New Mass Spectrometry Tools to Transform Metabolomics and Lipidomics

July.3.2013

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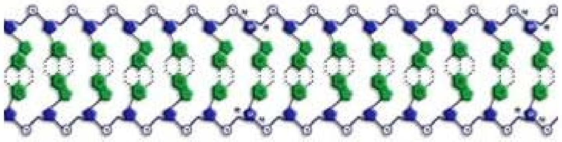
Vice President of Marketing,
Life Sciences Mass Spectrometry

Omics & the Central Dogma of Biology

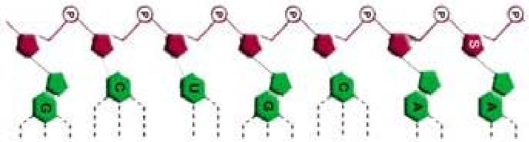
DNA



Genomics – 22,000 genes

What could occur

RNA



Transcriptomics – 100,000 transcripts

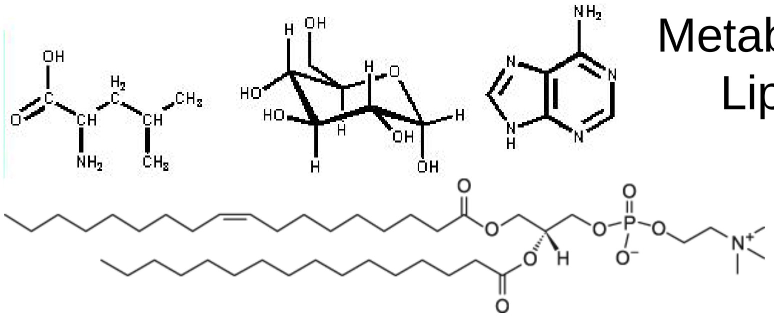
What may occur

Proteins

Proteomics – 1,000,000 proteoforms

What is occurring

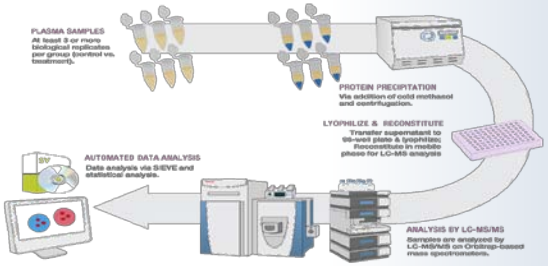
Metabolites
Lipids



Metabolomics – 2,000 compounds
Lipidomics – 10,000 species

What has occurred

Addressing the Metabolomics Challenges



Developing SOPs for major sample types

Innovating LC/MS to improve separation & detection

SIEVE

LipidSearch

TraceFinder™
The Power to Know

Developing streamlined, workflow-oriented software

m/z CLOUD

ChemSpider
Building community for chemists

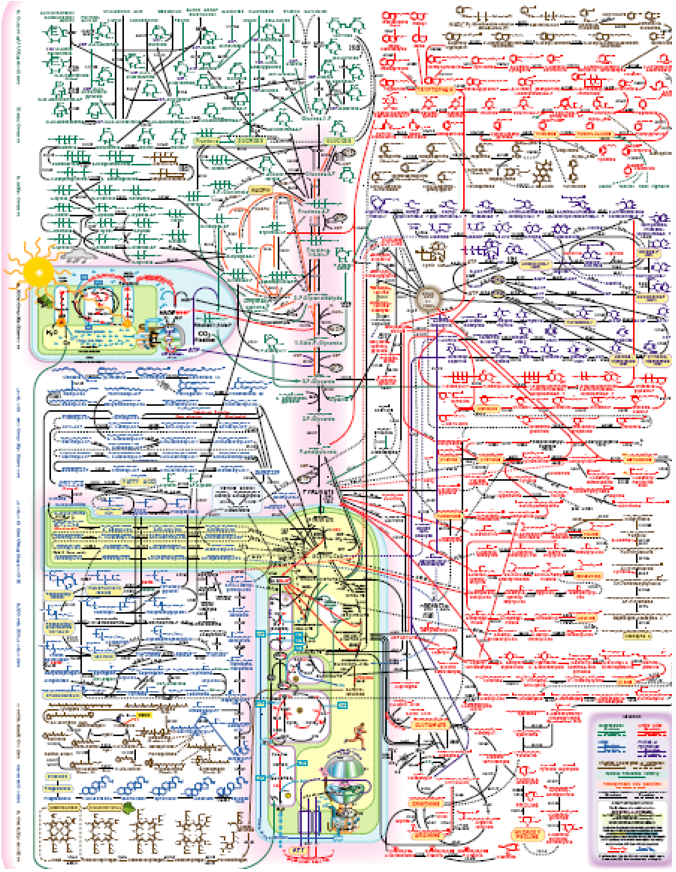
KEGG
Kyoto Encyclopedia of Genes and Genomes

Building and connecting to new and existing database

Orbitrap Delivers the Highest Quality Data

Better data, Better results!

- Highest Resolution
- Best Mass Accuracy
- Widest Dynamic Range
- Excellent Sensitivity



LCMS Metabolomics Offerings

Quan / Qual analysis of endogenous molecules of low molecular mass (<1200-1500Da)

Profile

- Detect, align, and quantify components, use statistical analysis to find key features

Identify

- Putative ID web or local DB search
- Confirm ID via MS^2
- *De novo* structural ID via MS^n

Quantify

- Rountinely quantify sets of putative biomarkers in large sample populations

Thermo Scientific™ Exactive™ Series MS

Thermo Scientific™ Orbitrap Fusion™ MS

TSQs

High-End

Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC

Thermo Scientific™ Q Exactive™ MS

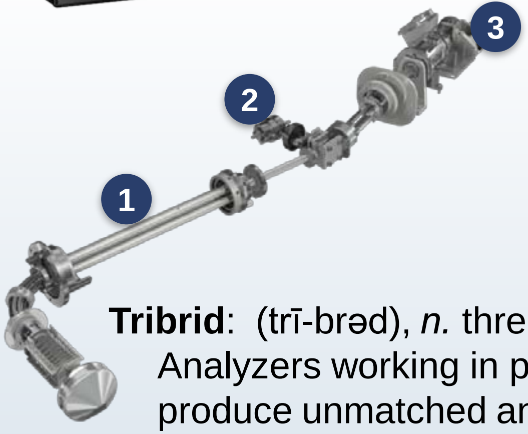
Budget Limited

SIEVE V1.3

m/z CLOUD

TraceFinder™ The Power to Know

Orbitrap Fusion Tribrid Mass Spectrometer



Tribrid: (trī-brəd), *n.* three Mass Analyzers working in parallel to produce unmatched analytical results

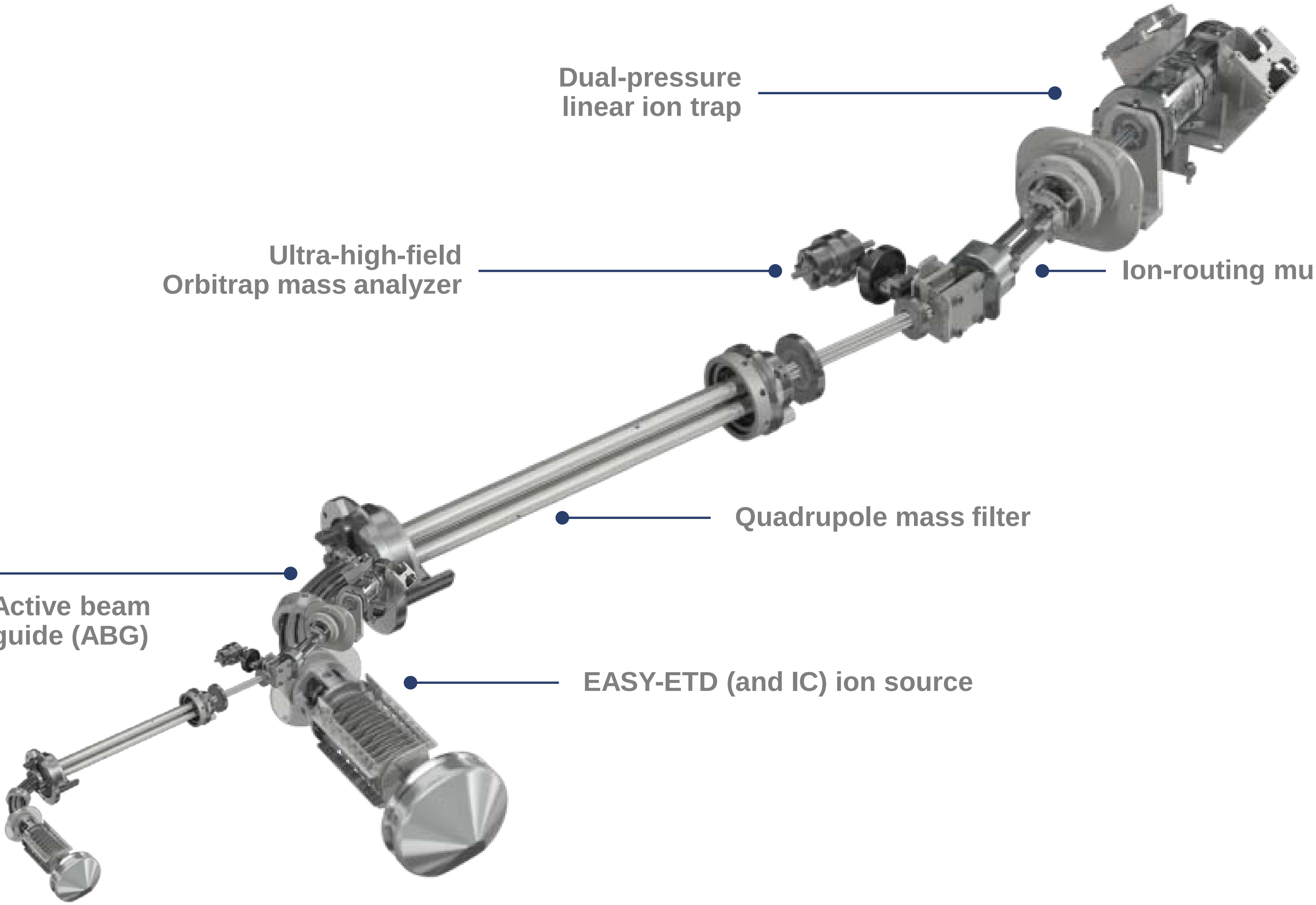
Unmatched Analytical Performance

Revolutionary performance

Unprecedented usability

Exceptional versatility

Orbitrap Fusion Tribrid Mass Spectrometer



Dual-pressure linear ion trap

Ultra-high-field Orbitrap mass analyzer

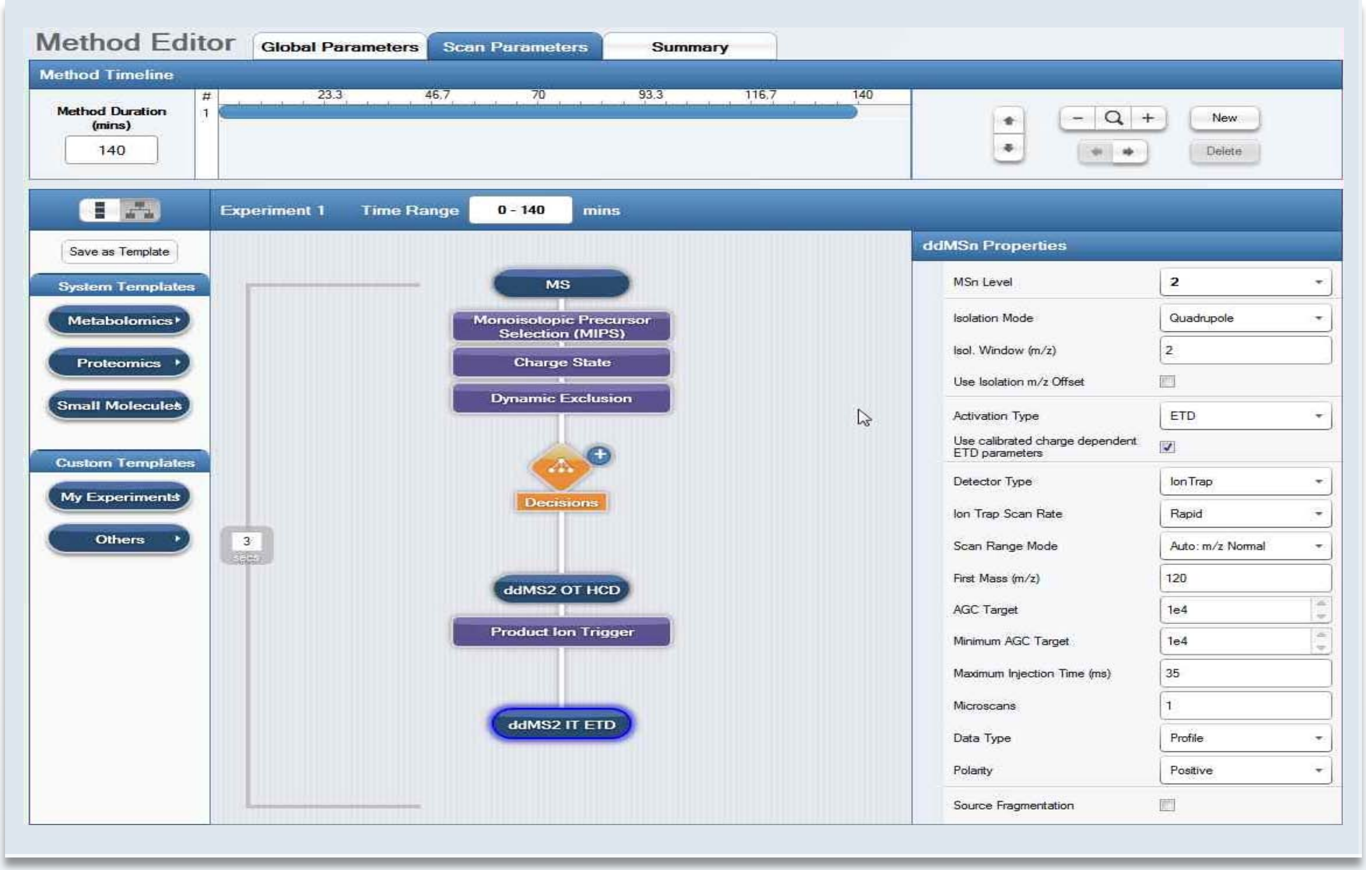
Ion-routing multipole

Quadrupole mass filter

Active beam guide (ABG)

EASY-ETD (and IC) ion source

Flexible, Intuitive, Easy to Learn Software



Method Duration (mins): 140

Experiment 1 Time Range 0 - 140 mins

MSn Level	2
Isolation Mode	Quadrupole
Isol. Window (m/z)	2
Use Isolation m/z Offset	☐
Activation Type	ETD
Use calibrated charge dependent ETD parameters	☑
Detector Type	Ion Trap
Ion Trap Scan Rate	Rapid
Scan Range Mode	Auto: m/z Normal
First Mass (m/z)	120
AGC Target	1e4
Minimum AGC Target	1e4
Maximum Injection Time (ms)	35
Microscans	1
Data Type	Profile
Polarity	Positive
Source Fragmentation	☐

The Thermo Scientific™ Orbitrap Fusion™ Method Editor allows for drag and drop method building.

Dynamic Scan Management for maximum data quality

Orbitrap Fusion Tribrid MS

Scan rate FTMS^2	15 Hz
Scan rate ITMS^2	20 Hz
Max resolution	450K at m/z 200
Quad isolation	down to 0.4 amu
Ion trap isolation	down to 0.2 amu
Mass Accuracy	1 ppm
Dissociation	CID, HCD, ETD
MSn	Up to MS^{10} in ion trap or Orbitrap
Analyzers	Q, FTMS, ITMS
Detectors	Ion Trap, Orbitrap
Compact	1186 x 674 x 650 mm (w, d, h)

Unmatched Analytical Performance

450,000 Resolution to remove spectral interferences

CID/HCD/ETD detected by the **Ion Trap or Orbitrap** at any level of MS^n for maximum experimental flexibility

Powered by the new **Dynamic Scan Management** architecture that lets every user easily get the maximum information from every sample

Mass Accuracy Matters!

Accurate mass improves the ability to confidently identify unknown compounds

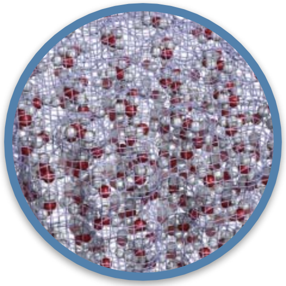
ppm error = (mass error / MW) x 10^6

EXAMPLE:

- Reserpine ($C_{33}H_{40}N_2O_9$) has a protonated ion at 609.28066

Mass Error **(ppm)**	**# of Possible Chemical Formulae** **(using only C, H, N & O)**
165 (0.1 daltons)	209
10	13
5	7
3	4
2	2
1	2
0.5 (0.0003 daltons)	1

Orbitrap Fusion Tribrid Mass Spectrometer



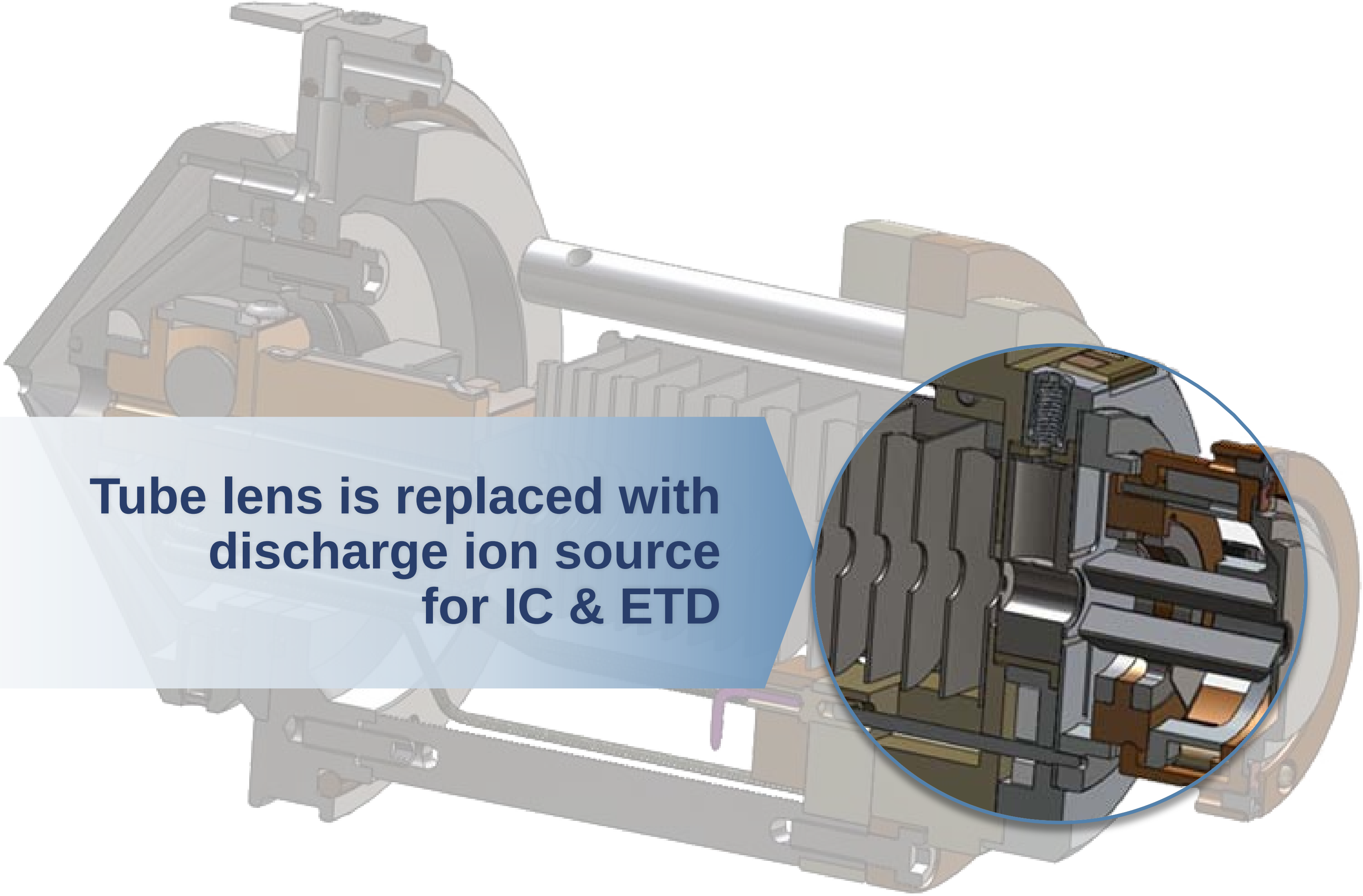
Challenge

Generate absolutely most accurate data to facilitate compound identification.

Orbitrap Fusion MS

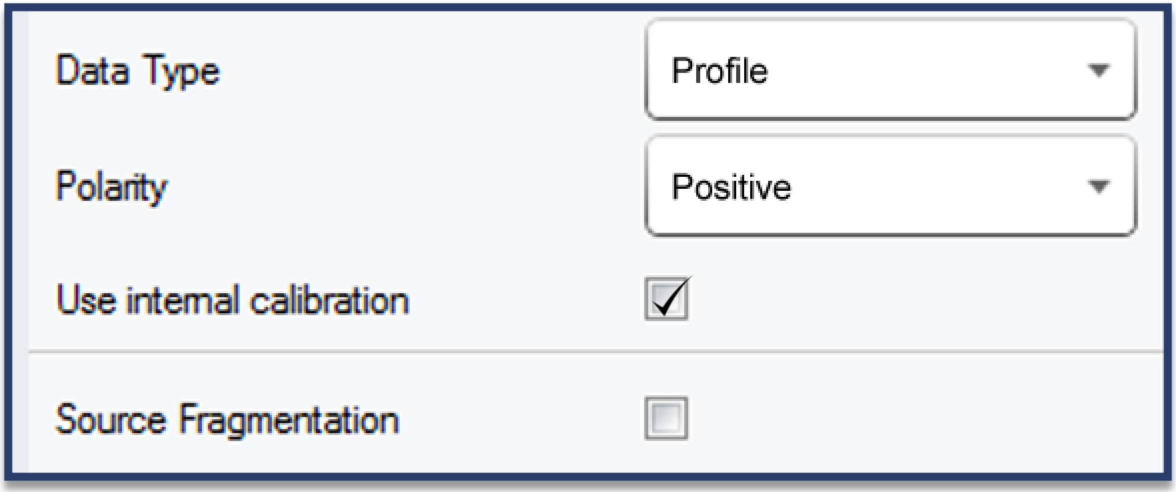
EASY IC (internal calibration): new ETD source allows use of internal calibration for sub-ppm mass accuracies. On/off toggle in SW. No ion suppression by IC.

Reagent/Internal Calibrant Source



Tube lens is replaced with discharge ion source for IC & ETD

Internal Calibration: LC/MS of Omeprazole Metabolites



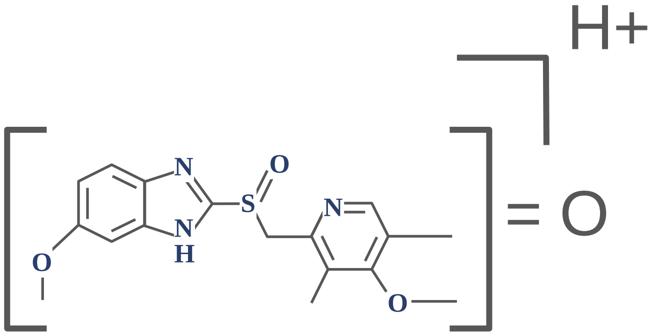
Data Type: Profile

Polarity: Positive

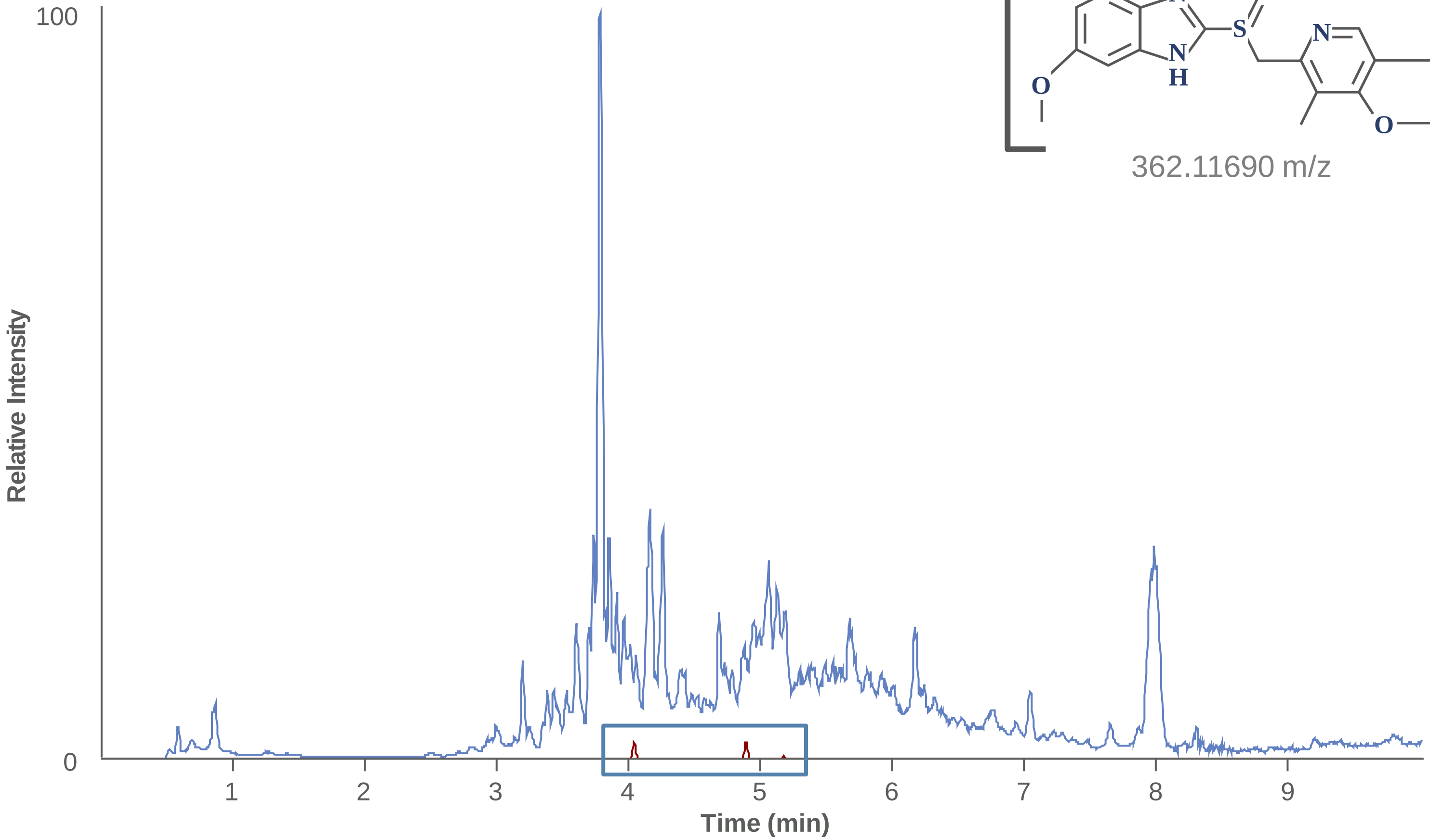
Use internal calibration ☑

Source Fragmentation ☐

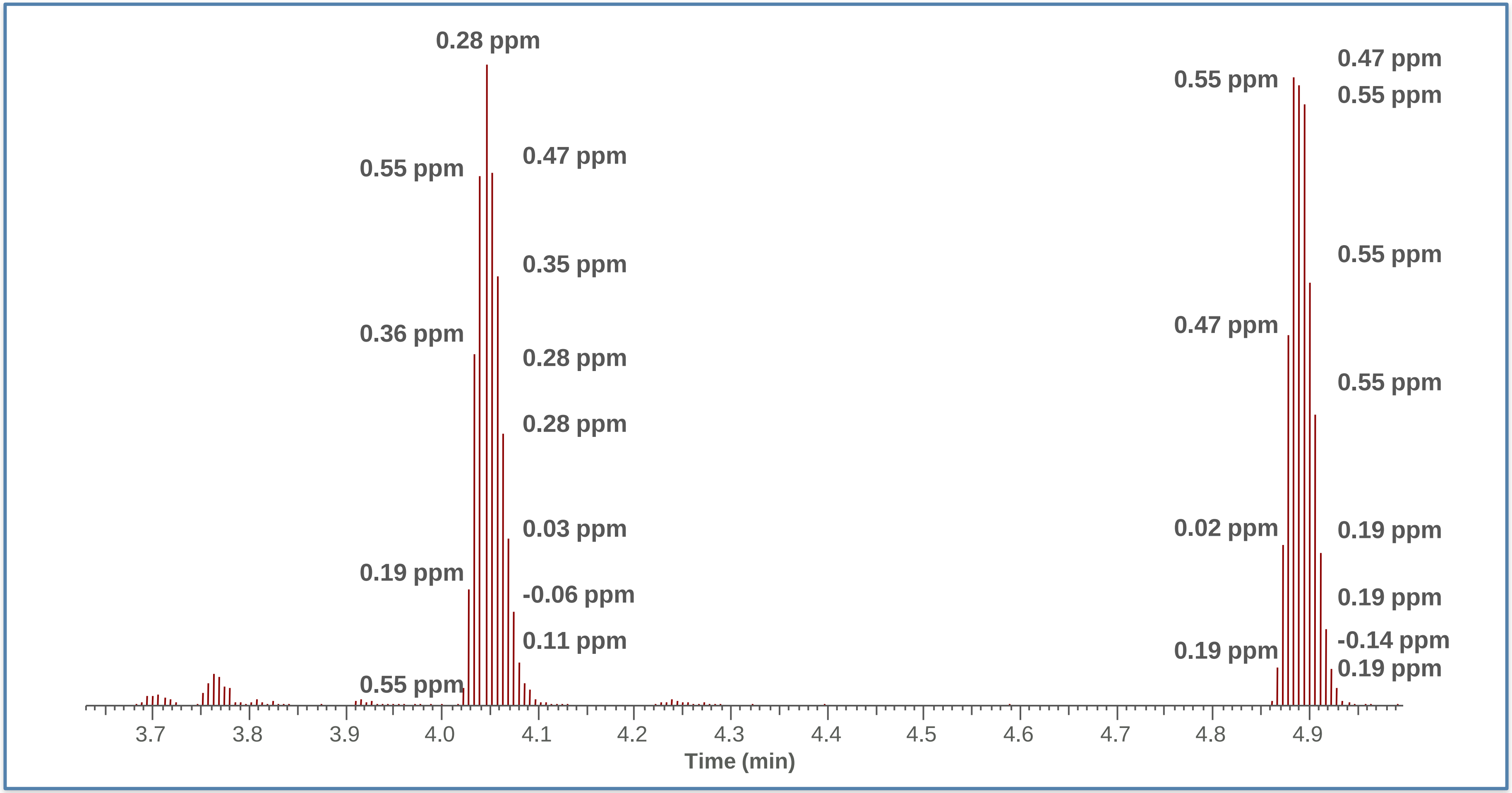
Internal Calibration: LC/MS of Omeprazole Metabolites



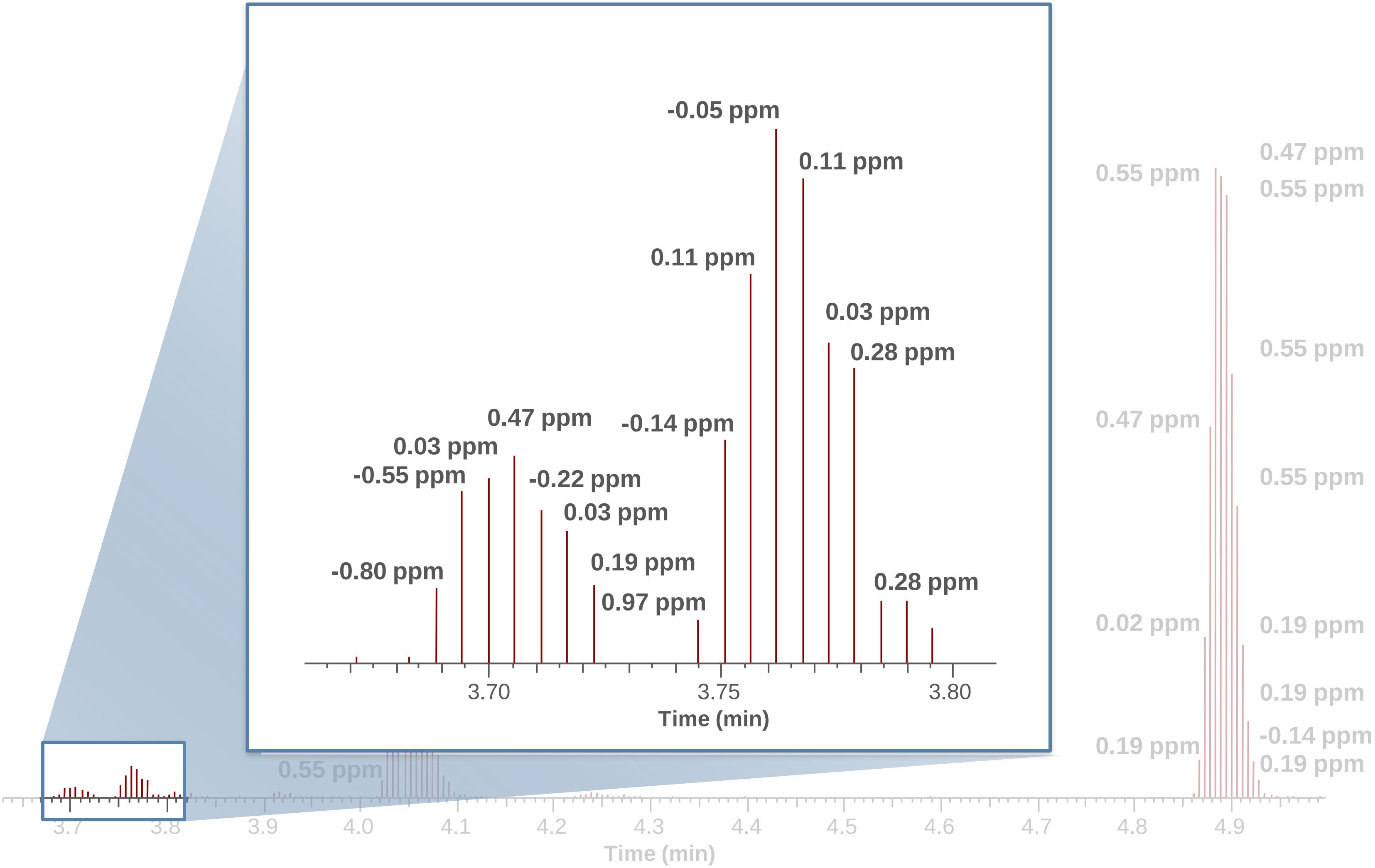
362.11690 m/z



Internal Calibration: LC/MS of Omeprazole Metabolites



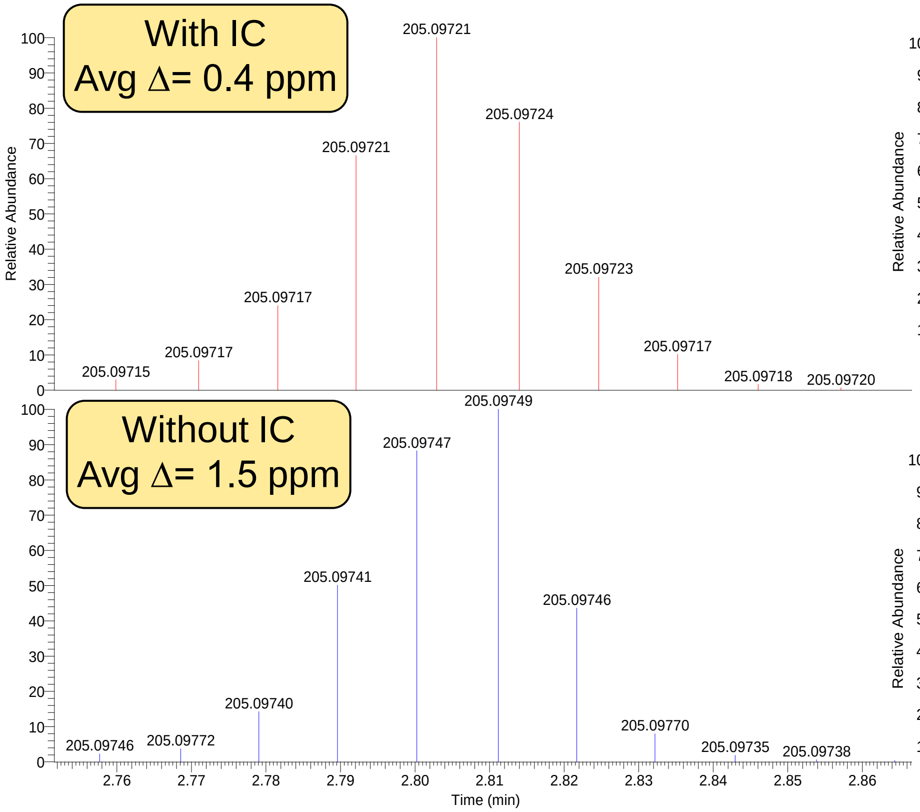
Internal Calibration: LC/MS of Omeprazole Metabolites



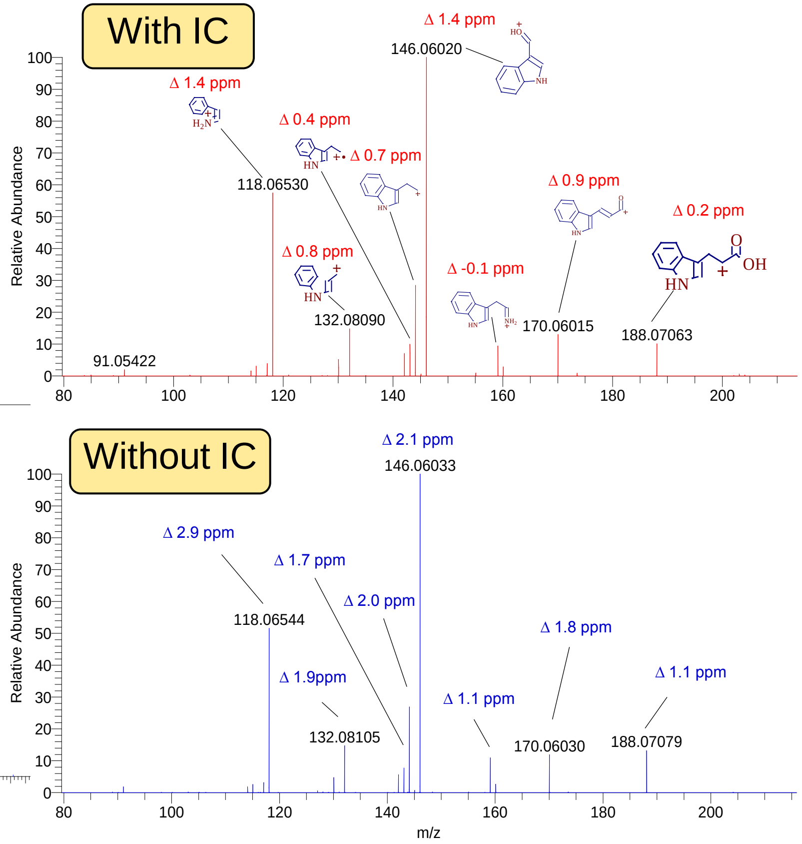
Easy-IC Providing Sub-ppm Mass Accuracy in MS and MS/MS

Orbitrap Fusion MS operating @120K for FS, 15k for HCD MS2, with UHPLC.
Sample, Urine. Analyte: L-Tryptophan, $C_{11}H_{12}N_2O_2$, $[M+H]^+=205.09715$

MS

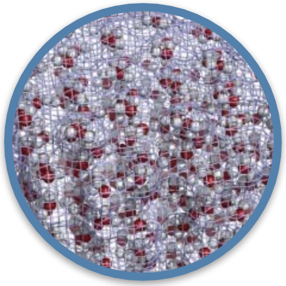


HCD MS/MS



ASMS 2013 poster **MP045** by Junhua Wang *et al.*

Orbitrap Fusion Tribrid Mass Spectrometer



Challenge

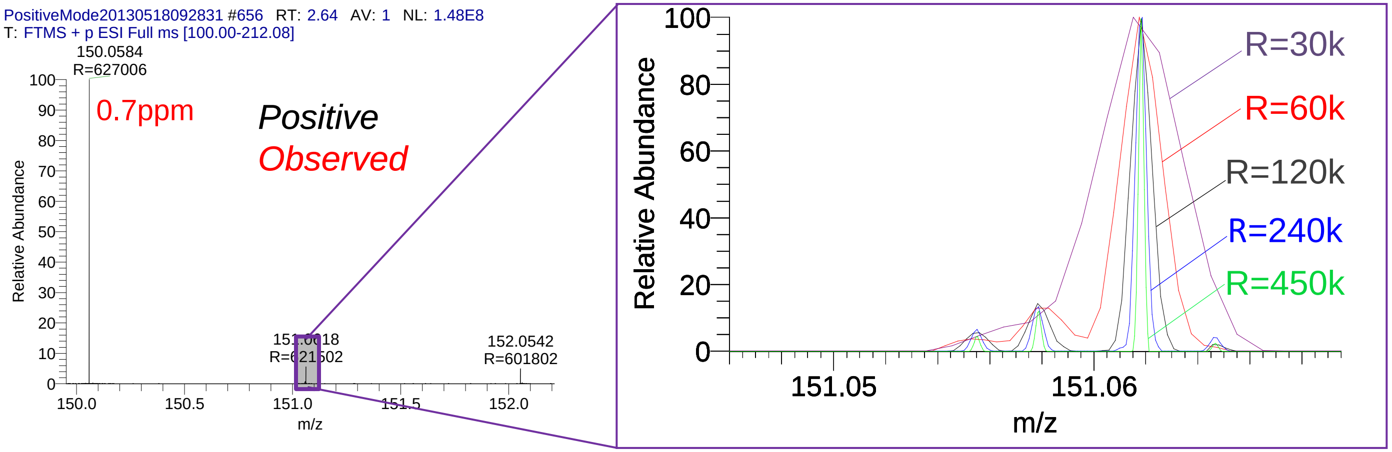
Separation of distinct isobars in complex mixtures

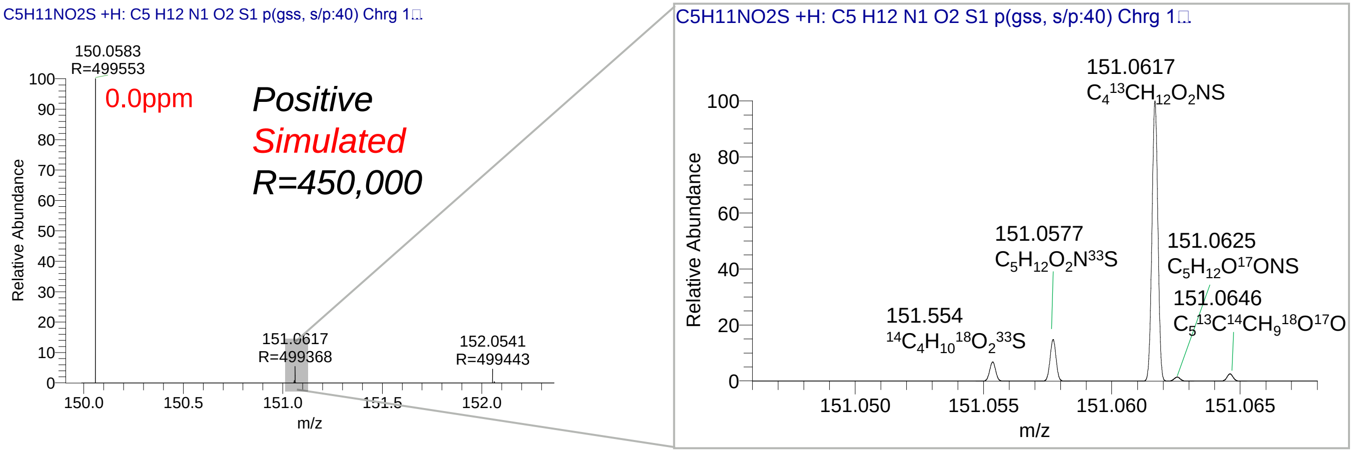
Orbitrap Fusion MS

Orbitrap Fusion can achieve up to 450,000 resolution.

See the Fine Isotope Pattern with Ultra-high Resolution

L-Methionine $C_5H_{11}NO_2S$

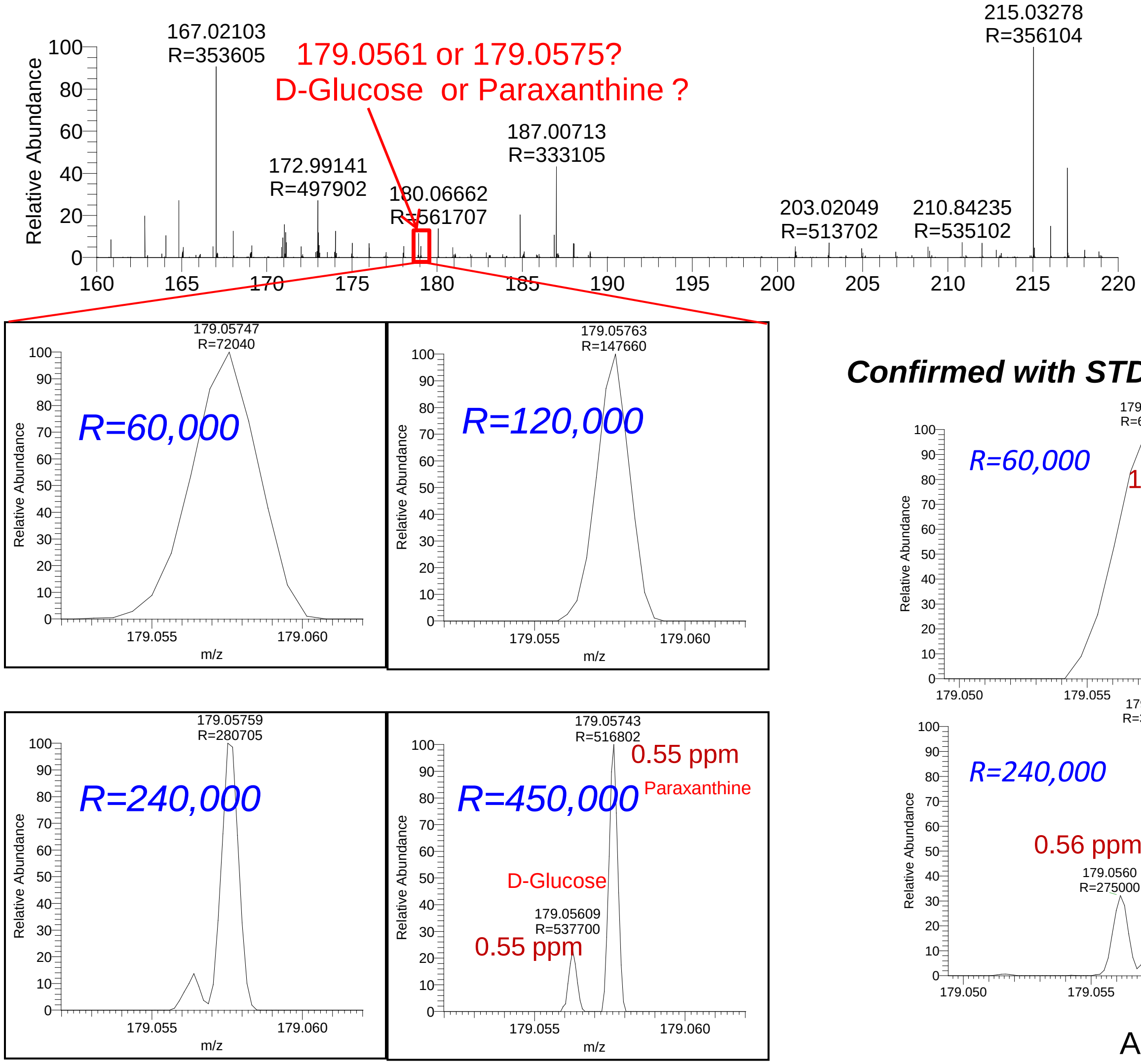


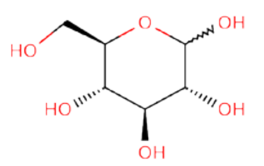


ASMS 2013 poster **MP045** by Junhua Wang *et al.*

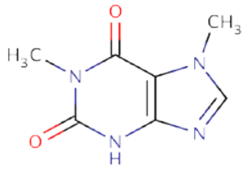
Resolving the Most Complex Sample

Nano-infusion of NIST SRM1950 plasma extract, Negative mode



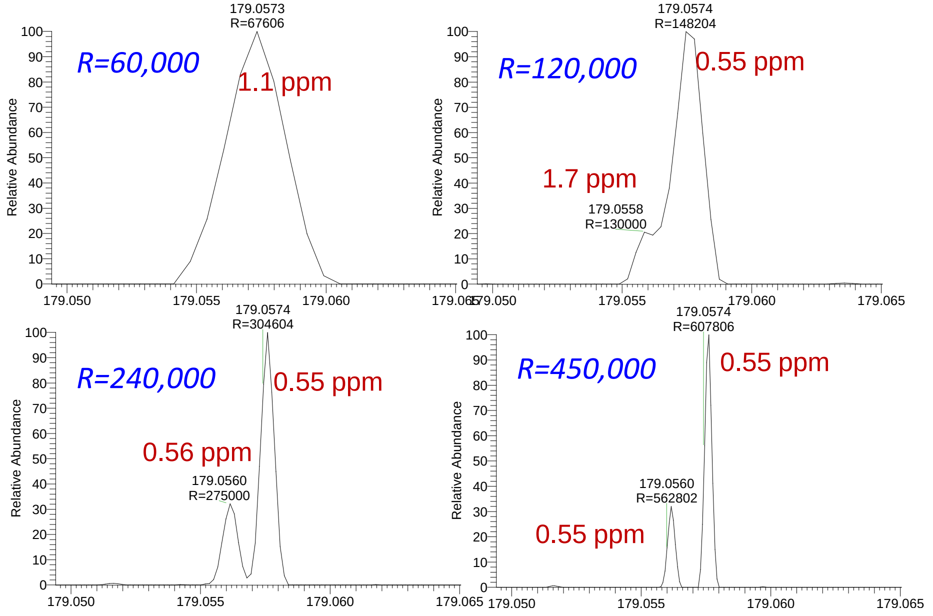


D-Glucose
$C_6H_{12}O_6$



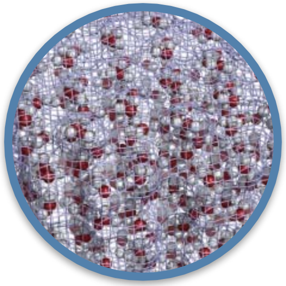
Paraxanthine
$C_7H_8N_4O_2$

Confirmed with STD mixture.



ASMS 2013 poster **MP045** by Junhua Wang *et al.*

Orbitrap Fusion Tribrid Mass Spectrometer



Challenge

Achieve maximum coverage of the metabolome/lipidome.

Many molecules require special fragmentation conditions to reveal their structural information.

Orbitrap Fusion MS

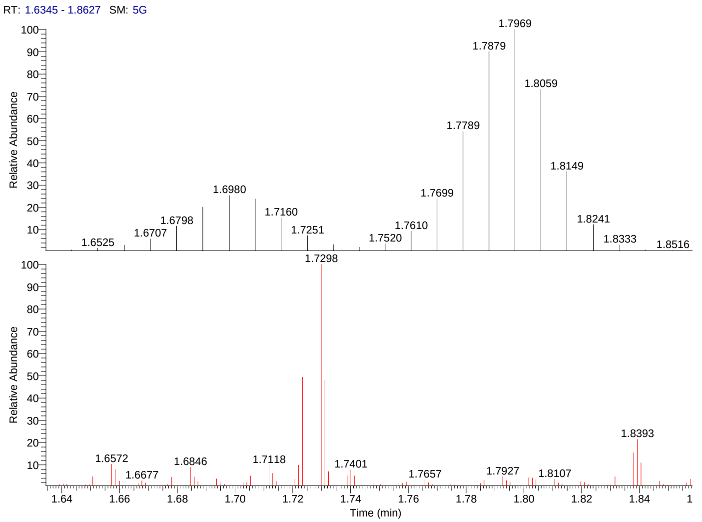
Instrument architecture allows for extremely fast MS/MS and full flexibility of MS^n experiments for structural analysis including novel workflows such as HCD^3 and MS^2 HCD followed by MS^3 CID.

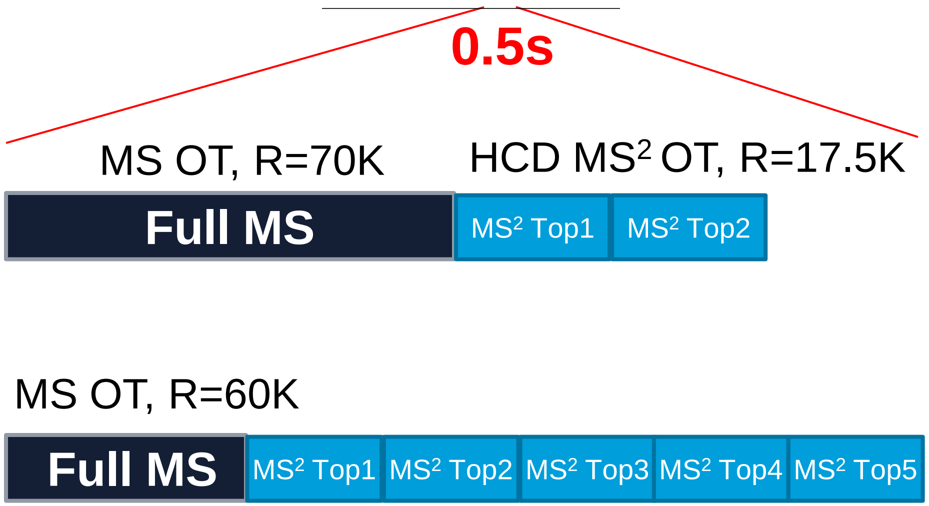
Method 1: The “Super” Q Exactive MS Mode

MS in Orbitrap

↓

Data dependent HCD MS/MS in Orbitrap





0.5s

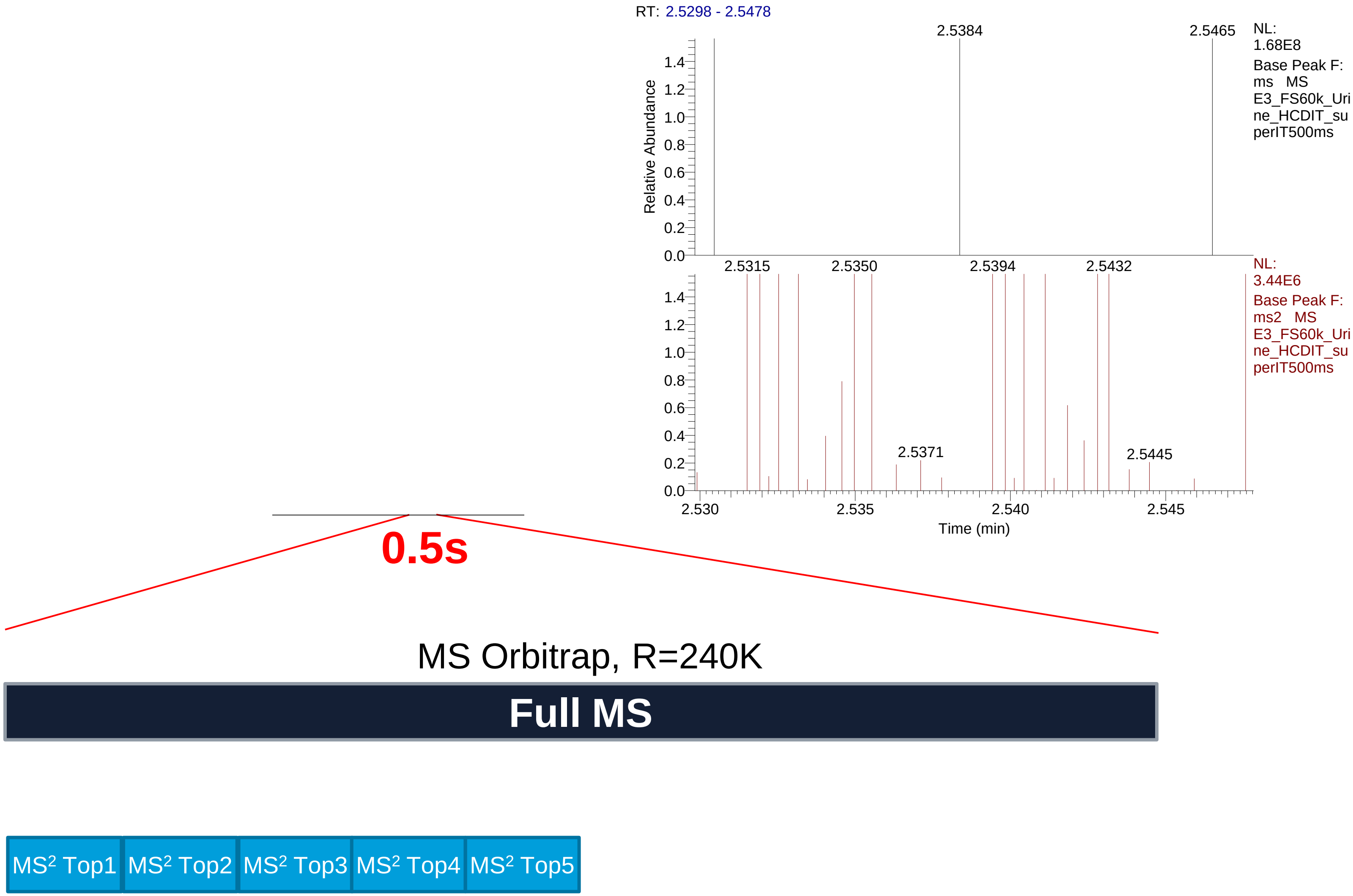
MS OT, R=70K

HCD MS^2 OT, R=17.5K

Full MS | MS^2 Top1 | MS^2 Top2

MS OT, R=60K

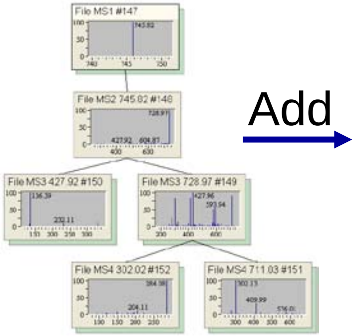
Full MS | MS^2 Top1 | MS^2 Top2 | MS^2 Top3 | MS^2 Top4 | MS^2 Top5

RT: 2.5298 - 2.5478
2.5384
2.5465
NL: 1.68E8
Base Peak F: ms MS E3_FS60k_Urine_HCDIT_superIT500ms
2.5315
2.5350
2.5394
2.5432
NL: 3.44E6
Base Peak F: ms2 MS E3_FS60k_Urine_HCDIT_superIT500ms
2.5371
2.5445
Relative Abundance
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2.530
2.535
2.540
2.545
Time (min)
0.5s
MS Orbitrap, R=240K
Full MS
MS² Top1
MS² Top2
MS² Top3
MS² Top4
MS² Top5

Method 3: MS^n Enables *de novo* Compound ID

Standards

Infusion (or LC)



Add

HR/AM MS^n Library

Match Score Above Threshold

IDs

Match Score Below Threshold

Individual Spectrum Search

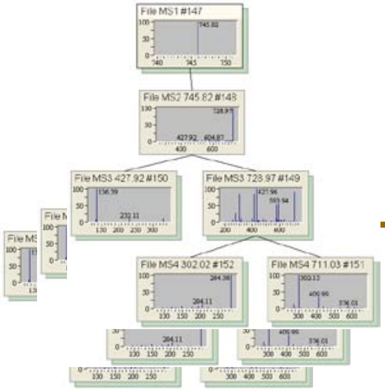
Partial Structures

Structure Candidates

- MS^n technology
- Spectral library technology
- Algorithms for structural-spectral investigation

Unknowns

LC-MS^n



Tree Search

Sheldon, Mistrik, **Croley**, *J Am Soc Mass Spectrom,* **2009**, 20: 370-376

Method 3: HR/AM Ion Trees for Unknown Identification

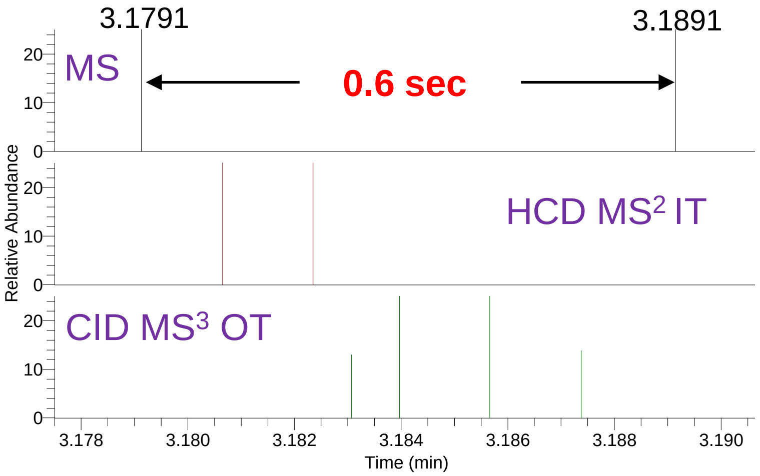
Full MS in Orbitrap

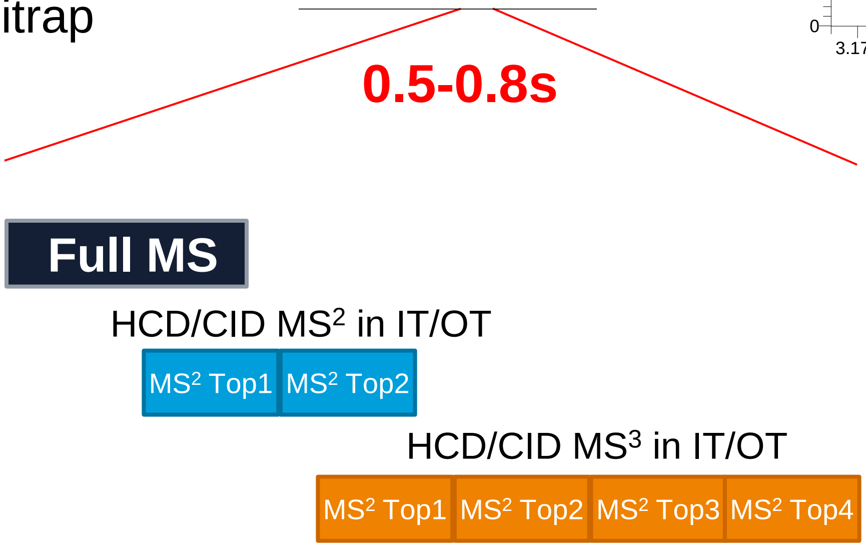
↓

Data dependent HCD MS^2 in ion trap

↓

Data dependent CID MS^3 in Orbitrap





0.5-0.8s

Full MS

HCD/CID MS^2 in IT/OT

MS^2 Top1	MS^2 Top2

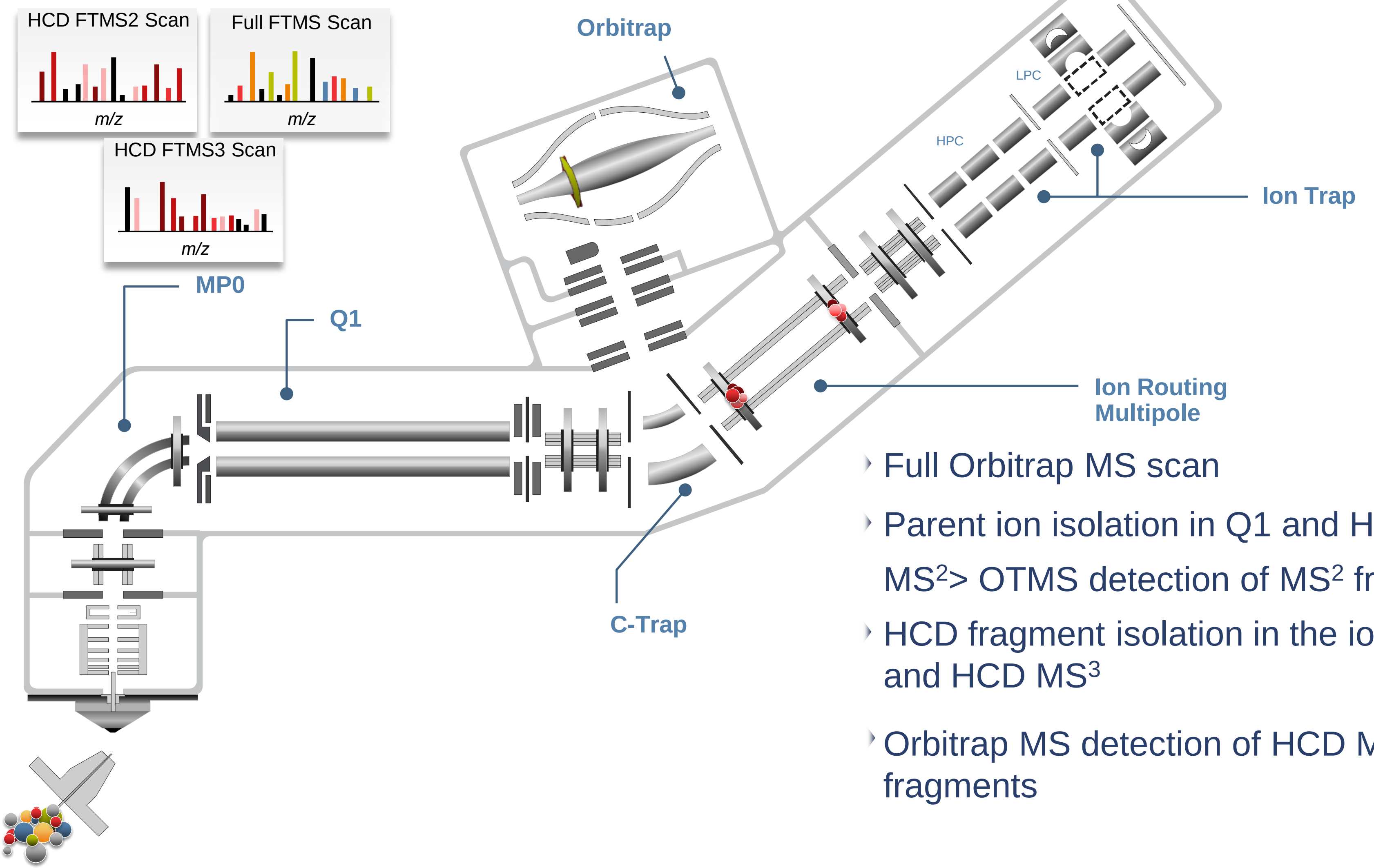
HCD/CID MS^3 in IT/OT

MS^2 Top1	MS^2 Top2	MS^2 Top3	MS^2 Top4

De novo structural elucidation: capture substructure with MS^n ion tree

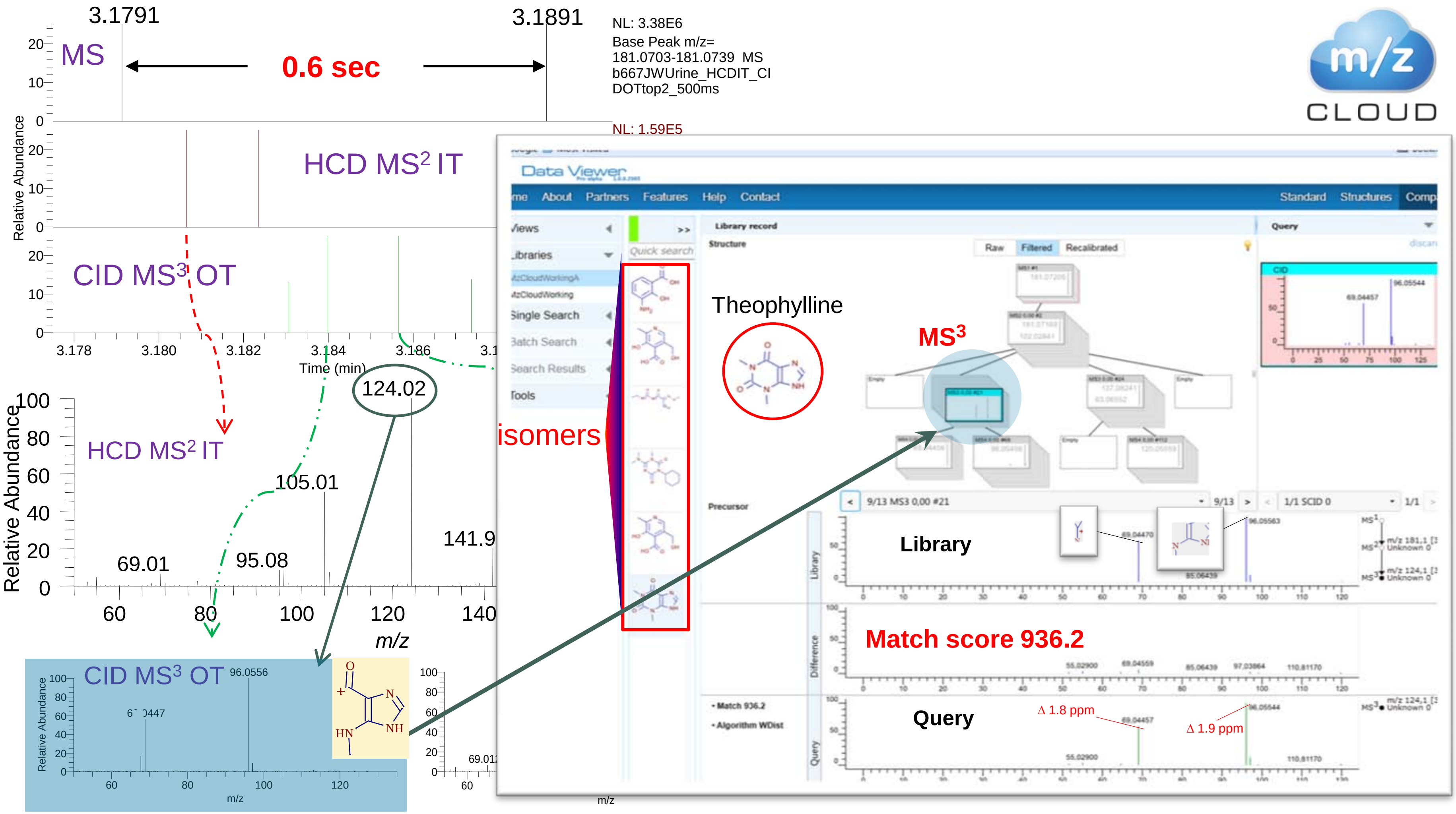
HCD MS^n is Unique to Orbitrap Fusion MS

OTMS> HCD OTMS^2> HCD OTMS^3



- Full Orbitrap MS scan
- Parent ion isolation in Q1 and HCD MS^2> OTMS detection of MS^2 fragments
- HCD fragment isolation in the ion trap and HCD MS^3
- Orbitrap MS detection of HCD MS^3 fragments

Structural Homology Identified by MS^n Ion Tree Search



The Industry's Leading Portfolio of MS Solutions

Exactive Series MS

Non-targeted Analysis

HR/AM

Tribrid Orbitrap MS

- Metabolomics
- Proteomics
- Bioanalysis

Quantitative

- Food Safety
- Environmental
- Clinical/Toxicology

Applied Markets

Transform Your Science

Research Markets

- Biomarker Discovery
- Proteomics
- Metabolism

Qualitative

- Metabolomics
- PTM Analysis
- Lipidomics

Triple Quads

MS, MS^n

Targeted Analysis

Ion Traps

Thermo
SCIENTIFIC

Transform Your Science