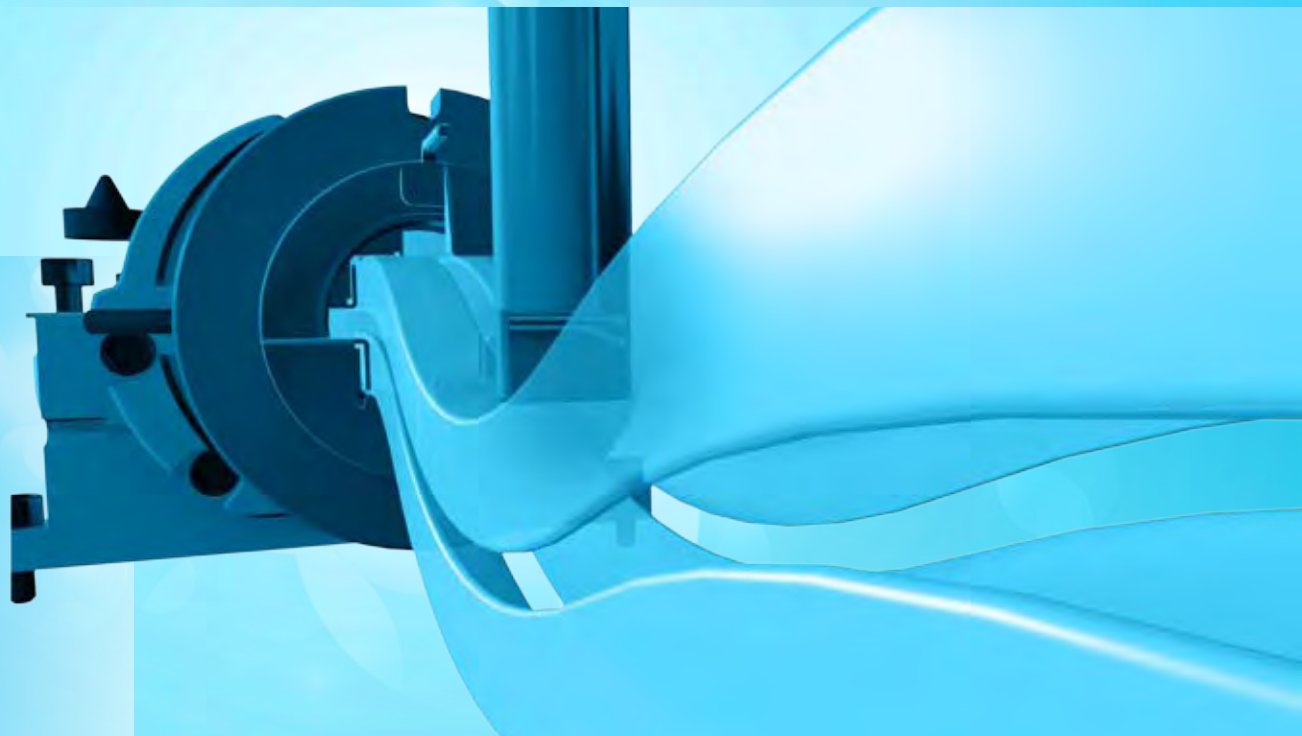**Thermo Scientific Velos Pro**
Ion Trap LC-MS^n

Dual-Pressure Linear Ion Trap
Accelerating Innovation

Designed for quantitation • UHPLC data rates
• Diversity in dissociation • Fast and sensitive MS^n

Thermo
S C I E N T I F I C

Accelerating
Innovation

The Thermo Scientific Velos Pro is the pinnacle of ion trap mass spectrometry that delivers enhanced performance for complex sample analyses. A novel wide dynamic range discrete dynode detection system offers the ultimate in identification and quantitation of low-abundance compounds and provides absolute confidence in every result. For the first time ever with an ion trap, the Velos Pro™ offers Trap-HCD (Higher-Energy Collisional Dissociation) combined with CID (Collision-Induced Dissociation), PQD (Pulsed-Q Dissociation) and ETD (Electron Transfer Dissociation) to solve the most difficult analytical problems. With the improved robustness of generation II/G2 ion optics, the Velos Pro delivers reliability on the fastest, most-sensitive, highest capability ion trap available today. The Velos Pro can also be enhanced with the ultra-high resolution and mass accuracy of Thermo Scientific Orbitrap Velos Pro or Orbitrap Elite™ technology.

Freedom of
Dissociation

The Velos Pro takes ion trap technology to new heights: with CID, PQD, ETD, and now Trap-HCD available at any level of MS^n, the Velos Pro is the most versatile MS instrument on the market.

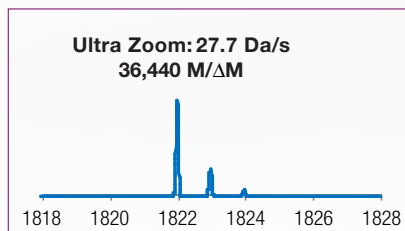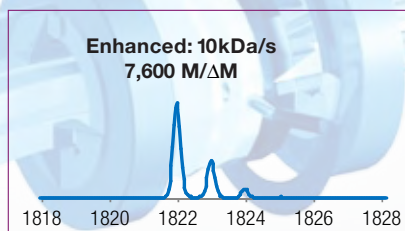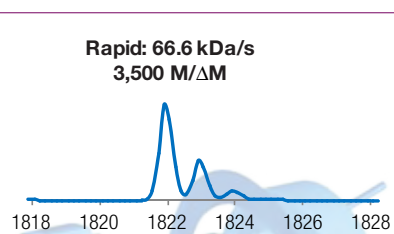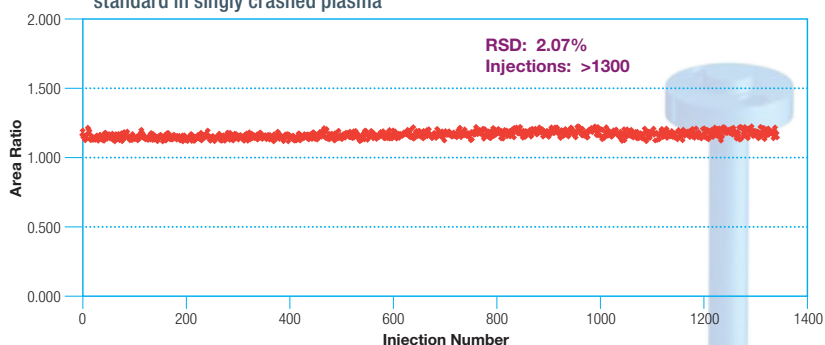**Trap-HCD of Caffeine**

CID of Caffeine
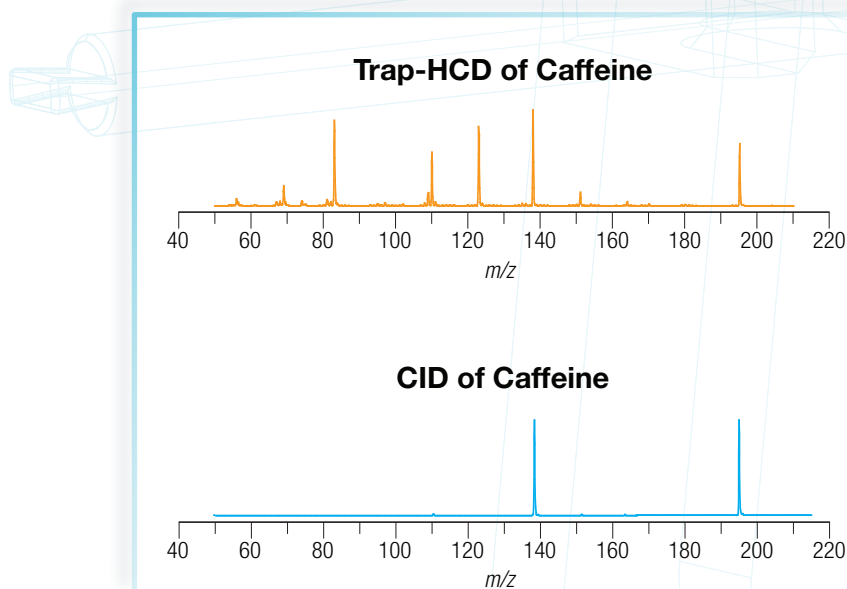
Triple quadrupole-like fragmentation with no precursor dependent low mass cutoff is now available on the Velos Pro. Precursor ions collide with N_2 in a high pressure octopole where collision energy can be tuned to maximize specific fragments at any mass-to-charge relative to parent. Collision energy is normalized for mass-to-charge and for charge state to deliver optimum fragmentation for all precursors across the entire mass range. Trap-HCD of small molecules furnishes higher energy transitions; trap-HCD of peptides offers strong y- and b-ion series as well as highly specific immonium ions. With CID, PQD, and ETD, Trap-HCD adds capability and flexibility to MS^n on the Velos Pro.

Amazing Sensitivity –
Maximum Robustness

The Velos Pro presents the latest advancements in ion optics design: the S-Lens delivers dramatically higher ion currents, and G2 Ion Optics provide a novel mechanism of blocking neutrals outside the path of the ion beam. These features in combination provide maximum uptime and total confidence for the most challenging applications.

50 pg on column of Alprazolam and internal standard in singly crashed plasma

RSD: 2.07%
Injections: >1300

Area Ratio (y-axis: 0.000, 0.500, 1.000, 1.500, 2.000)
Injection Number (x-axis: 0, 200, 400, 600, 800, 1000, 1200, 1400)

Rapid: 66.6 kDa/s
3,500 M/ΔM

(x-axis: 1818, 1820, 1822, 1824, 1826, 1828)

Enhanced: 10kDa/s
7,600 M/ΔM

(x-axis: 1818, 1820, 1822, 1824, 1826, 1828)

Ultra Zoom: 27.7 Da/s
36,440 M/ΔM

(x-axis: 1818, 1820, 1822, 1824, 1826, 1828)

Speed and
Selectivity

The revolutionary Velos Pro is an optimized dual-pressure ion trap with a high pressure and a low pressure cell. The high pressure cell offers maximum ion trapping, fragmentation, and isolation efficiency. The low pressure cells enable better resolution and higher scan rates than any other ion trap mass spectrometer. This dual-pressure trap technology in Velos Pro offers a scan speed for every application: from fast survey scans in the new Rapid Scan mode to maximum resolution in Ultra ZoomScan mode.
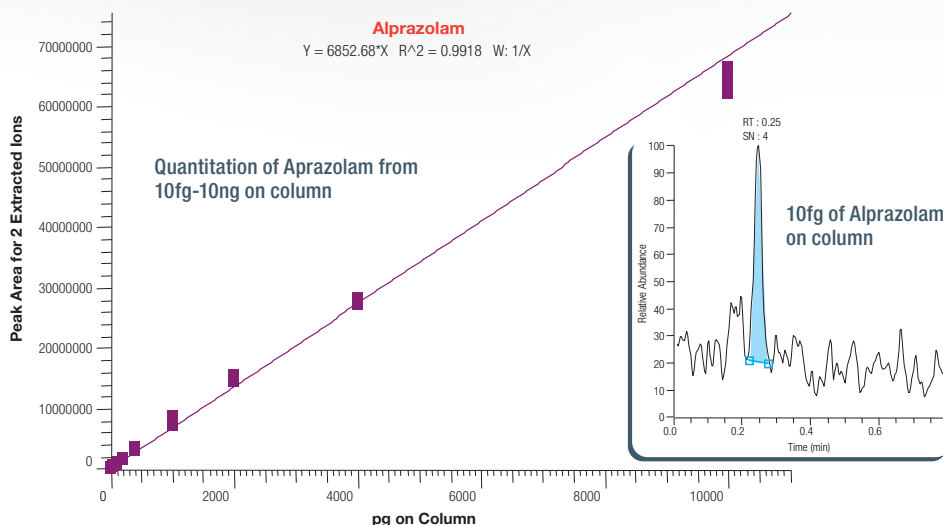
Quantitate Like
Never Before

The Velos Pro blurs the lines between traditional ion trap and triple quadrupole performance. The first trap of the dual-pressure cell is designed to act like a single quadrupole during the ion loading process, removing chemical background and minimizing space charge.
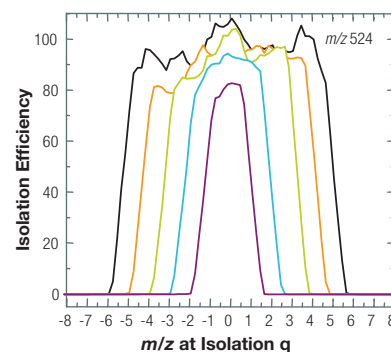Once trapped, precursor ions are fragmented and detected by a custom-designed

high-performance discrete dynode system optimized to capture the ultra-high flux of ions ejected from the Velos Pro. The quadrupole-like isolation combined with high data rate detection produces results only possible on the Velos Pro… designed with quantitation in mind.

Alprazolam
Y = 6852.68*X   R^2 = 0.9918   W: 1/X

Quantitation of Aprazolam from 10fg-10ng on column

RT : 0.25
SN : 4

10fg of Alprazolam on column

Isolation efficiency of the Velos Pro during the ion loading process

m/z 524

Maximum Identifications

Routine analysis of complex proteomics samples is often confounded by the inability to adequately detect low-abundance proteins, by the large number of PTMs (Post-Translational Modifications) possible, and because of the dynamic nature of the proteome. Ion trap-based mass spectrometry has demonstrated a superior ability to rapidly identify and characterize low-abundance proteins and determine their relative expression levels. It has become an essential tool in proteomics labs.

The Velos Pro enables a new level of proteomics sample interrogation, made possible by improvements in acquisition rates, MS^n sensitivity, selective accumulation of low-abundance ions and the addition of a novel fragmentation methodology: Higher-Energy Collisional Dissociation (HCD). HCD allows access to low mass fragments such as immonium ions which increases the confidence in and total number of peptide identifications.
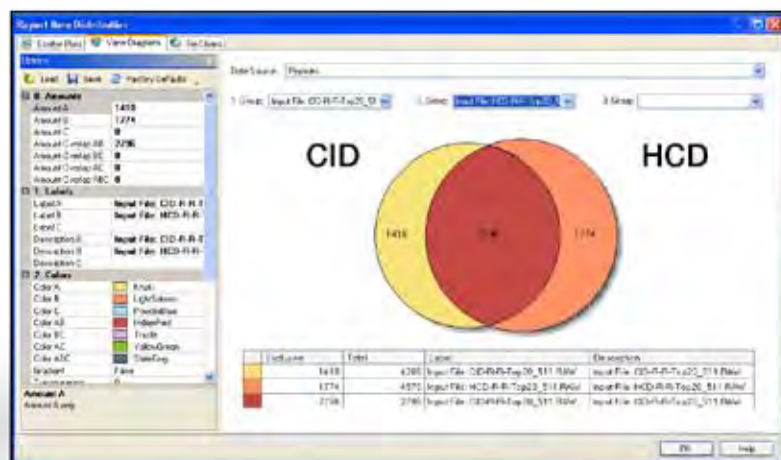
Venn diagram from Thermo Scientific Proteome Discoverer 1.3 software showing *C. elegans* peptide identifications by HCD and CID.

Maximum Coverage

Achieving complete coverage for the purpose of sequencing a protein, including its site-specific post-translational modifications, requires maximizing the number of peptides detected in a single experiment. The application of multiple activation methods, i.e. Collision-Induced Dissociation (CID), Electron Transfer Dissociation (ETD), and now the new Higher-Energy Collisional Dissociation (HCD) achieves this goal.
An all ion trap-based HCD/ETD decision tree is now possible on the Velos Pro providing maximum coverage and characterization of target proteins.
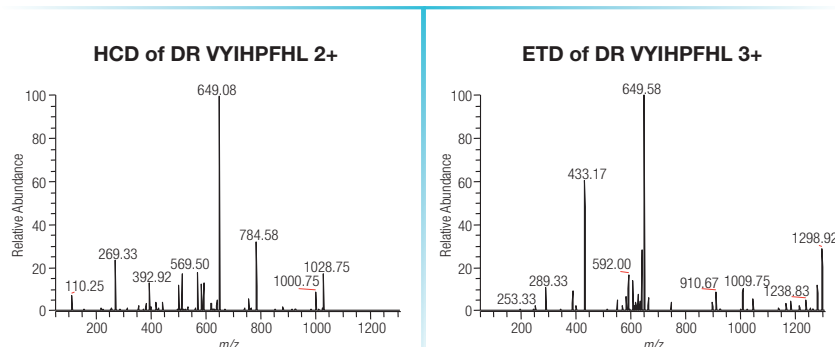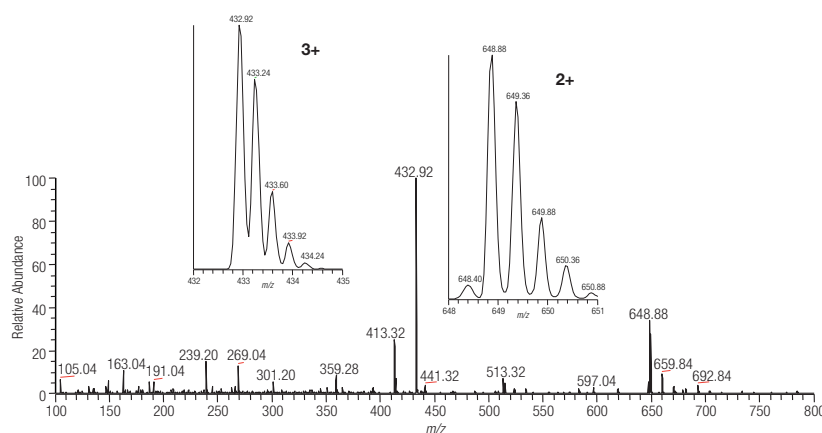

Full Scan MS followed by HCD for doubly charged precursor (bottom, left) and ETD for triplycharged precursor (bottom, right).

Every Target

Maximum Structural Characterization

Mass spectrometry has emerged as one of the most powerful tools for the structural elucidation of glycans due to its sensitivity and its ability to analyze complex mixtures. Ion trap mass spectrometry offers multistage fragmentation (MS^n) which is a critical tool for glycan structure elucidation as it allows for determination of structural heterogeneity and differentiation of isomers. It is commonly necessary to acquire six or seven levels of fragmentation (MS^6 or MS^7) to differentiate potential glycan structural isomers.

MS^n spectra and block structure for 9 unit glycan determined by LC-MS^n on the Velos Pro.
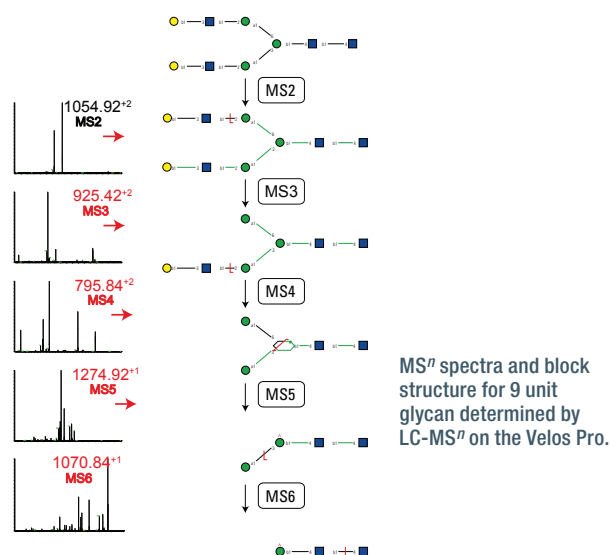
Relative Quantitation by TMT Labeling

Tandem Mass Tag (TMT) and other isobaric tagging technologies are based on the release of low-mass reporter ions during fragmentation, allowing simultaneous identification and quantitation of peptide ions. Both MS^3 and Pulsed-Q Dissociation (PQD) methods are well established linear ion trap applications of these technologies. The Velos Pro linear ion trap mass spectrometer features a new option. Higher-Energy Collision Induced Dissociation which utilizes a high-pressure RF-only octopole. HCD is a triple quadrupole-like fragmentation method which produces high intensity reporter ions resulting in enhanced accuracy and precise quantitation.

Results of Trap-HCD TMT Experiments	
Peptide Spectral Matches	4363
Peptides Quantified	3269
Percent Quantifiable	75%
Average Protein Level CVs	2.5%

Average of the coefficients of variation for each protein in a 12 protein mixture and number of IDs by trap-HCD fragmentation.

ITMS, HCD, z=+2, Mono m/z=688.02769 Da, MH+=1375.04810 Da, Match Tol.=1.2 Da
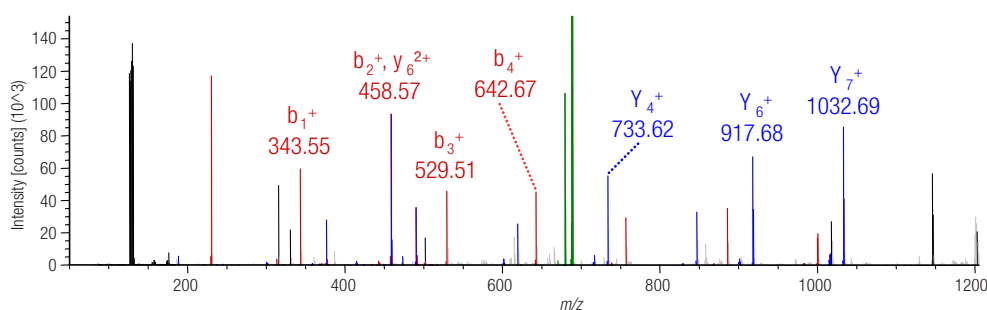
Sample HCD MS^2 spectrum for TMT 6-plex labeled protein digest.

Absolute Quantitation Using AQUA™ Peptides

Absolute quantitation strategies such as AQUA center around MS^2 detection of targeted peptides and their analogues labeled with stable isotope amino acids. The Velos Pro dual-pressure linear ion trap with ultra-fast scanning and multiple fragmentation techniques delivers high precision and accurate quantitation even over multiple orders of magnitude and in complex matrices.
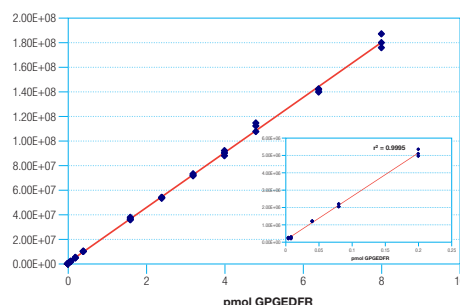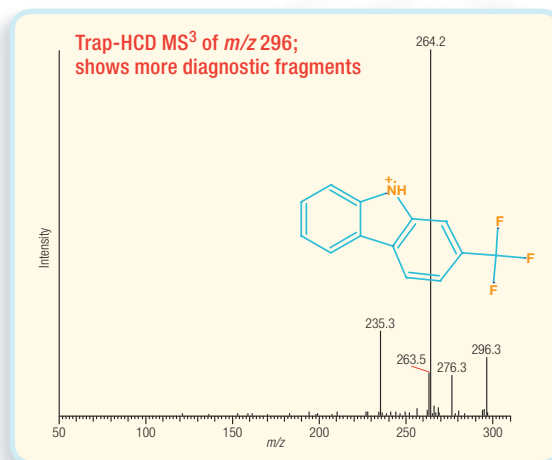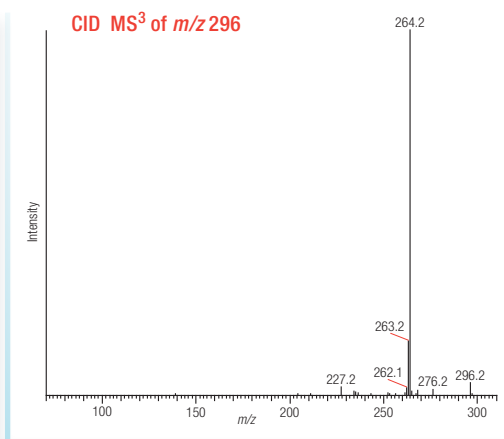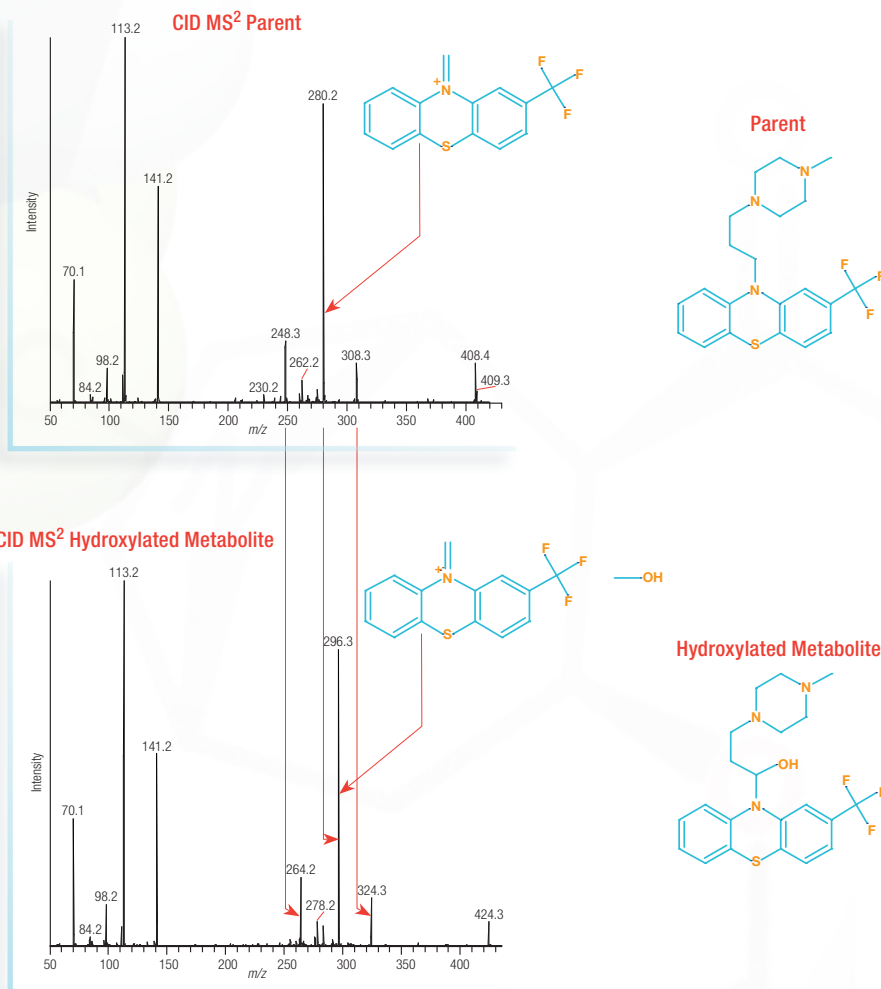

Peak area vs amount of GPGEDFR injected for 4 orders of magnitude change in concentration. GPGEDFR was spiked into unfractionated human cerebral spinal fluid.
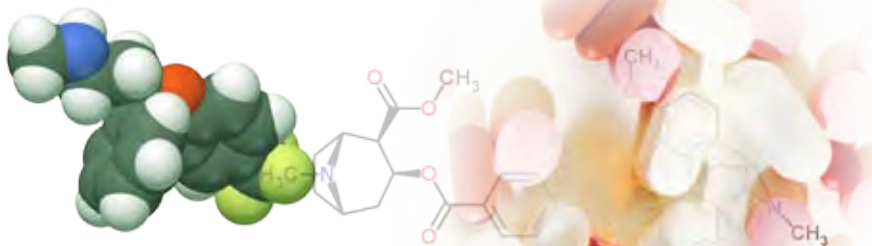
7

Maximum Productivity

The characterization of drug metabolites plays a pivotal role in pharmaceutical discovery and development. Parent drug and metabolites need to be identified in a variety of biological matrices over a wide range of concentrations for both *in vivo* and *in vitro* studies. Thermo Scientific MetWorks and Mass Frontier software, when used with the Velos Pro create a robust, sensitive, and integrated workflow for identification, characterization, and quantitation of drugs and their metabolites in biological matrices.
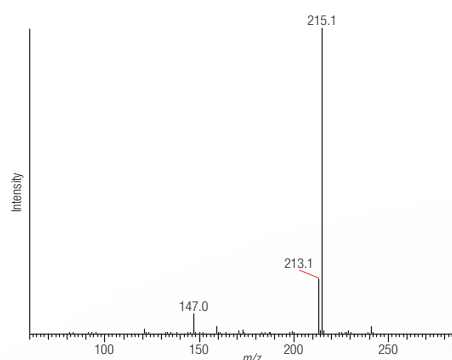
The power of MS^n can be observed with the fragmentation and interpretation of Trifluoperazine and its metabolites which include multiple oxidative metabolites. MS^3 data from HCD fragmentation provides useful diagnostic fragments not readily observed in a CID MS^3 scan.
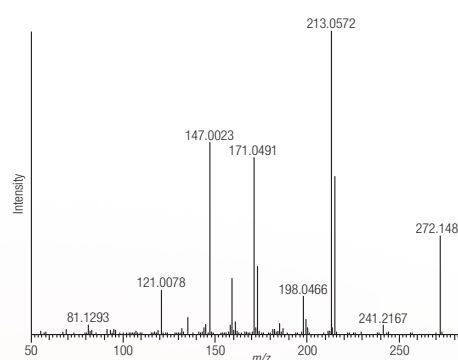
CID MS^2 Parent

Parent

CID MS^2 Hydroxylated Metabolite

Hydroxylated Metabolite

CID MS^3 of *m/z* 296

Trap-HCD MS^3 of *m/z* 296; shows more diagnostic fragments

Quantitation

etabolism Samples

### CID MS^2 Dextromethorphan

Trap-HCD MS^2 Dextromethorphan

With different fragmentation techniques available, researchers can tailor their choice to meet the demands of the analysis. For the quantitation of Dextromethorphan, CID generates an MS^2 spectrum with a single strong ion, ideal for quantitative sensitivity, while signal intensity following HCD fragmentation is spread over multiple MS^2 ions.

Maximum Performance

The increased sensitivity of the Velos Pro allows simultaneous qualitative and quantitative metabolite analyses to be carried out easily for both *in vivo* and *in vitro* studies. The faster scan rates of the Velos Pro allow the acquisition of more MS^n spectra in shorter chromatographic runs. With a full range of fragmentation techniques available for use at all MS^n stages, researchers can construct higher-quality ion trees for qualitative metabolite analysis and characterization. The use of Data Dependant acquisition coupled with Dynamic Exclusion provides a methodology for the analysis of both predicted and unexpected metabolites.

$y = 34201x + 289667$
$R^2 = 0.99974$
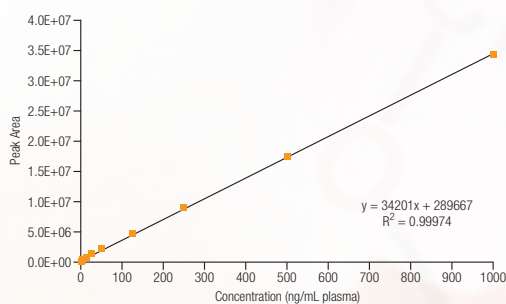
Thermo Scientific Application-Specific Software
Turning Data Into Information

Xcalibur™ data system
Stable operating platform

Thermo Scientific 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. The XReport package simplifies custom reporting with drag-and-drop functionality.

Proteome Discoverer software
Mass informatics platform for protein scientists

Thermo Scientific Proteome Discoverer software is a workflow-based proteomics data processing software for in-depth data mining of complex LC-MS^n 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.

ProteinCenter™ software
From data sets to meaningful biology

Thermo Scientific ProteinCenter is a web-based data interpretation tool that enables scientists to compare and interpret data sets in minutes instead of months. The software enables filtering, clustering and statistical bioinformatics analysis utilizing a regularly updated, consolidated protein sequence database containing >10 million non-redundant proteins.

Pinpoint software
Accelerating targeted protein quantitation in biological research

Thermo Scientific Pinpoint software facilitates the transition from early-stage biomarker discovery to larger-scale, quantitative verification of putative biomarkers and general quantitative proteomics.

MetWorks™ software
Simplify the interpretation of complex metabolism data

Thermo Scientific 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™ software
Confident path from spectra to structure

Thermo Scientific 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.

SIEVE™ software
Analysis of differential expression based on comparison of LC-MS datasets

Thermo Scientific 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/velospro

Thermo
S C I E N T I F I C

Part of Thermo Fisher Scientific