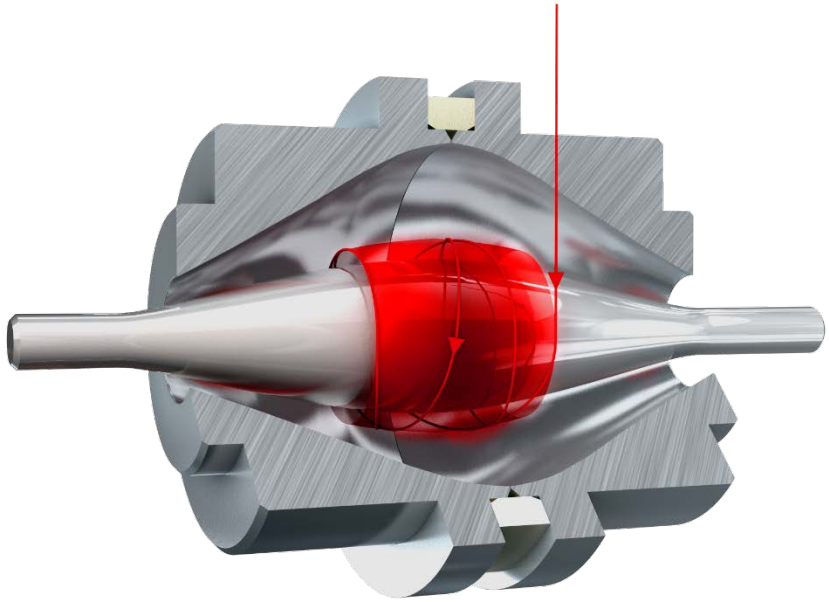
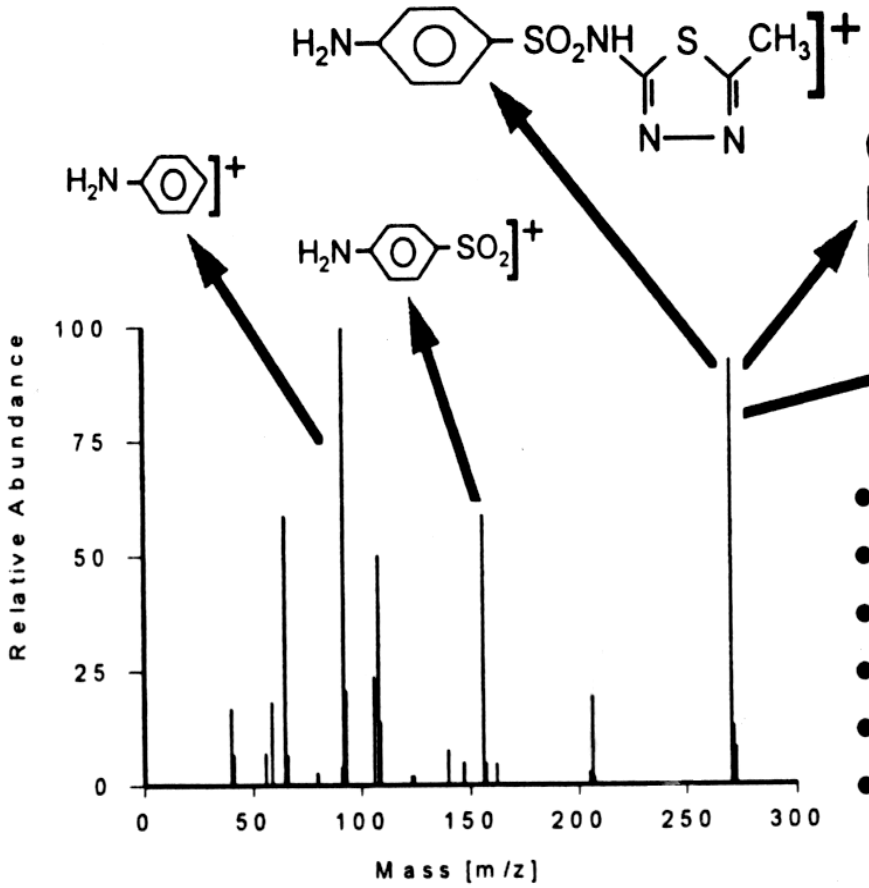


ORBITRAP Mass Spectrometer

An Ultimate Qual and Quan Machine

Pongsagon Pothavorn
Scispec Co., Ltd.

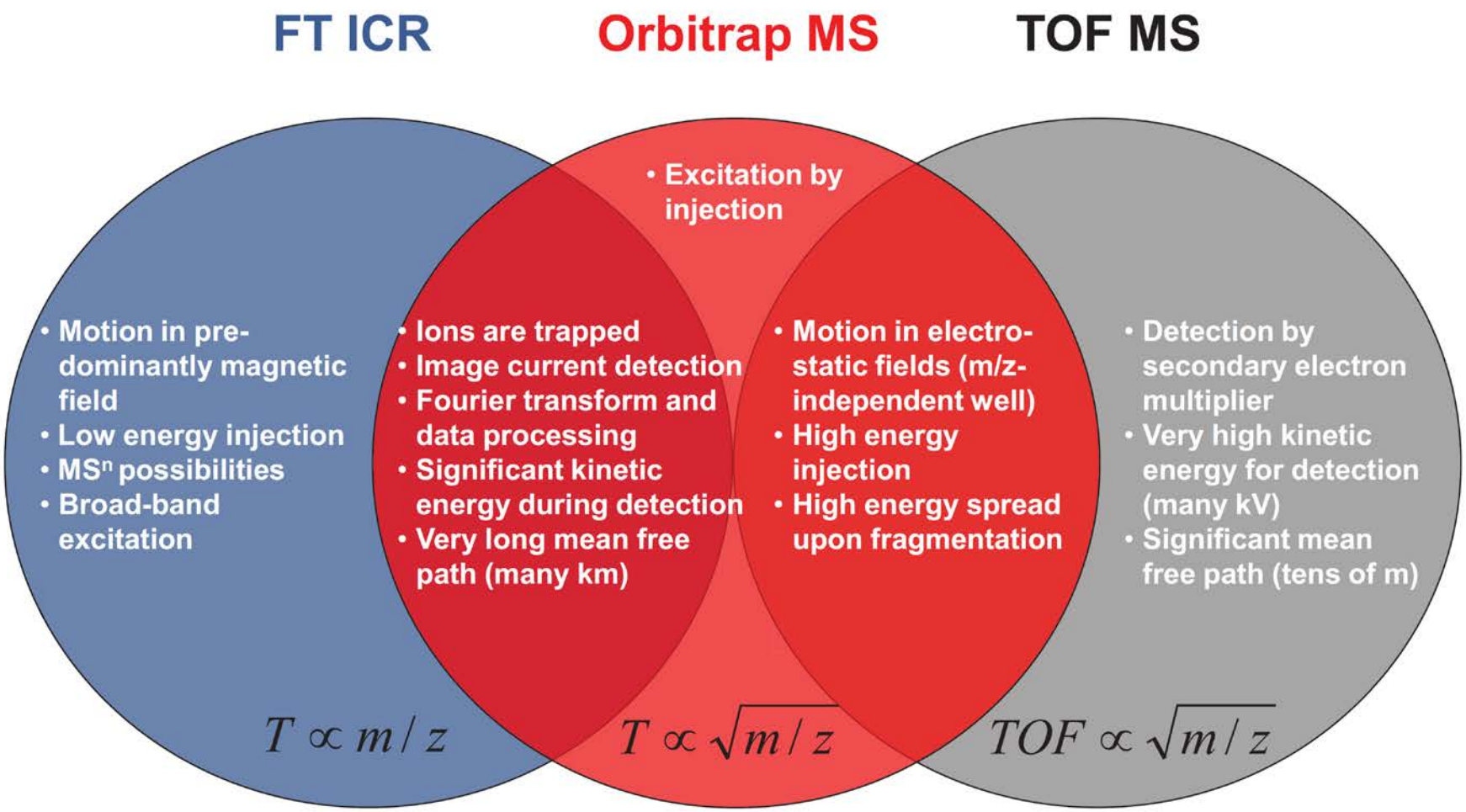
Information Rich Data



Compound: Sulfamethizole
MW: 270
Empirical Formula:
$C_9H_{10}N_4O_2S_2$

- *Molecular Weight*
- *Elemental Composition*
- *Empirical Formula*
- *Molecular Structure*
- *Unambiguous Identification*
- *Mass Specific Response*

Accurate Mass in Life Science

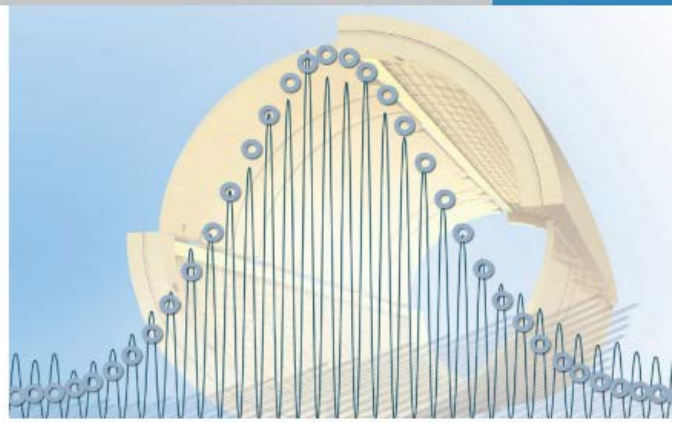


Resolution

FT Ultra - ICR

Thermo Scientific LTQ FT Ultra

Hybrid Mass Spectrometer
Unprecedented Analytical Power



- Concentration independent ppb mass accuracy
- Widest dynamic range
- Parallel detection
- MS^n
- ECD and IRMPD
- Ultra high resolution
- Intelligent Data Dependant acquisition

Unprecedented Analytical Power

The Thermo Scientific LTQ FT Ultra hybrid mass spectrometer delivers unprecedented analytical power for the most demanding applications.

The unmatched mass accuracy eliminates false positive identifications in bottom-up and middle-down proteomics and enables the unambiguous identification of unknown analytes with on-line LC-MS^n at any concentration.

Ultra high resolution is essential for the analysis of complex samples such as crude oil, Dissolved Organic Matter (DOM) or intact proteins. This is available with a single mouse click.

Brukers FT-ICR

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solariX MRMS

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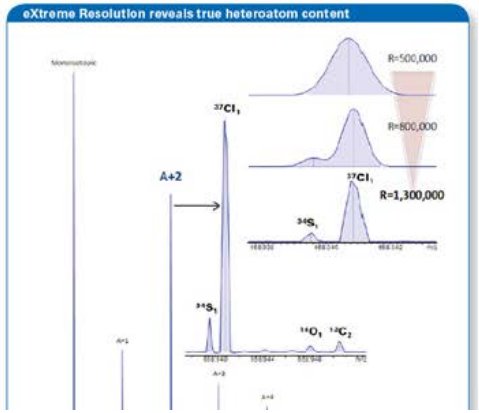
solariX MRMS - routine IFS for unambiguous results

The solariX MRMS (Magnetic Resonance Mass Spectrometry) instruments are available for different magnetic field strengths of 7T, 12T and 15T.

	7T solariX	12T solariX	15T solariX
maximum resolving power	>10M	>10M	>10M
Mass accuracy (internal)	600 ppb	300 ppb	250 ppb
ESI	yes	yes	yes
MALDI	optional	optional	optional
ETD	optional	optional	optional
detector	ParaCell XR or 2xR	ParaCell XR	ParaCell XR
magnetic field	7T	12T	15T
annual cryogen fill	yes	yes	yes
quench line required	yes	yes	yes

Key features of solariX series instruments



Waters Ion Mobility

Waters

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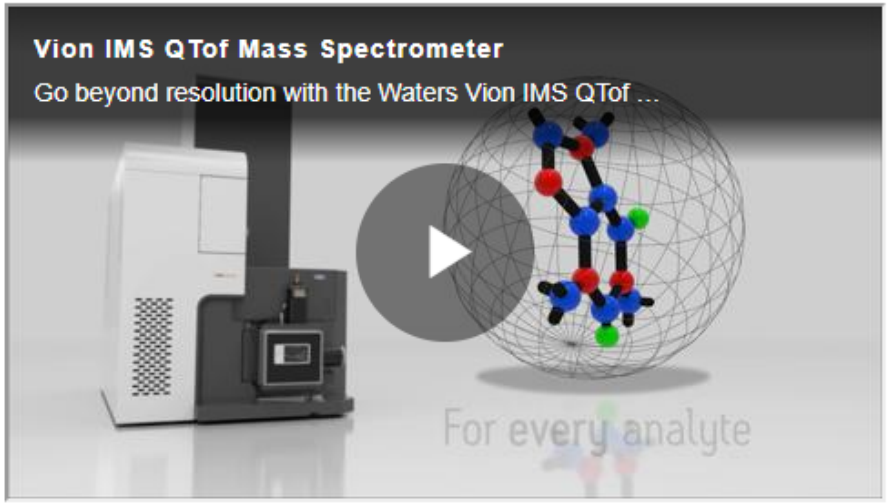
Vion IMS QTof Ion Mobility Quadrupole Time-of-flight Mass Spectrometry

Vion IMS QTof Ion Mobility Quadrupole Time-of-flight Mass Spectrometry

Beyond Resolution

Does high resolution, accurate mass data always lead to the correct answer first time? Avoid additional confirmatory experiments by utilizing the selectivity of ion mobility. Featuring new geometry and including XS Ion Optics and the QuanTof2 detection system, the Vion IMS QTof has the sensitivity and dynamic range to make ion mobility quantitative and routinely usable, enabling:

- easier data interpretation as ion mobility cleans up and simplifies every spectrum
- confident identification and quantification of analytes
- faster method development and higher sample throughput
- greater selectivity than conventional MS/MS with HDMS^E

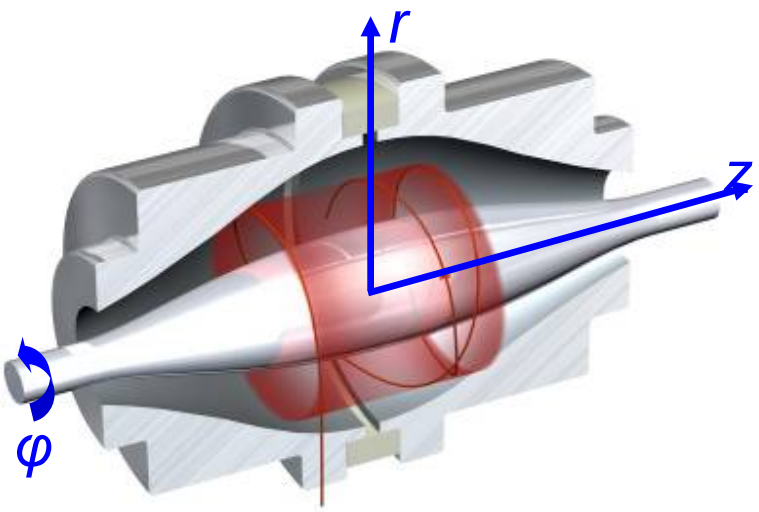


LC-MS solutions for all analytical challenges

- Best LC-MS Portfolio

Induced by ion packets moving inside the trap

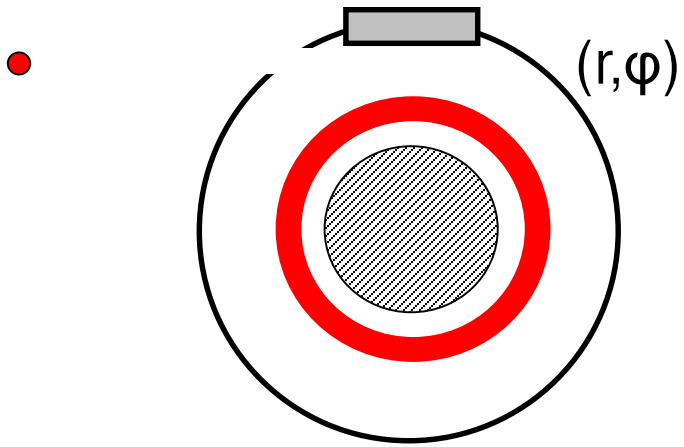
- Ions trapped in an electrostatic field
- Central electrode kept on high voltage
- Outer electrode is split and able to pick up an image current induced by ion packets moving inside the trap

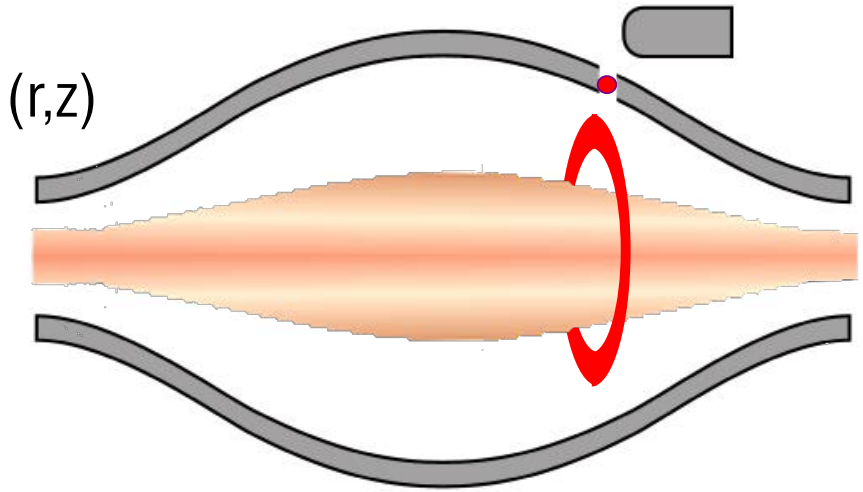


$$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2/2 + R_m^2 \cdot \ln(r/R_m) \right\}$$

Ion Injection and Formation of Ion Rings

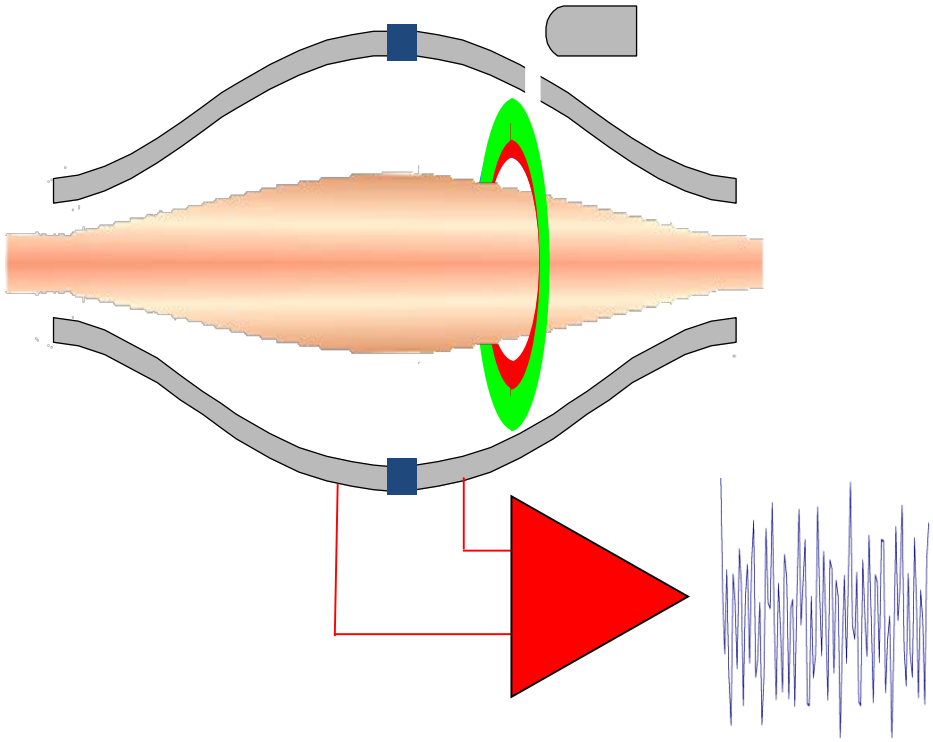
- An ion packet of a selected *m/z* enters the field
- Increasing voltage squeezes ions
- Voltage stabilises and ion trajectories are also stabilized
- Angular spreading forms a ROTATING RING





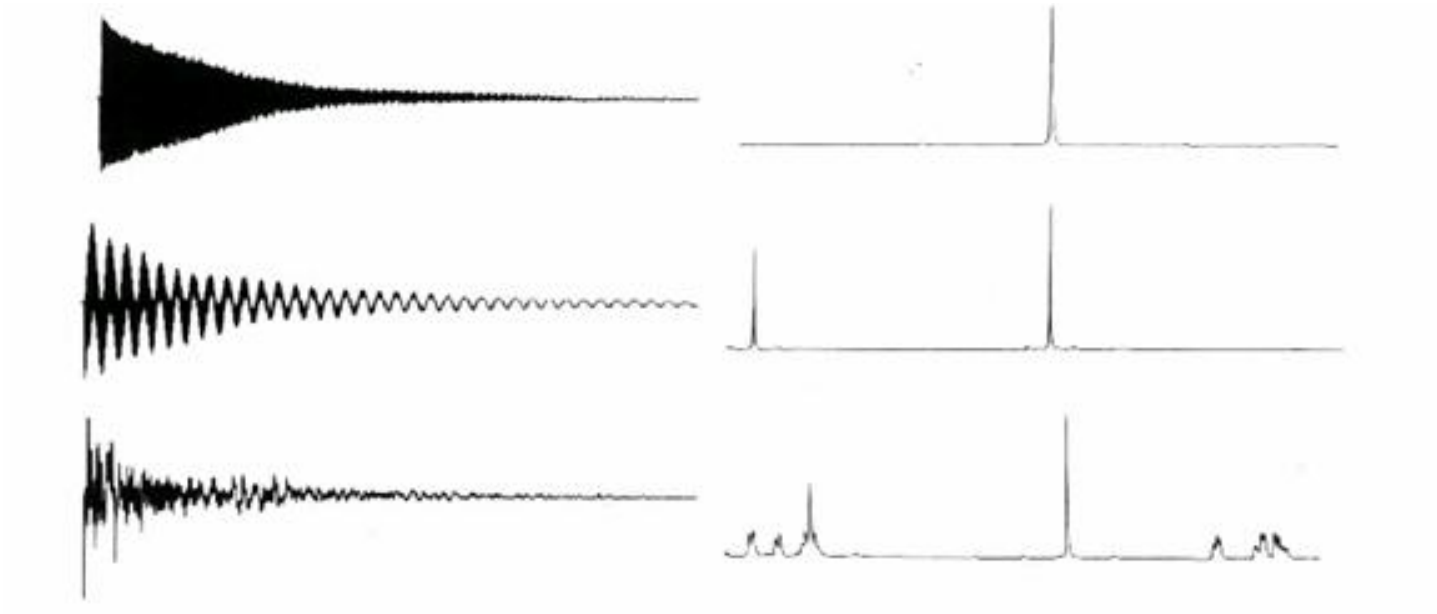
Fourier Transform-based

- The moving ion rings induce an image current on outer electrodes
- The frequency of harmonic oscillations is proportional to ions' *m/z*



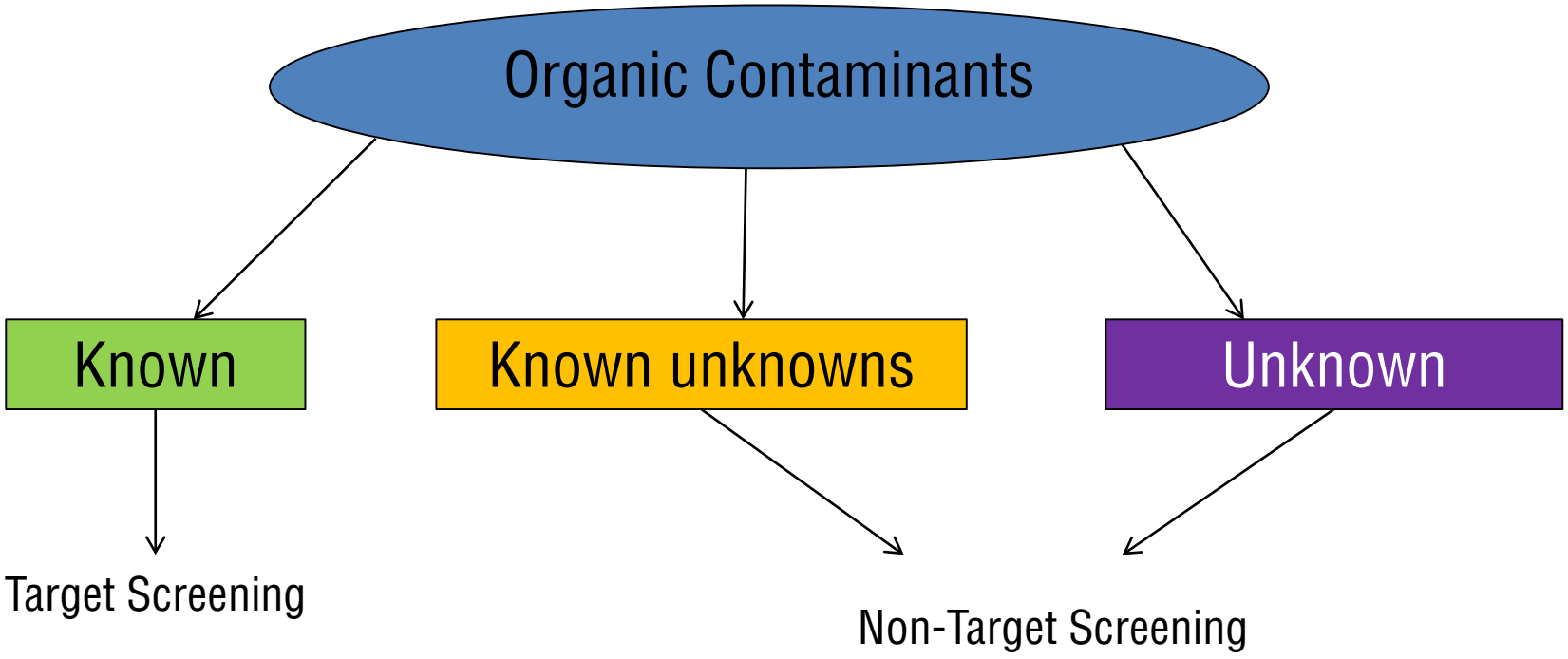
Orbitrap and Nuclear Magnetic Resonance (NMR)

- Free Induction Decay (FID)



Time Domain ->Fourier Transform -> Spectrum (Frequency Domain)

Strategies for Analysis



Rapid and sensitive screening methods able to assign positive hits undoubtedly to particular organic compounds

Typical Mass Accuracy

Type of MS	Mass accuracy	Utility for
Quadrupole	0.1 μ	Identify
Traps	0.1 μ	Identify
TOF	0.0001 μ	Empirical formula/ composition
Sector	0.0001 μ	Empirical formula/ composition
FT-MS	0.0001 μ	Empirical formula/ composition

Mass Accuracy

- The precision of which the mass is measured by the mass spectrometer.
- Typical way of reporting mass error in ppm (relative measure) or mDa (absolute measure)

$$\text{Mass error} = \left(\frac{\text{Measured} - \text{Exact Mass}}{\text{Exact Mass}} \right) \times 10^6$$

C = 12.0000

O = 15.9949

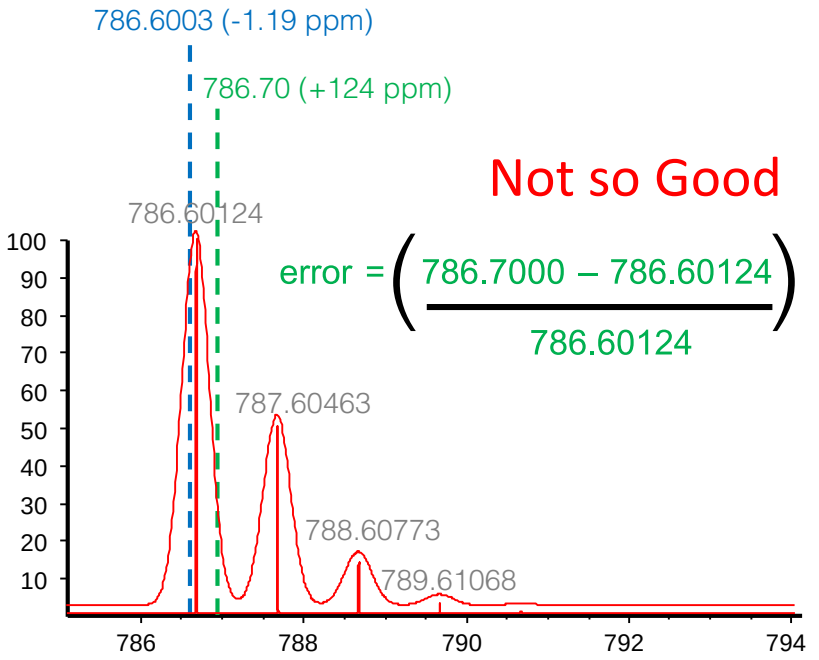
S = 31.9721

H = 1.0078

N = 14.0031

Good

$$\text{error} = \left(\frac{786.6003 - 786.60124}{786.60124} \right) \times 10^6$$



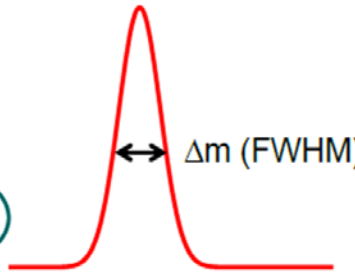
Not so Good

$$\text{error} = \left(\frac{786.7000 - 786.60124}{786.60124} \right) \times 10^6$$

Mass Resolution

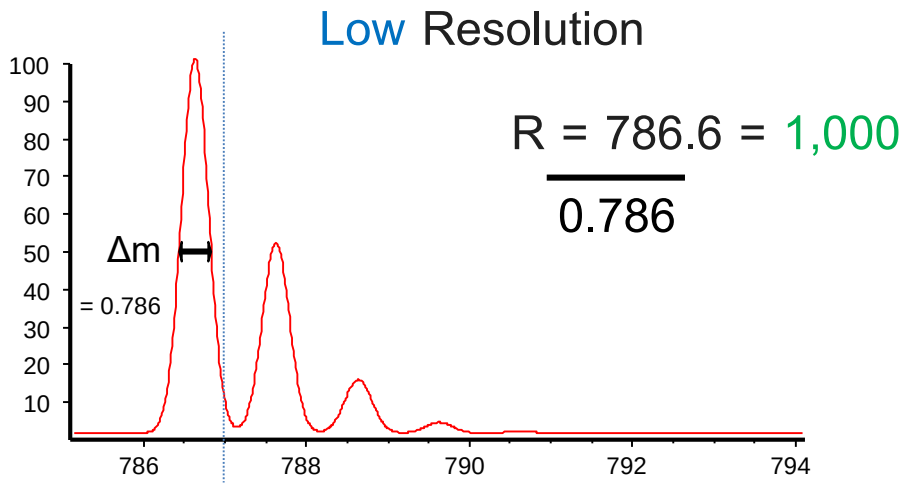
- Ability of a mass spectrometer to distinguish between ions of nearly equal m/z ratios (isobars).

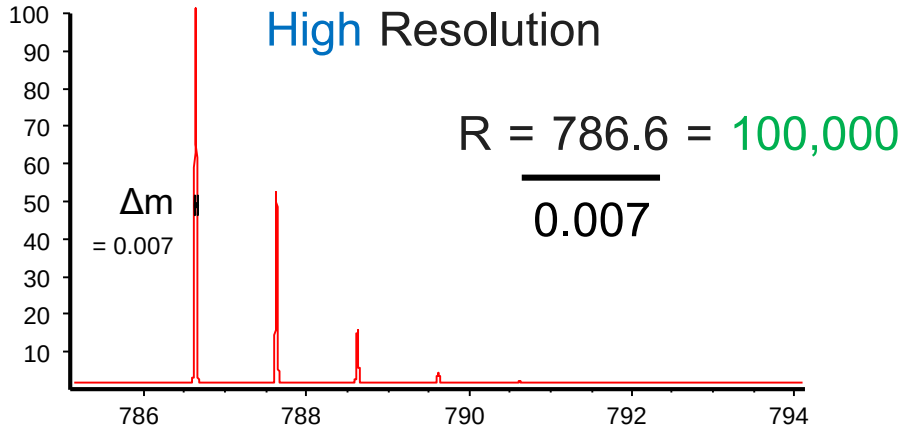
$$R = \frac{m}{\Delta m}$$



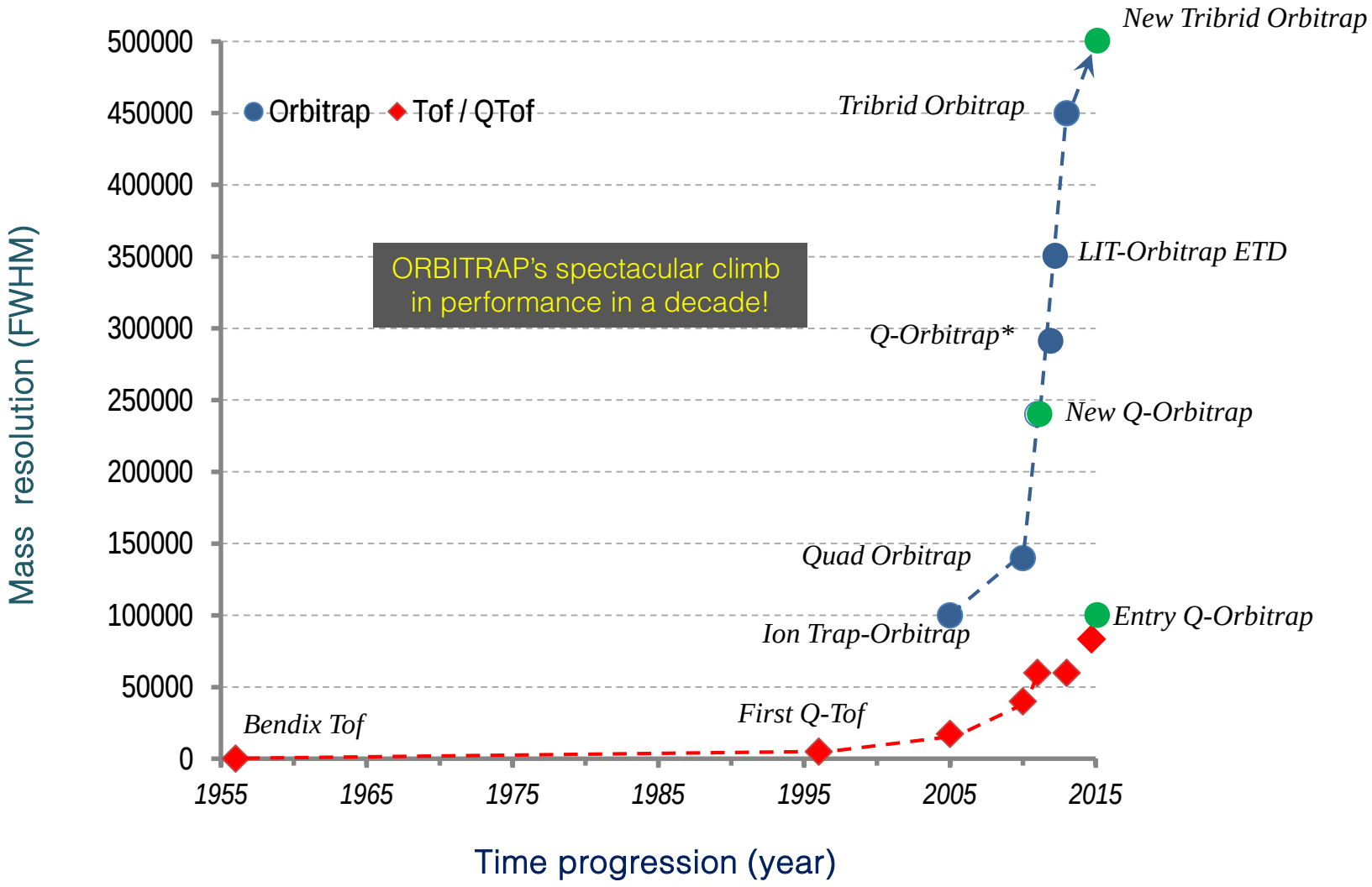
m - measured mass

Δm - peak width measured at 50% peak intensity (Full Width Half Maximum)



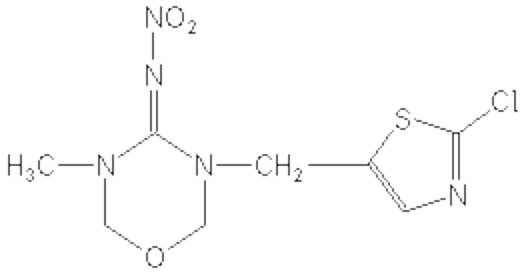


Commercial High Resolution MS Technology Race

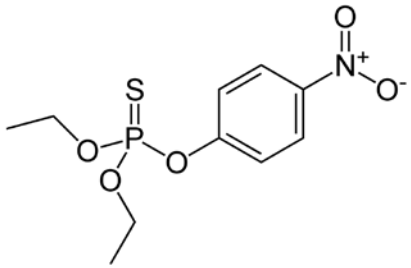


Isobaric Pesticides

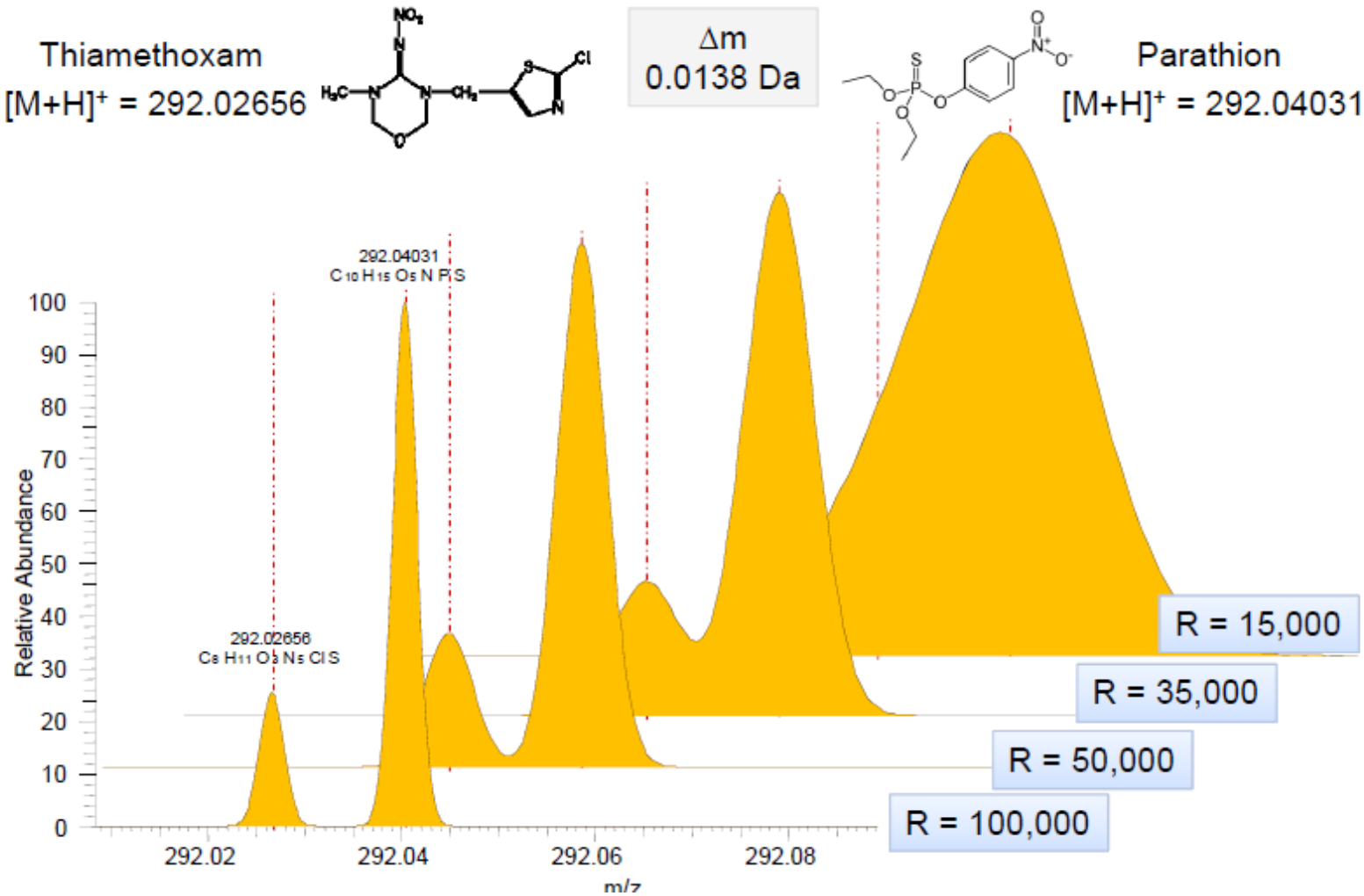
Thiamethoxam: $[M+H]^+ = C_8H_{11}ClN_5O_3S$ (292.02656)



Parathion: $[M+H]^+ = C_{10}H_{15}NO_5PS$ (292.04031)

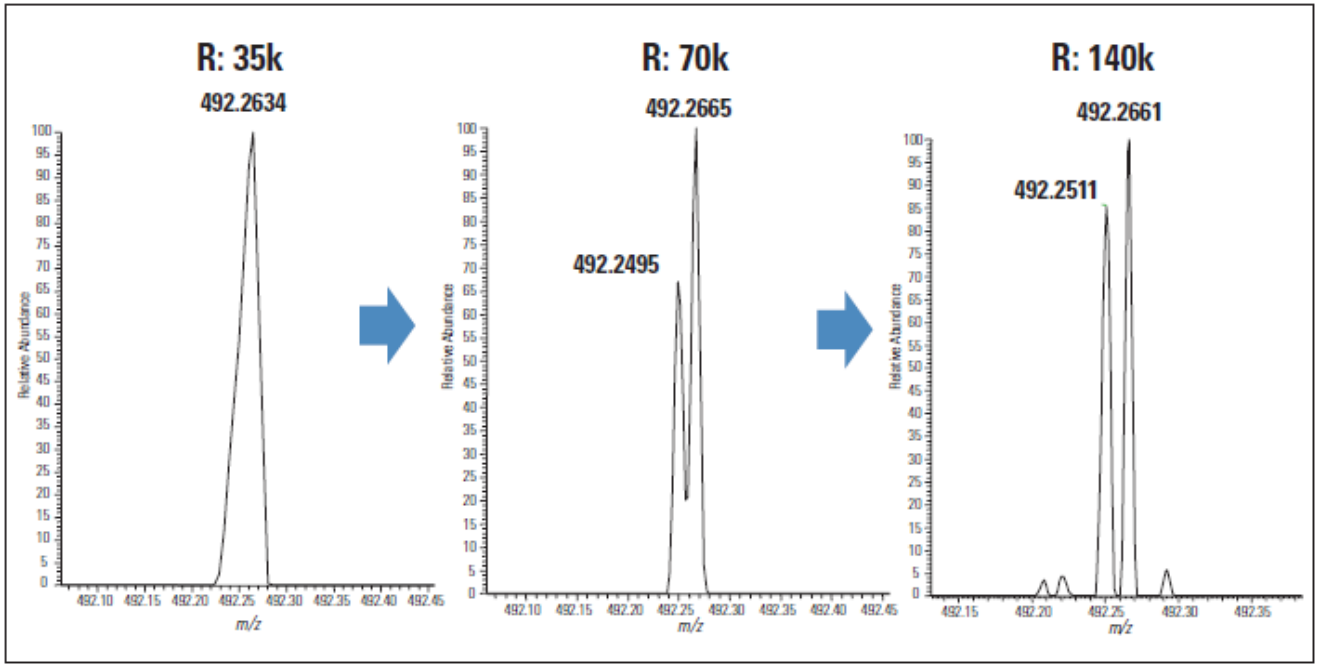


Isobaric Pesticides 3:1 Mix



Resolution – ***Why Is It Important?***

- Enables accurate mass
- Increases confidence of identification
- Improves quantitative accuracy
- Gives access to qualitatively different information



Periodic Table of Elements

Average Mass

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1 H Hydrogen 1.00794	Atomic # / Symbol / Name / Atomic Mass																2 He Helium 4.002602
2	3 Li Lithium 6.941	4 Be Beryllium 9.012182											5 B Boron 10.811	6 C Carbon 12.0107	7 N Nitrogen 14.0067	8 O Oxygen 15.9994	9 F Fluorine 18.9984032	10 Ne Neon 20.1797
3	11 Na Sodium 22.98976928	12 Mg Magnesium 24.3050											13 Al Aluminium 26.9815386	14 Si Silicon 28.0855	15 P Phosphorus 30.973762	16 S Sulfur 32.065	17 Cl Chlorine 35.453	18 Ar Argon 39.948
4	19 K Potassium 39.0983	20 Ca Calcium 40.078	21 Sc Scandium 44.955912	22 Ti Titanium 47.867	23 V Vanadium 50.9415	24 Cr Chromium 51.9961	25 Mn Manganese 54.938045	26 Fe Iron 55.845	27 Co Cobalt 58.933195	28 Ni Nickel 58.6934	29 Cu Copper 63.546	30 Zn Zinc 65.38	31 Ga Gallium 69.723	32 Ge Germanium 72.64	33 As Arsenic 74.92160	34 Se Selenium 78.96	35 Br Bromine 79.904	36 Kr Krypton 83.798
5	37 Rb Rubidium 85.4678	38 Sr Strontium 87.62	39 Y Yttrium 88.90585	40 Zr Zirconium 91.224	41 Nb Niobium 92.90638	42 Mo Molybdenum 95.96	43 Tc Technetium (97.9072)	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.90550	46 Pd Palladium 106.42	47 Ag Silver 107.8682	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.710	51 Sb Antimony 121.760	52 Te Tellurium 127.60	53 I Iodine 126.90447	54 Xe Xenon 131.293
6	55 Cs Caesium 132.9054519	56 Ba Barium 137.327	57–71	72 Hf Hafnium 178.49	73 Ta Tantalum 180.94788	74 W Tungsten 183.84	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.217	78 Pt Platinum 195.084	79 Au Gold 196.966569	80 Hg Mercury 200.59	81 Tl Thallium 204.3833	82 Pb Lead 207.2	83 Bi Bismuth 208.98040	84 Po Polonium (208.9824)	85 At Astatine (209.9871)	86 Rn Radon (222.0176)
7	87 Fr Francium (223)	88 Ra Radium (226)	89–103	104 Rf Rutherfordium (261)	105 Db Dubnium (262)	106 Sg Seaborgium (266)	107 Bh Bohrium (264)	108 Hs Hassium (277)	109 Mt Meitnerium (268)	110 Ds Darmstadtium (271)	111 Rg Roentgenium (272)	112 Uub Ununbium (285)	113 Uut Ununtrium (284)	114 Uuq Ununquadium (289)	115 Uup Ununpentium (288)	116 Uuh Ununhexium (292)	117 Uus Ununseptium	118 Uuo Ununoctium (294)

	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71
	La Lanthanum 138.90547	Ce Cerium 140.116	Pr Praseodymium 140.90765	Nd Neodymium 144.242	Pm Promethium (145)	Sm Samarium 150.36	Eu Europium 151.964	Gd Gadolinium 157.25	Tb Terbium 158.92535	Dy Dysprosium 162.500	Ho Holmium 164.93032	Er Erbium 167.259	Tm Thulium 168.93421	Yb Ytterbium 173.054	Lu Lutetium 174.9668
	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103
	Ac Actinium (227)	Th Thorium 232.03806	Pa Protactinium 231.03588	U Uranium 238.02891	Np Neptunium (237)	Pu Plutonium (244)	Am Americium (243)	Cm Curium (247)	Bk Berkelium (247)	Cf Californium (251)	Es Einsteinium (252)	Fm Fermium (257)	Md Mendelevium (258)	No Nobelium (259)	Lr Lawrencium (262)

C Solid
Hg Liquid
H Gas
Rf Unknown

Metals: Alkali metals; Alkaline earth metals; Lanthanoids; Actinoids; Transition metals; Poor metals
Nonmetals: Other nonmetals; Noble gases

For elements with no stable isotopes, the mass number of the isotope with the longest half-life is in parentheses.

Ptable.com

How's About Mass Accuracy

- Average Mass = summing the average atomic masses of the constituent elements, H_2O; 1.00794 + 1.00794 + 15.9994 = 18.01528.
- Exact Mass = summing the masses of the individual isotopes of the molecule, H2O; 1.0078 + 1.0078 + 15.9994 = 18.0106.

The Others Stories;

- Isotopomer (Isotopic Isomer) = same type of isotope but difference in position, CH_3CHDCH_3 vs $CH_3CH_2CH_2D$
- Isotopologues = difference in isotope in the molecules, H_2O HOD
- Monoisotopic = sum of masses in molecule. Using of most abundance or stable isotope.

Mass Accuracy – What for?

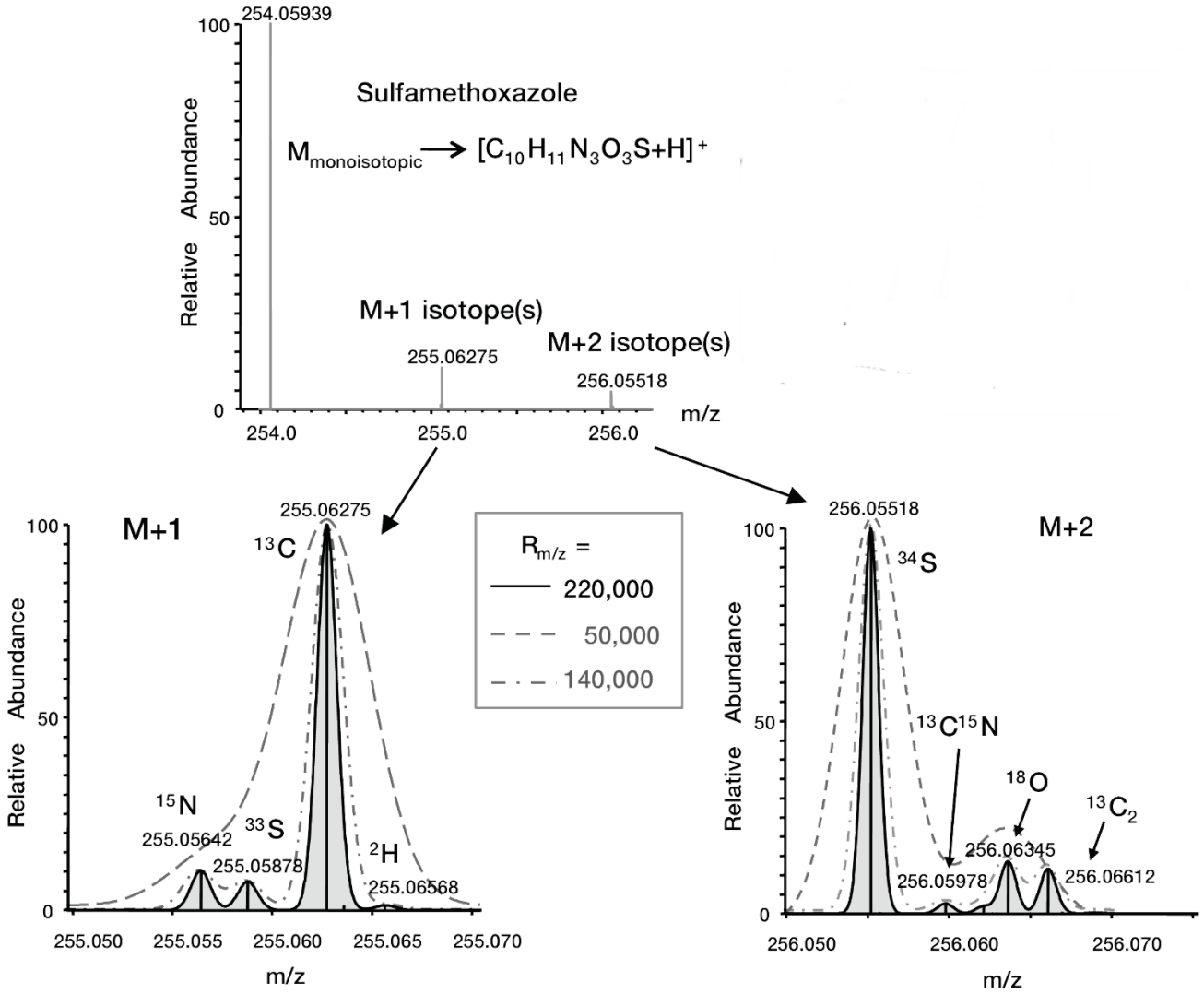
C = 12.0000

H = 1.0078
N = 14.0031

O = 15.9949
S = 31.9721

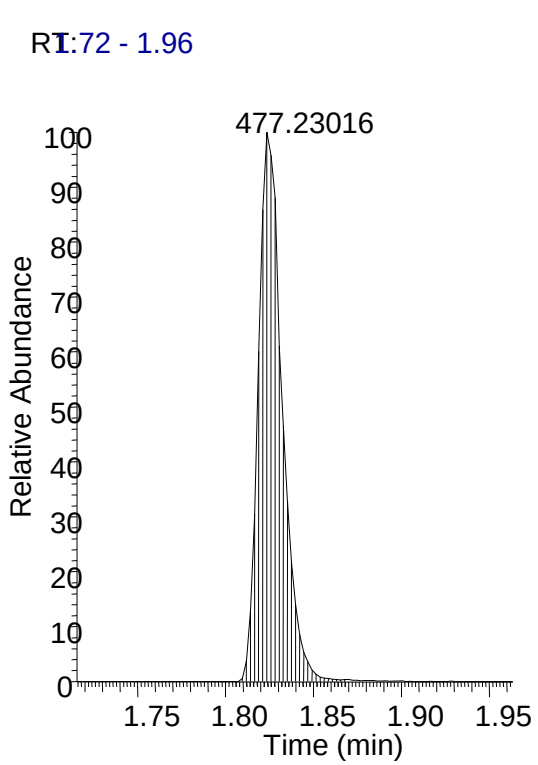
Mass measured	Tolerance [Da]	Suggestions	Calc Mass
32.0	+/- 0.2	O_2 CH_3OH N_2H_4 S	31.9898 32.0261 32.0374 31.9721
32.02	+/- 0.02	CH_3OH N_2H_4	32.0261 32.0374
32.0257	**+/- 0.002**	**CH_3OH**	**32.0261**

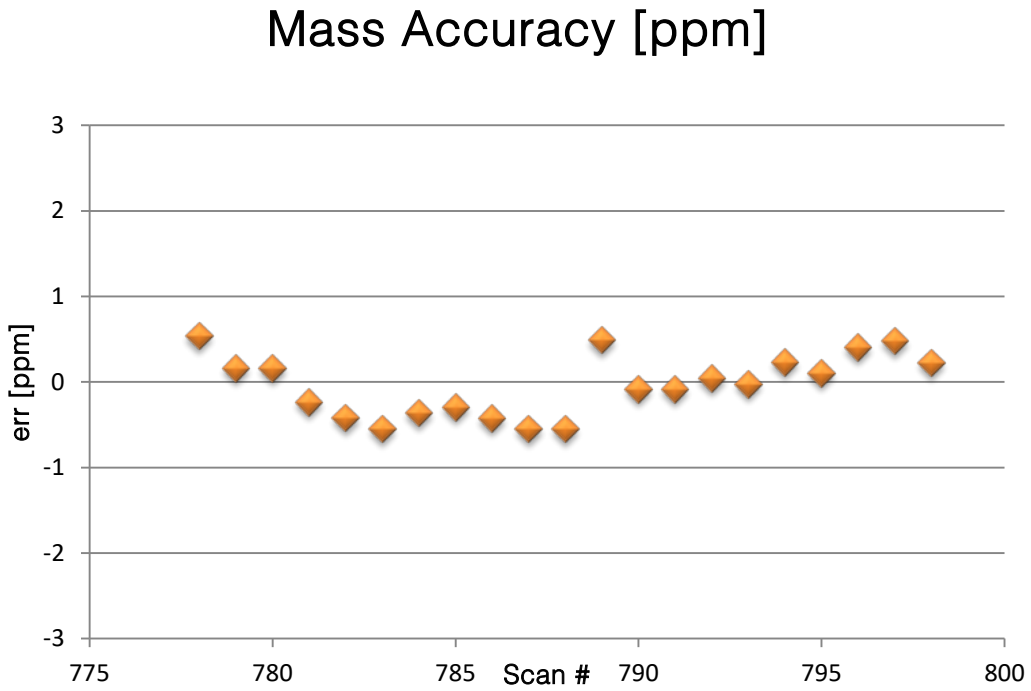
Determine Fine Isotopic Pattern



Mass Accuracy across the Elution Profile

- 21 scans per elution peak
- External calibration

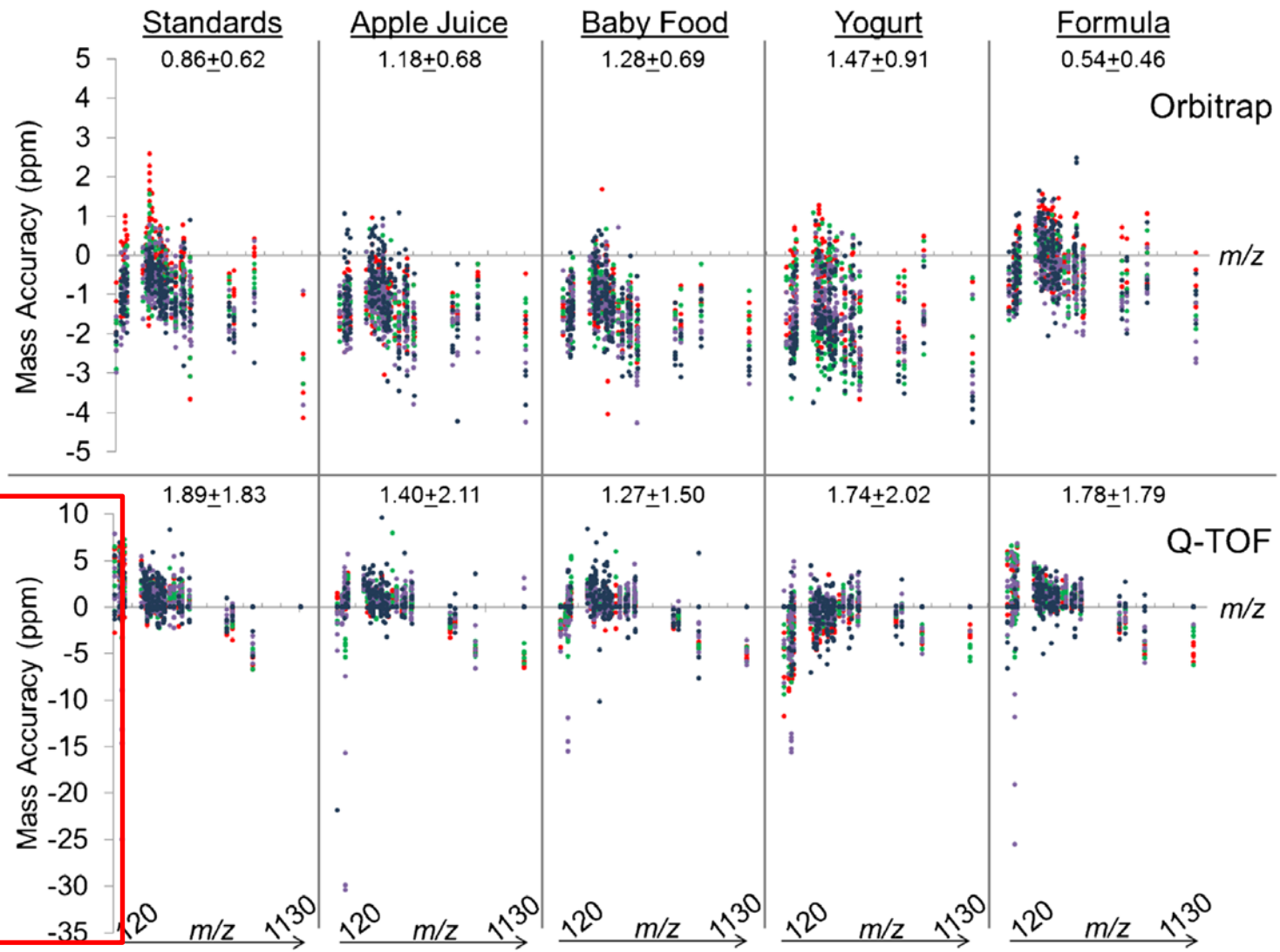




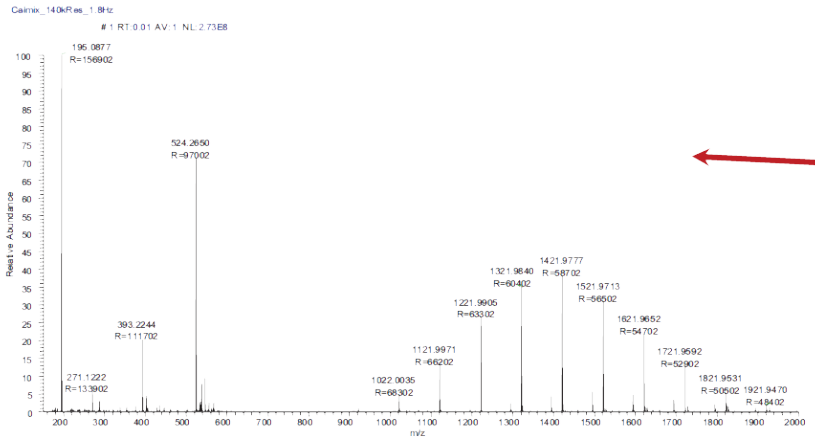
Average Isotope Ratio Variation

pg on column	Standards	Apple juice	Baby food	Yogurt	Formula
A + 1					
Q-Exactive, Overall: 1.69 ± 2.30					
10	1.95 ± 2.26	3.17 ± 3.27	3.67 ± 3.33	3.21 ± 2.83	2.18 ± 1.69
100	2.61 ± 4.81	1.95 ± 1.98	1.91 ± 2.19	1.95 ± 1.87	2.10 ± 2.08
500	0.86 ± 0.96	1.07 ± 1.05	1.07 ± 1.18	1.26 ± 1.47	1.18 ± 1.36
2000	1.02 ± 1.79	0.75 ± 0.96	0.89 ± 1.34	0.74 ± 0.97	0.66 ± 0.89
MaXis, Overall: 5.01 ± 7.53					
10	9.20 ± 7.07	13.47 ± 9.06	15.30 ± 11.03	11.78 ± 7.62	11.49 ± 9.44
100	4.85 ± 6.66	7.78 ± 13.99	6.79 ± 7.02	6.94 ± 7.91	5.99 ± 6.25
500	3.05 ± 6.45	5.22 ± 9.58	3.30 ± 3.85	3.23 ± 3.79	3.33 ± 4.34
2000	1.77 ± 2.36	2.79 ± 6.28	2.13 ± 3.13	1.88 ± 2.56	2.03 ± 2.62
A + 2					
Q-Exactive, Overall: 1.59 ± 4.33					
10	5.31 ± 18.09	3.36 ± 5.42	4.38 ± 9.08	5.15 ± 6.56	6.44 ± 5.03
100	1.75 ± 3.01	1.93 ± 2.91	2.24 ± 4.60	1.70 ± 2.37	1.57 ± 1.86
500	1.03 ± 1.26	0.91 ± 0.62	0.86 ± 0.59	1.05 ± 0.81	1.22 ± 1.94
2000	0.81 ± 1.05	0.86 ± 1.20	0.73 ± 0.56	0.82 ± 0.57	0.74 ± 0.53
MaXis, Overall: 3.67 ± 6.47					
10	10.96 ± 9.71	12.89 ± 6.70	19.43 ± 38.22	11.21 ± 5.68	14.92 ± 7.62
100	3.55 ± 4.75	6.09 ± 6.85	6.73 ± 7.02	4.67 ± 4.46	5.22 ± 5.24
500	2.13 ± 3.14	4.02 ± 7.02	3.02 ± 3.17	3.01 ± 4.27	2.78 ± 3.38
2000	1.24 ± 2.06	2.23 ± 4.56	1.69 ± 2.36	1.68 ± 2.57	1.94 ± 3.21

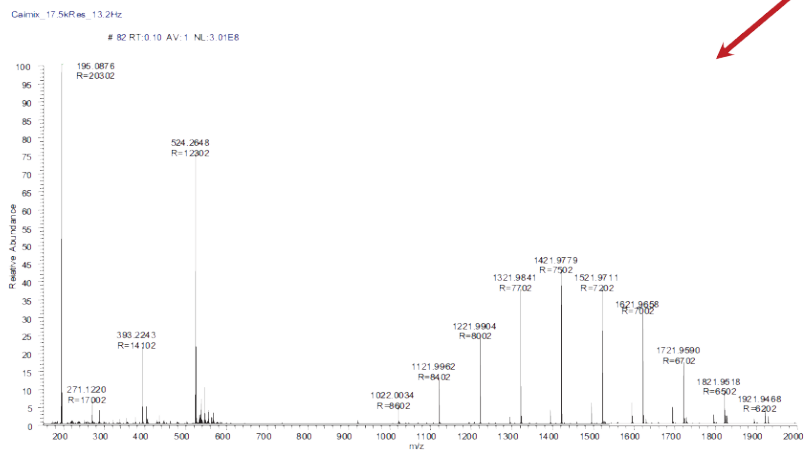
Mass Accuracy

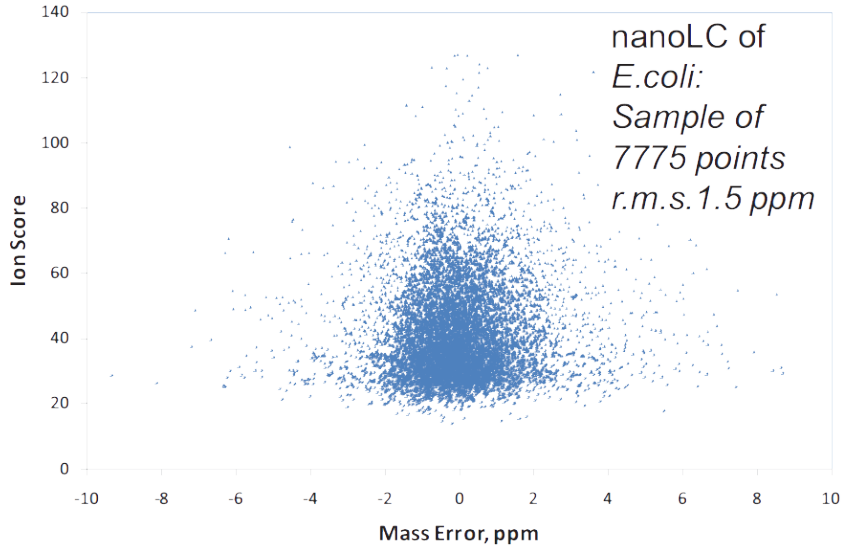


Resolving Power and Mass Accuracy

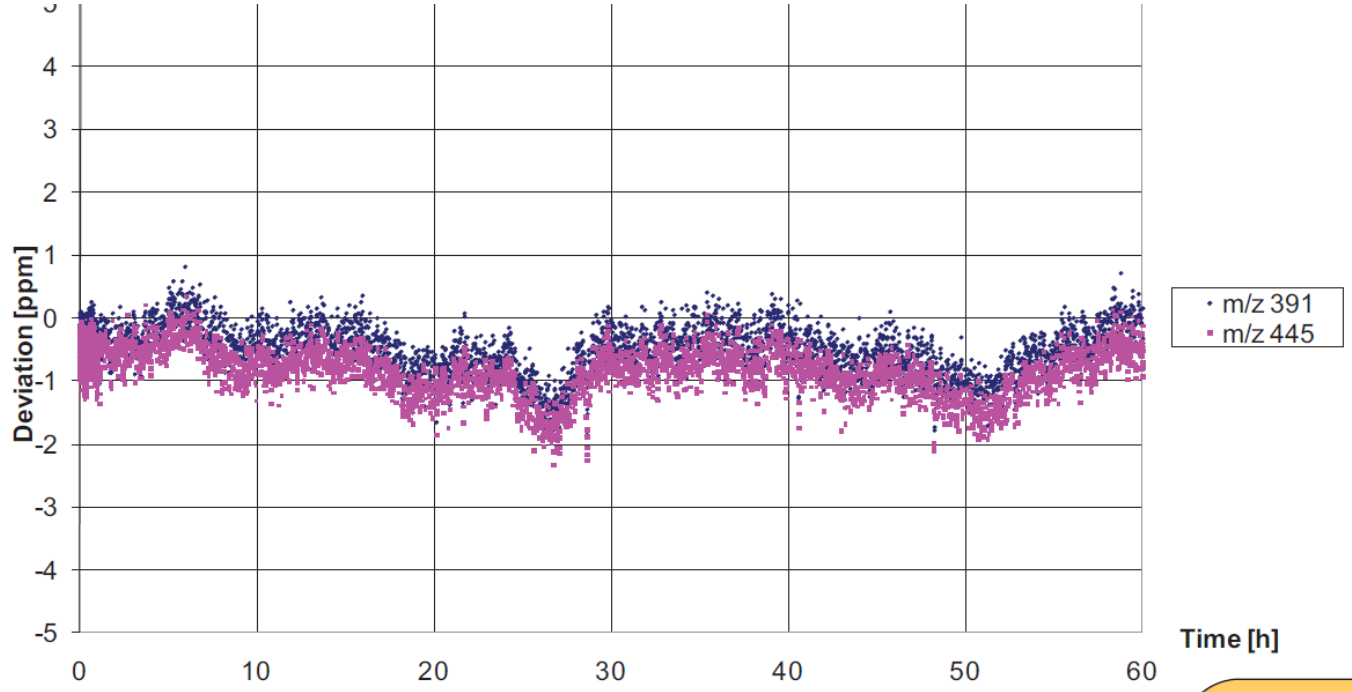


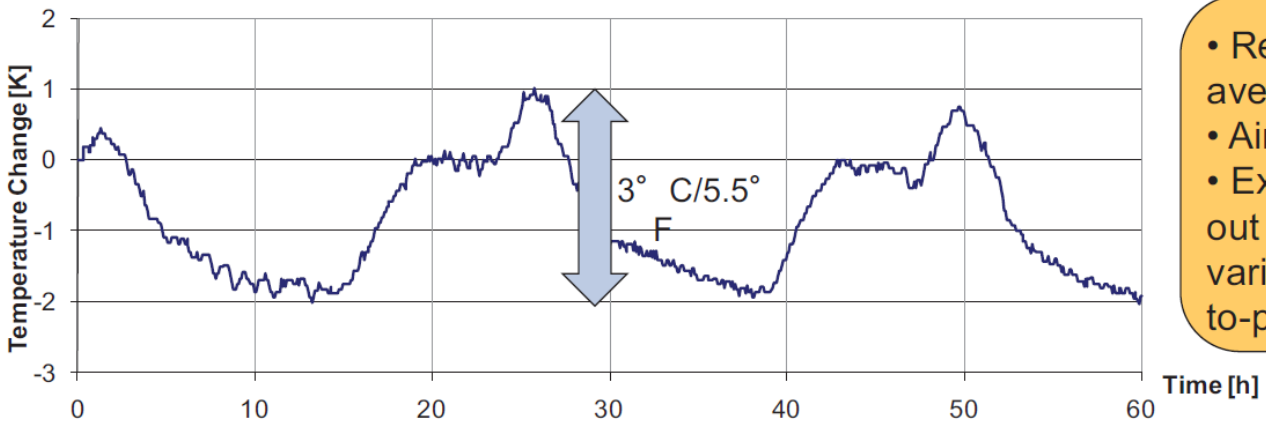
Res setting @ m/z 200	Transient length, ms	Max. scan speed, Hz
140,000	512	1.5
70,000	256	3
35,000	128	7
17,500	64	12





Long-term mass accuracy with external calibration





- Realistic conditions of an average lab
- Air cooling only!
- Experiment was carried out with temperature variations up to 3°C peak-to-peak, up to 1°C/hour

Advantage

- Easy method development for multi-residue analysis especially in complex matrices
- Easy troubleshooting with detection of all adducts, degradation and contaminants
- Higher detection specification
- Simultaneous Qual and Quan analysis

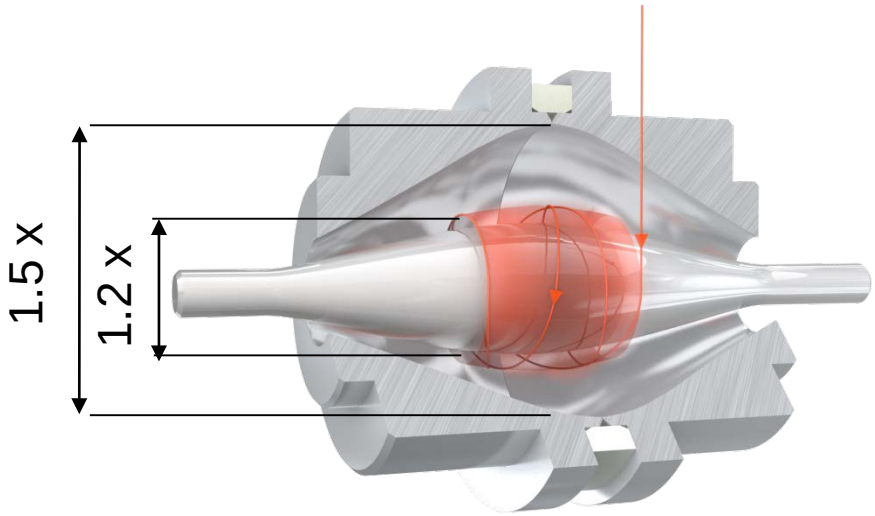
Comparison

	Orbitrap	QQQ	Q-TOF
Sensitivity			
Resolution			
Identification			
Unknowns			
Selectivity			
Quantitation			
Retrospective data mining			
Ease of troubleshooting			
Cost			

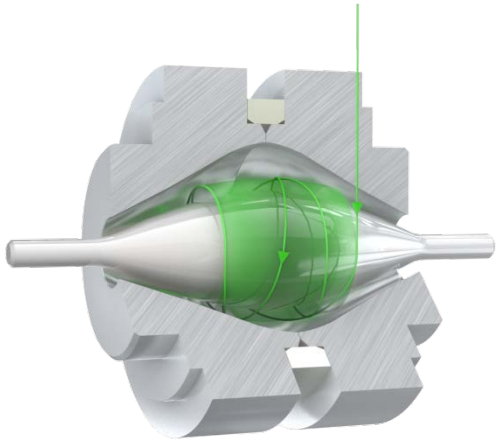
Non-Targeted Screening or Newborn Ideal

- High isolation power for higher discrimination
- High precision for accurate mass identification
- High resolution for more identification
- High mass stability for a long lasting mass calibration
- MS^n
- Library availability for easy interpretations

Orbitrap Analyzer - the ‘Heart’ of a Mass Spectrometer

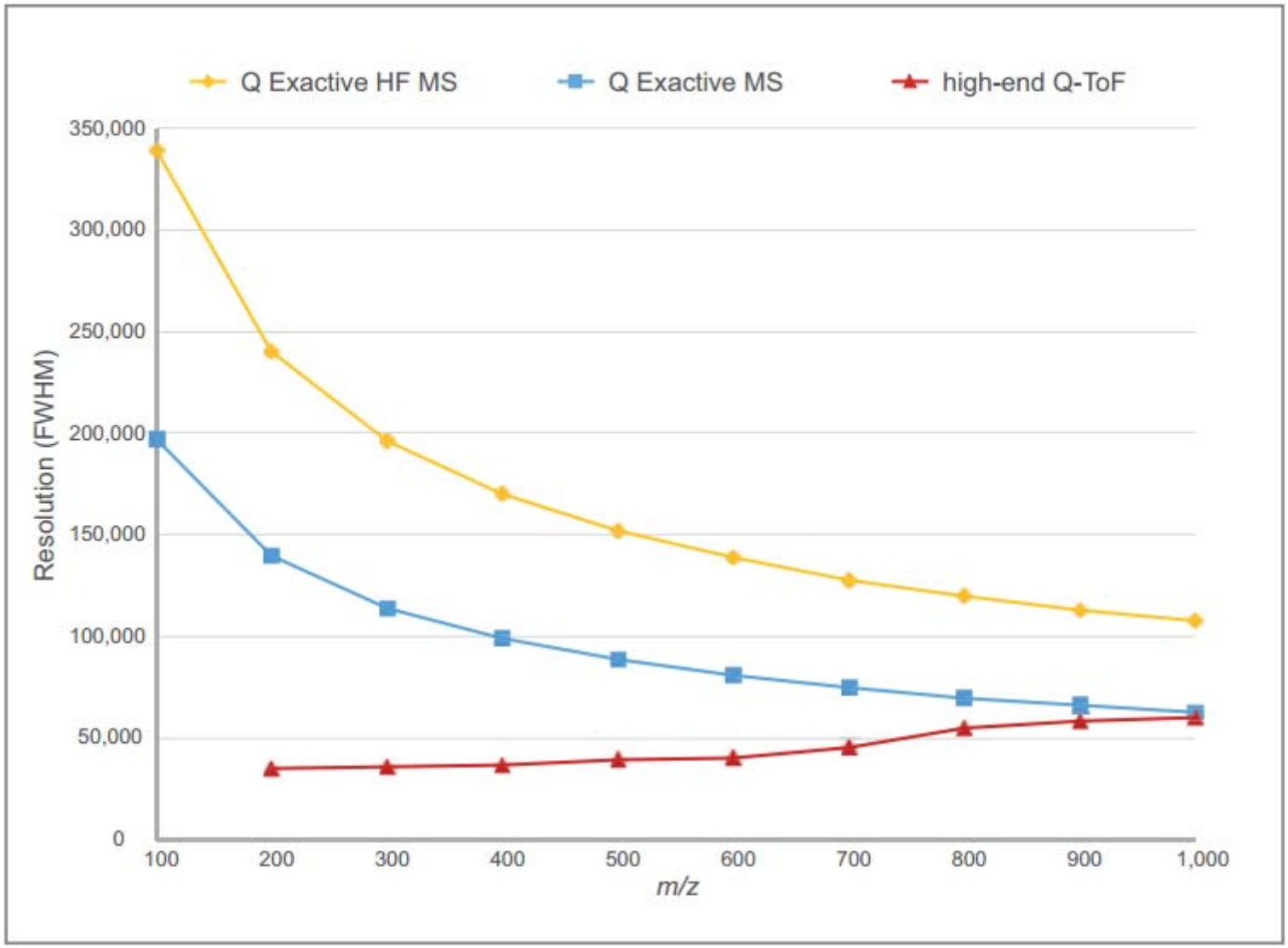


Standard Orbitrap

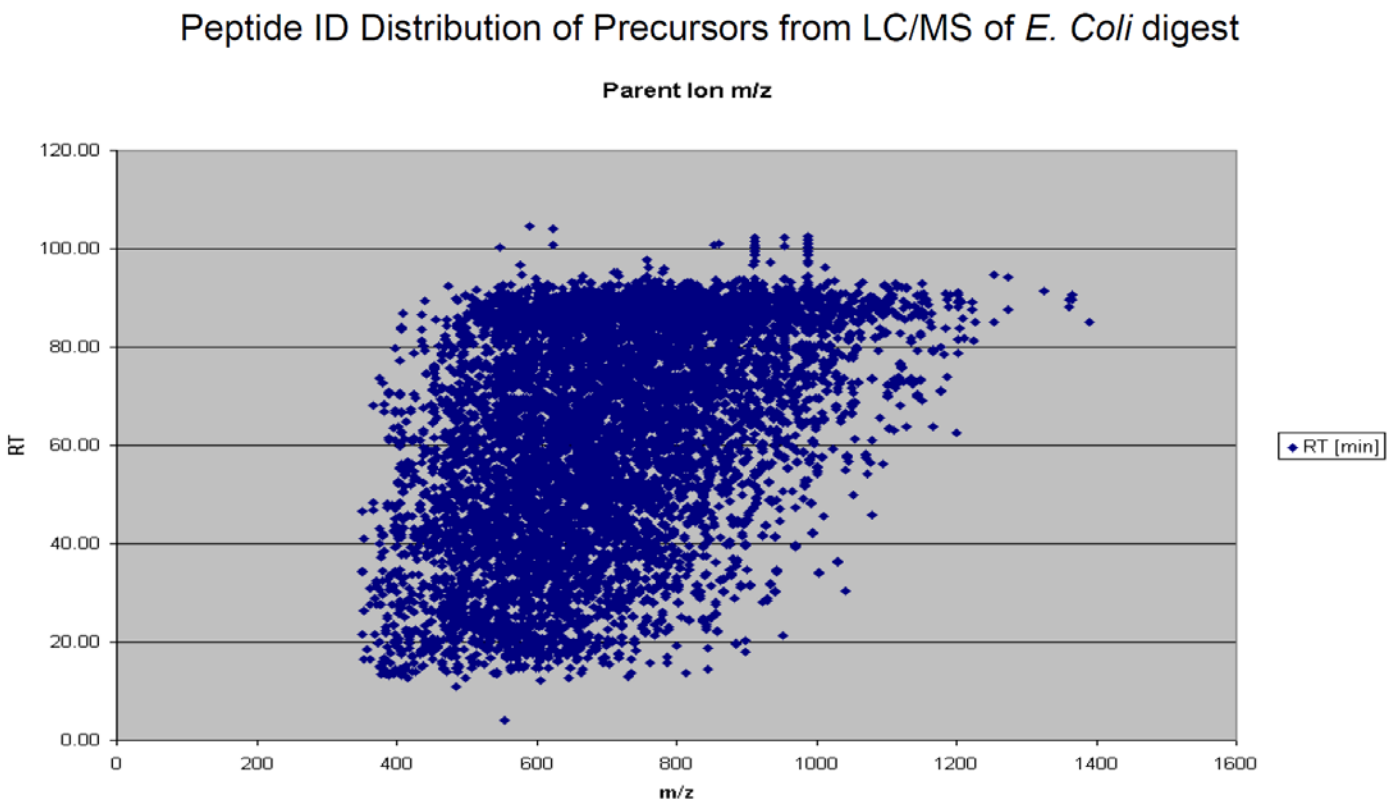


High-field Orbitrap

Resolution VS m/z

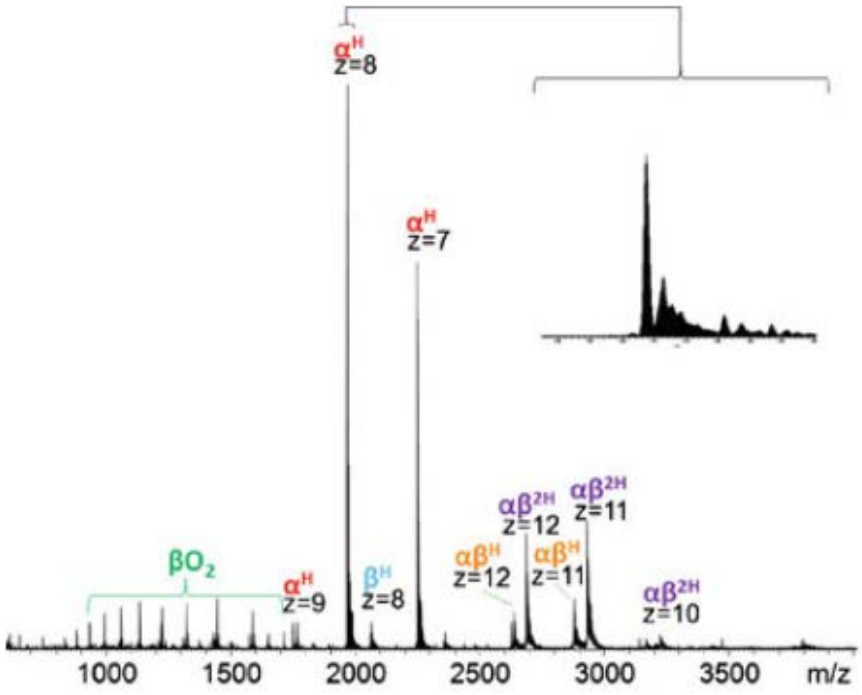


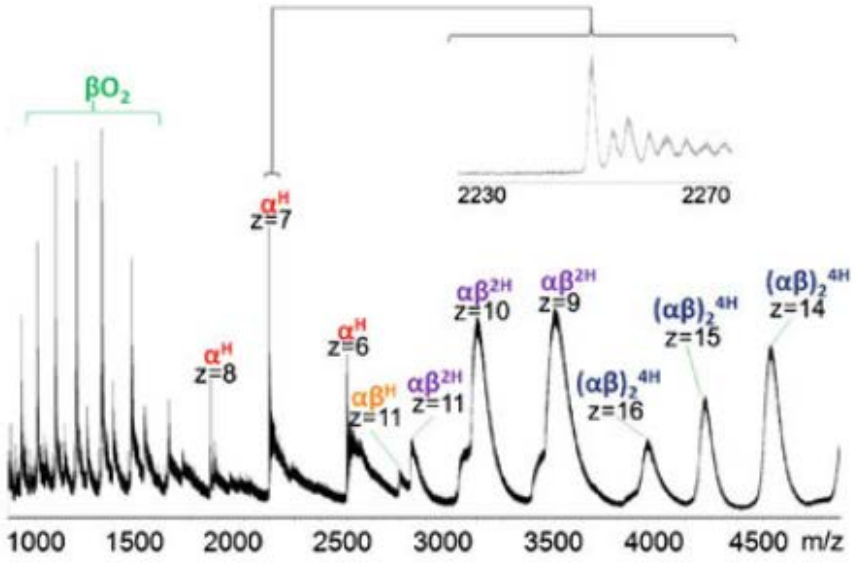
Resolving Power



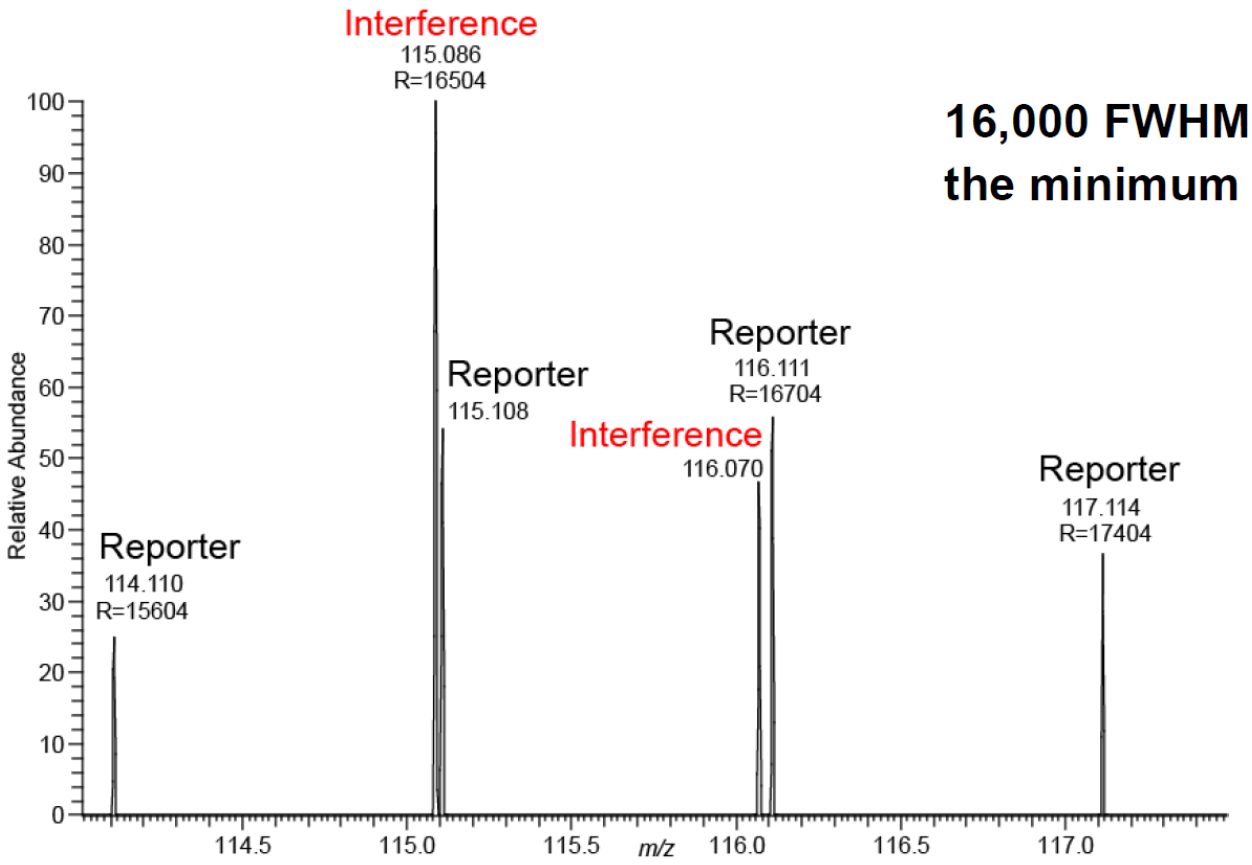
- **85% of the peptide parent ions (precursors) are below *m/z* 800**
- **Most of the chemical interferences are below *m/z* 600**
- **The highest resolution is needed below *m/z* 800 where Orbitrap technology has it and TOF technology doesn't!**

Orbitrap VS QToF





Labelling Techniques

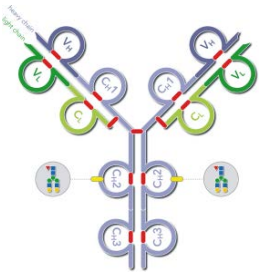


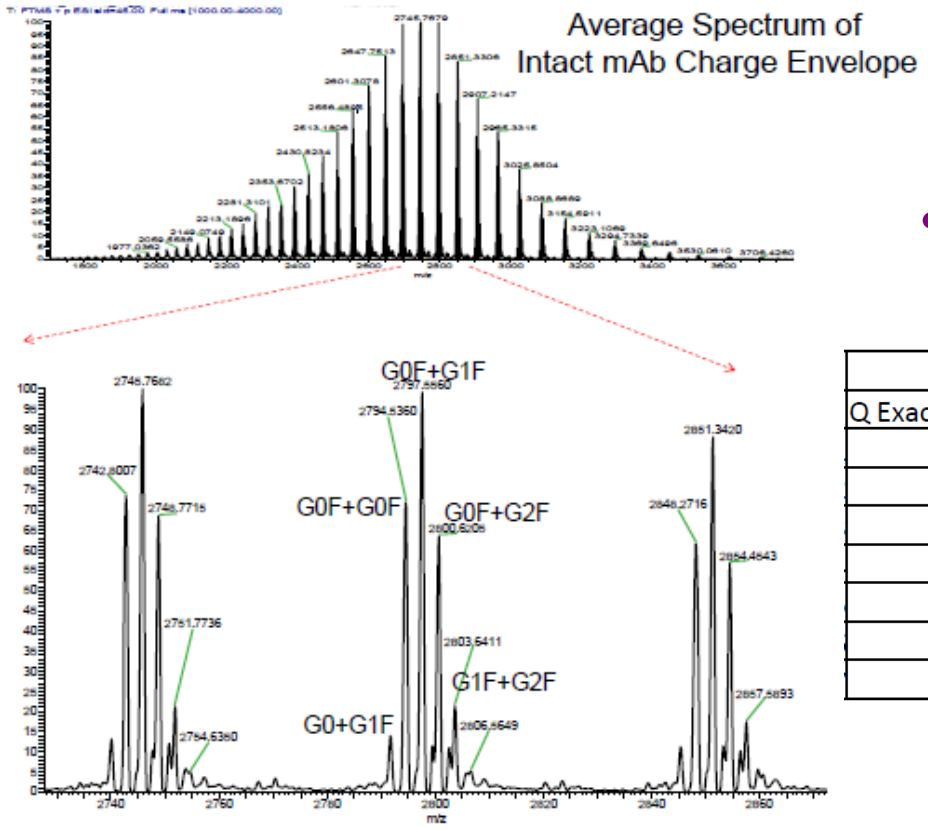
16,000 FWHM is the minimum required!

- **Isobaric labeling techniques (iTRAQ, TMT) need high resolution at low masses**
- **Chemical interferences are common when using collision cells!**

Intact Protein Analysis

- Complete charge state envelope of IgG ‘Humira’
- Major glycosylation forms are baseline separated



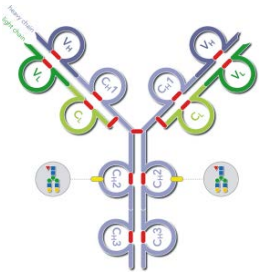


- Relative intensity reproducibility within a few percent

	Relative abundances				
Q Exactive	G0+G0F	G0F+G0F	G0F+G1F	G0F+G2F	G1F+G2F
1	12.9	74.1	100.0	67.0	23.4
1	12.3	76.0	100.0	71.4	29.8
1	12.0	72.8	100.0	66.2	22.0
1	12.2	75.0	100.0	67.0	23.6
2	12.7	75.7	100.0	63.6	21.6
2	13.2	75.4	100.0	64.8	21.0
2	12.9	76.6	100.0	64.7	21.6

Intact Protein Analysis

- Mass measurement accuracy
- Average error for 34 measurements 6.9 ppm
- Standard deviation 6.4 ppm



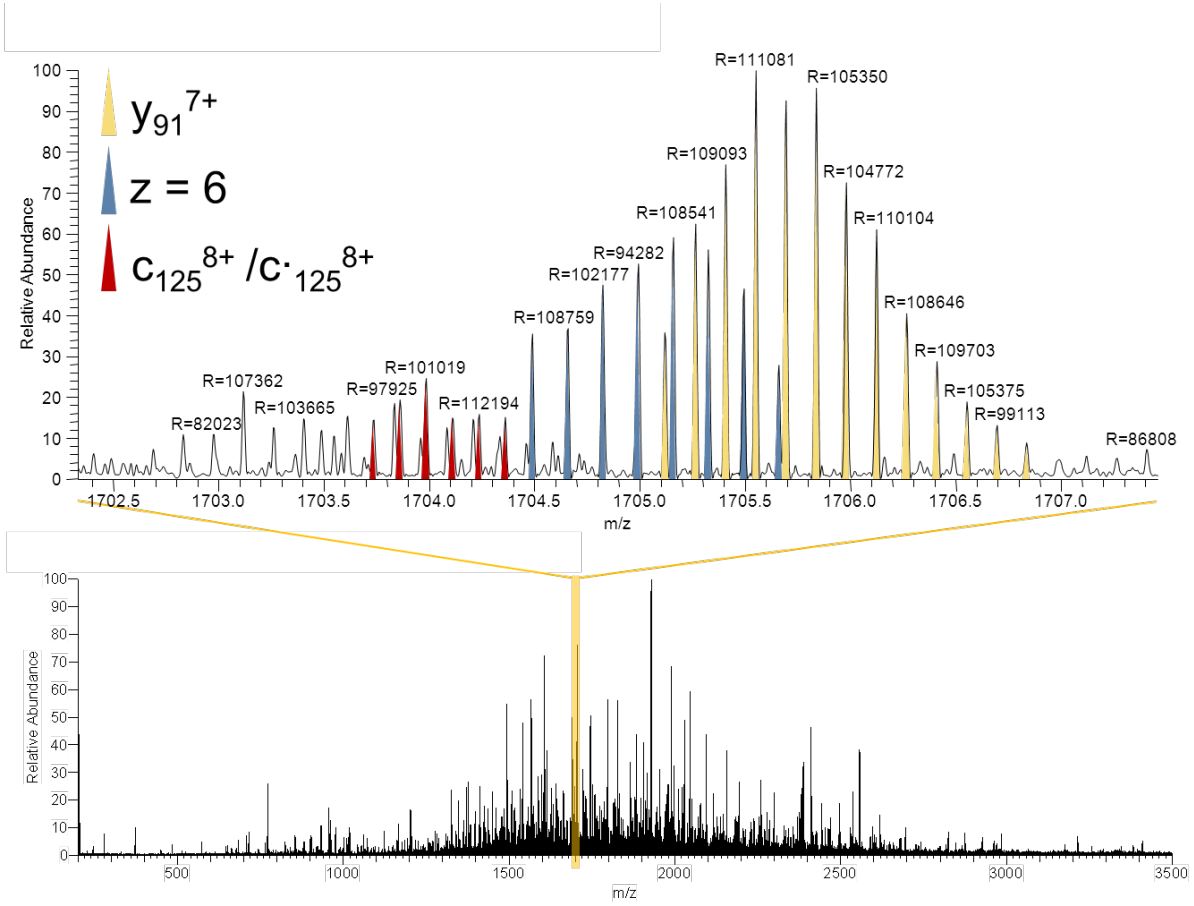
	ppm mass measurement errors				
Q Exactive	G0+G0F	G0F+G0F	G0F+G1F	G0F+G2F	G1F+G2F
1	-10.5	0.7	-10.5	-13.8	-18.0
1	-3.2	-4.3	-6.9	3.2	N/A
1	-11.6	-1.1	-8.8	-11.2	-12.0
1	5.1	-5.0	-2.6	5.1	5.6
2	-14.3	3.0	-6.9	-5.4	-5.9
2	-8.6	-2.2	-12.2	-12.5	-12.9
2	-14.3	-6.6	-12.3	-14.8	-10.1

Confirmation of protein primary structure

Sequence Confirmation of mAB

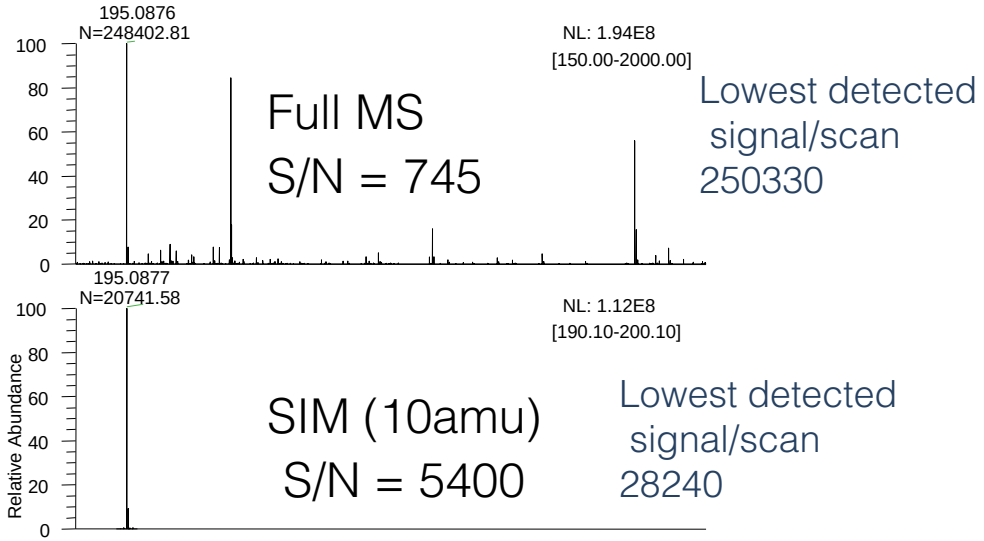
- ETD fragmentation of an intact IgG ‘Humira’

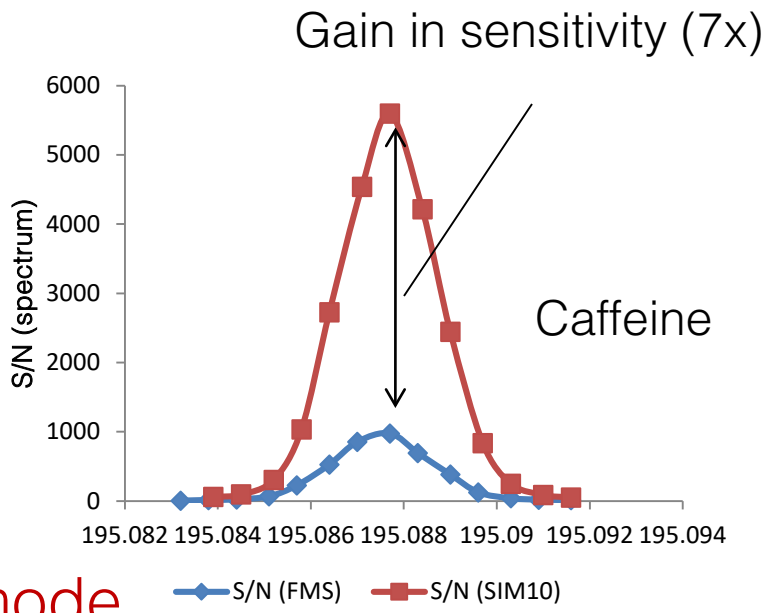
- Resolution settings 240,000 for fragment detection
- Increased sequence coverage
- Localization of modifications (deamidation)



What do we gain by selected ion monitoring?

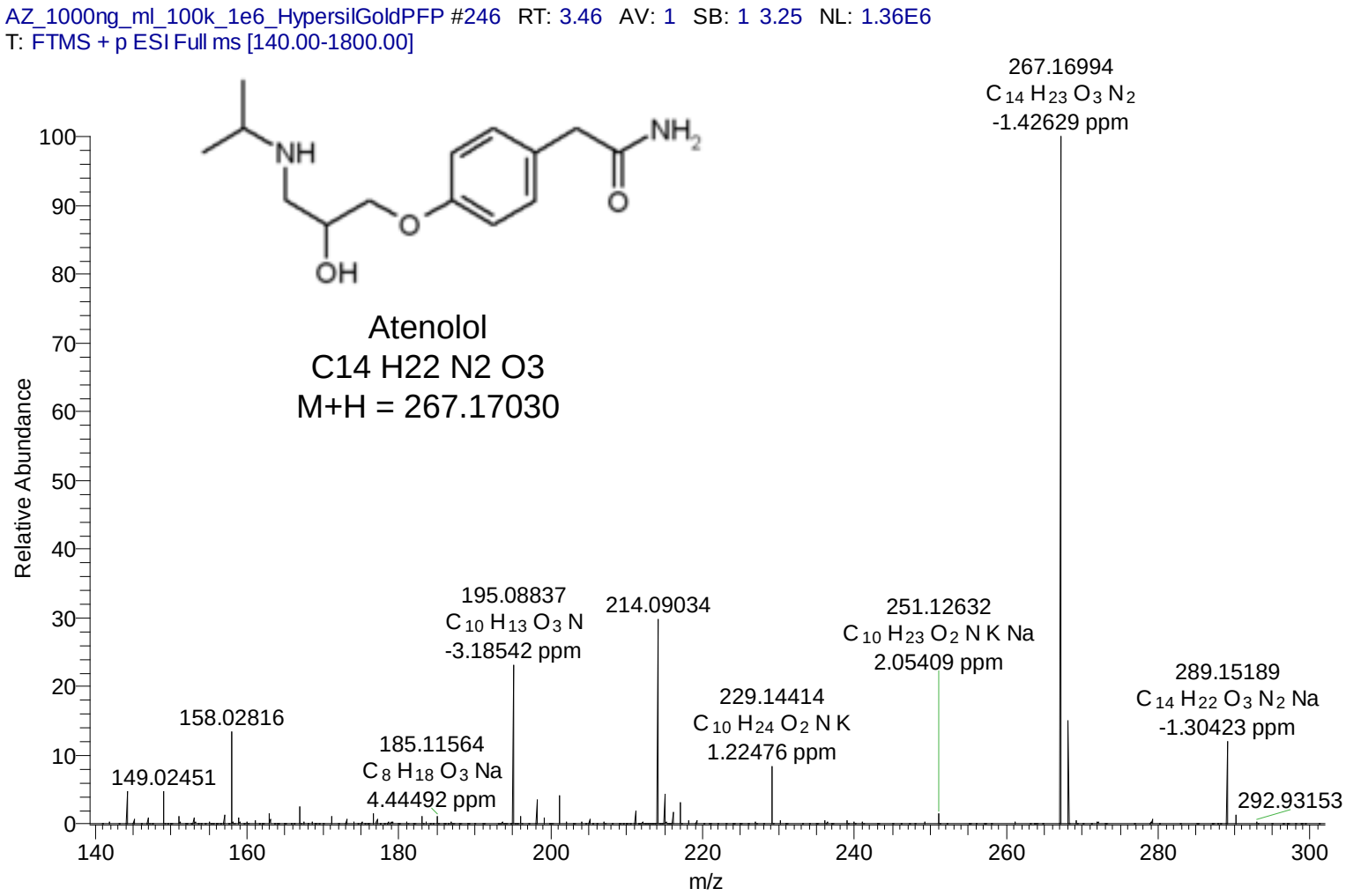
- Signal visibility is dependent, whether a signal is visible above the spectrum noise
- Spectrum noise is dependent on the ratio of compound within a certain ion population





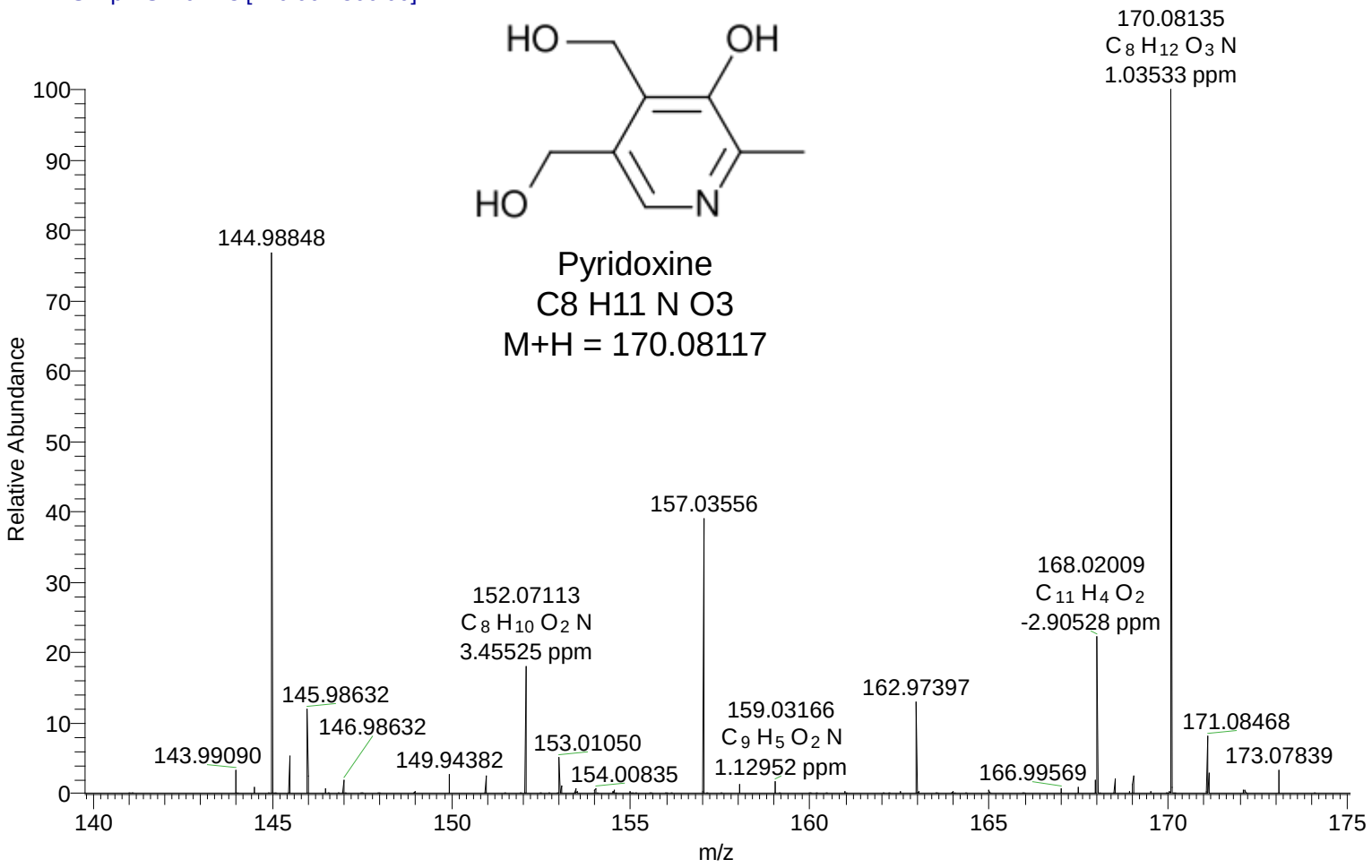
Sensitivity gain 5 – 10 x with SIM mode

Full Scan Spectrum of Atenolol



Full Scan Spectrum of Pyridoxine

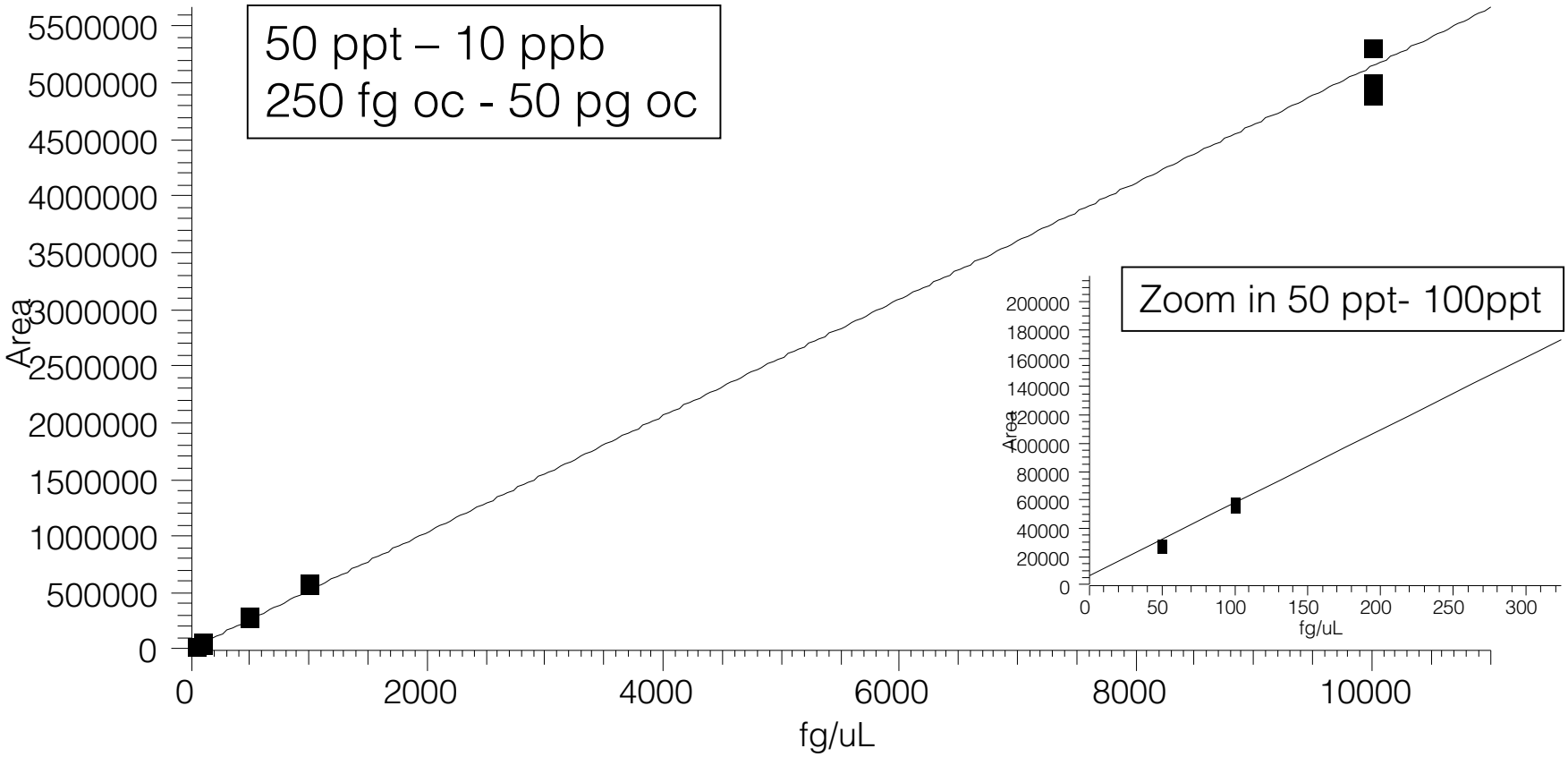
AZ_1000ng_ml_100k_1e6_HypersilGoldPFP #92 RT: 1.27 AV: 1 SB: 1 1.04 NL: 1.86E6
T: FTMS + p ESI Full ms [140.00-1800.00]



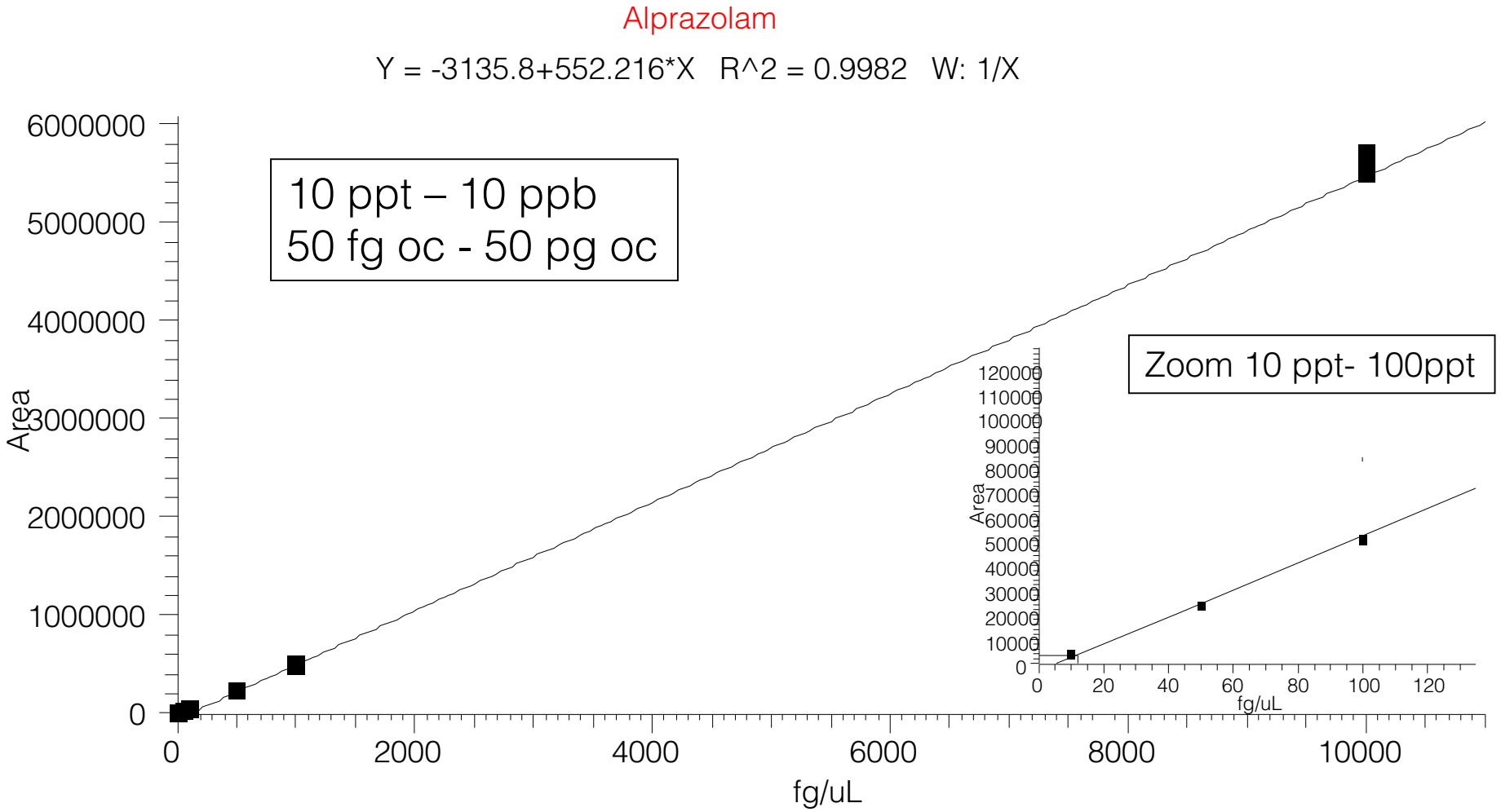
Alprazolam, Full Scan Experiment

Alprazolam

Y = 6366.31+514.015*X R^2 = 0.9967 W: 1/X



Alprazolam SIM Experiment



A Switch is on from QqQ to Orbitrap

Q Exactive Focus quadrupole-Orbitrap MS

TSQ Endura triple quadrupole MS

TABLE 6. Accuracy (% recovery, NIST standard concentration ng/mL) using PRM.

	25OH D2	25OH D3	epi-25OH D3
Level 1	BQL (0.54)	108 (28.8)	103 (1.84)
Level 2	BQL (0.81)	110 (18.1)	90.7 (1.29)
Level 3	97 (13.3)	104 (19.8)	114 (1.18)
Level 4	BQL (0.55)	106 (29.4)	90.9 (26.4)

TABLE 7. Accuracy (% recovery) using SRM.

	25OH D2	25OH D3	epi-25OH D3
Level 1	BQL	93.4	BQL
Level 2	BQL	102	BQL
Level 3	92.7	105	BQL
Level 4	BQL	98	100

TABLE 1. Linearity range (ng/mL)

	25OH D2	25OH D3	epi-25OH D3
PRM	1-100	1-100	1-100
SRM	2-100	2-100	2-100

	Amo	Butalbital	Pento	Pheno	Seco
PRM	5-2000	5-2000	5-2000	25-2000	5-2000
SRM	10-2000	5-2000	10-2000	25-2000	5-2000

TABLE 2. Inter-assay precision (% RSD, concentration ng/mL) using PRM (n=15). BQL, below quantitation limit.

	25OH D2	25OH D3	epi-25OH D3
QC0	BQL	6.4 (8.4)	BQL
QC1	5.3 (6.0)	4.1 (14.4)	4.4 (6.6)
QC2	3.1 (15.0)	3.2 (23.4)	2.9 (15.6)
QC3	4.2 (50.0)	3.5 (58.4)	2.5 (50.6)

TABLE 3. Inter-assay precision (% RSD, concentration is the same as above) using SRM (n=15).

	25OH D2	25OH D3	epi-25OH D3
QC0	BQL	7.6	BQL
QC1	7.6	8.6	6.9
QC2	6.4	4.8	9.4
QC3	5.2	3.4	6.9

Biocrates – 188 Metabolites

Key Features

Quantification of up to 188 Metabolites from Key Metabolite Classes

Metabolite Coverage

- Amino acids (21)
- Biogenic amines (21)
- Hexose (1)
- Acylcarnitines (40)
- Lysophosphatidylcholines (14)
- Phosphatidylcholines (76)
- Sphingolipids (15)

Kit Components

Reagents & Consumables
- Patented 96-well filter plate
- Internal & calibration standards
- Quality controls
- Testmix

Methods & Protocols
- Sample preparation protocol
- Instrument-specific acquisition & quantification methods
- System suitability test

Workflow Manager Met*IDQ*™
- Process guidance
- Automated technical validation
- Basic statistics (optional)

Instrument Platforms*

- Waters Xevo TQ-XS UHPLC
- Waters Xevo TQ-S UHPLC
- Waters Xevo TQ-S micro UHPLC
- SCIEX 6500+ (U)HPLC
- SCIEX 6500 (U)HPLC
- SCIEX 5500 (U)HPLC
- SCIEX 4500 (U)HPLC
- SCIEX 4000 HPLC
- Thermo TSQ Vantage (U)HPLC

Biocrates – ONLY Orbitrap 408 Metabolites

The **Absolute*IDQ*® p400 HR Kit** is a complete solution for **broad lipid and metabolic profiling** on high resolution, accurate mass (HRAM) Q Exactive™ mass spectrometers. It provides **quantification of a large range of analytes,** with high inter-laboratory, inter-instrument and longitudinal **reproducibility.**

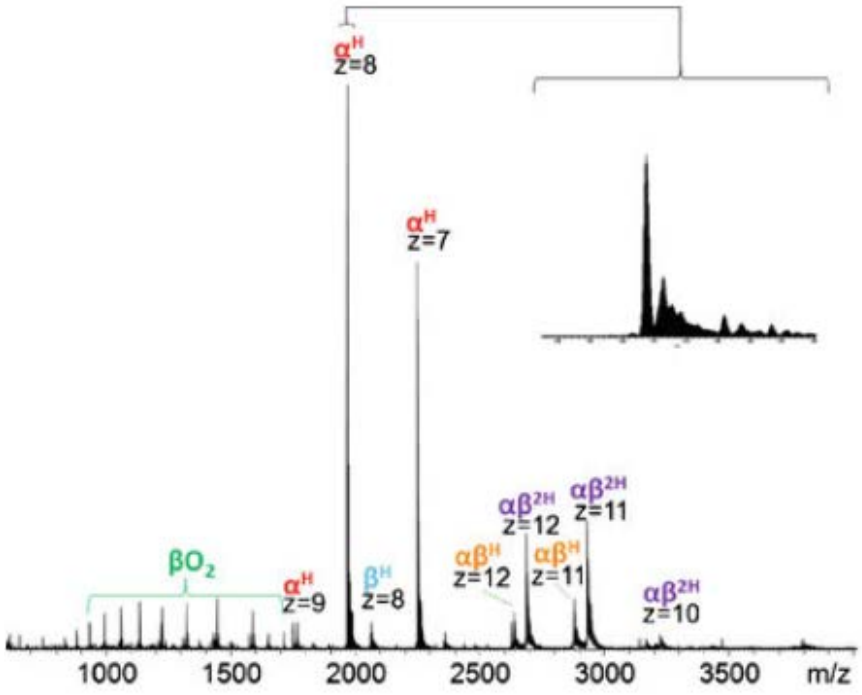
Tha kit covers **up to 408 metabolites** from **11 metabolite classes,** which are known to be relevant in a multitude of pathophysiological processes:

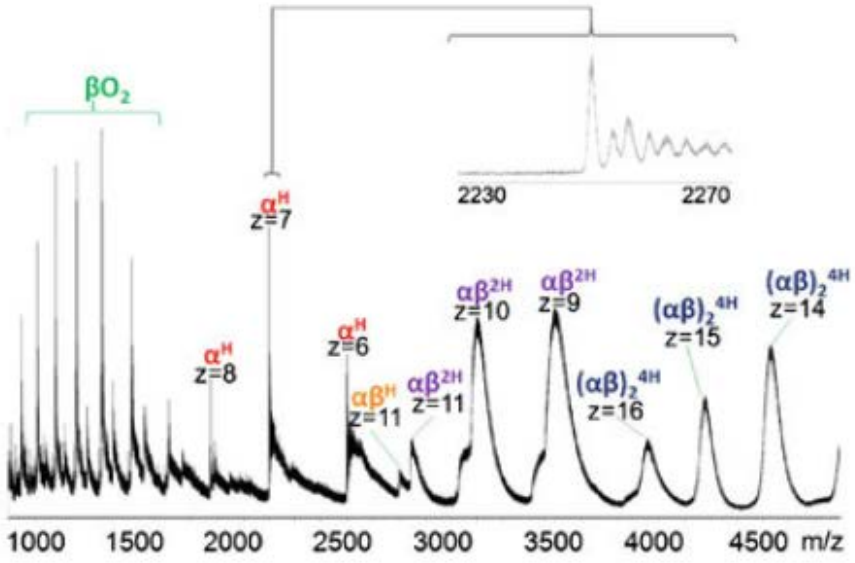
Small Molecules	**Polar Lipids**	**Neutral Lipids**
Amino Acids	Phosphatidylcholines	Acylcarnitines
Biogenic Amines	Lysophosphatidylcholines	Diglycerides
Hexoses	Sphingomyelins	Triglycerides
	Ceramides	Cholesteryl Esters

Non-Targeted Screening or Newborn Ideal

- High isolation power for higher discrimination
- High precision for accurate mass identification
- High resolution for more identification
- High mass stability for a long lasting mass calibration
- MS^n
- Library availability for easy interpretations

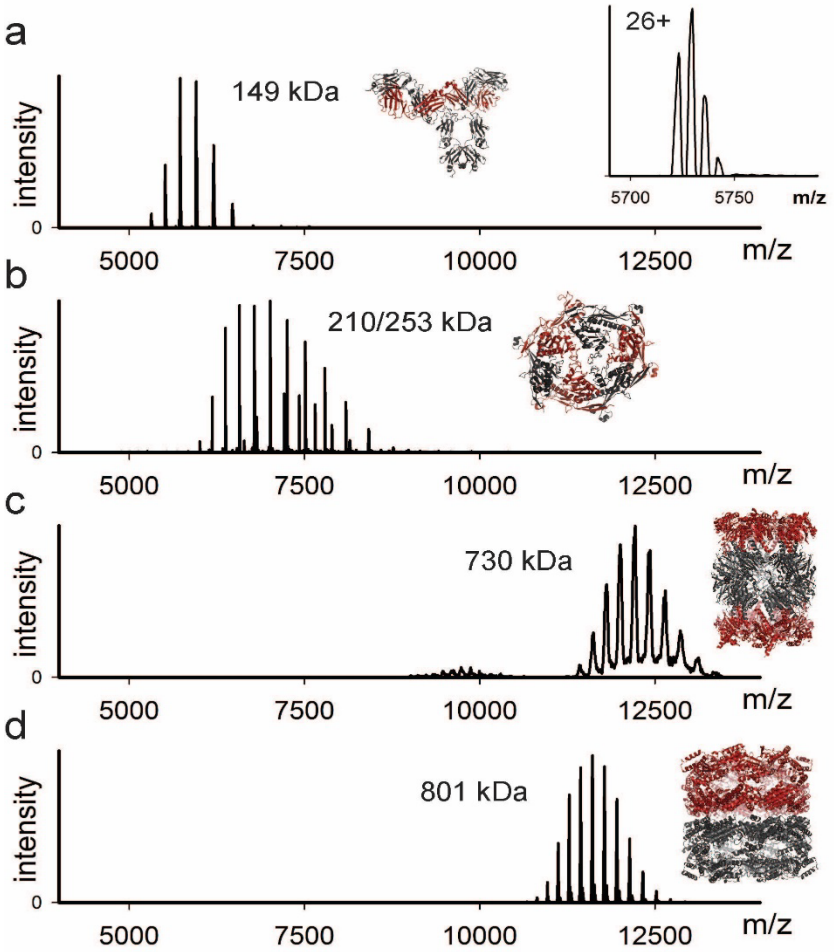
Orbitrap VS QToF





Analysis of Protein Complexes

- Extending the mass range
- Protein assemblies up to 1 million Da



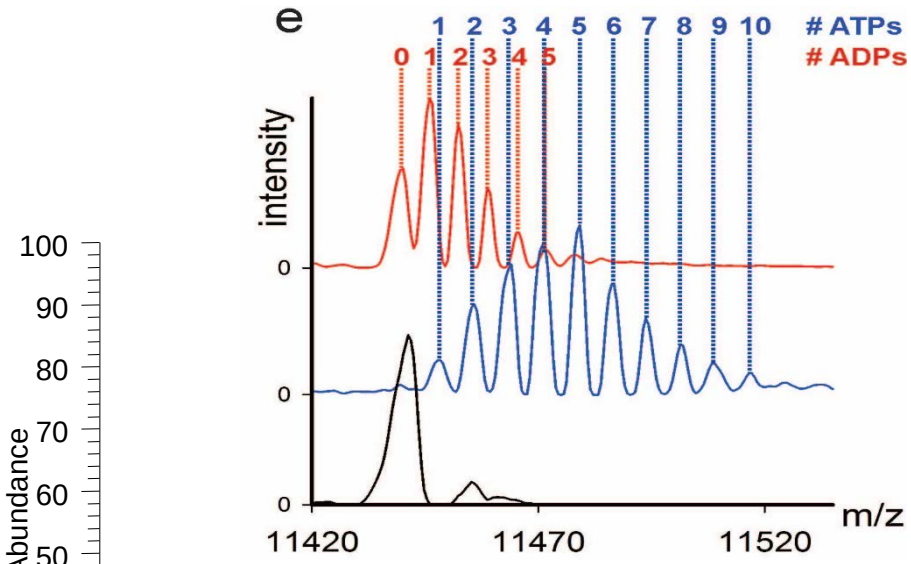
IgG antibody 150 kDa

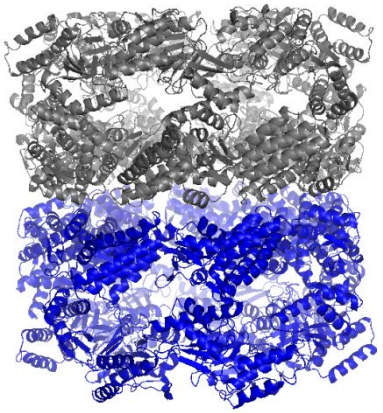
HK97 bacteriophage capsomers 253 kDa

Yeast proteasome 730 kDa

***E. coli* GroEl 801 kDa**

Ligand Binding Stoichiometry

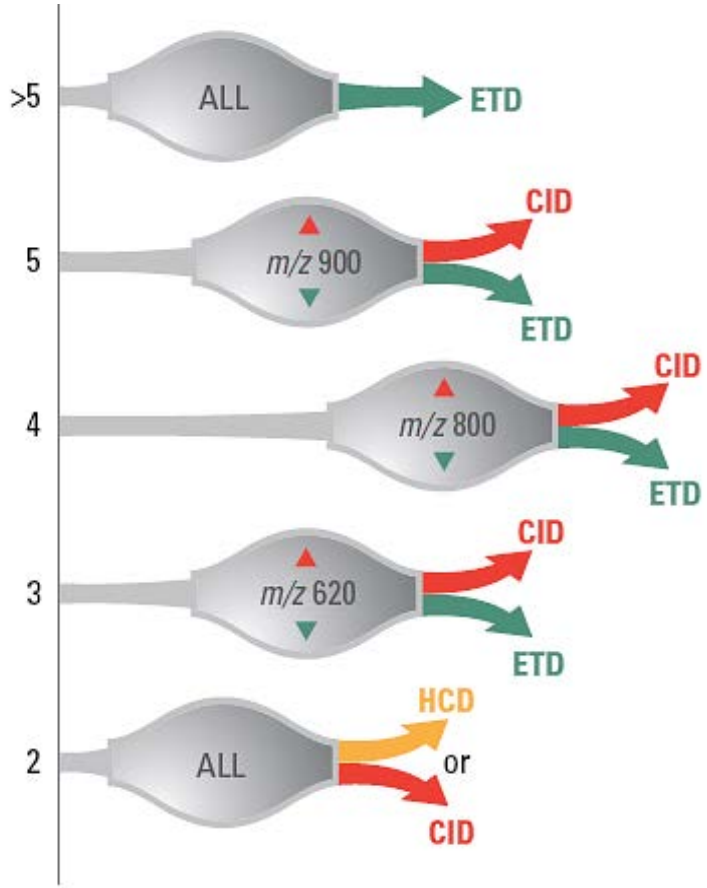


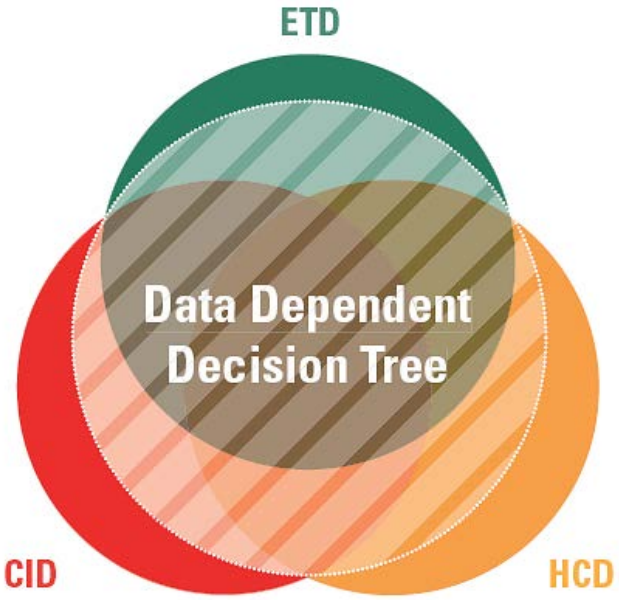


E. coli GroEl
801 kDa

Data Dependent Decision Tree

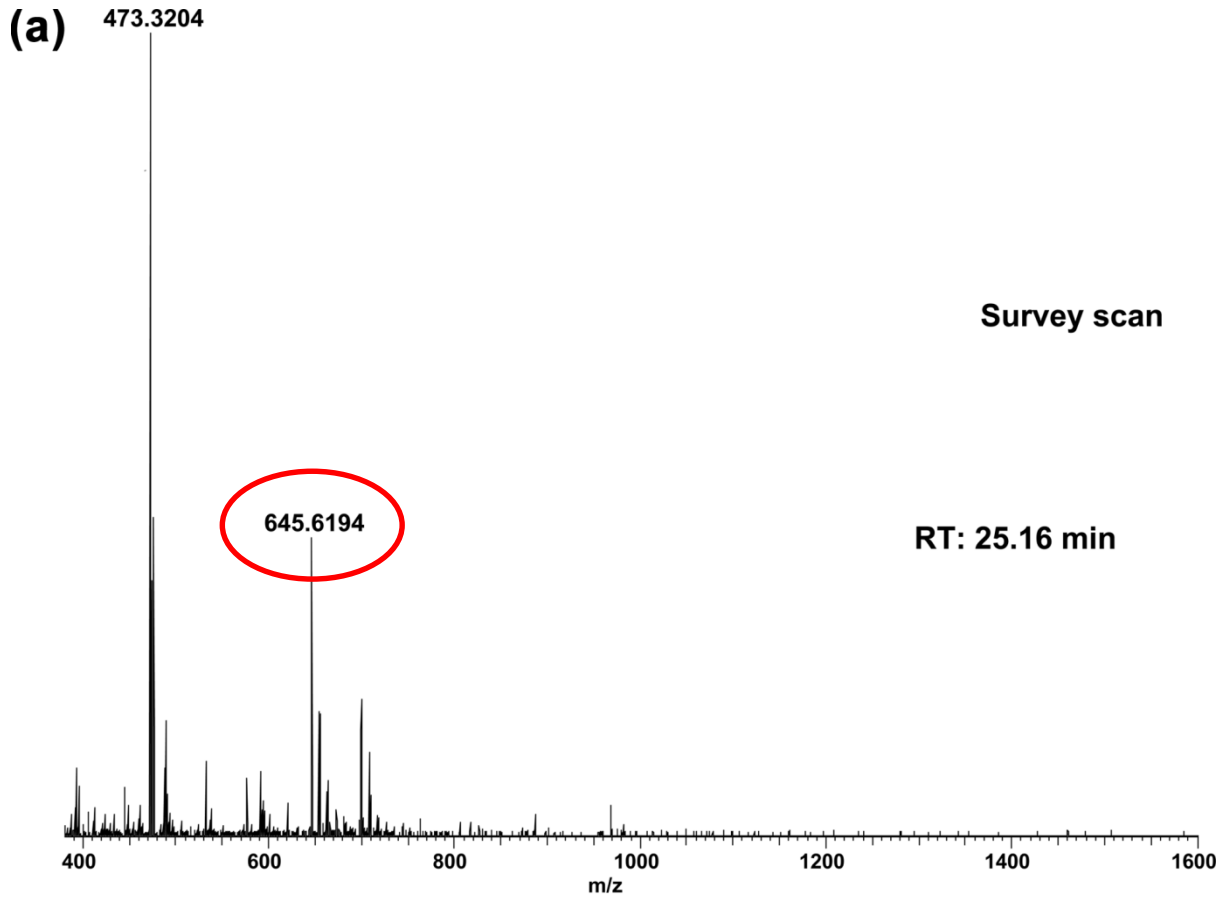
- Decision tree–driven tandem mass spectrometry for shotgun proteomics





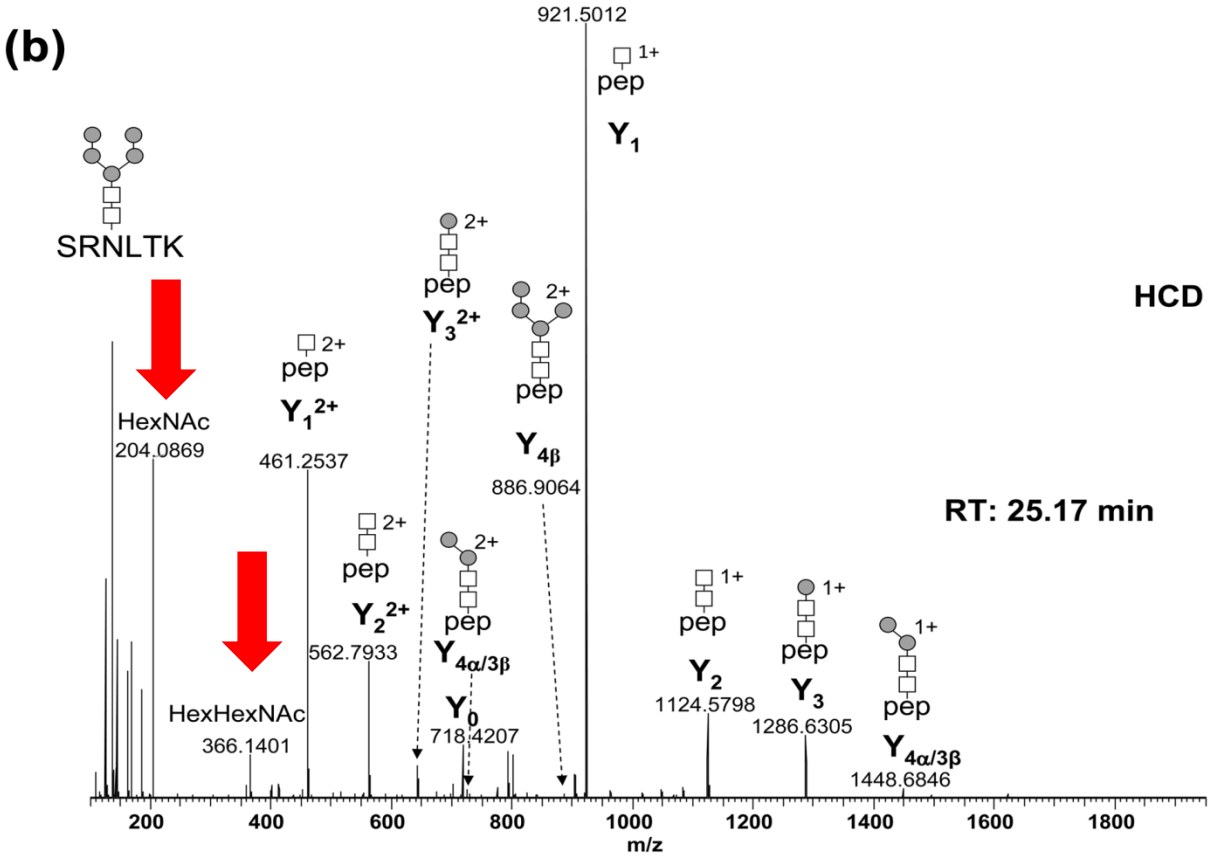
Product Dependent Trigger

- ZIC HILIC separation of a glycoprotein digest

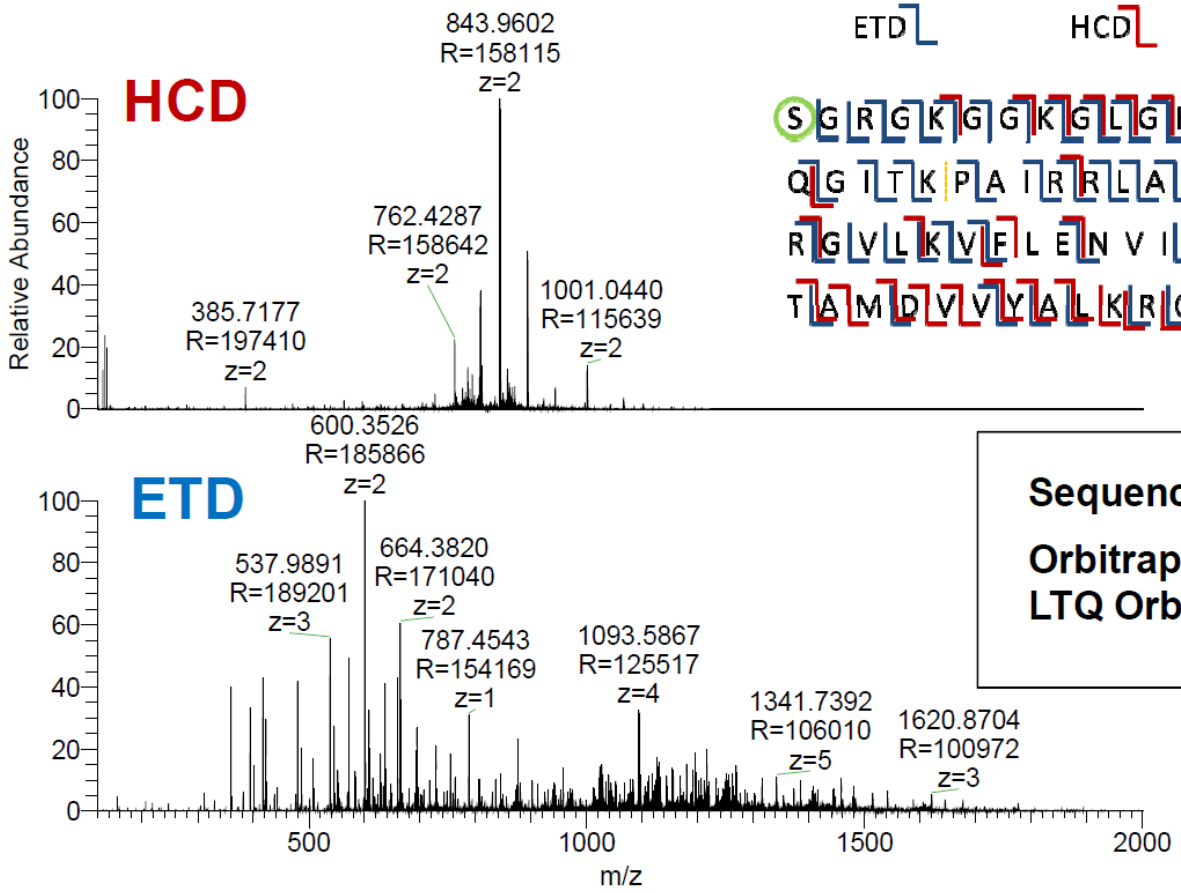


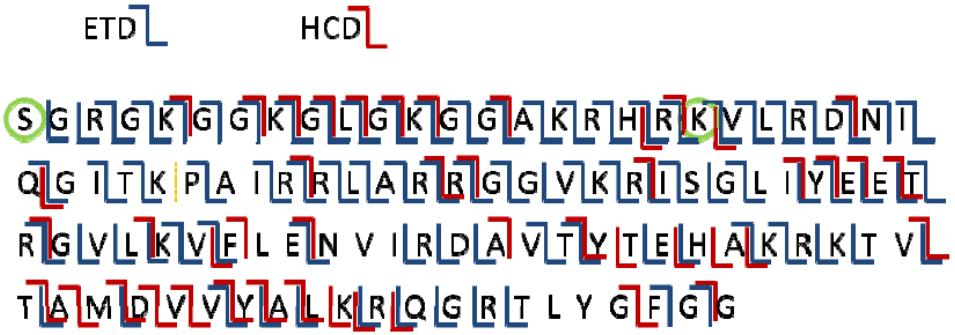
Product Dependent Trigger

- HCD fragmentation spectrum of *m/z* 645.6194
- Oxonium ions observed among top 20 peaks



Extended Top-down Capability



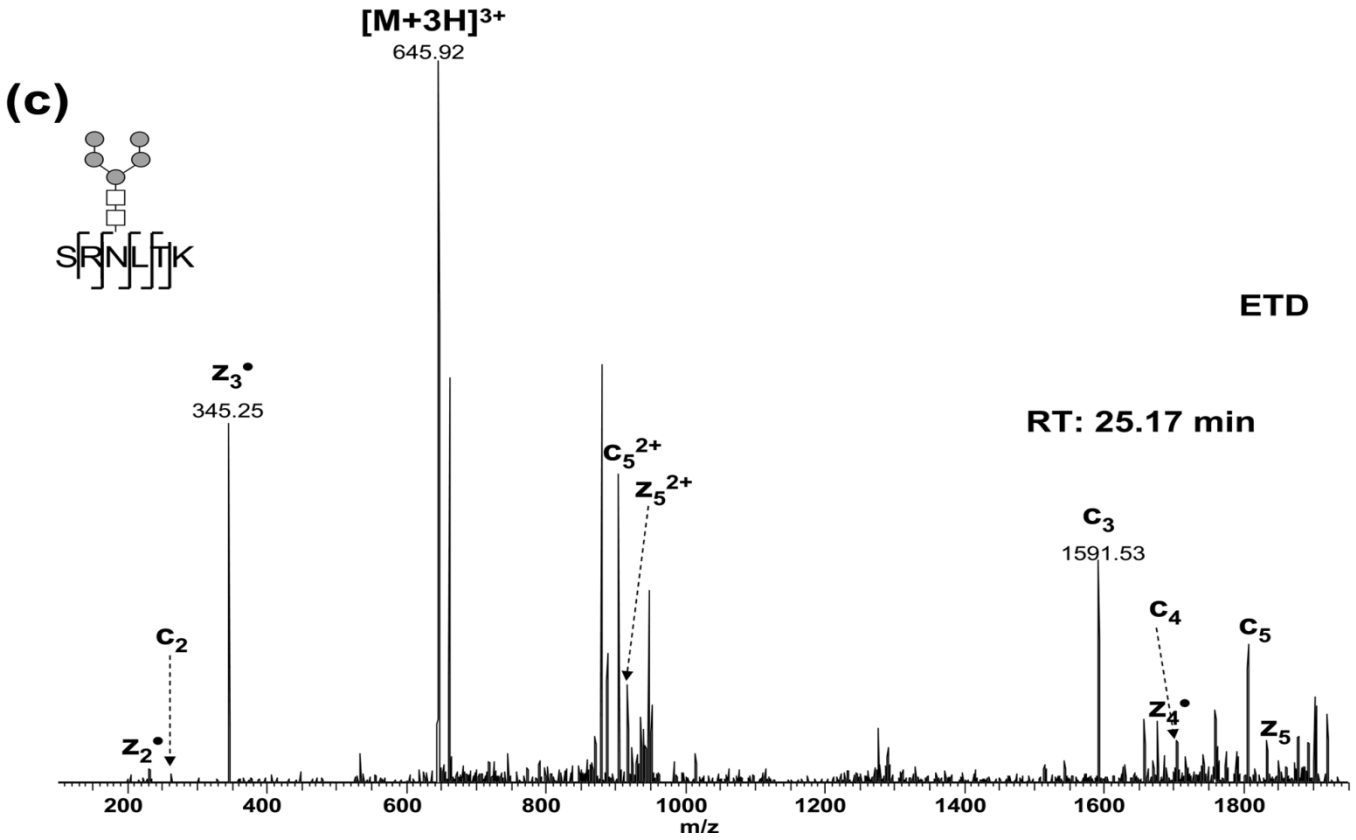


Sequence coverage

Orbitrap Elite using ETD: 81%
LTQ Orbitrap XL using ETD: 43%

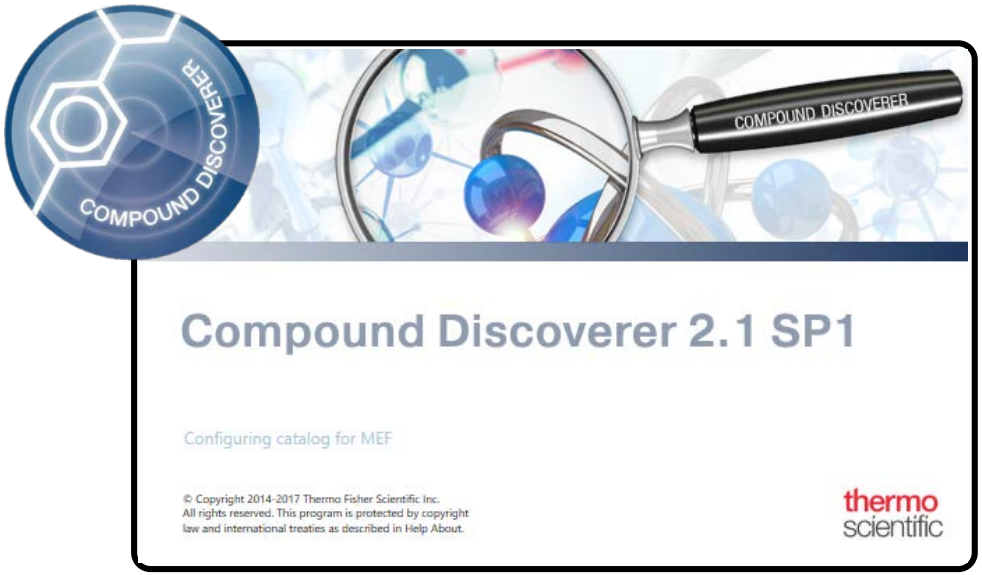
Product Dependent Trigger: HCD PD ETD

- ETD fragmentation triggered
  - Peptide sequence information
  - Glycosylation site localization

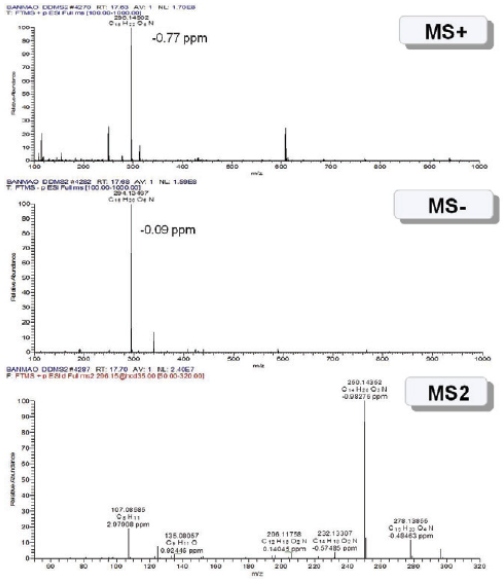


Structural Elucidation

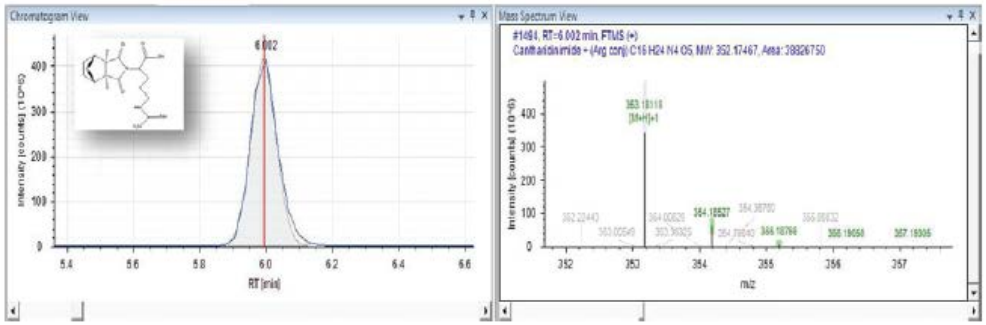
Discovery and Characterization of Components



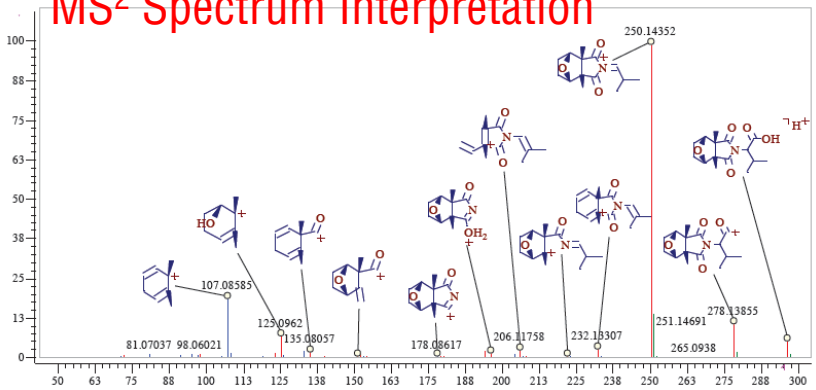
MS and MS² Spectrum



Extracted Ion Chromatogram and Isotopic Pattern



MS² Spectrum Interpretation

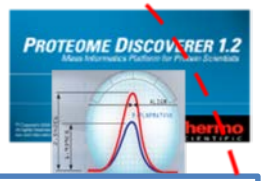


Elevator Speech

The orbitrap provides reproducible high resolution accurate mass with superior U-HPLC compatibility at resolution unattainable by QTOFs without compromising the sensitivity and dynamic range in MS or MS-MS data. With orbitrap, you will have fewer false positives, higher quality, better accuracy and more confidence in your quan/qual measurements.

From Discovery to Quantification - do it all with a Q Exactive

Qual-Quan



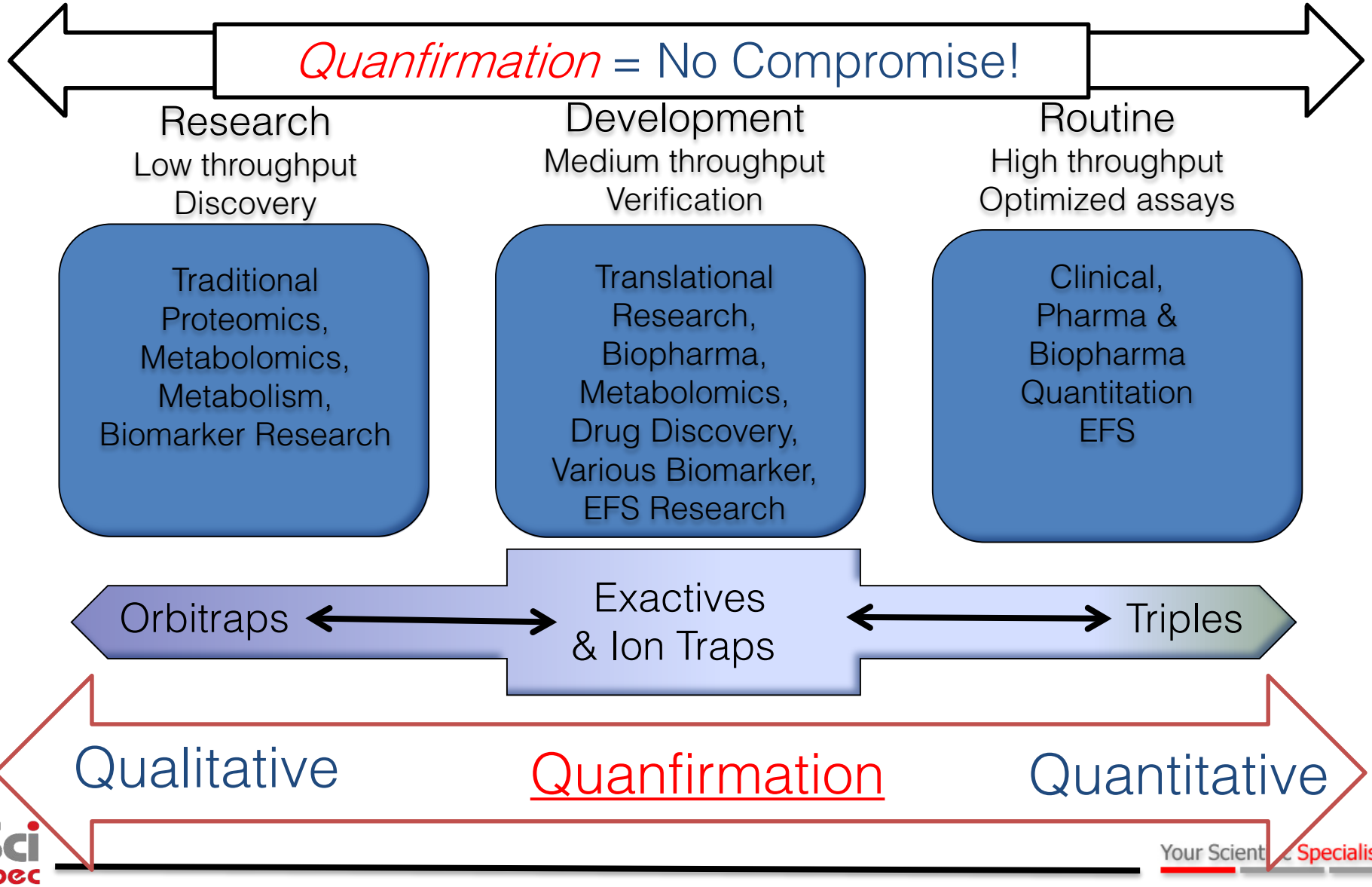
Discovery → HR/AM Targeted Quan ← Discovery Quan

HR/AM Targeted Quan:

- Target Confirmation
- Target Quantification

Discovery	Target Confirmation	Target Quantification	Discovery Quan
•Precursor accurate mass • MS/MS spectrum	•Accurate Mass (AM) • Retention Time (RT) • Isotope Pattern • MS/MS spectrum • MS/MS Transitions	• LC Peak Area • Precursor • MS/MS Transitions	•Precursor accurate mass • MS/MS spectrum
•Protein / peptide ID		•All Targeted Quan	•TMT •Label free •SILAC

Range of Experiments



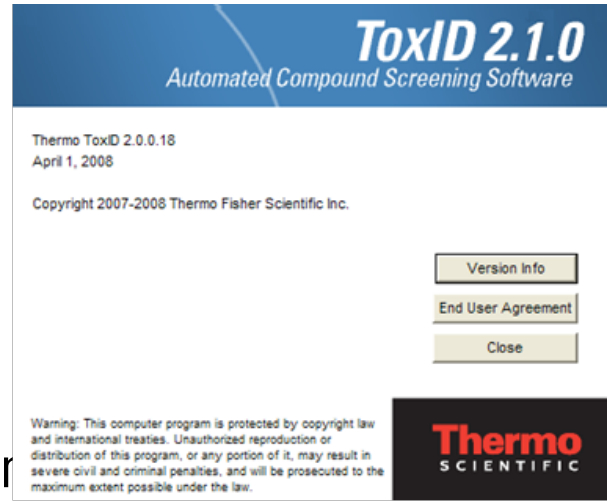
Linearity and Precision

Milk Samples	Non Fat				Low Fat (2%)				Whole Fat			
Fortification Levels	50	100	250	500	50	100	250	500	50	100	250	500
Sulphamethazine	0.9964				0.9908				0.9970			
(RSD %)	2.4	7.2	4.9	2.4	6.6	14.5	5.2	5.6	8.9	1.2	5.1	n/a
Oxytetracycline	0.9906				0.9931				0.9909			
(RSD %)	2.7	11.4	11.4	5.5	11.6	11.8	5.9	4.2	9.2	12.9	4.9	2.6
Tetracycline	0.9923				0.9948				0.9903			
(RSD %)	6.2	6.4	5.4	3.7	7.3	4.8	5.9	4.5	4.1	9.5	6.5	5.5
Enrofloxacin	0.9969				0.9969				0.9973			
(RSD %)	9.2	7.9	2.3	0.8	11.1	1.4	1.4	3.1	6.9	8.3	3.0	n/a
Difloxacin	0.9958				0.9907				0.9968			
(RSD %)	12.2	4.3	6.0	2.4	10.8	4.6	2.7	5.5	2.6	6.1	5.1	n/a
Spiramycin	0.9920				0.9740				0.9951			
(RSD %)	11.1	11.8	8.4	4.1	10.9	4.0	10.0	9.4	13.3	6.5	5.2	0.2
Albendazole	0.9984				0.9967				0.9928			
(RSD %)	1.6	1.7	1.7	2.7	6.3	3.2	3.6	4.2	2.6	6.2	1.2	2.9
Phenylbutazone	0.9947				0.9922				0.9663			
(RSD %)	2.9	3.3	0.8	2.6	8.1	4.1	4.9	3.1	0.6	0.9	0.7	0.3
Salinomycine Na	0.9993				0.9966				0.9984			
(RSD %)	1.2	0.7	1.2	1.5	1.5	0.8	3.1	0.4	2.8	3.1	1.3	1.2

Stolker, A.A.M. et al; Anal. and Bioanal. Chem. 2010 accepted for publication

Drug identification using ToxID™2.1.0

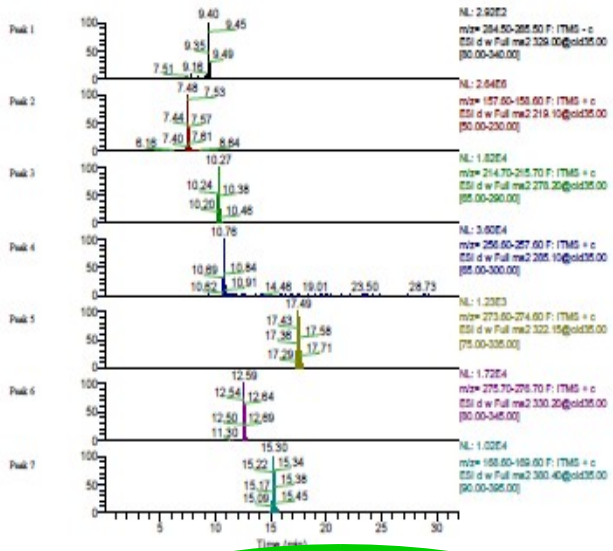
- Fully automated analysis and reporting
- Drug identification based on
- Molecular weight
- MS2 spectra
- Chromatographic retention time
- Built-in library of about 300 drugs
- Library spectra acquired under real world conditior for robust and accurate ID
- The software uses proven NIST search engine
- Feature to easily create and expand library
- Excellent results review and reporting
- Summary report
- Data review report
- Excel spreadsheet



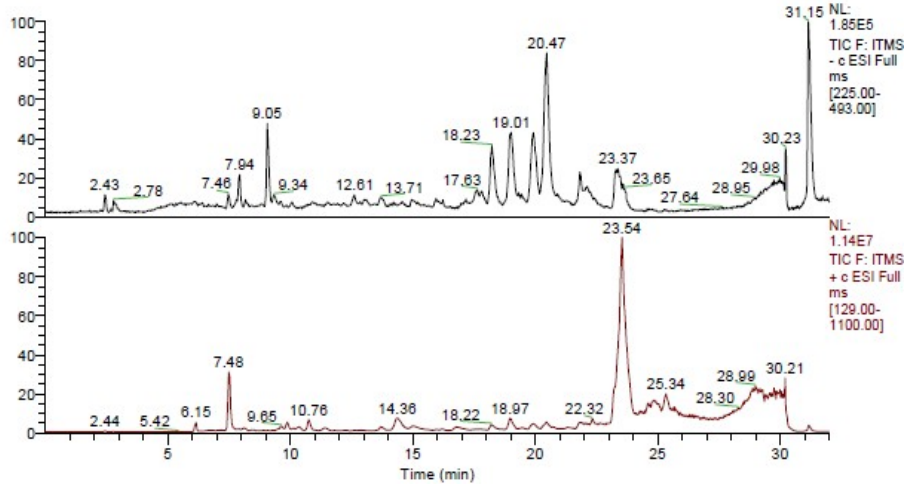
ToxID Summary Report

Your Company
Summary Report

Raw File Name: C:\Documents and Settings\benedicte.duret\Mes documents\My Data\ClinicalToxicologyForensic\Toxicology
Config File Name: C:\Documents and Settings\benedicte.duret\Mes documents\My Data\ClinicalToxicologyForensic\Toxicology
Sample Name: Laboratory:
Acquisition Start Time: 21/11/2008 11:43:54
Screening Conditions: Based on Full MS2 scans. m/z window (amu): 0.50, RT window (min): 1.00, MS2 Search libraries: Tox_Library_LXQ.Use full MS scan to confirm.



Peak Number	Compound Name	Code	SI	RSI	m/z	Expected RT	Actual RT	Concentration ng/ml	Library Name
1	Furosemide	p	999	999	329.00	8.70	9.40	0.03	Tox_Library_LXQ
2	Meprobamate	p	814	814	219.10	7.20	7.48	259.21	Tox_Library_LXQ
3	Venlafaxine	p	831	835	278.20	9.40	10.27	1.79	Tox_Library_LXQ
4	Diazepam	p	842	843	285.10	10.40	10.76	1.46	Tox_Library_LXQ
5	Chlorpromazine-D3	i	814	814	322.20	17.30	17.49	0.12	Tox_Library_LXQ
6	Prazepam-D5	i	911	916	330.20	11.70	12.59	1.69	Tox_Library_LXQ
7	Haloperidol-D4	i	806	826	380.40	14.90	15.30	1.00	Tox_Library_LXQ



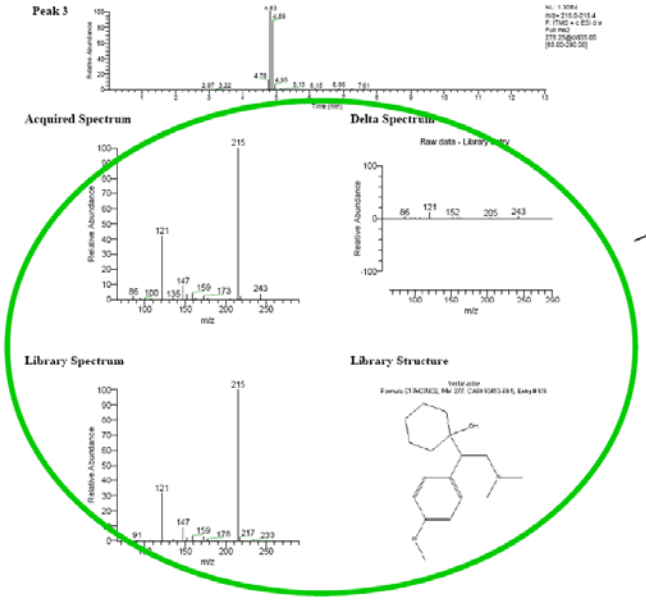
ToxID Review Report

Company Name ToxID Long Report

Raw File Name: C:\Documents and Settings\marta.kozak\Desktop\Desktop\Application_Notes\ToxID\2J.RAW
Config File Name: C:\Xcalibur\examples\ToxID\ToxID_config_13min.csv
Sample Name:
Laboratory: ChemLab
Acquisition Start Time: 2/13/2007 1:04:54 AM

Peak Number	Compound Name	Code	SI	RSI	m/z	Expected RT	Real RT	Intensity	Library Name
3	Venlafaxine	p	816	837	278.2	4.90	4.83	12964	Tox_Library

Peak 3



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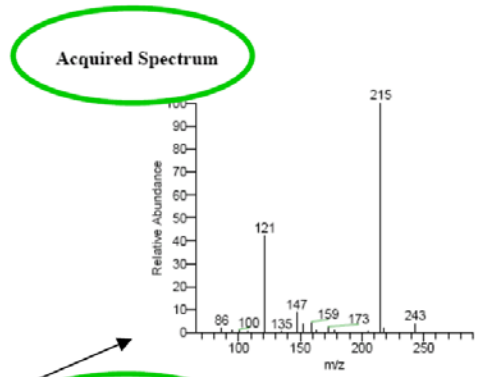
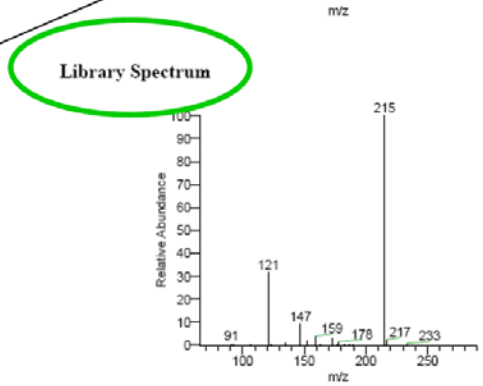
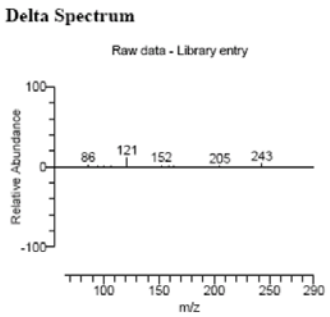






What is Mass Frontier?

- Software for small molecule structural elucidation via mass spectral interpretation
  - Predict fragmentation given a compound structure
  - Annotate spectra with fragment structures
  - Store MS^n spectra along with structures, peak annotations, ID numbers, pathway information, etc
  - Match unknown spectra against library entries
  - And MUCH more…

Tag Line:

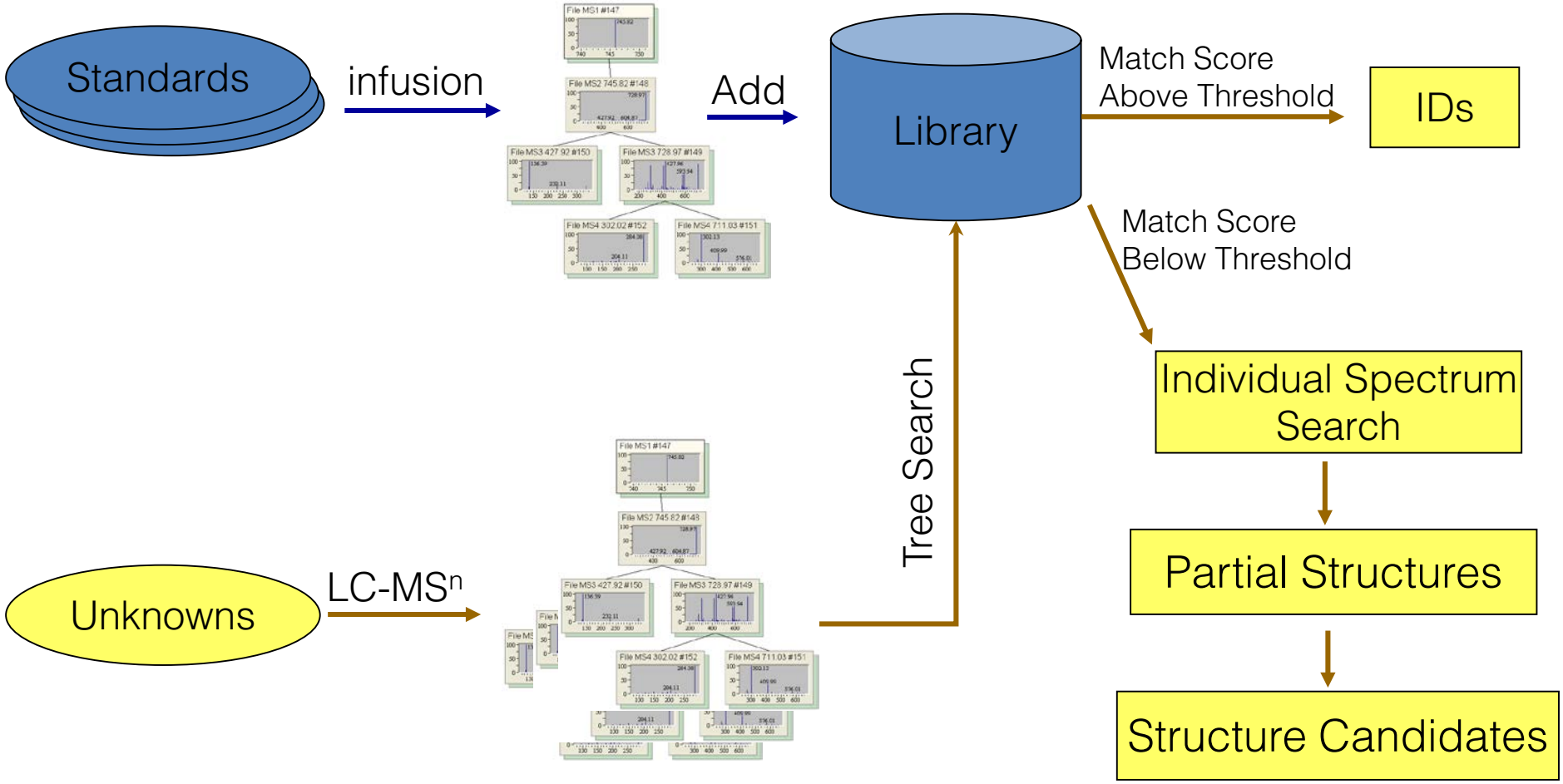
Mass Frontier helps you to go from SPECTRA

Who should get Mass Frontier?

- Anyone who is doing small molecule structural elucidation / confirmation *via* mass spectrometry

- Examples:
  - Metabolite Identification in Drug Metabolism
  - Impurity and Degrading analysis in QC/QA
  - Endogenous Metabolite Identification in Metabolomics
  - Forensic Analyses in Federal and State Agencies
  - Doping Control in Horse Racing
  - Chemistry/Biochemistry/Pharmacy Departments in Universities doing small molecule research
  - Service labs for synthetic chemists

General Unknown Screening using Mass Frontier



Sheldon et al. Determination of Ion Structures in Structurally Related Compounds Using Precursor Ion Fingerprinting. JASMS, ***2009****, 20, 370-376*

MS^n Spectral Trees—the ***ONLY*** Route to Unambiguous Structural Elucidation!

Accurate mass information is powerful – provides a potential formula

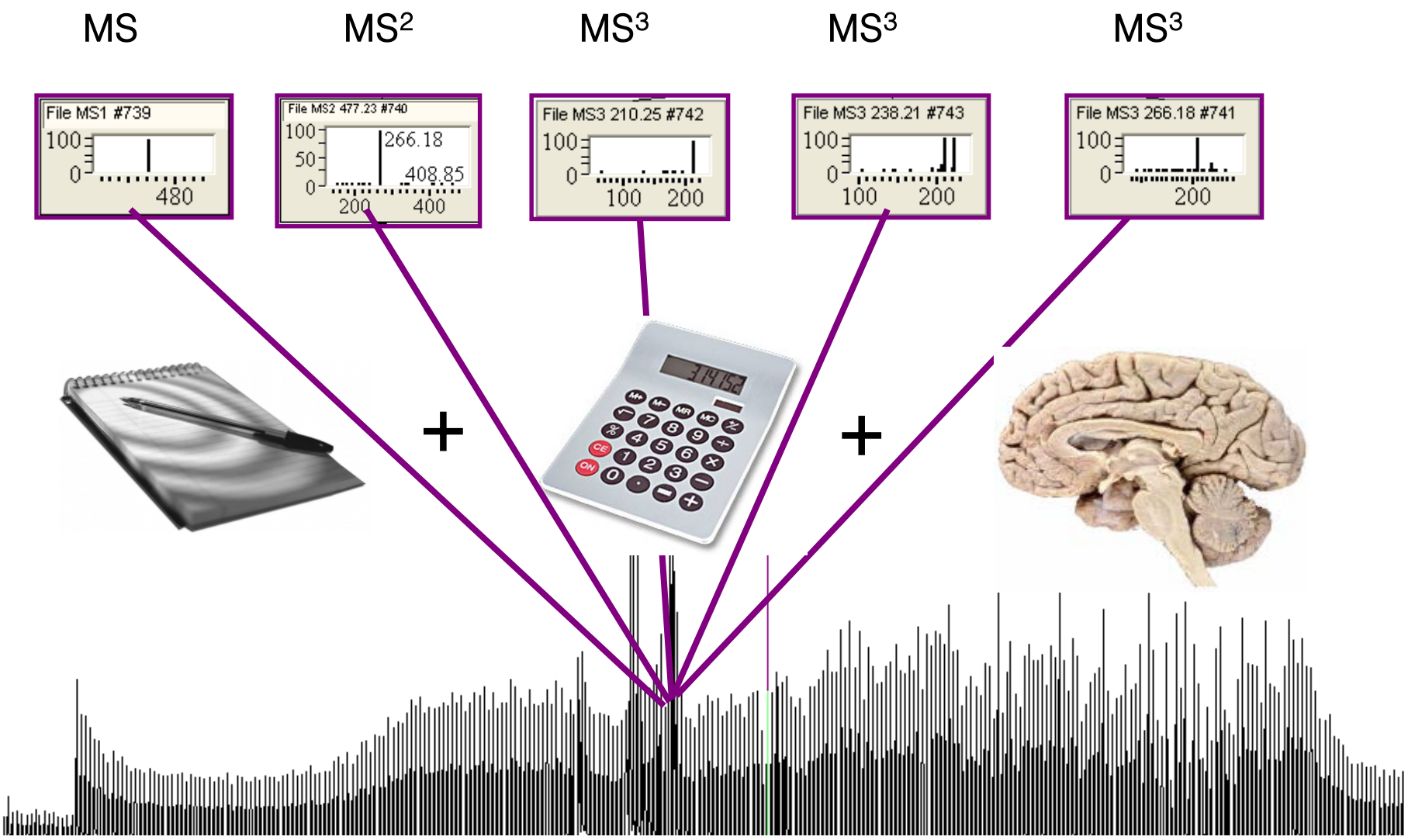
However MS^n information still necessary to distinguish between structural isomers

Trees can automatically be generated by Data Dependant LC-MS/MS runs on our instruments

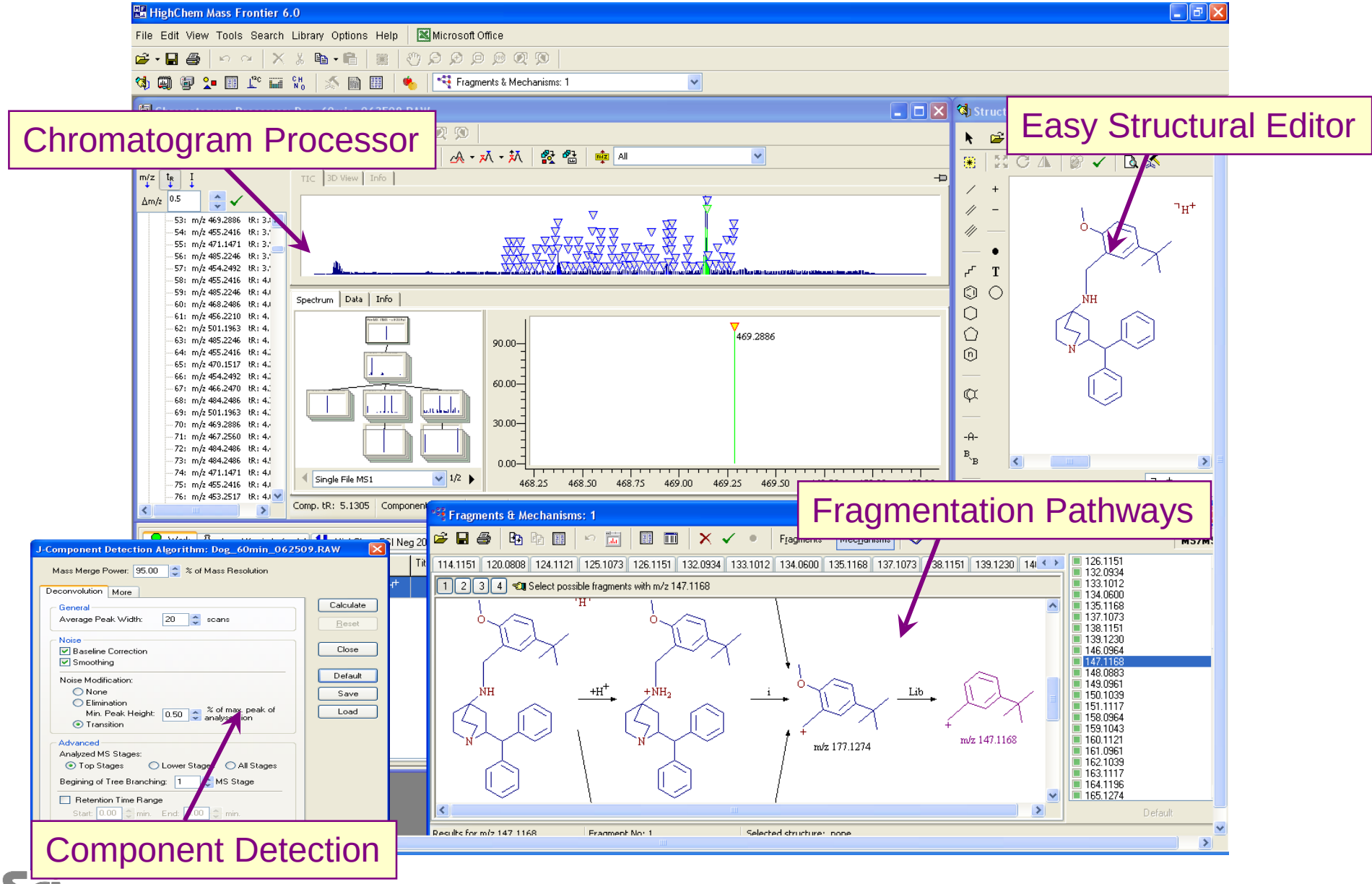
Component Detection from Mass Frontier can automatically deconvolute MS^n spectral trees!

This information collectively, uniquely defines the structure of the molecule

How Do You Get a Structure From MS Data?

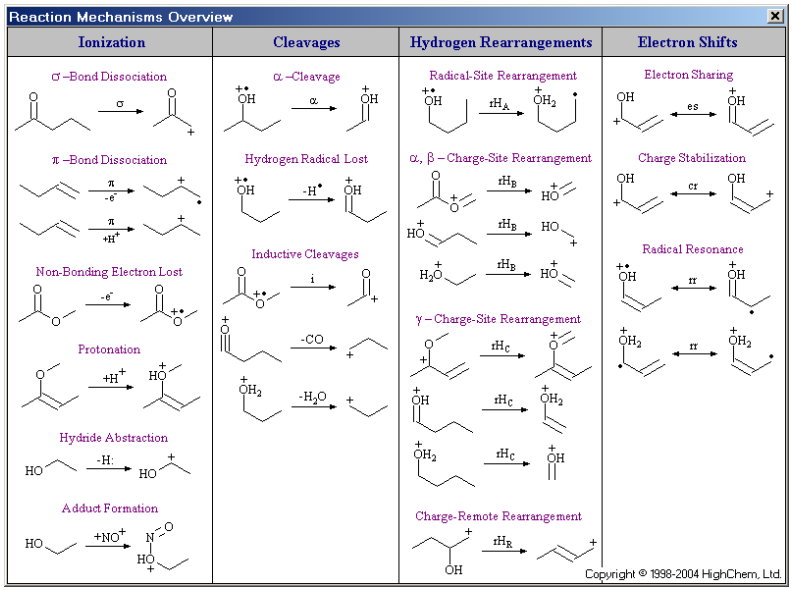


Mass Frontier: Toolbox for Structural Elucidation



Fragmentation Prediction: Three Knowledge Bases

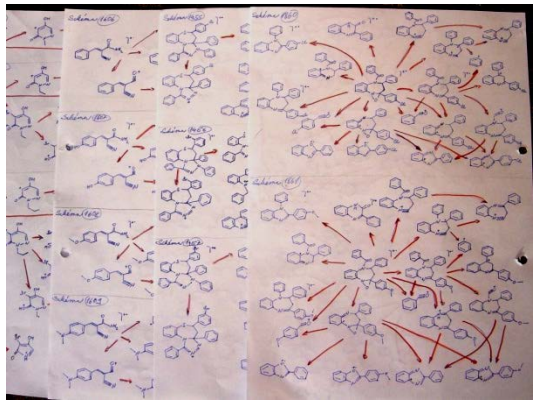
1. General fragmentation rules



2. Mass Frontier Fragmentation Library™

Total number of	Mass Frontier 6.0
Fragmentation Schemes	30.936
Individual Reactions	129.229
Chemical Structures	151.762
Decoded Mechanisms	120.029

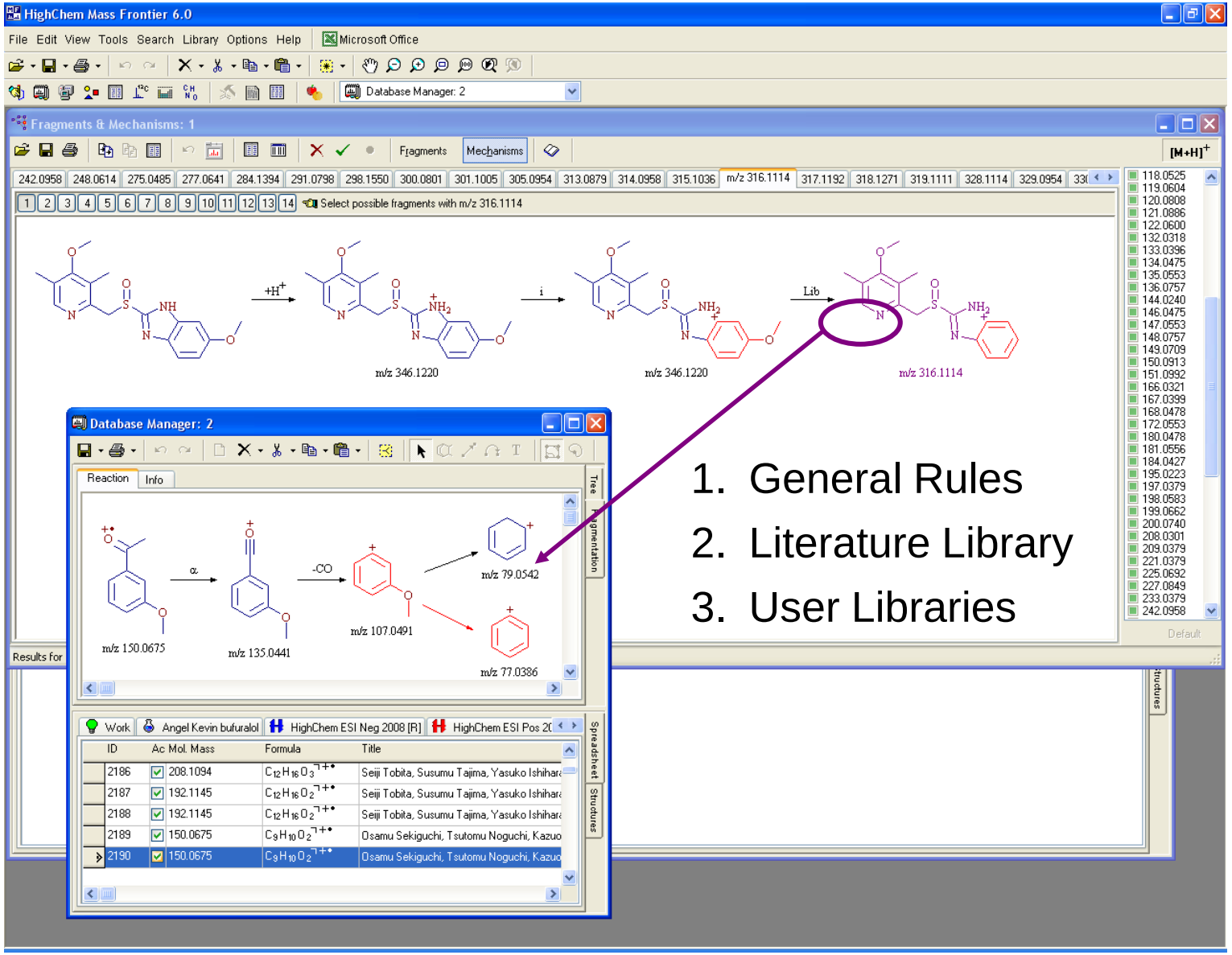
3. User Libraries



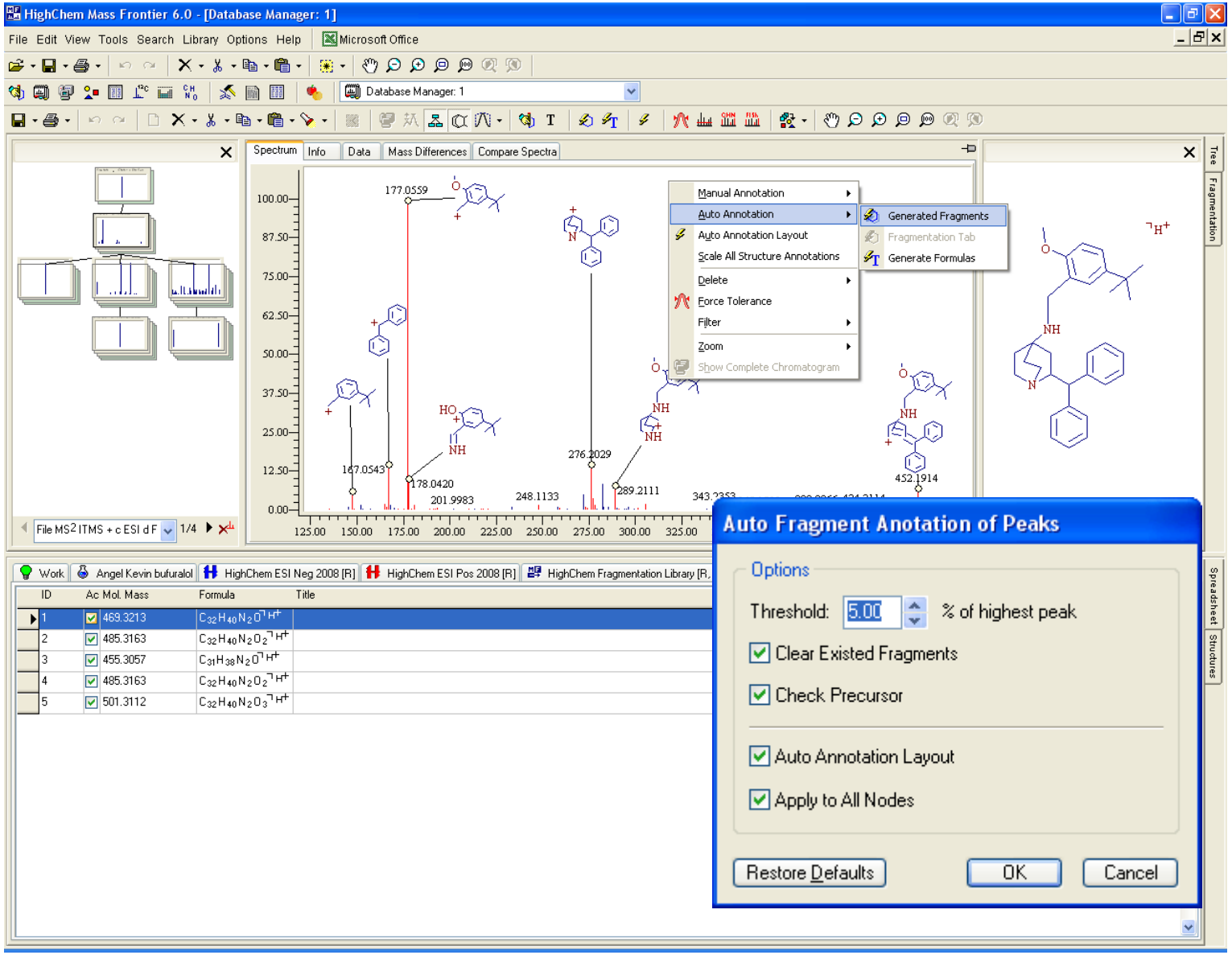
Fragmentation Library™ in 6.0 now covers >99% published literature

	Source	Volume	Year
1.	**JASMS** (Journal of the American Society for Mass Spectrometry)	1-17	1990-2006
2.	**IJMSIP** (International Journal of Mass Spectrometry and Ion Physics)	1-53	1968-1983
	IJMSIP (International Journal of Mass Spectrometry and Ion Processes)	54-175	1983-1998
	IJMS (International Journal of Mass Spectrometry)	176-255	1998-2006
3.	**RCM** (Rapid Communications in Mass Spectrometry)	1-20	1987-2006
4.	**JMS** (Journal of Mass Spectrometry)	30-41	1995-2006
5.	**OMS** (Organic Mass Spectrometry)	1-29	1968-1994
6.	**JMSSJ** (Journal of the Mass Spectrometry Society of Japan)	11-27 29-30 37-48 50-53	1964-1979 1981-1982 1989-2000 2002-2005
7.	**MSR** (Mass Spectrometry Reviews)	1-25	1981-2006
8.	**EJMSBMER** (European Journal of Mass Spectrometry in Biochemical, Medicine, and Environmental. Research)	1-2	1980-1982
9.	**BMS** (Biomedical Mass Spectrometry) **BEMS** (Biomedical and Environmental Mass Spectrometry) **BMS** (Biological Mass Spectrometry)	1-12 14-19 20-23	1974-1985 1987-1990 1991-1994
10.	**JC** (Journal of Chromatography)	181-536	1980-1991
11.	**EJMS** (European Journal of Mass Spectrometry)	4	1998

Predictive Fragmentation



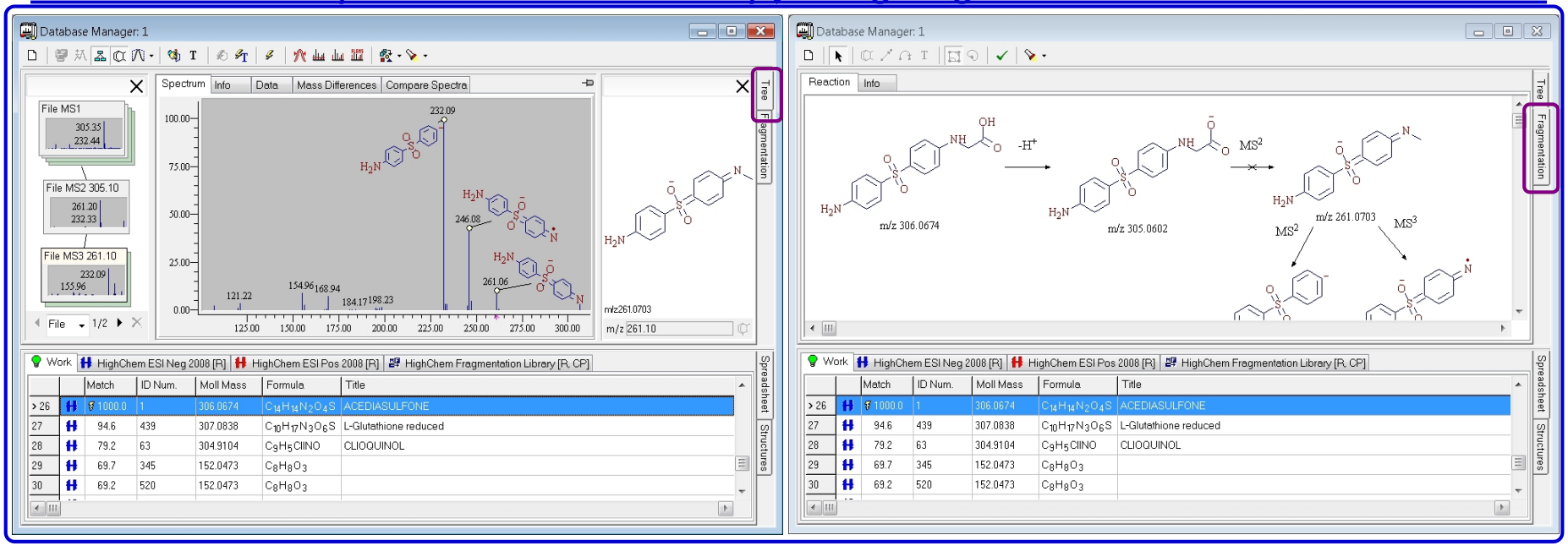
How Do I Annotate Spectral Trees? …Automatically



Database Manager: Integrated Knowledge Management

- All records of installed libraries are shown in Database Manager
- All records are accessible without querying
- Spectral and Fragmentation libraries are unified in Database Manager
- Searches are universal, independent of data type (structures, m/z values, names, CAS number, biological activity, etc)

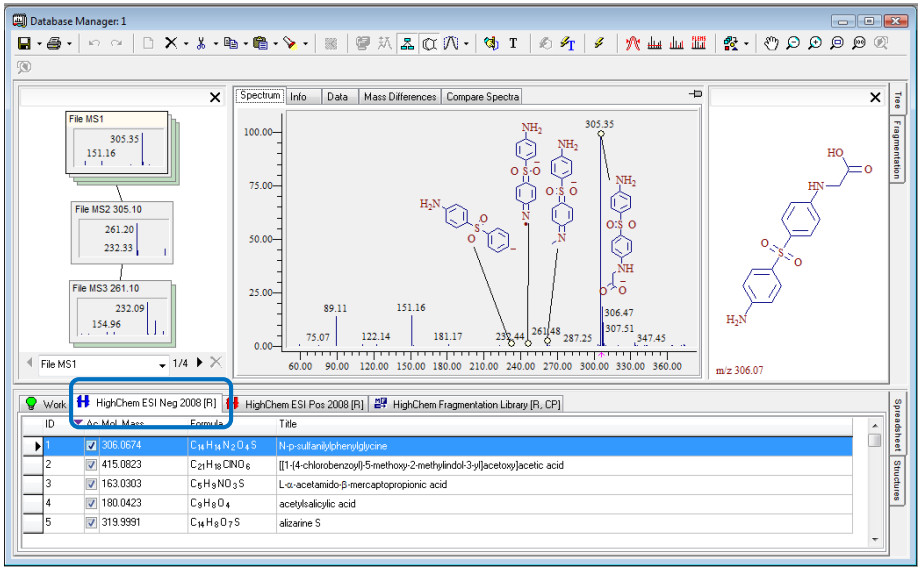
One Record: *Spectral tree with corresponding fragmentation mechanisms & more!*

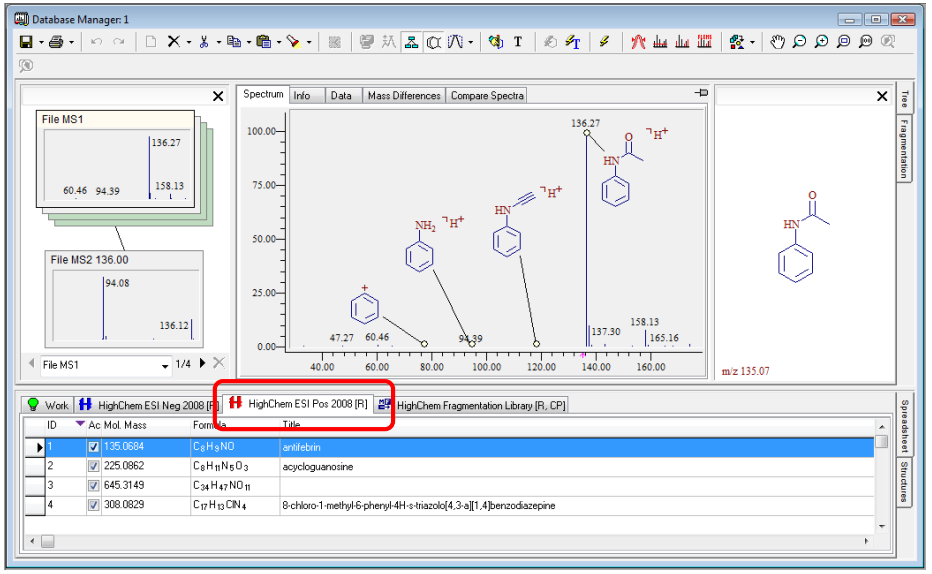


HighChem Spectral Tree Libraries—Free with the software!

Library: HighChem ESI **Neg** 2008
Tree Count: 524
Spectra Count: 3805
Fragmentation Schemes: 263

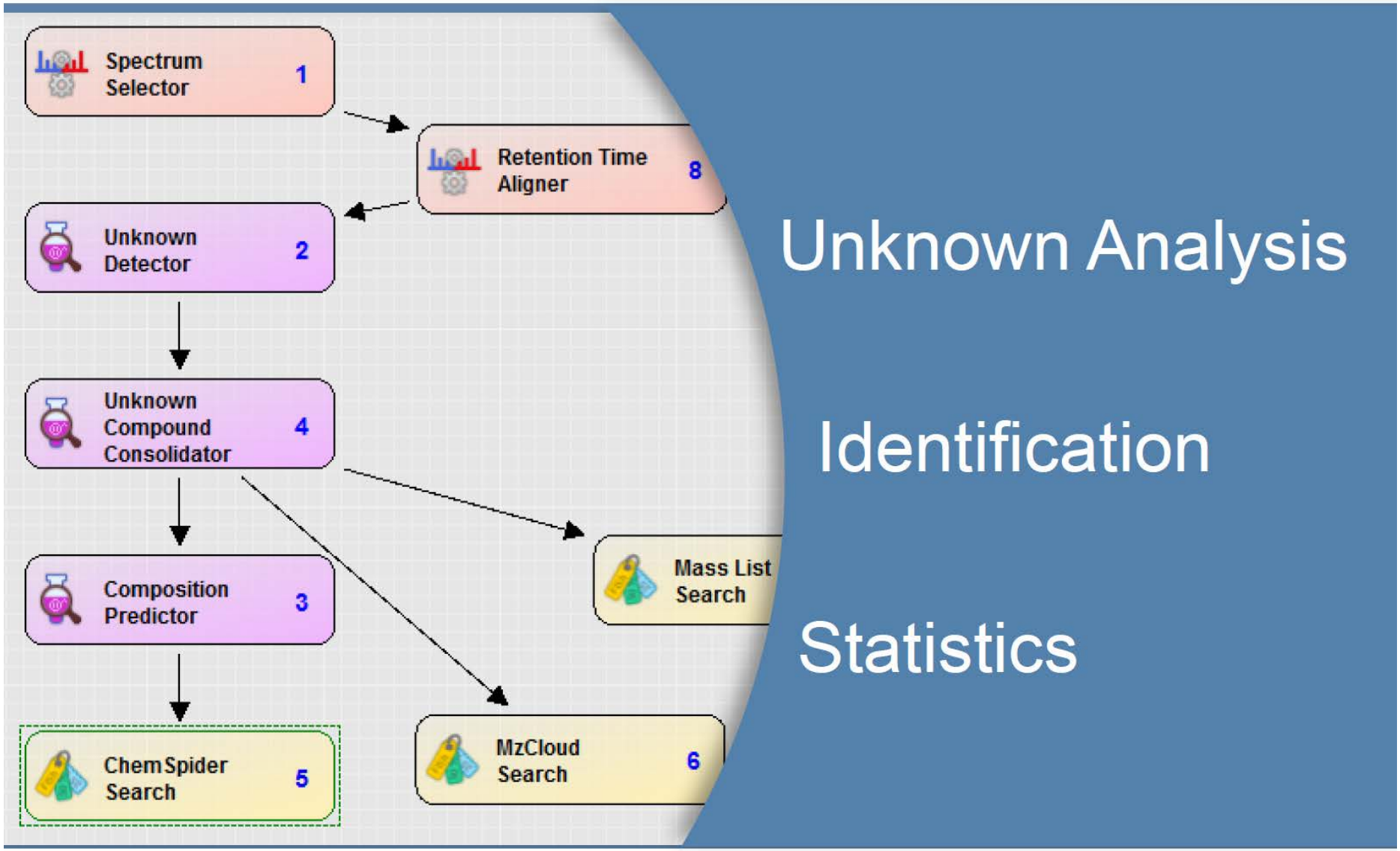
Library: HighChem ESI **Pos** 2008
Tree Count: 1251
Spectra Count: 10180
Fragmentation Schemes: 702



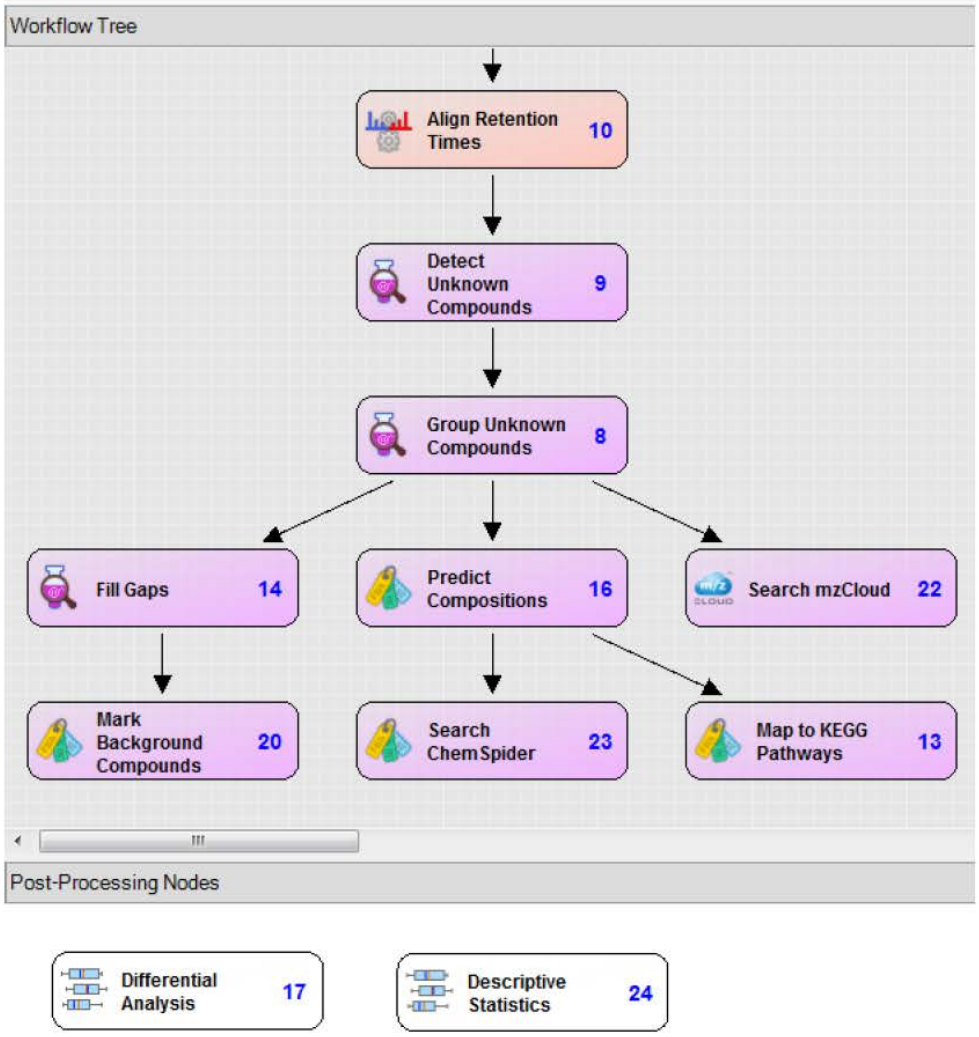


- Common pharmaceutical compounds and human metabolites
- Peaks manually annotated and fragmentation mechanism elucidated

Compound Discoverer

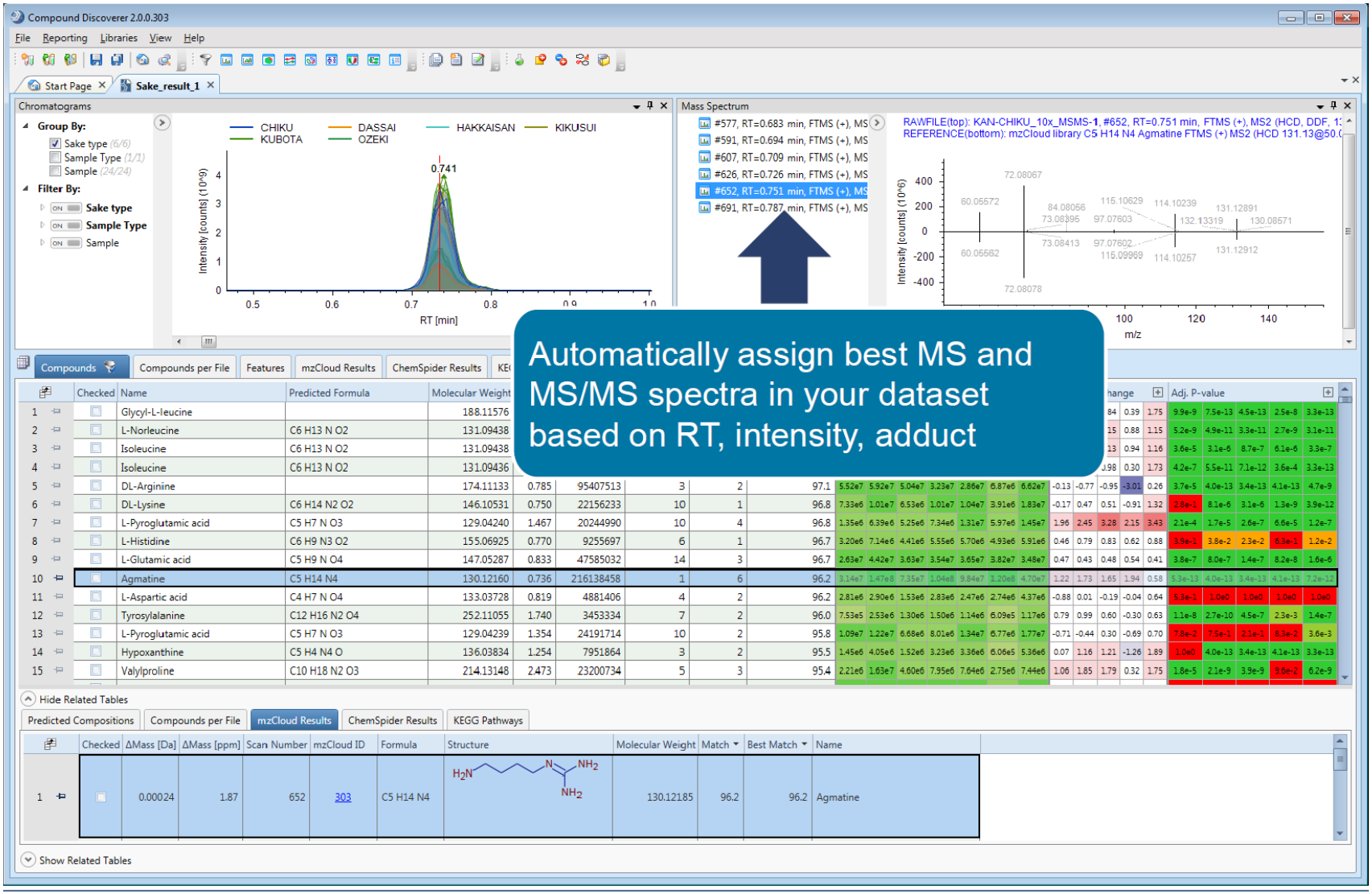


Flexible Workflow

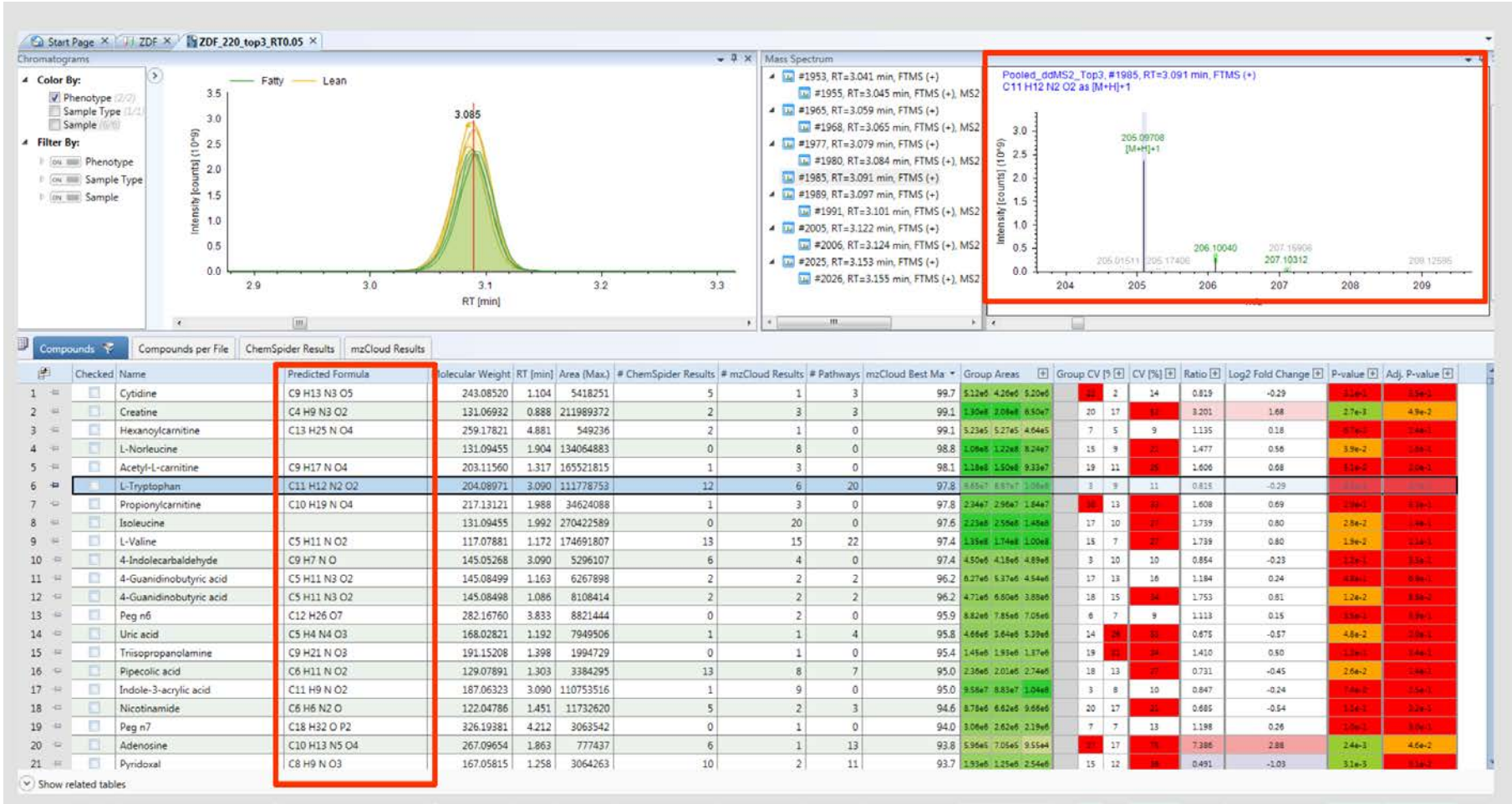


- Use common, predefined workflows or create your own
- Integrate your own nodes
- New in version 2.0: Nodes for Unknown Detection, Identification, Statistics

Identifying Unknown

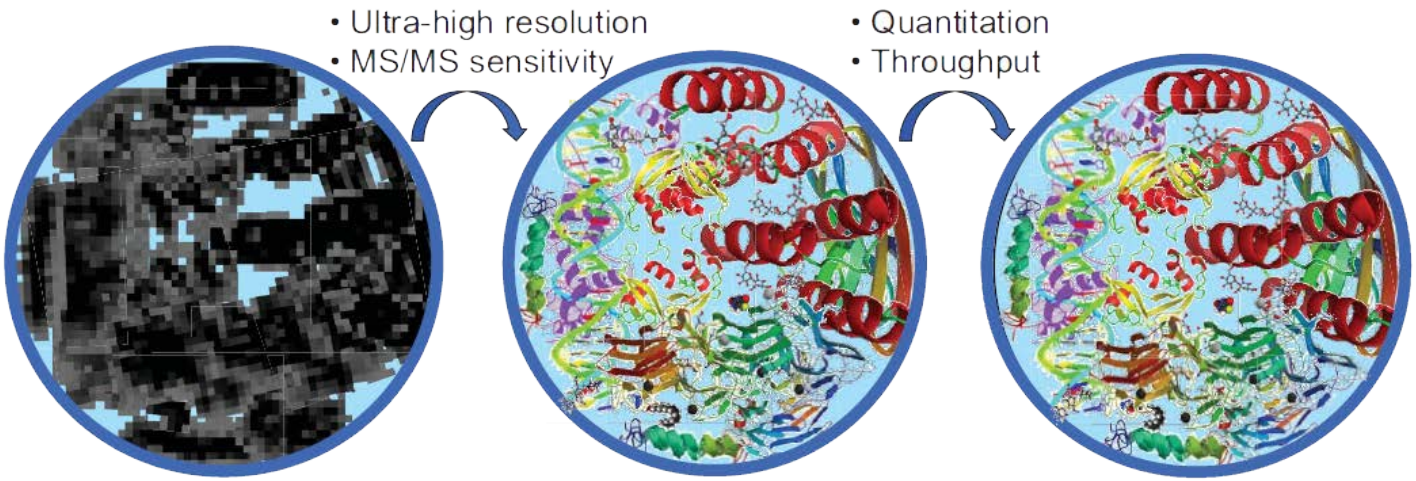


Predicted Composition



	Checked	Name	Predicted Formula	Molecular Weight	RT [min]	Area (Max.)	# ChemSpider Results	# mzCloud Results	# Pathways	mzCloud Best Ma	Group Areas	Group CV [%]	CV [%]	Ratio	Log2 Fold Change	P-value	Adj. P-value
1		Cytidine	C9 H13 N3 O5	243.08520	1.104	5418251	5	1	3	99.7	5.12e6 4.26e6 5.20e6	22	14	0.819	-0.29	3.1e-1	5.5e-1
2		Creatine	C4 H9 N3 O2	131.06932	0.888	211989372	2	3	3	99.1	1.90e8 2.08e8 6.50e7	20	17	1.201	1.68	2.7e-3	4.9e-2
3		Hexanoylcarnitine	C13 H25 N O4	259.17821	4.881	549236	2	1	0	99.1	5.23e5 5.27e5 4.64e5	7	5	9	1.135	0.18	6.7e-1	1.4e-1
4		L-Norleucine		131.09455	1.904	134064883	0	8	0	98.8	1.08e8 1.22e8 8.24e7	15	9	22	1.477	0.56	3.9e-2	1.8e-1
5		Acetyl-L-carnitine	C9 H17 N O4	203.11560	1.317	165521815	1	3	0	98.1	1.18e8 1.50e8 9.33e7	19	11	25	1.606	0.68	8.1e-2	2.0e-1
6		L-Tryptophan	C11 H12 N2 O2	204.08971	3.090	111778753	12	6	20	97.8	8.65e7 8.87e7 1.06e8	3	9	11	0.815	-0.29	3.1e-1	5.5e-1
7		Propionylcarnitine	C10 H19 N O4	217.13121	1.988	34624088	1	3	0	97.8	2.34e7 2.96e7 1.64e7	32	13	22	1.608	0.69	2.9e-1	5.1e-1
8		Isoleucine		131.09455	1.992	270422589	0	20	0	97.6	2.23e8 2.55e8 1.45e8	17	10	27	1.739	0.80	2.8e-2	1.4e-1
9		L-Valine	C5 H11 N O2	117.07881	1.172	174691807	13	15	22	97.4	1.35e8 1.74e8 1.00e8	15	7	27	1.739	0.80	1.9e-2	1.1e-1
10		4-Indolecarbaldehyde	C9 H7 N O	145.05268	3.090	5296107	6	4	0	97.4	4.50e6 4.15e6 4.89e6	3	10	10	0.854	-0.23	1.2e-1	3.3e-1
11		4-Guanidinobutyric acid	C5 H11 N3 O2	145.08499	1.163	6267898	2	2	2	96.2	6.27e6 5.37e6 4.54e6	17	13	16	1.184	0.24	4.8e-1	6.8e-1
12		4-Guanidinobutyric acid	C5 H11 N3 O2	145.08498	1.086	8108414	2	2	2	96.2	4.71e6 6.80e6 3.88e6	18	15	24	1.753	0.81	1.2e-2	8.3e-2
13		Peg n6	C12 H26 O7	282.16760	3.833	8821444	0	2	0	95.9	8.82e6 7.85e6 7.05e6	6	7	9	1.113	0.15	3.5e-1	5.9e-1
14		Uric acid	C5 H4 N4 O3	168.02821	1.192	7949506	1	1	4	95.8	4.66e6 5.64e6 5.39e6	14	24	33	0.675	-0.57	4.8e-2	2.0e-1
15		Triisopropanolamine	C9 H21 N O3	191.15208	1.398	1994729	0	1	0	95.4	1.45e6 1.93e6 1.37e6	19	21	24	1.410	0.50	1.2e-1	3.4e-1
16		Pipecolic acid	C6 H11 N O2	129.07891	1.303	3384295	13	8	7	95.0	2.36e6 2.01e6 2.74e6	18	13	27	0.731	-0.45	2.6e-2	1.4e-1
17		Indole-3-acrylic acid	C11 H9 N O2	187.06323	3.090	110753516	1	9	0	95.0	9.58e7 8.83e7 1.04e8	3	8	10	0.847	-0.24	7.4e-2	2.5e-1
18		Nicotinamide	C6 H6 N2 O	122.04786	1.451	11732620	5	2	3	94.6	8.78e6 6.62e6 9.66e6	20	17	21	0.685	-0.54	1.1e-1	3.2e-1
19		Peg n7	C18 H32 O P2	326.19381	4.212	3063542	0	1	0	94.0	3.06e6 2.62e6 2.19e6	7	7	13	1.198	0.26	1.9e-1	3.9e-1
20		Adenosine	C10 H13 N5 O4	267.09654	1.863	777437	6	1	13	93.8	5.96e5 7.05e5 9.55e4	87	17	75	7.386	2.88	2.4e-1	4.6e-2
21		Pyridoxal	C8 H9 N O3	167.05815	1.258	3064263	10	2	11	93.7	1.93e6 1.25e6 2.54e6	15	12	33	0.491	-1.03	3.1e-3	3.1e-2

Conclusion



- The Orbitrap Mass Analyzer is a new type of mass analyzers with its own unique combination of analytical parameters
- Orbitraps are still evolving…
  - Higher speed
  - Higher resolving power and mass accuracy
  - Higher sensitivity
  - More routine applications
- Exciting new applications continue to emerge

Summary

- **High resolution** is a key characteristics of MS data enabling
  - Mass accuracy
  - Confident identification
  - Reliable quantitation

- **Data dependent acquisition** offers an elegant simplicity and has proven highly useful for discovery-driven proteomics

- **Mass spectrometry technology** enables comprehensive analysis of proteomics samples
  - Multiple fragmentation techniques
  - MS^n capability

- **Quan&Qual** experiments done on a single platform