



**ThermoFisher**  
SCIENTIFIC

## What Are You Missing in Your Metabolome... *The Orbitrap Perspective*

The world leader in serving science

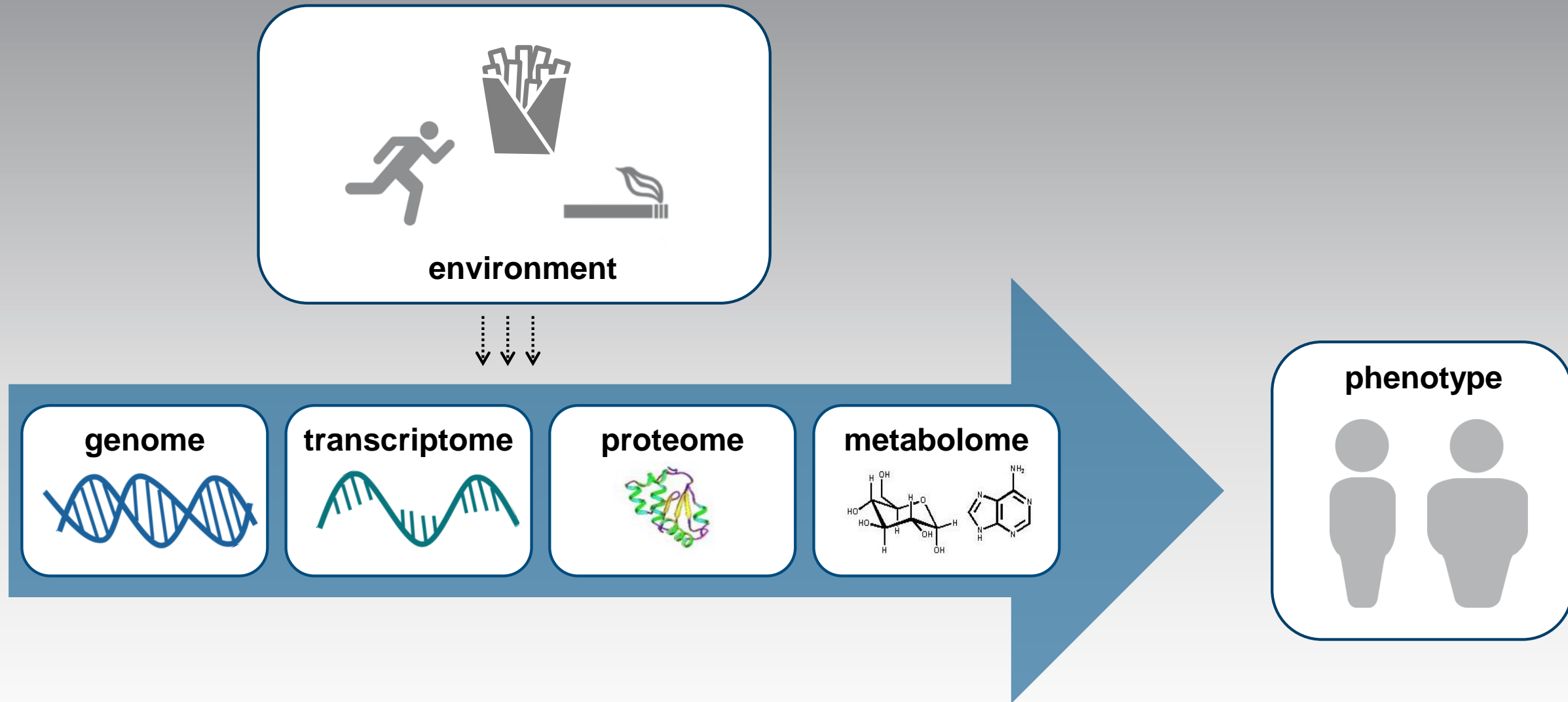


**1** Introduction to metabolomics

**2** Addressing the challenges with high resolution accurate mass spectrometry

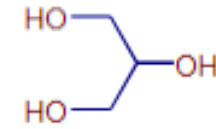
**3** Experimental design with Thermo Scientific™ Orbitrap™ mass spectrometers

# Metabolomics – the Link between Genotype and Phenotype

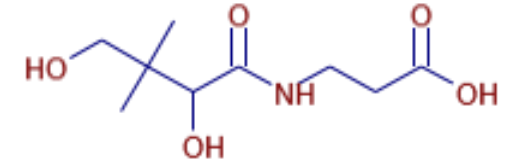




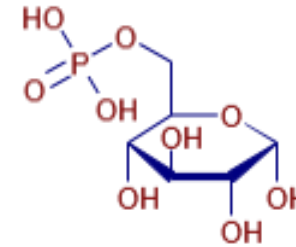
Why is exercise good for health?



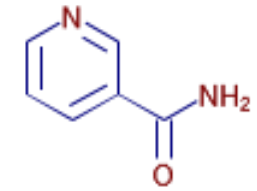
↑ glycerol



↑ pantothenate



↑ glucose-6-phosphate



↑ niacinamide

Lewis, G.D., Gerszten, R.E. et al (2010) Metabolic Signatures of Exercise in Human Plasma  
*Science Translational Medicine* 2(33): 33ra37.



# Untargeted Metabolomics: A Universal Application



Antimicrobial Agents  
and Chemotherapy



## Untargeted Meta

Isabel M. Vincent,<sup>a</sup> David E. Ehrlich,<sup>a</sup>  
University of Glasgow, Glasgow, United Kingdom

Deciphering the mode of action of antimicrobials is a major challenge in drug discovery. Here, we present a metabolomics approach that is blind to the composition of the cell wall metabolites. Using an untargeted metabolomics approach, we identified eight compounds that inhibit the growth of *Staphylococcus aureus*. These compounds were then tested against a panel of 100 strains of *S. aureus*, including those with different cell wall compositions. The results show that the compounds are active against all strains, suggesting that they target a common pathway. The analysis of the metabolomics data revealed that the compounds inhibit the synthesis of peptidoglycan, a key component of the cell wall.

## Amino acid metabolism is altered in adolescents with NAFLD - an untargeted, high resolution metabolomics study

Ran Jin, PhD<sup>1,\*</sup>, Sophia Shuzhao Li, PhD<sup>2</sup>, David

<sup>1</sup>Division of Pediatric

*Am J Physiol Endocrinol Metab* 311: E471–E479, 2016.  
First published June 28, 2016; doi:10.1152/ajpendo.00134.2016.

Boyles et al. *Particle and Fibre Toxicology* (2016) 13:49  
DOI 10.1186/s12989-016-0160-6

Particle and Fibre Toxicology

RESEARCH

Open Access

## Copper oxide nanoparticle toxicity profiling using untargeted metabolomics



Matthew S. P. Boyles<sup>1†</sup>, Christina Ranninger<sup>2†</sup>, Roland Reischl<sup>2</sup>, Marc Rurik<sup>3,4</sup>, Richard Tessadri<sup>5</sup>, Oliver Kohlbacher<sup>3,4,6,7,8</sup>, Albert Duschl<sup>1\*</sup> and Christian G. Huber<sup>2\*</sup>

### Abstract

**Background:** The rapidly increasing number of engineered nanoparticles (NPs), and products containing NPs, raises concerns for human exposure and safety. With this increasing, and ever changing, catalogue of NPs it is becoming

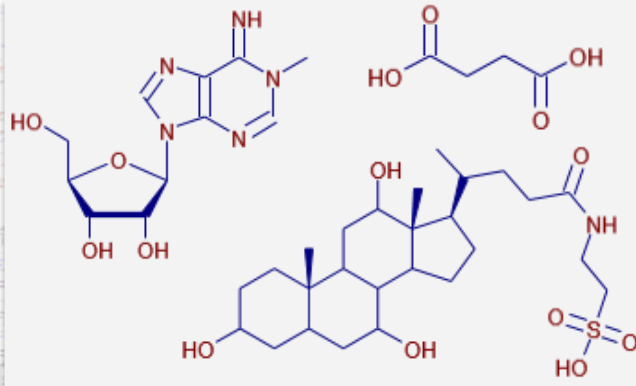
disorder provided  
diabetic detrusor

Endocrine/Cardiovascular Research, Lilly  
Biophysics, Albert Einstein College of

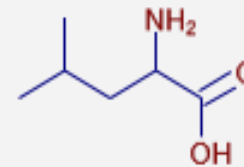
Detrusor determines the tone of the bladder, and the detrusor layer serves both as a barrier and in a detrusor tone. The differing roles of these layers are reflected by different pathophysiological conditions. The involvement of detrusor in the pathogenesis of several mechanisms that affect the development of smooth muscle tone; these include decreased muscarinic receptor density (31, 32), detrusor hypertrophy, increased calcium sensitivity, and decreased calcium channel activity (34), alteration

Downloaded from <http://ajf>

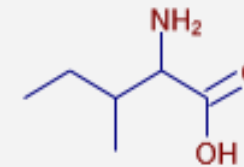
# Untargeted Metabolomics: Challenges



Structural and physical diversity

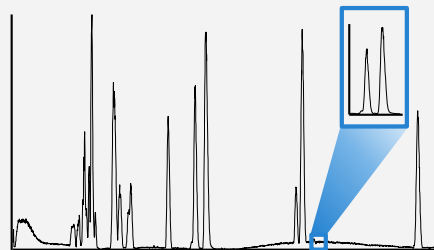


leucine



isoleucine

Isomeric / isobaric species



Dynamic range



Large sample sets

# High Quality Data for High Quality Results

- Complex matrix
- Differentiate similar masses
- Isobaric species
- Find isotopic pattern

High  
Resolution

- Identification of unknowns
- Narrow mass tolerance
- Mass stability from peak to peak and run to run

Mass  
Accuracy

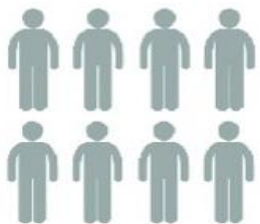
- Scan-to-scan consistency
- Injection-to-injection reproducibility
- Robustness over extended time periods

Instrument  
Performance

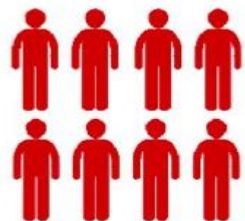


# Untargeted Metabolomics Workflow

## Sample Preparation



Collect and  
prepare  
samples



## Chromatography

## Mass Spectrometry

Untargeted discovery



IC-MS



LC-MS



GC-MS

## Data Analysis

Compound Detection,  
Quantitation, and  
Identification





# Thermo Scientific™ Q Exactive™ Series Portfolio for Metabolomics

PERFORMANCE



## Thermo Scientific™ Q Exactive™ Focus MS

- Orbitrap analyzer
- Mass Range  $m/z$  50 - 3000
- Mass Accuracy <1ppm
- Max. Mass Resolution >70,000
- Scan speed up to 12Hz
- Spectral multiplexing
- Polarity switching <1 sec



## Thermo Scientific™ Q Exactive™ MS

- Orbitrap analyzer
- Mass Range  $m/z$  50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >140,000
- Scan speed up to 12Hz
- Spectral Multiplexing
- Polarity switching <1 sec



## Thermo Scientific™ Q Exactive™ Plus MS

- Orbitrap analyzer
- Mass Range  $m/z$  50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >140,000
- Scan speed up to 12Hz
- Spectral multiplexing
- Polarity switching <1 sec
- Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Opt. Enh Res.Mode (280k)



## Thermo Scientific™ Q Exactive™ HF MS

- Ultra High Field Orbitrap analyzer
- Mass Range  $m/z$  50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >240,000
- Scan speed up to 18Hz
- Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Spectral Multiplexing
- Polarity switching <1 sec



## Thermo Scientific™ Q Exactive™ HF-X MS

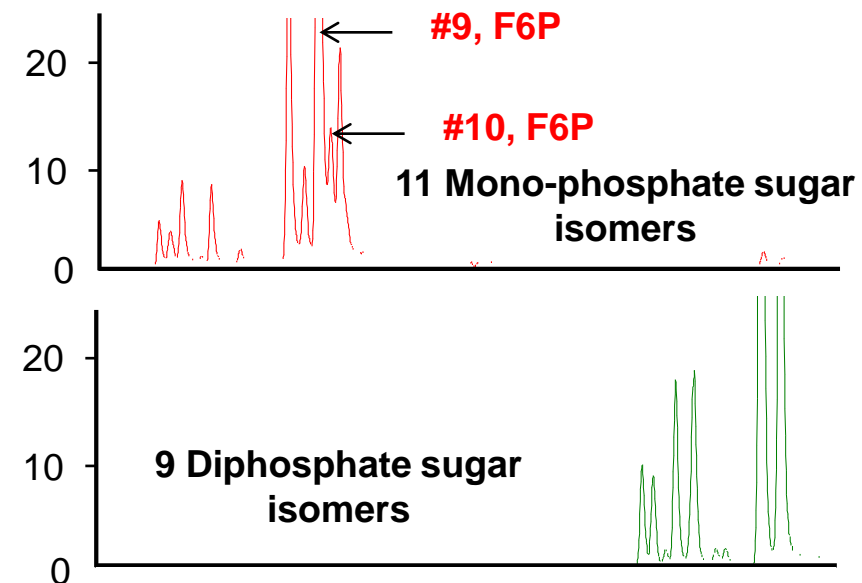
- Ultra High Field Orbitrap analyzer
- Mass Range  $m/z$  50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >240,000
- Scan speed up to 40Hz
- High capacity transfer tube
- Electrodynamic ion funnel
- Advanced Quadrupole Technology (AQT)
- Advanced Active Beam Guide (AABG)
- Spectral Multiplexing
- Polarity switching <1 sec
- Advanced ddHCD algorithm

VALUE

# Ion Chromatography (IC): Excellent Separation Tool for Polar Metabolites



Thermo Scientific™ Dionex™ ICS-5000+ HPIC System  
Thermo Scientific™ Q Exactive™ Orbitrap Series MS



- Efficient and sensitive separation for small isobaric polar metabolites
- Increased sensitivity, > 10-fold, as result of low chemical noise

## Thermo Scientific™ Q Exactive™ GC Hybrid Quadrupole-Orbitrap GC-MS/MS System

Offering Unprecedented Depth in GC-MS Analysis

- Resolution up to 100K
- Sub-ppm mass accuracy
- > 6 orders dynamic range
- Triple quad-like sensitivity



# Vanquish UHPLC Systems Bring You

## Confidence



### To ensure highest data quality

- Unmatched retention time precision with SmartInject
- Automation of workflows with barcode reading
- Predictive performance indicators

## Sensitivity



### To find the complete picture

- UV detection optimized for lowest dispersion and minimal baseline noise
- Highly sensitive and selective fluorescence detection
- Near universal charged aerosol detection



## Performance

### To improve separations

- Binary and Quaternary pumping systems
- Widest flow-pressure footprint
- Optimized system fluidics



## Accuracy

### To control experiments

- Unsurpassed sample dosage
- Active and passive pre-heating
- Multiple thermostating options







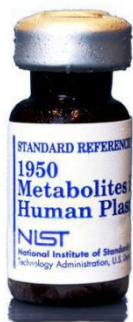
<http://srm1950.nist.gov/>

- Human plasma sample with certified values for most common metabolites.
- Organic solvent precipitation of proteins and extraction of small molecules.

# Untargeted Metabolomics Workflow

## Case Study: NIST SRM 1950 – a commercially available reference standard

### Sample Preparation



**NIST**  
National Institute of  
Standards and Technology  
U.S. Department of Commerce  
<http://srm1950.nist.gov/>

### Chromatography

### Mass Spectrometry

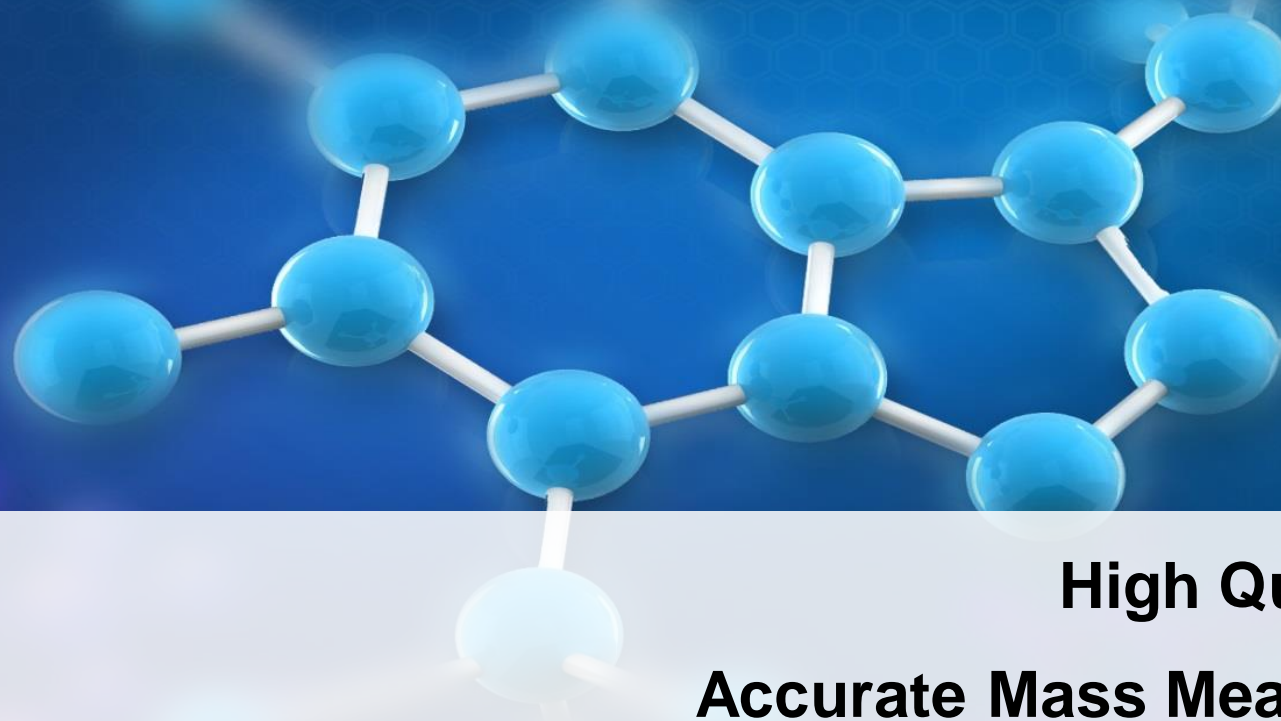


Thermo Scientific™ Q Exactive™ HF mass spectrometer  
Thermo Scientific™ Vanquish™ UHPLC system

### Data Analysis

Unbiased detection and  
confident identification  
tools





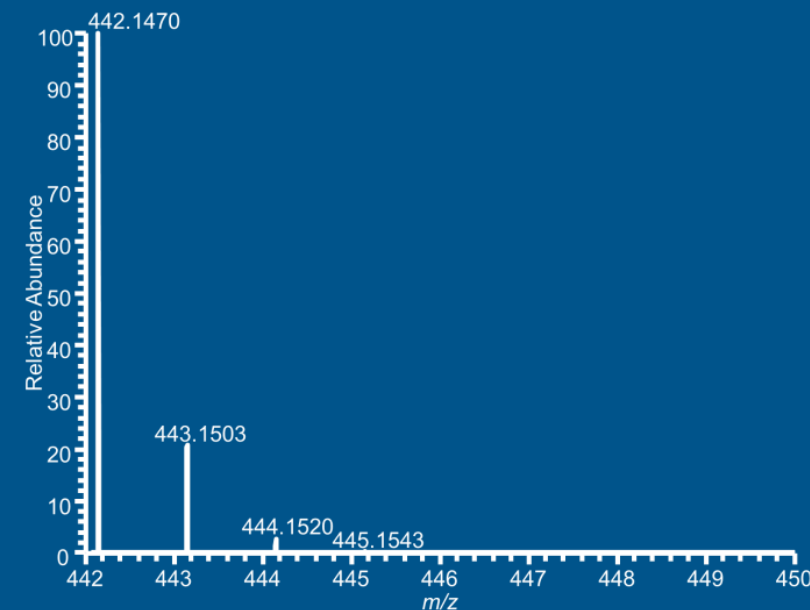
**High Quality Data:  
Accurate Mass Measurements**

# Mass Accuracy Provides Selectivity

| Mass Error<br>(tolerance) | # of Possible Chemical<br>Formulae<br>(using only C, H, N & O) |
|---------------------------|--|
| 0.1 Da                    | 319  |
| 10 ppm                    | 14   |
| 5 ppm                     | 6  |
| 2 ppm                     | 2  |

Mass error (ppm) =  $\frac{\text{exact mass} - \text{accurate mass}}{\text{MW}} \times 10^6$

Example: Folic acid ( $\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6$ )  
has a protonated ion at 442.1470

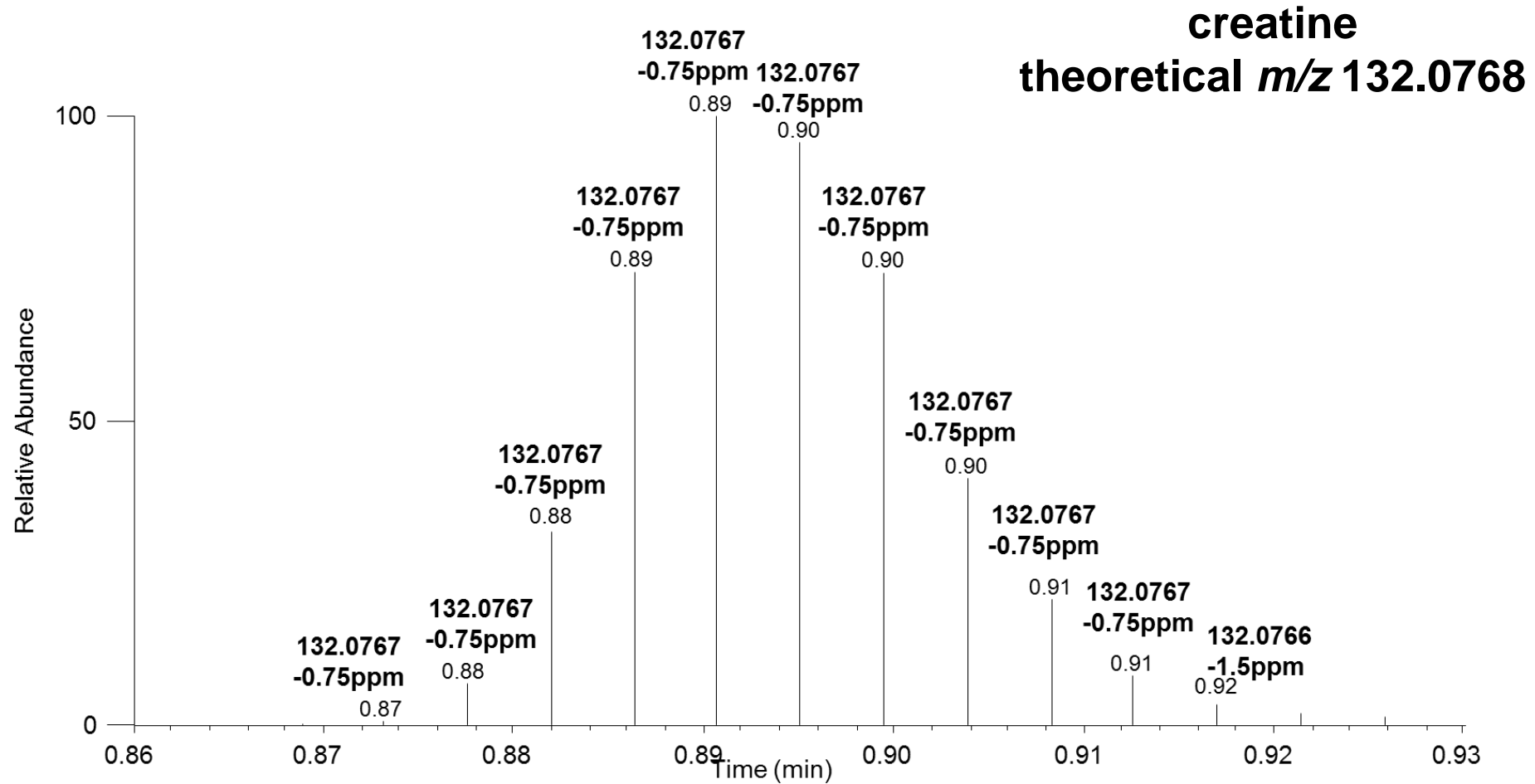


**Orbitrap HRAM MS delivers confident identification of unknowns**

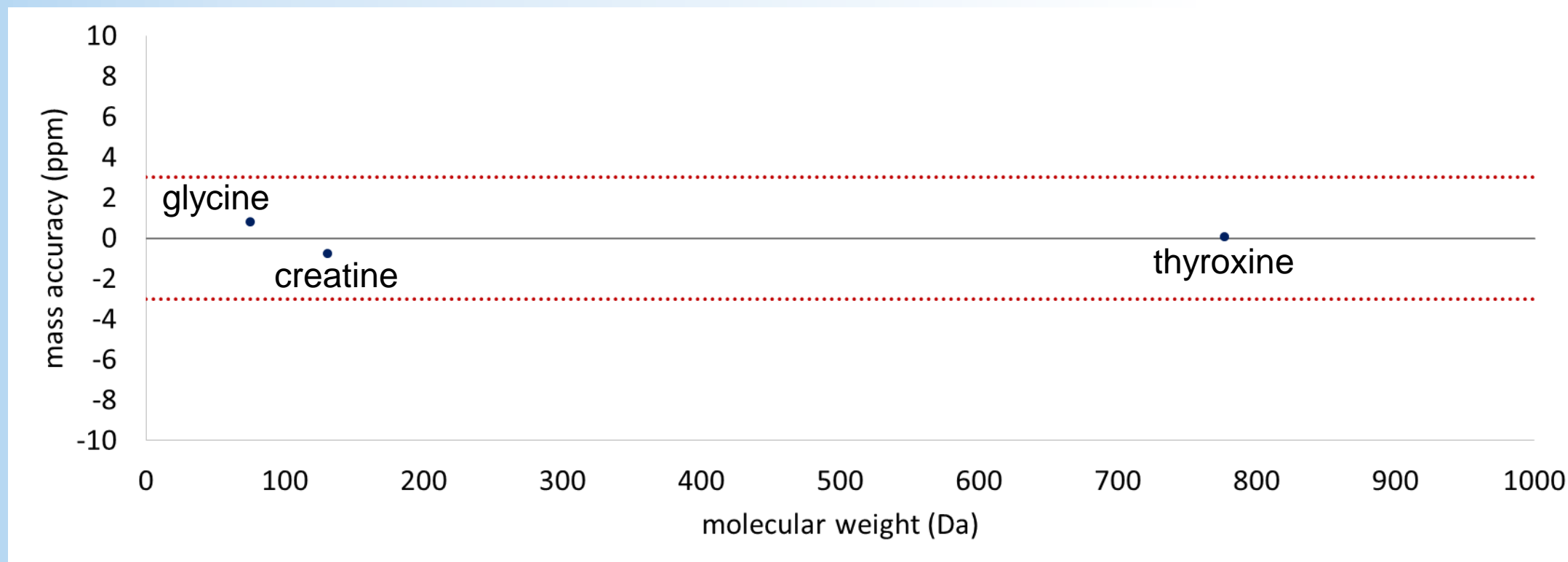


# Stable Mass Accuracy from Scan to Scan Across the Peak

## Orbitrap MS provides confidence in peak detection



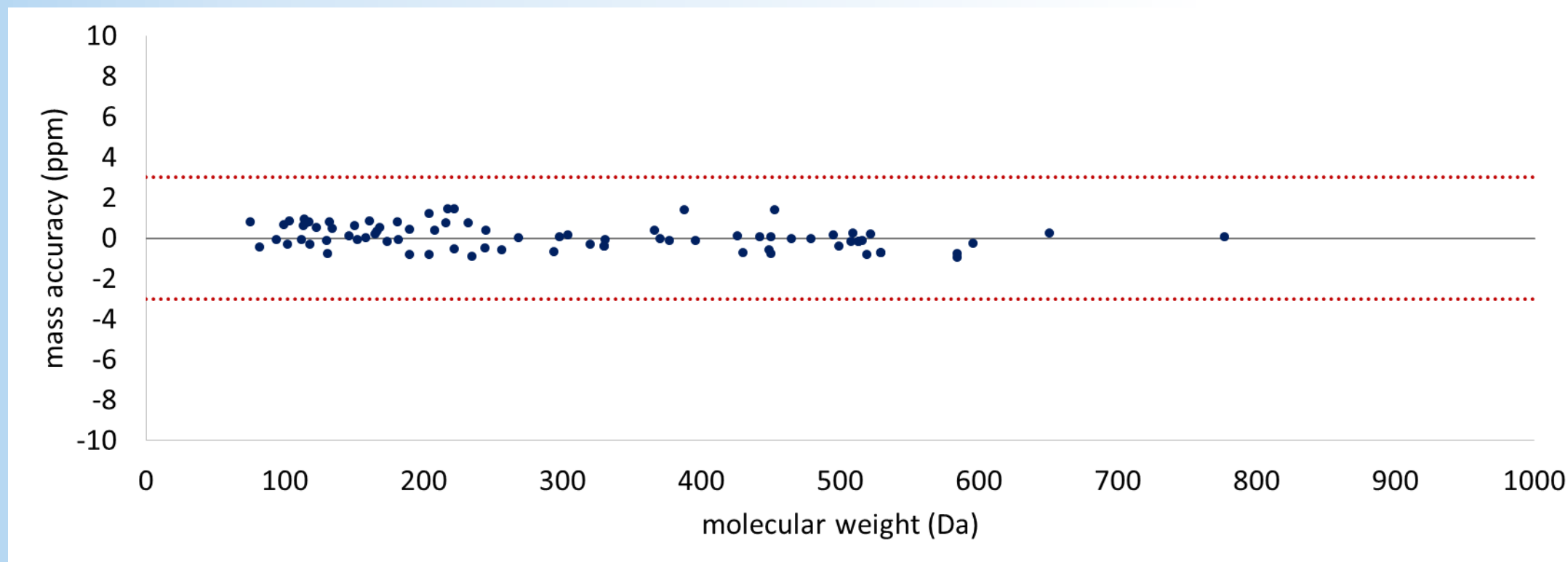
# Excellent Mass Accuracy Across the Molecular Weight Range



Metabolites identified from human plasma and verified against an authentic standard

**Orbitrap MS delivers accurate mass measurements for all metabolites**

# Excellent Mass Accuracy Across the Molecular Weight Range

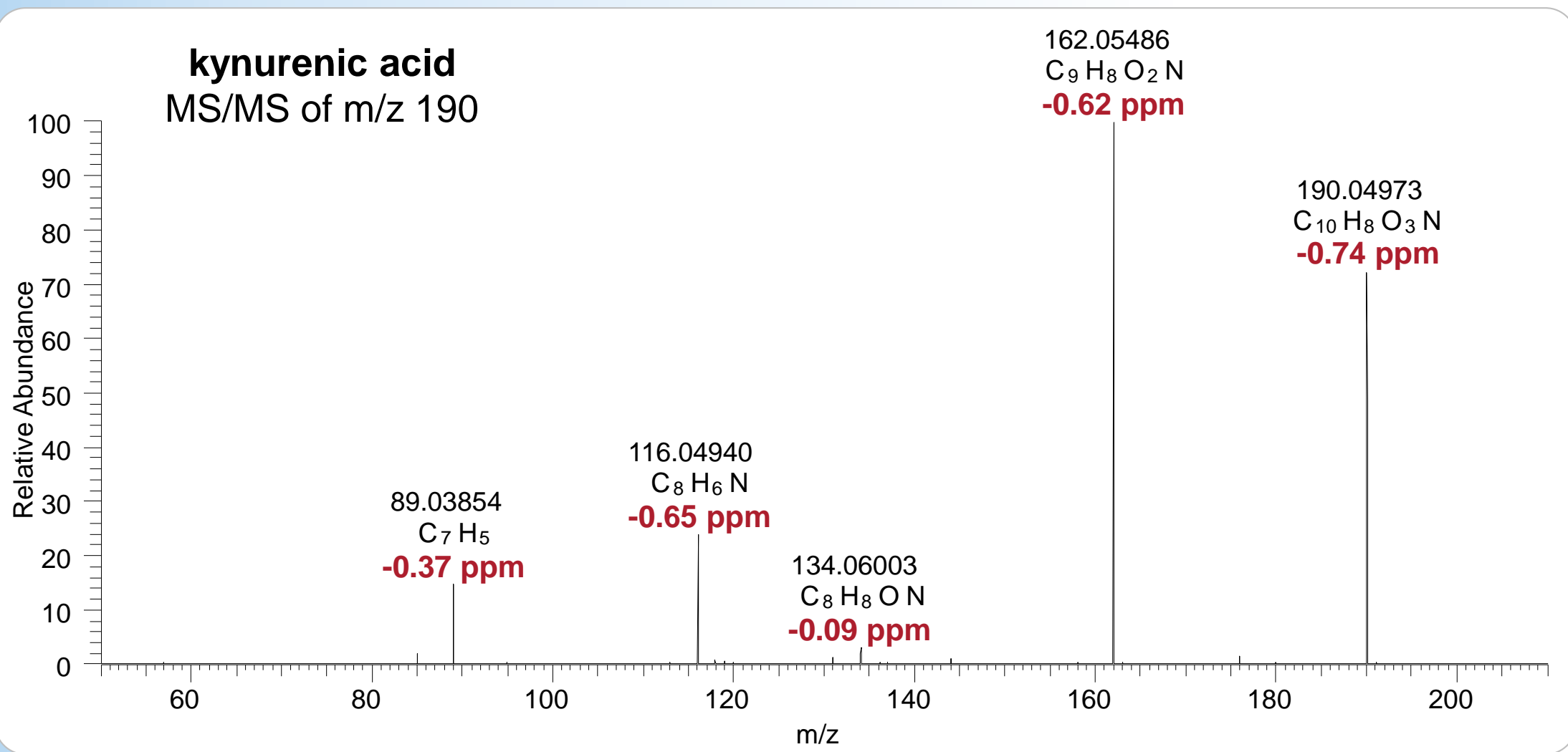


Metabolites identified from human plasma and verified against an authentic standard

**Orbitrap MS delivers accurate mass measurements for all metabolites**

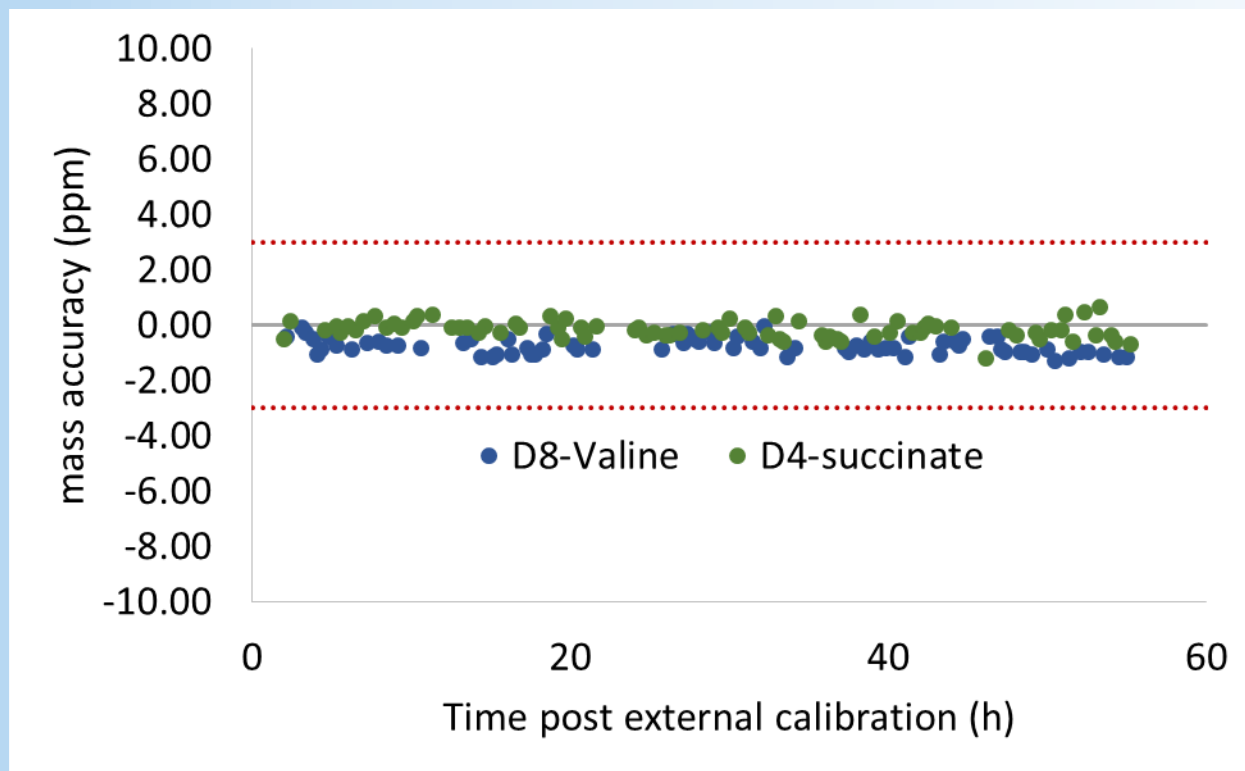
# Excellent Mass Accuracy During Fragmentation

Orbitrap MS provides high mass accuracy for confident structure annotation





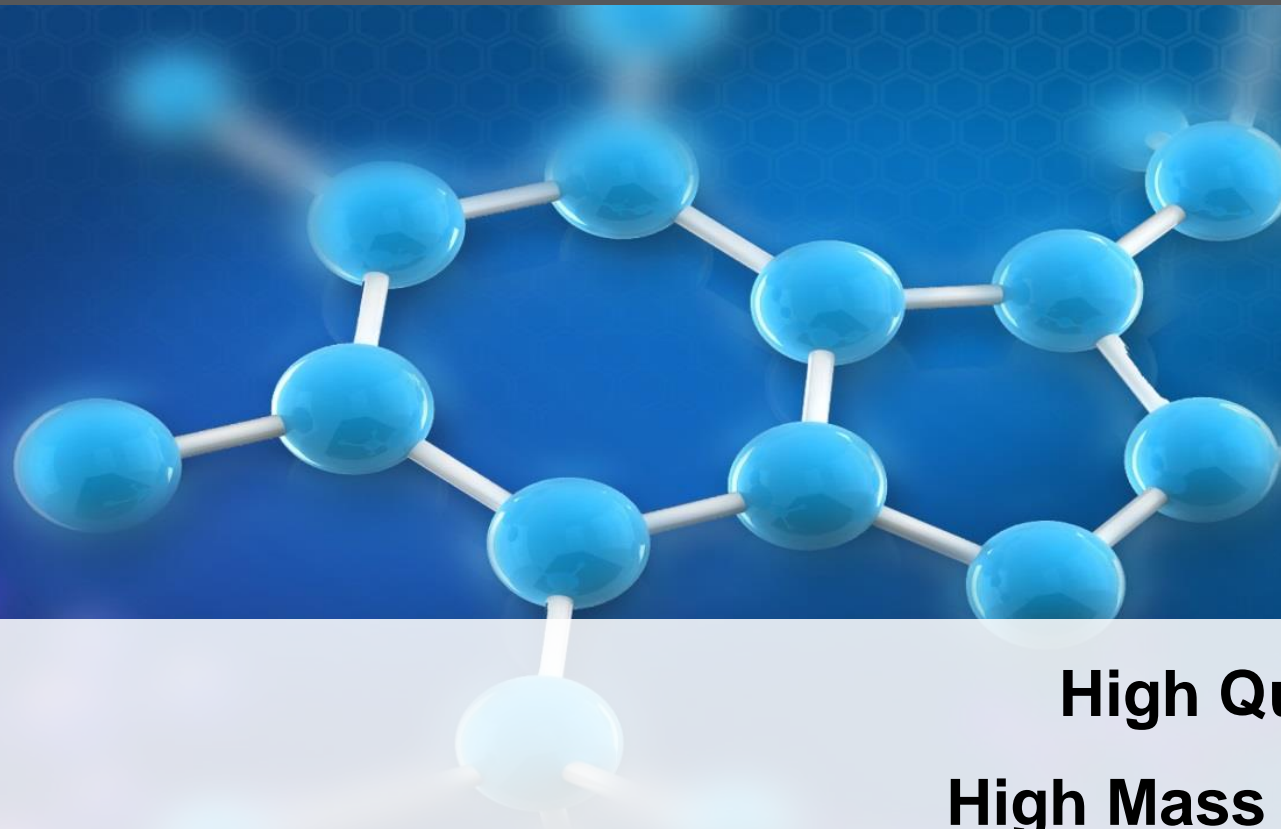
# Robust Mass Accuracy from Run to Run



Human plasma (NIST SRM1950)  
spiked with internal standards

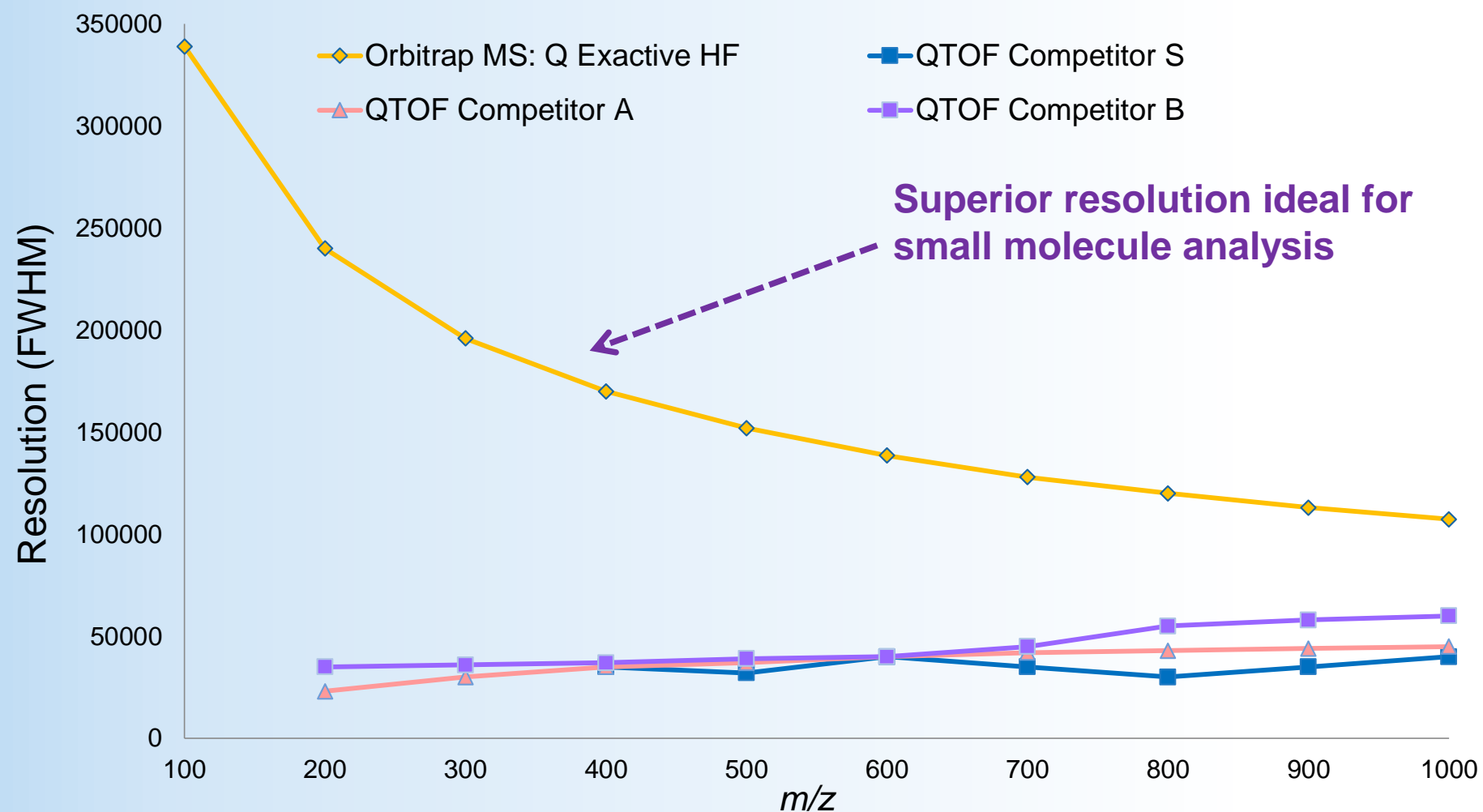
Mass accuracy <2ppm in both  
positive and negative modes  
during a 55 h analysis

**Orbitrap MS delivers unmatched mass accuracy over long periods of time**



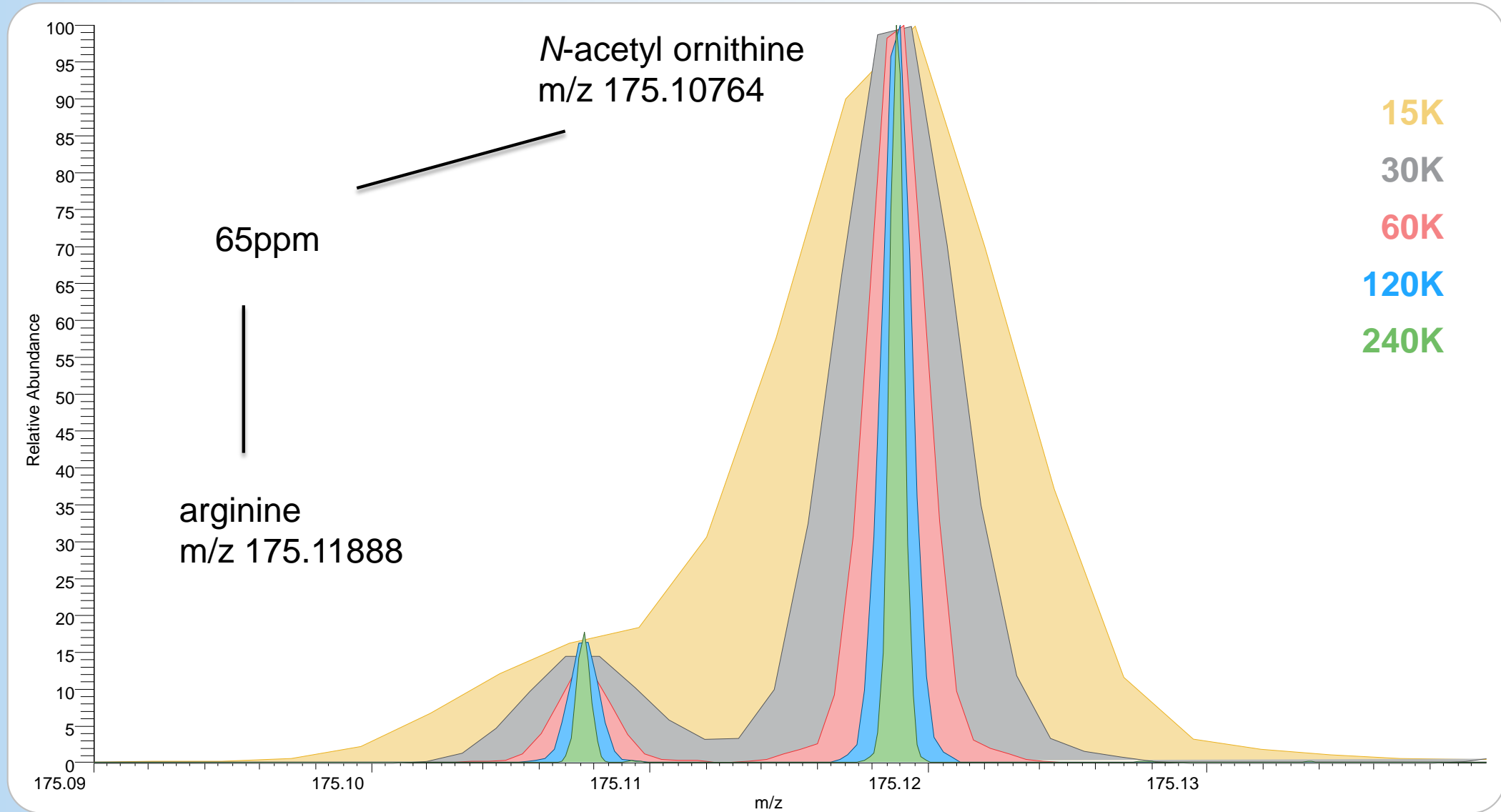
**High Quality Data:  
High Mass Resolution**

# Orbitrap MS: Unmatched Resolution



High Orbitrap MS resolving power for correct identification of isomeric and isobaric species

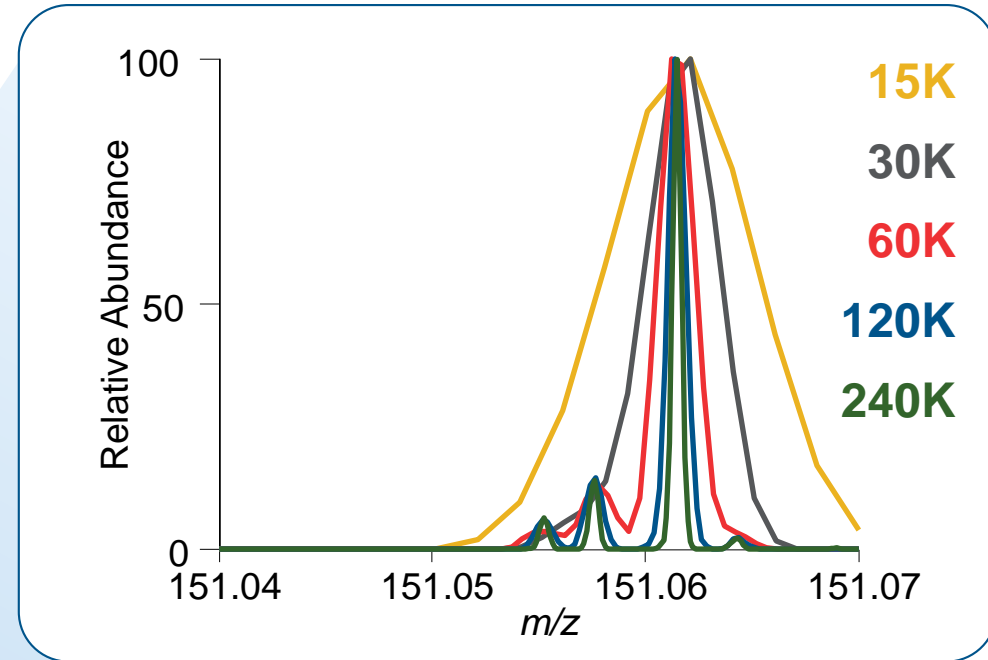
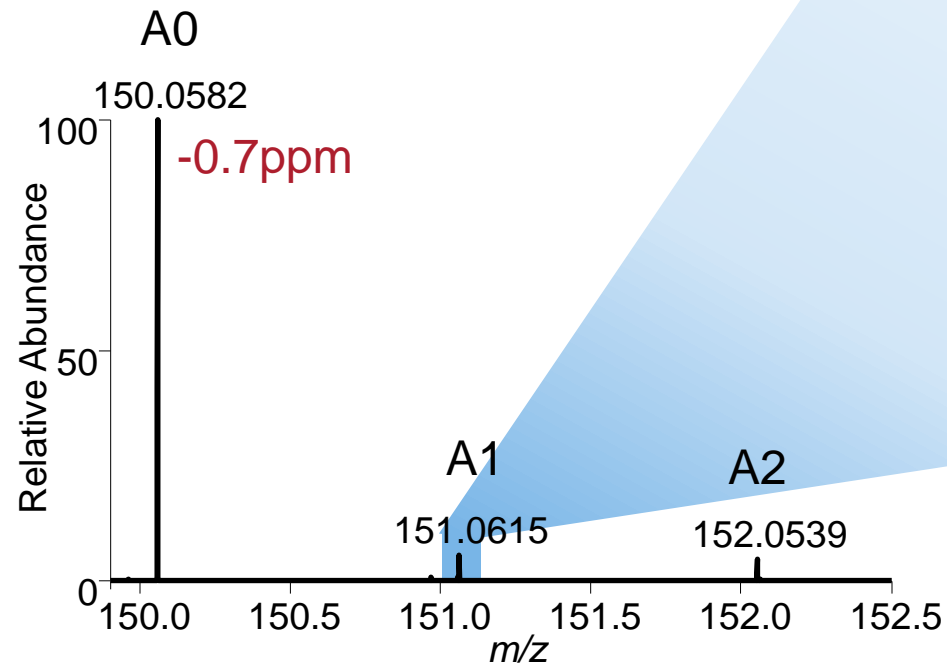
# Resolving Isobaric Metabolites in Human Plasmas with Orbitrap High Resolution





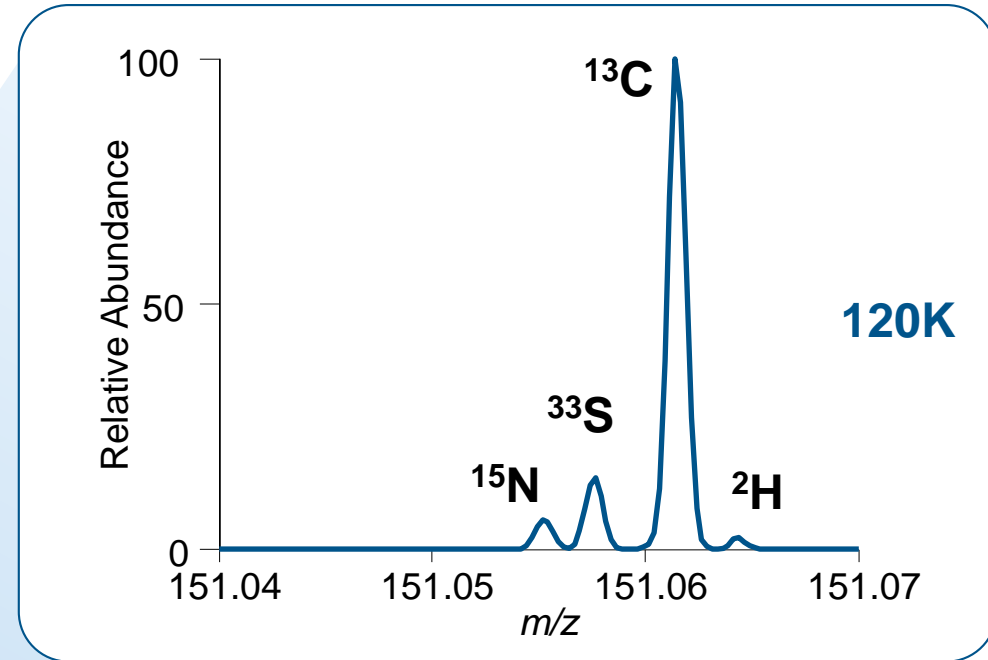
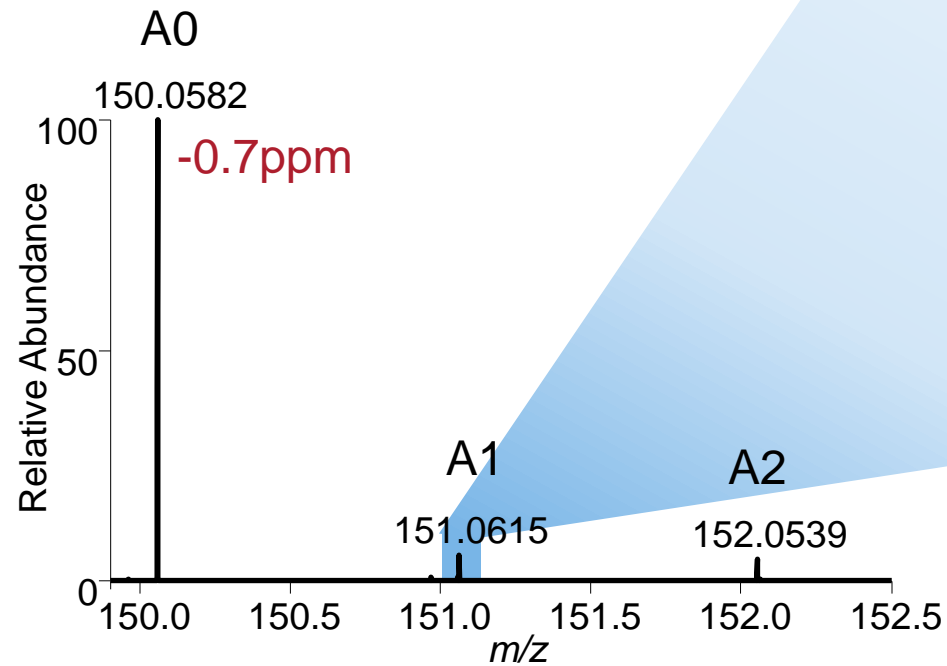
# High Resolution is Essential for Fine Isotopic Pattern Determination

L-Methionine  $\text{C}_5\text{H}_{11}\text{NO}_2\text{S}$   
Positive mode



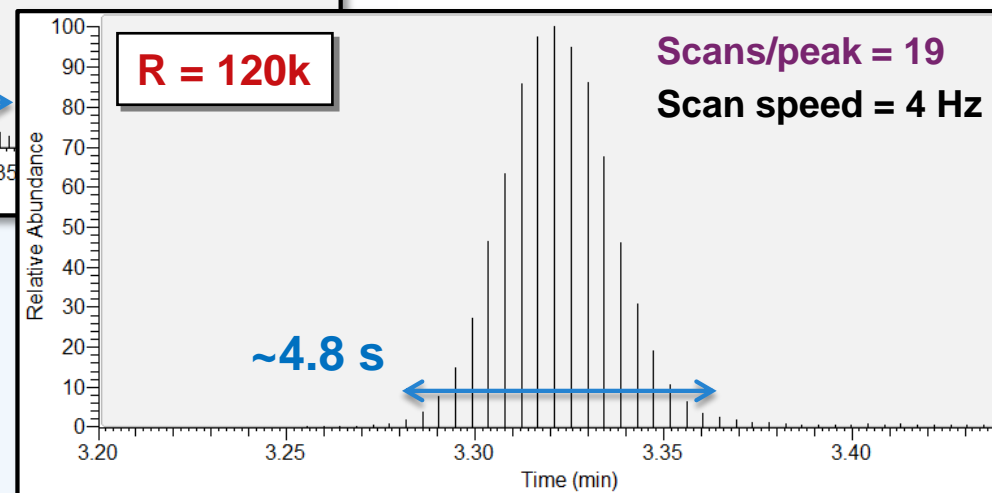
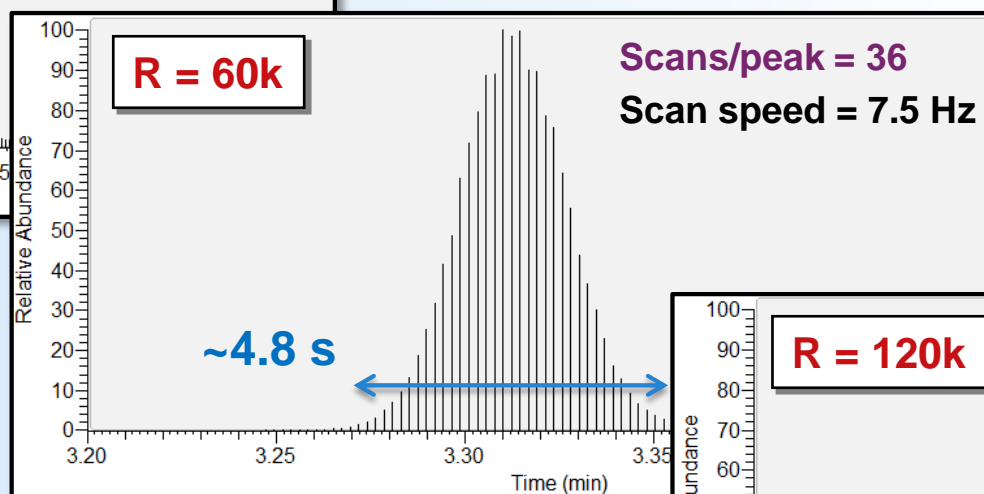
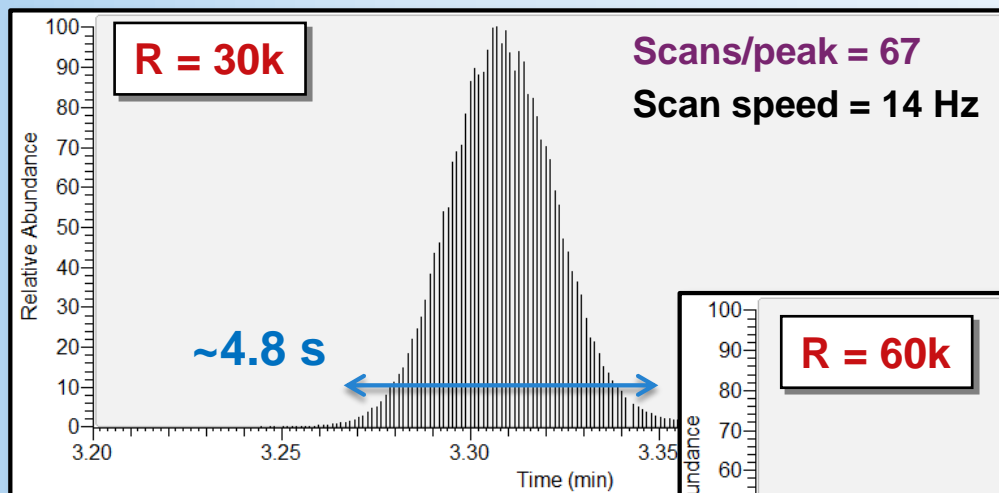
# High Resolution is Essential for Fine Isotopic Pattern Determination

L-Methionine  $\text{C}_5\text{H}_{11}\text{NO}_2\text{S}$   
Positive mode



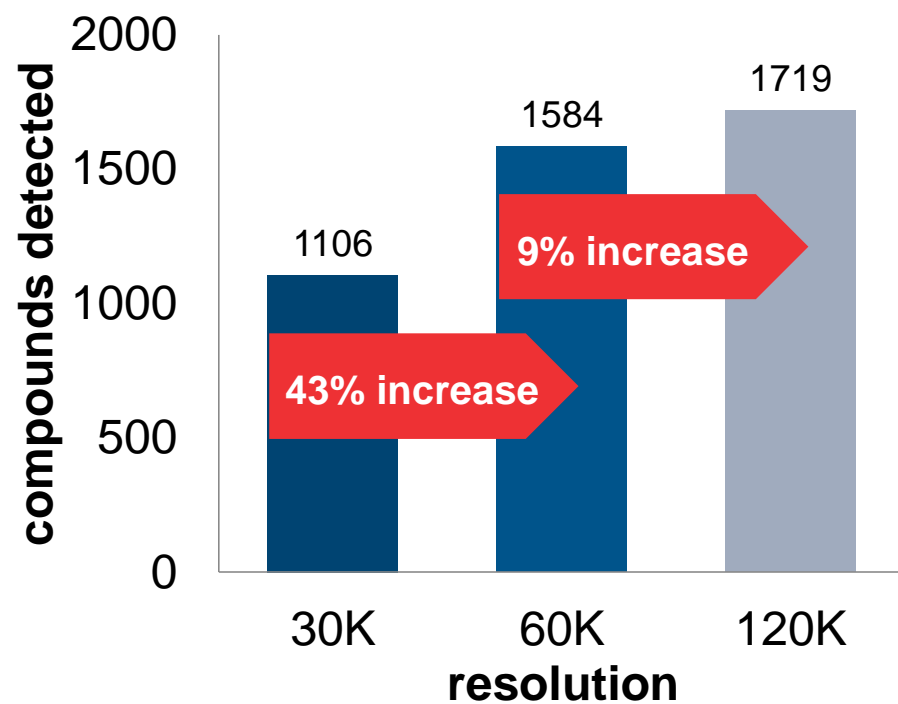
# Mass Resolution and Scan Speed

Vanquish UHPLC and Q Exactive HF MS  
D8-Phenylalanine spiked in human plasma

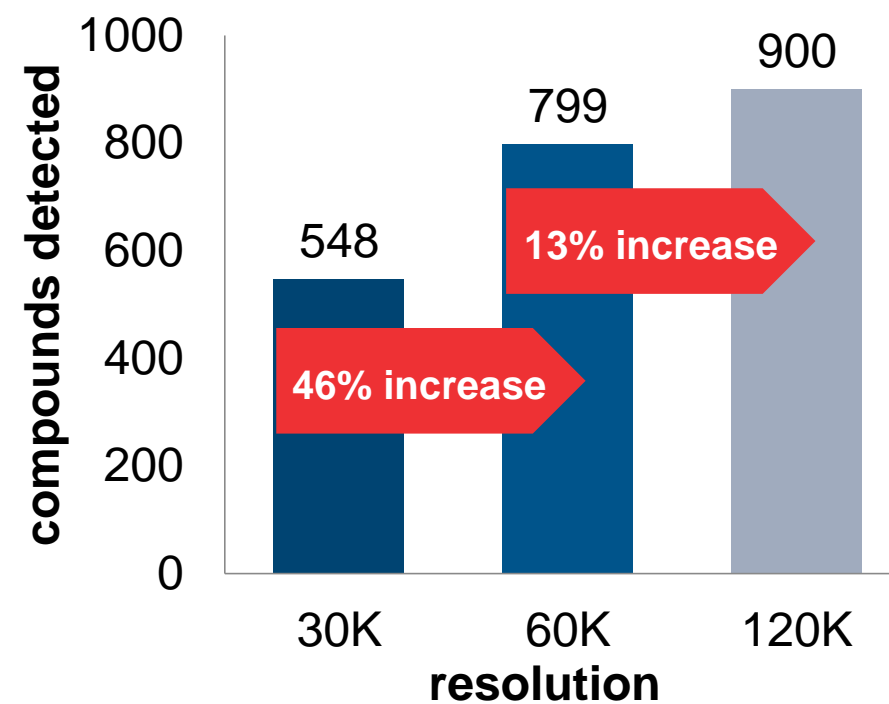


# High Resolving Power Increases Metabolome Coverage

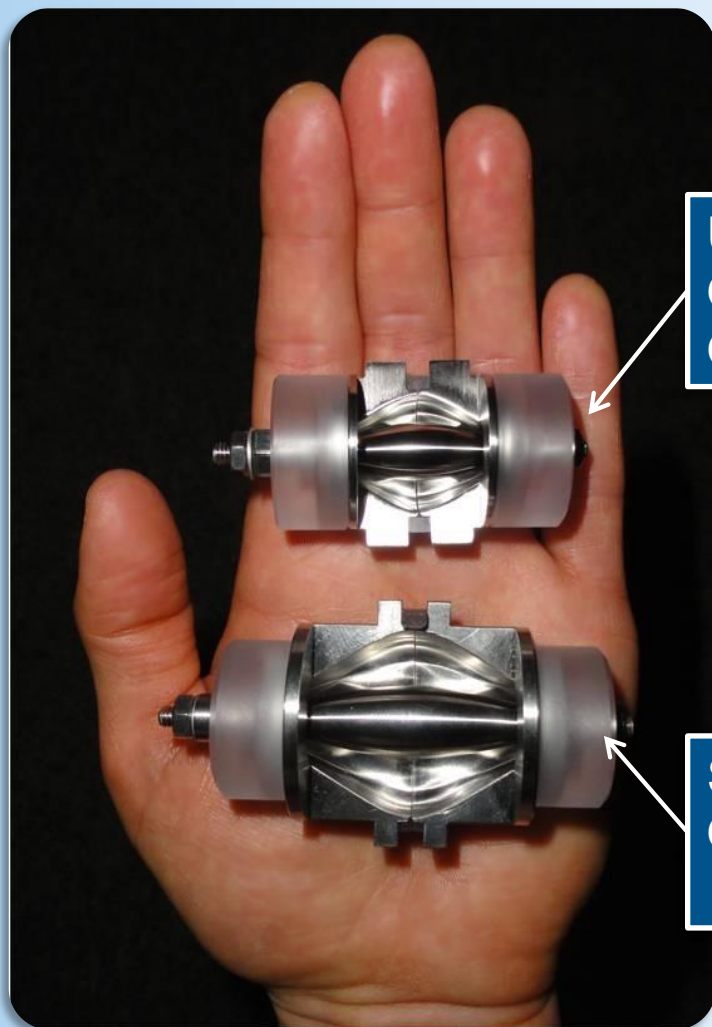
**Human plasma metabolites  
(positive mode)**



**Human plasma metabolites  
(negative mode)**



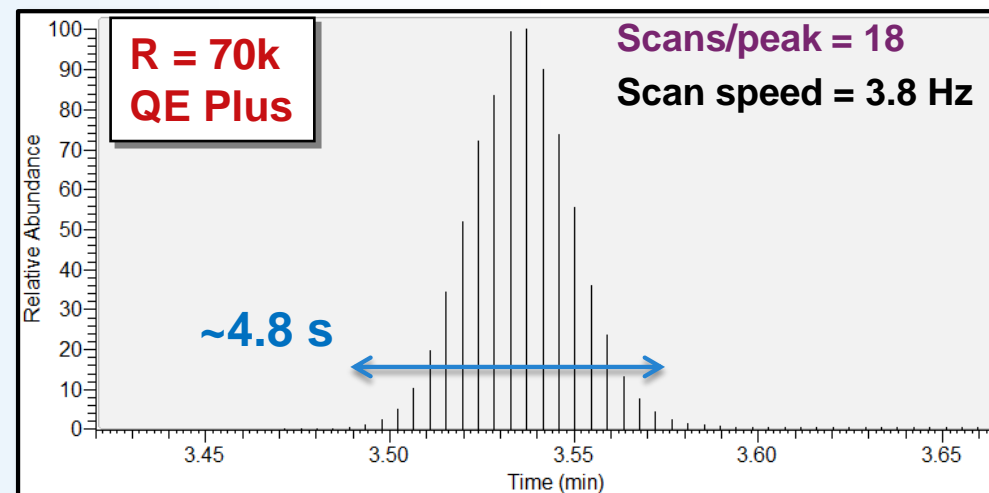
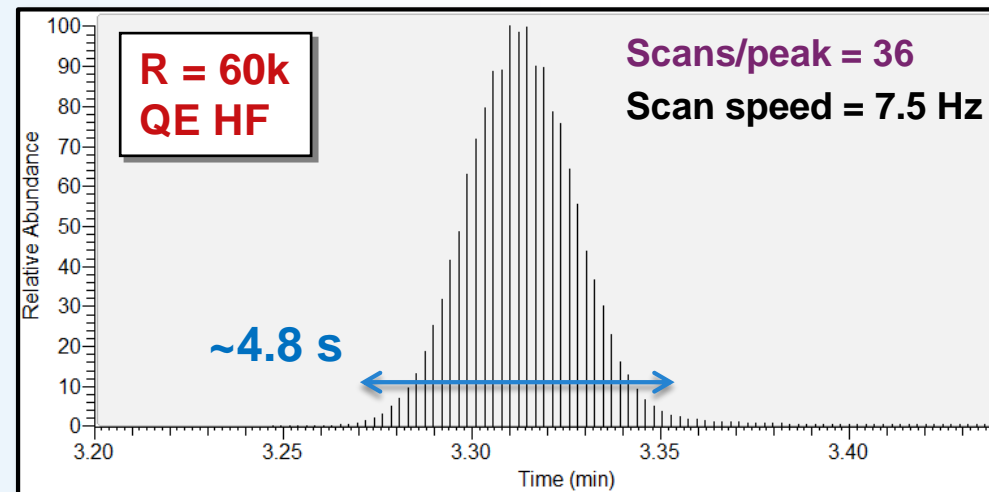
# Improved Metabolome Coverage with the Q Exactive HF MS



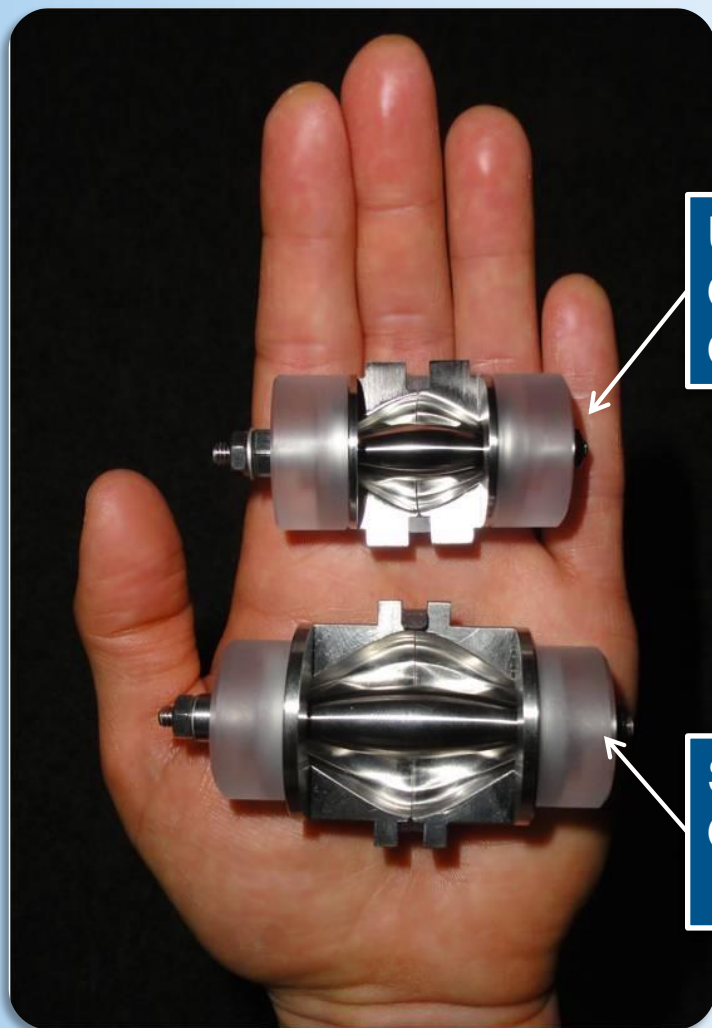
Ultra-High Field  
Orbitrap  
Q Exactive HF MS

Standard Orbitrap  
Q Exactive Plus MS

D8-Phenylalanine spiked in human plasma



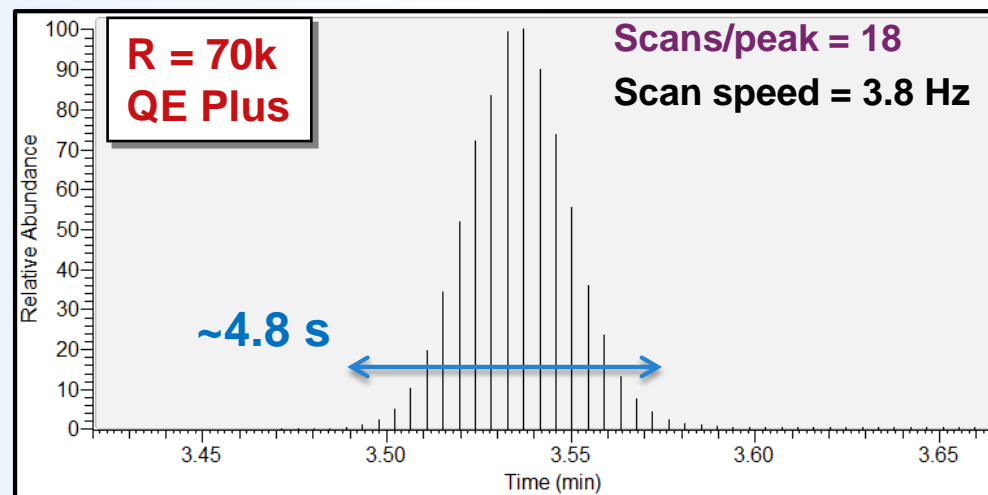
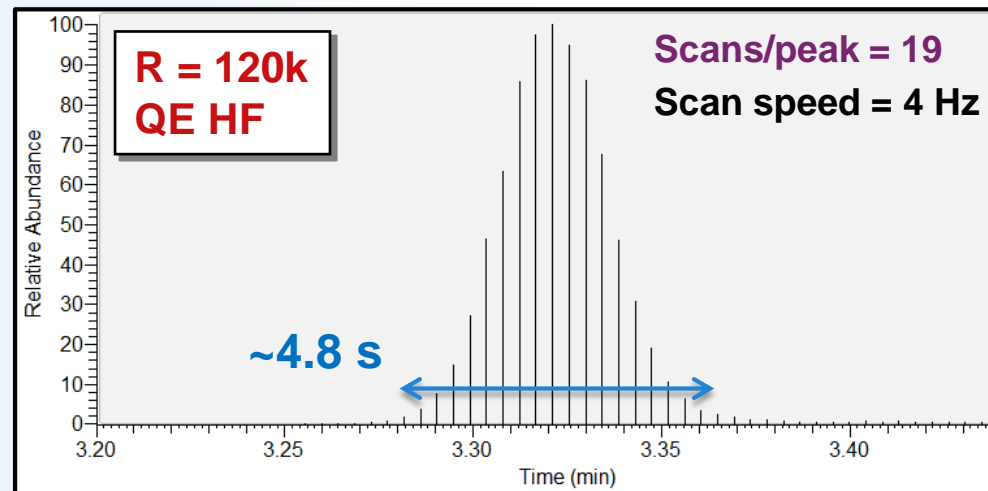
# Improved Metabolome Coverage with the Q Exactive HF MS



Ultra-High Field  
Orbitrap  
Q Exactive HF MS

Standard Orbitrap  
Q Exactive Plus MS

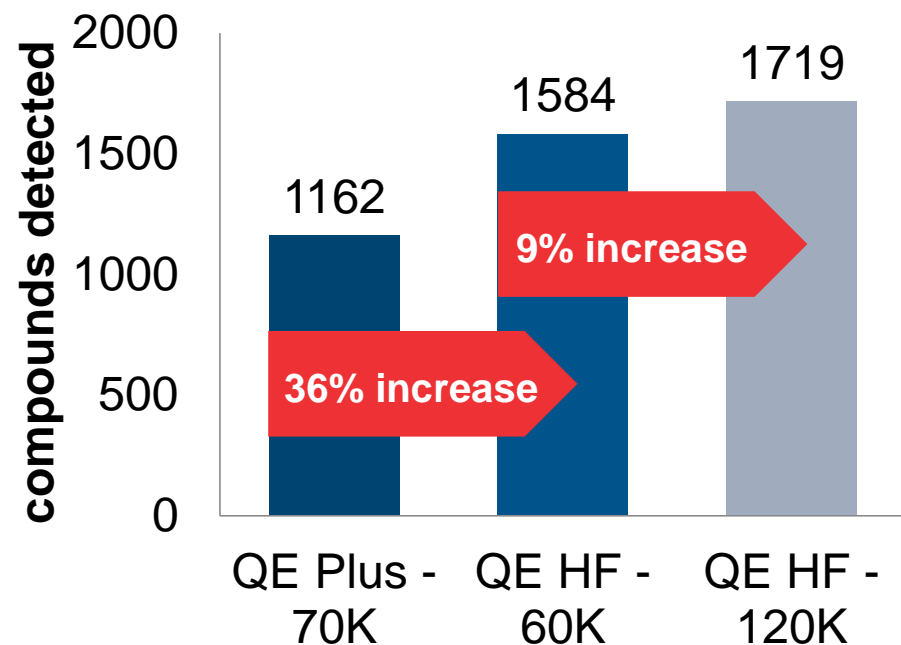
D8-Phenylalanine spiked in human plasma



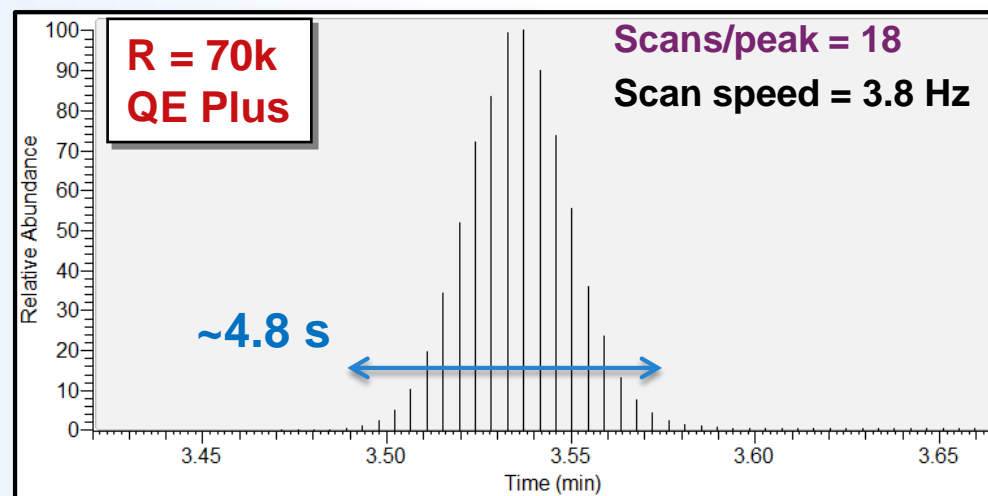
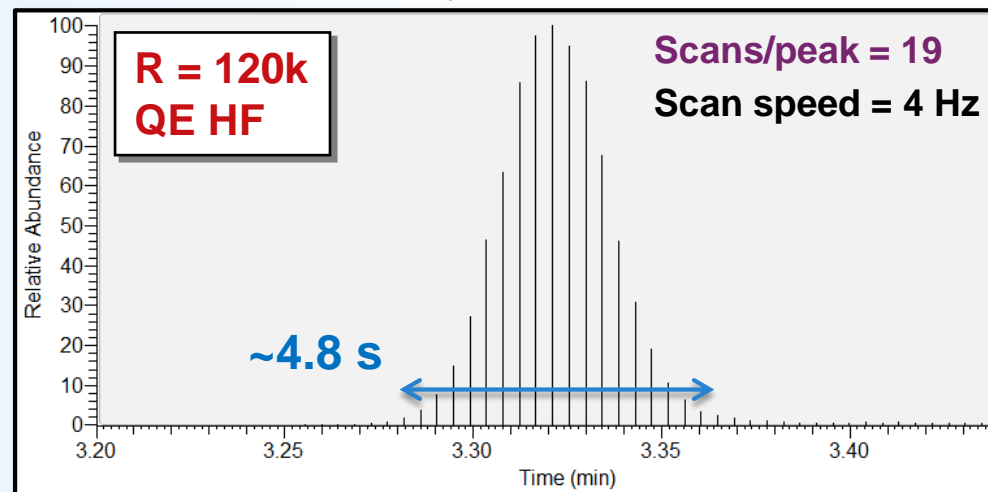


# Improved Metabolome Coverage with the Q Exactive HF MS

## Human plasma metabolites (positive mode)



## D8-Phenylalanine spiked in human plasma





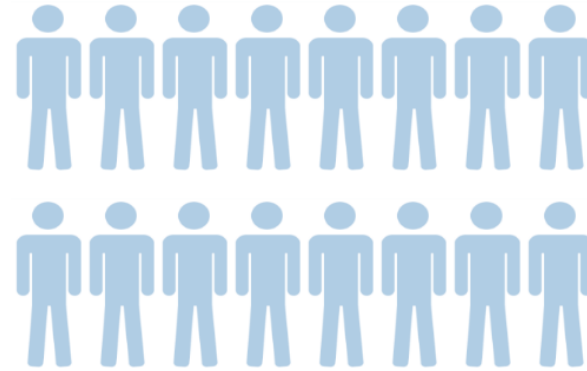
## **High Quality Data: Experimental Design for Discovery Metabolomics**

# Unknown Discovery Metabolomics Workflow

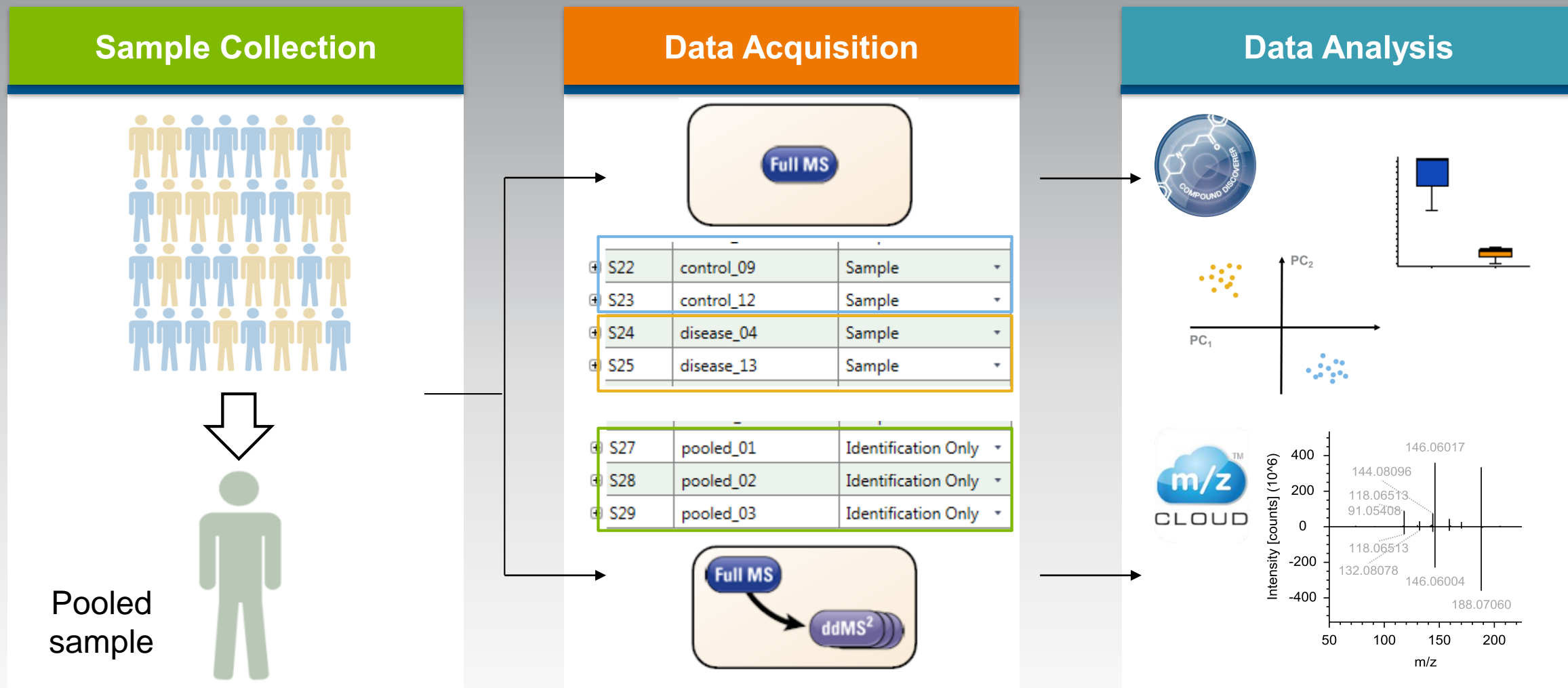
## Disease



## Matched Controls



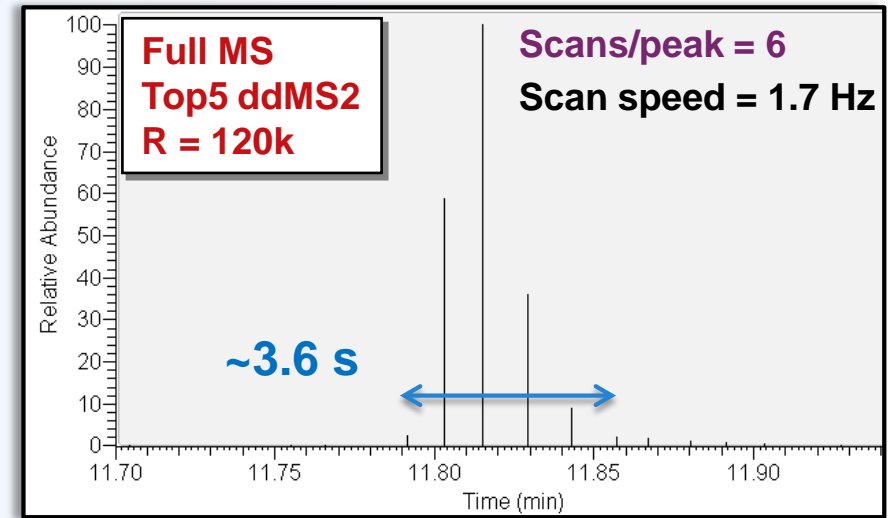
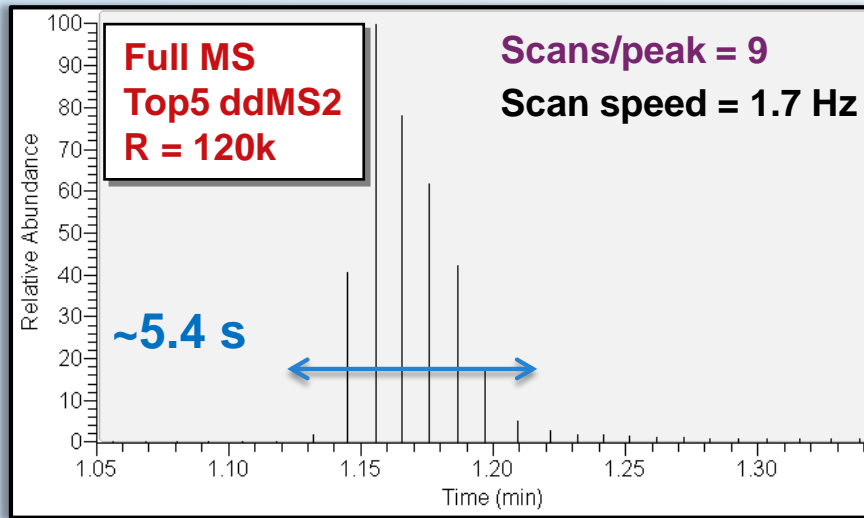
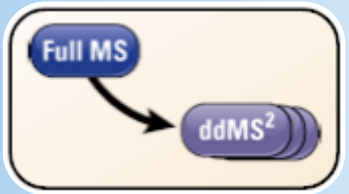
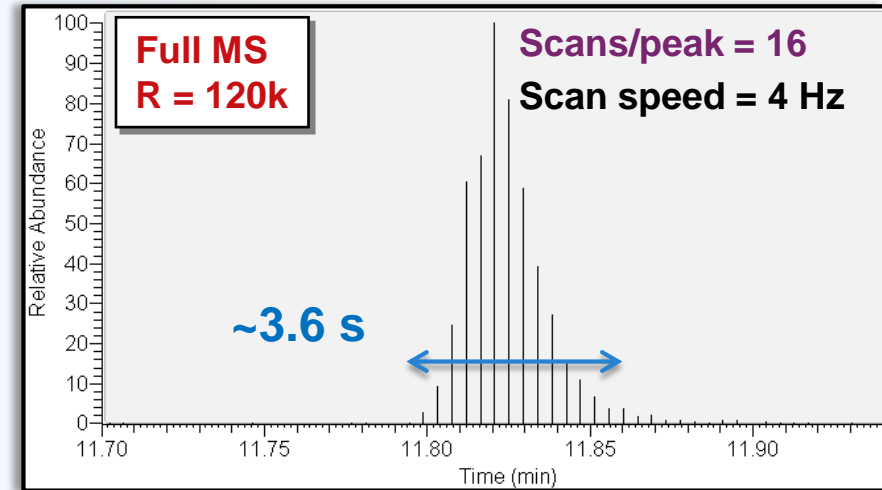
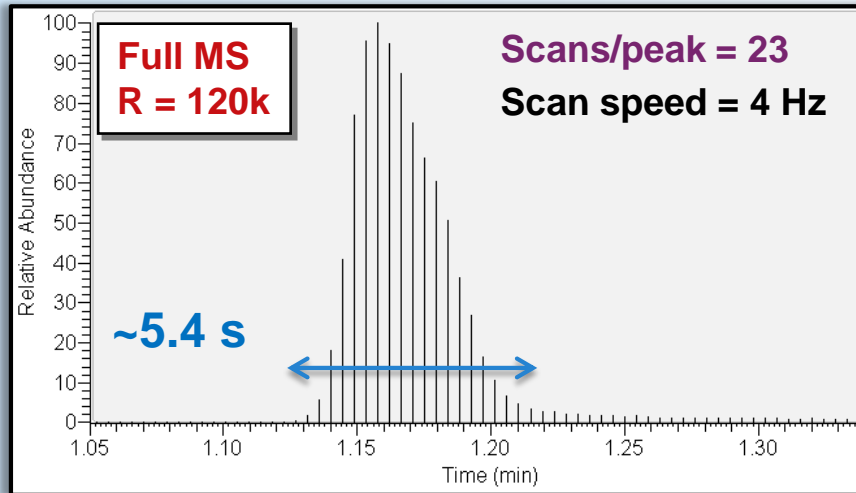
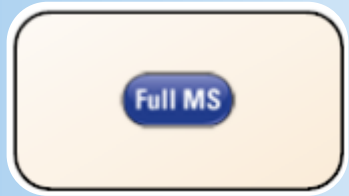
# Discovery Metabolomics Workflow



# Maximize Unknown Compound Detection with MS1 Only Acquisition

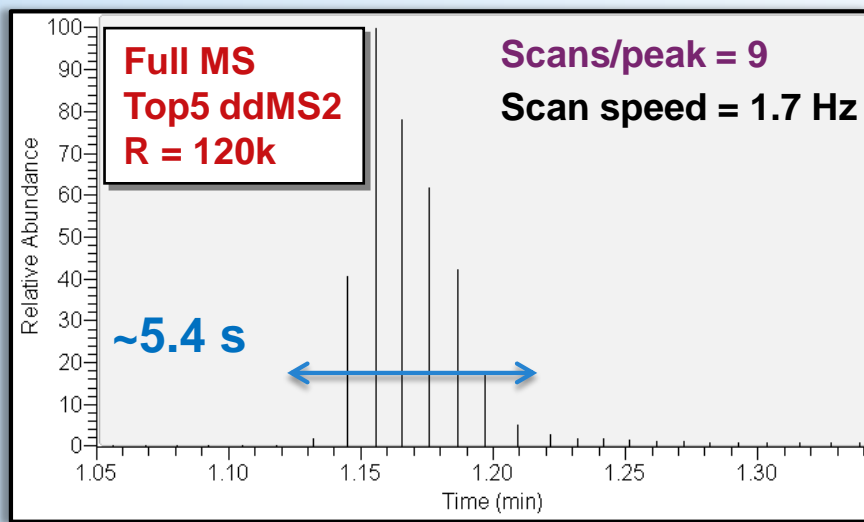
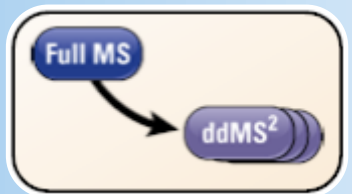
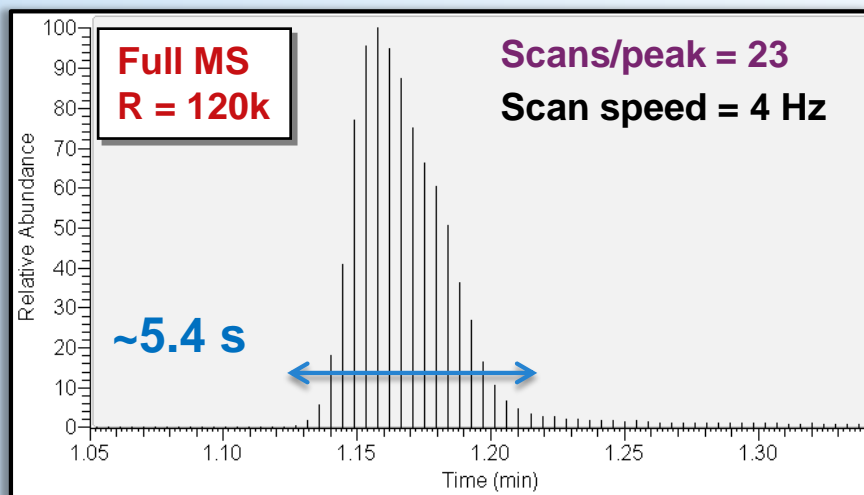
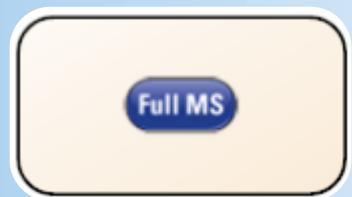
D8-Valine spiked in human plasma

Endogenous palmitoylcarnitine in human plasma

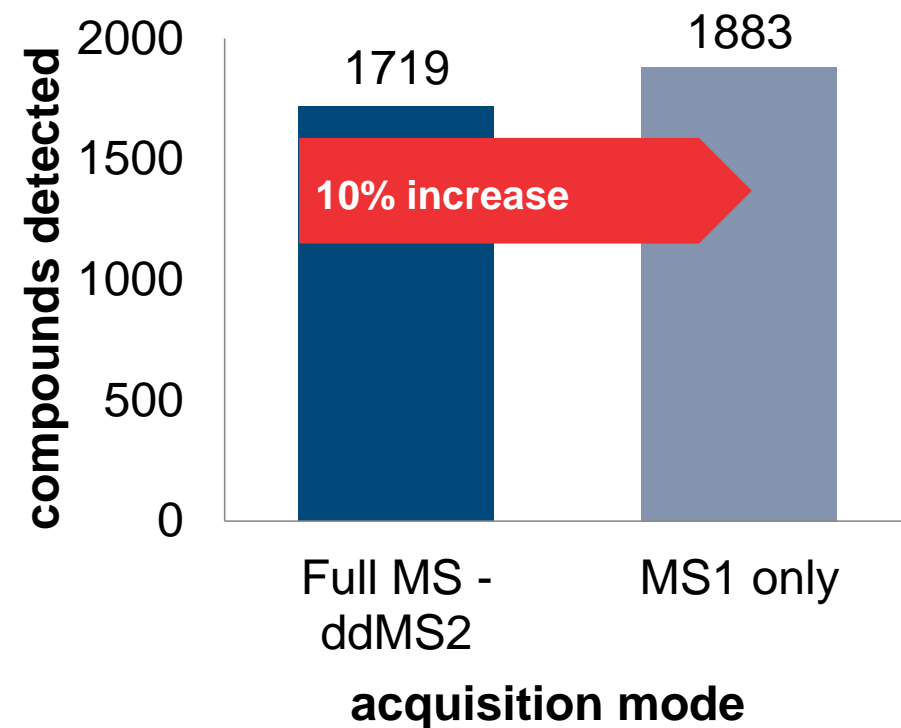


# Maximize Unknown Compound Detection with MS1 Only Acquisition

D8-Valine spiked in human plasma

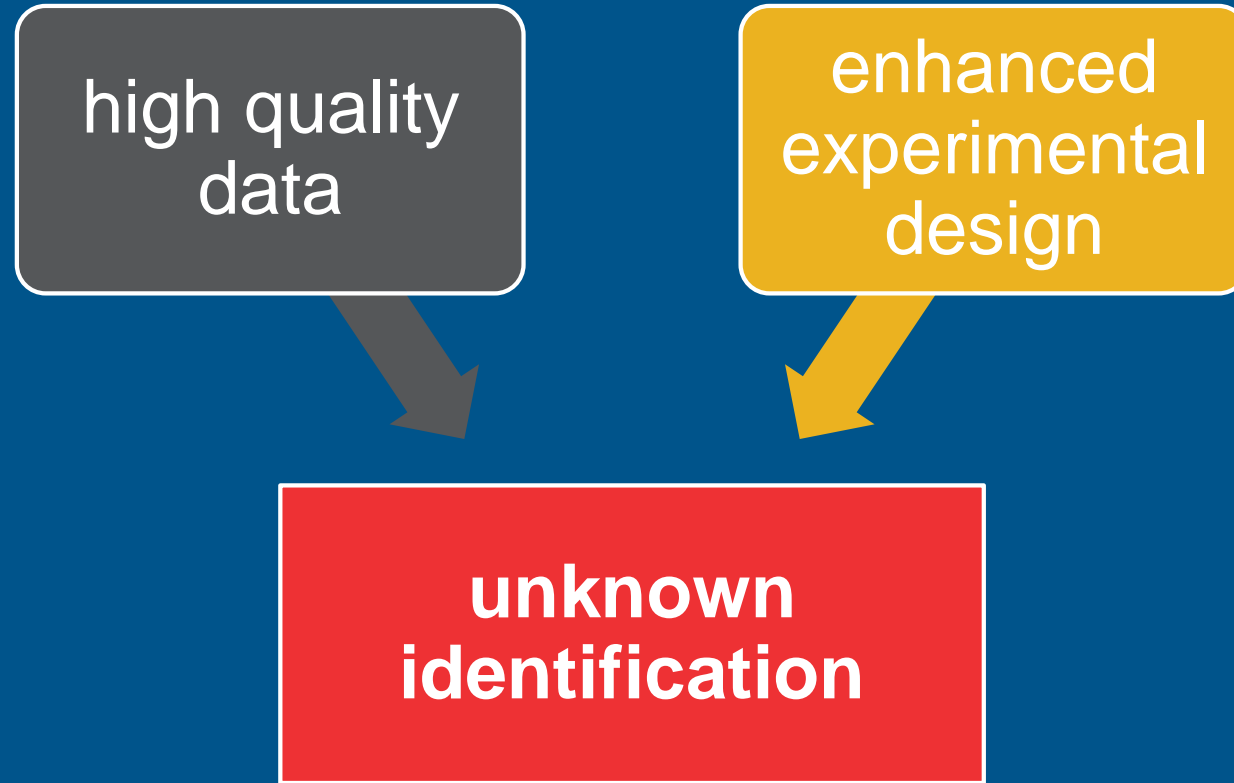
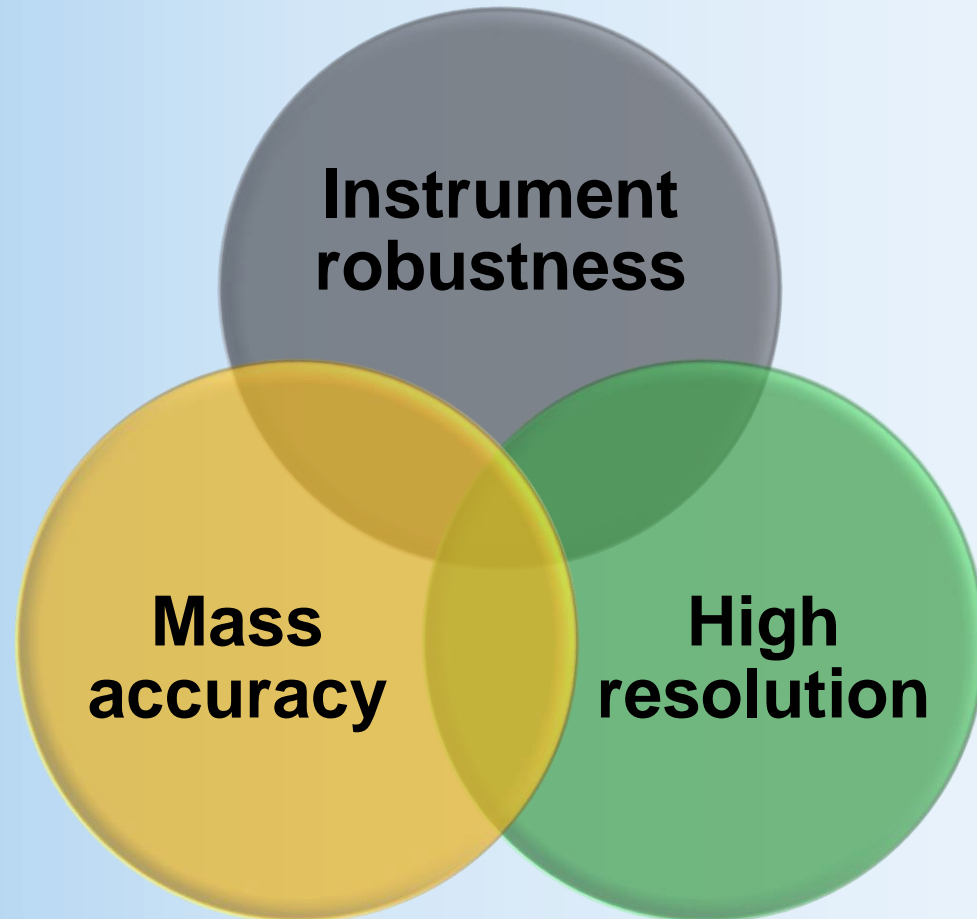


Human plasma metabolites  
(positive mode)





# High Quality Data Ensure High Quality Results





# Questions?