



**Thermo Scientific
Orbitrap Velos Pro**
Hybrid Mass Spectrometer



**Benchmark platform for confident
Structural Elucidation**

Thermo
SCIENTIFIC

Take the Express Route to Success

The Thermo Scientific Orbitrap Velos Pro hybrid mass spectrometer blends the unsurpassed speed and sensitivity of the Thermo Scientific Velos Pro dual-pressure ion trap with the high resolution and outstanding mass accuracy of Orbitrap™ technology.

The high mass accuracy increases the speed and confidence of protein identification in complex samples by minimizing false positives. The high resolution provides certainty in analytical results by enabling molecular weight determination for intact proteins and in-depth analysis of isobaric species.

The Orbitrap Velos Pro

Hybrid MS provides

- More unique proteins identified in a single experiment
- Multiple fragmentation techniques deliver optimal structural characterization
- Rapid quantitation of low level isobarically labeled peptides
- Precise intact protein analysis workflow-driven data mining
- Workflow-driven data analysis



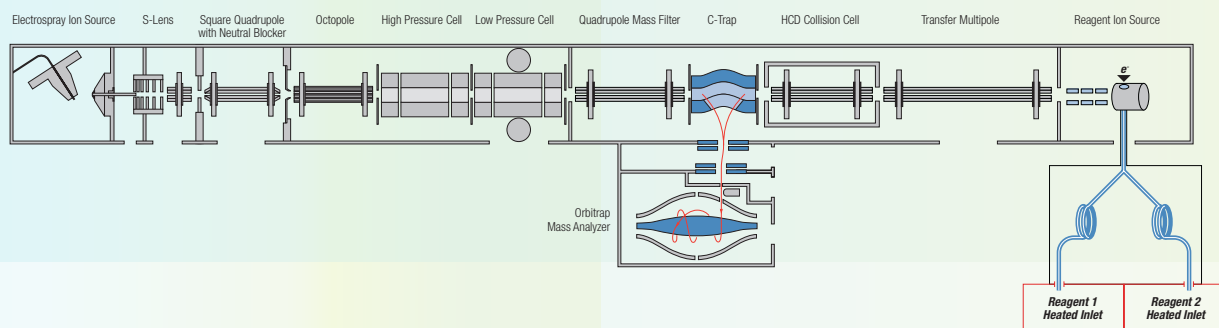
The HCD (higher energy collisional dissociation) cell in the Orbitrap Velos Pro mass spectrometer is more efficient, resulting in improved quantitation of isobarically labeled peptides, such as applications requiring Tandem Mass Tags (TMT). Electron Transfer Dissociation (ETD) generates complementary information for highly sensitive Post Translational Modification (PTM) analyses and *de novo* sequencing.

Whether your challenge is discovering low-abundance proteins or profiling and identifying metabolites in complex biological matrices, the Orbitrap Velos Pro hybrid MS lets you meet it with an unrivaled combination of mass spectrometric performance, speed, flexibility, and confidence.

Orbitrap Velos Pro Hybrid MS

- Generation II ion optics with neutral blocking technology for improved robustness
- Faster scanning without loss of sensitivity means more compounds identified and characterized in less time
- Novel new detection system with higher linear dynamic range
- High resolution and outstanding mass accuracy provide greater analytical confidence without the need for an internal lock mass
- More efficient HCD improves quantitation of isobarically labeled peptides
- Wide selection of fragmentation modes – CID, HCD, PQD, and optional ETD – allows precise matching of technique and analytical objective

Schematic of the Orbitrap Velos Pro Hybrid Mass Spectrometer with ETD



Identify More Proteins

with Increased Sequence Coverage in Less Time

The comprehensive analysis of complex protein samples is one of the most challenging tasks in proteomics. The Orbitrap Velos Pro hybrid MS combines the best aspects of ion trap and Orbitrap mass analyzer performance to give researchers the confidence and power to tackle the full complexity of the proteome.

Parallel Acquisition

Increases Throughput

The ability to acquire data in parallel in the ion trap and in the Orbitrap mass analyzer increases the amount, quality, and variety of data acquired in a given time. It provides high-throughput sequencing of a quality that no other mass spectrometers can match.

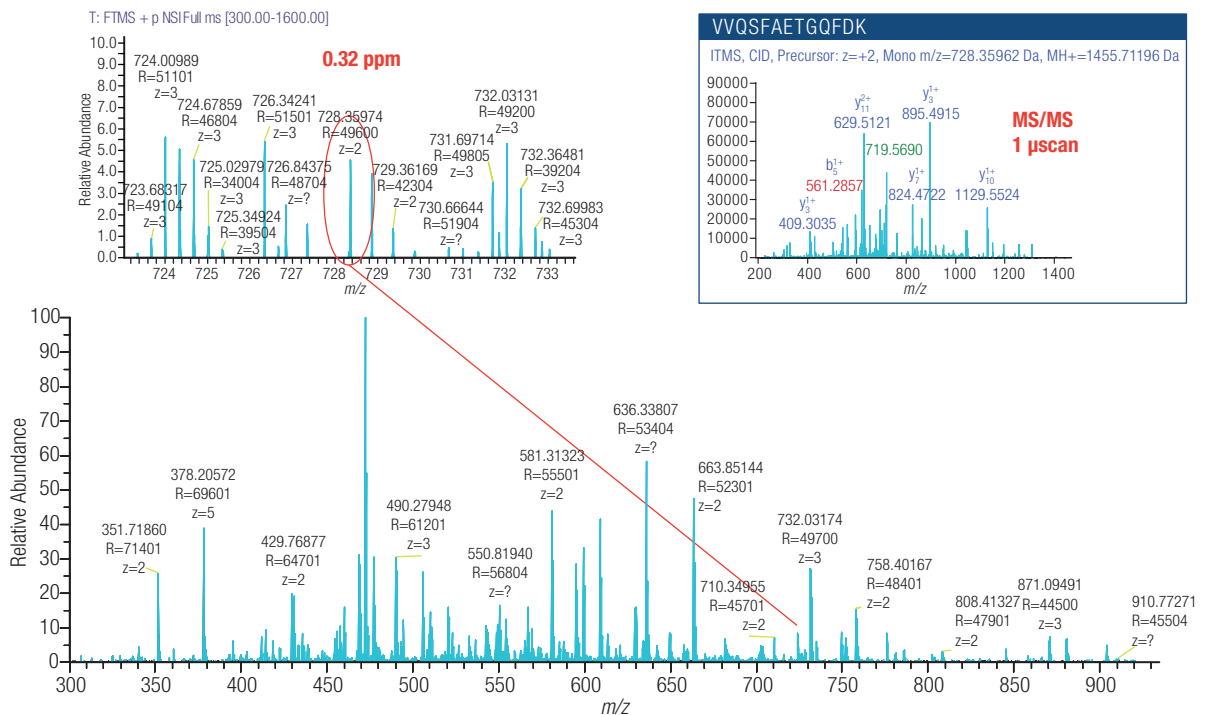
Sensitivity Enables

Detection of Low-Level Proteins

Ion trap detection provides the outstanding sensitivity and superior dynamic range required to find low-level biomarkers or post-translational modifications of low stoichiometric abundance. These species would be lost to description in a lesser instrument.

Targeting Small but Significant Features

in Complex Full Scan Spectra



The speed, sensitivity, and mass accuracy of the Orbitrap Velos Pro hybrid MS deliver high-quality MS and MS/MS spectra needed for high-confidence identification of low-level analytes.

The technological advances are improved ion transmission for greater sensitivity, faster scanning without loss of sensitivity, and greater fragmentation efficiency. All contribute to the identification of more proteins in less time.

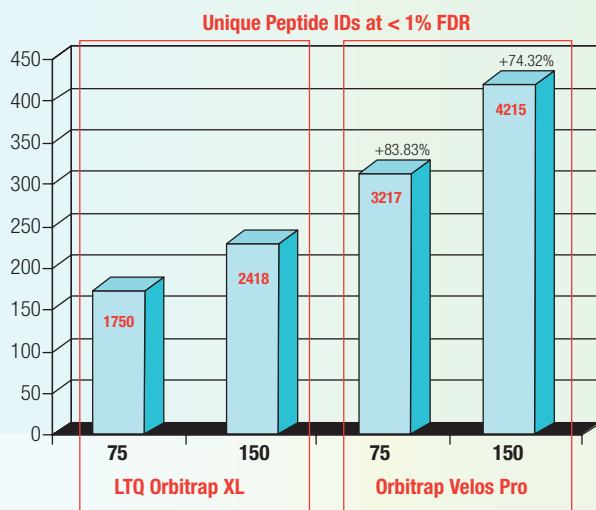
Specificity

for Confident Identification

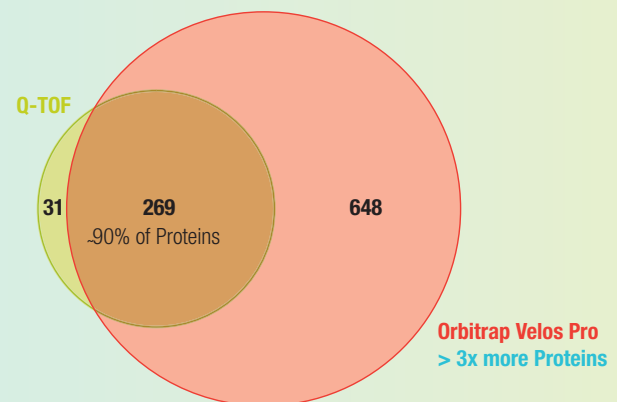
The high mass accuracy and mass resolution of Orbitrap technology yield unrivaled specificity and more confident identifications. It is particularly beneficial for complex searches involving post-translational modifications and non-specific cleavages.

Orbitrap Velos Pro Hybrid MS

- More IDs in less time – Top 12 experiment in 1.7 sec
- Mass Accuracy for minimized false positive rate for Protein ID
- Improved duty cycle for increased productivity for Protein ID experiments
- Sensitivity resulting in detection of low-abundance peptides/proteins
- Dual-pressure Ion Trap Technology:
 - High Pressure Cell for fragmentation efficiency leading to more peptide identifications
 - Low Pressure Cell for increased scan speed resulting in higher numbers of identified peptides
- Thermo Scientific Proteome Discoverer software for comprehensive workflow-driven data mining



In identical analyses performed by hybrid MS, the Orbitrap Velos Pro identifies almost twice as many unique peptides as the previous state-of-the-art Thermo Scientific LTQ Orbitrap XL.



Analysis of 1 µg of *C. elegans* digest demonstrates the superiority of the Orbitrap Velos Pro hybrid MS for protein identification: it identified more than 3x the number of proteins identified by a Q-TOF and identified ~90% of the same proteins as the Q-TOF.

Rapid, Comprehensive, Quantitative Cellular Proteome Analysis

Insights into biological systems frequently require comparison of protein abundances between multiple biological states. The unsurpassed no-compromise performance of the Orbitrap Velos Pro hybrid MS make it the ideal tool for common quantitation strategies, both labeled and non-labeled, relative and absolute.

Stability and Mass Accuracy

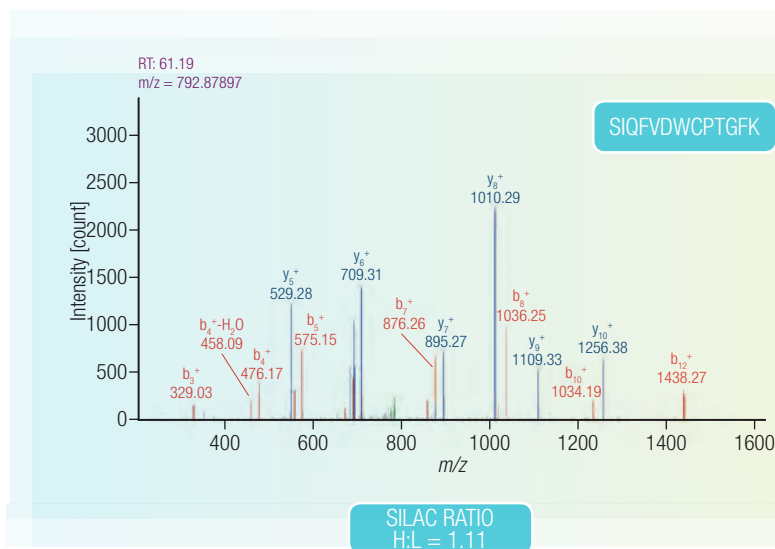
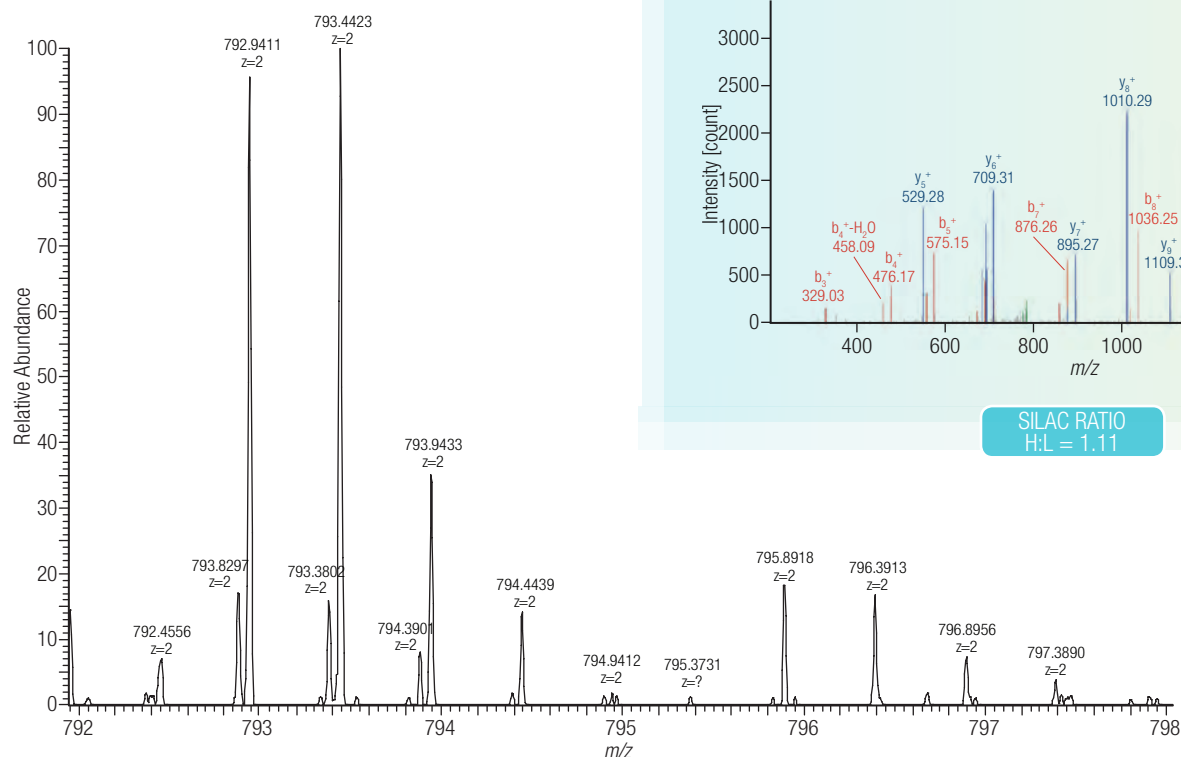
for Label-Free Quantitation

Due to its simplicity and cost-effectiveness, label-free quantitation has gained favor for large-scale biomarker discovery and studies of disease-related changes, protein interaction networks, and cell signaling dynamics. The Orbitrap Velos Pro Hybrid MS has the stability, mass accuracy, and wide dynamic range that are essential for successful label-free analyses.

Simultaneous Sequence Confirmation

for Unparalleled Confidence

Parallel acquisition allows the simultaneous acquisition of high-resolution data for accurate assessment of relative abundances and of the peptide sequence data that confirms identification. The result is unparalleled confidence in quantitative results.



The Orbitrap Velos Pro hybrid MS reveals even small changes in abundance as demonstrated in this stable isotopic labeling experiment. Parallel acquisition capabilities provide simultaneous relative quantification with sequence confirmation.

High Resolution at Lower Masses

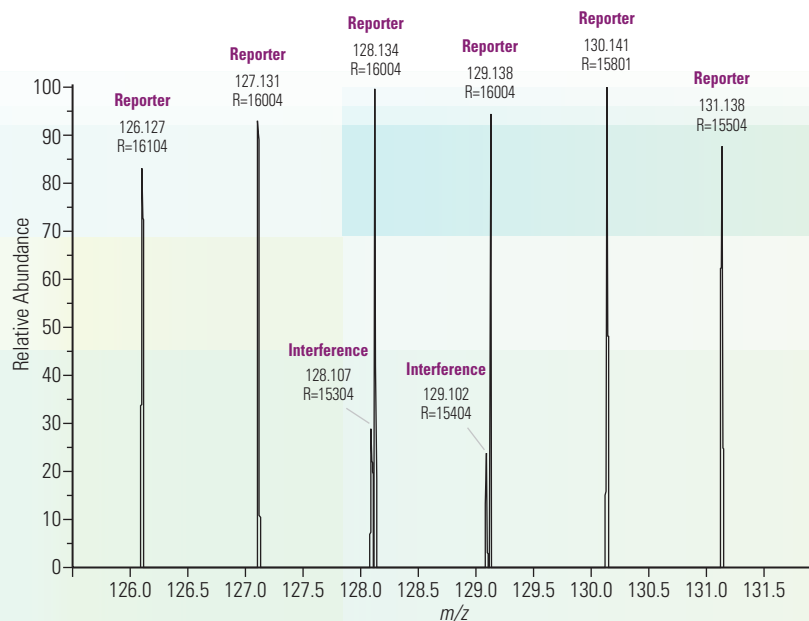
Separates Reporter Ions from Interferences

For multiplexed quantification experiments using isobaric labels, such as Thermo Scientific Isobaric Mass Tagging Kits (TMT), it is essential that the mass spectrometer is able to separate reporter ion signals from interferences. Unlike TOF technologies, which suffer a significant fall-off in mass resolution at lower masses, the Orbitrap technology provides outstanding resolution in the low mass range where reporter ions are found.

The same outstanding mass resolution and mass accuracy allow the Orbitrap Velos Pro MS to distinguish between heavy and light peptide ion pairs and interferences in SILAC experiments.



Isobaric chemical tags are powerful tools that enable concurrent identification and quantitation of proteins in different samples using tandem mass spectrometry.



High mass resolution at low m/z separates reporter ion signal from interferences, providing quantification accuracy and a large dynamic range for relative quantification with isobaric mass tags.

Comprehensive Characterization of Intact Proteins

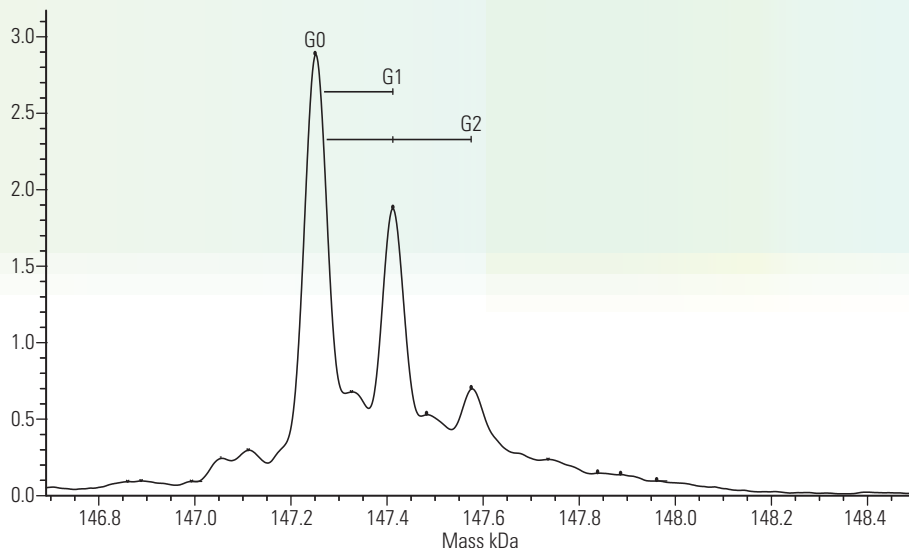
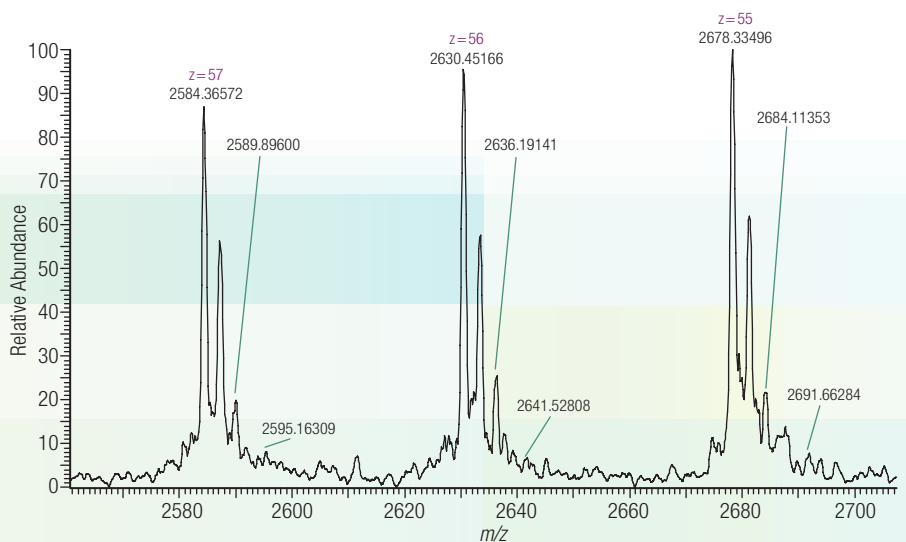
Orbitrap technology reignited general interest in characterization of intact proteins by making ICR-MS levels of performance far more accessible. The performance of the Orbitrap Velos Pro hybrid MS promises even better results for applications such as top-down analysis of monoclonal antibodies.

Benefits of the Orbitrap Velos Pro MS

- Improved vacuum in the Orbitrap for precise intact protein analysis
- Highest resolving power for intact protein analysis molecular weight determination
- Complementary fragmentation techniques – CID, HCD, ETD – for comprehensive sequence coverage of intact proteins

Resolve Highly Charged Intact Proteins

The Orbitrap Velos Pro Hybrid MS provides the high mass resolution (>100,000) and mass accuracy (1 ppm) necessary to accurately measure and identify intact proteins. The improved vacuum system facilitates detection of proteins as large as 150 kDa.



Analysis of immunoglobulin. Top: zoomed section of electrospray mass spectrum of IgG, bottom: deconvoluted spectrum of IgG, with 6 ppm product ion mass accuracy on largest antibody peak.

Complementary Fragmentation Techniques

Access a Wider Range of Proteins

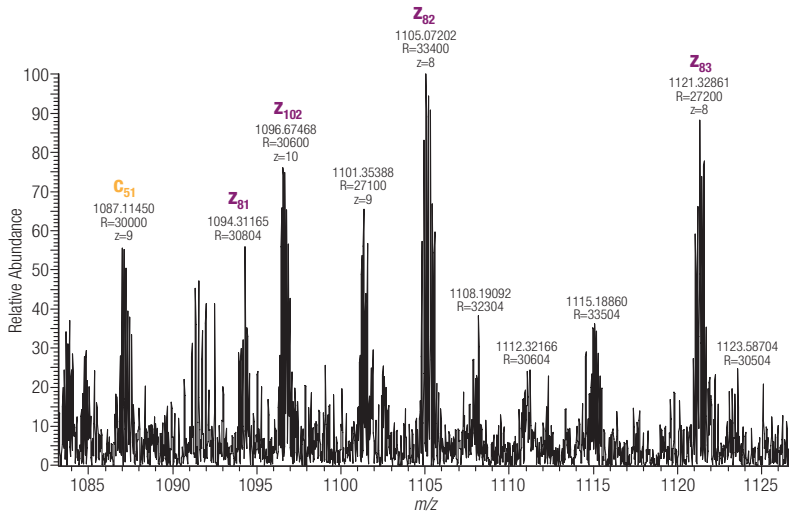
Complementary fragmentation techniques: CID, HCD, and optional ETD, provide optimum fragmentation for a wider range of proteins, including phosphorylated and glycosylated species. In addition, to ensure better fragmentation efficiency and more simple operation for bottom-up approaches, data dependent decision tree (DDDT) capability automates the selection of the best possible fragmentation technique for each peptide.

Proteomics Software

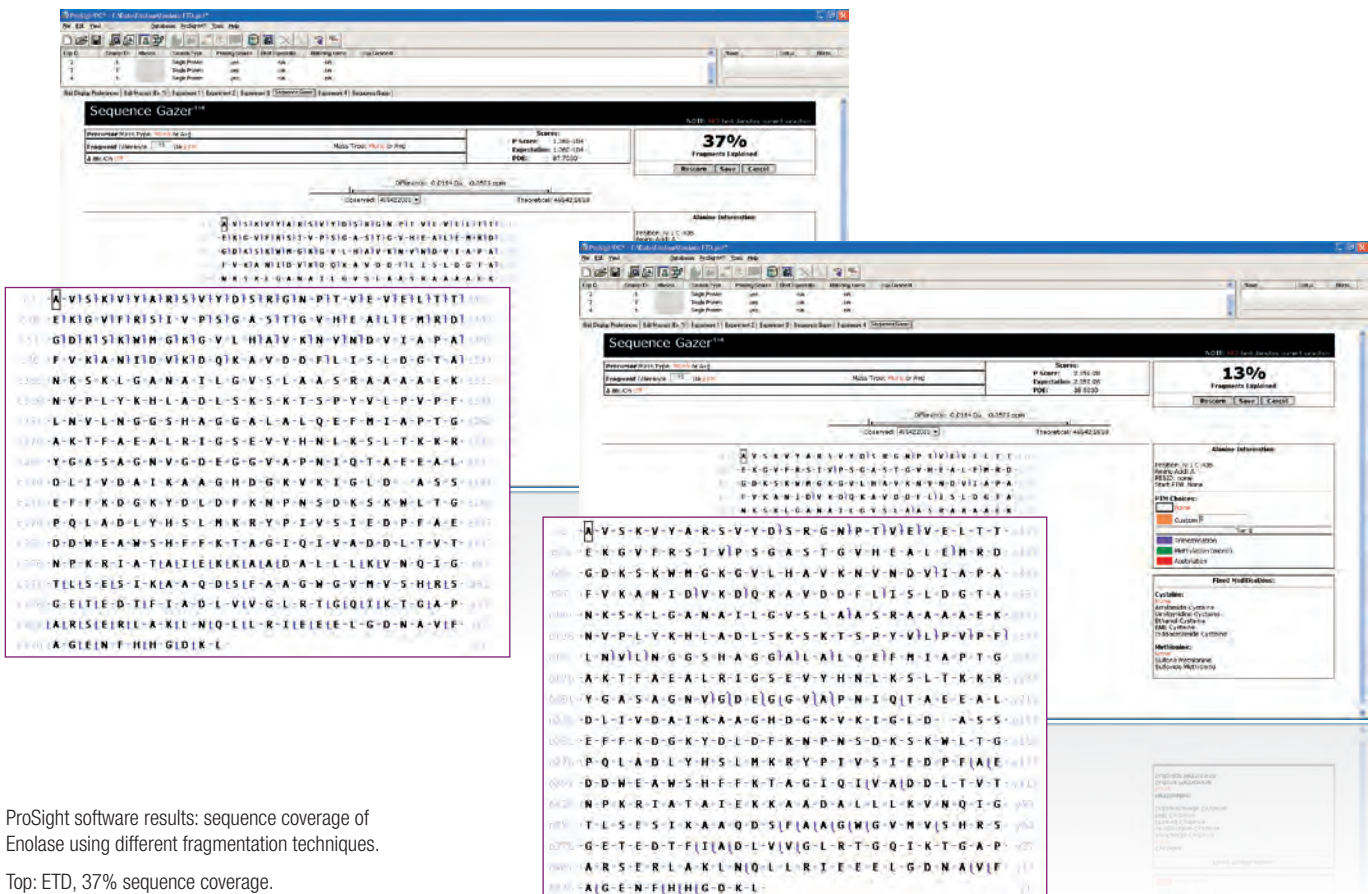
Turns Mass Spectrometry Data into Answers

In addition to the Thermo Scientific Proteome Discoverer software, a number of other software packages facilitate top-down analysis of intact proteins.

Thermo Scientific Xtract and ProMass software assign molecular weights and deconvolute spectra, respectively. Thermo Scientific ProSightPC software takes advantage of high-resolution, accurate-mass MS/MS data to provide more confident identification and characterization.



High-resolution ETD spectrum of Enolase (47 kDa).



Confident Metabolite Identification and Profiling

For small molecule applications, including metabolism and metabolomics, the Orbitrap Velos Pro hybrid MS is the benchmark platform for structural identification and quantitation experiments. Its superior resolving power, mass accuracy, and dynamic range enable researchers to identify low-level metabolites in complex biological matrices with high confidence.

Enhanced MS Performance

Lowers Limits of Detection and Quantitation

The increased ion transmission, improved fragmentation efficiency, and shorter overall cycle times increase the sensitivity 2 - 5 times for lower limits of detection and quantitation. They also improve the quality of the ion-tree experiments for structural elucidation.

MetWorks Software

Accelerates Metabolic Analyses

The Thermo Scientific MetWorks software integrates acquisition, processing, and reporting for metabolic LC/MSⁿ analyses. It simplifies and accelerates the search for expected and unexpected biotransformations. A novel technique of using MSⁿ spectral ion trees in conjunction with *m/z* and RT provides unsurpassed confidence when comparing sample data versus control.

Multiple Mass Defect Filtering

Captures More Metabolites in a Single Analysis

Multiple mass defect filtering (MMDF) allows users to capture metabolites from Phase I and Phase II, specifically and concurrently. At the same time, it can capture metabolites from hydrolysis or dealkylation, even when the products from such processes have mass defects significantly different from the parent.

Advanced HCD Collision Cell

for More Comprehensive Analyses

The HCD collision cell with axial field gradient in the Orbitrap Velos Pro MS complements the CID fragmentation of the linear ion trap at comparable sensitivity. Together, these techniques make the Orbitrap Velos Pro a versatile and sensitive mass spectrometer for small molecule structural analysis, offering comprehensive fragmentation information.

The screenshot displays the Thermo MetWorks software interface for Multiple Mass Defect Filtering (MMDF). The main window is titled "Multiple Mass Defect Filtering [...samples\MetWorks\Mass Defect Filter\Haloperidol_bile_Top1_MS3_PL_MDF.RAW]".

Original Raw File: A callout points to the "Original Raw File" icon in the top toolbar.

MMDF Raw File: A callout points to the "MMDF Raw File" icon in the top toolbar.

Actual Filter Values: A callout points to the table of filter parameters:

Filter #	Mass Range Lower Limit (amu)	Mass Range Upper Limit (amu)	Mass Defect Lower Limit (mmu)	Mass Defect Upper Limit (mmu)
Filter #1	338.0714	384.4037	37.000	189.000
Filter #2	509.8506	509.8703	119.500	223.750

Original Chromatogram: A callout points to the "Original Chromatogram" plot showing relative intensity vs. time (min).

MMDF Chromatogram: A callout points to the "MMDF Chromatogram" plot showing relative intensity vs. time (min) with filtered peaks.

MDF Plot showing the two Filters used: A callout points to the "MDF Plot showing the two Filters used" plot, which shows Mass Defect (mmu) vs. Nominal Mass (amu). It highlights the "Parent" peak and "Possible Metabolites" within the filter windows.

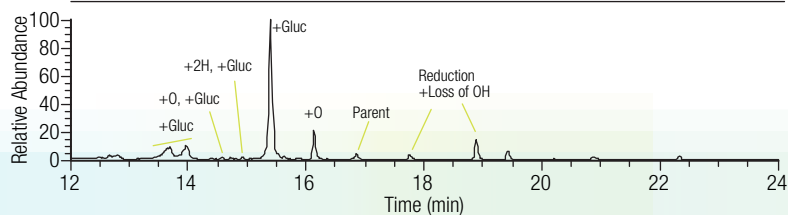
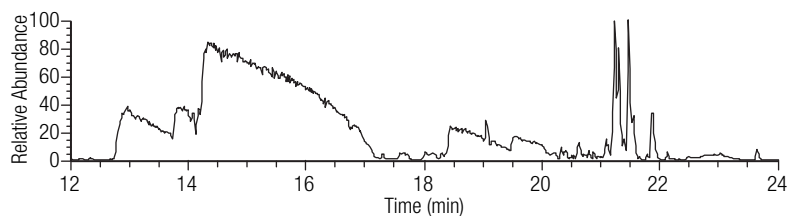
This will create the MMDF Raw File: A callout points to the "Create File" button at the bottom of the window.

Multiple mass defect filter in the MetWorks™ software allows users to capture metabolites from Phase I, Phase II, hydrolysis, and *N*-dealkylation, specifically and concurrently.

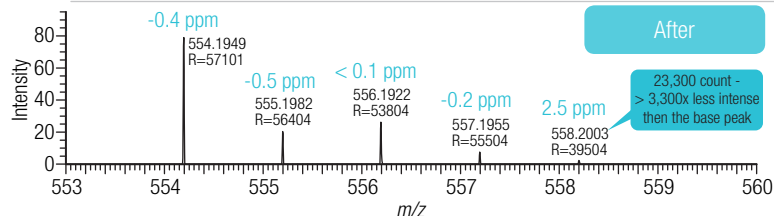
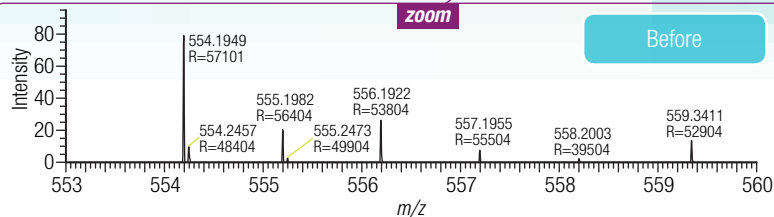
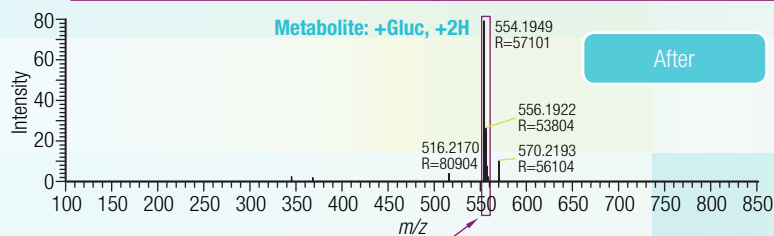
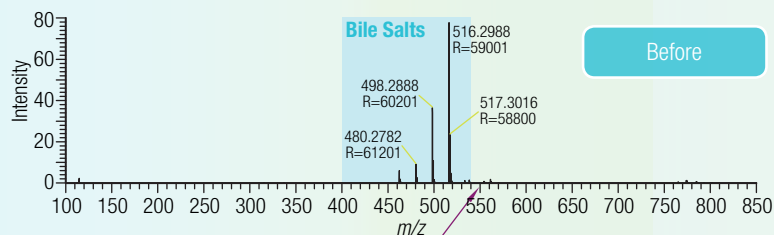
Identification of Haloperidol Metabolites in Rat Bile:

Base Peak Chromatograms Before and After MMDF Processing

RT: 12.00 - 24.00



RT: 14.92



Orbitrap Velos Pro Hybrid MS

- Resolving power of >100,000 for eliminating matrix interferences
- Complementary dissociation methods: CID MSⁿ spectral trees and/or HCD MS/MS for unambiguous structural elucidation
- S-lens/sensitivity resulting in detection of low-abundance metabolites or impurities
- Dual-pressure ion trap with higher scan speed and better fragmentation efficiency leading to more information for structural ID

Application-Specific Software

Turning Data Into Information

Thermo Scientific Xcalibur Data System

Stable operating platform

Xcalibur™ software is the versatile, easy-to-use data system that controls all Thermo Scientific MS systems. The home page of the Xcalibur software offers easy navigation through the process of instrument setup, sequence setup, and data acquisition.

Xtract

Xtract software deconvolutes isotopically resolved data for intact protein analysis and complex MS/MS spectra acquired in top-down analysis.

Thermo Scientific Proteome Discoverer Software

Mass informatics platform for protein scientists

Proteome Discoverer™ software is a workflow-based proteomics data processing software for in-depth data mining of complex LC-MSⁿ data sets. With the ability to exploit data from different dissociation techniques (CID, HCD, IRMPD, ETD and ECD), Proteome Discoverer software provides extra certainty for peptide and protein identifications. Optional inclusion of multiple search algorithms increases analytical flexibility, and results can now be merged into a single report for easier interpretation.

ProSightPC™ Software

ProSightPC software was developed to address the specific requirements of a top-down and middle-down proteomics strategy. The fragmentation spectra of multiply charged, intact proteins are exceedingly complicated. The software processes high resolution, accurate mass data from the Orbitrap Elite MS to produce a list of neutral fragment masses and performs comparisons to proteome databases to identify and characterize proteins.

Thermo Scientific ProteinCenter Software

Revealing meaningful biological information

ProteinCenter™ software is a web-based data interpretation tool that enables scientists to compare and interpret proteomic data sets and extract meaningful biological information quickly and easily. It provides access to a single database consolidated from more than 20 public databases, including all historical data.

Thermo Scientific Pinpoint Software

Facilitating quantitative proteomics

Pinpoint™ software facilitates the transition from early-stage biomarker discovery to larger-scale, quantitative verification of putative biomarkers and general quantitative proteomics. Pinpoint software simplifies the creation of targeted quantitative assays. It allows researchers to leverage previously acquired data from discovery experiments. Pinpoint software largely automates the development of preliminary methods. It enables acquisition and analysis of preliminary data, which is in turn used to optimize the method.

SimGlycan™ Software

Predicting glycan and glycopeptide structure

SimGlycan software analyzes mass spectrometric data to predict the structure of glycans and glycopeptides. It compares experimental MS/MS data against its own database of theoretical fragments and generates a list of probable candidate structures. Each candidate structure is scored to reflect how closely it matches the experimental data. The software also provides additional biological information for probable glycans and identified glycopeptides.

Thermo Scientific MetWorks

Drug Metabolism Software

Simplify the interpretation of complex metabolism data

MetWorks software simultaneously searches multiple modifications of one or more parent drugs and interprets simple to complex isotope patterns, or unexpected or low-abundance metabolites.

Mass Frontier

Spectral Interpretation Software

Confident path from spectra to structure

Mass Frontier software allows confident structural elucidation through chemically intelligent spectral annotation, state-of-the-art fragmentation prediction, and unparalleled spectral and fragmentation mechanism knowledge management.

Thermo Scientific SIEVE Differential Expression Software

Analysis of differential expression based on comparison of LC-MS datasets

SIEVE software provides label-free, semi-quantitative differential expression analysis of proteins, peptides, or metabolites from the comparison of multiple LC-MS data sets. It is a statistically rigorous tool for analyzing data from metabolism or biomarker discovery experiments.

www.thermoscientific.com/OrbitrapVelosPro

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