



ASMS 2017

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Petr Verner

Thermo Fisher Scientific (Praha) s.r.o.

Ozvěny ASMS 2017 - Novinky Thermo Scientific

Discovery Day 2017



Precision Mass Spectrometry

AGENDA

- Pár faktů o ASMS 2017/Indianapolis
- Q Exactive HF-X
- Orbitrap Fusion Update
- TSQ Product Line Update
- MS Software Update

65th ASMS Conference on Mass Spectrometry and Allied Topics

<https://www.asms.org/about/history>

Overview

Users' Meeting

Breakfast Workshops

Software Users' Meeting

Customer Appreciation

Posters

Oral Presentations

ASMS 2017

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ASMS 2017

June 4-8 | Indianapolis, IN



Pár faktů o ASMS (Indianapolis)

- Máte-li s sebou děti mladší 14 let, mohou zaplatit za každé slovo klení pokutu 3 USD, max. však 10 USD/den
- Velice známý je závod Indianapolis 500. Automobily projedou 200 kol (500 mil) na slavné Indianapolis Motor Speedway. První závod se zde konal 30.5.1911, vítěz jel průměrnou rychlostí 75 mil/h (cca 115 km/h) a vyhrál cenu 14000\$. Méně známé je, že vůbec první závod na nově postavené dráze (3,2 mil. Cihel, přezdívka „brickyard“) byl závod heliem plněných balonů v roce 1909.
- Jay Stokes of Indianapolis drží Guinness World Record za nejvíce skoků padákem za den: 640.
- Firma Wonder Bread of Indianapolis uvedla jako první na trh toustový (plátkový) chléb
- The inventor of the Gatling gun, Richard Gatling, was born in Indianapolis.





Food and Beverage

Environmental

Biopharmaceuticals

**Translational Research/
Precision Medicine**

**Life Science Research
"omics"**

Instruments

Orbitrap HRAM LC-MS



**Orbitrap Fusion™
Lumos™ MS – Options**

- UVPD
- 1M
- APD



**Q Exactive™ HF-X
LC-MS**



**Exactive™ GC
Orbitrap™ GC-MS**

Triple Quadrupole LC-MS



TSQ Altis™ MS



TSQ Quantis™ MS

Software

- Small molecule analysis
- Protein analysis
- Cloud Applications
- Spectral Libraries



**Proteome Discoverer™
Software**



**Compound Discoverer™
Software**

Sample Prep, Kits and Consumables

- MS Crosslinking reagents
- Capillary flow HPLC columns
- Metabolomics Standards Kits



**Biocrates
AbsoluteIDQ
p400HR Kit**



**DSSO and
DSBU MS-
Cleavable
Linkers**



**Easy-Spray
PepMap
150µm
Column**



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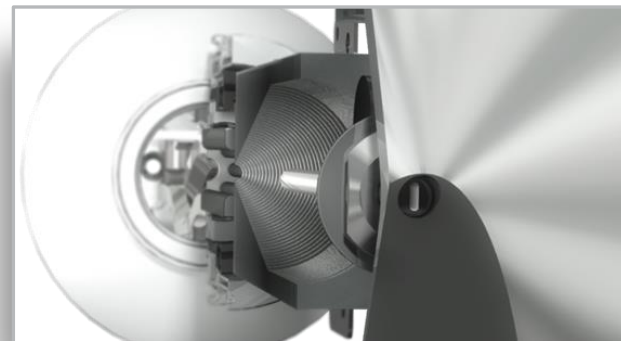
Q Exactive HF-X

The world leader in serving science

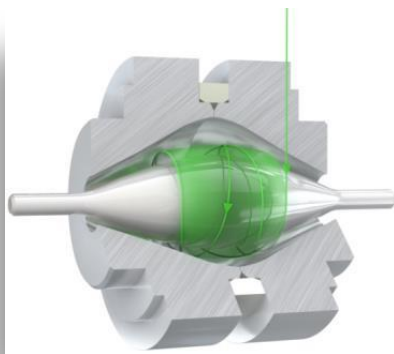
Key Technologies of Q Exactive HF-X



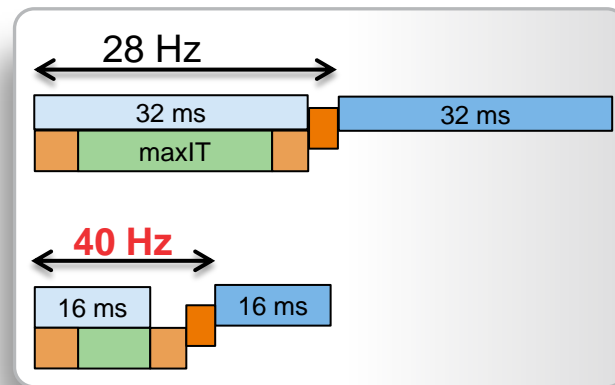
High Capacity Transfer Tube



Electrodynamic Ion Funnel

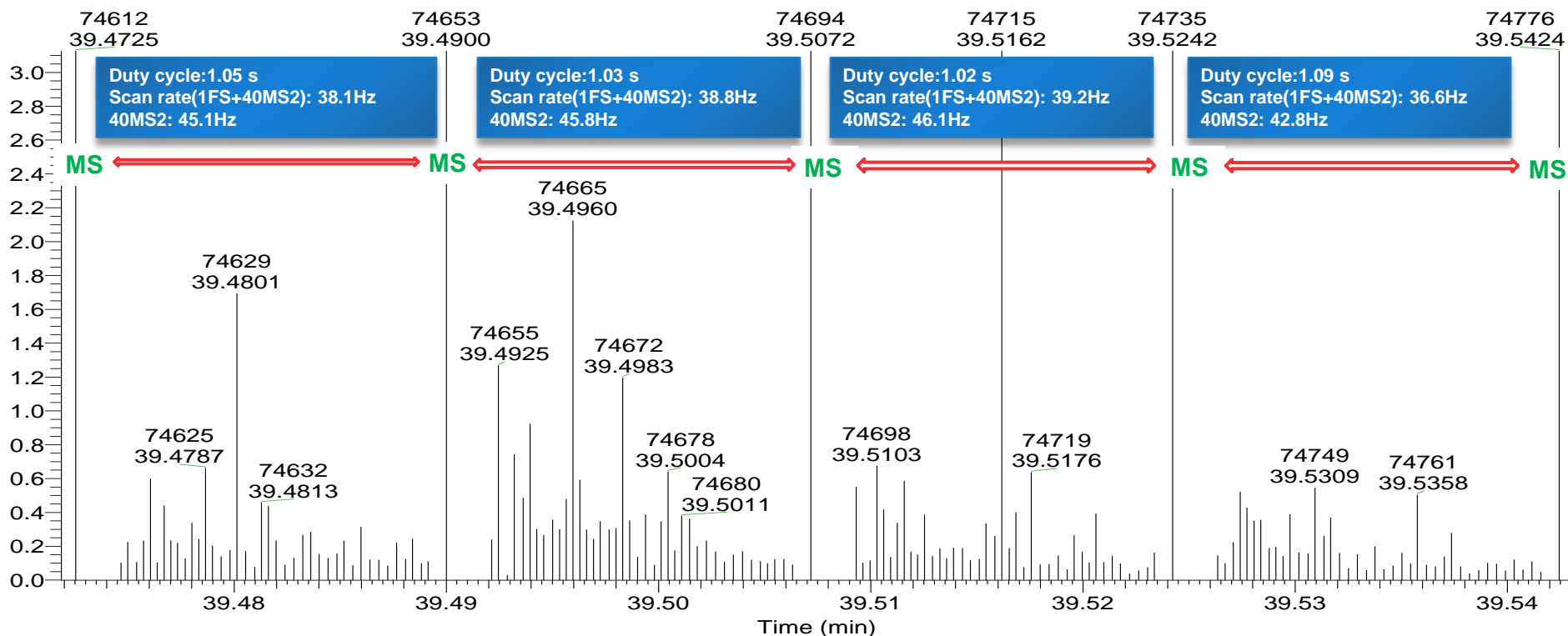


Ultra-High Field Orbitrap



Optimized Scan Matrix and new Transients

Ultra Fast MS/MS Scan Speed > 40Hz

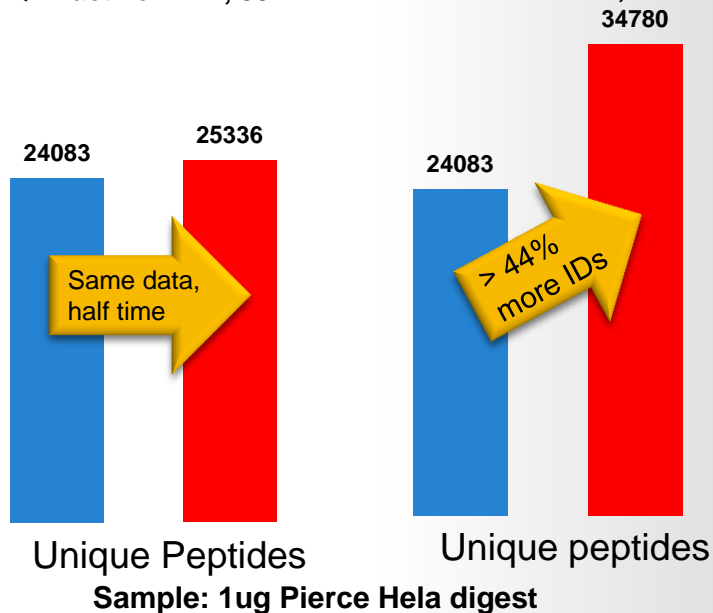


**1 full scan (60,000 @ m/z 200) and 40 MS² scans 7,500 @ m/z 200) at LC time scale in 1 second.
30 min gradient, MS² max IT: 11 ms**

ASMS'17: TP 389, T.N. Arrey et al. New innovations implemented on the Q Exactive HF mass spectrometer.

Productivity increase in DDA

■ Q Exactive HF, 60 min ■ Q Exactive HF, 60 min
■ Q Exactive HF-X, 30 min ■ Q Exactive HF-X, 60min

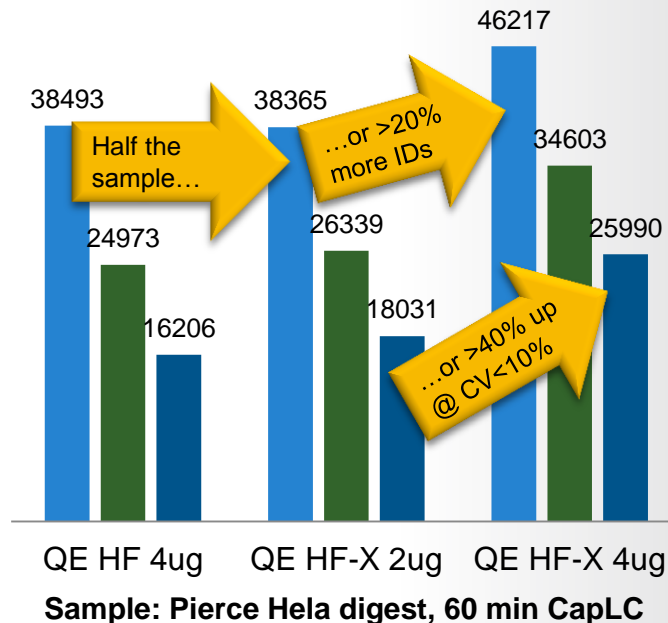


ASMS'17: TP 389, T.N. Arrey et al.

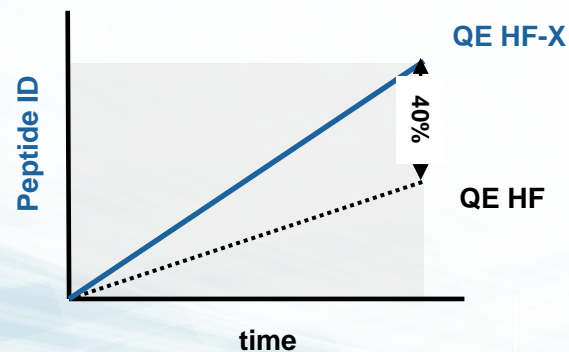
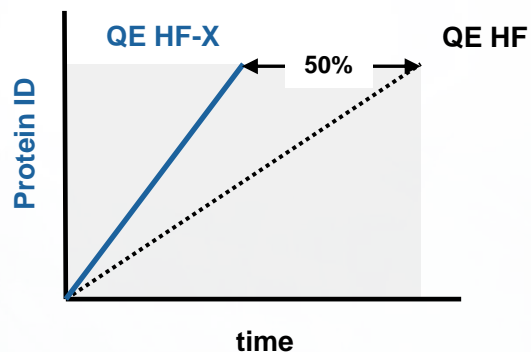
Peptide quantitation in DIA

Peptide Precursors

■ ID ■ CV% <20% ■ CV% < 10%



ASMS'17: ThP237, Y. Xuan et al.



Value

Maximizing protein identifications

- Quick screening of complex samples
- Quality control of complex samples
- Assessment of sample concentration

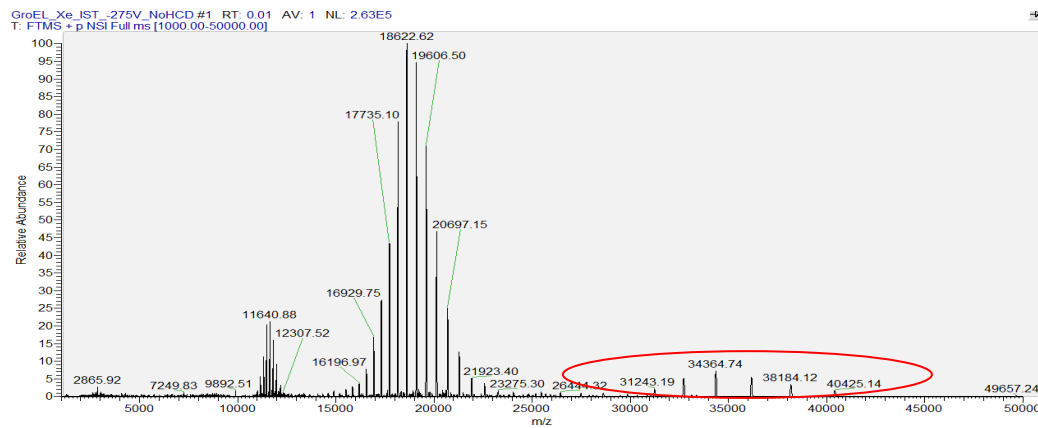
Maximizing peptide identifications

- Highest peptide coverage
- Deep proteome analysis
- Spectral library building

Saving time and sample in large-scale proteomics efforts

Functionalities of the Upgrade:

- Mass-range (mass of ions that pass the whole ion path): 2,000m/z – **80,000 m/z**
- Lowest measurable mass of fragments from the HCD: 400m/z
- In-Source Trapping for desolvating ions before they pass the Quadrupol
- Can be used for Q Ex Plus or HF
- **Normal mode of operation requires replacement of boards!**

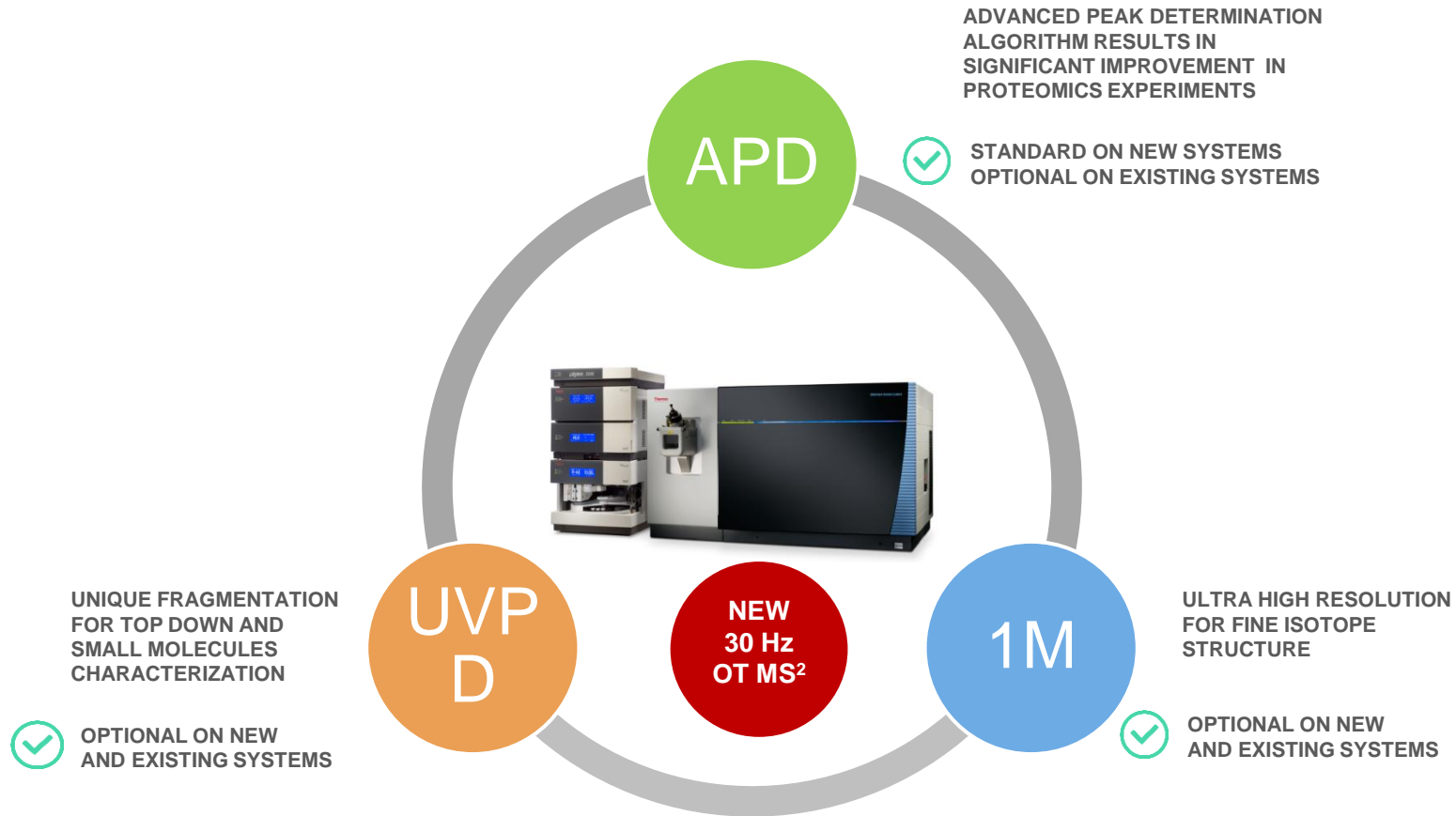




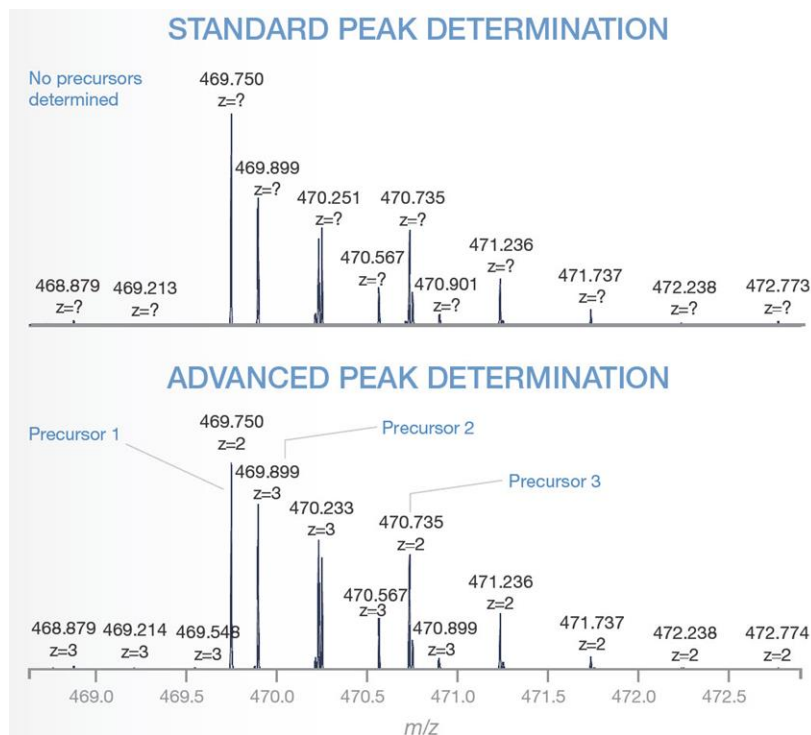
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Orbitrap Fusion Lumos Options
ASMS 2017

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Advanced Peak Determination: Reading Between The Lines



APD Details

- The Advanced Peak Determination (APD) algorithm identifies the charge states and monoisotopic m/z values of isotopic envelopes at greatly improved peak depths.
- This significantly increases the population of precursors available for data-dependent analysis, which in turn results in more MS^n spectra, PSMs, and unique peptide identifications.
- APD has been evaluated for peptides/proteins only. Improvements for small molecule analysis are still under investigation.



TP 144 McAllister et al.; Tue breakfast workshop

Example Applications Of APD

BOTTOM UP PROTEOMICS

Data-dependent experiments with complex peptide mixtures

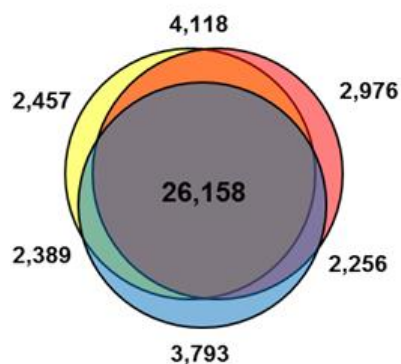
TOP DOWN PROTEOMICS

Identification and characterization of intact proteins by MS

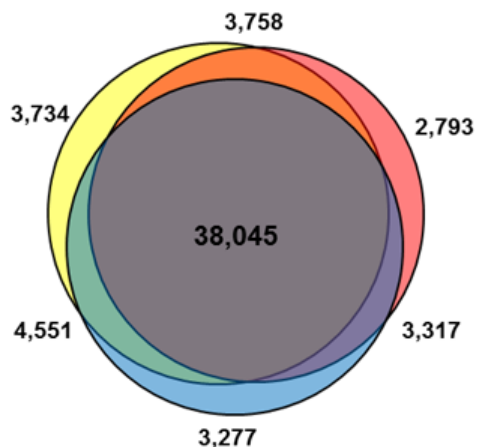


Large Increase In Reproducibly Identified Unique Peptides

Standard Peak Determination



Advanced Peak Determination

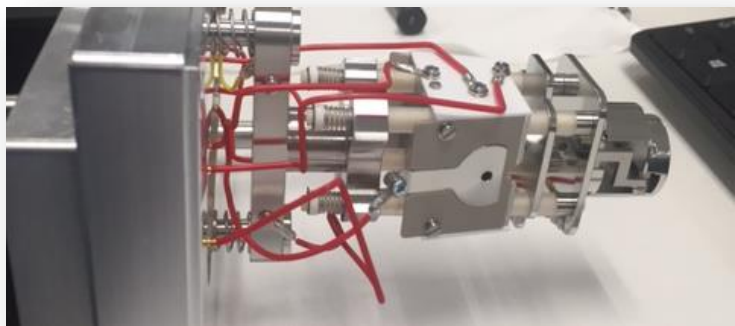


APD Performance On A Tribrid

- 1 μ g HeLa, 2 h gradient, n=3
- OT MS at 120K (SPD) and 240K (APD)
- LT MS/MS with 20 ms max IT
- The number of unique peptides detected by all three replicates increased by **46%** with APD (26,158 vs. 38,045).
- The number of proteins identified in all three replicates increased by **12%** with APD (5317 vs. 4715)



TP 144 McAlister et al.; Tue breakfast workshop



← Matching C-trap

1M Orbitrap →



A Kit Comprising An Orbitrap And A C-trap Assembly

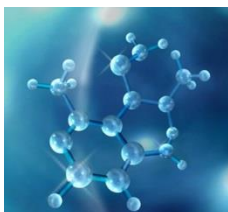
- Ultra high resolution (1,000,000 at m/z 200, 2 sec transient), improved dynamic range and isotope ratio specs
- A matching C-trap and a high performance Thermo Scientific™ Orbitrap™ mass analyzer, available for factory installations or field upgrades



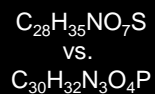
Example Applications Of 1M



Fluxomics: Stable isotope labeling and measuring turnover rates



Metabolomics: Selected screening of compounds of interest based on fine isotope structure



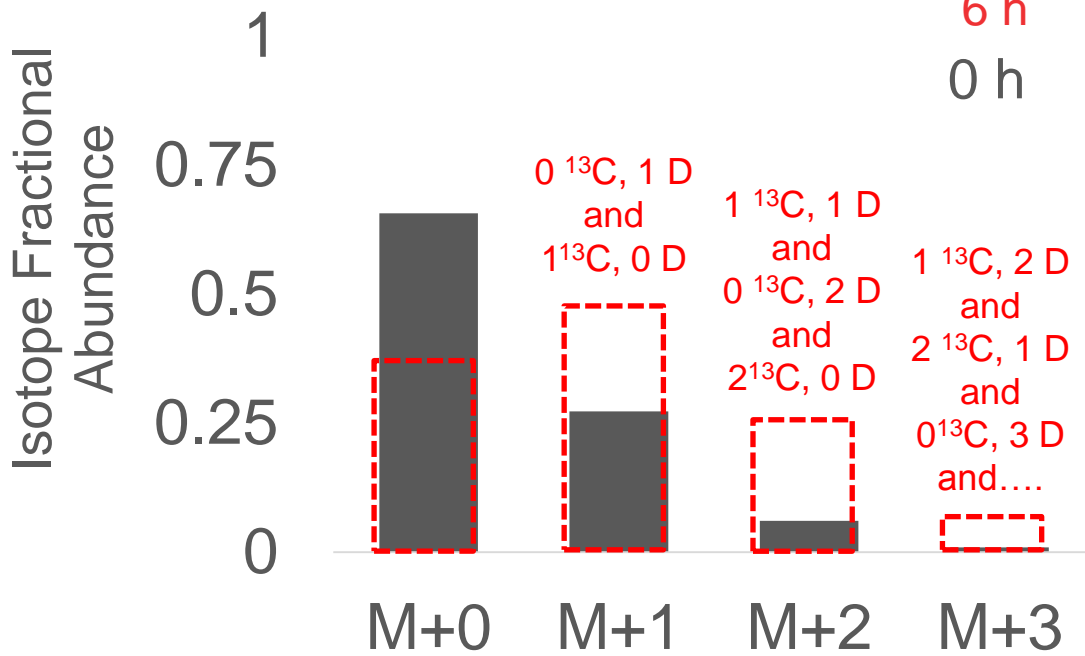
Extractables and Leachables:
High confidence elemental composition determination



Basic Stable Isotope Labeling Experiment To Measure Biosynthetic Rates/Flux



Distribution of Natural Triacylglyceride Isotopes, $C_{37}H_{83}O_4$

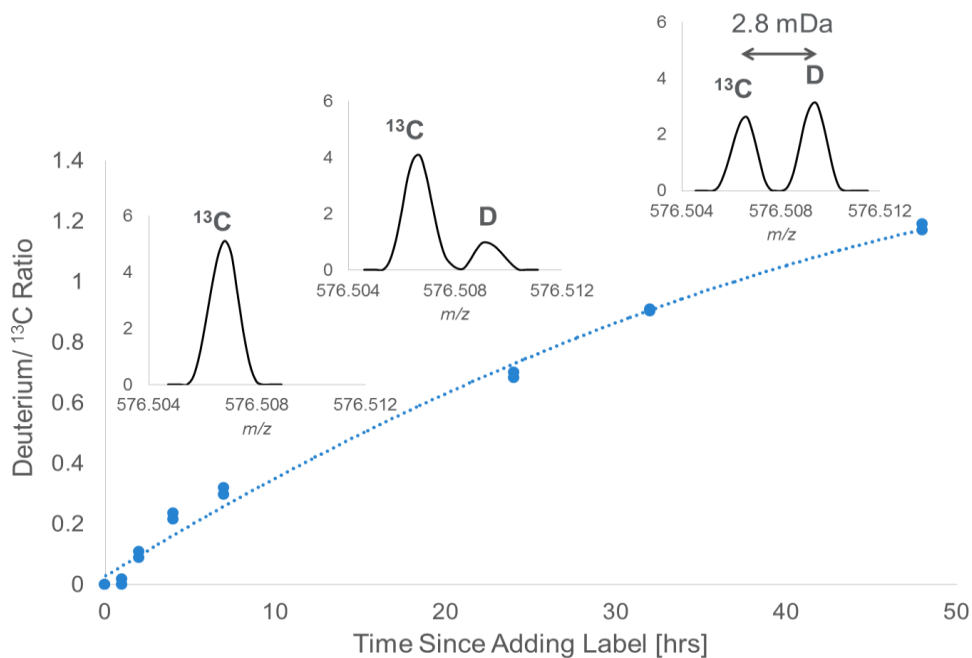


Isotope Dilution Quantitation Using a Low Resolution Instrument

- Grow cells on natural isotope background
- Change media to 5% D_2O ; cells will start using D_2O in biosynthesis alongside of H_2O
- Collect cells at time points
- Extract lipids
- Measure change in isotope ratios by MS
- Calculate **Synthesis Rates/Flux** based on the $D/^{13}C$ ratio change



New 1M Ultra-High Resolution For Lipid Flux Analysis



Rate of deuterium incorporation for TAG 52:3 in human hepatoma cells (HuH7) labeled with 5% D₂O

MOB am 10:10, M. Mitsche

Resolving Natural ¹³C From Labeled Deuterium Isotope

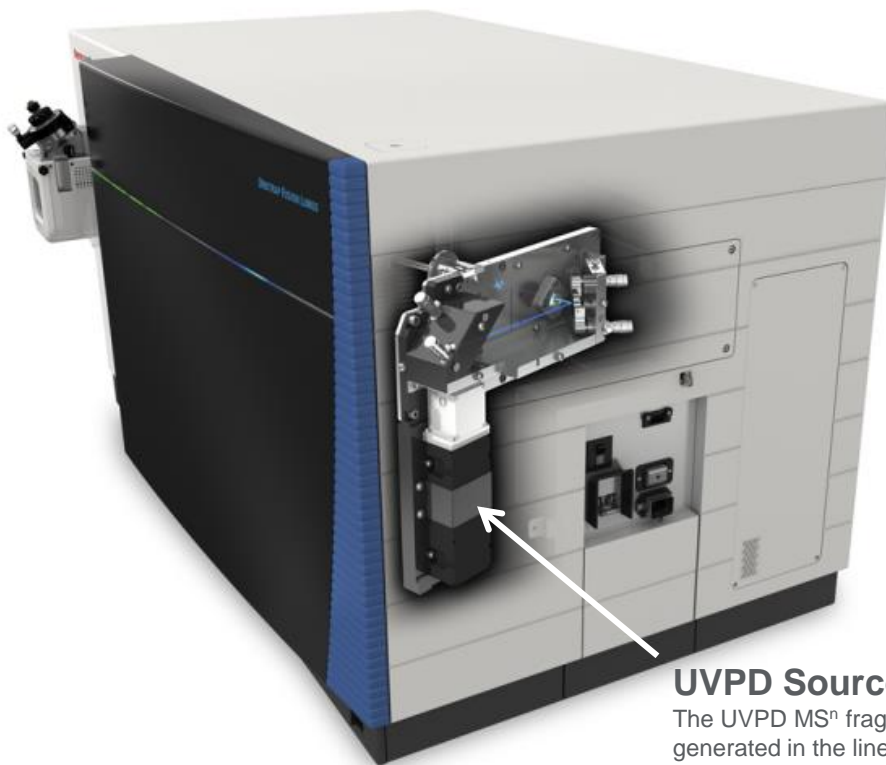
- The Triacylglyceride precursor ion was fragmented using high energy collisional activation
- Deuterium incorporation levels were plotted for the fragment ion at *m/z* 576.5





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UVPD Option



UVPD Source

The UVPD MSⁿ fragments are generated in the linear ion trap and can be detected by either ion trap or Orbitrap

Compact Footprint

- UVPD source is embedded inside the instrument, directly connected to the dual-pressure linear ion trap
- UVPD source employs a 213 nm laser with 2.5 kHz repetition rate delivering >1.2 μ J
- UVPD is a field upgradable option



Example Applications Of UVPD



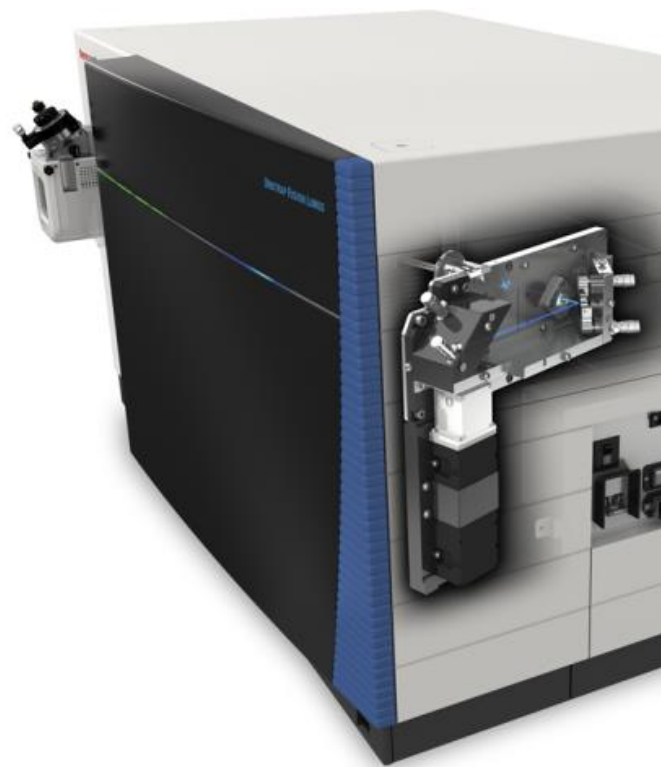
**Comprehensive sequence
characterization/confirmation of
protein drugs**



**Identification and characterization
of intact proteins by MS**

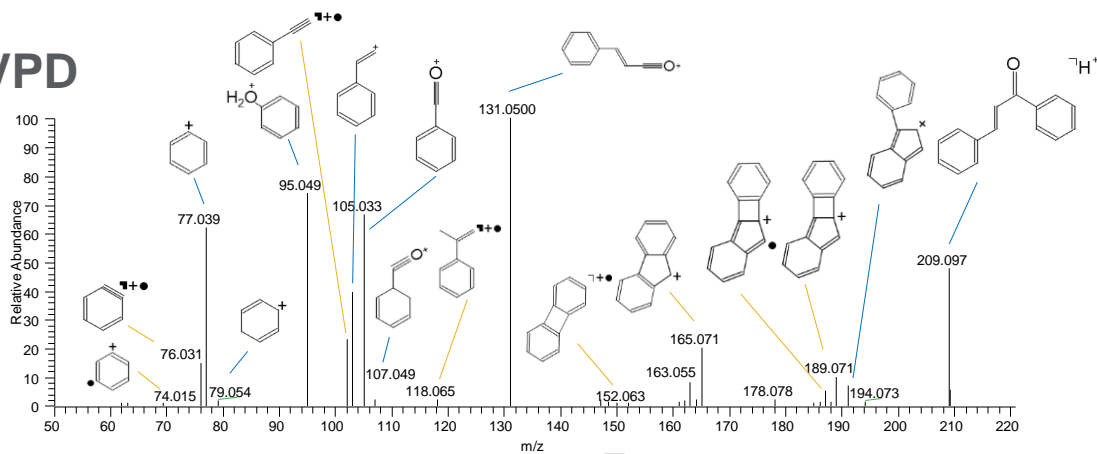


**Structural elucidation of lipids,
metabolites, xenobiotics and others**

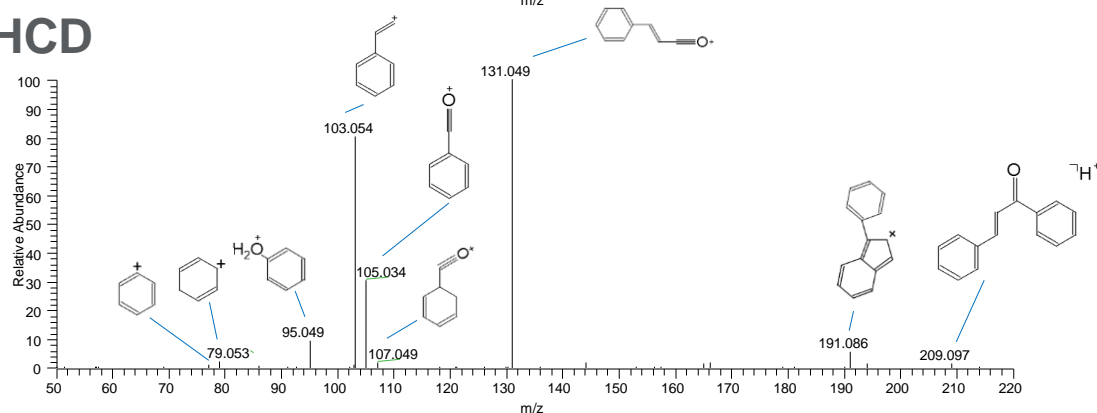


Rich Fragmentation Pattern For Small Molecule Structural Analysis

UVPD

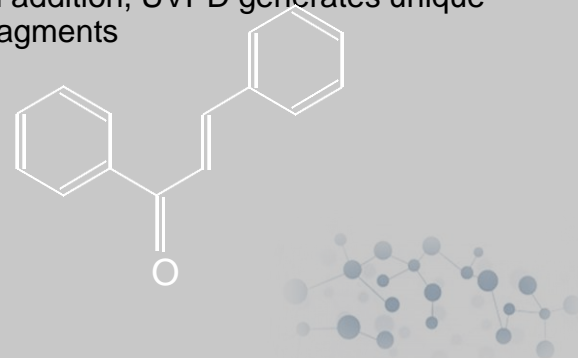


HCD



Unique Fragmentation Channels

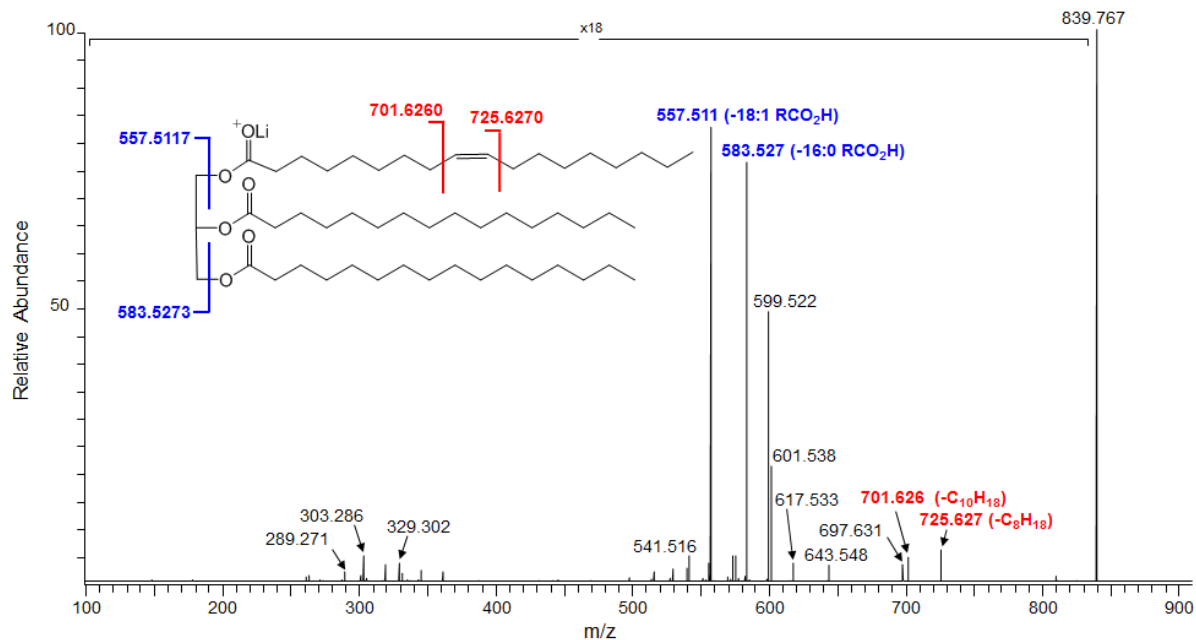
- HRAM UVPD and HCD LC/MS² spectrum of **CHALCONE** (an intermediate in plant flavonoid biosynthesis)
- UVPD accesses the same fragmentation channels as HCD
- In addition, UVPD generates unique fragments



TP 703: Huguet et al.

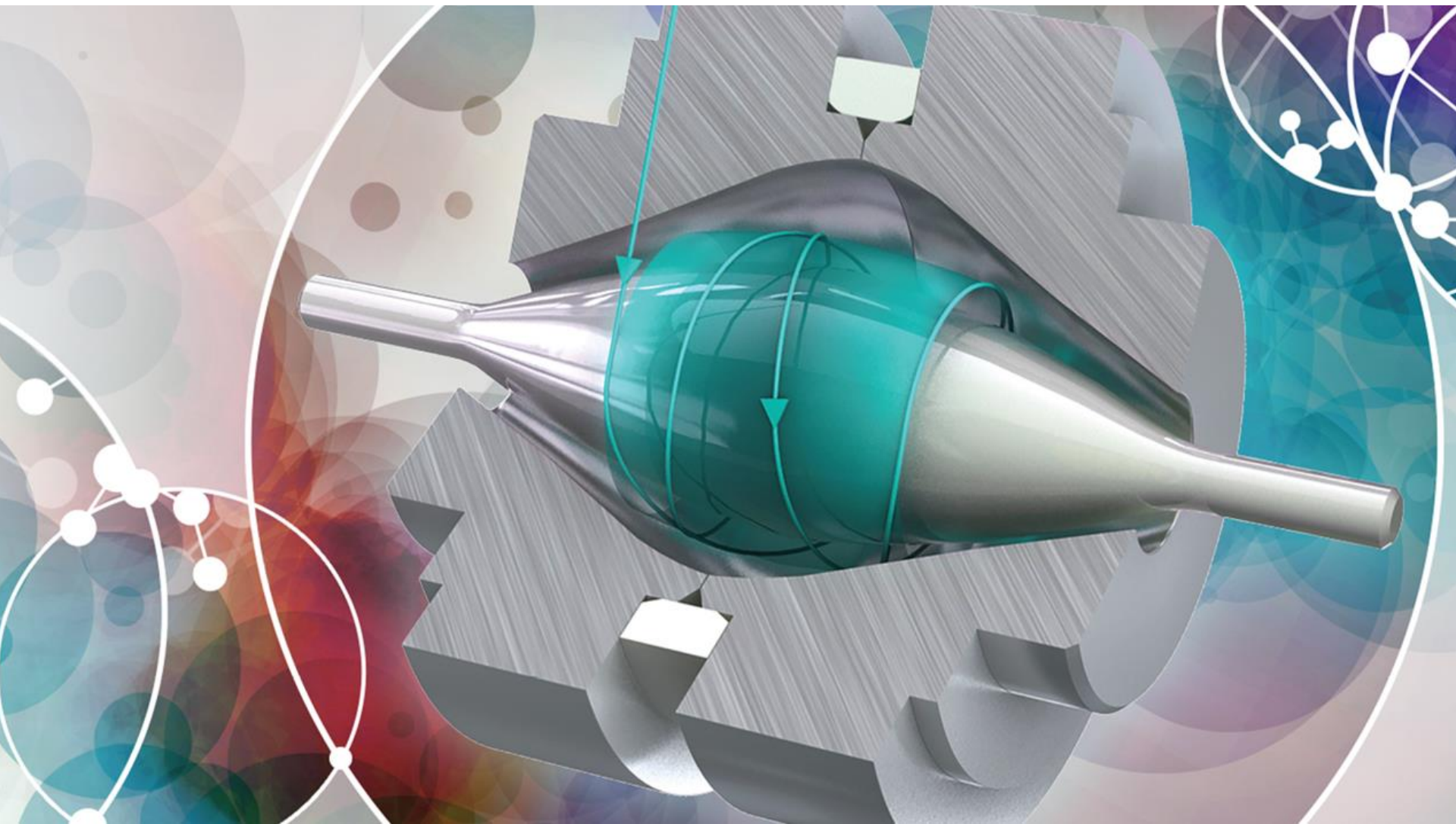
Locating Double Bonds

- HRAM UVPD MS² spectrum of [M+Li]⁺ precursor ions of TG 16:0/16:0/18:1
- Fragments identify acyl chains
- UVPD unique fragments identify location of double bonds within the acyl chains



WOD 03:10 pm : Reid G. et al.







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Confident Quantitation: TSQ Altis and TSQ Quantis

- **Development of Hardware and Software with focus on market requirements:**
 - Robustness (Reliability), Sensitivity (& Reproducibility), Speed, Ease-of-use (maintenance and flexibility), Selectivity (H-SRM), Automated compound Optimization, SRM visualization
- **Development and Testing performed in collaboration with customers and internal partners:**
 - 14 customer projects finalized in San Jose (Peptide Quan, Lipidomics/Metabolomics, EFS, Clinical Research, Forensic, Pharma)
 - 6 TSQs installed and running: New Jersey, Yokohama, SSC Dreieich, Hemel Hempstead and University of Washington – Mike MacCoss
 - This week (end MAY):
 - Installation of TSQ Altis - Hendrik Neubert (Pfizer)
 - TSQ Altis and TSQ Quantis shipped to Shanghai and Beijing demo labs



TSQ Altis



TSQ Quantis



Performance: Sensitivity, Selectivity (H-SRM)

Robustness, Reproducibility, Speed, Ease-of-Use, Flexibility

	<i>TSQ Altis</i> High-end	<i>TSQ Quantis</i> Mid-tier
Mass Range	5-2000	5-3000
SRM/sec	600	600
Selectivity (H-SRM)	0.2 Da FWHM	0.4 Da FWHM
Sensitivity (HESI Reserpine 1 pg)	500,000:1	150,000:1
Targeted Market	Omics, Research, Pharma/Biopharma, Forensic Toxicology	Environmental and Food Safety, Clinical Research, and Forensic Toxicology

TSQ Altis: Sensitivity with Robustness, No Compromises

AIM+
TECHNOLOGY

Active Ion Management Plus (AIM+)

Ion beam guide with neutral blocker
Reduces chemical background

High capacity ion transfer tube (HCTT)
Increases ion flux

Electrodynamic ion funnel (EDIF)
Increases ion flux

OptaMax™ NG
APCI ready

NEW!

Segmented Quadrupoles
with hyperbolic surface for enhanced performance with both SRM and H-SRM (0.2 FWHM)

NEW!

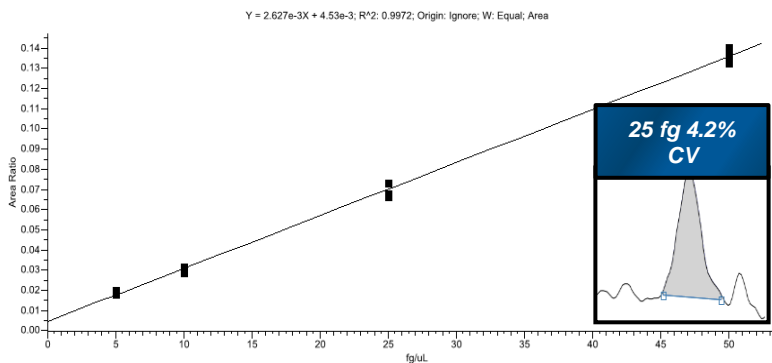
Active collision cell with axial DC field
facilitates more SRMs/sec

Enhanced dual-mode electron multiplier detector
Ensures excellent linearity and dynamic range

NEW!

TSQ Altis: Ultra High Sensitivity with Excellent Reproducibility!

Fluticasone Propionate
Corticosteroid in matrix

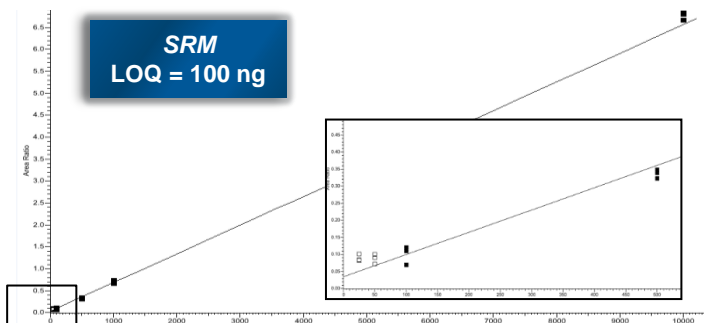
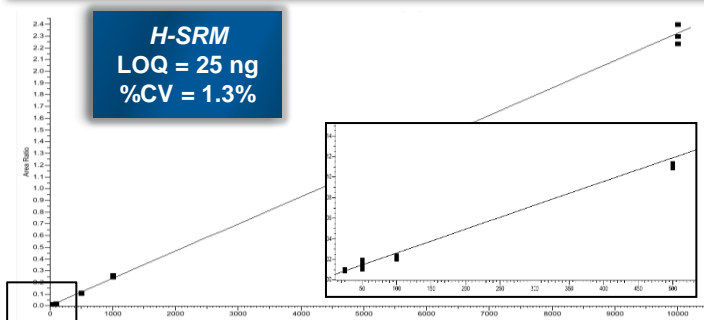


Results obtained with customer samples during Internal Beta Testing

TSQ Altis 25 fg 4.2% CV

Sensitivity with Reproducibility!

GPSVFPLAPSSK
Peptide from monoclonal antibody hinge region



H-SRM for the most complex applications!

Differentiators

We Offer:

Sensitivity

Sensitivity with reproducibility
Better Dynamic Range

Robustness

Easier routine maintenance (5 min operation)

Selectivity

Better performance at 0.4/0.2 Da FWHM
Sensitivity with selectivity

Speed

We are faster – 600 SRMs/sec!
We have better performance at lower dwell times!

Ease-of-use

Market Specific Software (w/databases and libraries) – TraceFinder
Intuitive user interface – Automated Compound Optimization
Dynamic Retention Time

Flexibility

High Sample Throughput and High Performance LC
Multi-channel and UHPLC
Ion Sources
Robust/reliable high and nano-flow options

Complete Solution

Single Vendor provider - complete solutions
(Chromatography, Mass Spectrometry – GC/LC (Orbitrap and QQQ) and Software)



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Applications Software Update



Proteome Discoverer 2.2

- Label-free quan
- Cross linking
- New ProSight nodes



ProSightPC 4.0

- C-score for modification confidence
- Automated disulfide recognition



Xcalibur 4.1

- New instrument & UVPD Support



FreeStyle 1.3

- Elemental composition using MSMS
- Improved usability



Compound Discoverer 2.1

- Elemental composition using MSMS
- Identifies significantly more components
- Mass-structure correlation



ProteinCenter 4.0 (Q4 2017)

- Custom proteoform searches (UVPD)
- Track observed PTMs



BioPharma Finder 3.0 (Q4 2017)

- Top down workflow
- HDX visualizations



TraceFinder 5.0 (Q4 2017)

- GLP compliance capable
- Configurability for ease of use
- Improved processing speed



MS Instrument Connect

Remote monitoring dashboard for multiple instruments
Mobile app for Android and iOS



Sample Profiler

Create profile of authentic products
Compare new samples, determine trends and differences



Pathway Overrepresentation

Find significant pathways from gene or protein lists

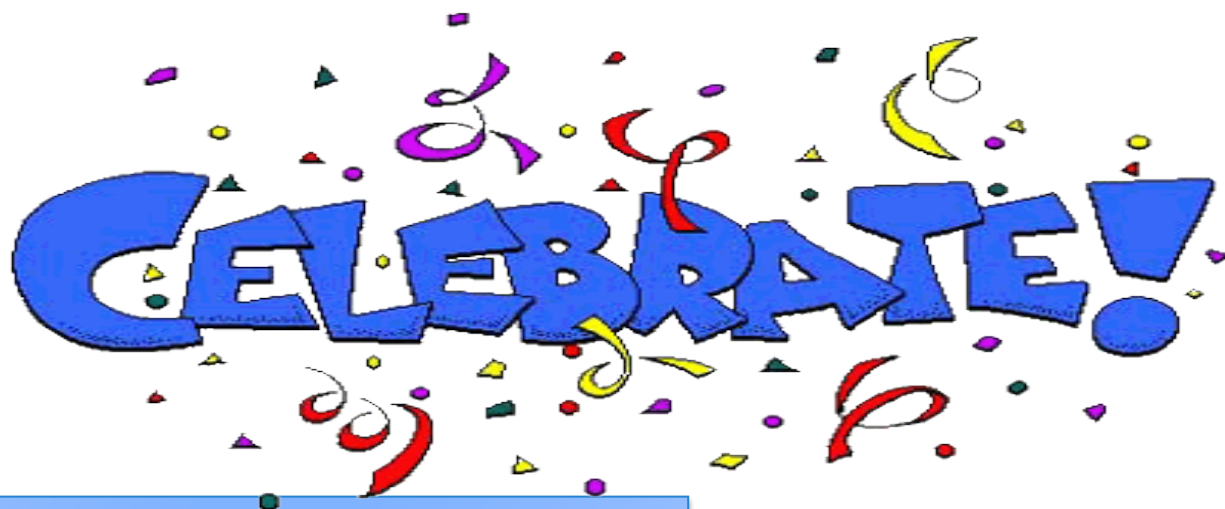


Omics Comparator

Quickly see overlap and uniqueness comparing protein or gene lists



Thermo Fisher **Cloud**



- In 2017 Thermo Scientific first introduced 1 000 000 resolution and 45 Hz scan rate benchmark using ORBITRAP technology.

