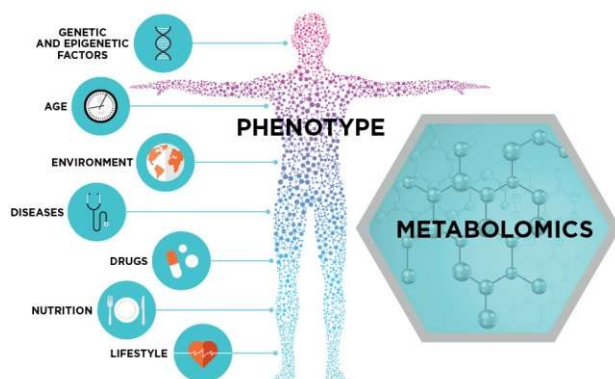# SYNAPT™ XS: Metabolomics, Lipidomics and Proteomics

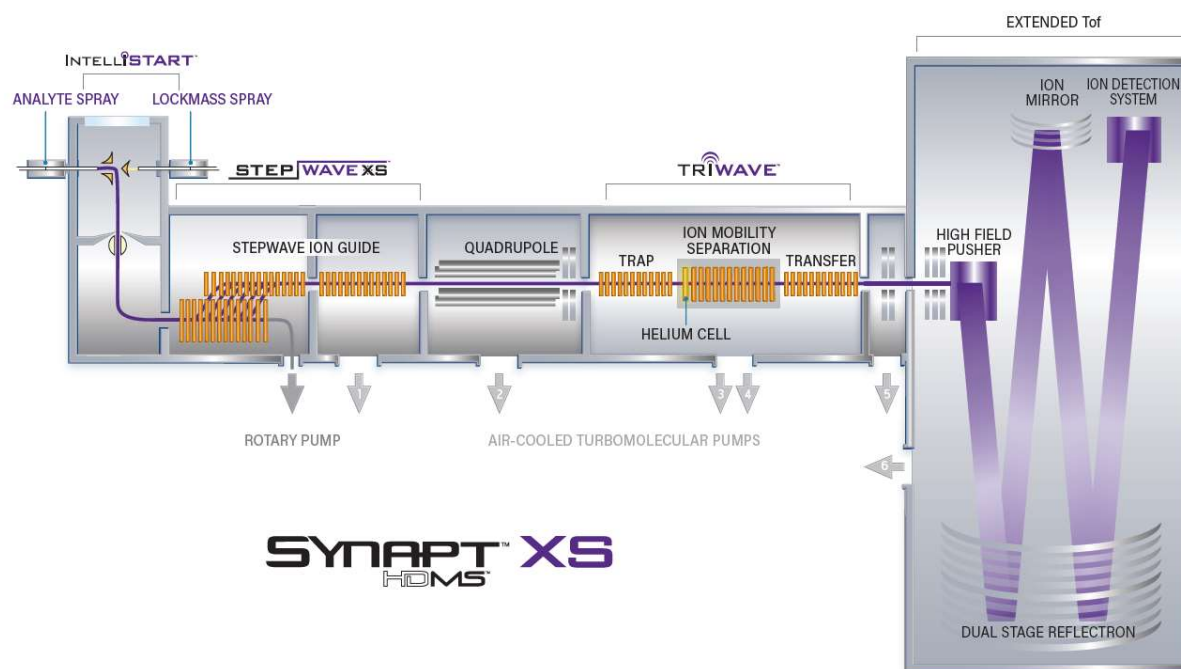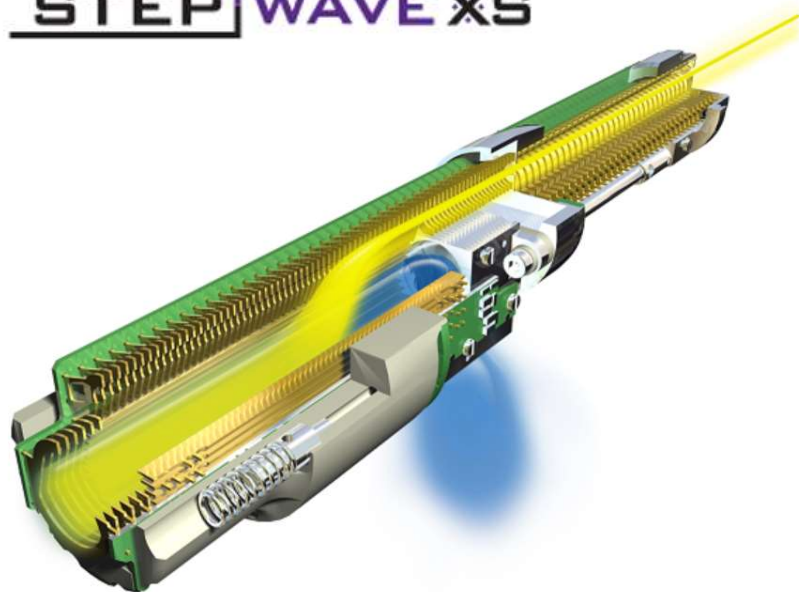## Robert Plumb, FRSC.

### Director Omics Science, Scientific Operations

SYNAPT XS

>80K Resolution

Labile compound transmission

SONAR enabled

Increased Sensitivity
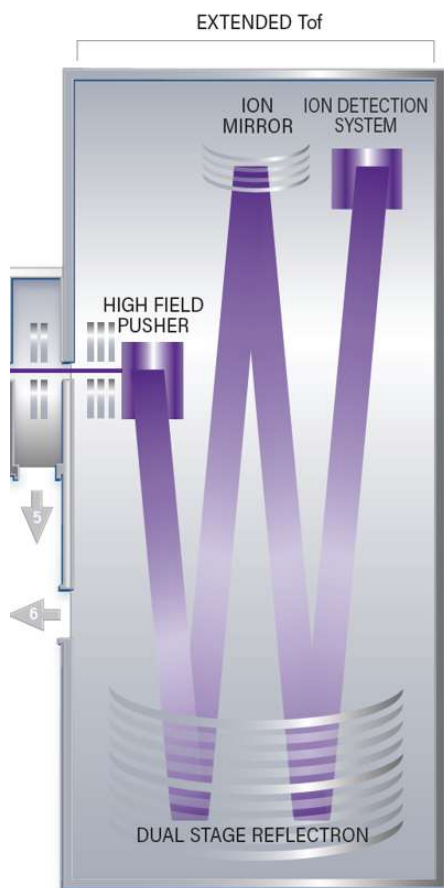
Flexibility, DESI, LC/MS, IMS

Enhanced Performance:
Stepwave XS

- Improved overall sensitivity

- Reproducible and reliable quantification

- Enhanced active ion transmission

- Neutrals and gas load removed

Sensitivity Comparison
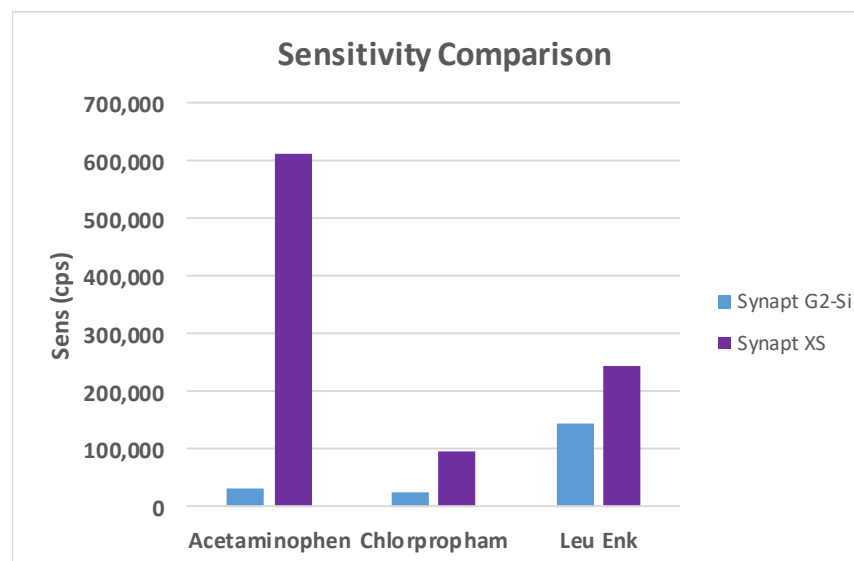
Enhanced Performance:
## Extended Flight Tube

- Improved mass resolution in all modes of analysis

- Superior quantification and qualitative information

- Four modes of resolution to suit analytical needs

- Improved selectivity and more confident identification

EXTENDED Tof

ION MIRROR ION DETECTION SYSTEM

HIGH FIELD PUSHER

DUAL STAGE REFLECTRON
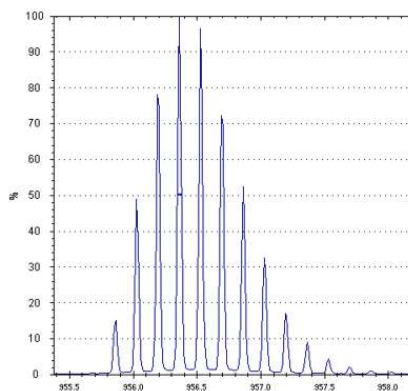
Resolution Mode (V)

Enhanced Res Mode (W)
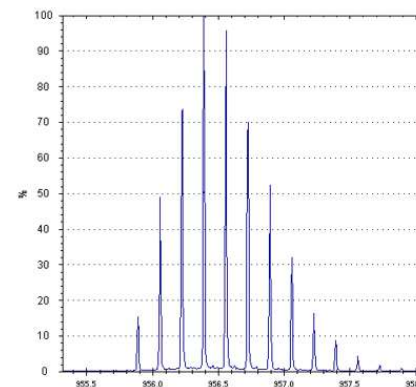
Complementary Acquisition Strategies
Unravel Sample Complexity

<table>
<tr><th>HDMS^E</th><th>SONAR</th></tr>
</table>

HDMS^E

- Transmitting quadrupole
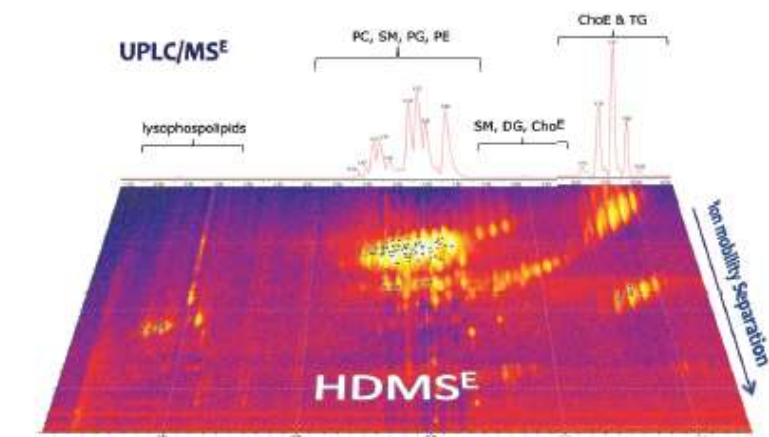- Separation by size, shape and charge
- Resolution of isobaric species

SONAR

- Scanning quadrupole
- Separation by RT and *m/z*
- Clean and clear fragmentation spectra

UPLC/MS^E

lysophospolipids

PC, SM, PG, PE

SM, DG, ChoE

ChoE & TG

Ion mobility Separation

HDMS^E

Figure 2. Increased peak capacity through the added dimension of Ion Mobility Separation.

m/z · m/z · m/z · m/z

m/z

Quad Transmission window

Time

SONAR

m/z
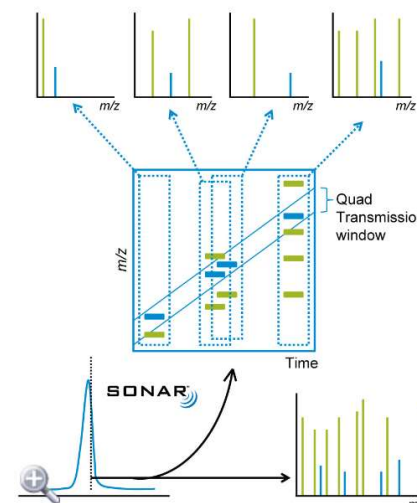
SYNAPT XS is a Highly Flexible Platform
Meeting the Needs of the Modern Research Scientist
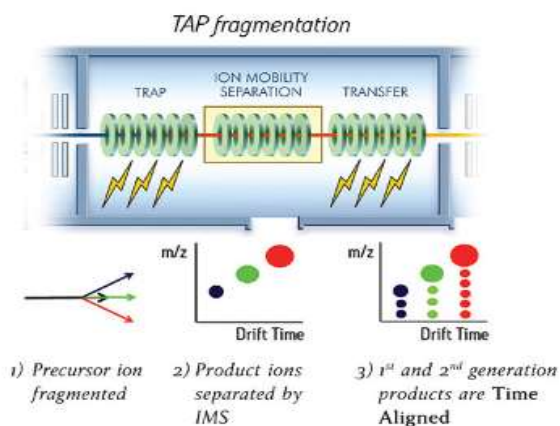
Triwave IMS	Acquisition Modes	Options
	■ Fast-DDA ■ HD-DDA ■ Tof-MRM ■ HD-MRM ■ MS^E ■ $HDMS^E$ ■ $UDMS^E$ ■ SONAR ■ TAP ■ ETD* *option	■ UPLC ■ nano/micro UPLC ■ UPC^2 ■ 2D-LC ■ APGC ■ HDX ■ DESI ■ MALDI ■ REIMS ■ ASAP ■ UniSpray

TAP fragmentation

1) Precursor ion fragmented
2) Product ions separated by IMS
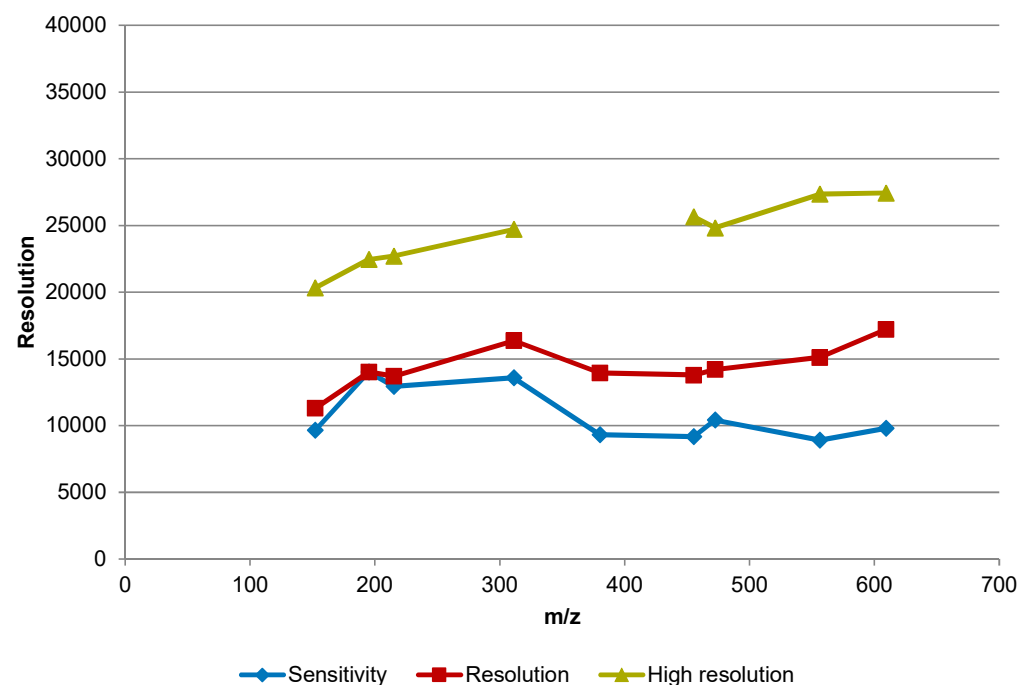3) 1st and 2nd generation products are **Time Aligned**

SYNAPT XS: Metabolomics

Resolution Comparison – Positive Ion
LC-MS QC Mix compounds over different acquisition mode (XS vs. G2-Si)

SYNAPT XS

SYNAPT G2-Si

COMPANY CONFIDENTIAL
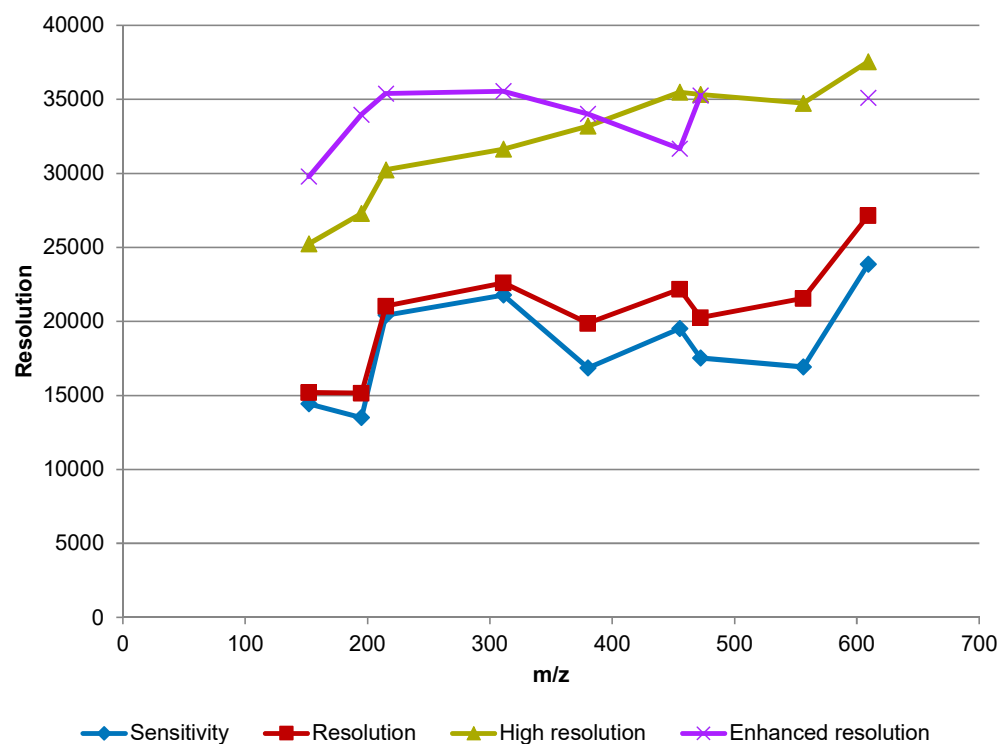
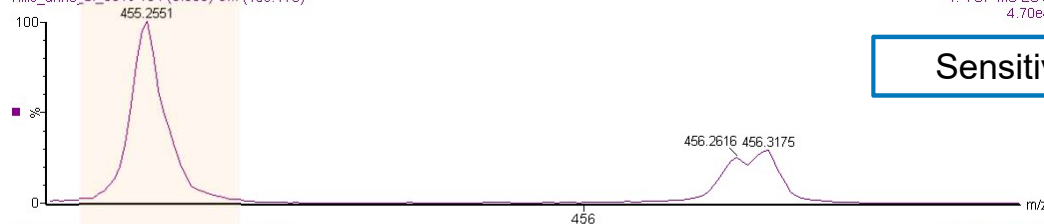# Example Benefit Of Increased Resolution
Verapamil MS spectra
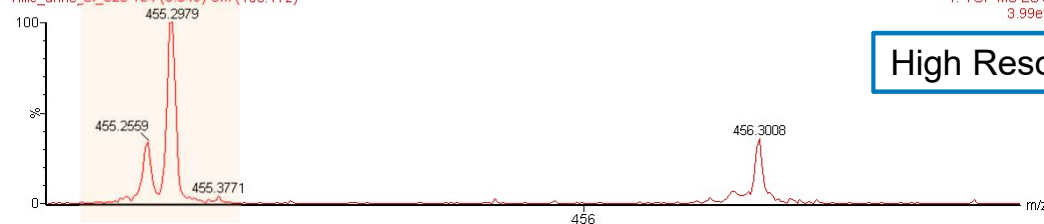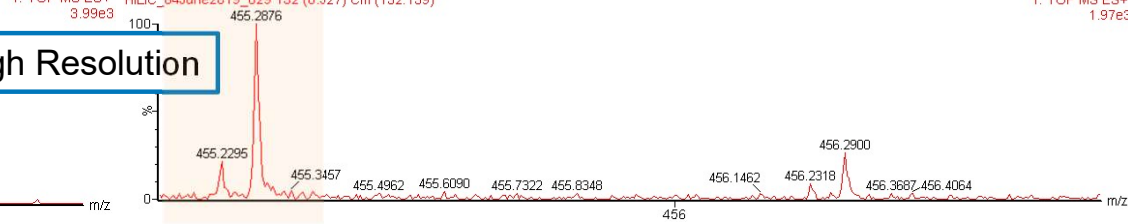
Resolution is sufficient on the SYNAPT-XS for all acquisition mode to separate Verapamil (m/z = 455.29) from the additional component (m/z = 455.24). On the SYNAPT G2-Si, these two species can only be separated in high resolution mode.

Increase In Sensitivity
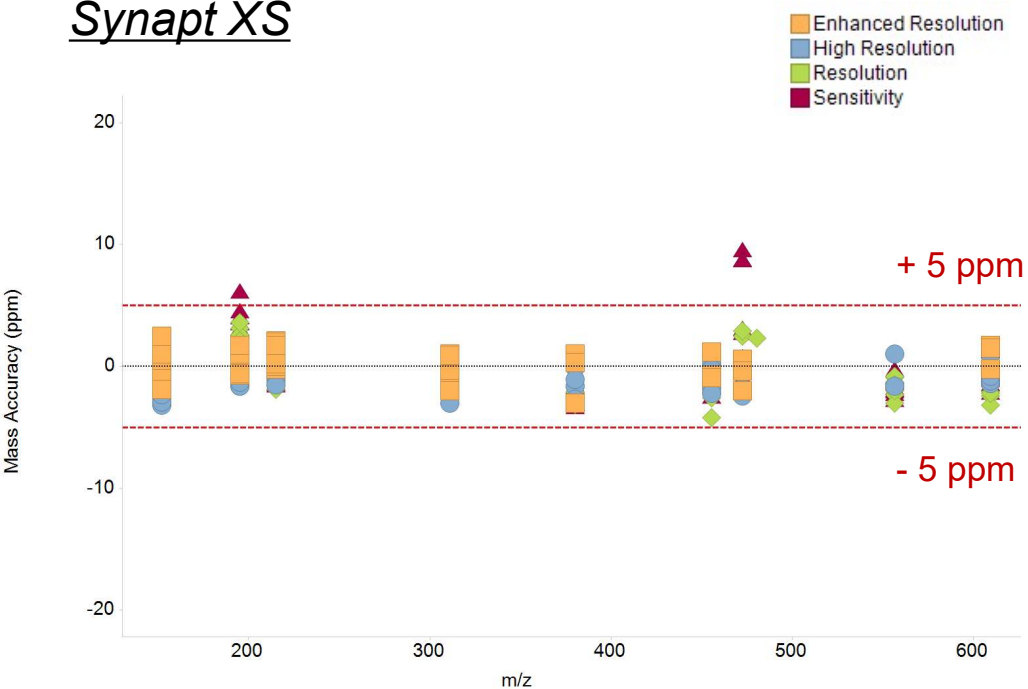## SYNAPT XS vs. SYNAPT G2-Si - Positive
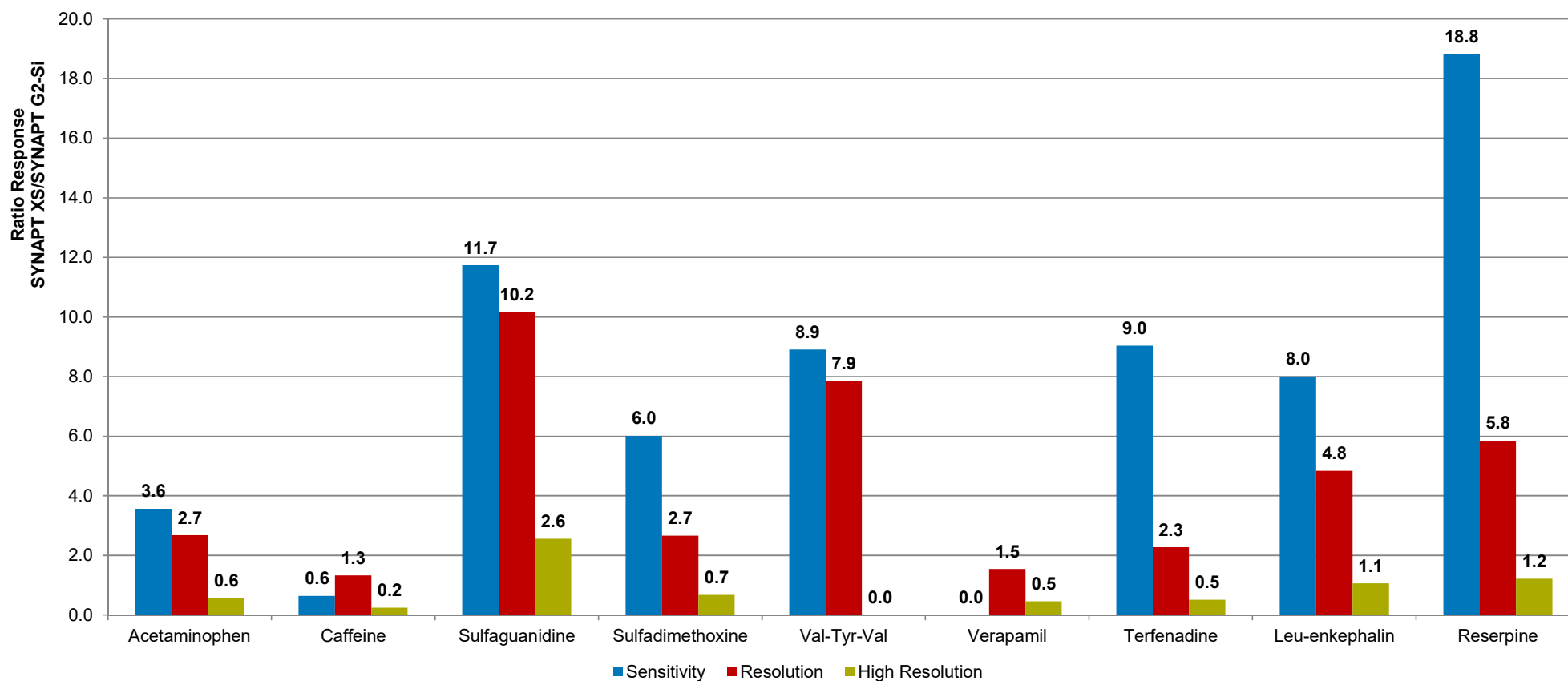
Mass Accuracy
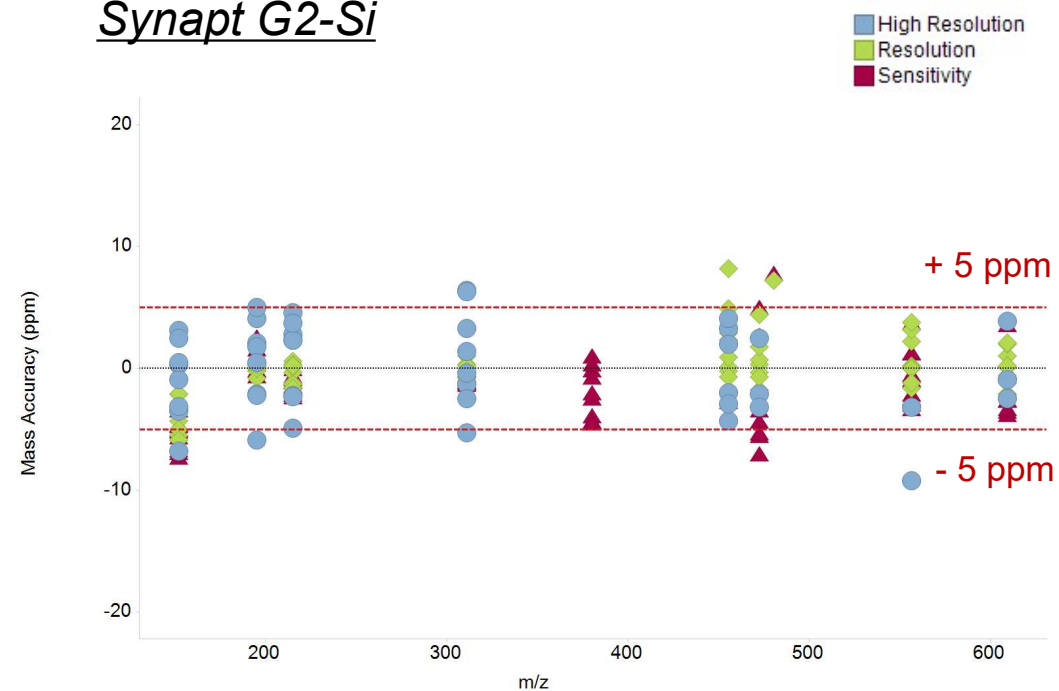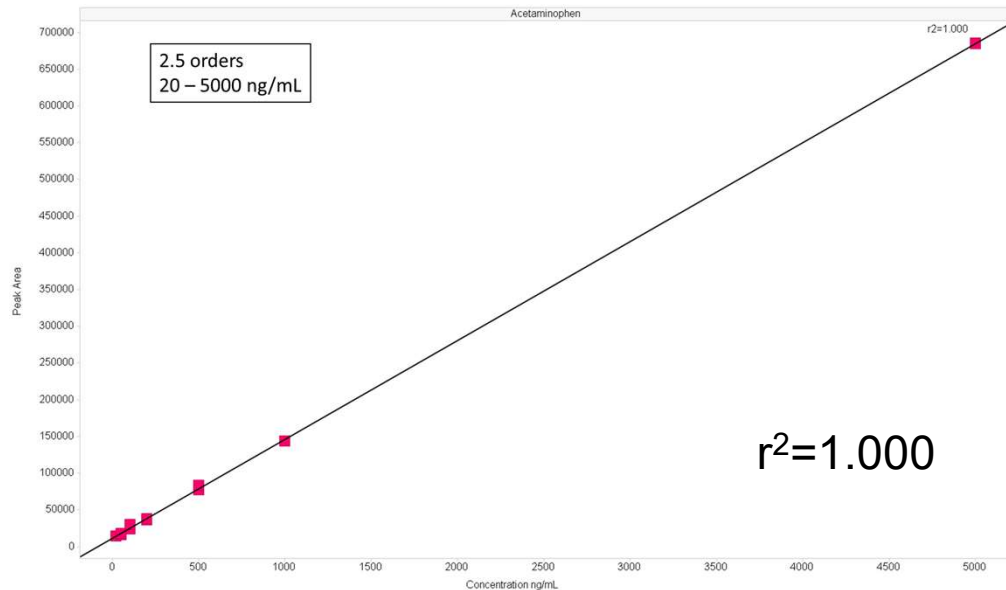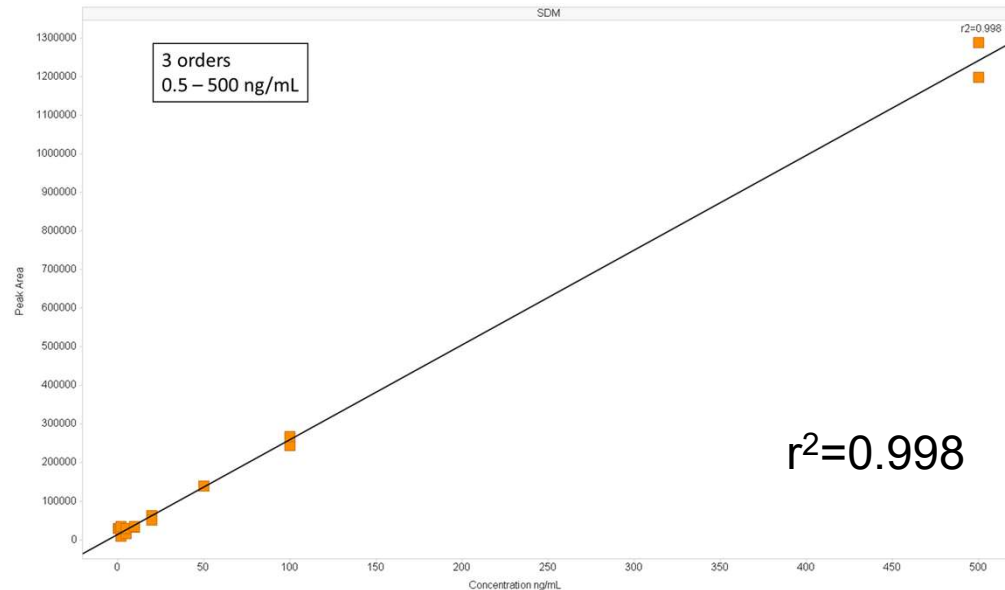## LC-MS QC Mix compounds – Positive ion
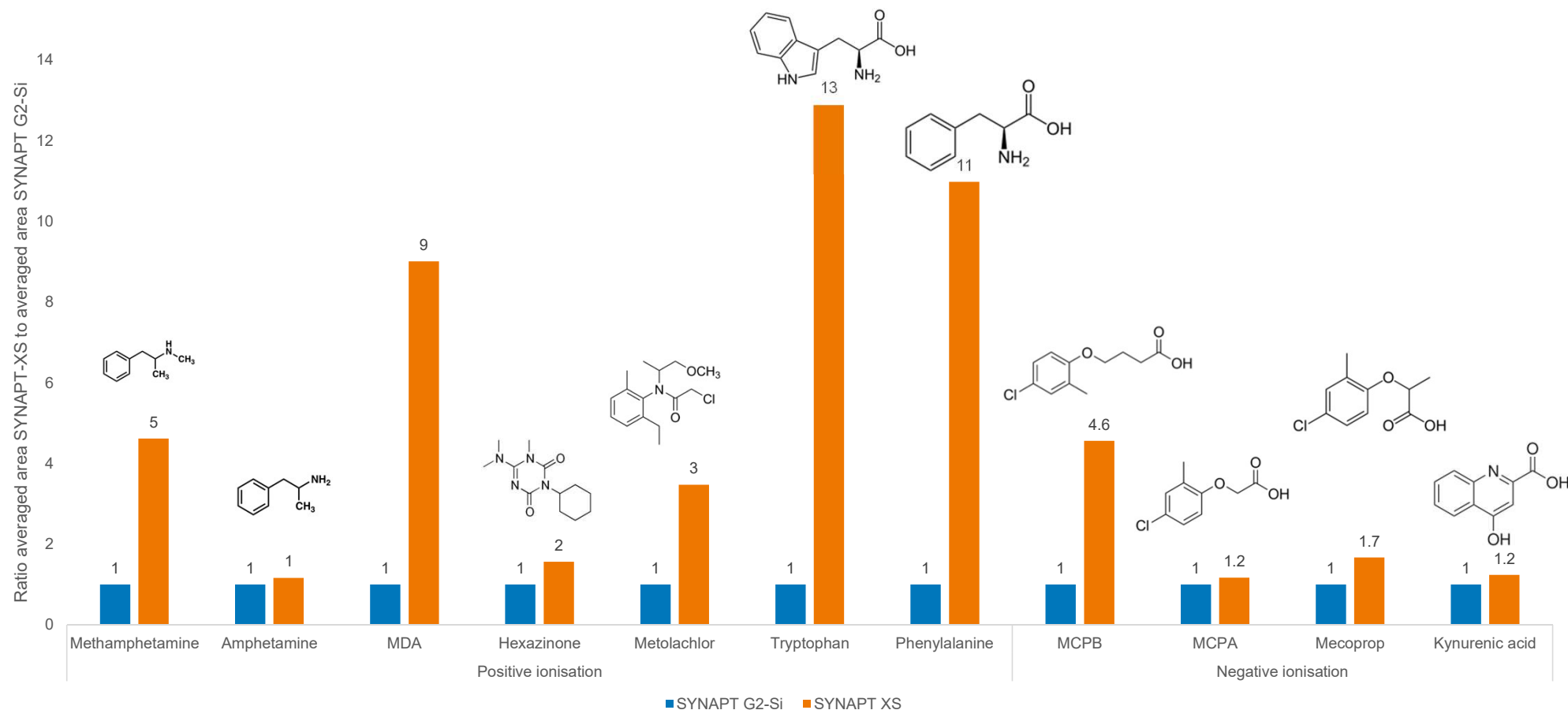
Dynamic Range In MS^e Mode

Acetaminophen

SDM

Transmission of labile species

Increase of sensitivity for labile compounds on the SYNAPT XS
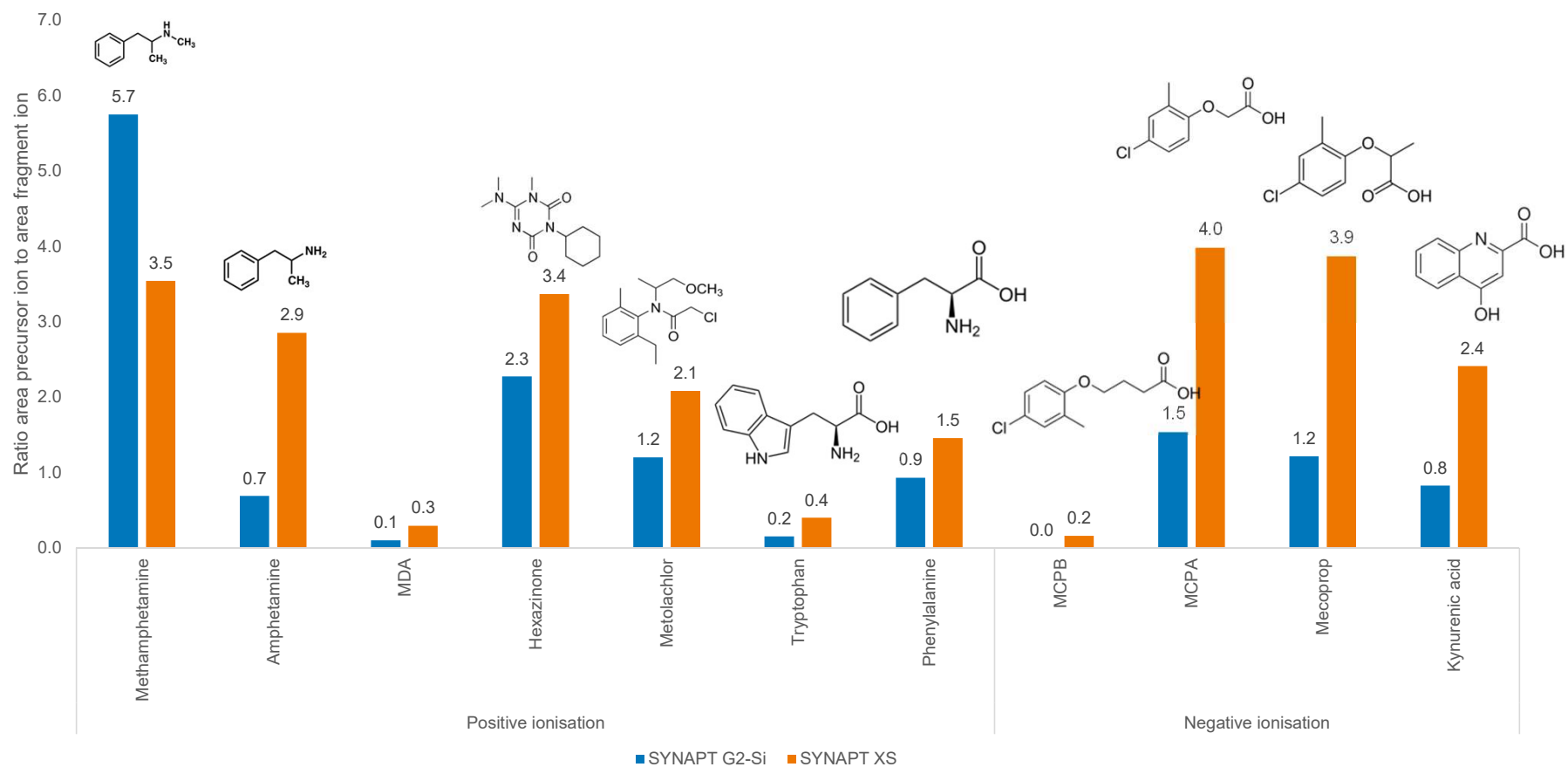## Sensitivity SYNAPT XS vs. SYNAPT G2-*Si*

Decreased fragmentation of labile compounds on the SYNAPT-XS

Ratio area precursor ion to area fragment ion on the SYNAPT XS vs. SYNAPT G2-*Si*

Example chromatograms
Precursor ion area vs. fragment ion area on the SYNAPT XS and SYNAPT G2-*Si*

Amphetamine

SYNAPT XS
Ratio= 3

SYNAPT G2-*Si*
Ratio= 0.7
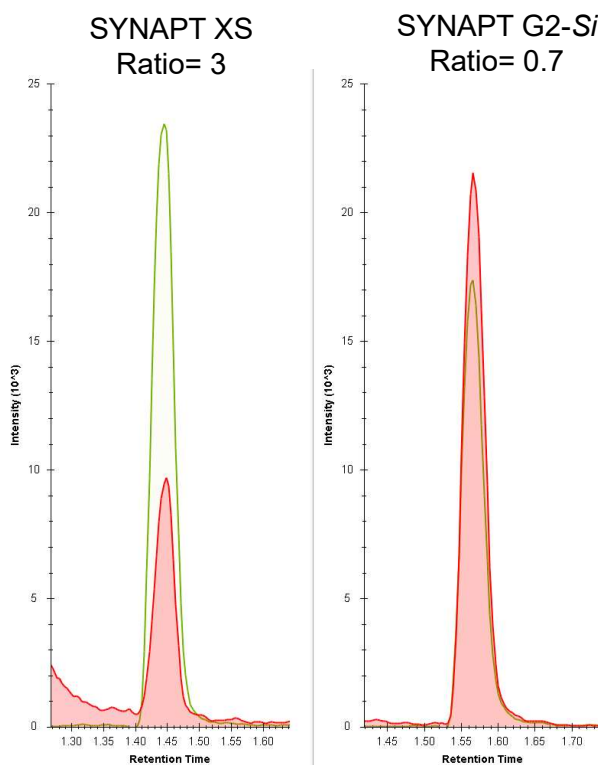
Fragment (*m/z* = 91.052)
Precursor (*m/z* = 136.1121)

Metolachlor

SYNAPT XS
Ratio= 2

SYNAPT G2-*Si*
Ratio= 1.2
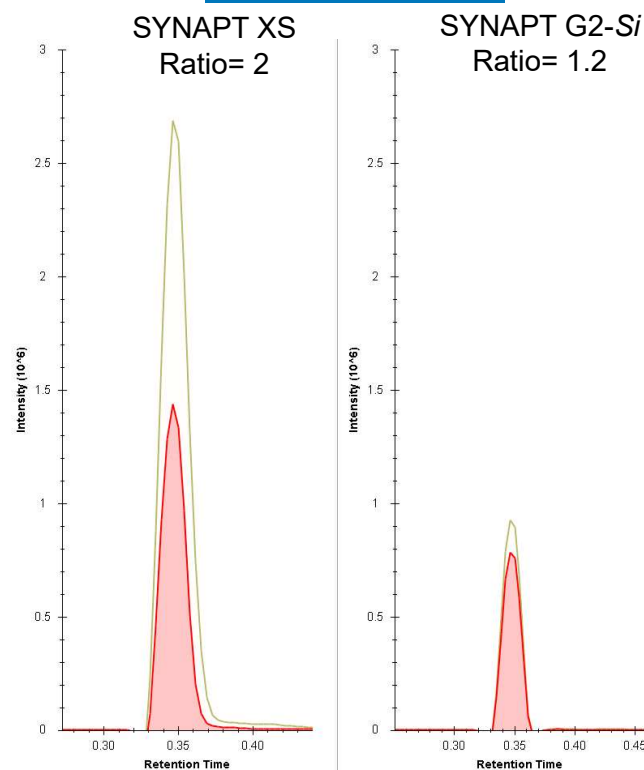
Fragment (*m/z* = 252.1150)
Precursor (*m/z* = 284.1412)

Phenylalanine

SYNAPT XS
Ratio= 1.5

SYNAPT G2-*Si*
Ratio= 0.9
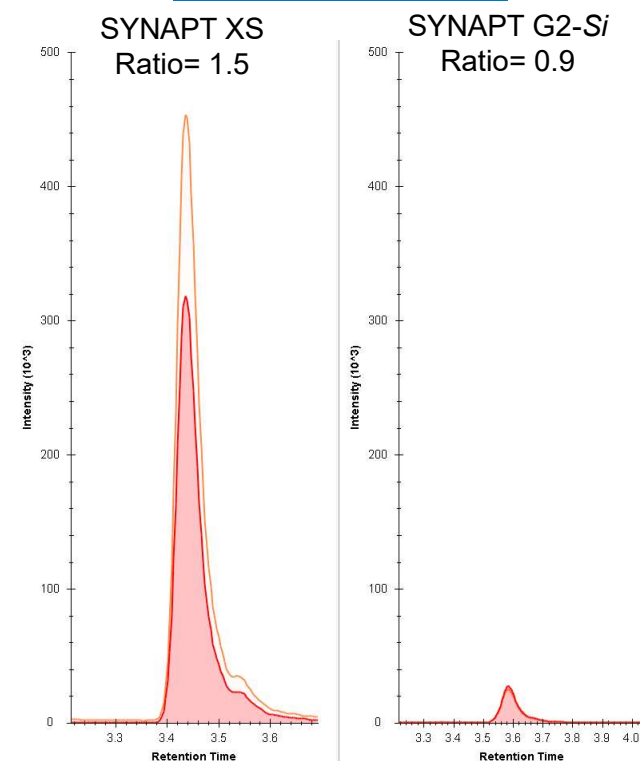
Fragment (*m/z* = 120.0808)
Precursor (*m/z* = 166.0863)

Example labile compounds
Positive ionisation

Tryptophan

SYNAPT G2-Si Ratio P/F= 0.07	SYNAPT G2 XS Ratio P/F=0.3

Kynurenic acid

SYNAPT G2-Si Ratio P/F= 12	SYNAPT G2 XS Ratio P/F=18
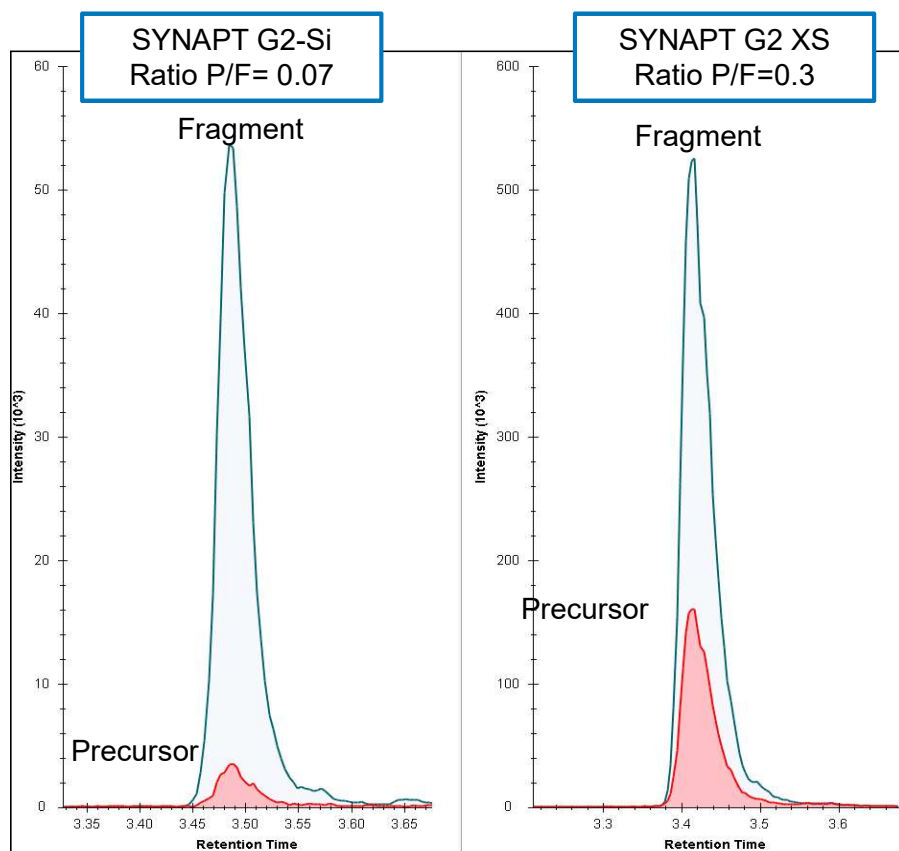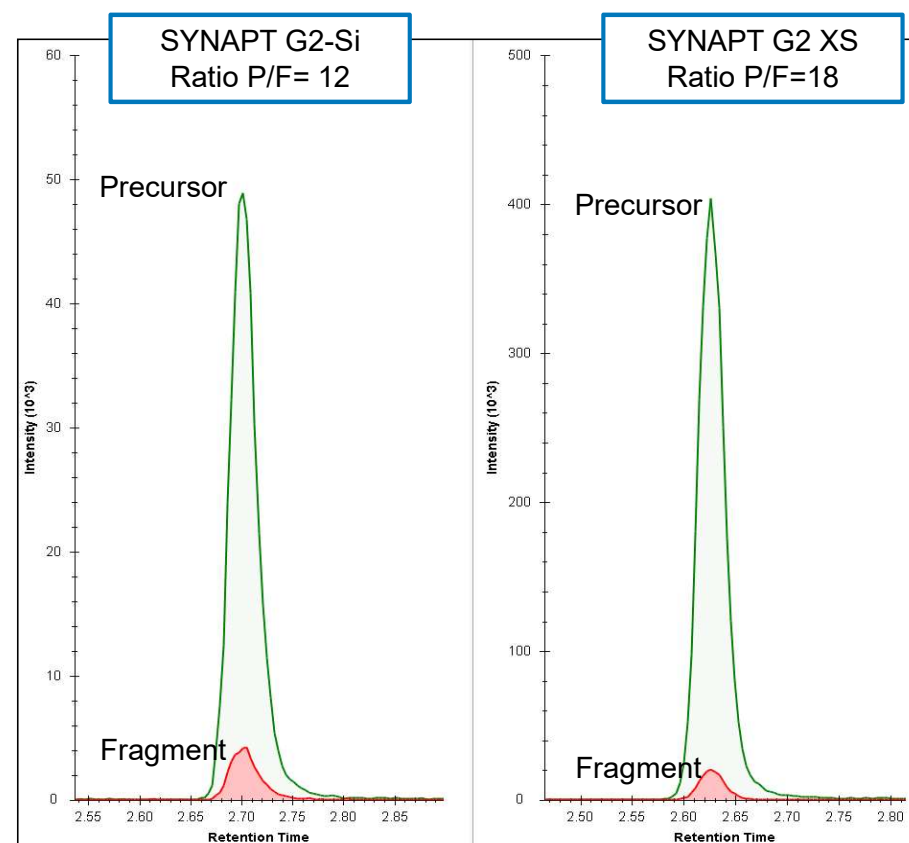
Intelligent Data Capture Phase II

Item name: Sample 16
Channel name: 2: HD TOF MSe (50-1200) 6eV ESI+

A: Without IDC

Item name: Sample 20
Channel name: 2: HD TOF MSe (50-1200) 6eV ESI+

B: With IDC

Up to 90% File Size Reduction

SYNAPT XS & Proteomics

SYNAPT XS: Proteomics
K562 Protein Identifications (25, 50, 100 & 150ng loadings; sensitivity & resolution mode)

Average values calculated on the basis of duplicate technical replicates
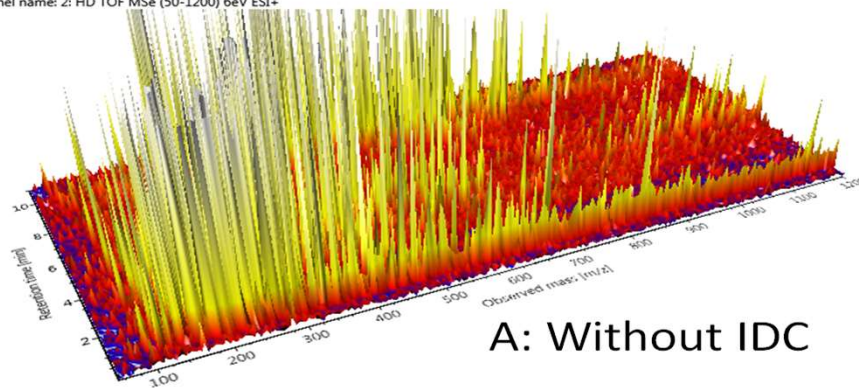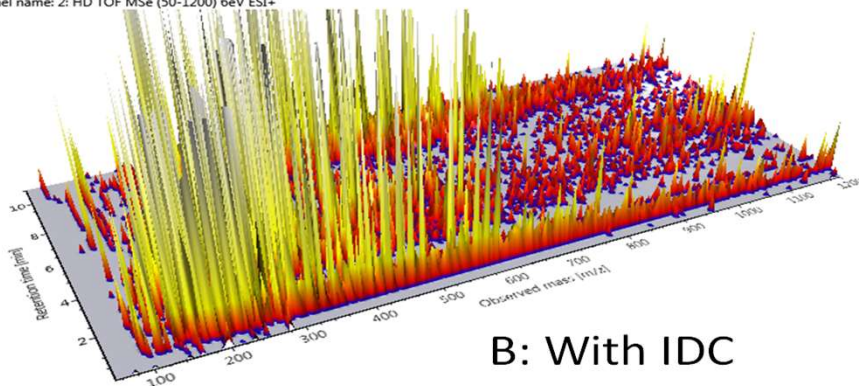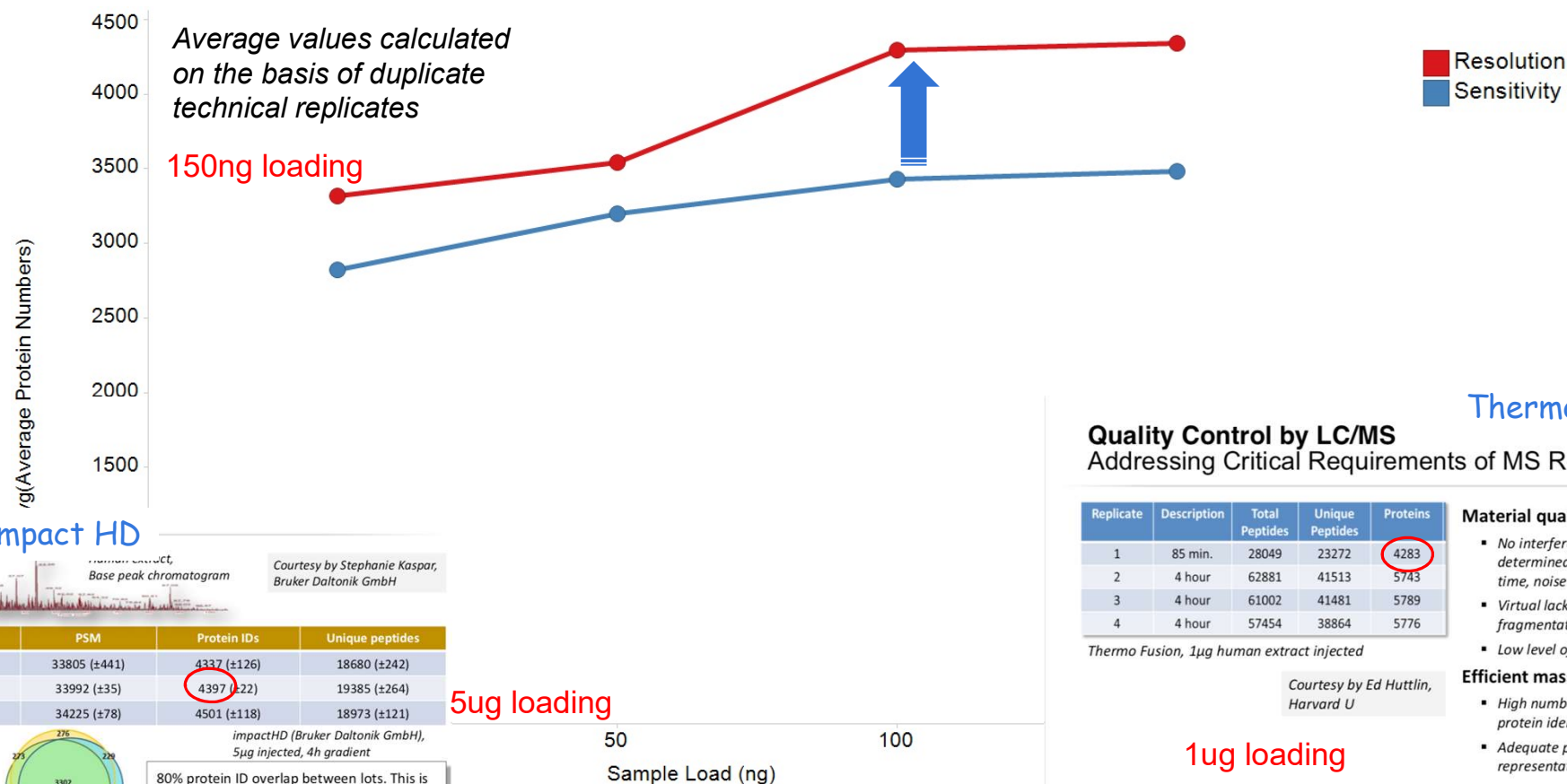
150ng loading

Resolution
Sensitivity

Bruker Impact HD

Thermo Fusion

Quality Control by LC/MS
Addressing Critical Requirements of MS Ref. Material

Replicate	Description	Total Peptides	Unique Peptides	Proteins
1	85 min.	28049	23272	4283
2	4 hour	62881	41513	5743
3	4 hour	61002	41481	5789
4	4 hour	57454	38864	5776

Thermo Fusion, 1µg human extract injected

Courtesy by Ed Huttlin, Harvard U

Material quality
- *No interference with LC/MS as determined by peptide retention time, noise and signal intensity*
- *Virtual lack of protein fragmentation*
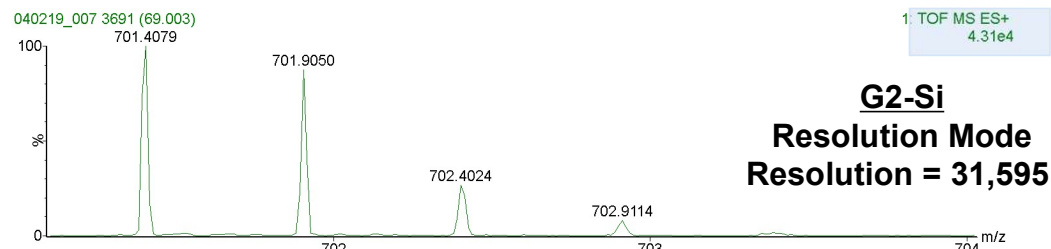- *Low level of non-biological PTMs*

Efficient mass spec analysis
- *High number of peptide and protein identifications*
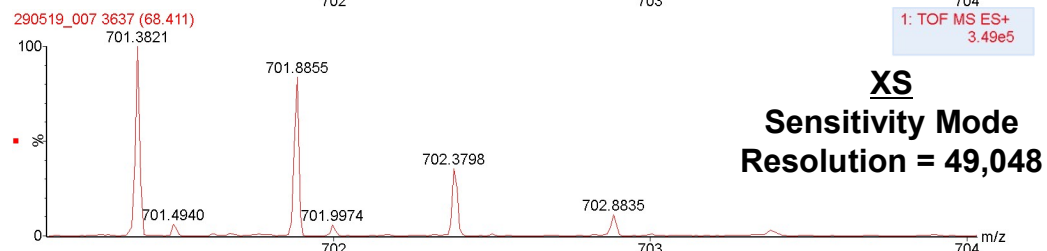- *Adequate proteome representation*

1ug loading

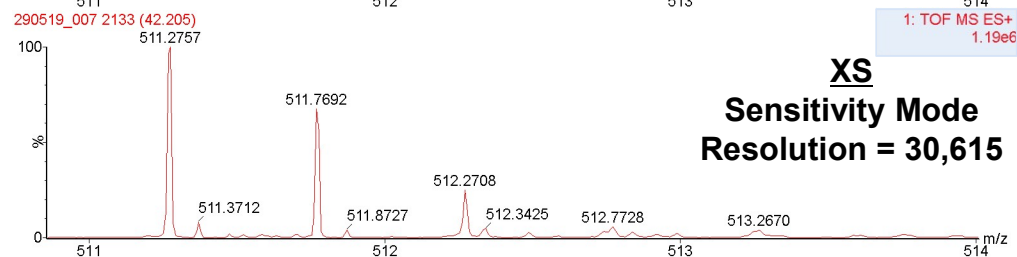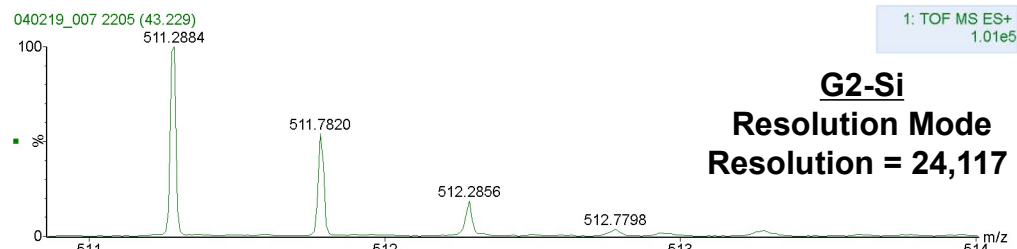*Deeper proteome coverage can be achieved with a topline instrument and longer run.*

Human extract,
Base peak chromatogram

Courtesy by Stephanie Kaspar, Bruker Daltonik GmbH

Human extract lot	PSM	Protein IDs	Unique peptides
Lot A	33805 (±441)	4337 (±126)	18680 (±242)
Lot B	33992 (±35)	4397 (±22)	19385 (±264)
Lot C	34225 (±78)	4501 (±118)	18973 (±121)

impactHD (Bruker Daltonik GmbH), 5µg injected, 4h gradient

5ug loading

Protein ID overlap between three human extract lots
Venn diagram
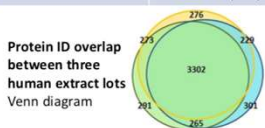
80% protein ID overlap between lots. This is comparable to the protein overlaps between technical runs.

Sample Load (ng)

Example of increased sensitivity (100ng Ecoli)

G2-Si
Resolution Mode
Resolution = 31,595

8x increased sensitivity

XS
Sensitivity Mode
Resolution = 49,048

G2-Si
Resolution Mode
Resolution = 24,117

12x increased sensitivity

XS
Sensitivity Mode
Resolution = 30,615

Protein Identifications – 100ng K562; _Resolution Mode_
(G2-Si vs. XS; duplicate injections)

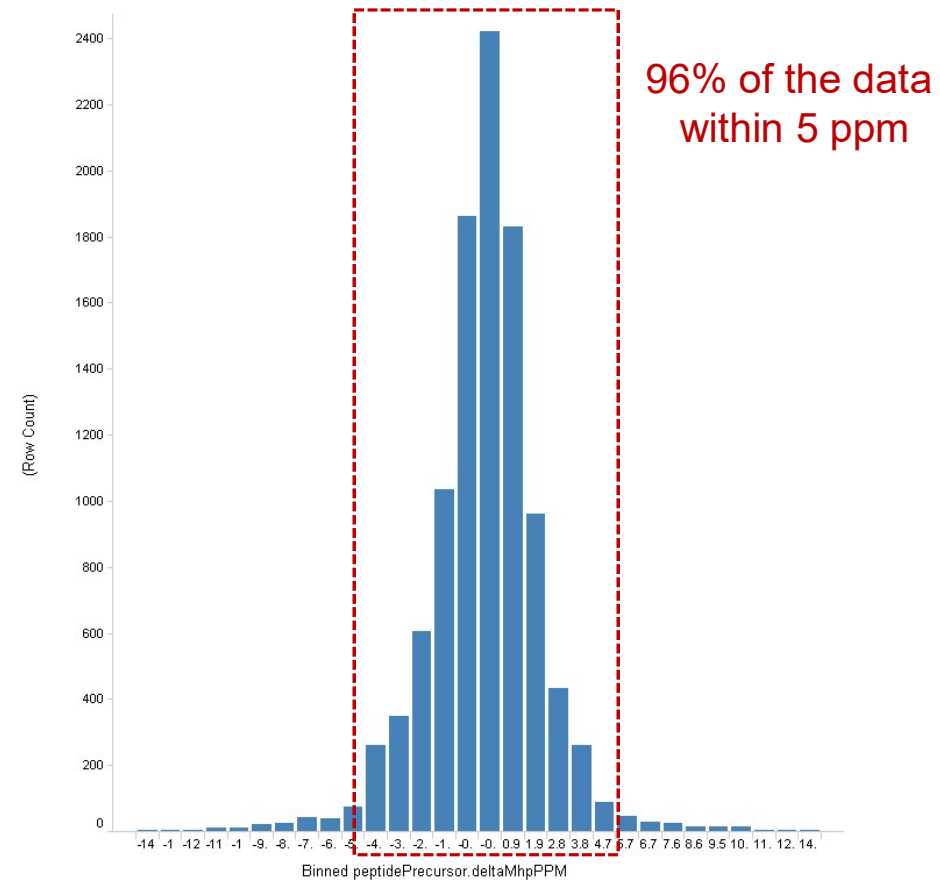24% increase in average protein numbers

Legend: G2-Si, XS
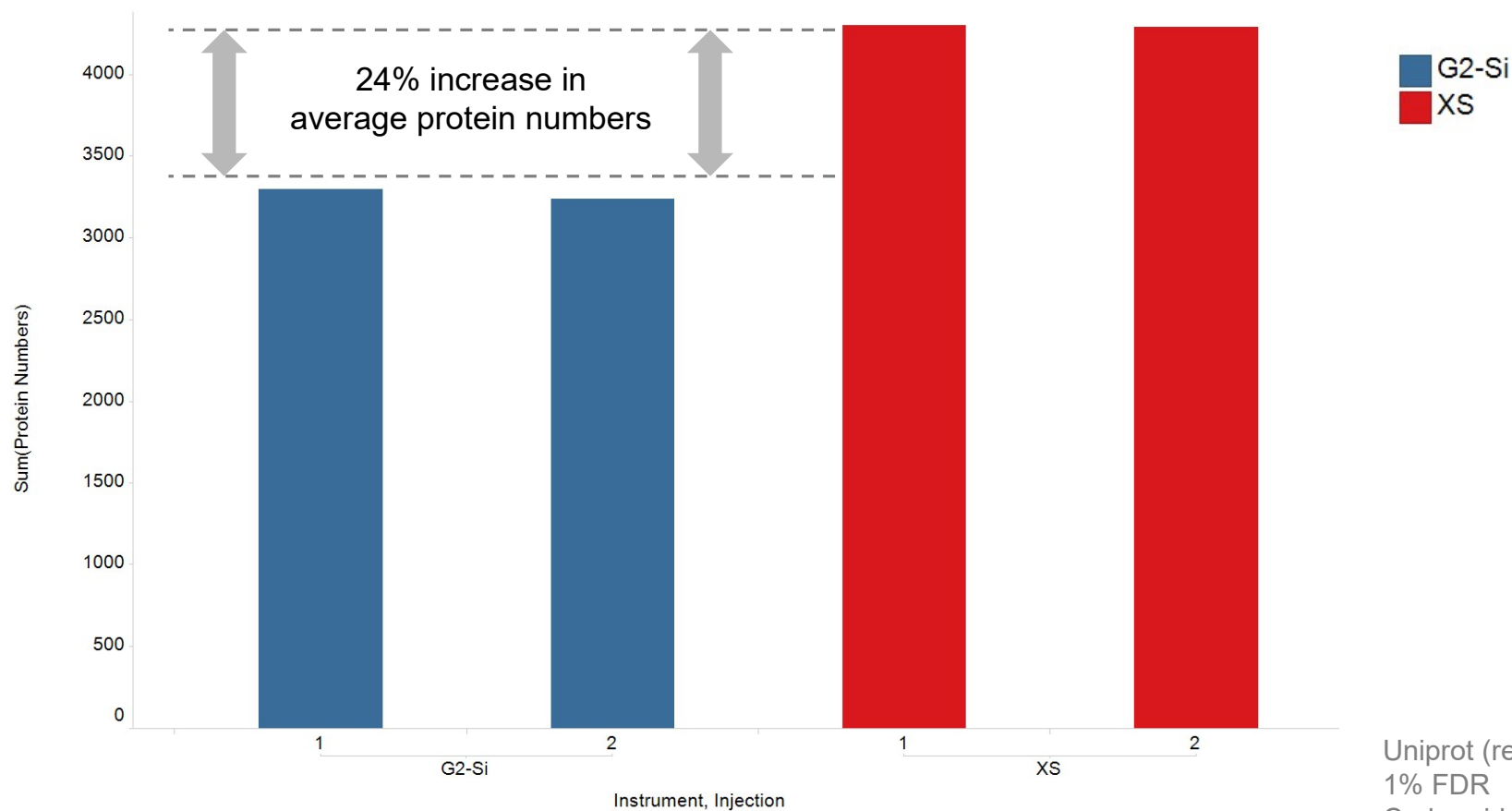
Uniprot (reviewed human entries)
1% FDR
Carbamidomethyl (C) – Fixed modification
Oxidation (M) – Variable modification

Proteome Dynamic Range & Mass Accuracy
(*E.coli*, sensitivity mode)
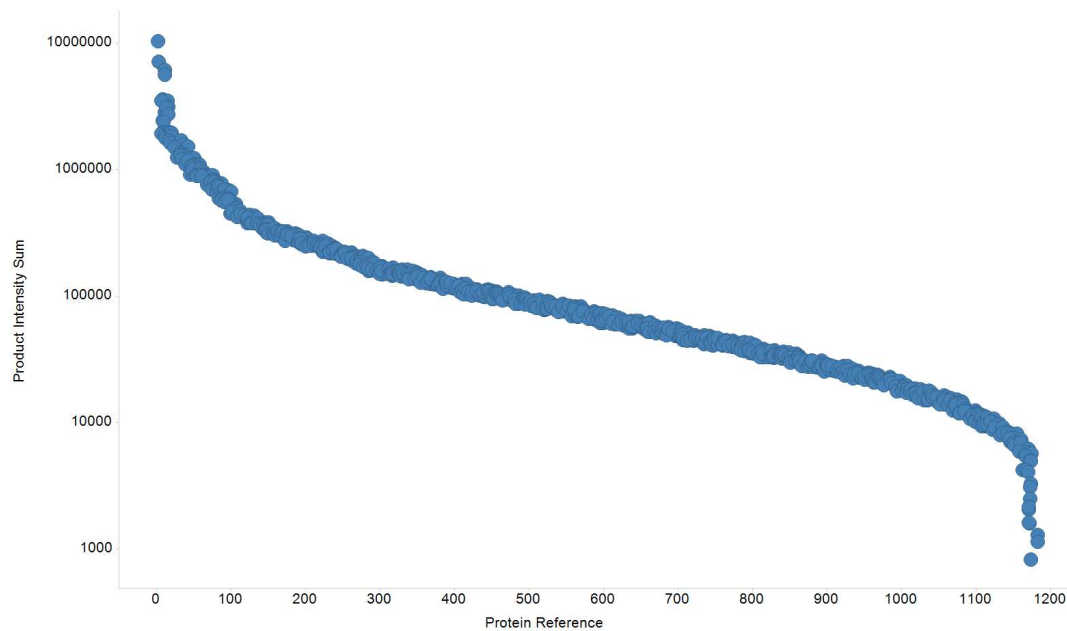
4-orders of dynamic range

96% of the data within 5 ppm
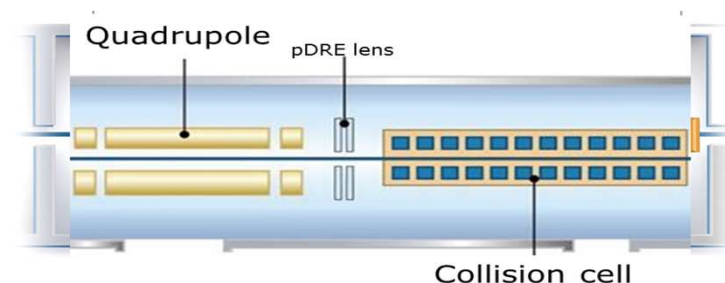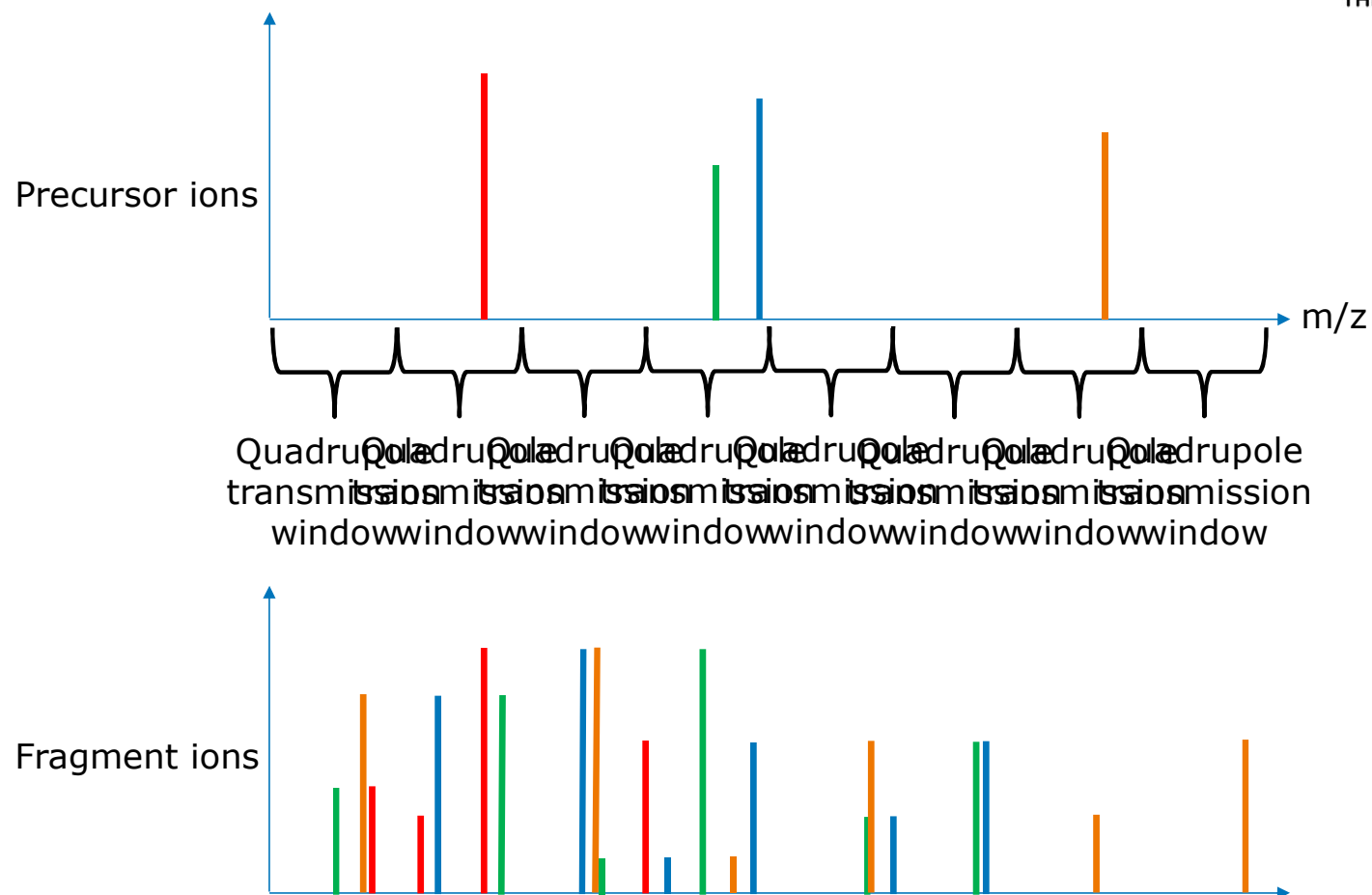
SONAR Enabled Proteomics

SONAR™ - an alternative DIA method
(introduced by Richardson et al. ASMS 2015)

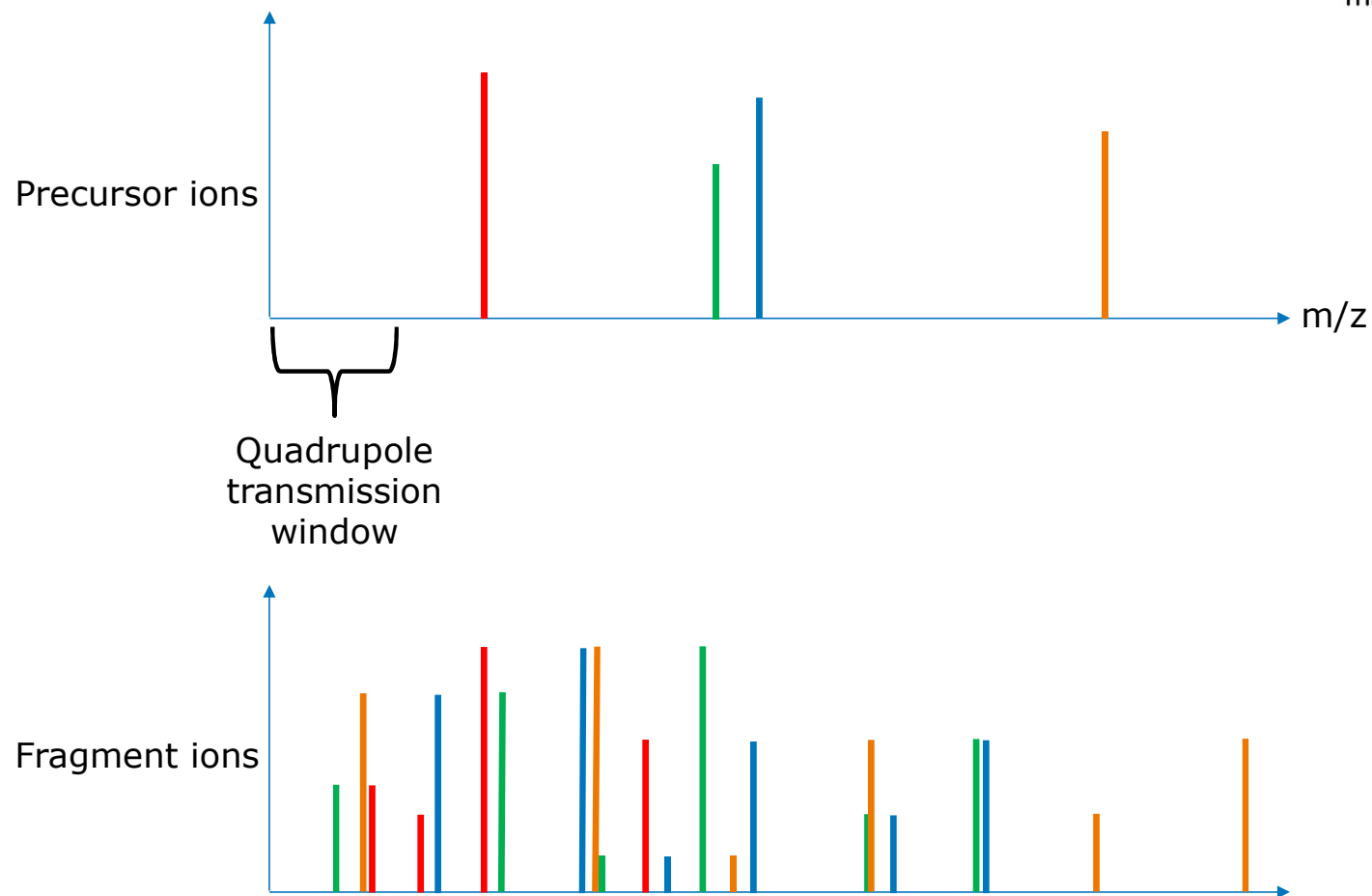The quadrupole is rapidly scanned across the m/z range of interest

- typically quadrupole m/z window
    - Proteomics (28 Da)
    - Small molecules (5-10 Da)

- alternating scans of low energy and high energy
    - qualitative and quantitative data for all precursors and all products

- rapid scanning electronics – repurposed from Ion Mobility Mass
  Spectrometers (SYNAPT)
    - samples 200 quadrupole positions per scan
    - typical scan times 0.1 to 0.5 sec (each energy level)
    - quadrupole can scan at >10,000 amu/sec; > 2000 spectra/second

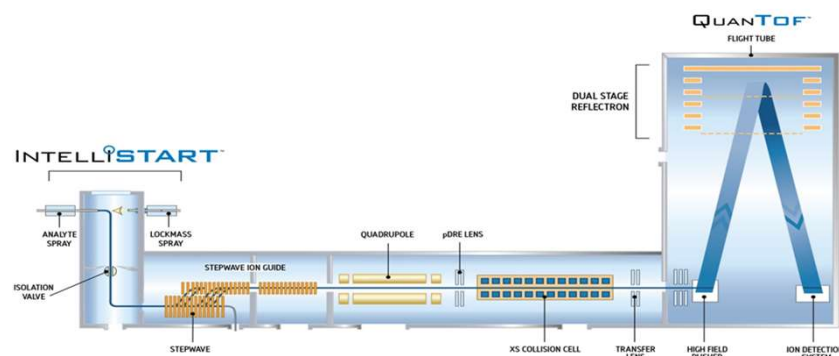- **compatible with high speed chromatographic
  and electrophoretic separations**

Quadrupole pDRE lens
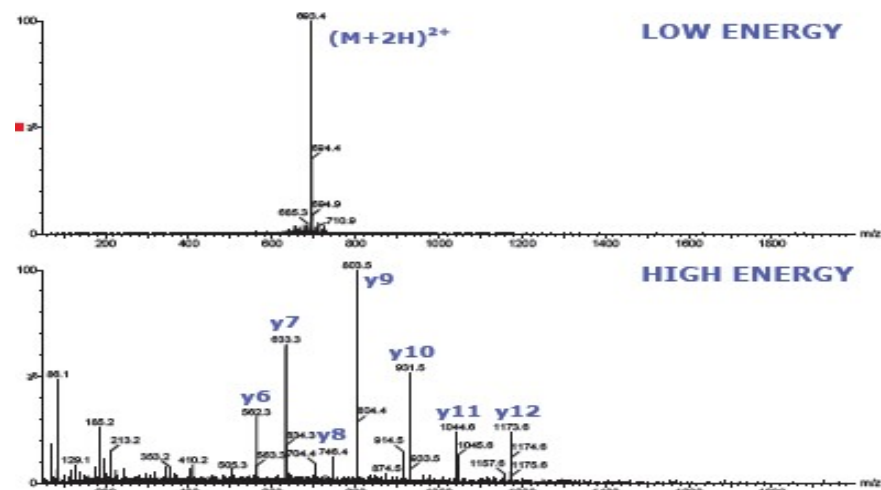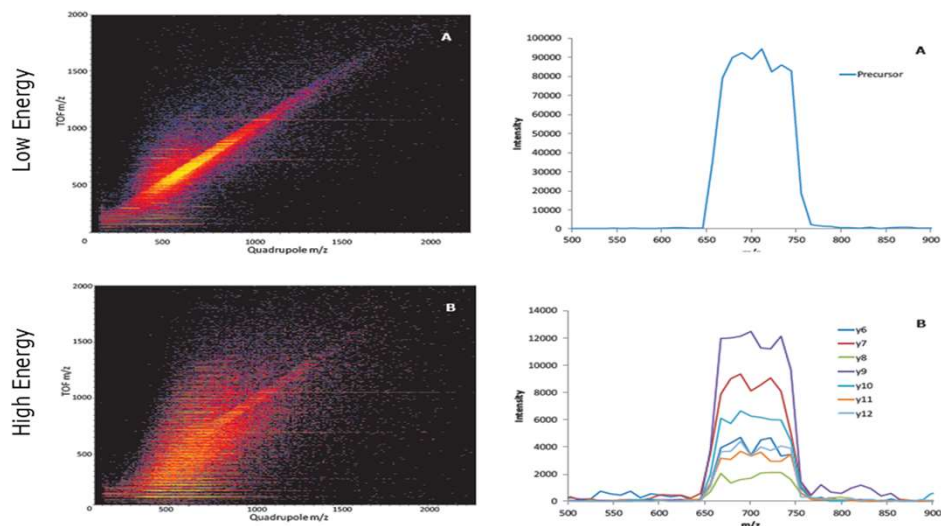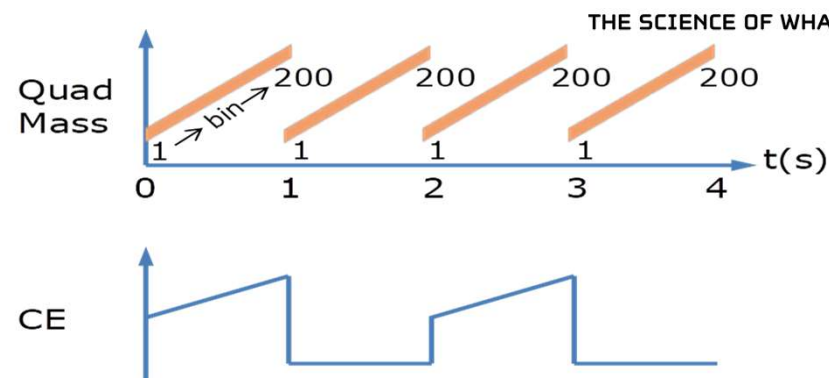
Collision cell

SWATH

Precursor ions

m/z

Quadrupole transmission window Quadrupole transmission window Quadrupole transmission window Quadrupole transmission window Quadrupole transmission window Quadrupole transmission window Quadrupole transmission window Quadrupole transmission window

Fragment ions

SONAR

Precursor ions

Quadrupole
transmission
window

Fragment ions

SONAR™ workflow/principle

SONAR Deliver Improved MS/MS Spectra

Simplified Spectra
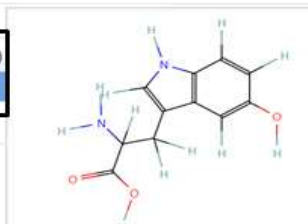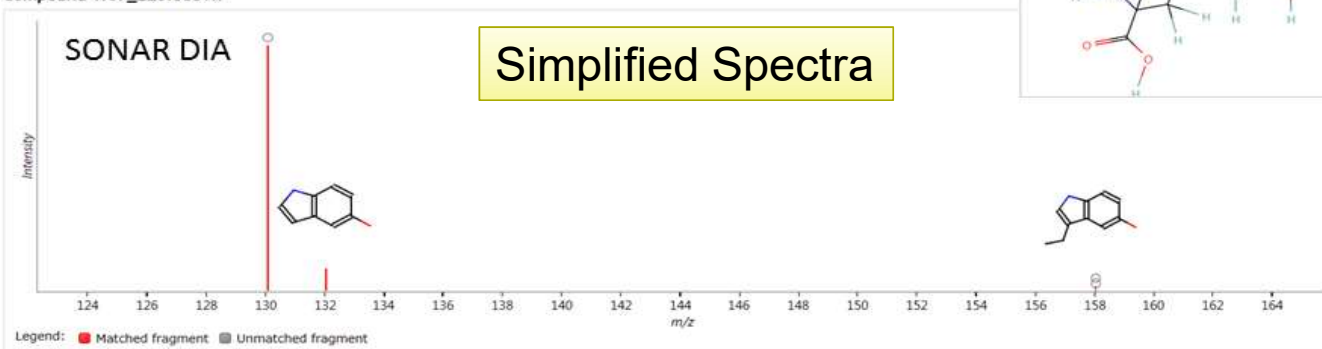
Improved Spectral Score

Selectivity Enhancements with SONAR - Proteomics

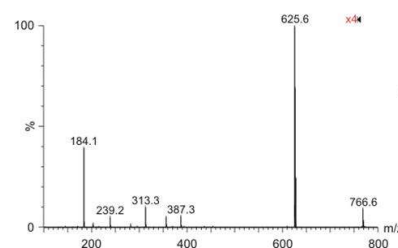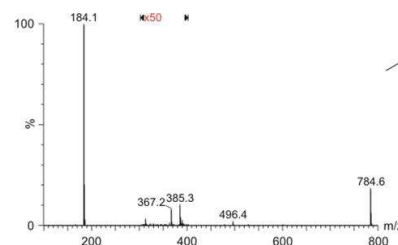

Low Energy

Elevated Energy
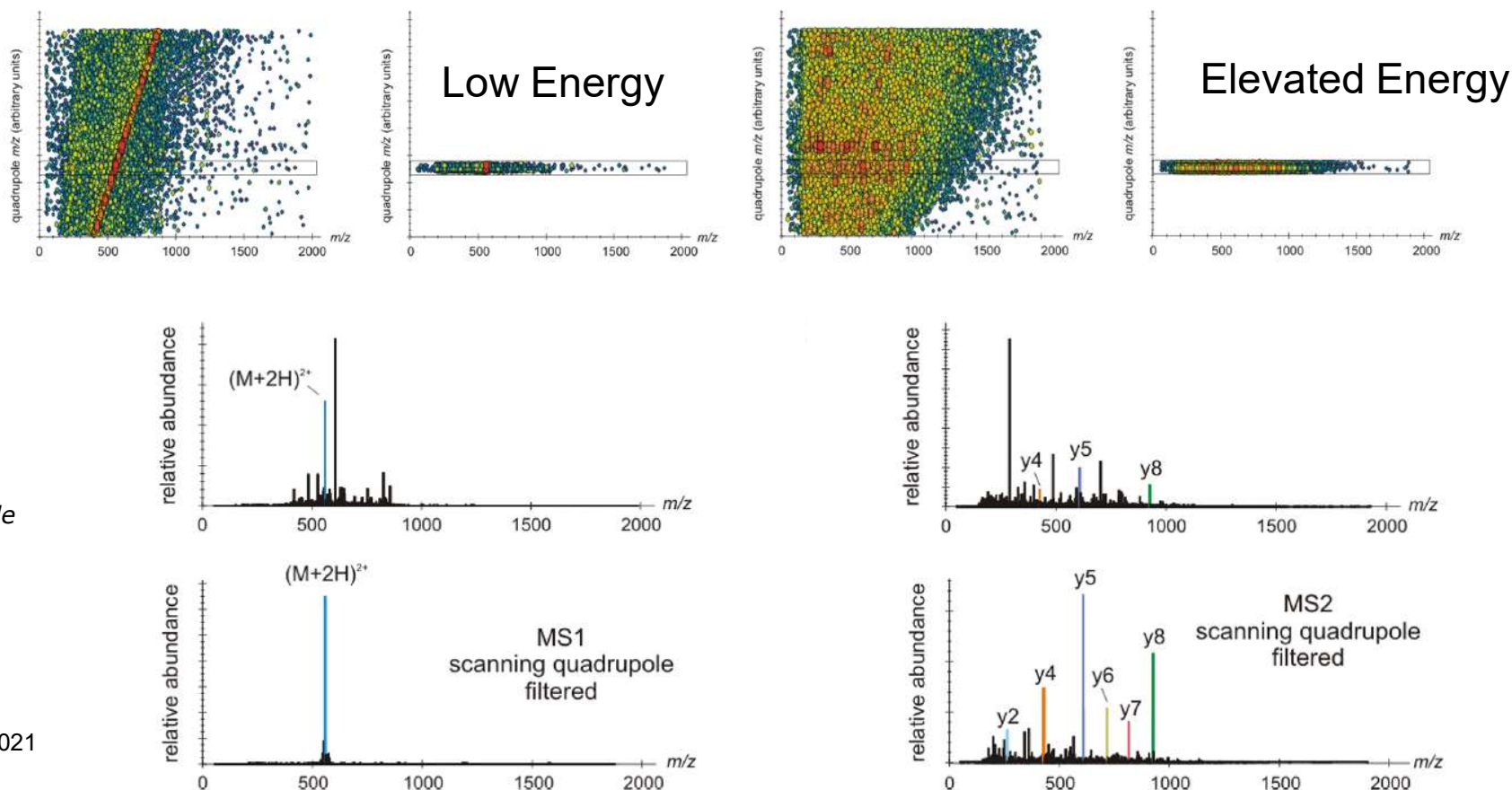
Scanning Quadrupole Data-Independent Acquisition, Part A: Qualitative and Quantitative Characterization MA Moseley et al, J Proteome Res. 2017 Dec 29. doi: 10.1021
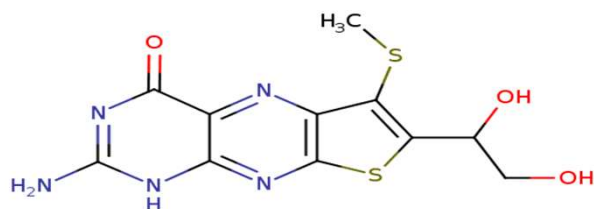
Selectivity Enhancements with SONAR – Small Molecules
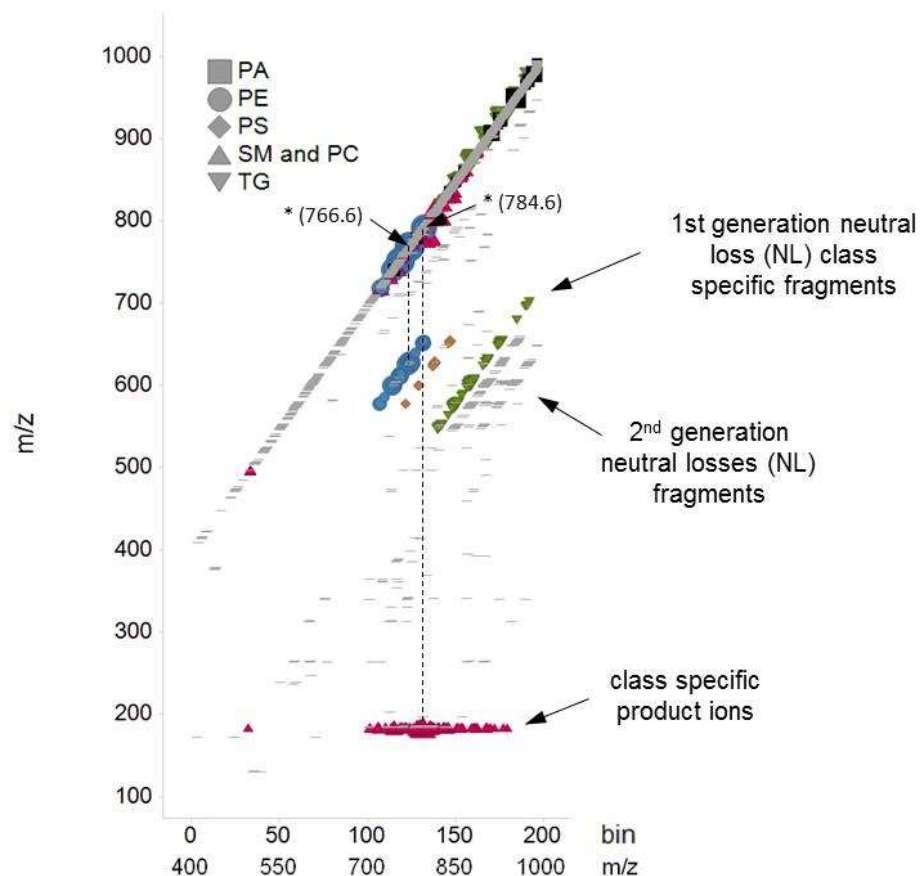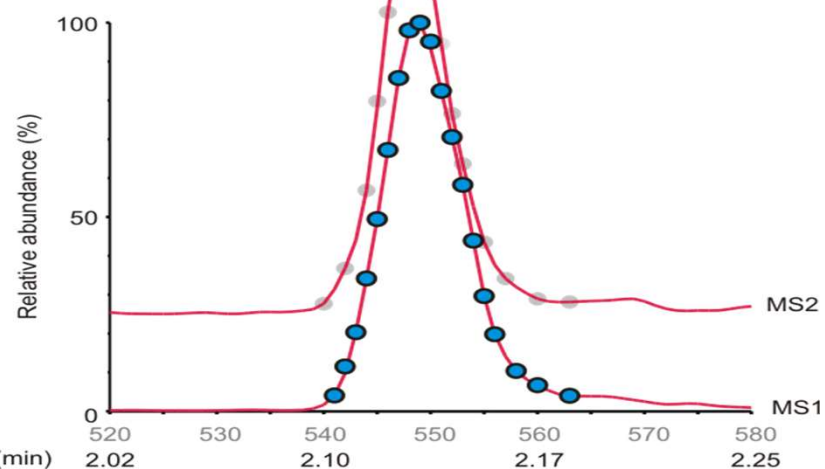

- searchable MS1 and MS2 data
- 'digital' precursor/product, neutral loss and product ion discovery maps
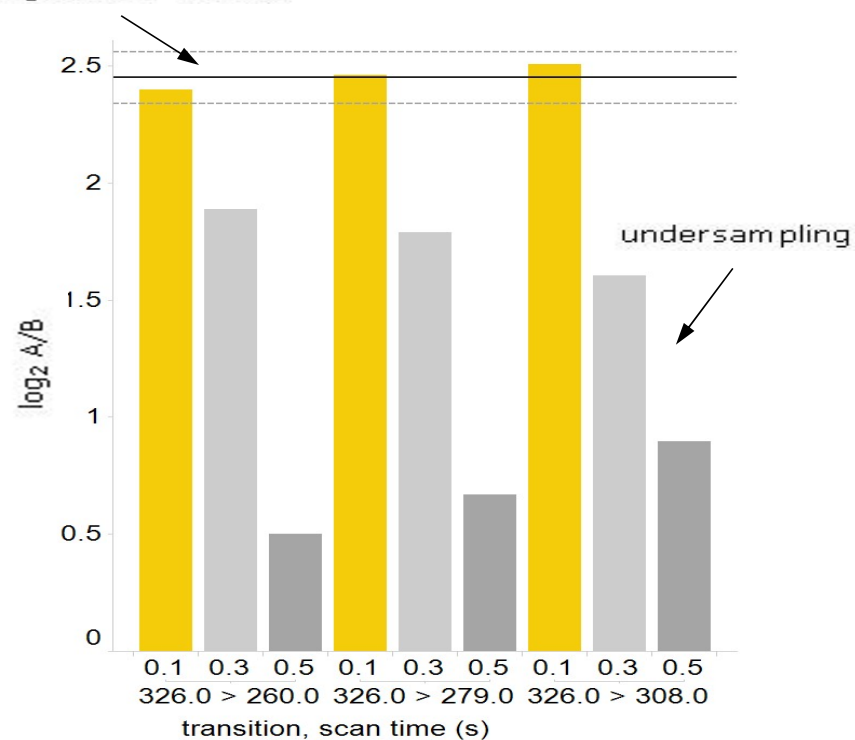
Quantitative precision *vs.* acquisition speed

1.7 s W$_{0.5}$
0.1 s scan (integration)
> 10 MS1 scans
> 10 MS2 scans

SONAR SPEED For Discovery Lipidomics/Metabolomics

- **RAMMP**
  - Rapid UPLC separation
  - Speed/high throughput
  - Quantitative precision accuracy
  - Large cohort analysis

1.7 s wide UPLC peak
100 ms SONAR scan
m/z 450 – 850
> 20 scans across peak

m/z 280.0327 Fragment

Elevated energy

m/z 326.0382 Precursor

Low energy

12 min

2.5 min

QC
Control
Low dose
High dose

Gray *et al.* Anal Chem 2016; 88(11):5742-51
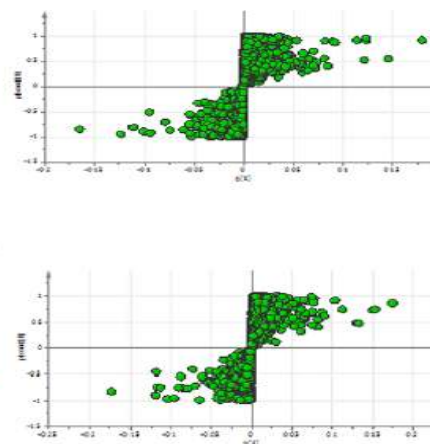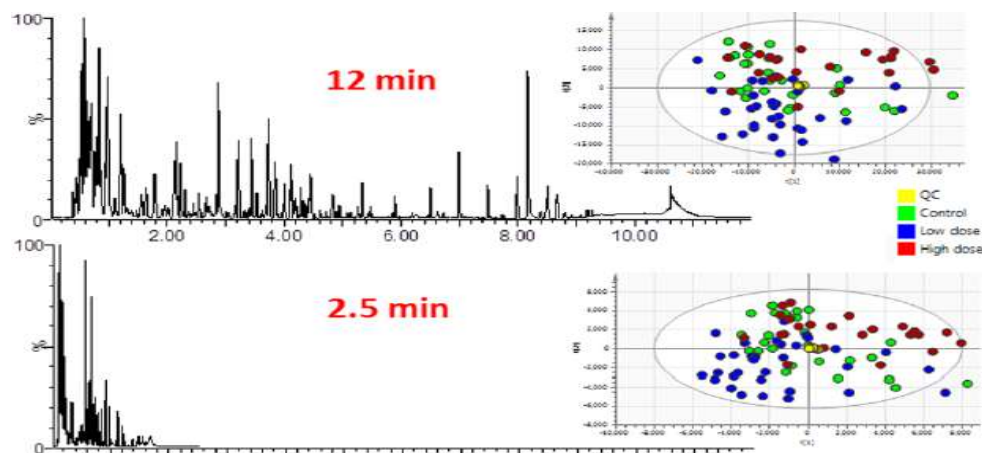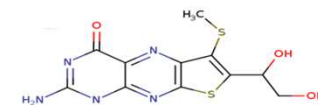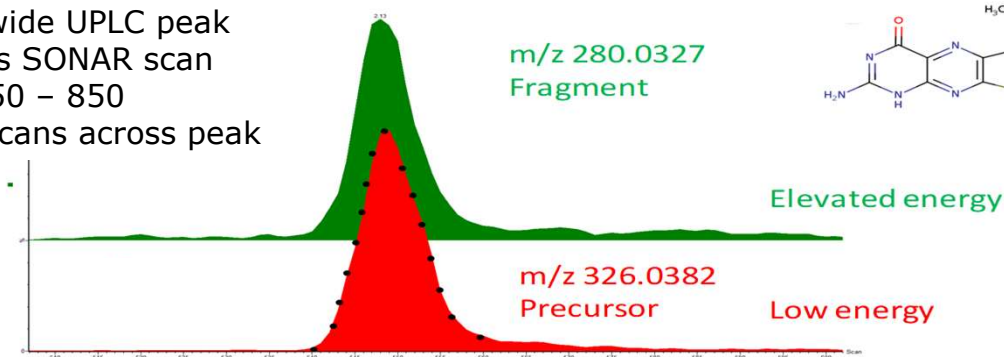
Comparative Results of SONAR and HDMS^E

	HDMS^E	MS^E	SONAR
Mass Range m/z	50-1000	50-1200	50-1200
Gradient length min	7	7	7
Loading per inj μL	3 (1:4)	3 (1:20)	3(1:4)
QI features	2890	3435	1184
Total identified features HMDB 3.0 5/10 ppm	1006	592	1097
Frag score Ellagic acid	53.2 -2.57ppm	38.1 -3.85ppm	92.8 -1.85ppm
Frag score chlorogenic acid	98.1 -1.79ppm	52.8 -2.25ppm	98.1 -0.04ppm
Frag score quercetin	84.1 -1.30ppm	20.7 -1.10ppm	92.1 -0.32ppm

[APPLICATION NOTE]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

A Non-Targeted Metabolomic Study of Pomegranate Juice to Investigate the Nutritional and Quality Characteristics Using Novel SONAR DIA Acquisition and Vion Ion Mobility QTof MS

Sara Stead and Joanne Connolly
Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS
Benefits of SONAR for the application of fruit juice metabolomic profiling for quality control and authenticity purposes include:

- Cleaner product ion spectra compared to other DIA methods
- High spectra/second scan rate compatible with HRMS
- Increased confidence in identification through selectivity
- Cataloging of a complex food sample within a single experiment
- Unbiased data acquisition within a targeted mass range

WATERS SOLUTIONS
ACQUITY™ UPLC™ I-Class System
Xevo™ G2-XS QTof
Vion™ IMS QTof Mass Spectrometer
Progenesis™ QI Software
SONAR™ Data Independent Acquisition Mode

KEYWORDS
Metabolomics, foodomics, food profiling, food authenticity, SONAR, MS/MS data acquisition

INTRODUCTION
Comprehensive identification of phytoactive compounds is a critical starting point for assessing the biological and technological properties in food matrices. Due to the complexity of plant secondary metabolism the full characterization of phytochemicals in fruits and vegetables is recognized as a significant analytical challenge and requires sensitive and accurate techniques to be employed. Pomegranate fruit (Punica granatum L.) is commonly reported as a rich dietary source of phenolic compounds with regular consumption being linked to a wide range of associated health benefits. Phenolic compounds are also known to play an important role in the quality and sensorial performance of fruit juice products and as such of value to the food industry.[1]

In this application note, we report the potential of a new data independent acquisition (DIA) mode (SONAR) on a QTof instrument in combination with a scanning quadrupole mass filter and ultra-fast detection system.[2-3] This methodology along with Vion ion mobility enabled QTof-MS (IM-QTof-MS) were used as tools to improve analytical selectivity and facilitate the process of marker identification in complex juice samples following a simple sample preparation step, as illustrated in Figure 1. The resulting information was further subjected to database searching which indicates the presence of several significant polyphenolic compounds and processing additives in a selection of commercially available processed juice products in the UK.
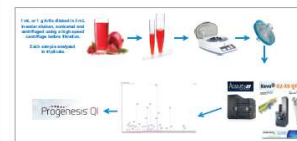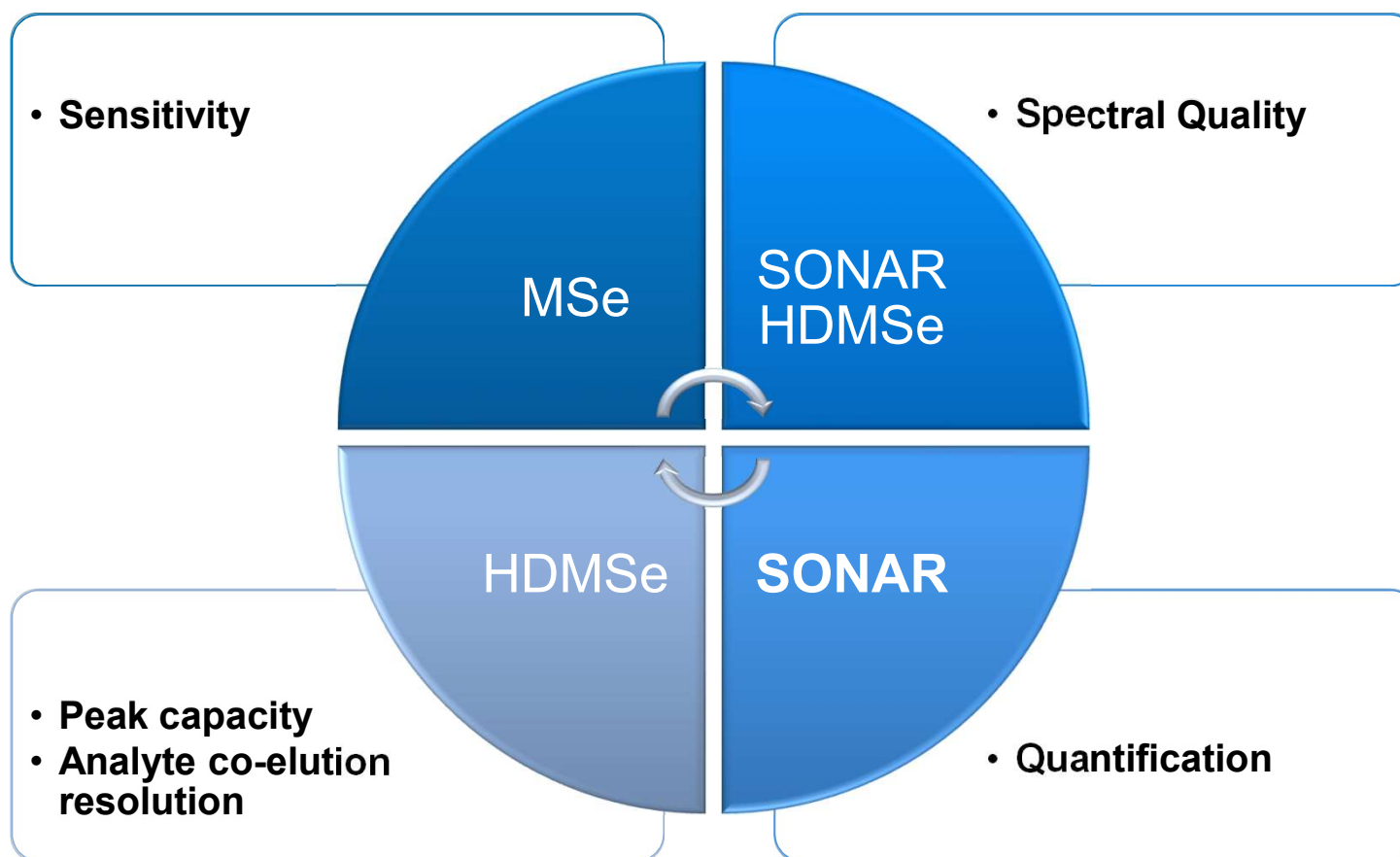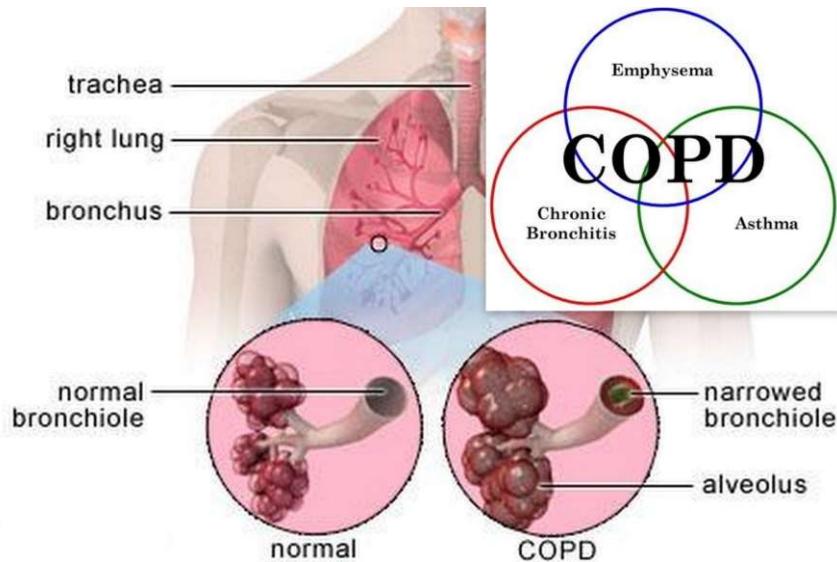
Figure I. Overall workflow schematic.

MS^e – SONAR - HDMS^e

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- **Sensitivity**

- **Spectral Quality**

MSe

SONAR
HDMSe

HDMSe

SONAR

- **Peak capacity**
- **Analyte co-elution resolution**

- Quantification
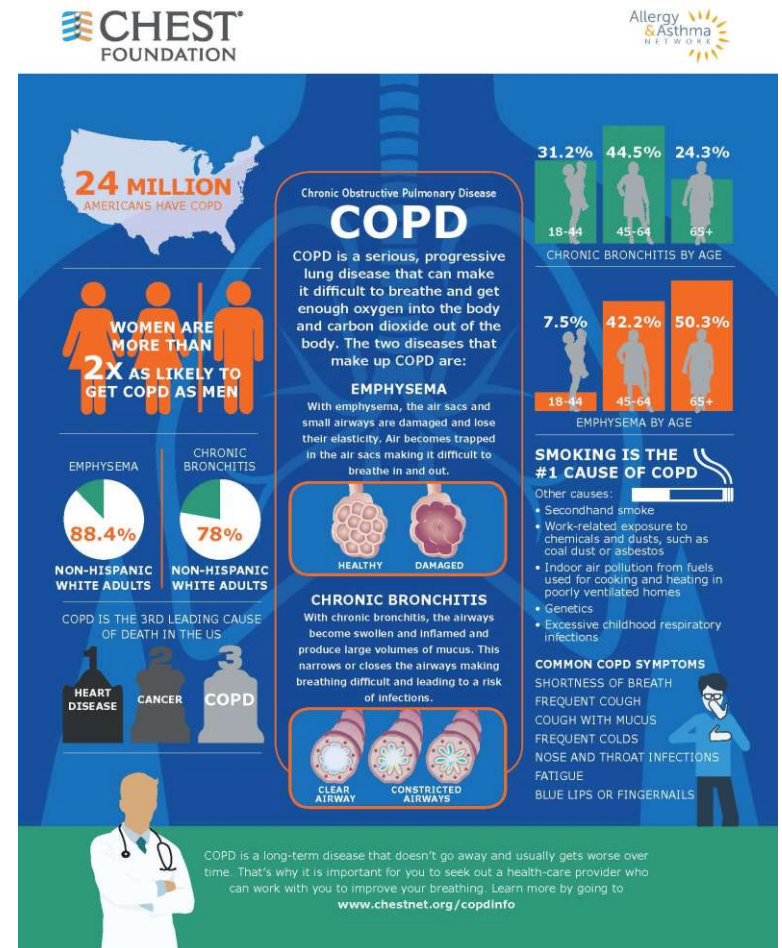
COPD and Asthma Case Study
Multi-Omic, single platform strategy

- COPD is 3rd leading cause of death in US
- Qualitative/Quantitative analysis of the proteome and lipidome

SONAR Proteomic Workflow with Spectronaut & Spectral library building

Differential Analysis Sequence db & Spectral Library Search

Search Results & Spectral Library output .mzIdentML

RAW Data

Samples + Biognosys iRT peptides or PQ500 Ref Peptides

Spectronaut™ Pulsar X

HRM-MS™ & Spectral Library Searching
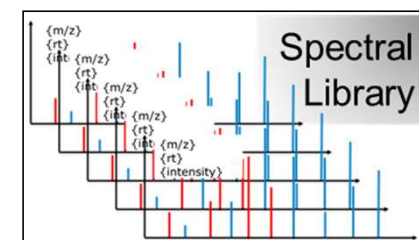
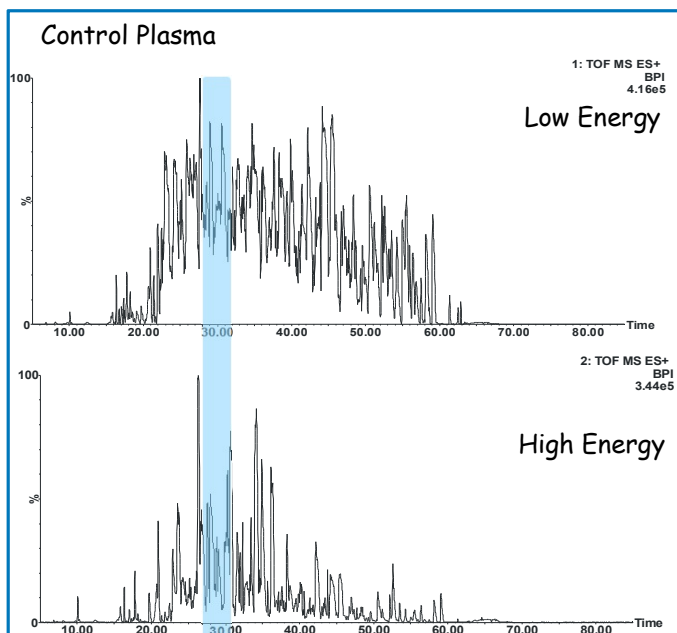# High throughput – 1mm chromatography
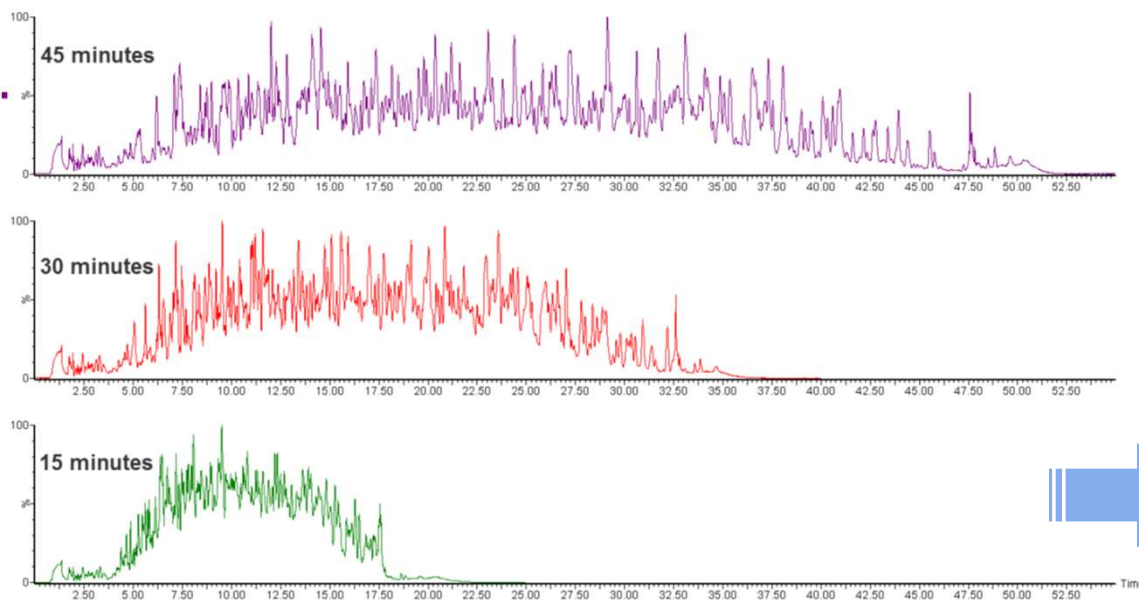
3x increase in throughput
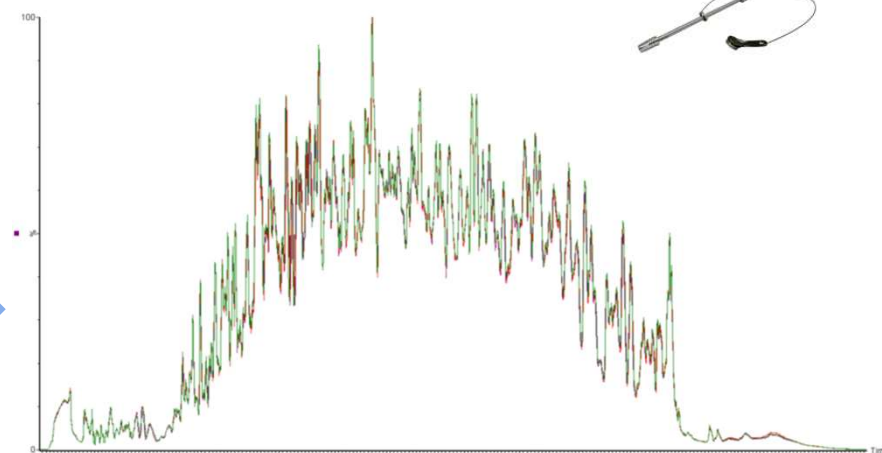
*Highly reproducible 15 min gradient
(3 technical replicates overlaid)*

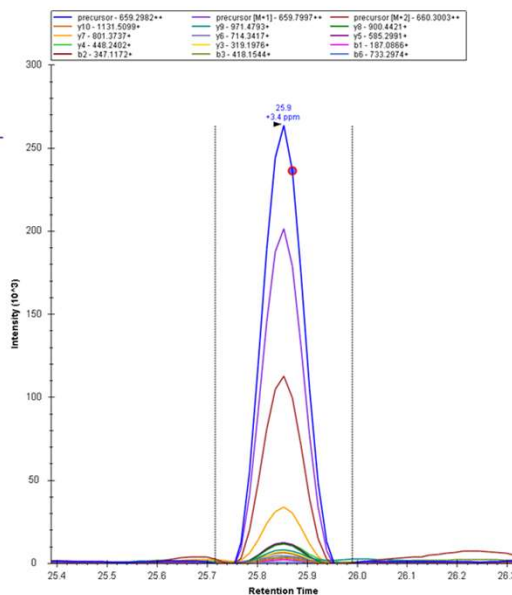# SONAR Delivers Increased Specificity For Co-eluting Peptides
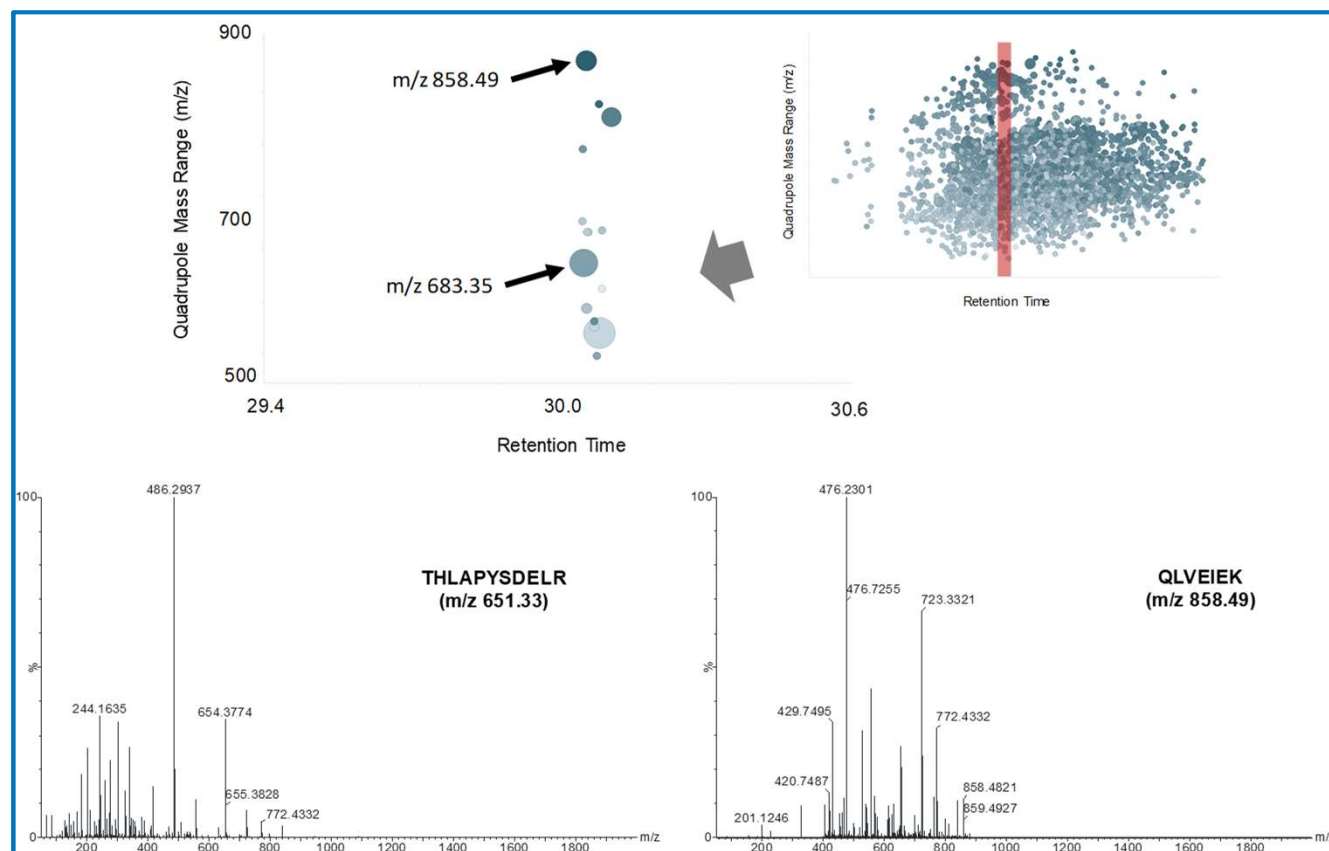
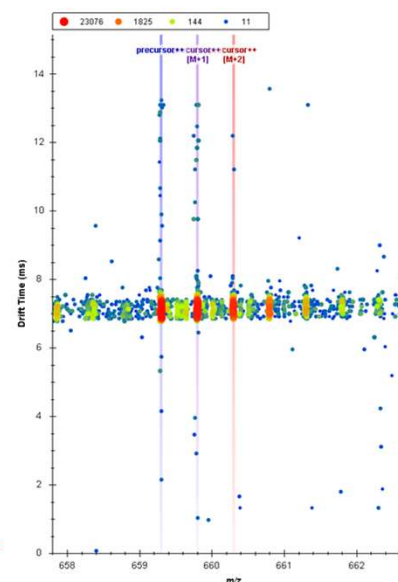# High **Quantitative Accuracy** Combined With High Specificity

>10 points across the chromatographic peak
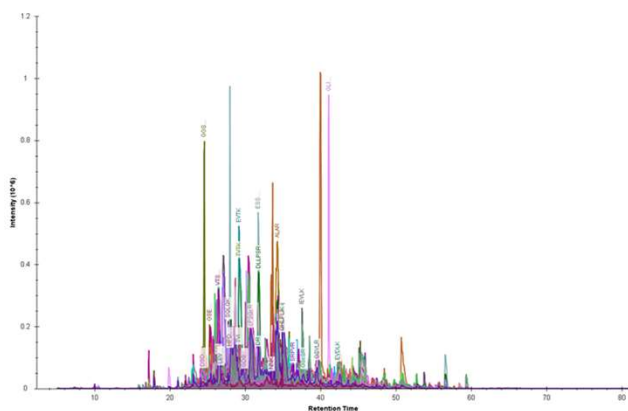
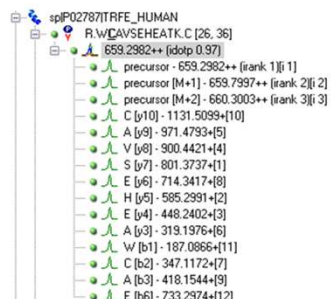*Multiple transitions – peptide (WCAVSEHEATK)*

Improved SONAR specificity

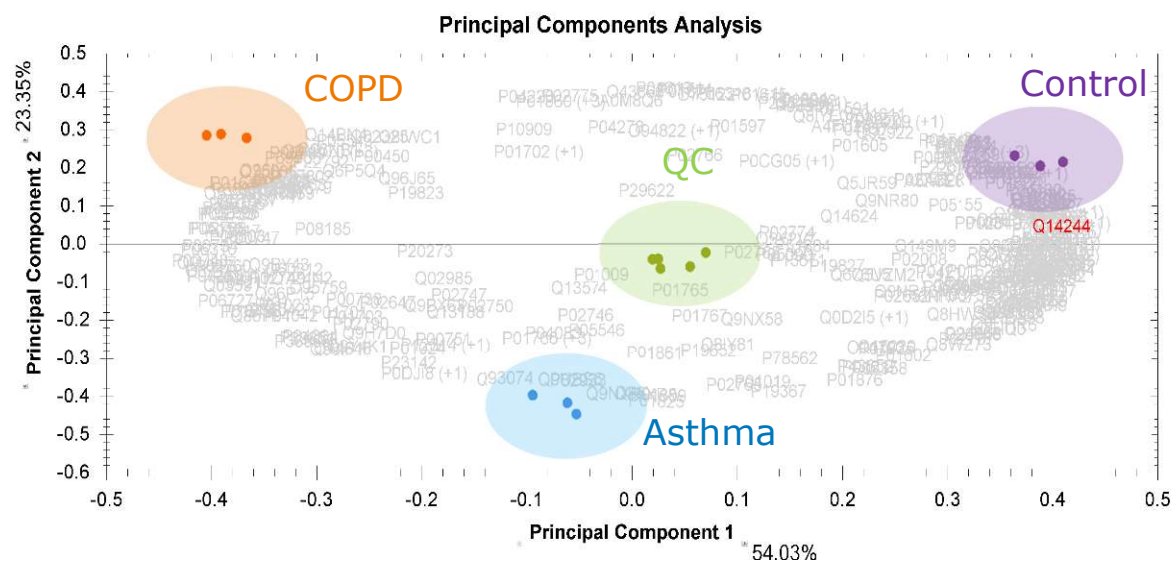SONAR provides both high specificity and high quantitative accuracy for the most complex of samples

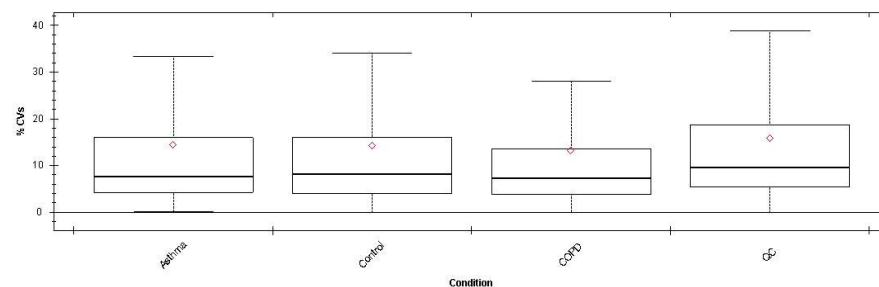*Serotransferrin*
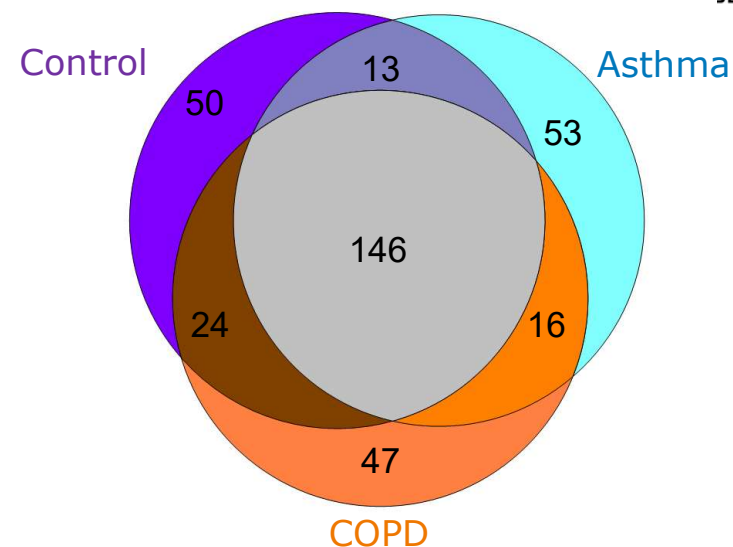*Retention Time = 25.9 mins*

Statistical Analysis/Qualitative Assessment
…15 min gradient proteomics

Protein Identifications

Unsupervised PCA

%CV's <10% over all conditions

What Next!!

Libraries and Predictive MS

Predicting CCS From Structure

Analytica Chimica Acta
Volume 924, 14 June 2016, Pages 68-76

Collision cross section prediction of deprotonated phenolics in a travelling-wave ion mobility spectrometer using molecular descriptors and chemometrics

Gerard Bryan Gonzales [a, b, d ✉], Guy Smagghe [b], Sofie Coelus [a], Dieter Adriaenssens [a], Karel De Winter [c], Tom Desmet [c], Katleen Raes [d], John Van Camp [a ✉]

Current Opinion in Chemical Biology
Volume 42, February 2018, Pages 34-41

Advancing the large-scale CCS database for metabolomics and lipidomics at the machine-learning era

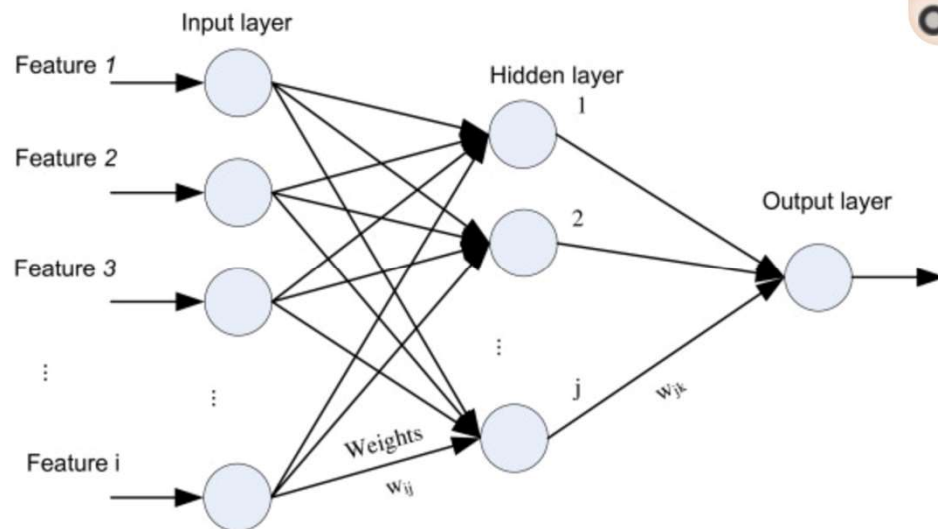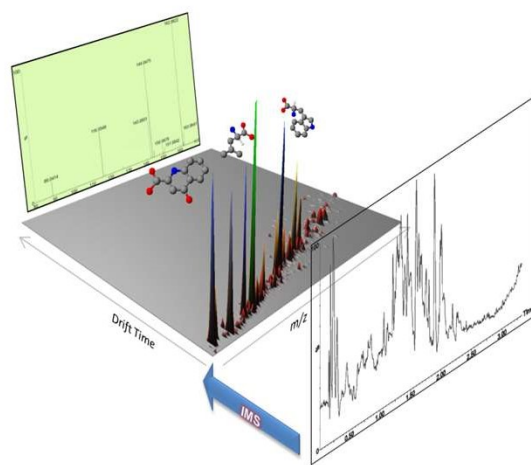Zhiwei Zhou [1, 2, 3], Jia Tu [1, 2, 3], Zheng-Jiang Zhu [1 ✉]

Artificial neural networks for the prediction of peptide drift time in ion mobility mass spectrometry

Bing Wang, [✉1,2] Steve Valentine, [3] Manolo Plasencia, [3] Sriram Raghuraman, [4] and Xiang Zhang [✉2]

Predictive CCS and Metabolomics

Identification of Metabolites From Predicted CCS Library
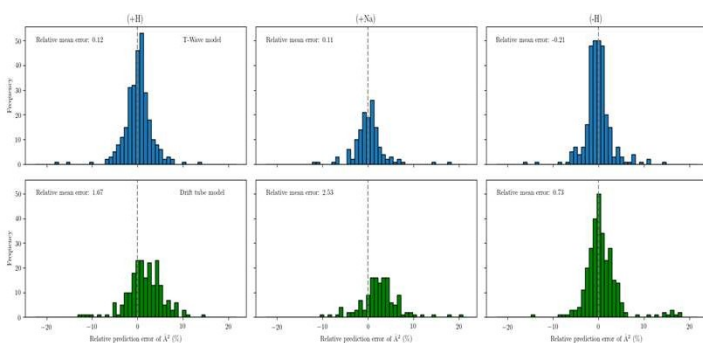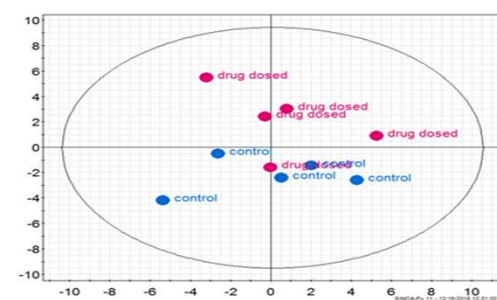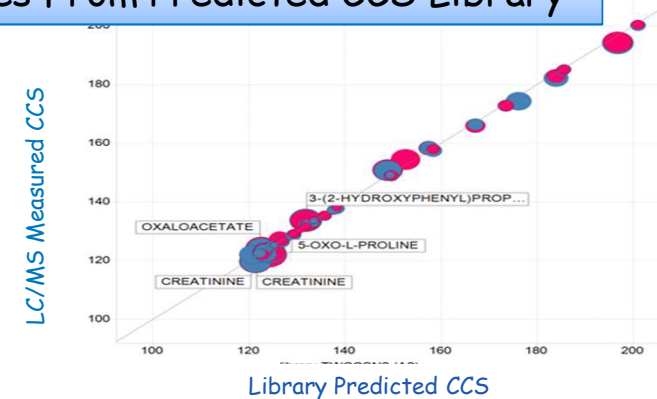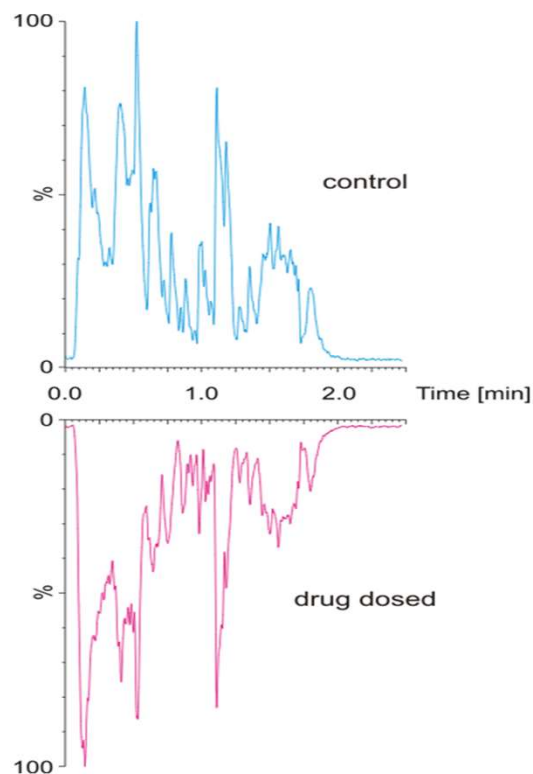
$^{TW}CCS_{N2}$ machine-learning prediction (blue)
and MetCCS (Green)

CCS Prediction Tool

CCS on demand   Home   Prediction   Links

Choose Files   No file chosen
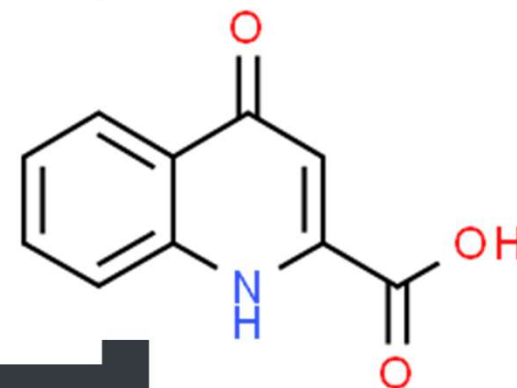
on demand   Home   Prediction   Links

Choose Files   No file chosen

	+H	+Na	+K	+NH4	-H	+HCOOH-H	-CH3COO
1	134.5	145.6	139	135.1	132.7	131.9	143.2

COMPANY CONFIDENTIAL

Summary

Outcome
- More competitive proteomics position
- Biomarker ID via CCS prediction

Omics Science
- Quantification
- High Throughput analysis
- Improved Peak detection
- CCS Measurement

Synapt XS Features
- Enhanced Resolution
- Approx 10X sensitivity
- Improved Labile Ion Transmission
- SONAR Enabled

The Biomedical Research Global Team
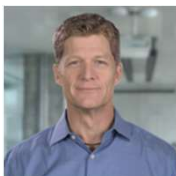
Market Development Team
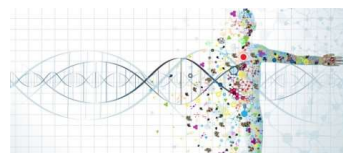
Suraj Dhungana
Manager
Biomedical Research

David Heywood
Senior Marketing Manager
Discovery Omics

Andrew Peck, Ph.D.
Senior Marketing Manager
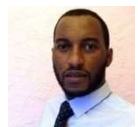Clinical Research

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Acknowledgements

Waters Omics Team:, Drs, Lee Gethings, Chris Hughes, Nyasha Munjoma, Gorgis Isaacs, Lisa Reid, Adam King, Lauren Mullin, Billy Molloy, Sarah Lennon, Nikunj Tanna, Hans Vissers,

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