thermo scientific

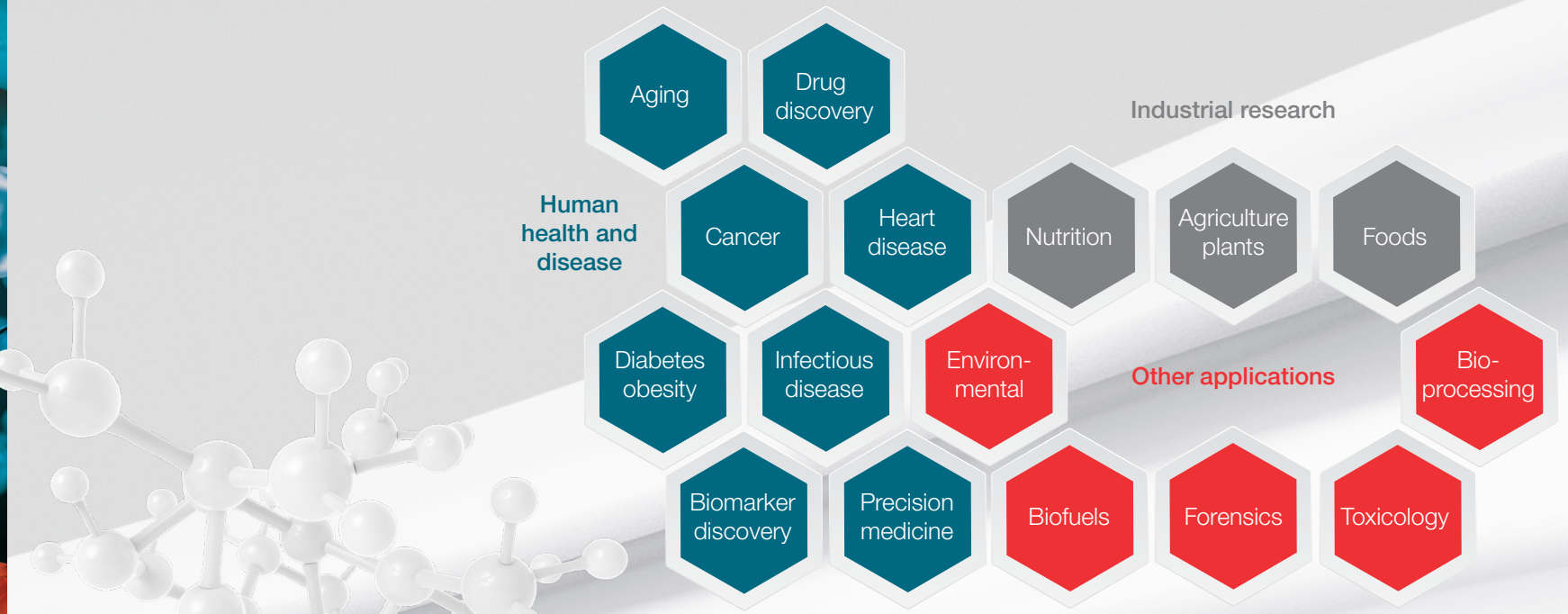
Go beyond

Harness the power of metabolomics

ThermoFisher SCIENTIFIC

The total package for metabolomics

By collaborating with the scientific community, we've developed pioneering metabolomics solutions that combine Thermo Scientific™ Orbitrap™ LC-MS instruments with powerful data analysis software and novel spectral libraries. With built-in, intelligent data acquisition and flexible workflows, we deliver a comprehensive package to perform increasingly complex analyses from confident compound annotation to accurate quantitation, so you can go beyond the edge of what was ever thought possible.



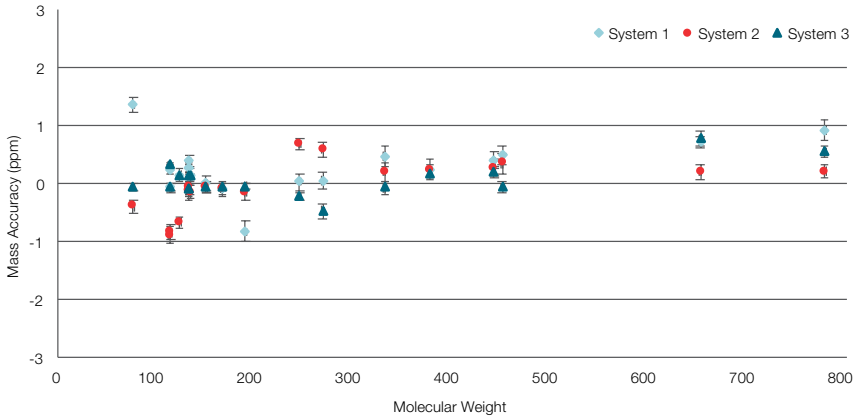
Metabolomics can be applied to many areas of scientific research such as human health and disease, industrial research, and other applications.

Setting new standards in metabolomics

Regardless of your metabolomics application and sample complexity, Thermo Scientific™ Orbitrap™ technology provides the highest quality, intelligently collected high-resolution accurate-mass (HRAM) LC-MS data to confidently power your science.

Consistent mass accuracy

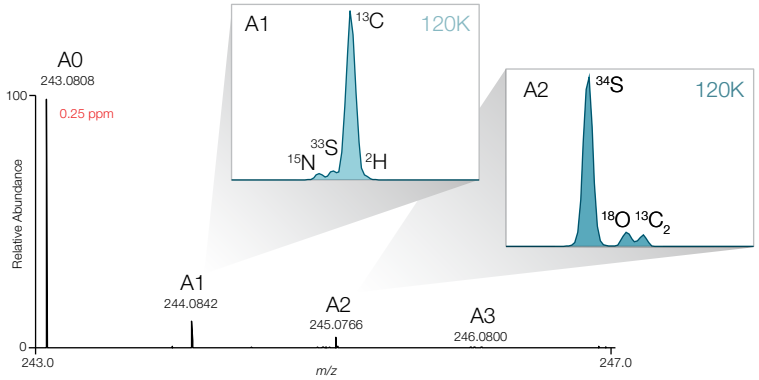
Orbitrap instruments provide consistent sub-ppm mass accuracy from run-to-run across the entire mass range without the need to average several scans, providing high-confidence, in-depth metabolome and lipidome coverage.



Analysis of 25 components using the optional Thermo Scientific™ EASY-IC™ ion source with internal calibration. Independent of molecular weight, all standards (MW 75–776) were measured with excellent mass accuracy over six days on three different Orbitrap Exploris 240 mass spectrometer systems.

Ultra-high resolution (UHRAM) provides high confidence

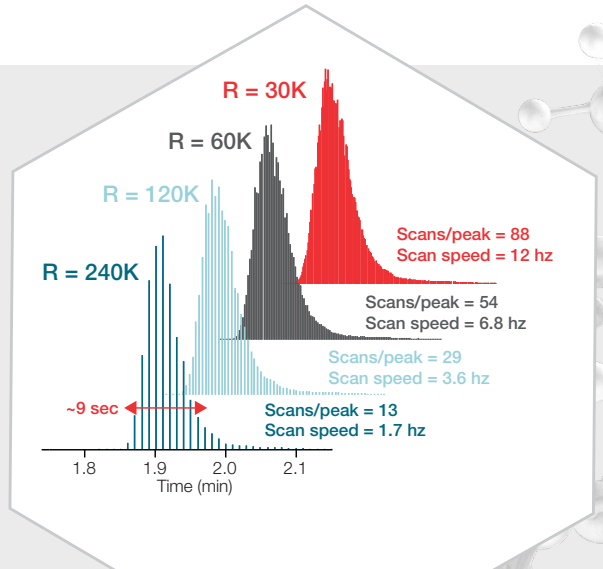
Increased resolving power provides higher confidence in elemental composition determined from UHRAM data. The combination of isotope fine structure with high mass accuracy reduces possible candidates and ensures correct formula assignment.



Biotin at 120K resolving power. A1 and A2 isotope fine structure (^{13}C, ^{15}N, ^{18}O, ^{33}S and ^{34}S).

Rapid instrument scan speed

Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer scan speeds provide sufficient data points across every chromatographic peak to enhance quality of results. Higher resolution increases peak area precision and selectivity, ensuring accurate quantitative performance.



Effect of resolution and scan speed for acetylcarnitine, *m/z* 204.1230. As resolution was increased from 30K to 240K, scan speed decreased, yet sufficient scans were maintained across the peak.

Collect more meaningful, high-quality data with ease

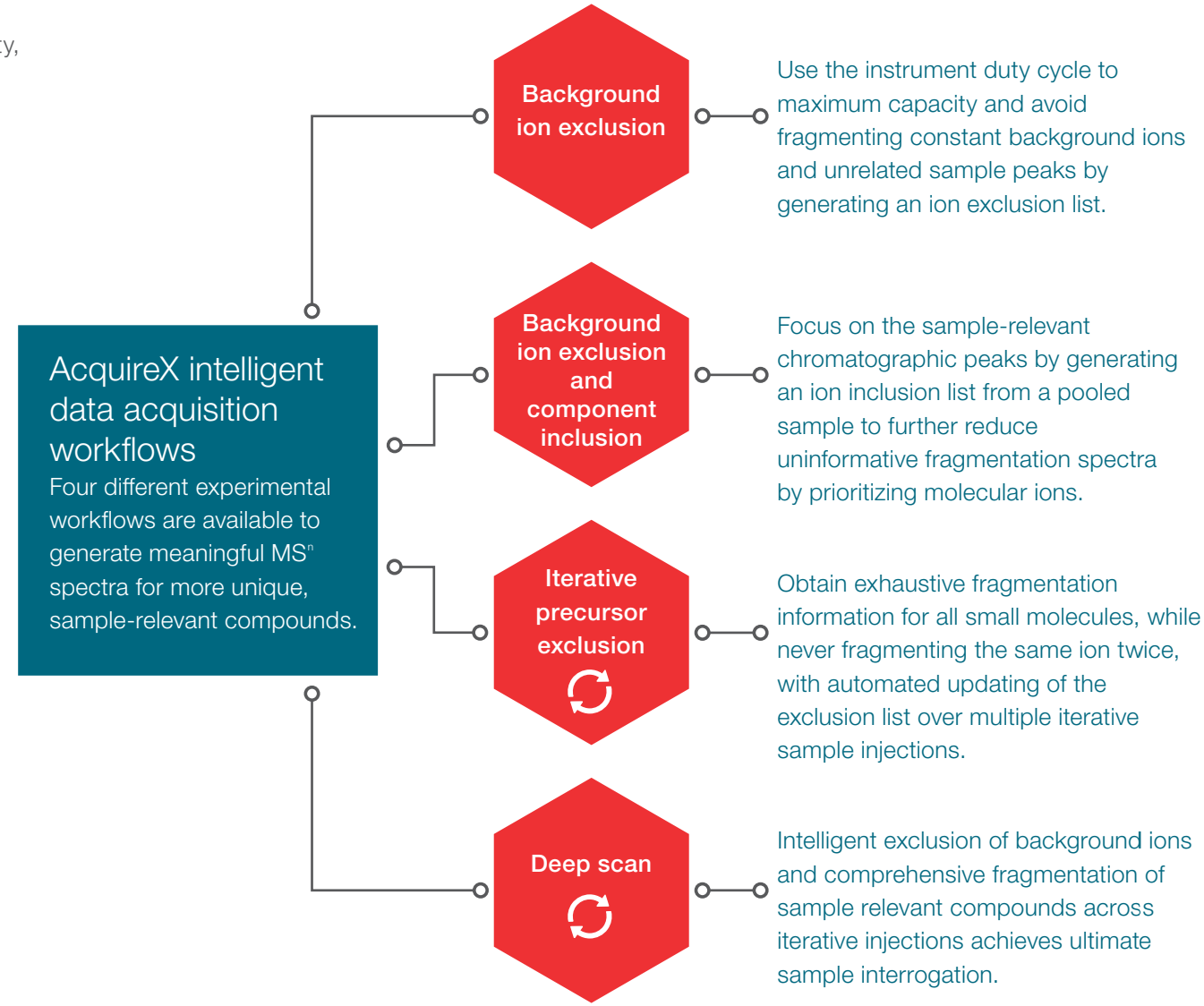
Small-molecule analysis demands the reliable acquisition of high-quality MS and MS^n data to facilitate confident compound annotation and subsequent identification. High sample complexity can limit the utility of the two most common strategies—data-dependent acquisition (DDA) and data-independent acquisition (DIA)—due to irrelevant spectra from chemical background or redundant spectra from adducts, isotopes, and in-source fragment ions. Thermo Scientific™ AcquireX intelligent data acquisition workflows automatically collect more informative high-quality MS^n data, increasing profiling efficiency while minimizing manual experimental setup and expediting subsequent data interpretation.

The challenge of fragmenting everything

DDA isolates a single precursor ion with high purity, generating easily-interpretable fragmentation spectra. With DDA, low-abundance species can be missed.

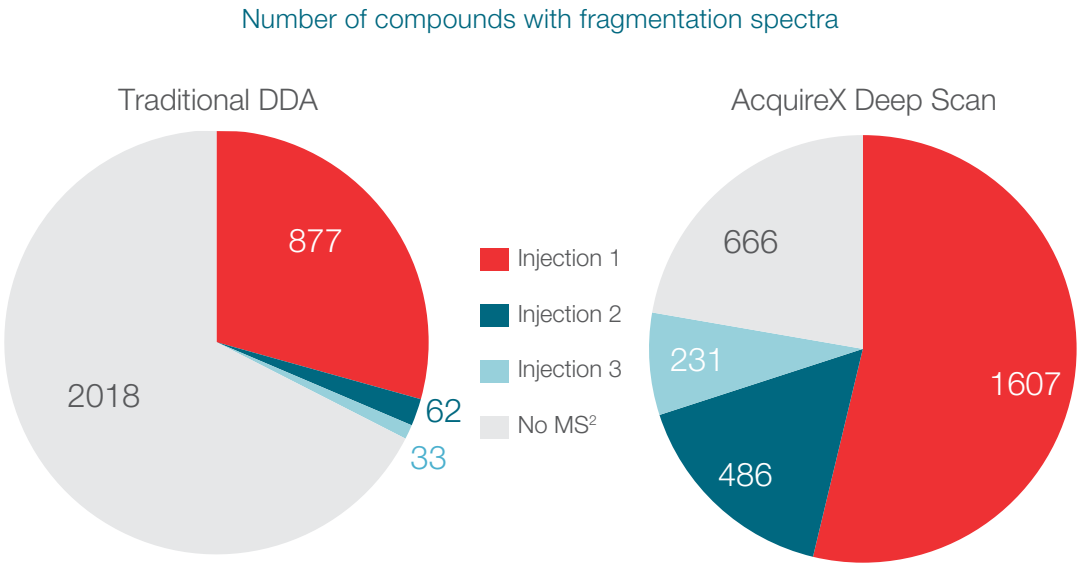
DIA fragments all ions for a given precursor mass window, including background ions, resulting in complex fragmentation spectra which require deconvolution to relate fragment ions back to their precursor ion.

AcquireX intelligent data acquisition takes advantage of DDA selectivity, focusing acquisition on what's important and enabling the fragmentation of more unique precursors, including low-abundance species.

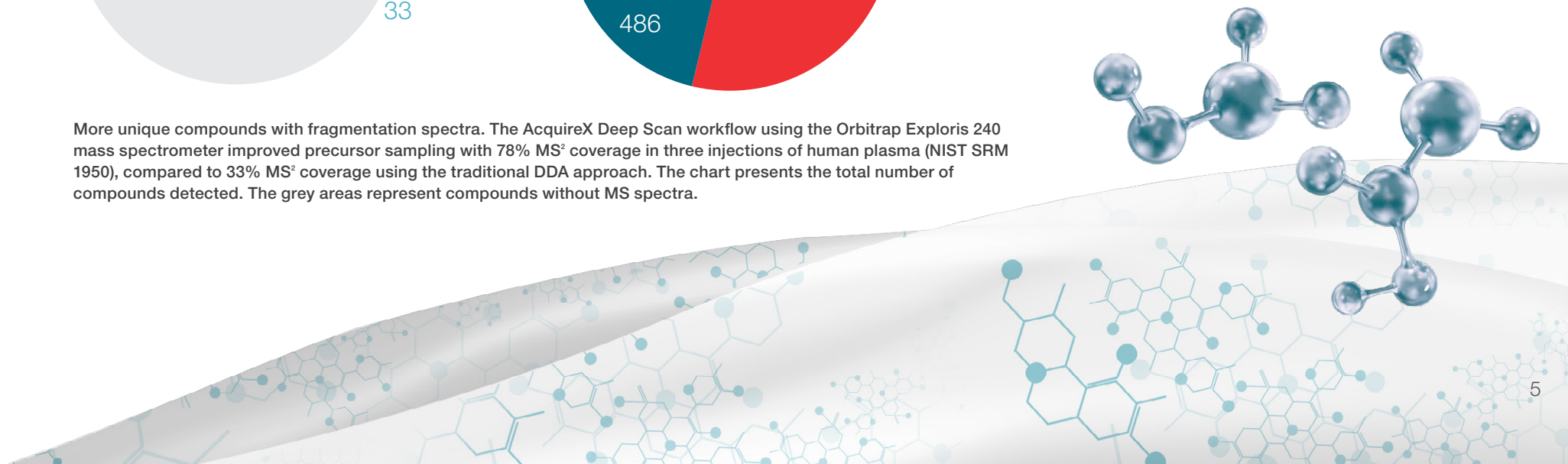


> Confident metabolite identification remains a challenging step in the untargeted metabolomics workflow where the data acquired are applied to derive the structure of all metabolites detected. Progress has been made to putatively annotate metabolites using MS or MS/MS data using established UHPLC/MS assays through the use of metabolomics database and mass spectral library searching workflows. The Orbitrap ID-X Tribrid MS delivers complementary information with multiple dissociation techniques and robust MS^n data required to deduce structural information and increase confidence in metabolite annotation. With built-in intelligent data acquisition, AcquireX, we now collect more informative data, and not just more data, we can maximize metabolome coverage and increase confidence in the identification on unknowns applying intelligent-DDA approaches."
>
> Dr. Warwick Dunn, PhD, Professor in Analytical and Clinical Metabolomics, University of Birmingham, UK

Higher fragmentation efficiency



More unique compounds with fragmentation spectra. The AcquireX Deep Scan workflow using the Orbitrap Exploris 240 mass spectrometer improved precursor sampling with 78% MS^2 coverage in three injections of human plasma (NIST SRM 1950), compared to 33% MS^2 coverage using the traditional DDA approach. The chart presents the total number of compounds detected. The grey areas represent compounds without MS spectra.



Semi-targeted metabolomics: the complete picture in one experiment

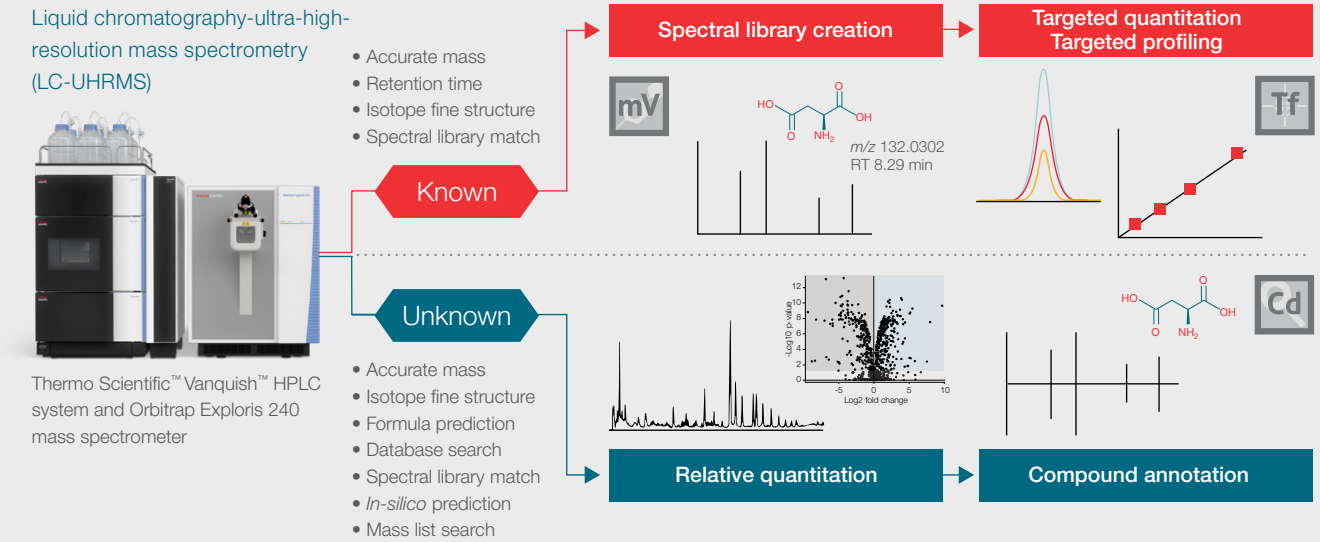
Traditionally, separate LC-MS instruments and methods are used for targeted analysis and untargeted profiling. Now, you can simultaneously measure known metabolites and discover unknown compounds in a single, semi-targeted workflow.

Quantify known metabolites of interest

Reference standards establish retention time, mass measurement, isotopic fine structure, and MS^2 spectral confirmation against an in-house library for the identification and quantification of target metabolites.

Discover novel metabolic signatures

As part of the same workflow, statistical analysis is applied to unknown compounds, focusing on relevant biological changes. Annotation tools, including formula prediction, database searches, spectral library searches and *in-silico* prediction, are used to increase annotation confidence.

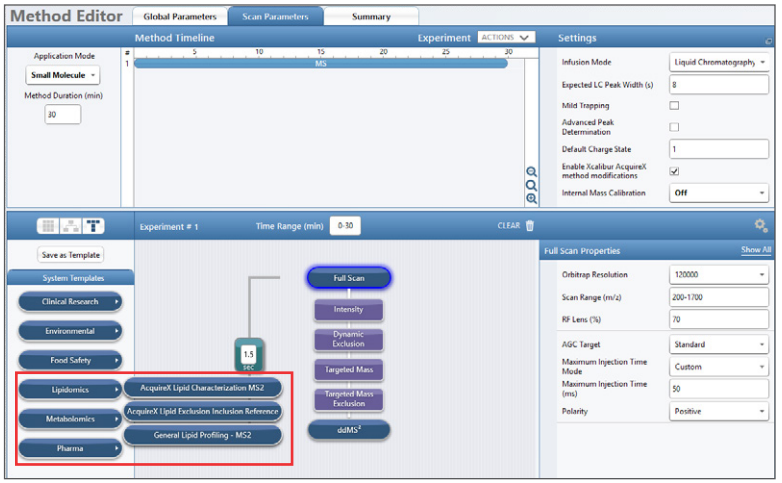


“Using the Orbitrap Exploris 240 mass spectrometer has been extremely user friendly, with one-click calibration and method templates for fast instrument setup. The system reproducibility in terms of mass accuracy and peak integrated area over several sample batches and several days of acquisition has been impressive. This is very important for our large-scale metabolomics projects, enabling minimum post-acquisition data manipulation to yield high quality results.”

Dr. Timothy J. Garrett, Associate Professor, Department of Pathology, Immunology and Laboratory Medicine
Co-Director of the Southeast Center for Integrated Metabolomics, University of Florida

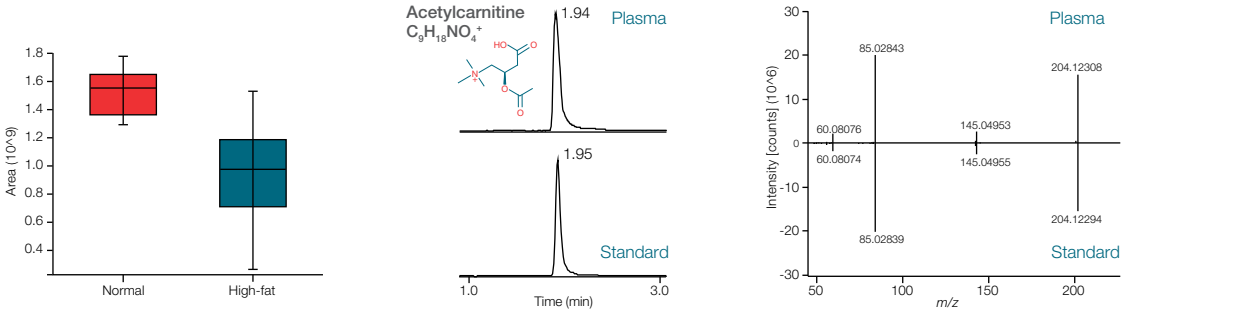
Intuitive user interface

The semi-targeted workflow is powered by ready-to-use Orbitrap Exploris 240 mass spectrometer method templates for analyses of polar metabolites and lipid species. Accessed in the Method Editor, the method templates serve as a good starting point for developing customized methods.

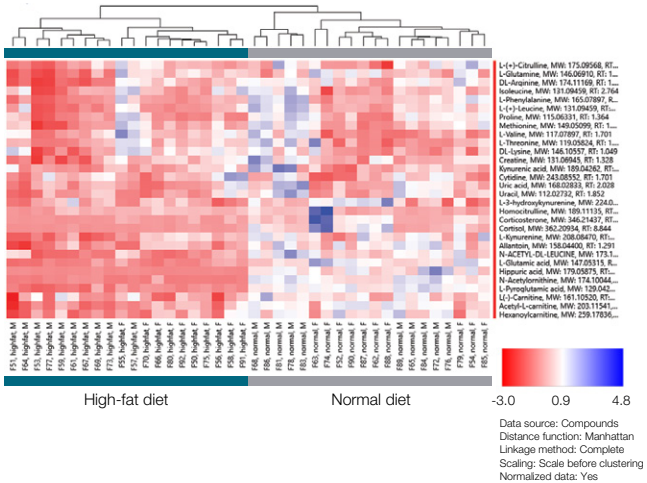


Detection of known and unknown markers

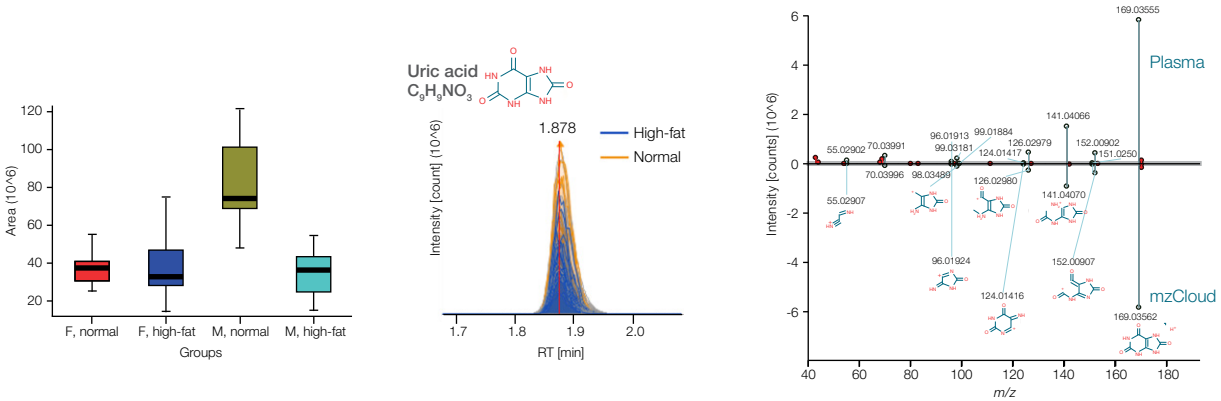
A semi-targeted workflow was developed for detection of known obesity markers with simultaneous comprehensive metabolic phenotyping of mouse plasma samples. Over 4,000 metabolites were detected, and statistical analysis revealed that diet and sex contributed to significantly different metabolite levels.



Targeted analysis of known biomarkers. Changes in acetylcarnitine abundance were accurately quantified in mice fed a normal vs. high-fat diet (left), identified in plasma with accurate mass and retention time (center), and MS^2 spectrum (right) matching the authentic standard.



Benefit of semi-targeted approach. Combining data from known and unknown discriminants provided a comprehensive picture of the metabolic effects of a high-fat diet.



Unbiased discovery of unknown biomarkers. The unknown *m/z* 169.0356 at 1.88 min, was down-regulated in male mice under a high-fat diet and putatively annotated as uric acid ($C_5H_4N_4O_3$) by a high-confidence MS^2 spectral match from the mzCloud mass spectral library.

Thermo Scientific™ mzVault™ library

Create, modify, and customize mass spectral libraries of UHRAM data

Library application for semi-targeted and targeted analysis of metabolites using standard reference compounds.

Thermo Scientific™ mzCloud™ mass spectral library

Increase confidence in unknown annotation and identification

The gold standard in mass spectral libraries for annotation and identification of known and unknown metabolites.

Thermo Scientific™ Compound Discoverer™ software

The ultimate toolbox to analyze untargeted metabolomics data

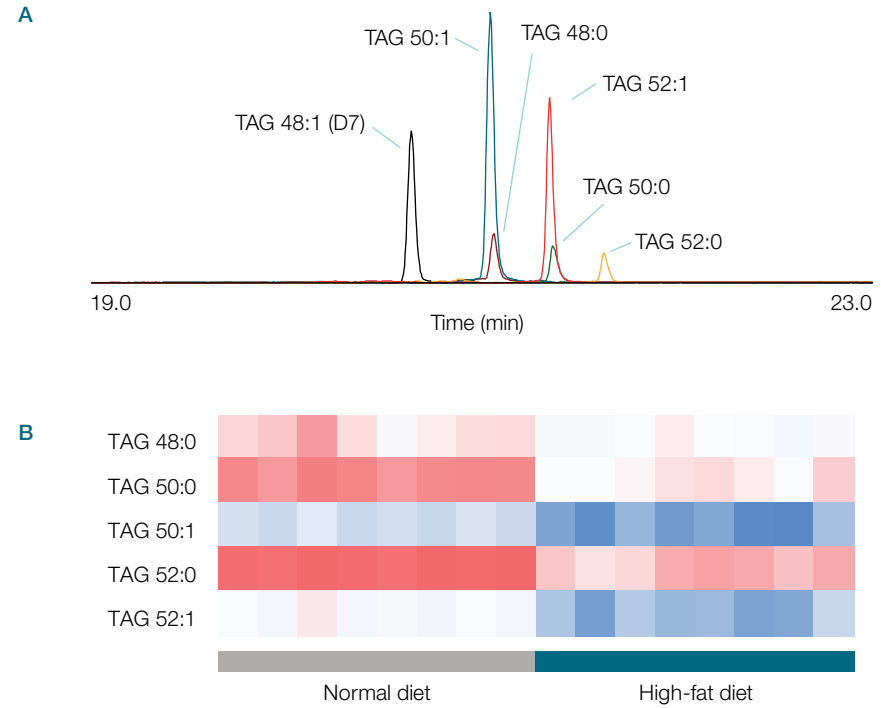
Compound Discoverer software supports untargeted metabolomics: differential analysis, statistics, elemental composition determination, database, pathway and mass spectral library searches and stable isotope labeling.

Targeted metabolomics: the new standard

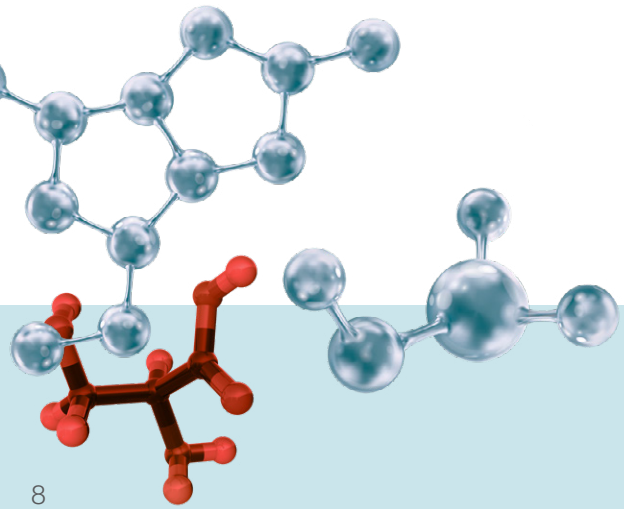
Targeted metabolomics aims to provide quantitative information for metabolites and lipids. Depending on the goal of the experiment, this may include relative or absolute quantitation using nominal mass or high-resolution mass spectrometers. At Thermo Fisher Scientific, we have invested in developing leading-edge software and workflows that, in combination with our robust instrumentation, are the new standard in quantitative metabolomics.

UHRAM targeted profiling using Orbitrap instruments

Targeted profiling requires high sensitivity, dynamic range, and selectivity due to the high complexity of sample extracts. In addition to these attributes, Orbitrap-based instruments provide robust ultra-high resolution allowing extraction of ion chromatograms using a very narrow mass window thus greatly reducing interferences from chemical backgrounds. All these factors combined make an Orbitrap mass spectrometer the perfect choice for targeted profiling of metabolites.



UHRAM targeted profiling of triglyceride levels in female mice fed a high-fat diet using an Orbitrap Exploris 240 mass spectrometer. Lipids were accurately and confidently quantitated with full scan 120K resolution. [A] Extracted ion chromatograms showing separation of target lipids and d7-48:1 TAG internal standard in a pooled sample. [B] Heatmap illustrating the increased levels of triglycerides (blue) in female mice fed a high-fat diet compared to a normal diet. Lipids were quantified using TraceFinder software at the "sum composition" level.



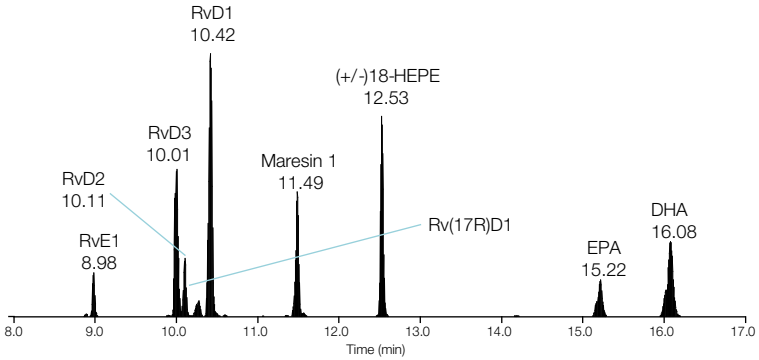
Thermo Scientific™ TraceFinder™ software

Data analysis for quantitation, semi-targeted profiling, and QA/QC monitoring

TraceFinder software supports absolute and semi-quantitative approaches with customizable options for different user experience levels. Target metabolites with accurate mass, isotopic pattern, and retention time. Confirm identifications with mzVault MS^2 spectral library matching.

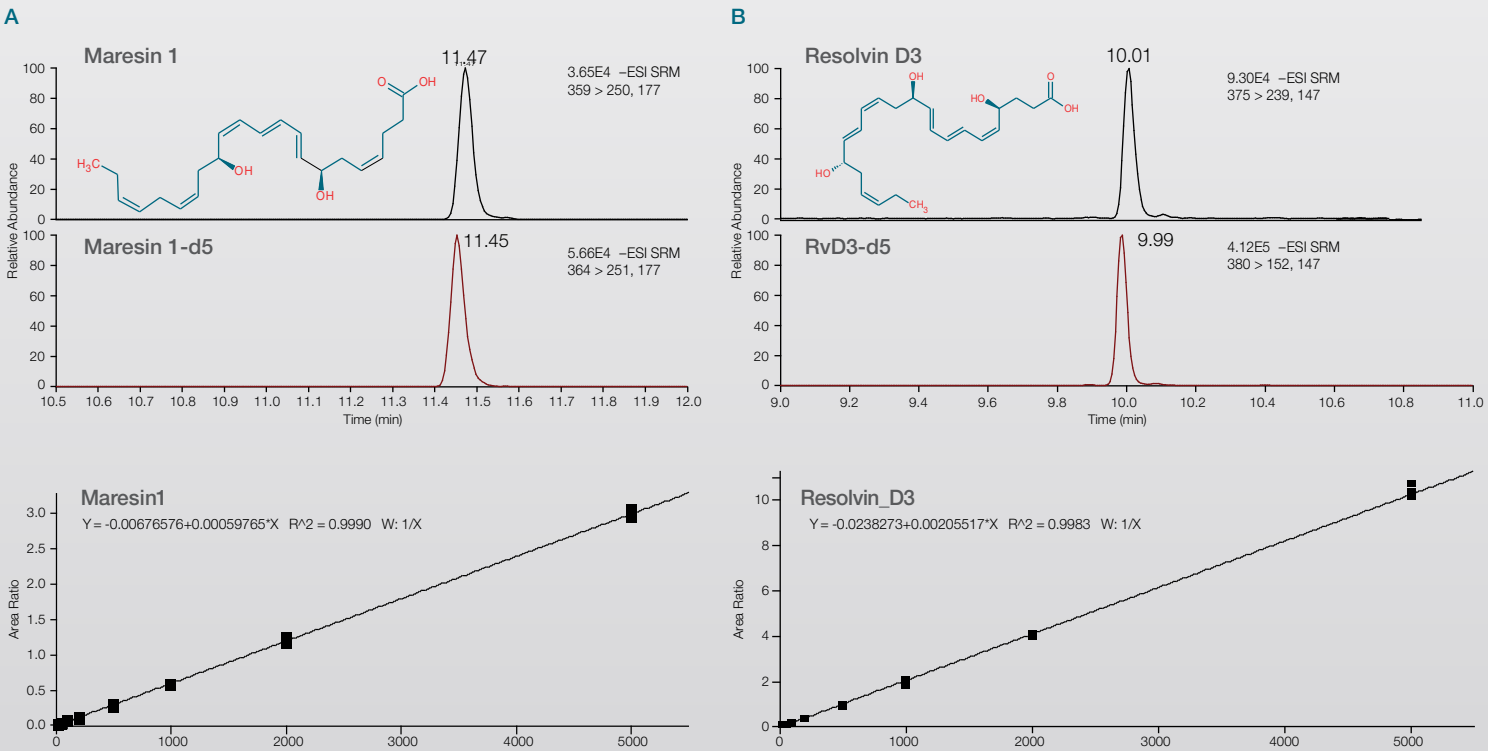
Targeted quantitation by triple quadrupole LC-MS²

Routine analysis of lipid mediators, resolvins, and hydroxylated polyunsaturated ω-3 fatty acids is challenging due to the presence of many isomeric species and their low concentration in biological fluids. For assays demanding ultimate sensitivity, Thermo Scientific™ Vanquish™ UHPLC separation and Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer selected reaction monitoring (UHPLC-SRM) detection is an ideal choice for targeted, absolute quantitation of polar metabolites and lipids.



Representative total ion current of lipid mediator standards. Negative polarity analysis of Resolvin E1, D1, (17R) D1, D2, D3, Maresin 1, 18-hydroxy eicosapentaenoic acid (18-HEPE), docosahexaenoic acid (EPA), and docosahexaenoic acid (DHA).

Vanquish UHPLC system and TSQ Altis LC-MS system.



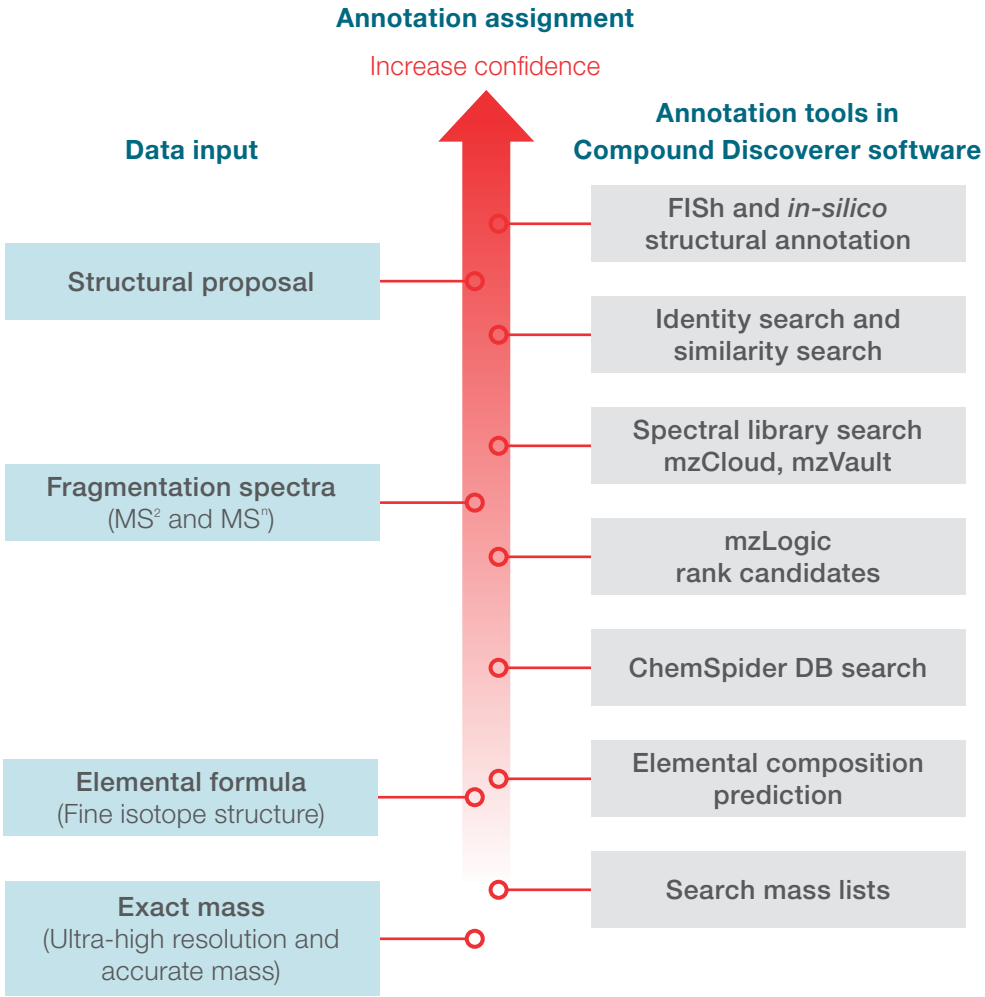
Calibration curve from 25 to 5,000 pg/mL. [A] SRM results for 5,000 pg/mL Maresin 1 and d5-MaR1; [B] SRM results for 5,000 pg/mL Resolvin D3 and d5-RvD3. Data courtesy of Professor Timothy Garrett, University of Florida.

Transform mass spectra into biological knowledge

Untargeted metabolomics provides the broad metabolome coverage needed for hypothesis generation. For untargeted analysis to provide biological insights of the metabolome and associated biology, raw mass spectra must be transformed into meaningful data, including the ability to confidently annotate and identify unknown compounds. Thermo Fisher Scientific leads small-molecule identification with an unprecedented workflow to confidently annotate unknowns by combining high-quality Orbitrap mass spectrometer data, intelligent acquisition, and powerful processing software.

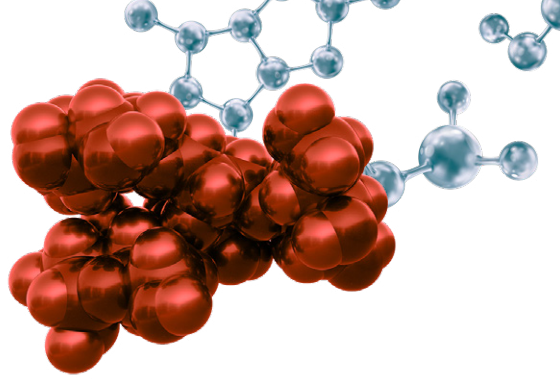
Increase annotation confidence with Compound Discoverer software

Multiple analytical measurements reduce the number of candidate structures and increases annotation confidence. The annotation tools in Compound Discoverer software support accurate mass, isotopic pattern, and fine structure to confirm elemental formulas for database searching. Fragmentation spectra can be searched against the mzCloud mass spectral library.

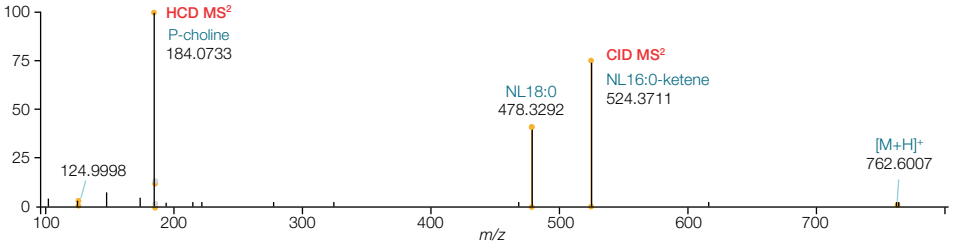


Multiple dissociation modes for structure elucidation and confident annotation

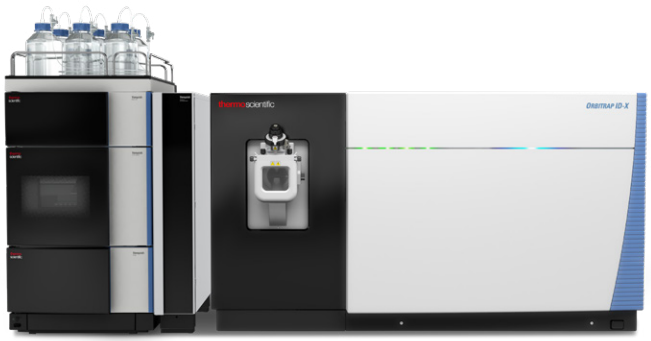
Structural elucidation of unknowns and confident annotation of metabolites is facilitated using stepwise MS^n and complementary fragmentation methods available on the Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass specrometer, including collision induced dissociation (CID) and higher collision energy dissociation (HCD).



Improved annotation of lipids by product ion dependent acquisition



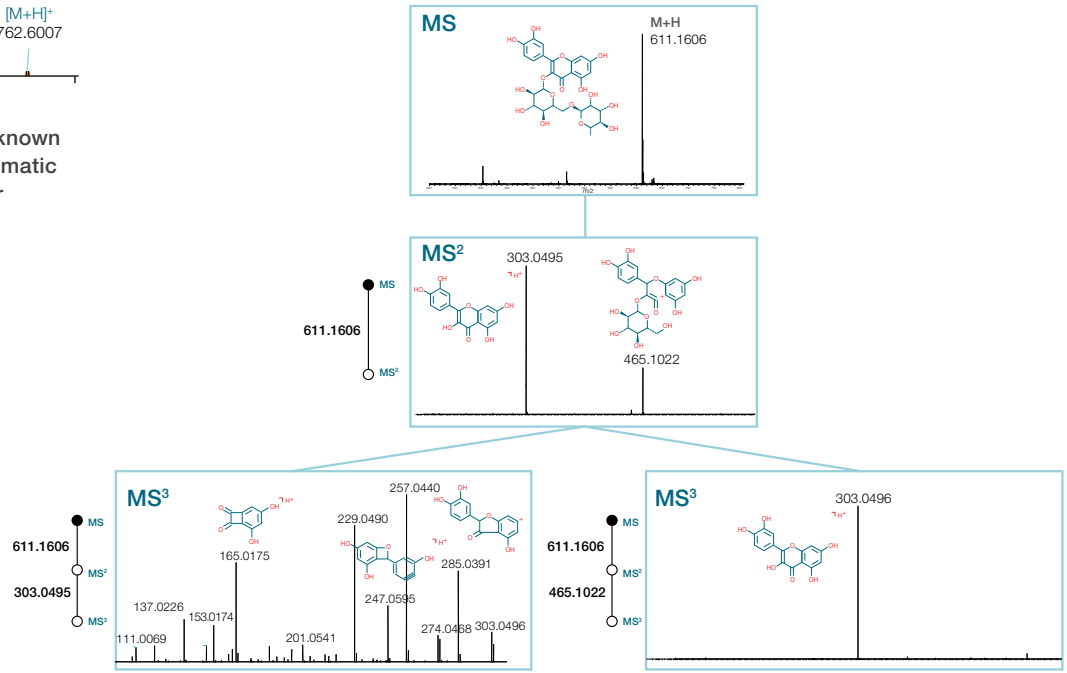
PC 34:0 annotation with LipidSearch software using HCD and CID. HCD MS^2 of an unknown lipid produced the characteristic phosphatidylcholine product ion *m/z* 184.0733. Automatic triggering of complementary CID fragmentation generated additional product ions for confident annotation using LipidSearch software.



Vanquish UHPLC system and Orbitrap ID-X LC-MS system for untargeted metabolomics and lipidomics.

Improved annotation of flavonoids by neutral loss dependent CID MS^n acquisition

Improved annotation of flavonoid metabolites was obtained by searching mzCloud MS^n substructural trees using Mass Frontier software to detect known CID product ions and neutral losses (NL).



Thermo Scientific™ LipidSearch™ software

Untargeted analysis using high-resolution LC-MS^n data for confident lipid annotation and relative quantitation

Predicts lipid fragmentation, identifies and correlates lipids in complex data sets, and offers tools for exporting filtered results.

Thermo Scientific™ Mass Frontier™ spectral interpretation software

Search mzCloud mass spectral library; curate MS^n libraries

Provides substructure/subtree searches, FISh, fragment ion search, and mzLogic for structure proposals.

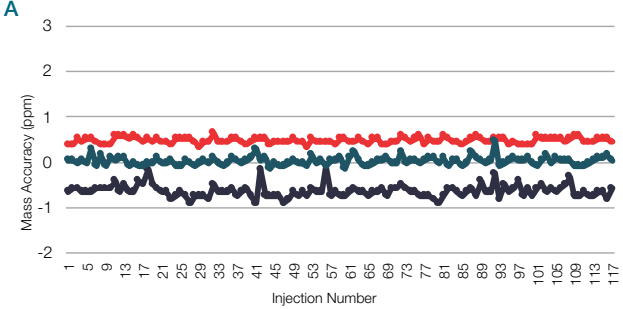
Highest quality and reliability of data

Ultra-high resolution Orbitrap MS technology provides robust, highest-quality metabolomics data sets. Adding QA/QC measures to metabolomics workflows provides increased confidence in metabolomics data, from small-scale projects to large sample cohorts, garnering true biological insights.

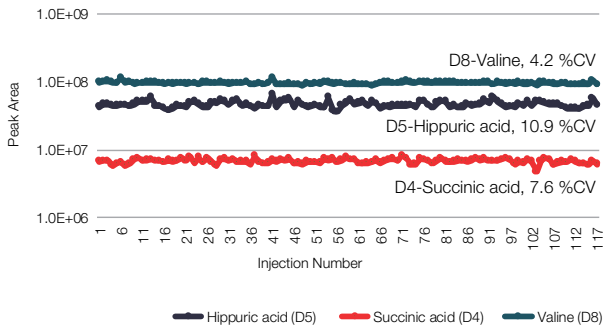
Exceptional LC-MS instrument performance

Mass accuracy and peak area reproducibility of internal standards demonstrate the excellent stability of the Vanquish UHPLC system coupled to the Orbitrap Exploris 240 mass spectrometer.

A



B

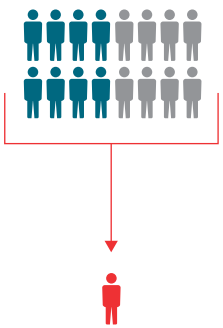


[A] Mass measurement accuracy within ±1 ppm for D5-Hippuric acid, D4-Succinic acid and D8-Valine internal standards during 117 injections of plasma extract. [B] Excellent peak area reproducibility for D5-Hippuric acid, D4-Succinic acid and D8-Valine internal standards during 117 injections of plasma extract. Data courtesy of Dr. Elizabeth O'Day at Olaris, Inc.

QA/QC for increased confidence and data reliability

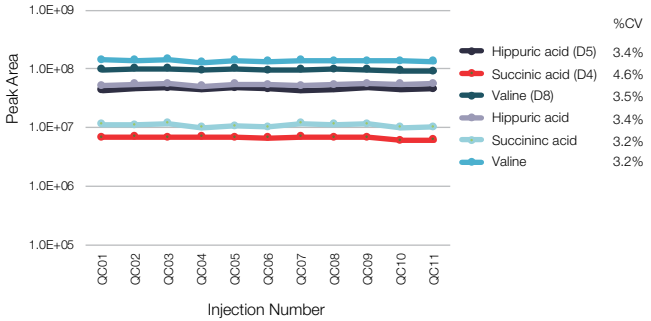
QA/QC measures such as internal standards and pooled QC samples ensure instrument performance and increased confidence in data reliability for metabolomics studies.

Comparison groups

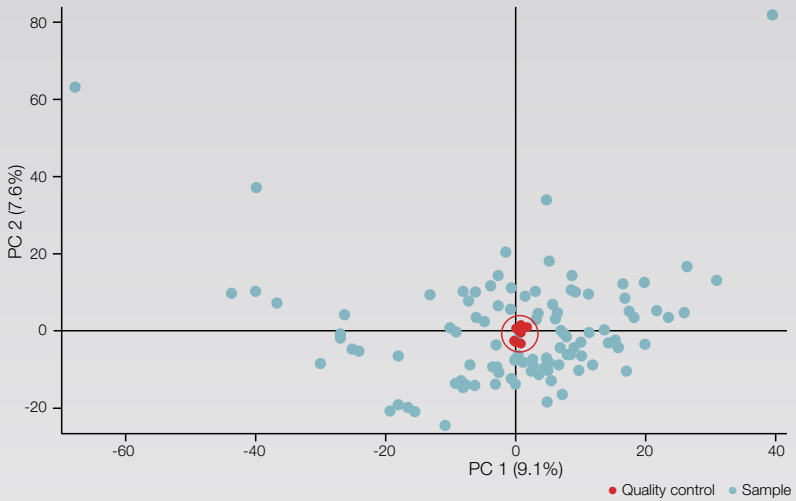


The pooled QC sample was injected repeatedly for every 15 experimental samples.

Internal standards and corresponding endogenous metabolites



Pooled QC samples and internal standards confirmed highly reproducible data with no outliers and good CV's.



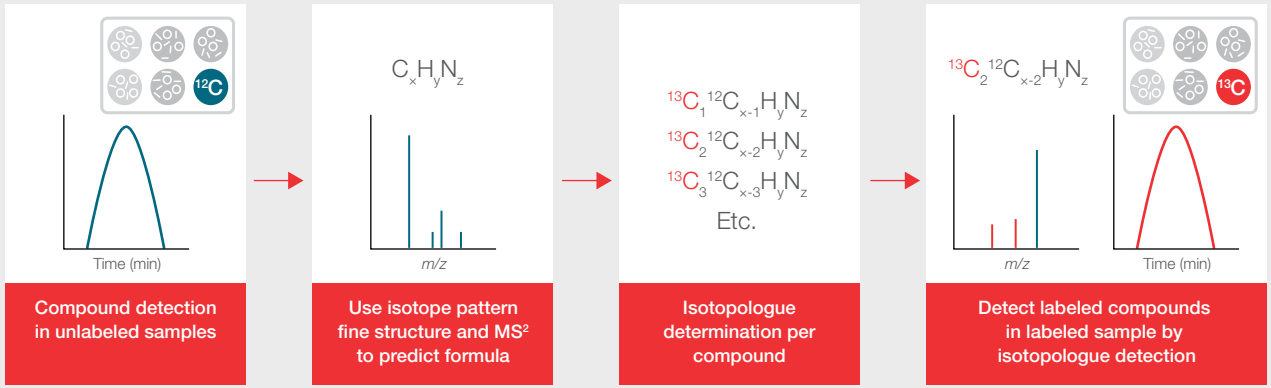
Principal component analysis for monitoring quality control

Principal component analysis (PCA) showed the pooled QC samples clustered tightly in the plot center, indicating reliable data quality.

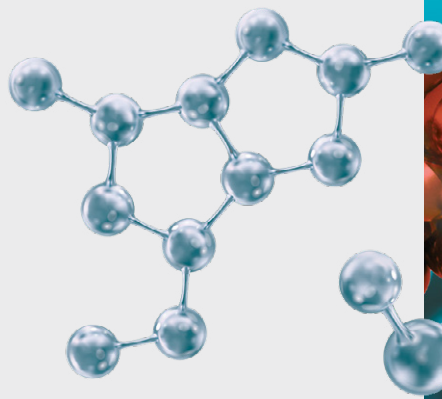
Understand underlying mechanisms with stable isotope labeling

Stable isotope labeling (SIL) allows metabolic substrates to be followed through downstream biochemical reactions, thereby providing insights into the dynamics of cell metabolism. Orbitrap mass spectrometers are ideally suited to SIL techniques due to their leading UHRAM capability. Together with Compound Discoverer software, Orbitrap instruments enable the detection of label incorporation without a priori biological knowledge, making it a discovery tool. Analytical methods employing SIL typically focus on defined pathways of interest using a targeted approach.

SIL workflow enabled by Compound Discoverer software



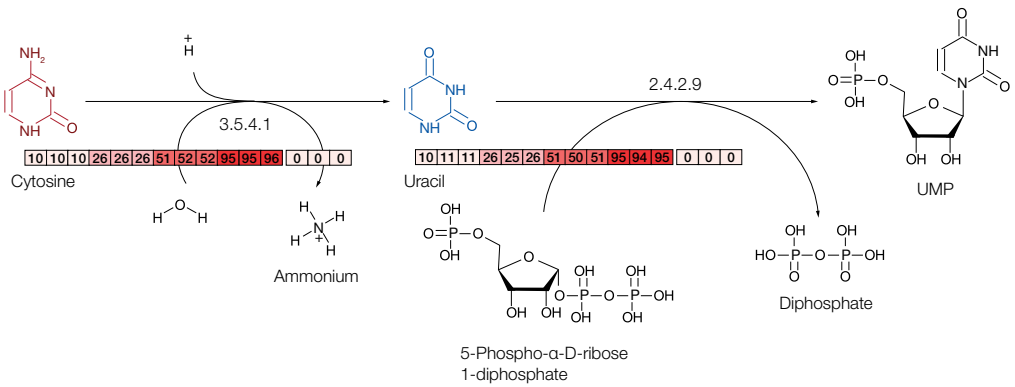
Compound detection in unlabeled samples	Use isotope pattern fine structure and MS² to predict formula	Isotopologue determination per compound	Detect labeled compounds in labeled sample by isotopologue detection



Reproducibility of isotopologue determination using an Orbitrap Exploris 240 mass spectrometer

Uracil	^{13}C-Isotopologue
M+0	$C_4H_4N_2O_2$
M+1	$^{13}CC_3H_4N_2O_2$
M+2	$^{13}C_2C_2H_4N_2O_2$
M+3	$^{13}C_3CH_4N_2O_2$
M+4	$^{13}C_4H_4N_2O_2$

Exchange Rate [%]					Rel. Exchange [%]	Triplicate Injections	% CV
0	1	2	3	4			
100	0	0	0	0	0	0% Label Incorporation	
100	0	0	0	0	0		
100	0	0	0	0	0		
89	0	0	1	9	10	10% Label Incorporation	5.4%
89	0	0	1	10	11		
89	0	0	1	10	11		
73	0	0	4	23	26	25% Label Incorporation	1.2%
74	0	0	4	22	25		
73	0	0	4	23	26		
46	0	1	7	46	51	50% Label Incorporation	1.1%
47	0	1	7	45	50		
47	0	1	8	45	51		
1	0	1	14	84	95	100% Label Incorporation	
2	0	1	14	83	94		
1	0	1	14	84	95		



Reproducibility of isotopologue determination at different levels of ^{13}C incorporation. Uracil contains four carbons with a potential of five isotopologues. Relative exchange was computed for each replicate injection while displaying incorporation for individual isotopologues. Low % CVs were observed for all ratio mixtures.

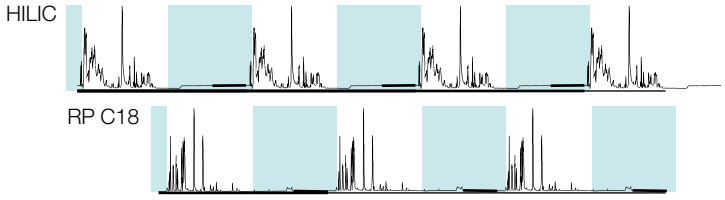
An untargeted approach with SIL allowed the detection of unexpected metabolites. Cytosine and Uracil were detected in the pyrimidine nucleobase salvage pathway using Metabolika pathways. The relative exchange is shown overlaid for both detected metabolites in this pathway.

Comprehensive metabolomics coverage with orthogonal techniques

Comprehensive metabolomics requires alternative strategies to separate metabolites with differing physico-chemical properties. Thermo Fisher Scientific products deliver a breadth in analyte separations with dual liquid and gas chromatography systems. When combined with Orbitrap mass spectrometers, our technologies enable deeper metabolome analysis with excellent robustness and reproducibility, providing novel results for high-impact discoveries.

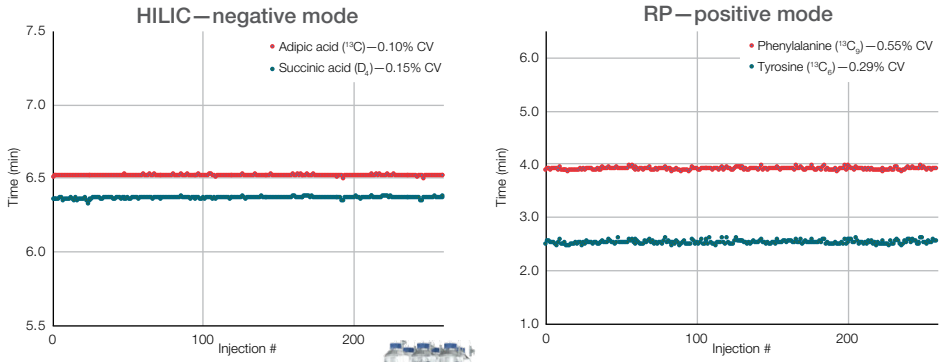
Maximum productivity with the Vanquish Duo system

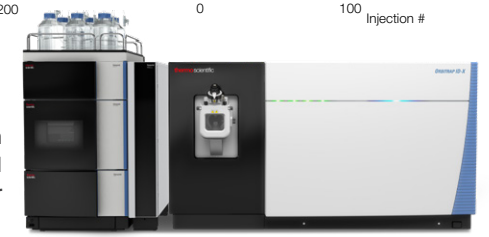
The Thermo Scientific™ Vanquish™ Duo UHPLC system has two independent flow paths that provide reproducible dual orthogonal separations, enabling LC-MS data to be acquired with high throughput.



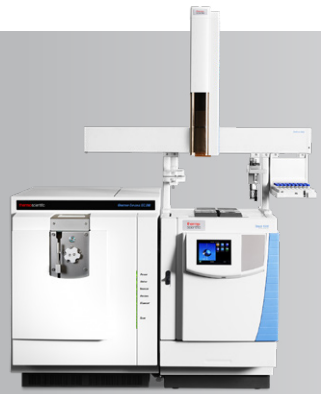
Reversed phase (Thermo Scientific™ Hypersil GOLD™ column) and HILIC (Thermo Scientific™ Accucore™ Amide column) LC-MS data was acquired using a Vanquish Duo system and an Orbitrap ID-X Tribrid mass spectrometer. Throughput increased by 30%, while maintaining retention time stability and chromatographic robustness.

Reproducibility of HILIC and RP retention times





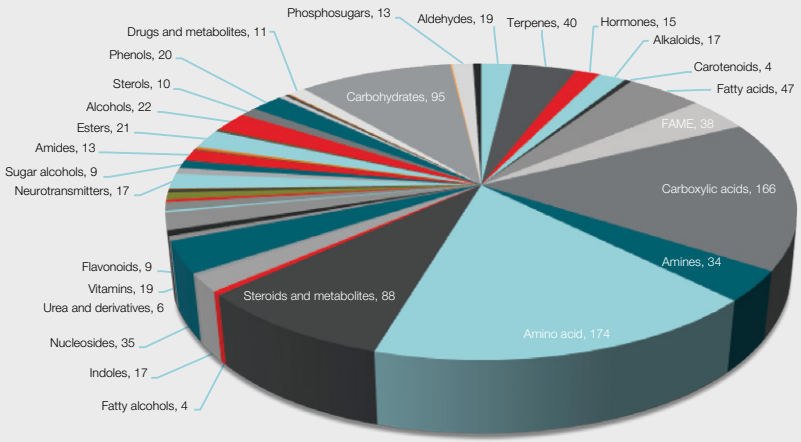
Vanquish Duo UHPLC system and Orbitrap ID-X Tribrid mass spectrometer



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

Explore deeper into the metabolome

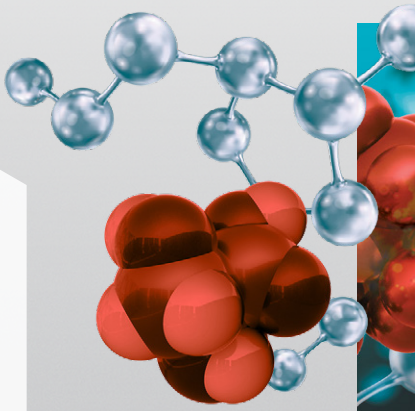
The Thermo Scientific™ Orbitrap Exploris™ GC 240 mass spectrometer provides unrivaled power to discover metabolites that go unnoticed using other GC-MS technologies. Identify and quantify more metabolites with the combination of high-resolution gas chromatographic separation, high dynamic range HRAM acquisition, and identification powered with Compound Discoverer or TraceFinder software.



Identify and quantify more metabolites. The Thermo Scientific™ Orbitrap™ GC-MS HRAM Metabolomics Library contains retention-indexed unique entries from more than 1000 metabolites, providing broad coverage of primary and secondary metabolites, including volatiles in plants, animals, and microbes.

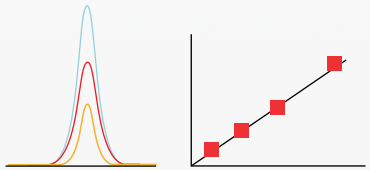
Streamline your path to metabolomics insights

Thermo Fisher Scientific has invested heavily in developing metabolomics software that lives up to the high standards set by our leading instrumentation, promising the same standards of quality, usability, and data integrity. Now you can have the total package needed to perform increasingly complex analysis and lead the way to high-impact discoveries. Our suite of integrated applications is built to take you quickly from data acquisition to interactive analysis and interpretation of results. Customize your workflow with flexible solutions and simplify analysis of large-scale data.



TraceFinder software

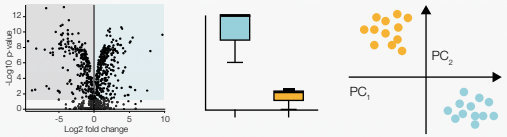
Rapidly detect targeted knowns
for QA/QC, profiling, and absolute quantitation



Tf

Compound Discoverer software

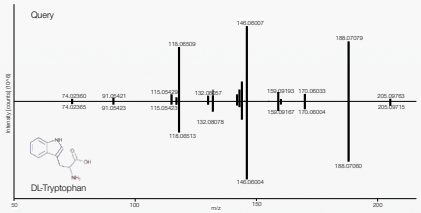
Ultimate toolbox for complete
analysis of untargeted metabolomics data



Cd

mzCloud mass spectral library

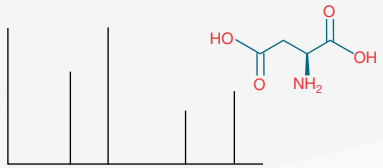
MS^n reference spectra for confident unknown
annotation and identification



m/z CLOUD

mzVault library

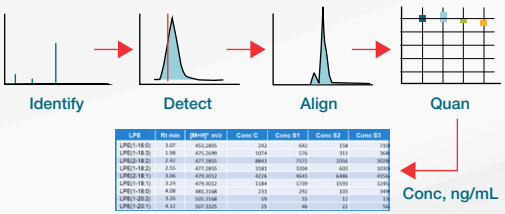
Create mass spectral libraries for
metabolite annotation and identification



mV

LipidSearch software

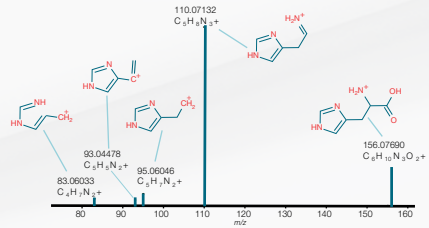
Lipid-specific untargeted analysis for
confident lipid annotation and quantitation



Ls

Mass Frontier software

MS^n spectral tree searching, interpretation
and in-silico fragment ion prediction



Mf

thermo scientific

Almanac web-based monitoring and management

Stay connected to your science. See how the Thermo Scientific™ Almanac™ application can help you get the most out of your instruments.

thermofisher.com/almanac

Technical and online support: peak performance for your instruments

Helping you keep your instruments running at peak performance is our goal. Whether you're looking for an instrument manual or spare parts, want to submit a repair request, or check on the status of your warranty or service contract, we have every support option you're looking for.

thermofisher.com/technicalresources

Protecting your investments: unparalleled laboratory services

Unity™ Lab Services provides a single source for integrated lab service, support, and supply management. Our customized service offerings and world-class service experts have the flexibility and experience to address your laboratory's needs. We provide a complete portfolio of services and support solutions designed to help you improve productivity, reduce total cost of ownership, and ensure performance throughout your laboratory.

unitylabservices.com

Thermo Scientific Data Acquisition software
thermofisher.com/InstrumentControl

Thermo Scientific Data Processing software
thermofisher.com/DataProcessing

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

ThermoFisher SCIENTIFIC