvements to Advanced Precursor Determination (APD) enhances detection and monoisotopic mass assignment for halogenated compounds, leading to fewer redundancies in acquisition.

Expanded AcquireX workflows

AcquireX data acquisition workflows provide comprehensive small molecule sample profiling on an LC timescale using automated, logic-based sample re-injection with inter-run inclusion and exclusion lists. The expanded AcquireX data acquisition now includes multiple unique workflows to benefit a variety of applications and experimental hypothesis, including:

- Background Exclusion AcquireX workflow utilizes an exclusion reference to automatically generate an exclusion list that directs the MS away from interrogating background ions in favor of sample specific compounds.

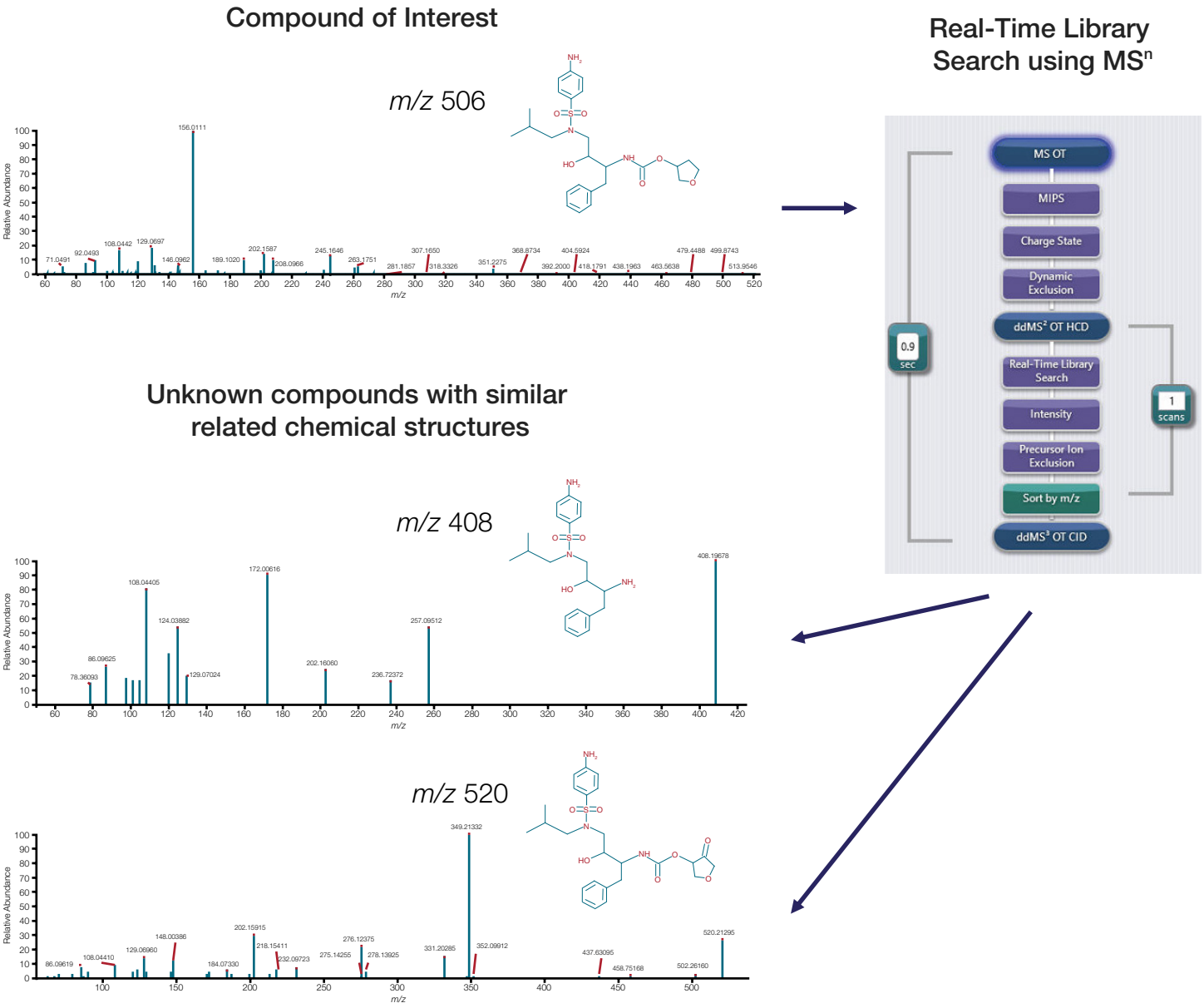

Figure 3. The Real-Time Library Search provides intelligent data acquisition of small molecule compounds for confident annotation, using a similarity search workflow to identify all the possible transformation products for a compound of interest, based upon related chemical structures with spectral similarities.

- Exclusion and Component Inclusion AcquireX workflow utilizes a reference sample to automatically generate the corresponding exclusion and inclusion lists allowing the triggering of MS^n acquisitions on unique precursor ions that are only present in the sample of interest.
- The Iterative Precursor workflow creates an exclusion list based upon the HRAM MS analysis of a representative blank sample, with the exclusion list being automatically updated based upon the precursor ions being selected. As additional iterative samples are analyzed, the exclusion list is continually updated and the process repeated until a user-defined number of replicate injections have been performed, or no more precursor ions exist for analysis.
- Deep Scan AcquireX workflow provides higher depth and coverage for complex samples by combining the efficiency of the inclusion and exclusion list-based MS^n triggering with automated re-injections, where each re-injection is informed by the preceding LC-MS analysis through dynamic updates of the exclusion and inclusion lists
- New Advanced Deep Scan AcquireX workflow provides ultimate depth and coverage for small molecule analysis with increased flexibility to submit several experiments from a single sequence, automatically acquire and combine multiple blank or exclusion lists using improved algorithms for background exclusion/component detection. Additionally, the user interface has been enhanced to increase ease-of-use with copy/fill-down, export/import sequence, and insert blank/wash functionalities.

EASY-IC source comes standard

An Orbitrap IQ-X Tribrid MS includes the EASY-IC (Internal Calibration) source to deliver superior mass accuracy in every scan. Using a second ion source to generate specific calibrant ions, the instrument software uses the known mass-to-charge ratio of the calibration ions to provide real-time adjustment of the instrument's mass accuracy to less than 1 ppm and enabling corrections for scan-to-scan variations for positive and negative modes. Combined with the Auto-Ready ion source, mass calibration is now fully automated, increasing productivity.

Auto-Ready ion source

The Auto-Ready ion source standardizes calibration and maintenance by automatically performing instrument calibration at chosen intervals. It features a dedicated emitter and ion transfer tube hardware that support consistent calibration performance over time. Calibration and maintenance results are reported directly in the instrument control software interface, and the internal Thermo Scientific™ Pierce™ FlexMix™ Calibration solution consumable lasts ≥3 months with regular use.

Expanded experimental flexibility with new optional components

The Orbitrap IQ-X Tribrid MS provides expanded experimental flexibility with new optional components, which are field upgradeable, enabling upgrades before or after installation.

1M resolution

The Orbitrap IQ-X Tribrid MS with the optional 1 million resolution provides fine isotope information with high isotopic fidelity. Resolving fine isotope structure allows confident assignment of the elemental composition of small molecule analytes. Ultra-high resolution, along with MS^n capability and the availability of multiple fragmentation modes, enable new characterization of analytes (Figure 4). Particularly in the low *m/z* range this is critical for the confident characterization of both parent and fragment ions.

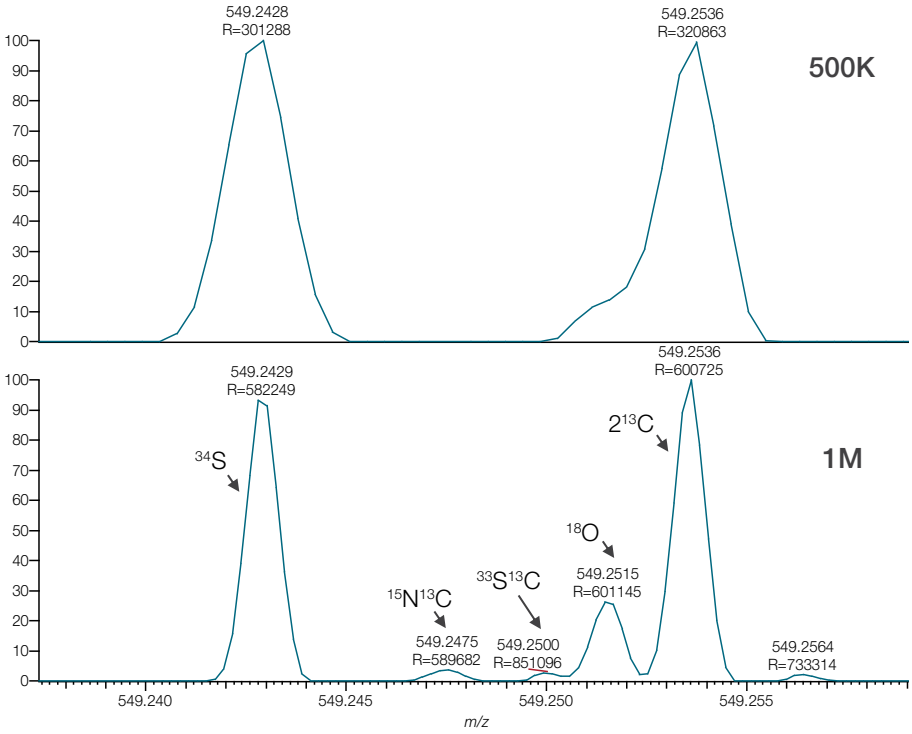

Figure 4. 1 million (1M) resolution option enables for fine isotope structure allowing confident assignment of the elemental composition of a small molecule analyte. Targeted precursors of interest were detected using an ultra-high resolution SIM scan, where the 500K resolution SIM spectrum (top) does not show resolved fine structure for M+2, whereas 1M resolution SIM spectrum (bottom) shows resolved fine isotope structure for M+2. The selected composition along with MS^2 fragment analysis allowed proposed structure elucidation for this unknown extractable.

UVPD

Ultraviolet Photodissociation (UVPD) is a unique fragmentation mode for the unambiguous characterization of various lipid classes and improved analysis of unknowns. The Orbitrap IQ-X Tribrid MS with optional UVPD using a 213 nm laser system with 2.5 kHz repetition rate delivers unique fragments, unique structurally diagnostic fragments otherwise unobtainable (Figure 5). With its compact size, UVPD is located entirely within the footprint of the instrument.

FAIMS Pro Duo interface

Differential ion mobility with the optional Thermo Scientific FAIMS Pro Duo interface enables improved detection and measurement of small molecules, down to the Limits of Quantitation (LOQ). Field Asymmetric Ion Mobility Spectrometry (FAIMS) enables online gas-phase separation for orthogonal selectivity, with optimized performance for 100 nL/min to 1 mL/min flow rates.

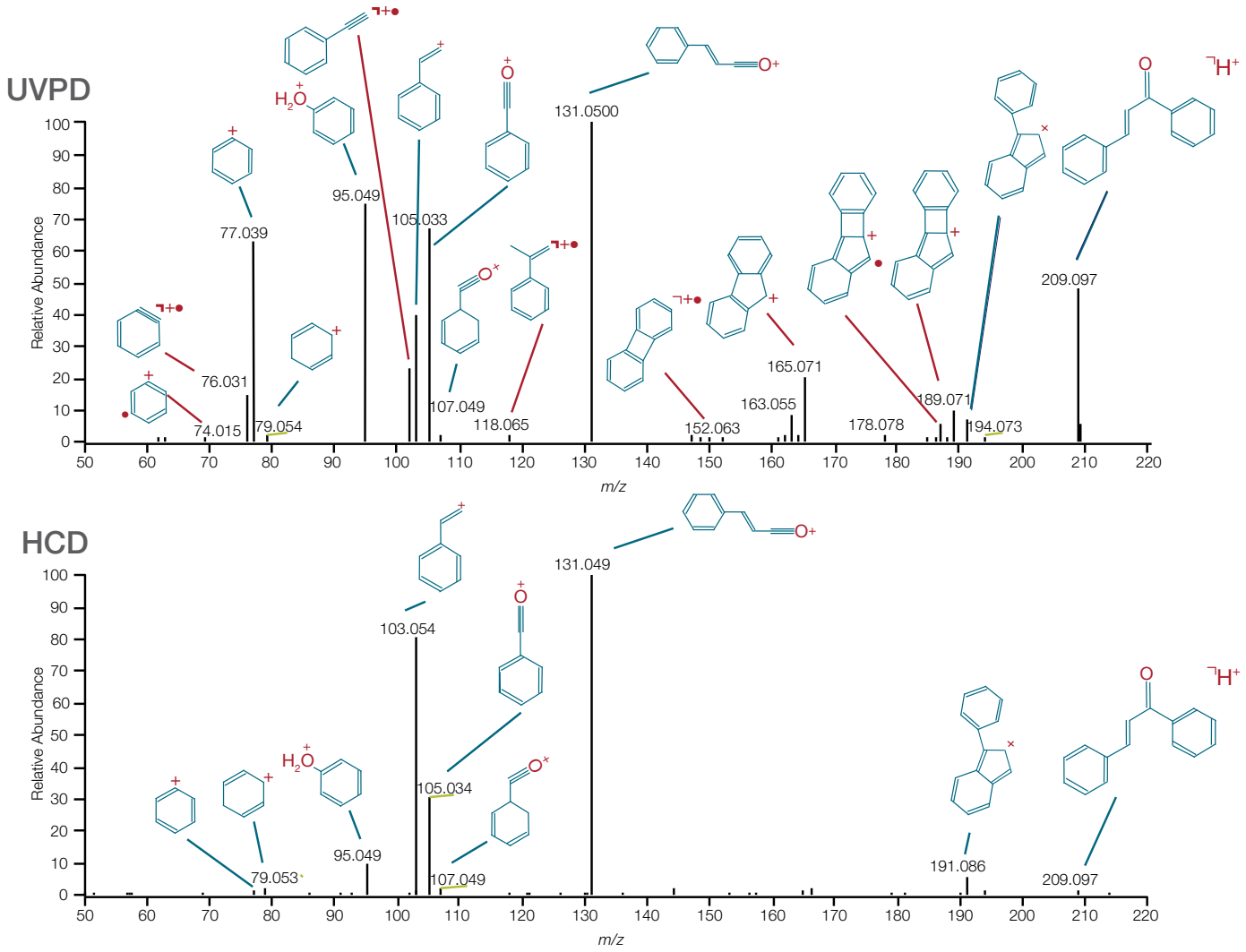

Figure 5. UVPD provides rich fragmentation pattern and unique fragments for small molecule structural analysis. Shown is spectrum of Chalcone, an intermediate in plant flavonoid biosynthesis, analyzed using UVPD (Top) or HCD (Bottom)

Additional features that benefit small-molecule analysis

Building upon the success of the original Orbitrap ID-X Tribrid MS for small-molecule research, the following additional features were previously available and are also included on the next generation Orbitrap IQ-X Tribrid MS.

- Orbitrap instrument control software that has the same user interface across all next-generation Thermo Scientific mass spectrometers, accelerating familiarization and reducing training requirements. The Tune software enables instrument control, system calibration, diagnostics, and manual data acquisition. The Method Editor software uses a drag-and drop user interface with comprehensive small-molecule, pre-built templates that can be fully customized for applications including metabolomics, lipidomics, metabolite ID, impurities, extractables and leachables, and more

- Assisted CE mode utilizes hidden ion trap acquisitions to assess the optimal collision energy in real-time, automatically generating precursor ion specific breakdown curves to select the collision energy required to sufficiently deplete a given precursor

- MS^n Library Builder method acquires high-quality, fragmentation-rich MS^n product ion spectra utilizing Assisted CE for improved signal to noise for the HCD and CID product ions

Increased insight to small molecule characterization with optional application-specific software

Enable greater insight to your small-molecule, compounds of interest by combining the high quality, HRAM data acquisition on the Orbitrap IQ-X Tribrid MS with application specific software to annotate and identify with confidence.

- Thermo Scientific™ mzCloud™ spectral library—Mass spectral libraries and databases may be required to identify unknown compounds when reference standards are not available. This is particularly true for studies in areas such as metabolomics, forensic toxicology, food & beverage, natural products, environmental emerging contaminants or extractables & leachables. In these situations, a mass spectral library, particularly a high-resolution and accurate-mass MS^n fragmentation library, such as the mzCloud mass spectral library, can provide the information required to confidently identify unknown compounds. For Real-Time Library Search methods, an active license for Compound Discoverer software or Tracefinder software is required to use the mzCloud Offline library. To create custom spectra libraries, mzVault is used and can be downloaded for free from https://thermo.flexnetoperations.com/

- Thermo Scientific™ Compound Discoverer™ software—No matter what your small molecule research application, Compound Discoverer software offers a unique approach to small molecule structural identification. Compound Discoverer software efficiently extracts high-confidence insights from information-rich small molecule HRAM data. This mass spectrometry data analysis software offers a full suite of tools to address your challenges with small molecule data analysis, including automated MS^n tree search against mzCloud. The software streamlines unknown identification, determination of real differences between samples, and elucidation of biological pathways.

- Thermo Scientific™ Mass Frontier™ software—Confident small molecule unknown compound identification and structural elucidation can be labor intensive and time consuming. Simplify and streamline this challenge through the use of Thermo Scientific Mass Frontier software which utilizes the most high quality, fully curated experimental spectral and fragmentation libraries available to provide greater insights, understanding and confidence when turning your data into actionable knowledge.

- Thermo Scientific™ LipidSearch™ Software— Lipidomics is crucial to understanding cellular physiology and pathology, and lipid profiling for disease phenotype analysis is a rapidly growing area in translational medical research. LC/MS is a powerful technique for the identification and quantitation of cellular lipids. The LipidSearch software processes LC-MS data from high-resolution accurate-mass data generated on the Orbitrap IQ-X Tribrid MS, to make provide accurate lipid identification, while automatically integrating complex data into reports and dramatically reduces data analysis time.

Summary

In conclusion, the Orbitrap IQ-X MS provides superior benefits for small molecule research over previous generation Orbitrap instruments including Q Exactive instruments and quadrupole time-of-flight (QTOF) mass analyzers. These benefits positively impact research for metabolite identification, untargeted metabolomics, lipidomics, impurity analysis, and leachables/extractables research, but also benefit structural elucidation for natural products or even oligonucleotides (Figure 6). The Orbitrap IQ-X Tribrid MS combines with powerful software for streamlined data processing and interpretation; capturing, identifying, and quantifying more low-abundance analytes for unrivaled small-molecule analysis.

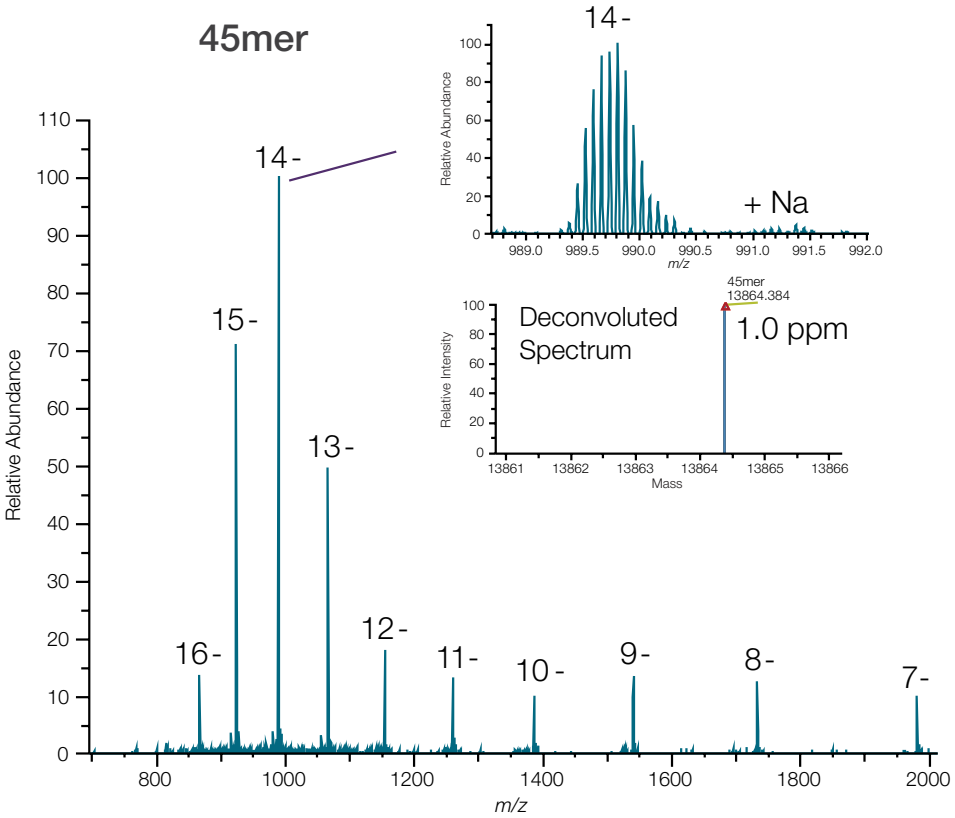

Figure 6. ESI MS of oligonucleotides reveals high resolution, mass accurate measurements that are nearly free of salt adduct using ion paring reverse phase liquid chromatography. Isotopically resolved spectra were obtained for 45mer oligonucleotides using a resolution setting of 120,000 at *m/z* 200 with high accuracy (<1 ppm). The Orbitrap IQ-X Tribrid MS enables analysis of 14kDa oligonucleotides without additional adjustment to pressure or ion optics typical for larger molecules.

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