Setting a New Standard for Analytical Rigor and Confident Identifications in Metabolomics

Anas Kamleh, PhD

7th Scientific Conference of Polish Metabolomics Society

November 2020

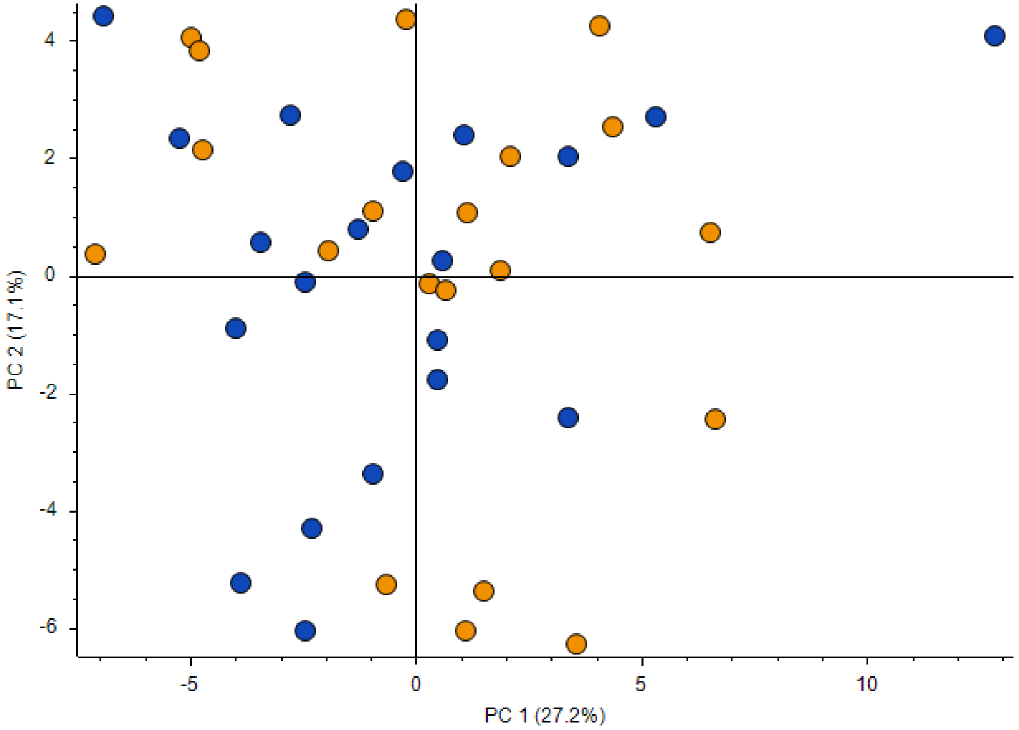
The world leader in serving science

Delivering a New Standard in Metabolomics

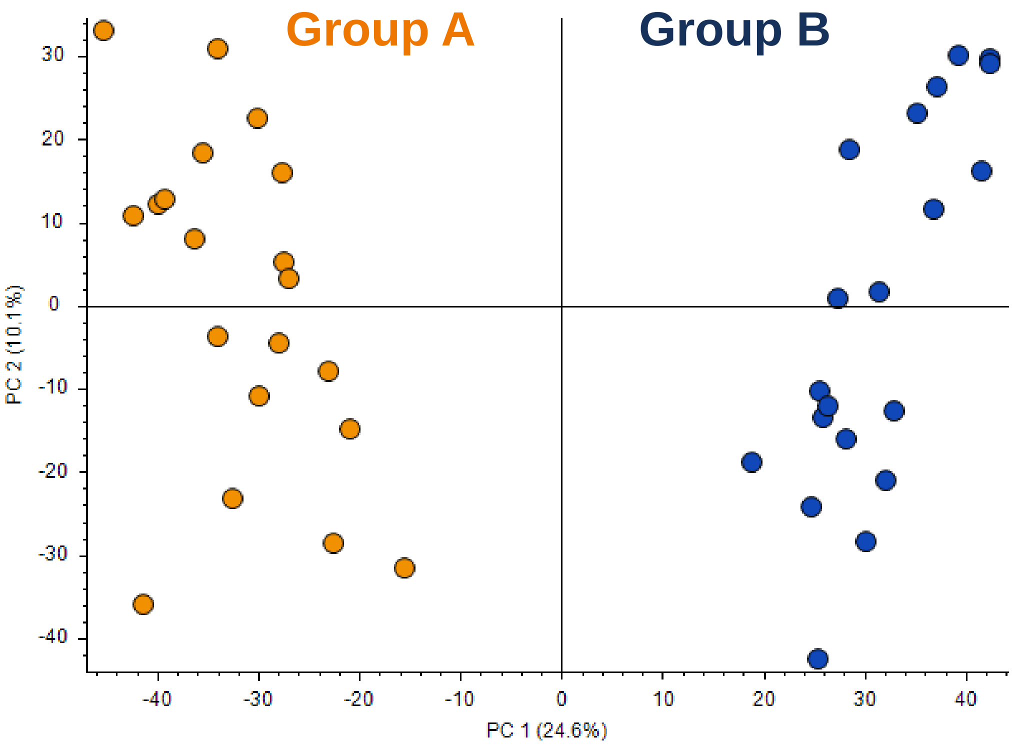
Challenge	Answer
Metabolomics entails comprehensive analysis	Ultra-High Resolution MS
Large scale analysis demands analytical rigor	Thermo Scientific™ Orbitrap Exploris™ 240 MS
Biological interpretation requires confident identification	Semi-targeted Approach

Comprehensive Metabolite Profiling Enables Differentiation

Targeted Analysis (60 metabolites)



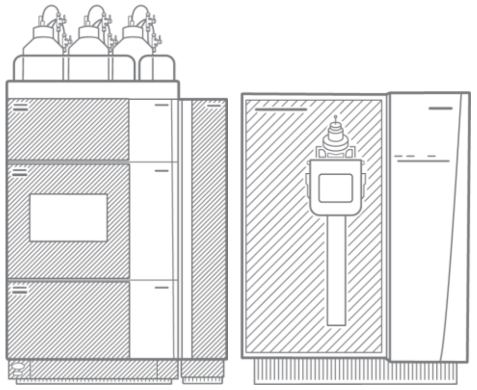
Untargeted Analysis



Orbitrap Exploris 240 MS
40 Mouse Plasma Extracts

Semi-targeted Metabolomics to Detect Knowns and Discover Unknowns

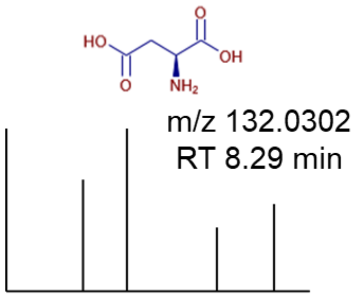
LC-UHRMS



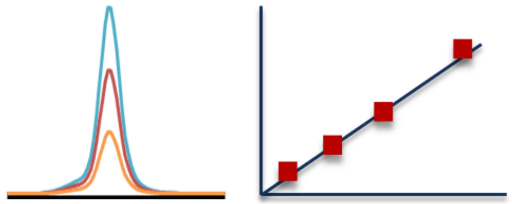
Known

Accurate Mass
Retention Time
Isotope Fine Structure
Spectral Library Match

Spectral Library Creation



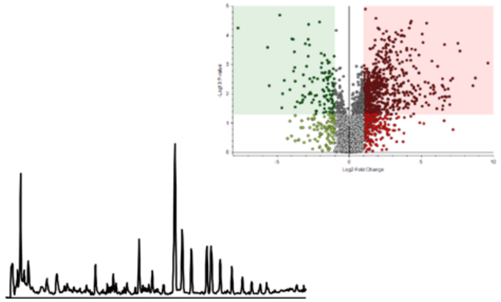
Targeted Quantitation
Targeted Profiling



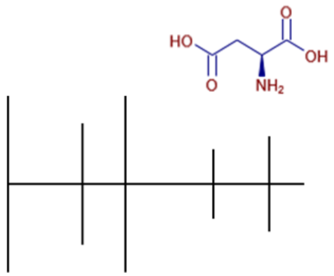
Unknown

Accurate Mass
Isotope Fine Structure
Formula Prediction
Database Search
Spectral Library Match
In silico Prediction
Mass List Search

Relative Quantitation



Compound Annotation



Analytical Requirements for Semi-targeted Analysis

High Resolution

Accurate Mass

Analytical Robustness

Intelligent Data Acquisition

Thermo Scientific Orbitrap Exploris 240 Mass Spectrometer

Leading Performance

With leading performance, application versatility, and operational simplicity, the Thermo Scientific Orbitrap Exploris 240 mass spectrometer fast tracks your path to high-confidence discovery and identification.

Application Modes: Small Molecule, Peptide, Intact Protein

Mass range: 40 - 6000 *m/z* (8000 *m/z* optional)

Quad isolation: down to 0.4 u & up to *m/z* 2500

Max resolution: 240,000 at *m/z* 200

Mass Accuracy: 3 ppm RMS ext., 1 ppm RMS int., Thermo Scientific™ EASY-IC™ Source

Polarity Switching: 125 ms

Dissociation: Higher energy Collisional Dissociation (HCD)

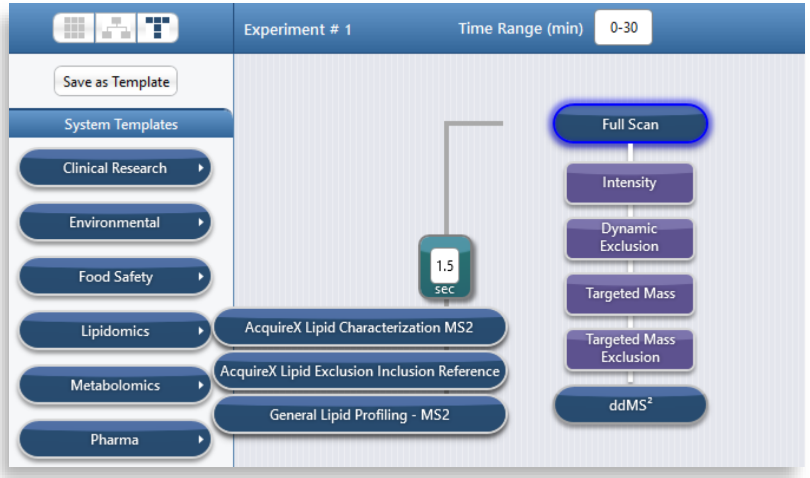
Scan Analysis: dd-MS2 Top N, Thermo Scientific™ AcquireX Intelligent Acquisition

Orbitrap Exploris Platform – Exceptional Simplicity and Usability

One Click Calibration

Positive & negative polarity
Low mass calibration

Method Templates



Software Harmonization

Thermo Scientific^{TM} TSQ Triple Quad MS

Thermo Scientific^{TM} Orbitrap Exploris^{TM} MS

Thermo Scientific^{TM} Orbitrap Tribrid^{TM} MS

Smaller Footprint

Thermo Scientific™ Orbitrap Exploris™ 120

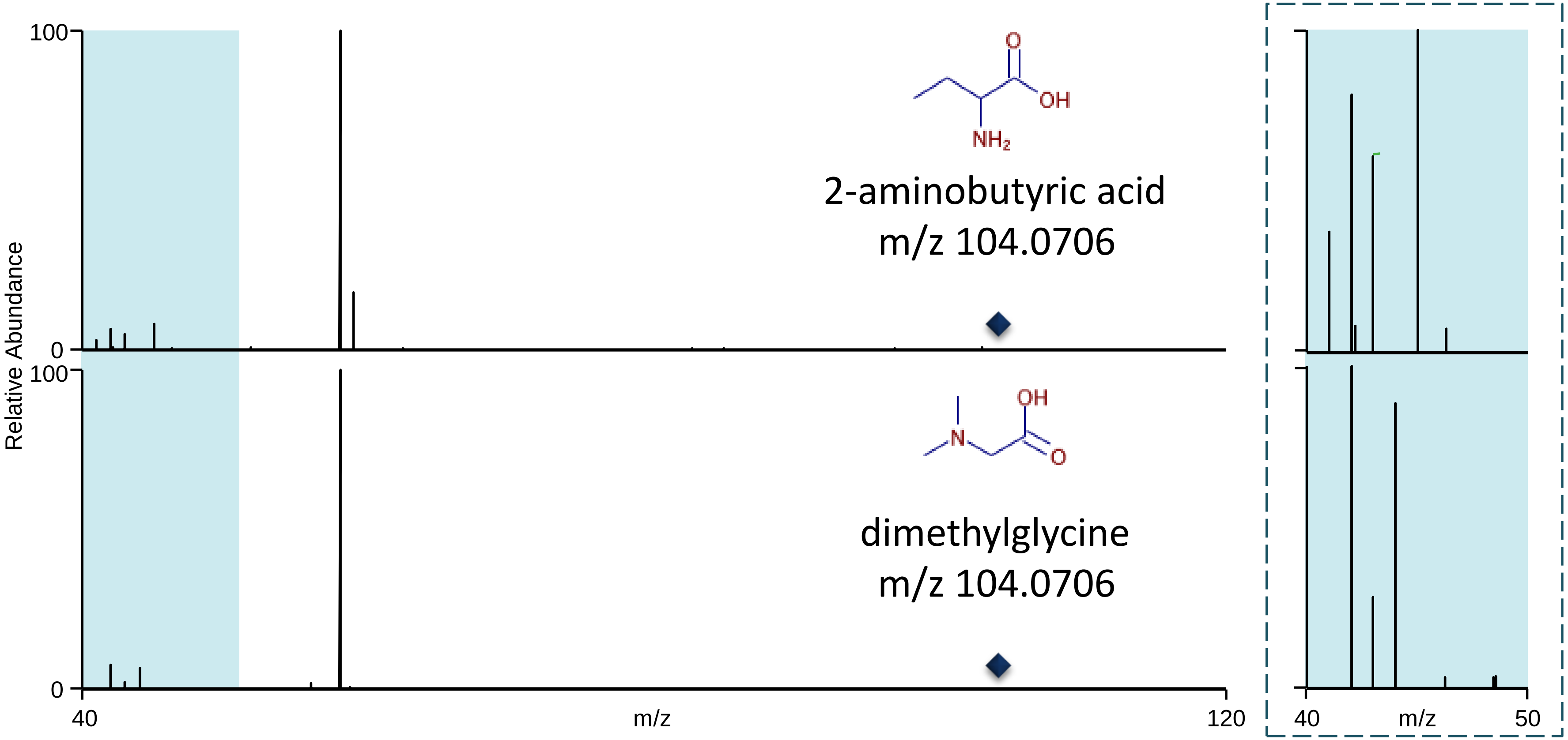
Thermo Scientific™ Q Exactive™ HF

Confident Identification | Increased Specificity of Isomers

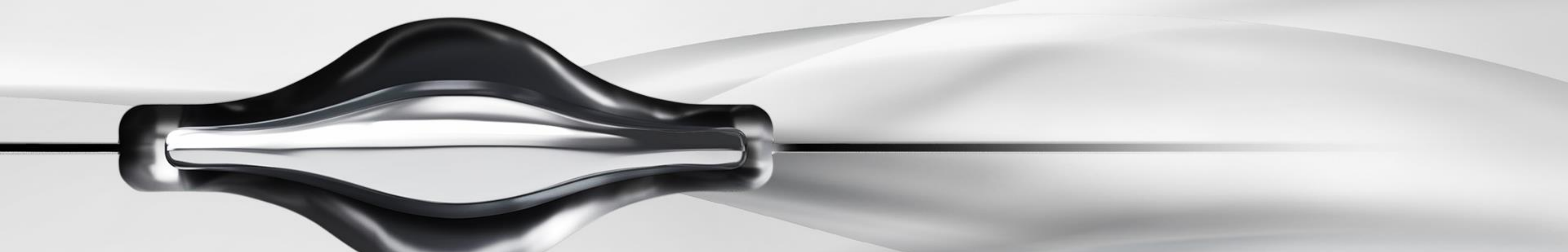
Extended Low Mass Range Starting at *m/z* 40

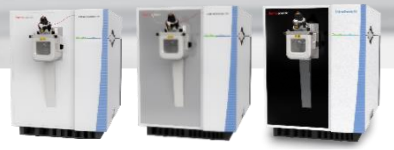
MS^2
30K Resolution

Five additional fragment ions detected



Orbitrap Exploris 240 MS
Neat Standard Reference Mixture

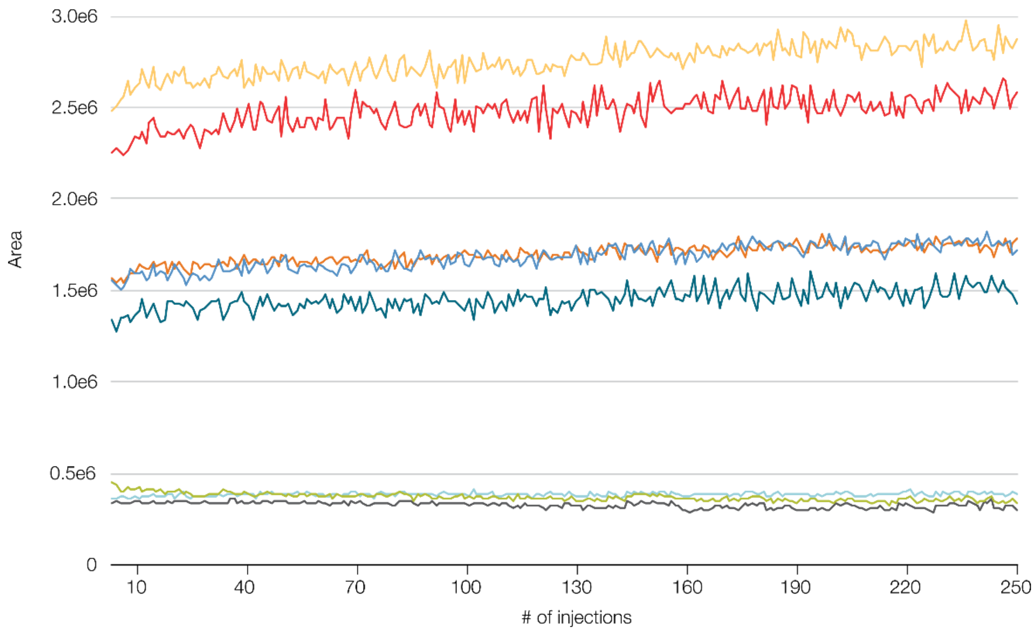




Ruggedness for Seamless Data Acquisition

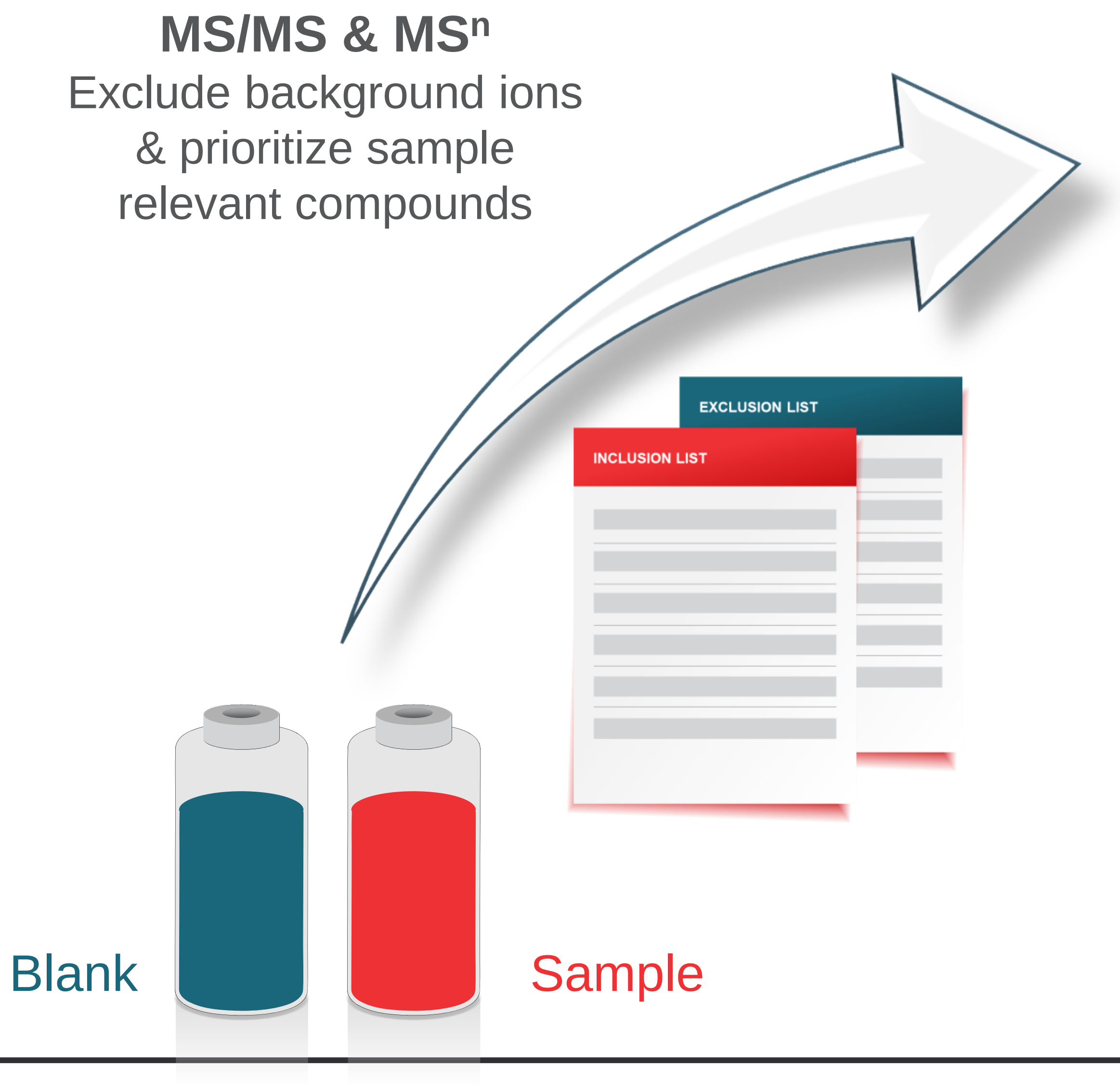
- Long term system robustness for the analysis of pesticides in **olive oil matrix** is demonstrated over **250 injections**
- Acquired with **polarity switching**
- Excellent peak area **RSDs of <6%**, with an **average of 2.3%**

Peak area stability over 250 injections



	Compound name	Average %RSD for 250 injections
🟧	Atrazine	1.6
🟦	Azoxystrobin	2.0
⬛	Bentazone neg	2.5
🟦	Bromoxynil neg	1.2
🟥	Carbendazim	2.0
🟨	Cycluron	1.6
🟦	Dicrotophos	2.2
🟩	Fluazinam neg	5.5

Thermo Scientific AcquireX Acquisition | Collect More Meaningful Data

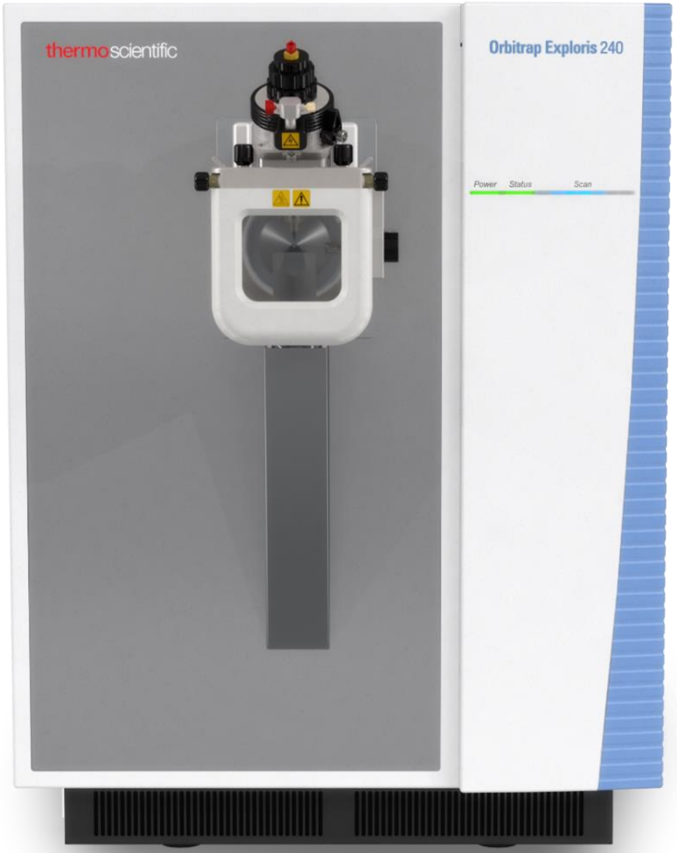


Knowledge Driven Real-time Precursor Selection

- Fully automated to save time
- Avoid unrelated background ions and remove redundancy
- Fragment more sample relevant compounds
- Go deeper by fragmenting low abundant ions

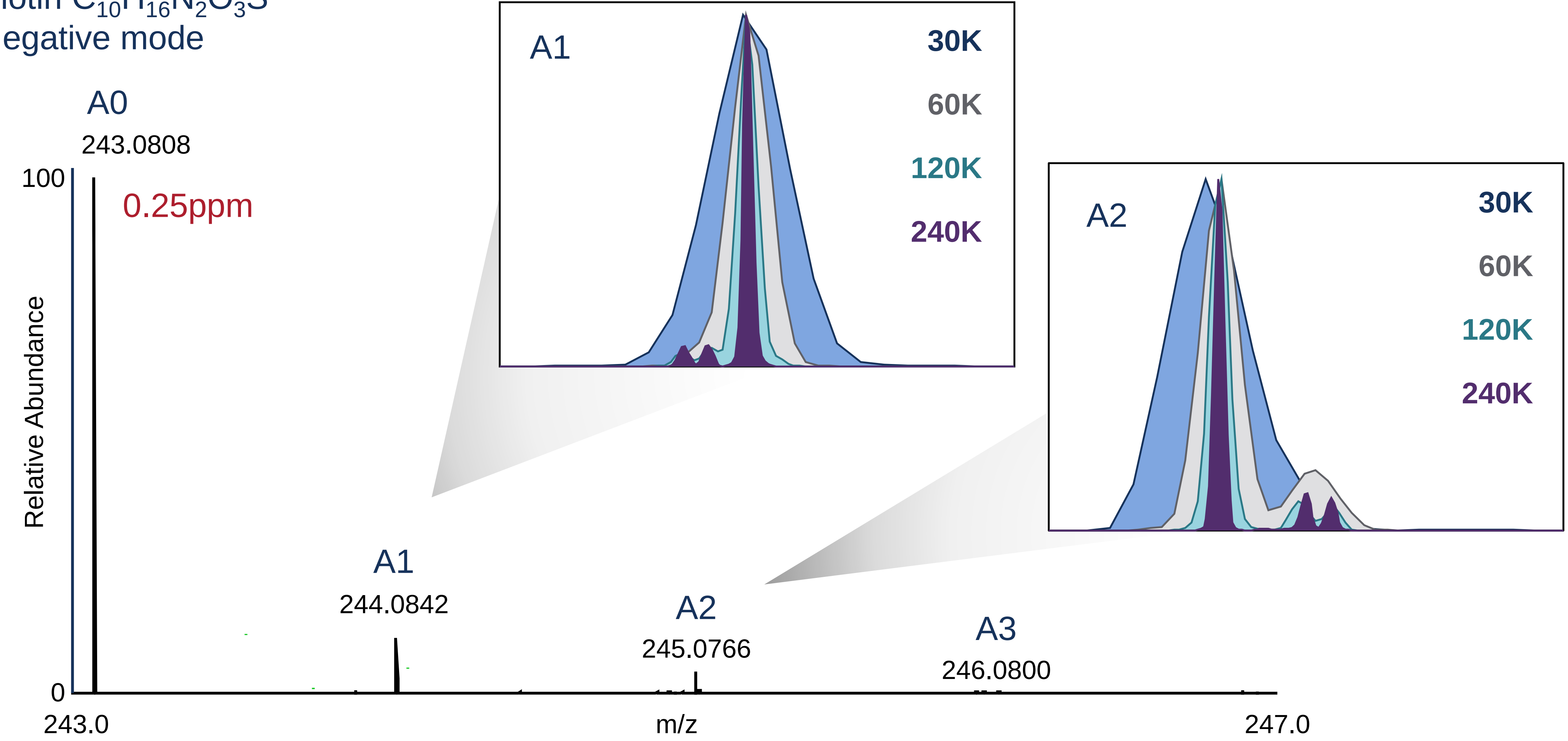
Orbitrap Exploris 240 MS

Instrument Performance for Metabolomics Applications



High Resolution for Fine Isotope Pattern Determination

Biotin $C_{10}H_{16}N_2O_3S$
Negative mode



High Resolution for Fine Isotope Pattern Determination

Biotin $C_{10}H_{16}N_2O_3S$
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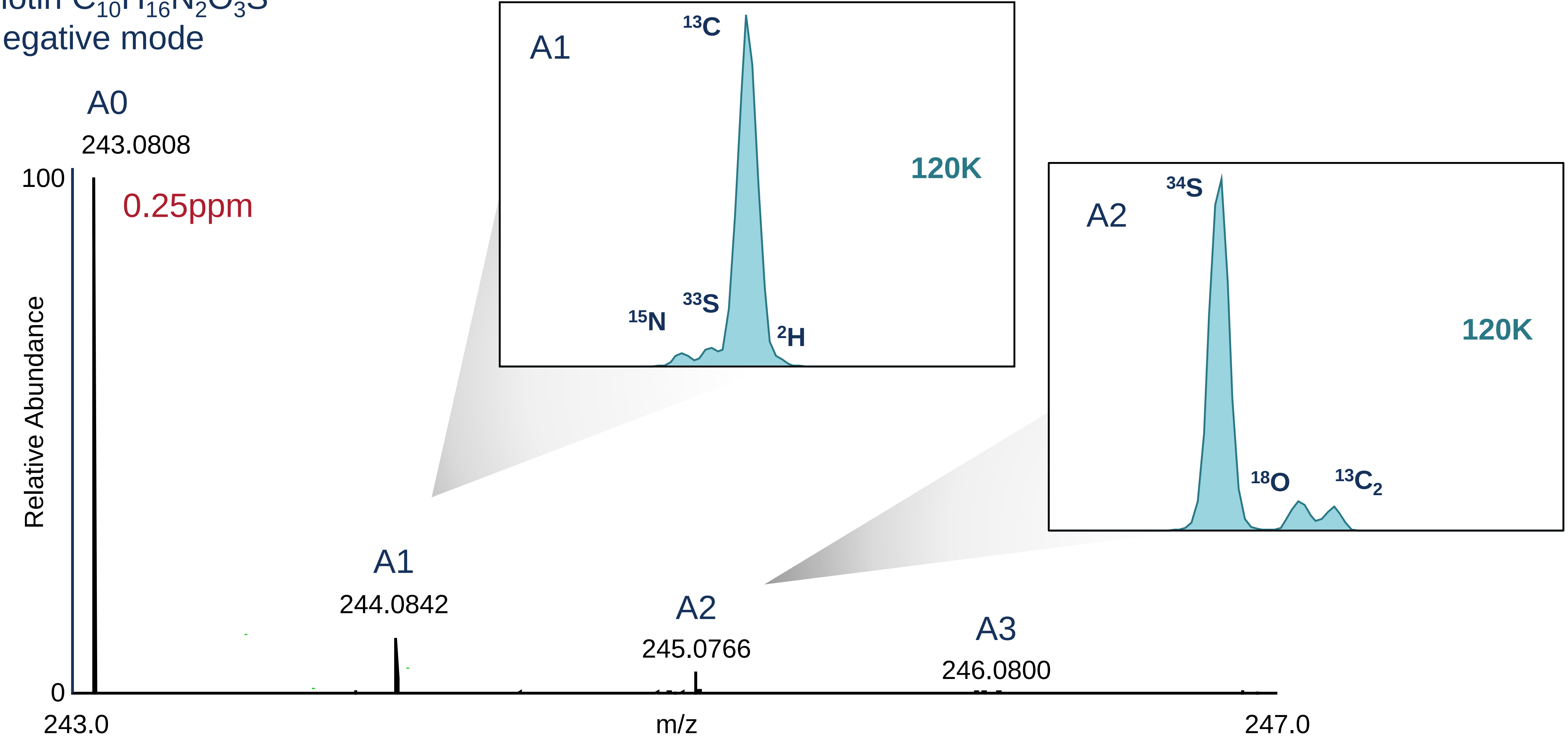
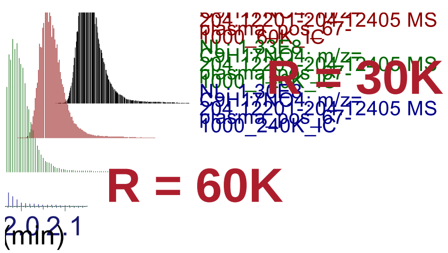


Resolution vs Scan Speed

NIST SRM 1950
Human Plasma
Reference
C18, 15min gradient

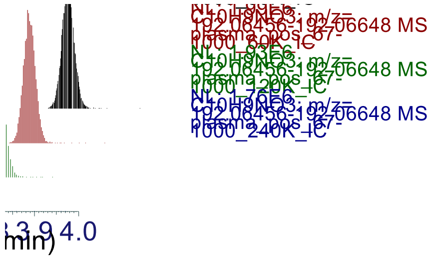


R = 30K

R = 60K

R = 120K

R = 240K



Scans/peak = 88
Scan speed = 12 Hz

Scans/peak = 54
Scan speed = 6.8 Hz

Scans/peak = 29
Scan speed = 3.6 Hz

~9 sec ⟷

Scans/peak = 13
Scan speed = 1.7 Hz

Scans/peak = 59
Scan speed = 12 Hz

Scans/peak = 30
Scan speed = 6.8 Hz

Scans/peak = 19
Scan speed = 3.6 Hz

~4.8 sec ⟷

Scans/peak = 7
Scan speed = 1.7 Hz

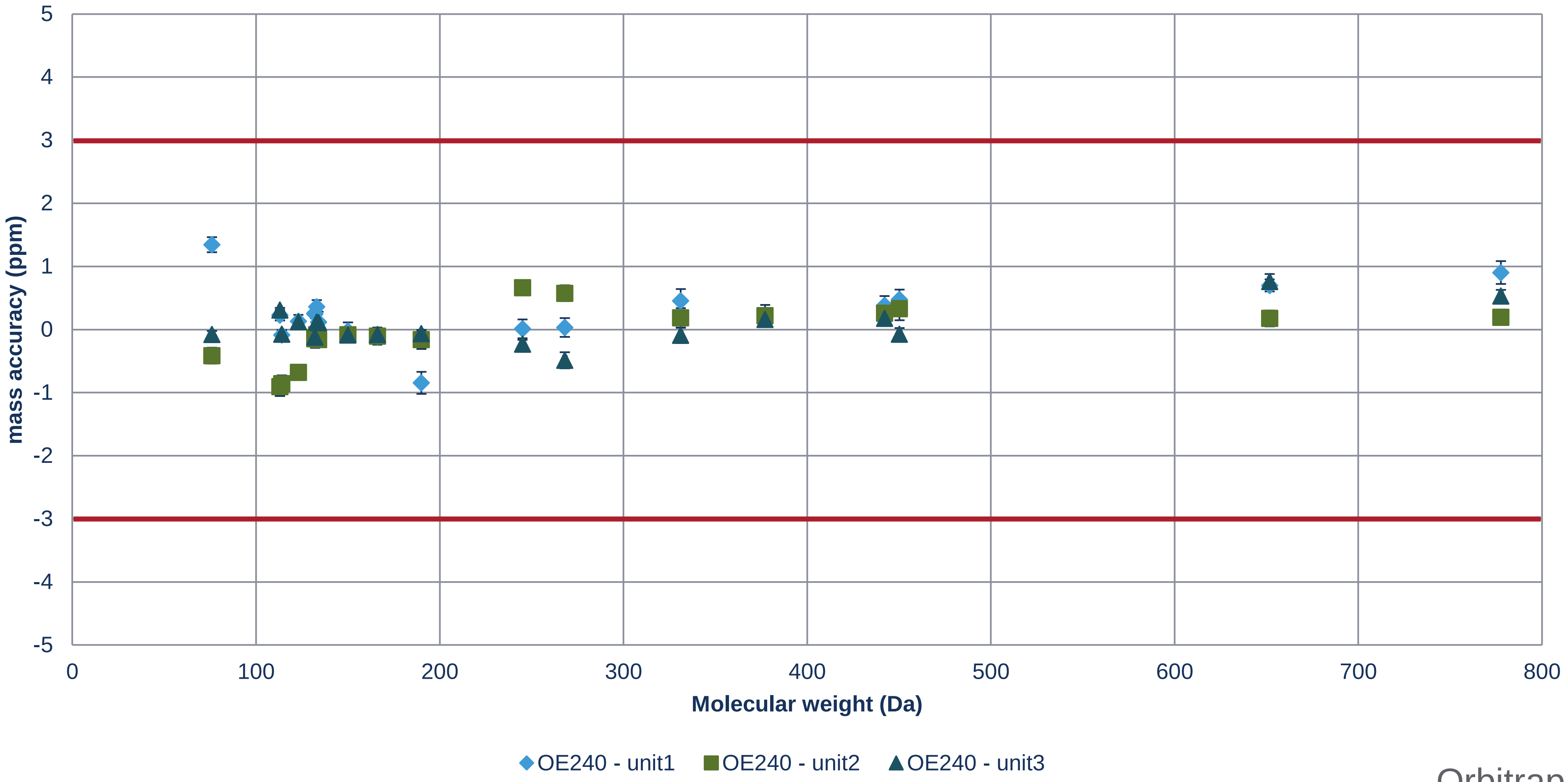
Acetylcarnitine
$C_9H_{17}NO_4$, *m/z* 204.1230

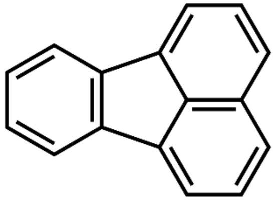
5-HIAA
$C_{10}H_9NO_3$, *m/z* 192.0655

ThermoFisher SCIENTIFIC

Exceptional Mass Accuracy with Thermo Scientific EASY-IC Option

Mass Accuracy Within 6 Days of Calibration on 3 Different Instruments





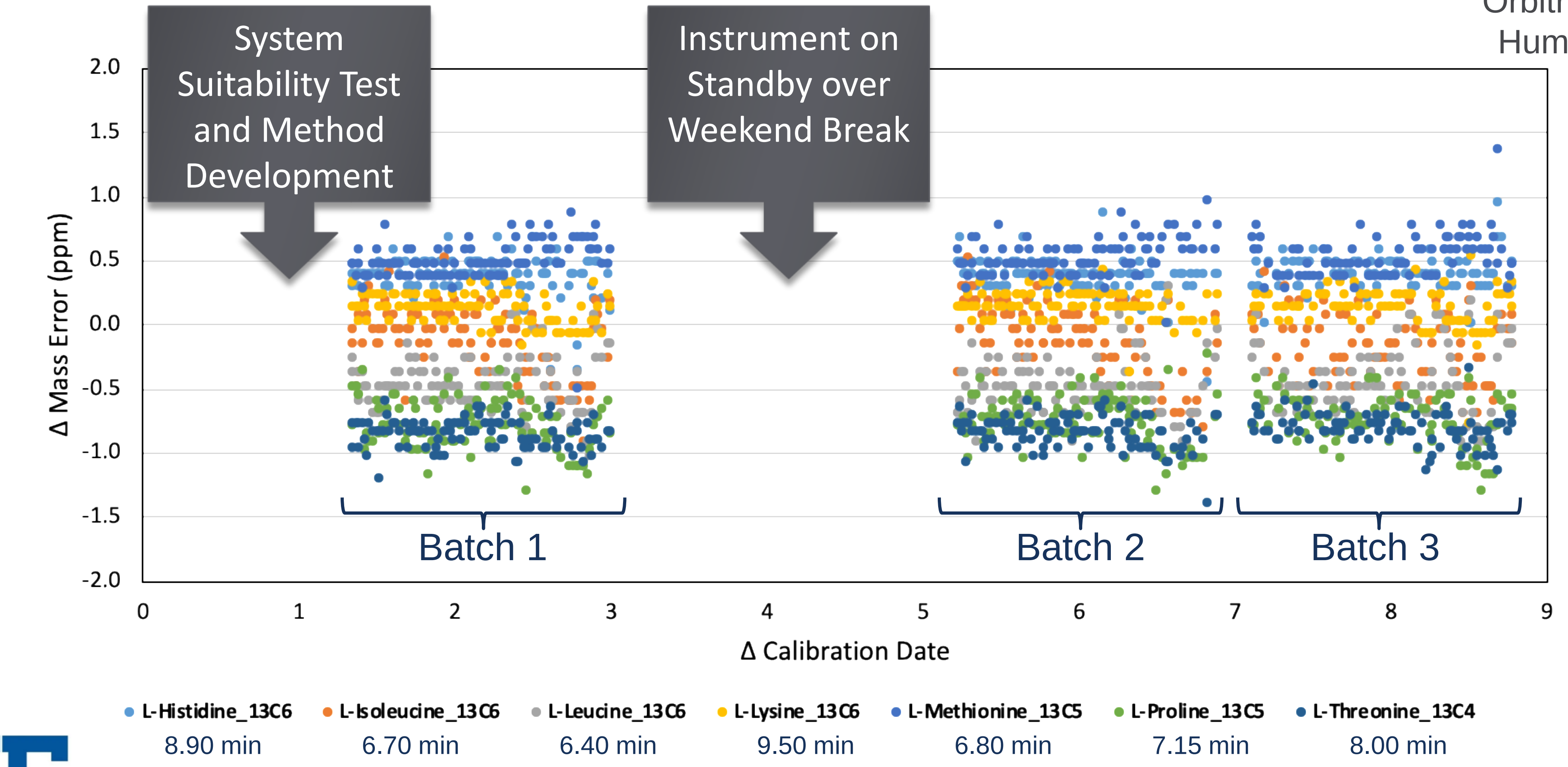
Fluoranthene
$C_{16}H_{10}$
MW 202.07825

Orbitrap Exploris 240 MS
Neat Standard Reference Mixture
Positive Polarity

Exceptional Instrument Performance

Outstanding Mass Accuracy Across 300 Injections, 9 Days Post Calibration

Orbitrap Exploris 240 MS
Human Plasma Extracts
120K Resolution



System Suitability Test and Method Development

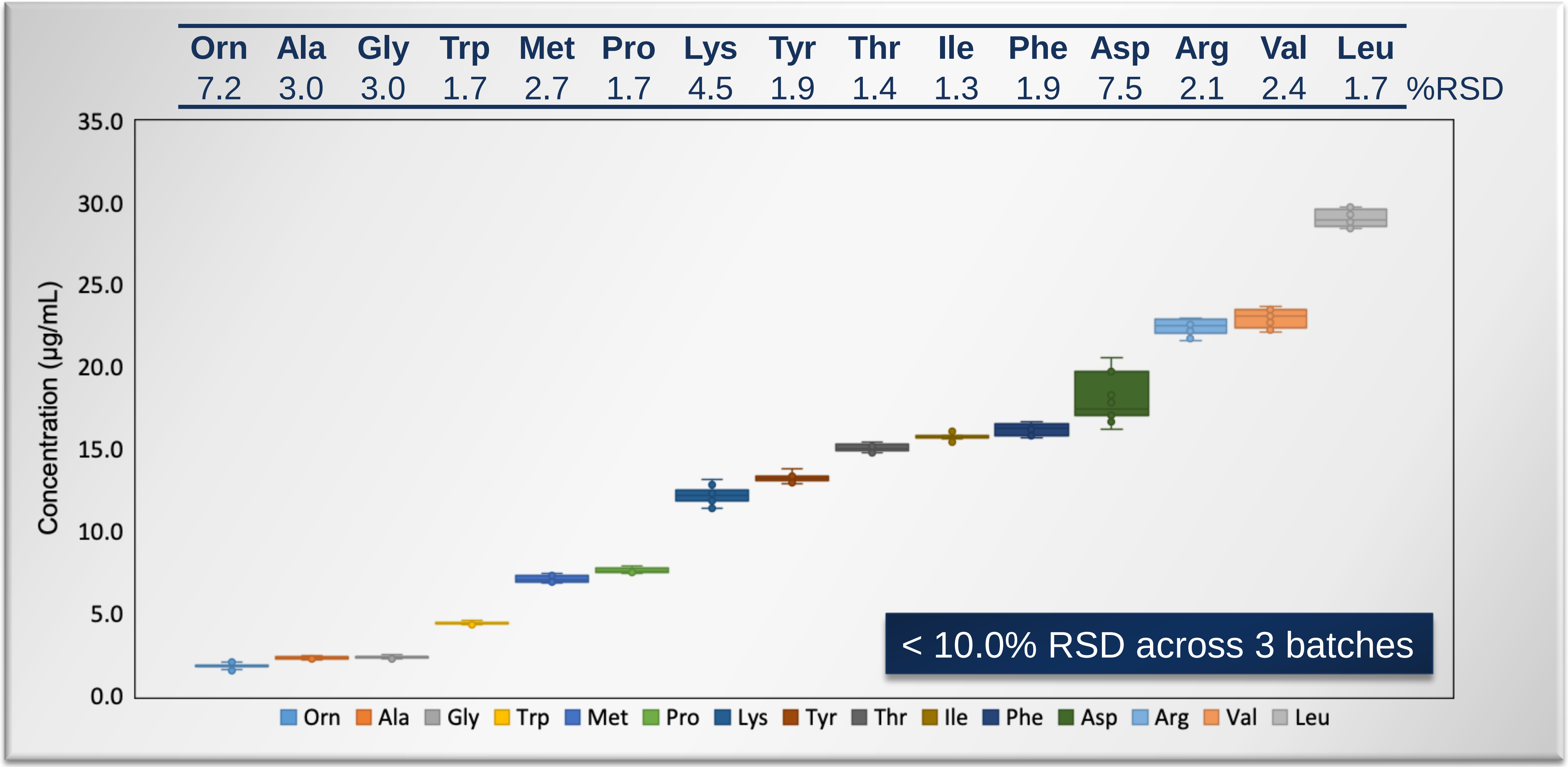
Instrument on Standby over Weekend Break

8.90 min
6.70 min
6.40 min
9.50 min
6.80 min
7.15 min
8.00 min

For General Laboratory Use Only – Not for Diagnostic Procedures.

Robust Quantitative Performance

Confidently Measure Known Compounds Over 9 Days of Consistent Instrument Response

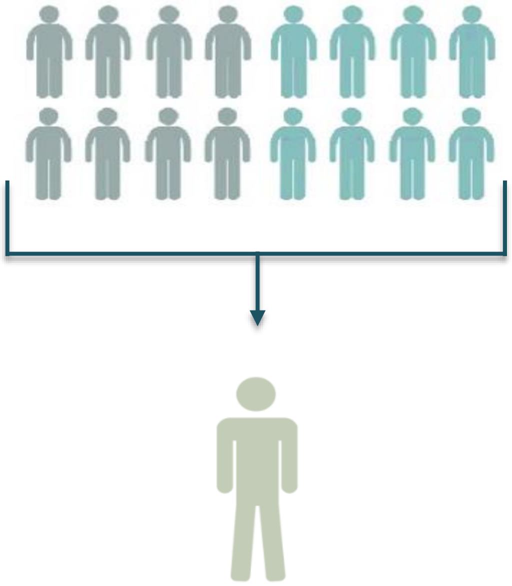


Orn	Ala	Gly	Trp	Met	Pro	Lys	Tyr	Thr	Ile	Phe	Asp	Arg	Val	Leu	
7.2	3.0	3.0	1.7	2.7	1.7	4.5	1.9	1.4	1.3	1.9	7.5	2.1	2.4	1.7	%RSD

< 10.0% RSD across 3 batches

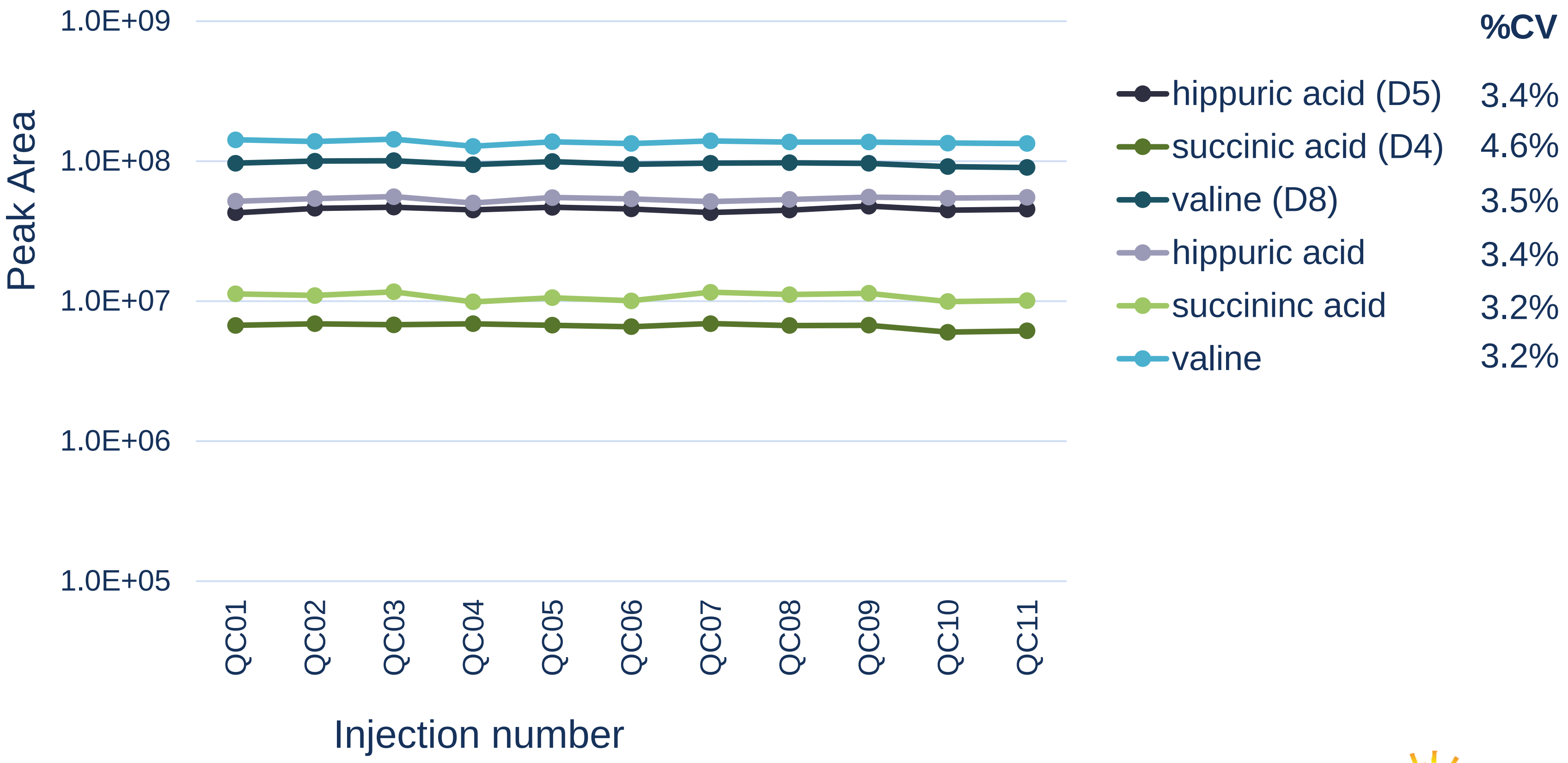
Reproducibility Throughout the Experiment | Pooled QC Samples

Comparison Groups



The pooled QC sample was injected repeatedly for every 15 experimental samples

Internal Standards and Corresponding Endogenous Metabolites



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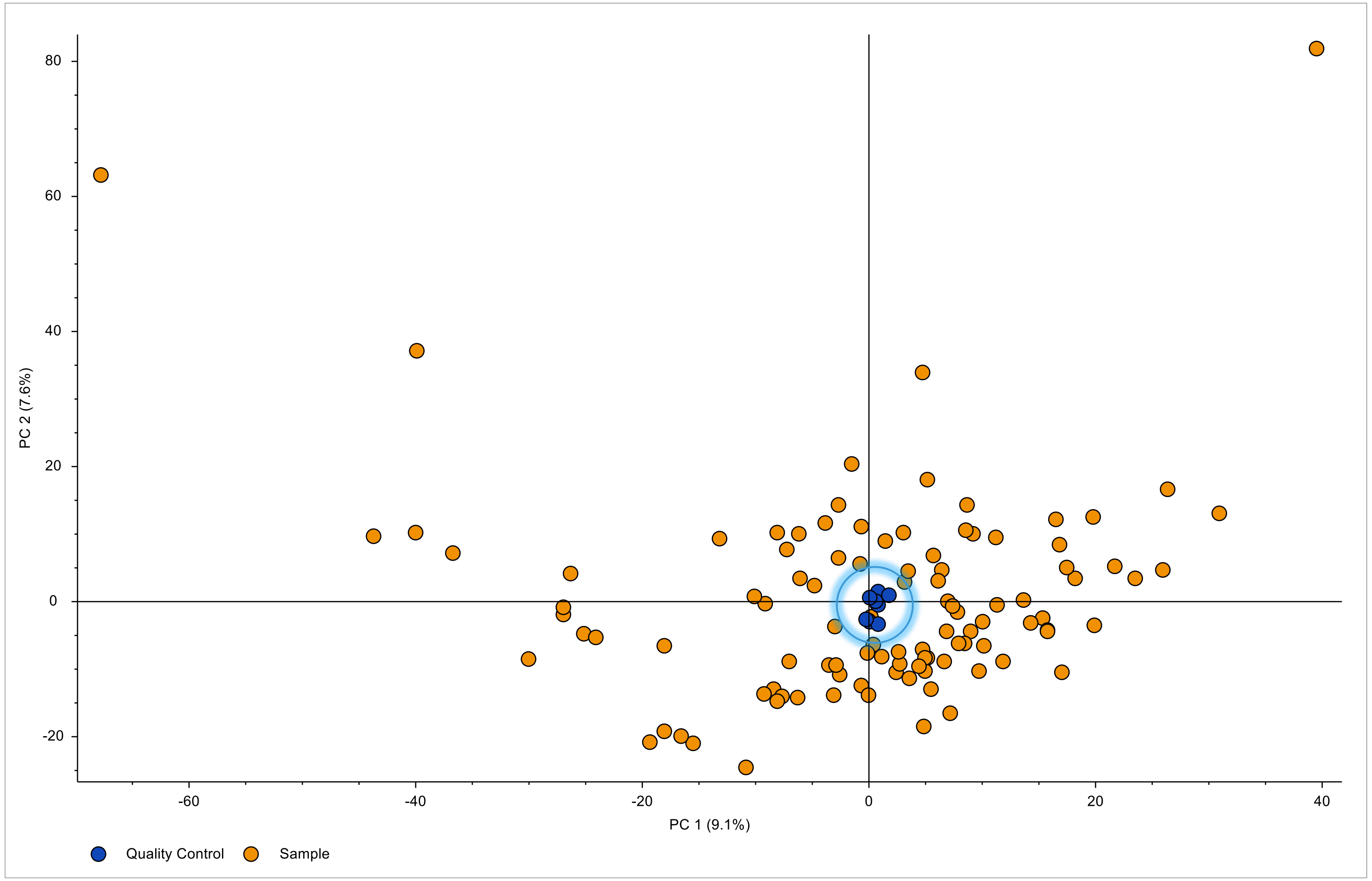
Confirming High Quality Data | Pooled QC Samples

Quality Control Samples to Assess Data Quality via PCA plot

- ✓ QC samples are highly correlated indicated by tight clustering
- ✓ QC samples are clustered at the center of all experimental samples
- ✓ QC samples are reproducible and represent all experimental samples



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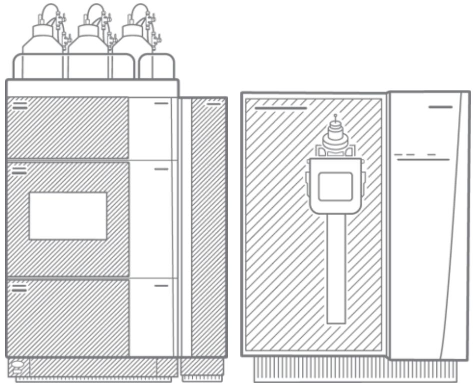
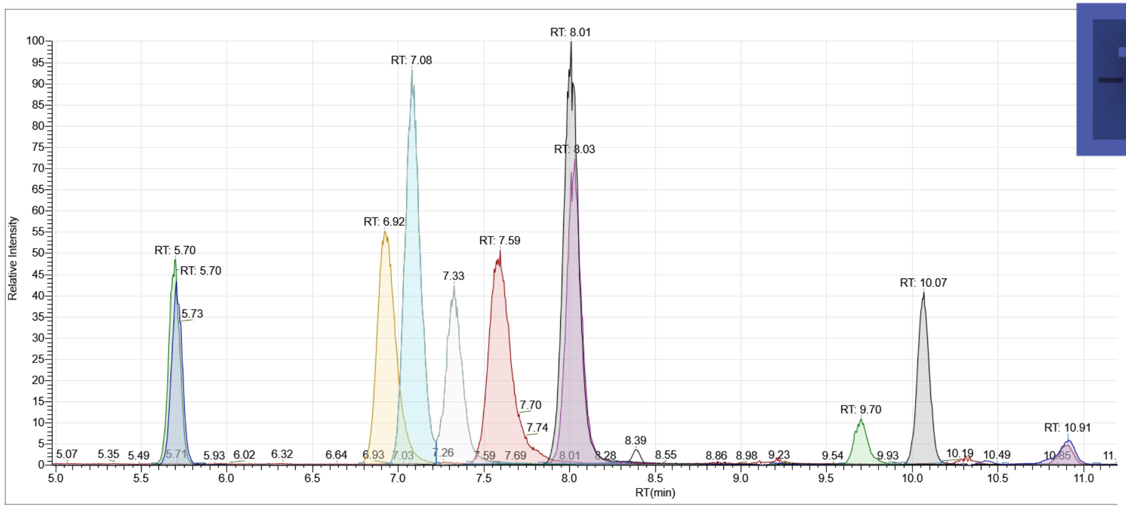
Semi-targeted Metabolomics to Detect Knowns and Discover Unknowns

LC-MS



Known

Accurate Mass
Retention Time
Isotope Fine Structure
Spectral Library Match



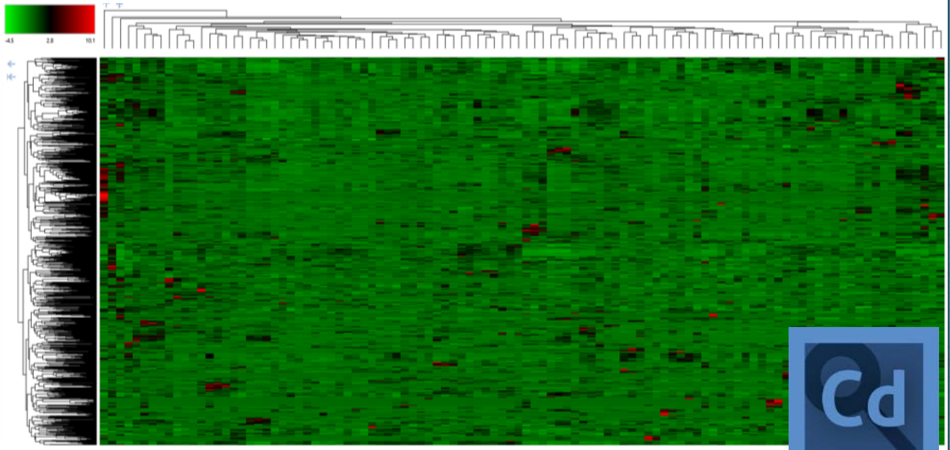
Accurate Mass
Isotope Fine Structure
Formula Prediction
Database Search
Spectral Library Match
In silico Prediction
Mass List Search

Unknown

Unbiased Peak Detection

>121,000 unique features
2,707 unique compounds
2,511 annotated
100 identified

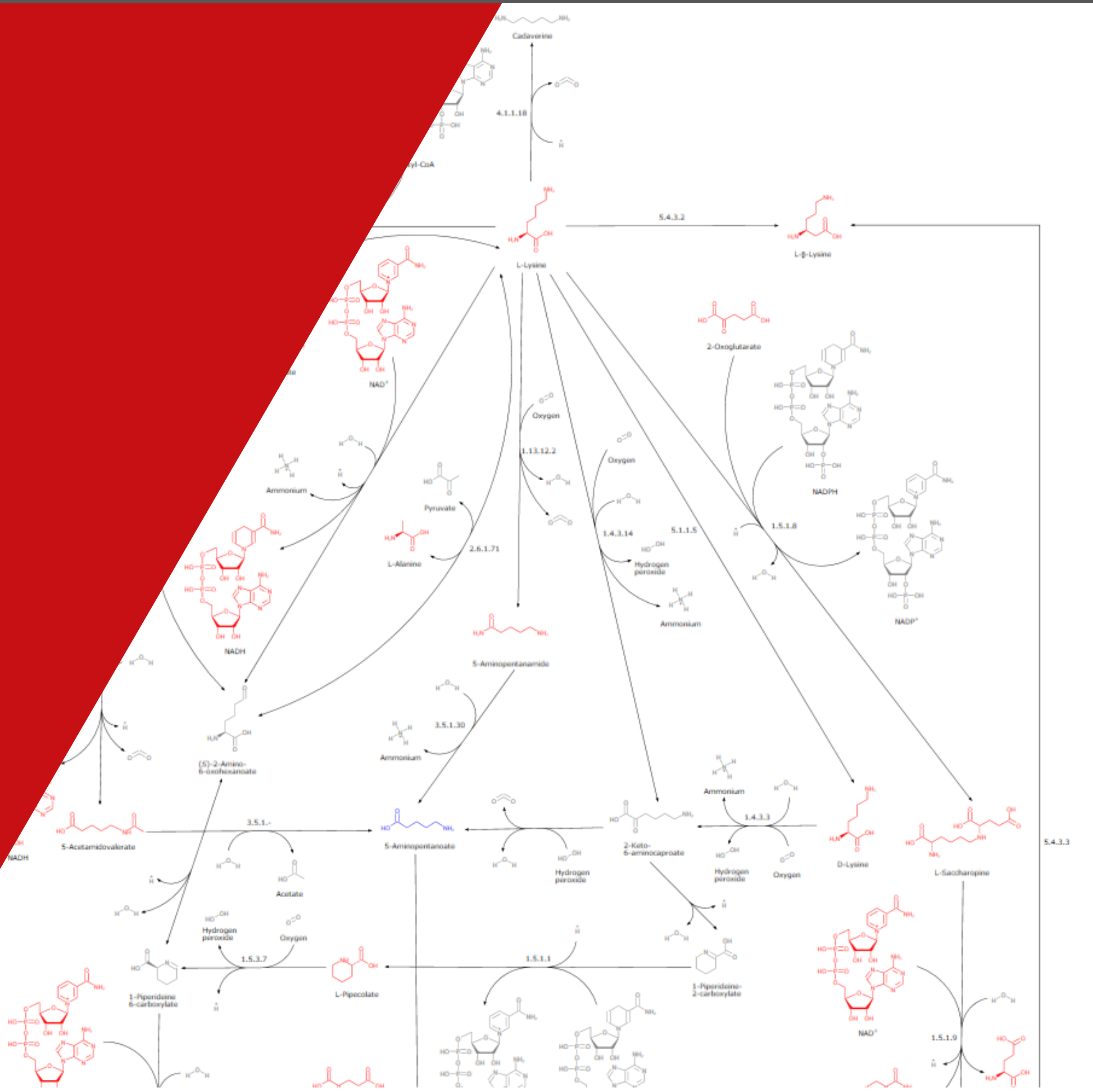
Hierarchical Clustering Analysis



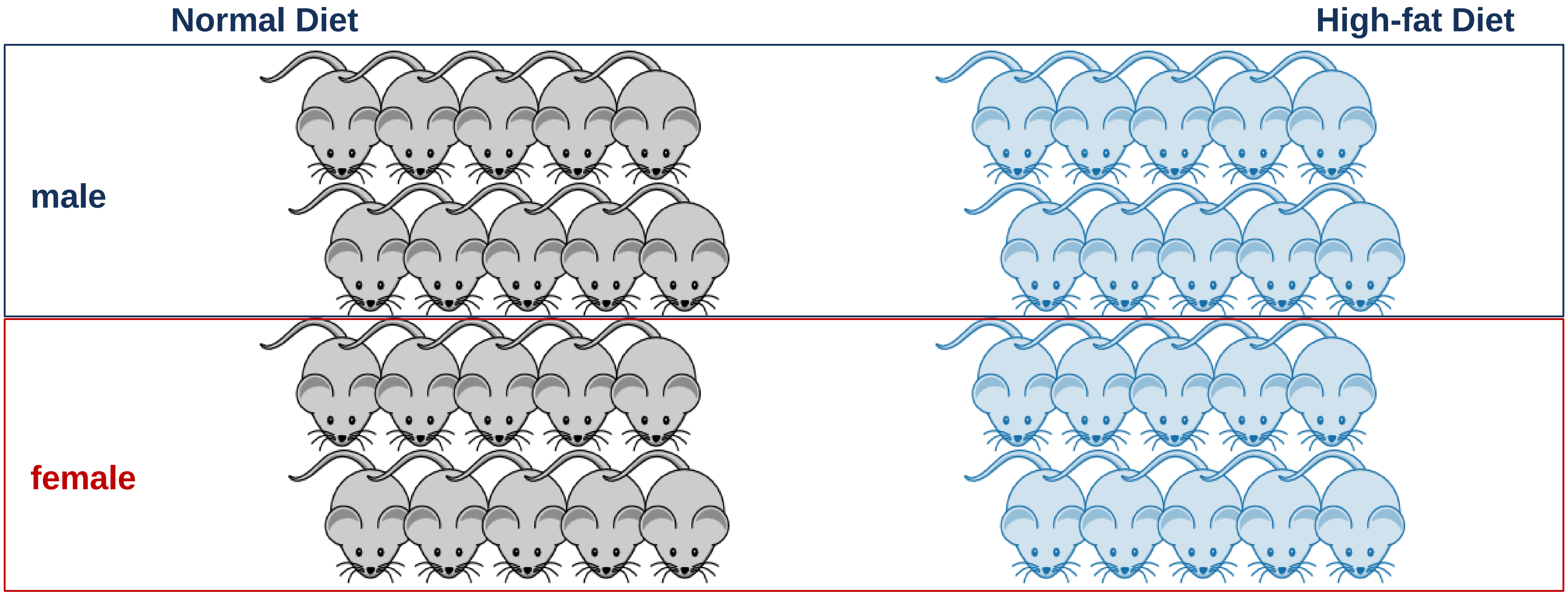
For General Laboratory Use Only – Not for Diagnostic Procedures.

Metabolomics Case Study:

Metabolic alterations observed in plasma of mice fed high-fat diet



Comparative Study | Diet Induced Changes in Mouse Plasma Metabolome



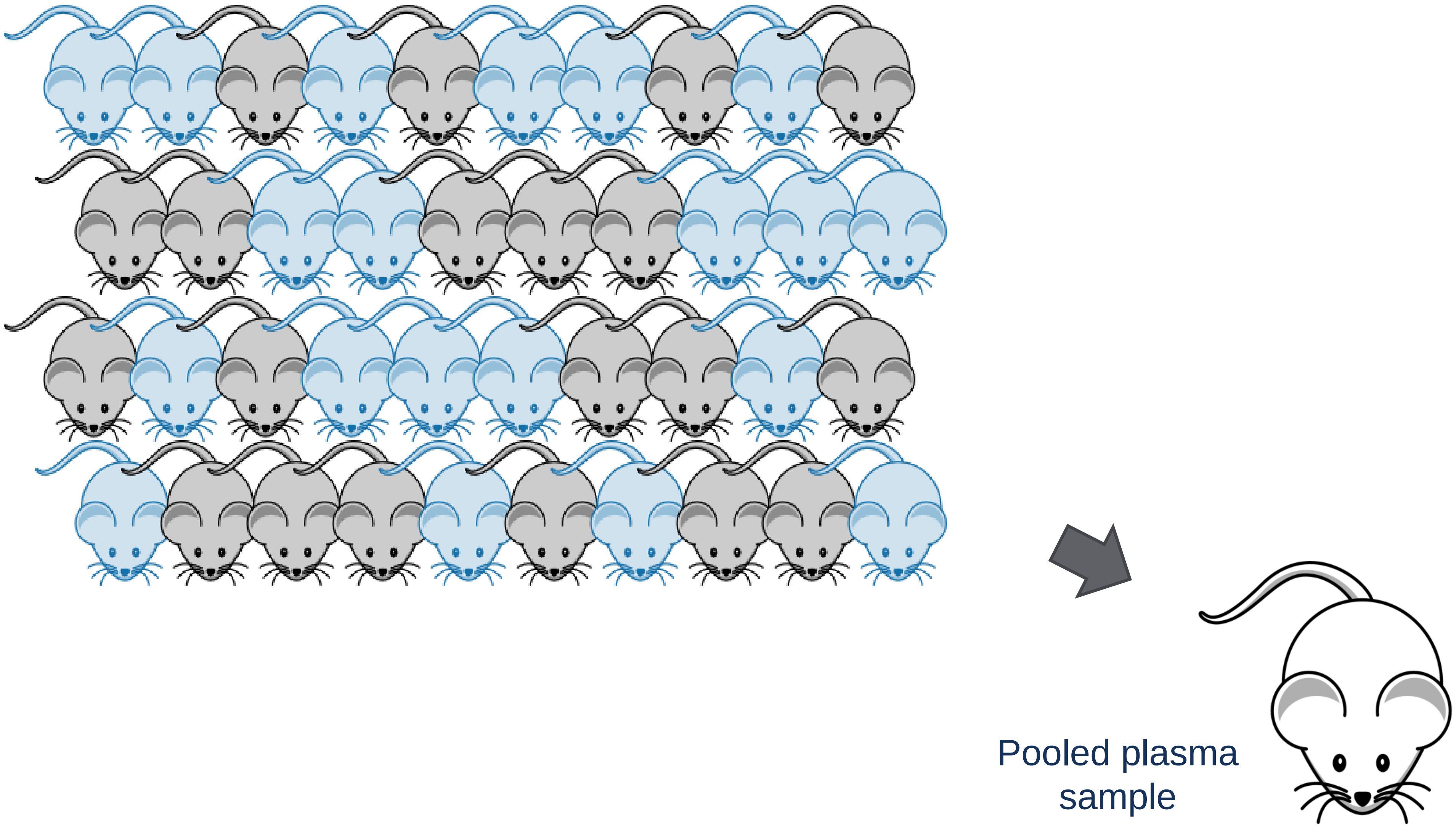
Normal Diet

High-fat Diet

male

female

Comparative Study | Diet Induced Changes in Mouse Plasma Metabolome



Pooled plasma sample

Sample Preparation for Comprehensive Metabolome Coverage

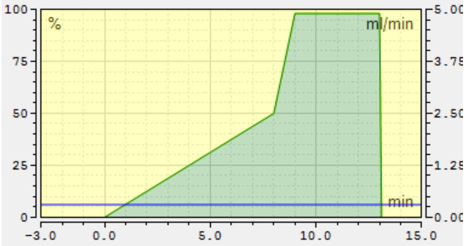
Sample

Extraction

LC method

Polar

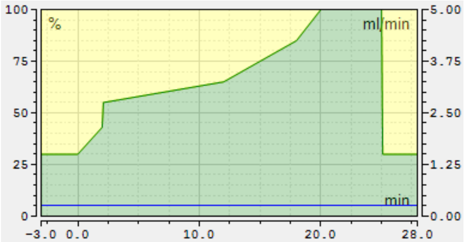
Methanol (3:1)
+ internal standards



Thermo Scientific™ Hypersil GOLD™ C18 column

Lipid

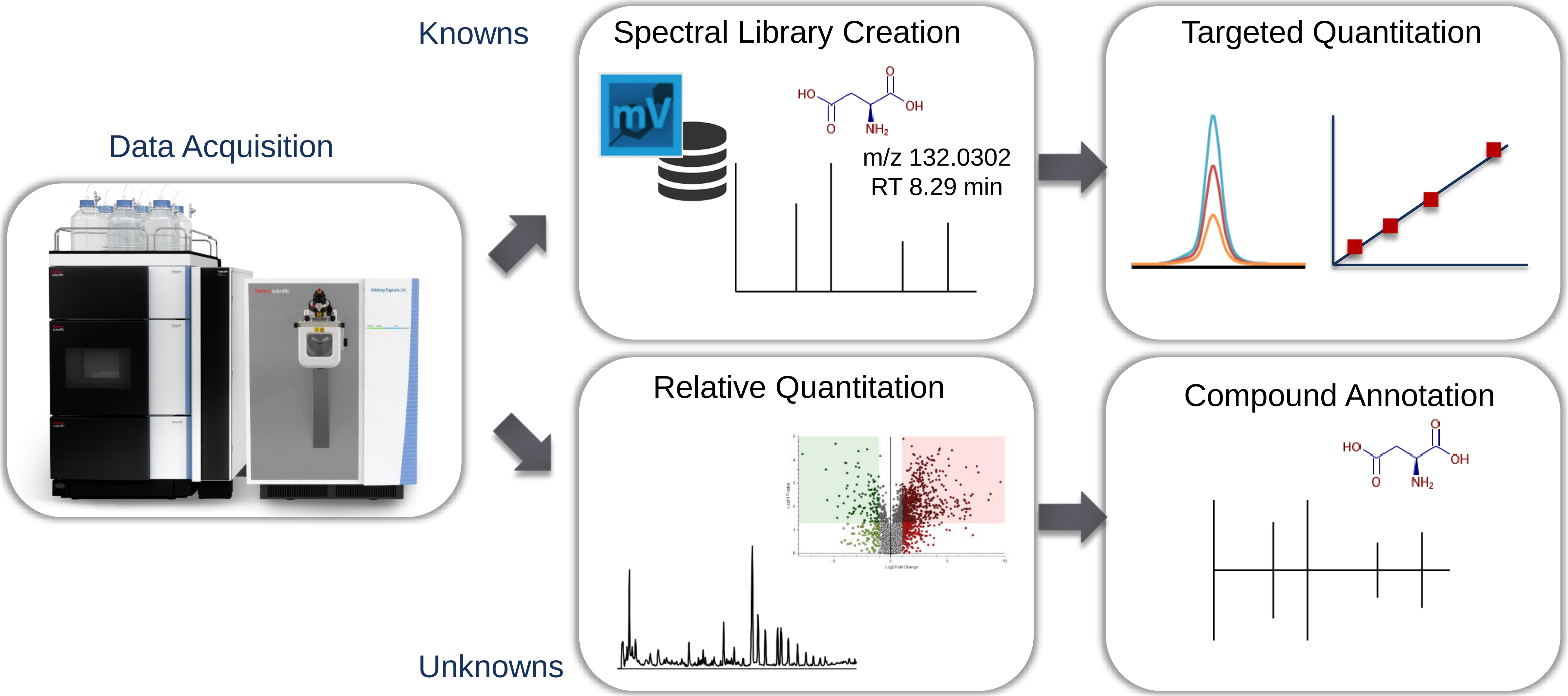
Isopropanol (3:1)
+ internal standards



Thermo Scientific™ Accucore™ C30 column

Semi-targeted Metabolomics | Diet Induced Changes in Mouse Plasma Metabolome

Verifying Known Differentiators and Identifying New Markers



Knowns

Data Acquisition

Unknowns

Spectral Library Creation

m/z 132.0302
RT 8.29 min

Targeted Quantitation

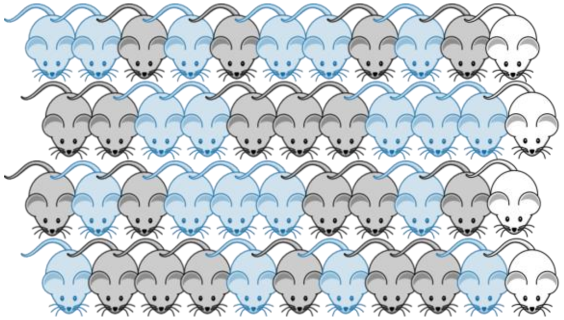
Relative Quantitation

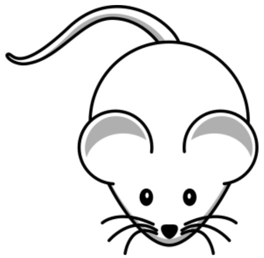
Compound Annotation

A Universal Metabolomics Workflow | Polar Metabolites

Sample Collection

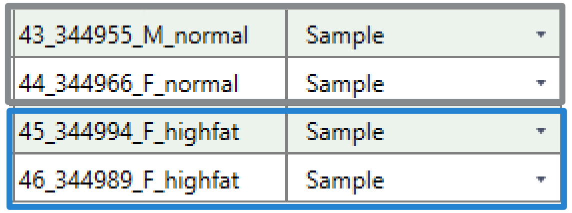
Mouse plasma samples



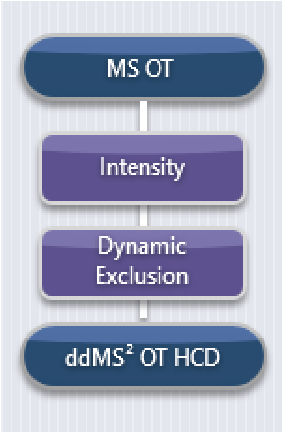


Pooled plasma sample

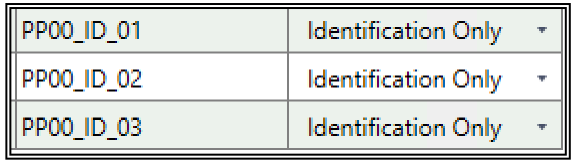
Data Acquisition



43_344955_M_normal	Sample
44_344966_F_normal	Sample
45_344994_F_highfat	Sample
46_344989_F_highfat	Sample



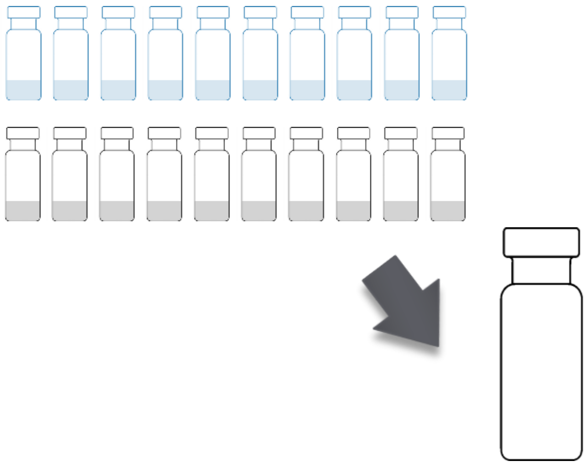
MS^2 for identification



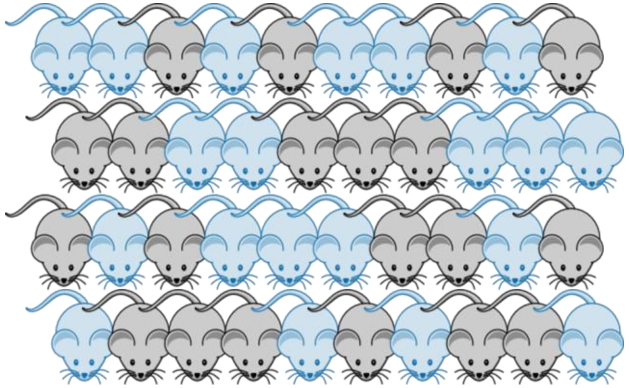
PP00_ID_01	Identification Only
PP00_ID_02	Identification Only
PP00_ID_03	Identification Only

Comparative Study | Experimental Setup

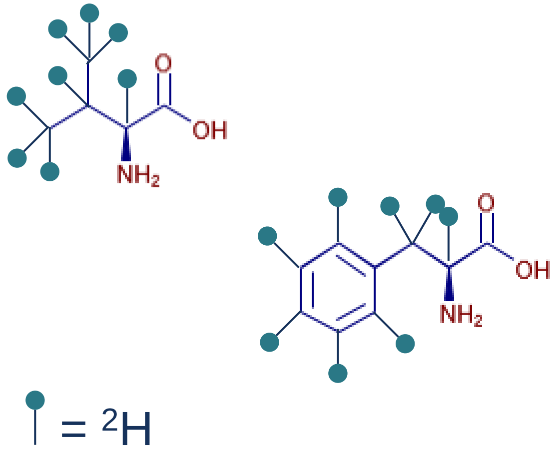
Building QA/QC Into the Experiment



Pooled QC samples



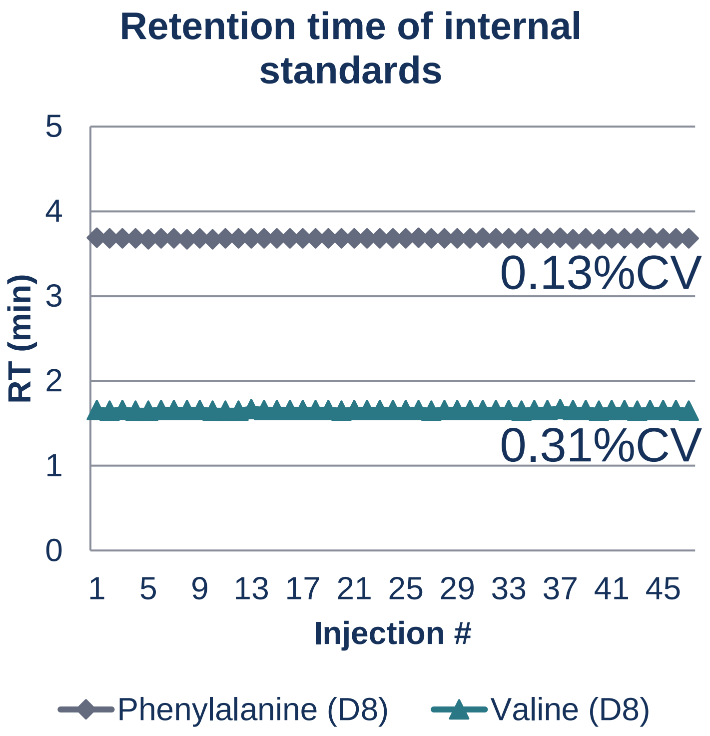
Randomized order

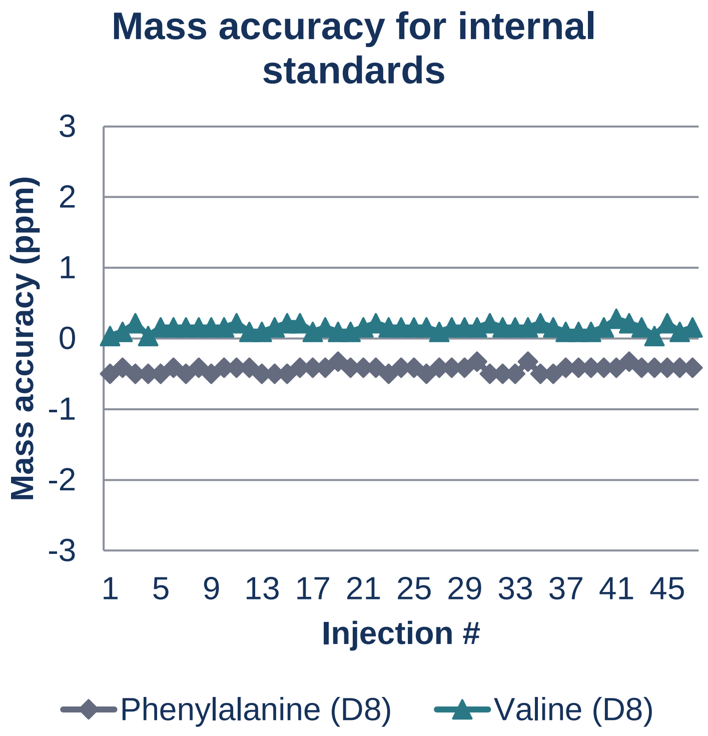


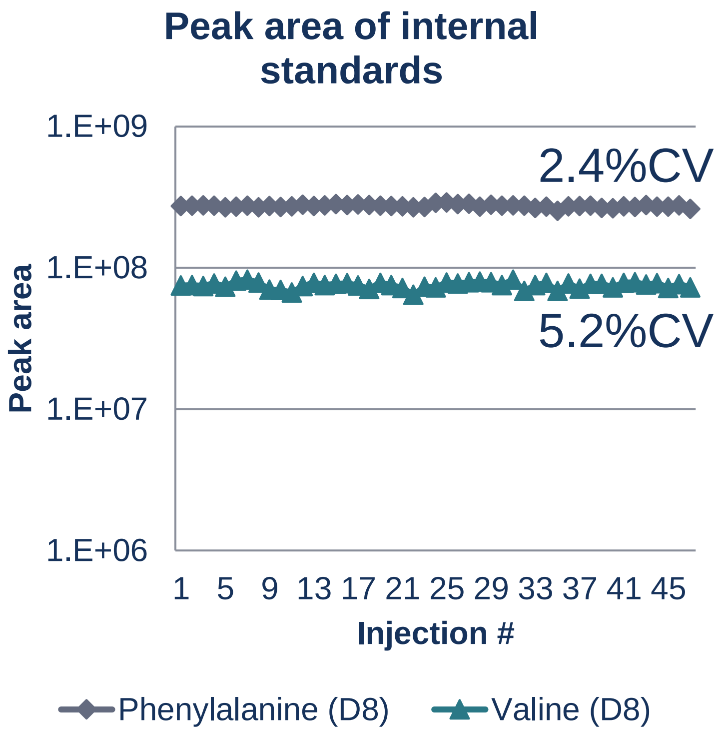
Internal standards

Achieving High Quality Data

Instrument Stability Across 47 Injections


Retention time of internal standards


Mass accuracy for internal standards

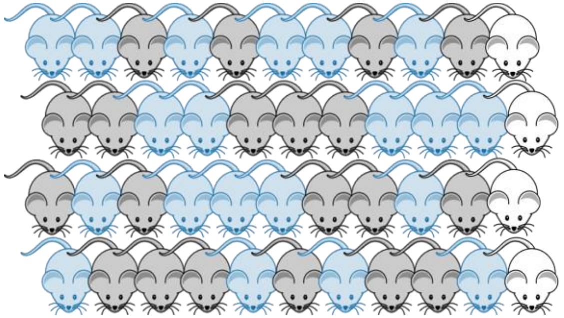

Peak area of internal standards

Orbitrap Exploris 240 MS
Mouse Plasma Extracts
15h of Data Acquisition

A Universal Metabolomics Workflow | Polar Metabolites

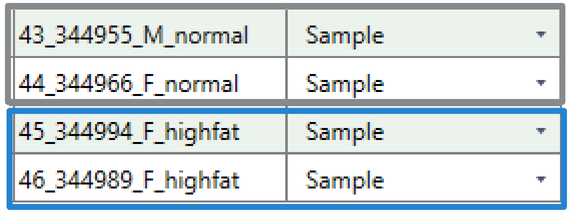
Sample Collection

Mouse plasma samples



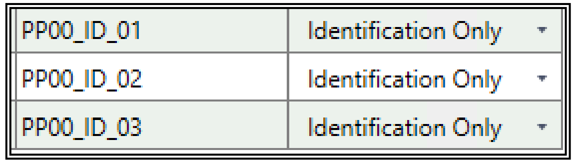
Pooled plasma sample

Data Acquisition



43_344955_M_normal	Sample
44_344966_F_normal	Sample
45_344994_F_highfat	Sample
46_344989_F_highfat	Sample

AcquireX Deep Scan
on Pooled Sample

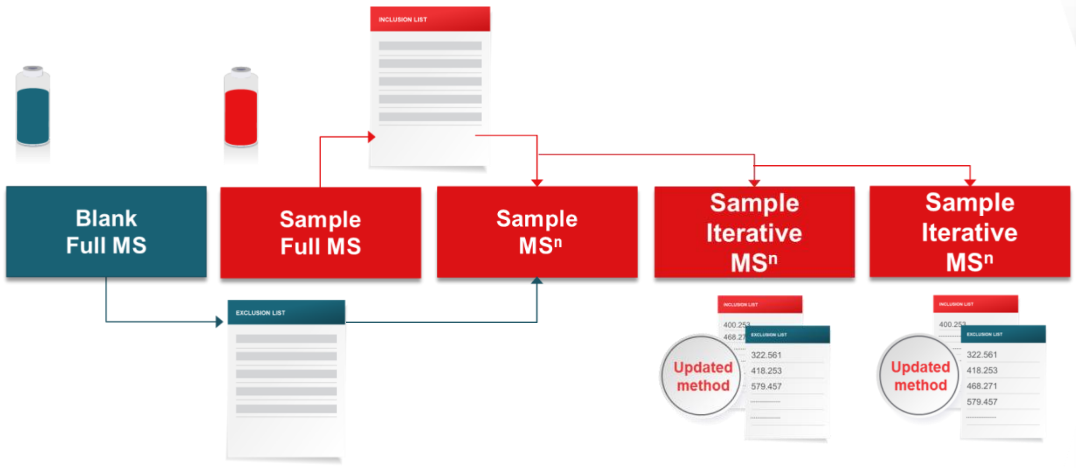


PP00_ID_01	Identification Only
PP00_ID_02	Identification Only
PP00_ID_03	Identification Only

AcquireX Deep Scan – Intelligent Data Acquisition

Collect More Meaningful Data, Not Just More Data

Maximize productivity with automation for deep coverage of unknown compounds



Blank Full MS

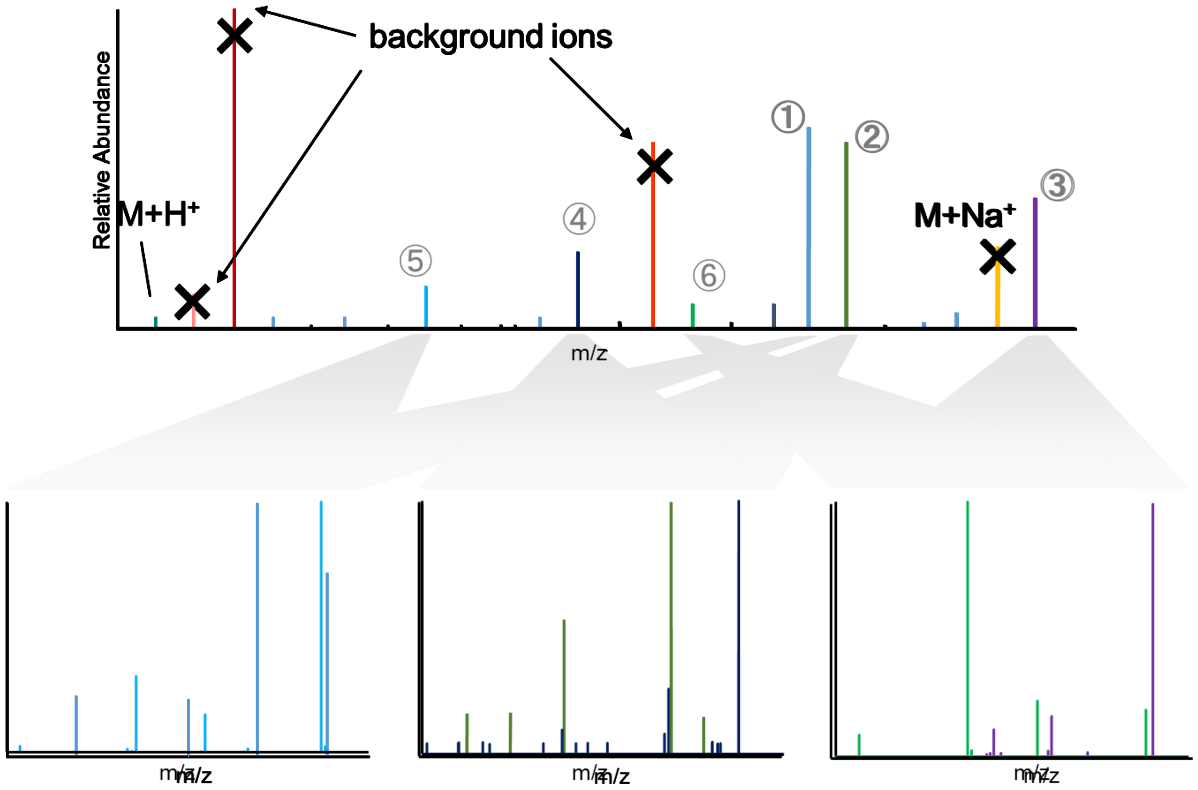
Sample Full MS

Sample MS^n

Sample Iterative MS^n

Sample Iterative MS^n

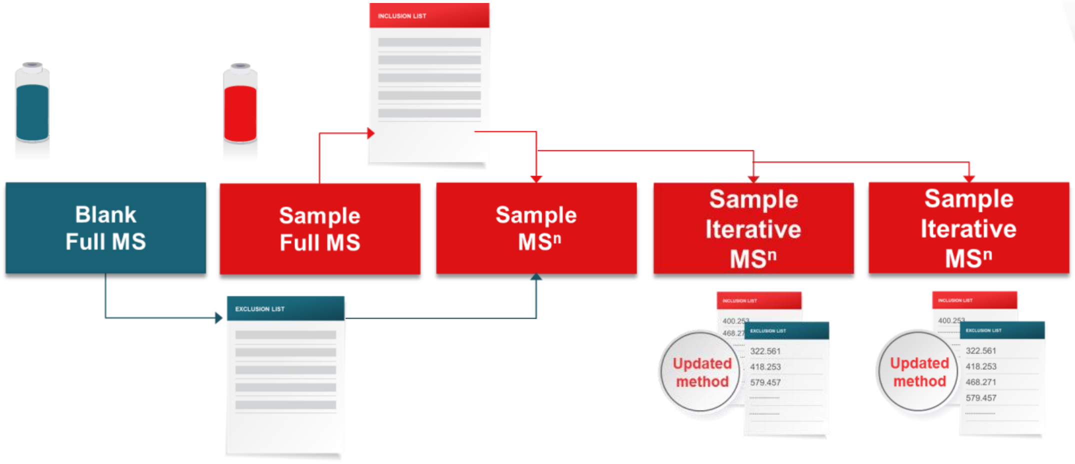
Automatically updated run-to-run inclusion/exclusion



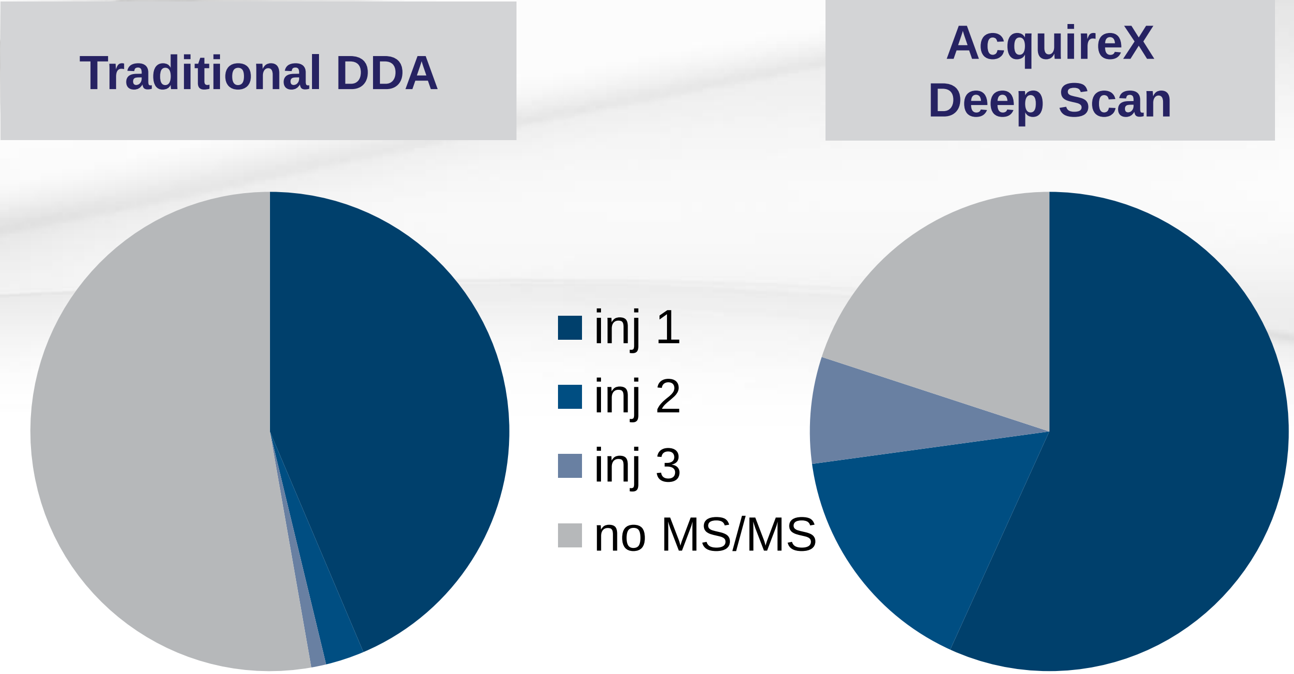
AcquireX Deep Scan – Intelligent Data Acquisition

Collect More Meaningful Data, Not Just More Data

Maximize productivity with automation for deep coverage of unknown compounds



Automatically updated run-to-run inclusion/exclusion

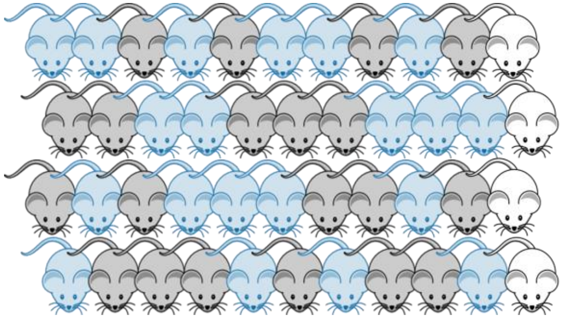


Mouse pooled plasma, C18, 15min gradient

A Universal Metabolomics Workflow | Polar Metabolites

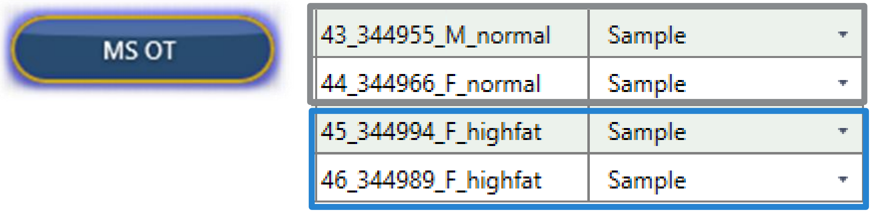
Sample Collection

Mouse plasma samples



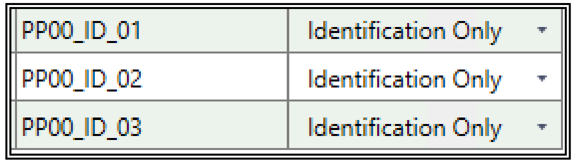
Pooled plasma sample

Data Acquisition



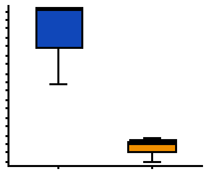
43_344955_M_normal	Sample
44_344966_F_normal	Sample
45_344994_F_highfat	Sample
46_344989_F_highfat	Sample

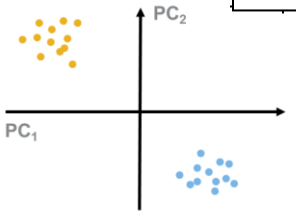
AcquireX Deep Scan
on Pooled Sample

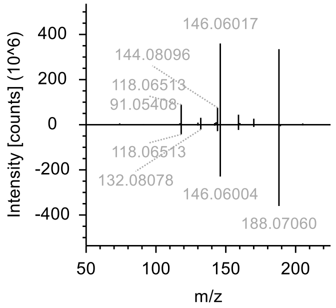


PP00_ID_01	Identification Only
PP00_ID_02	Identification Only
PP00_ID_03	Identification Only

Data Analysis

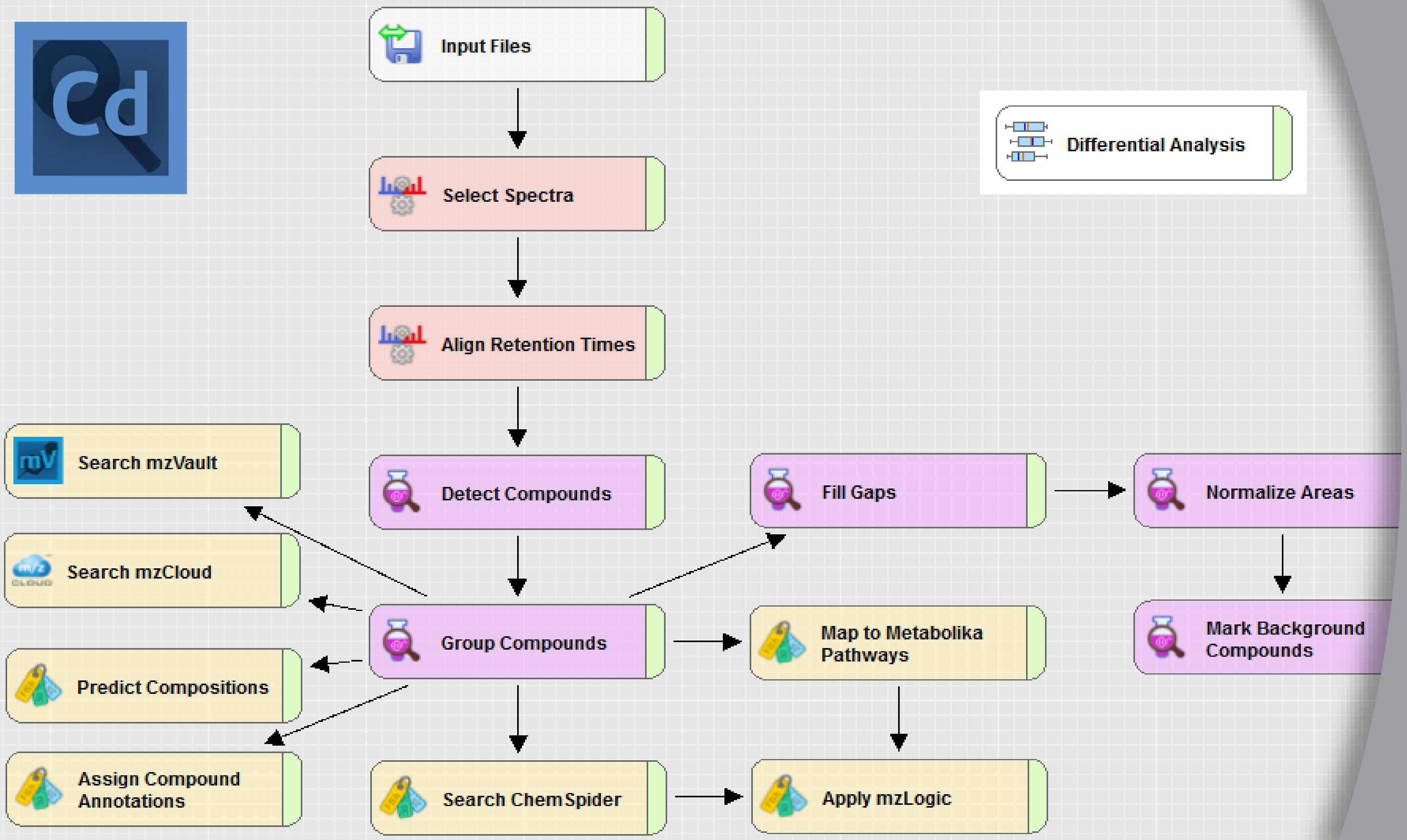






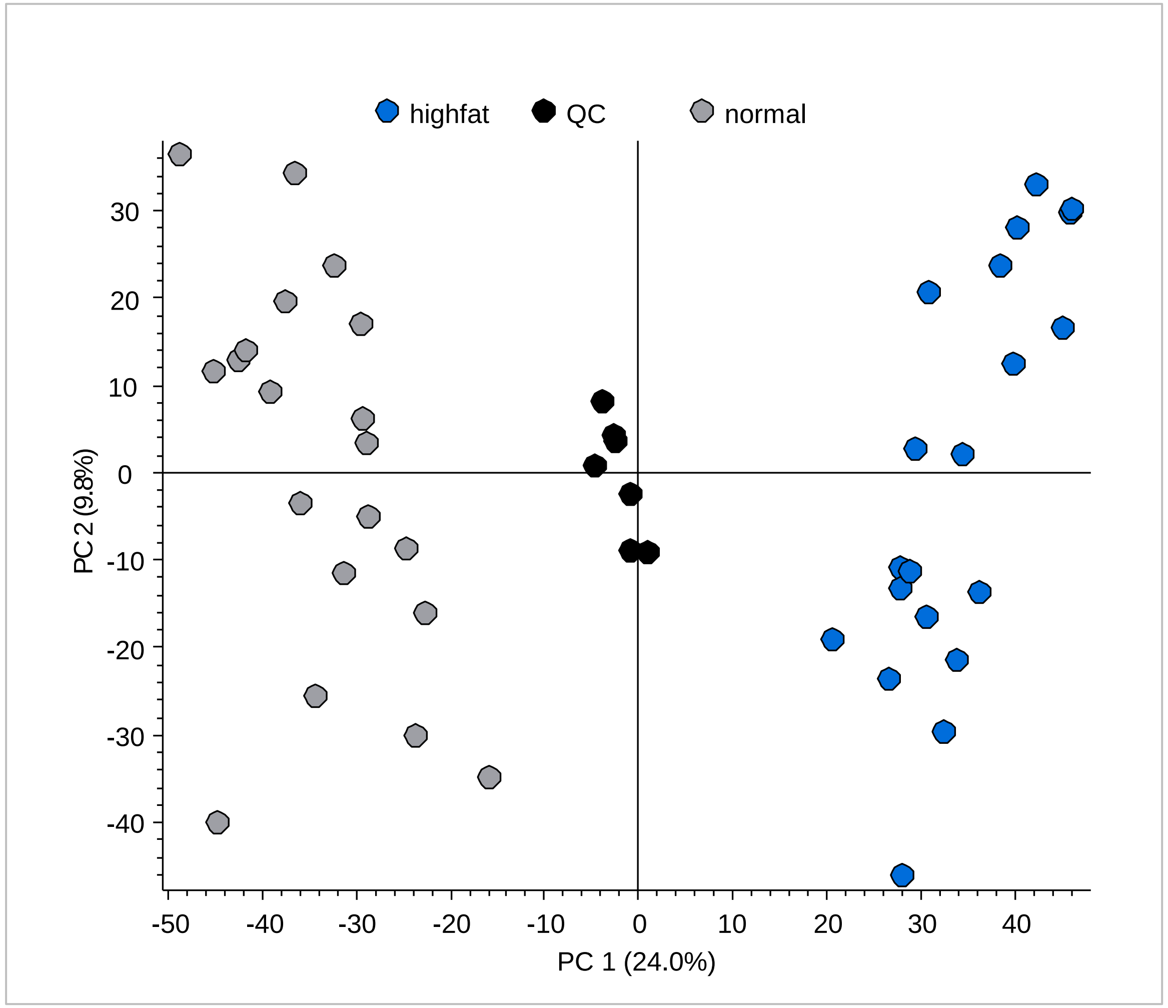
The Ultimate Toolbox for Metabolomics Data Analysis

Thermo Scientific™ Compound Discoverer™ 3.1 Software

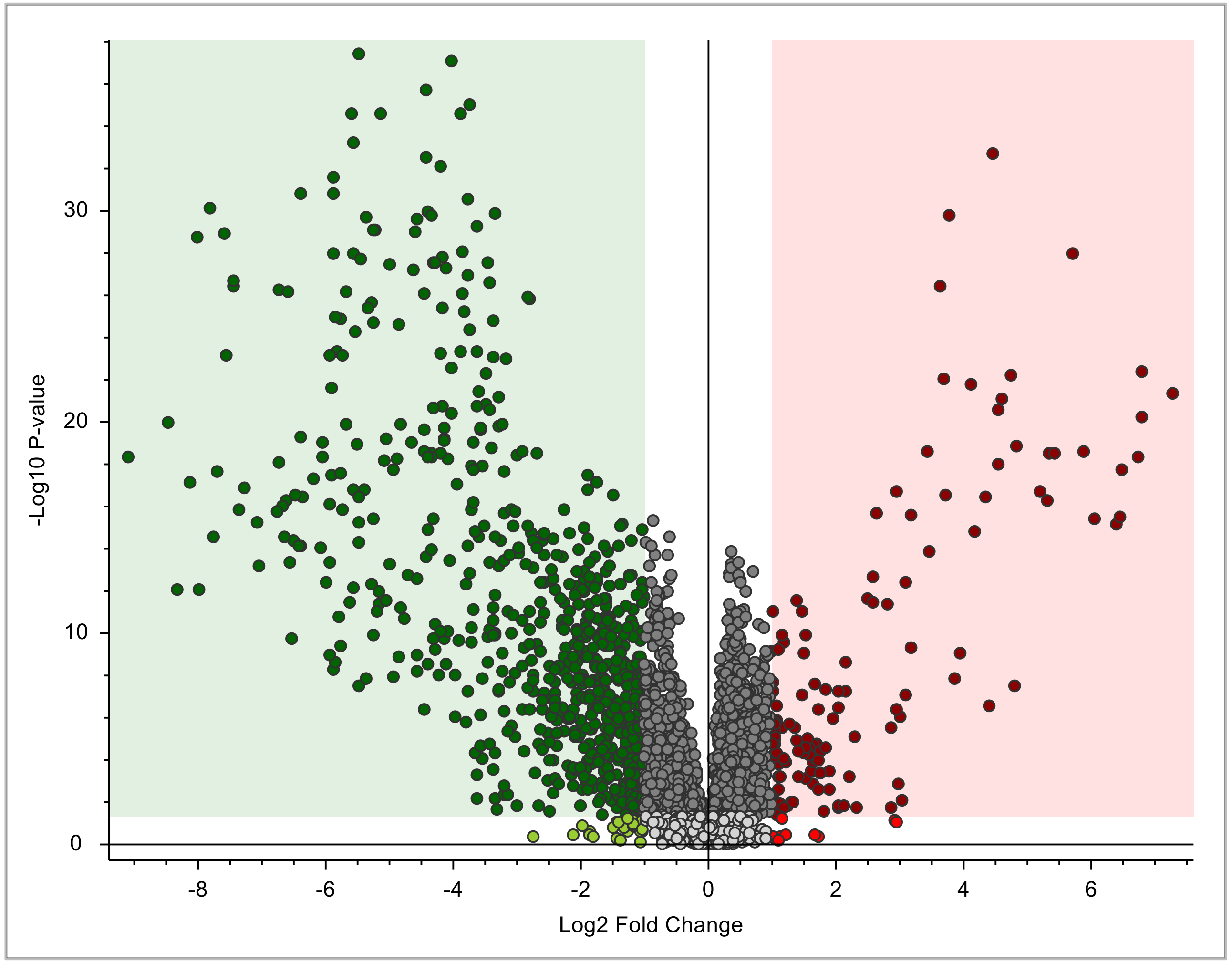


- Feature Detection
- Data Reduction
- Unknown Annotation
- Statistics
- Pathway Mapping

Differential Analysis | Diet Induced Changes in Mouse Plasma Metabolome



PCA plot

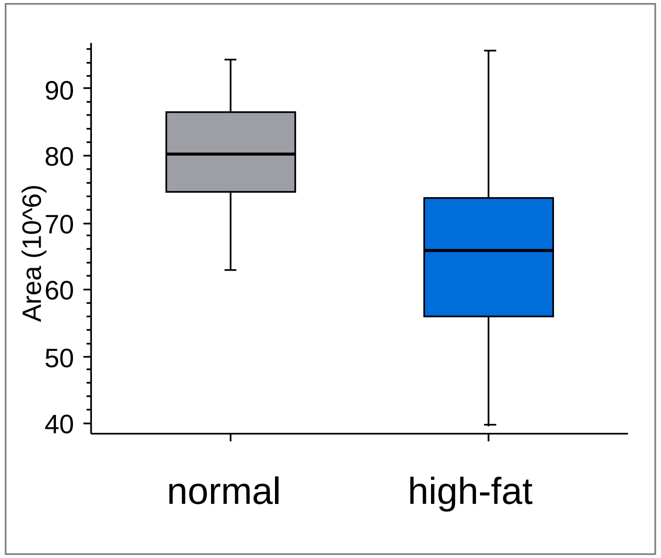


Volcano plot

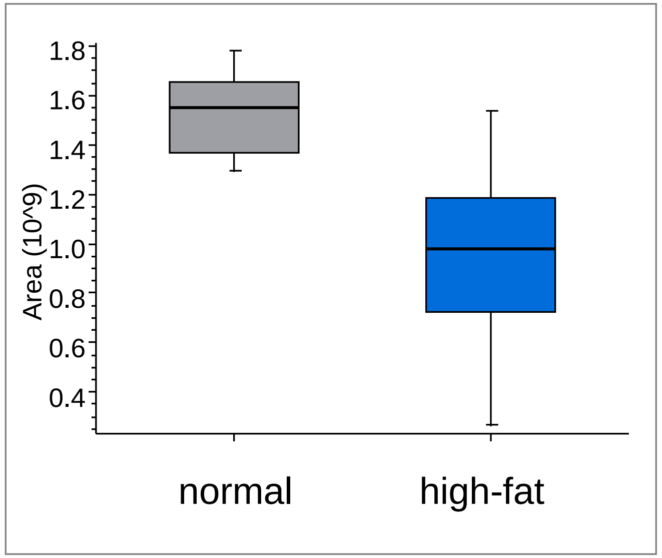
Known Metabolic Markers | Diet Induced Changes in Mouse Plasma Metabolome

Carnitine Levels Are Affected by High-fat Diet

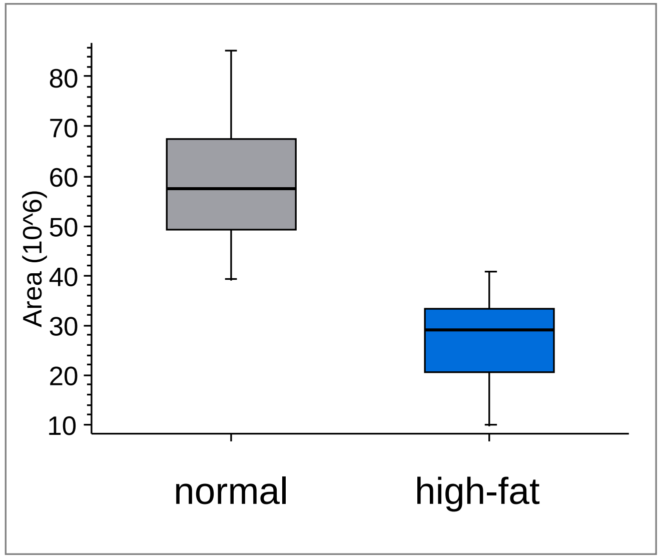
carnitine



acetylcarnitine

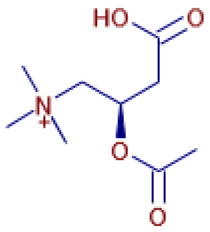


propionylcarnitine



Known Metabolic Markers | Diet Induced Changes in Mouse Plasma Metabolome

Identification of Acetylcarnitine Against Authentic Standard

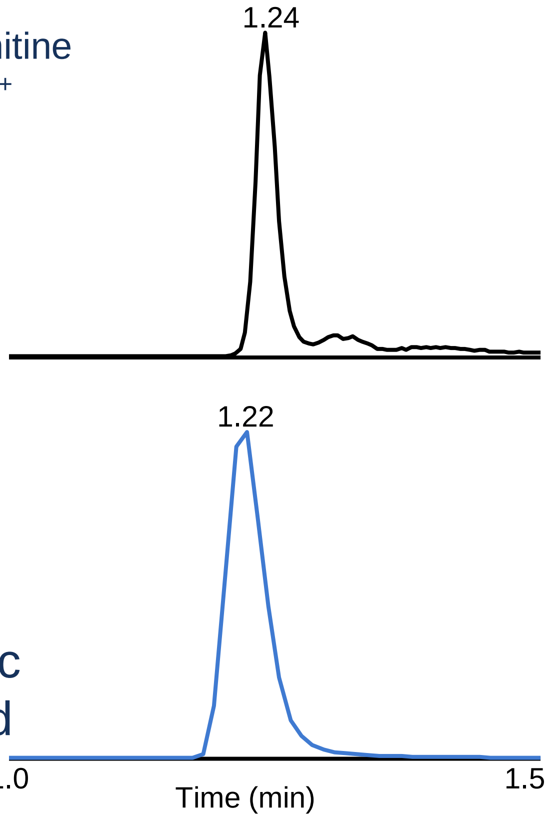


acetylcarnitine
$C_9H_{18}NO_4^+$

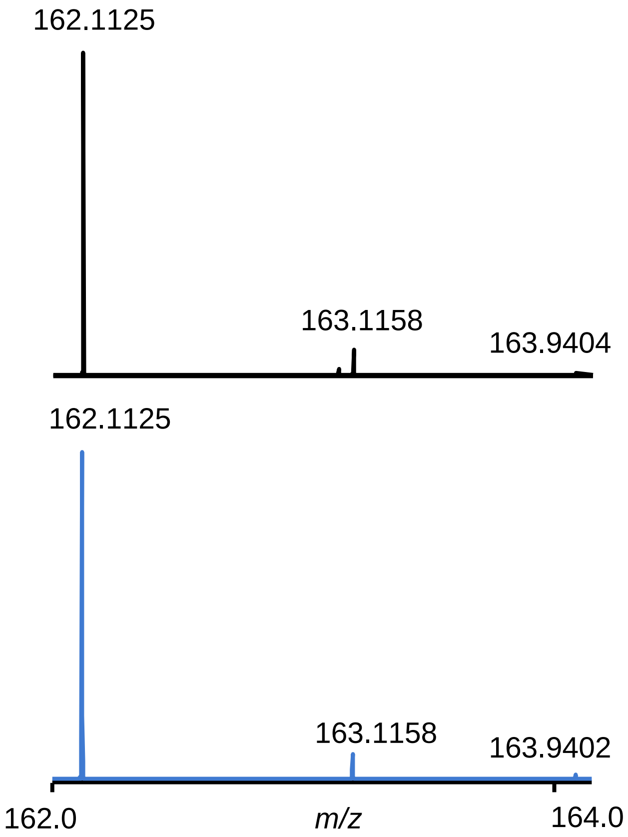
pooled plasma

authentic standard

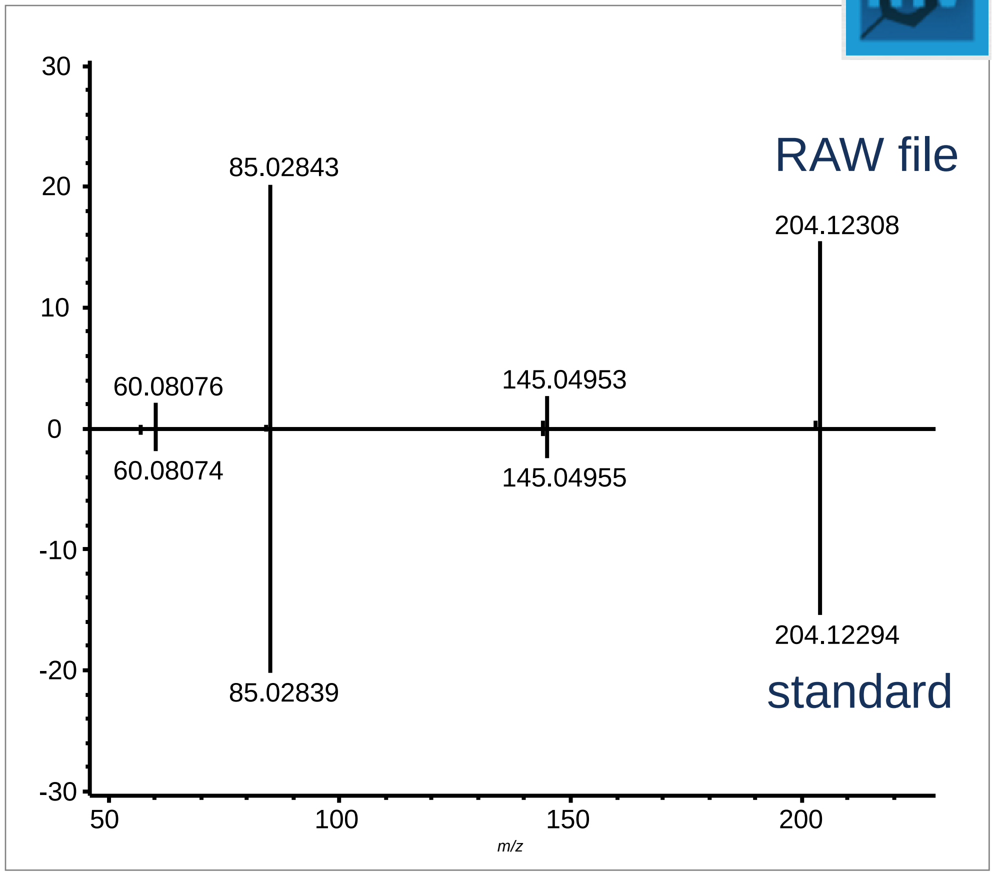
Retention time



m/z (MS¹)



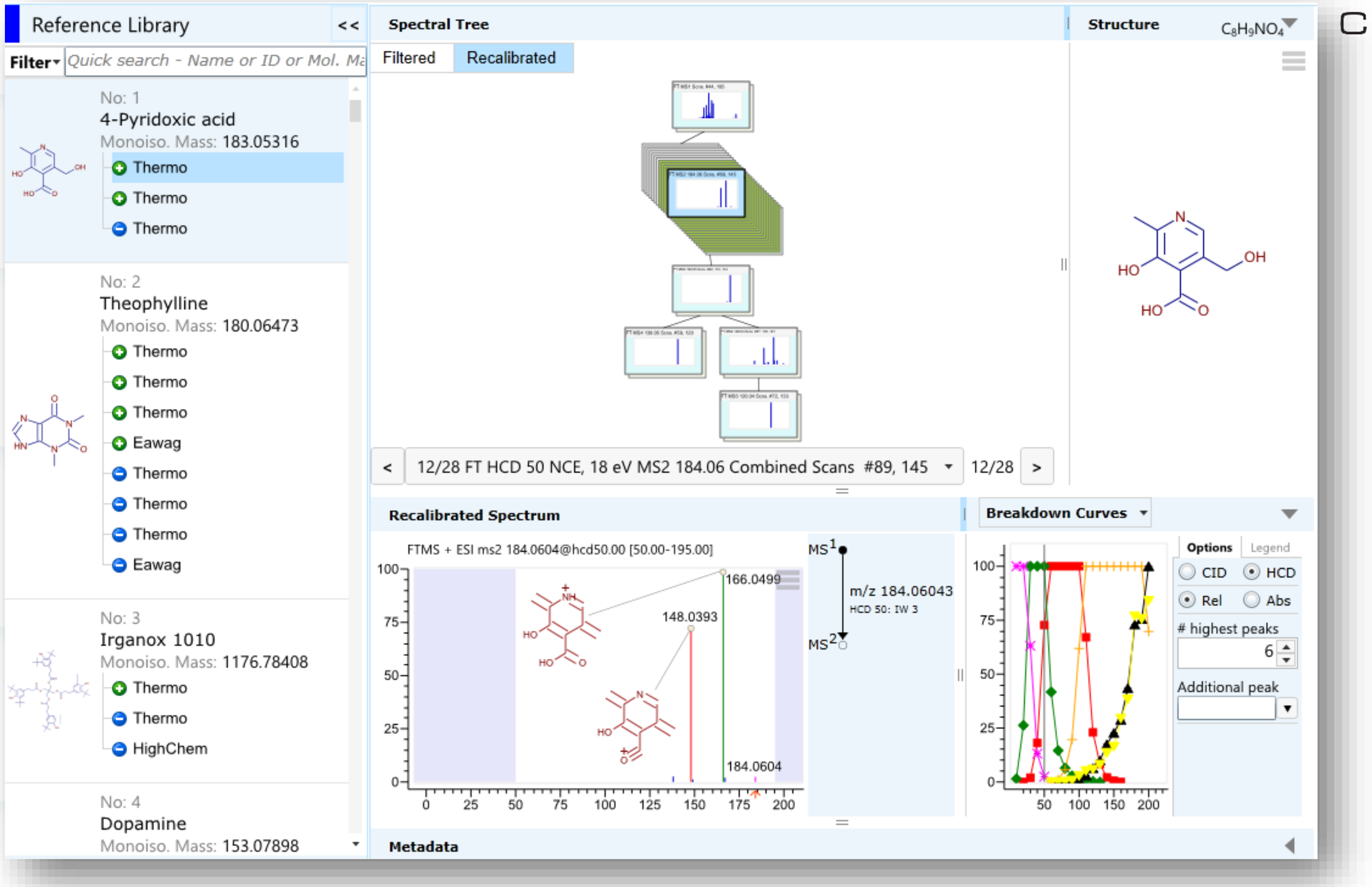
MS² spectrum



Thermo Scientific mzCloud Spectral Library

Ultra High-quality Online MS/MS and MS^n Spectral Library

- **Professionally Curated**
- **Multi-Stage Fragmentation**
- **HCD/CID Dissociation**
- **Multiple Collision Energies**
- **Structural Annotations**



No: 1
4-Pyridoxic acid
Monoiso. Mass: 183.05316
Thermo
Thermo
Thermo

No: 2
Theophylline
Monoiso. Mass: 180.06473
Thermo
Thermo
Thermo
Eawag
Thermo
Thermo
Thermo
Eawag

No: 3
Irganox 1010
Monoiso. Mass: 1176.78408
Thermo
Thermo
HighChem

No: 4
Dopamine
Monoiso. Mass: 153.07898

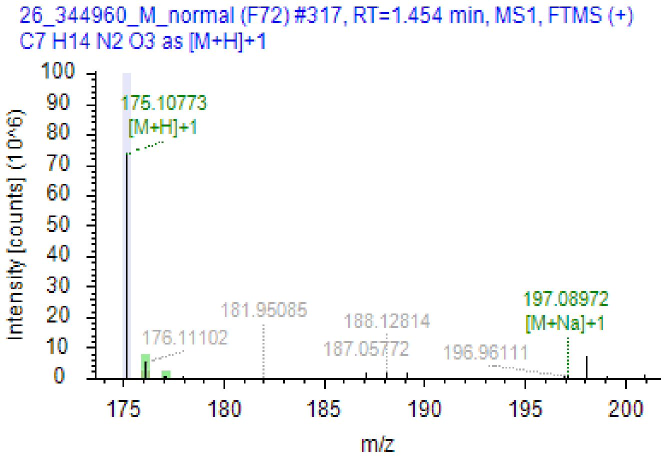
< 12/28 FT HCD 50 NCE, 18 eV MS2 184.06 Combined Scans #89, 145 12/28 >

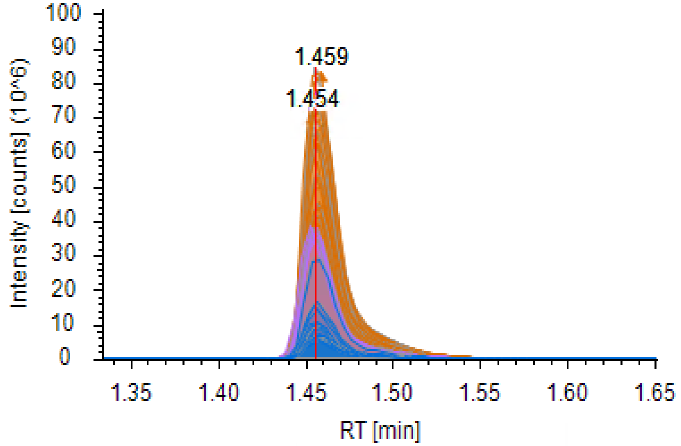
FTMS + ESI ms2 184.0604@hcd50.00 [50.00-195.00]

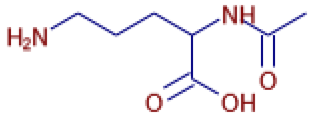
Unknown Discriminants | Diet Induced Changes in Mouse Plasma Metabolome

Metabolic Markers Specific to Diet

m/z 175.1077, 1.45 min

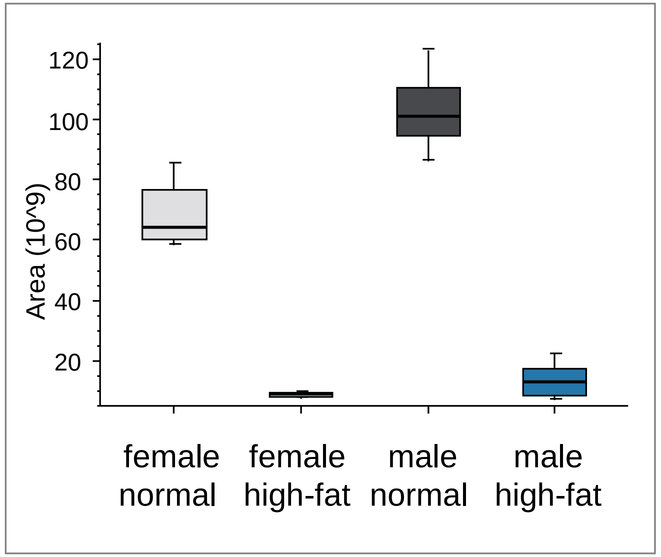


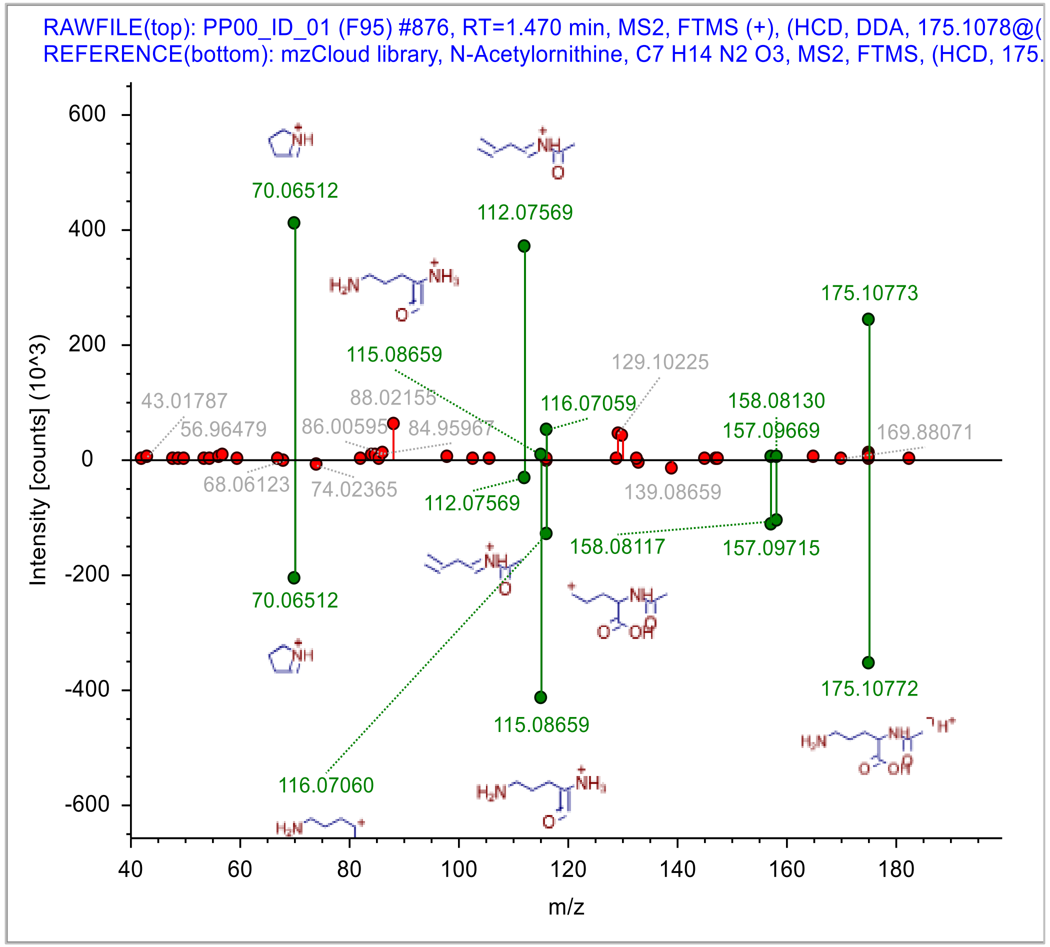




N-acetylornithine

$C_7H_{14}N_2O_3$

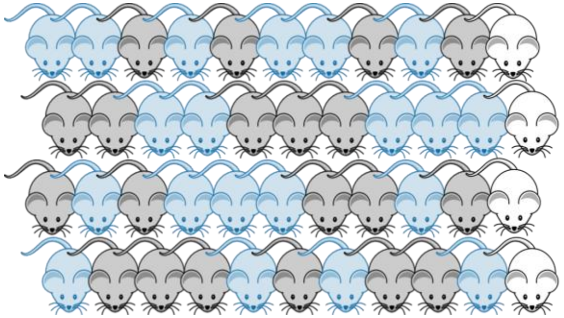




A Universal Metabolomics Workflow | Lipid Species

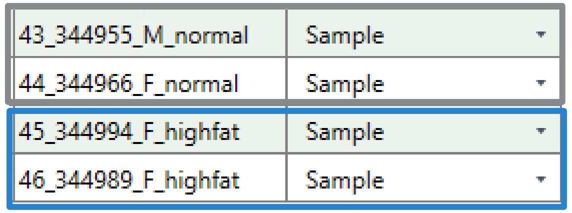
Sample Collection

Mouse plasma samples



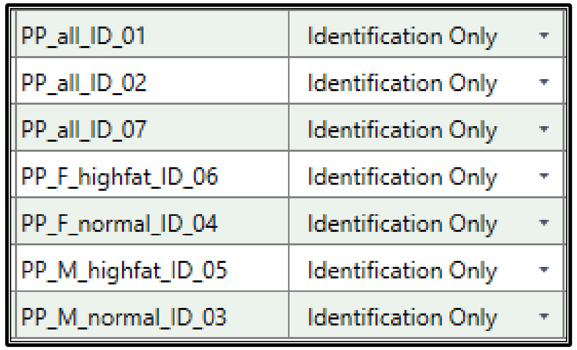
Pooled plasma sample

Data Acquisition



43_344955_M_normal	Sample
44_344966_F_normal	Sample
45_344994_F_highfat	Sample
46_344989_F_highfat	Sample

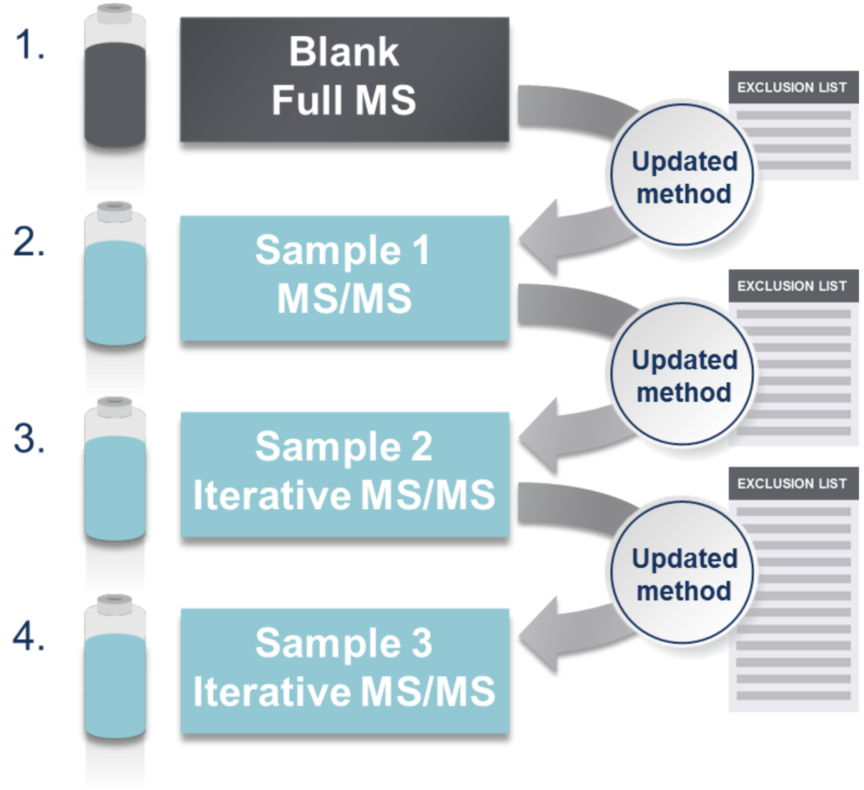
AcquireX Iterative Precursor Workflow on Pooled Samples



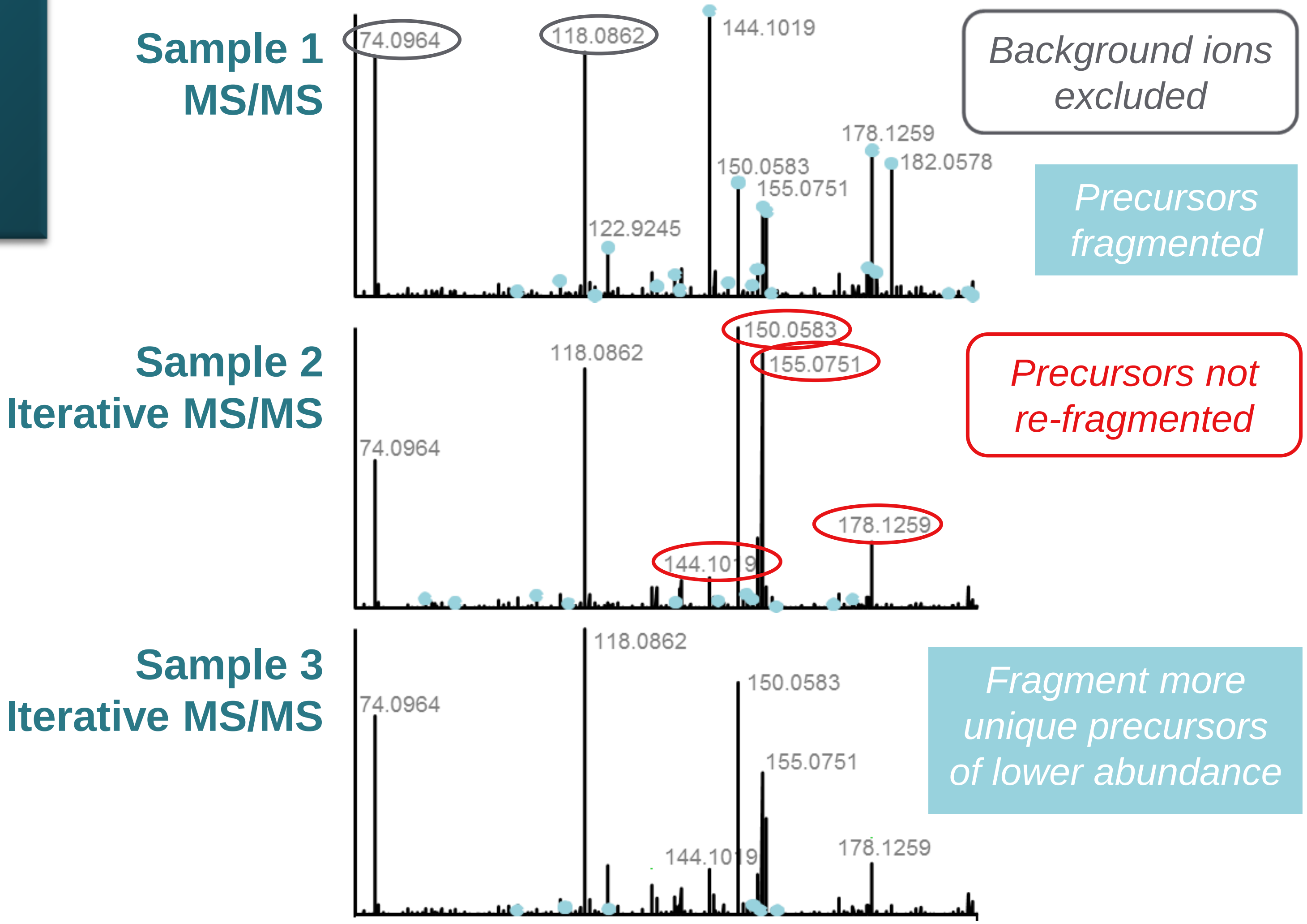
PP_all_ID_01	Identification Only
PP_all_ID_02	Identification Only
PP_all_ID_07	Identification Only
PP_F_highfat_ID_06	Identification Only
PP_F_normal_ID_04	Identification Only
PP_M_highfat_ID_05	Identification Only
PP_M_normal_ID_03	Identification Only

Intelligent Data Acquisition | AcquireX Iterative Precursor Exclusion Workflow

Never fragment the same ion twice by automatically updating the exclusion list over multiple iterative injections for obtaining exhaustive fragmentation of all small molecules in the study.



1. **Blank Full MS**
2. **Sample 1 MS/MS**
3. **Sample 2 Iterative MS/MS**
4. **Sample 3 Iterative MS/MS**



Sample 1 MS/MS

Sample 2 Iterative MS/MS

Sample 3 Iterative MS/MS

Background ions excluded

Precursors fragmented

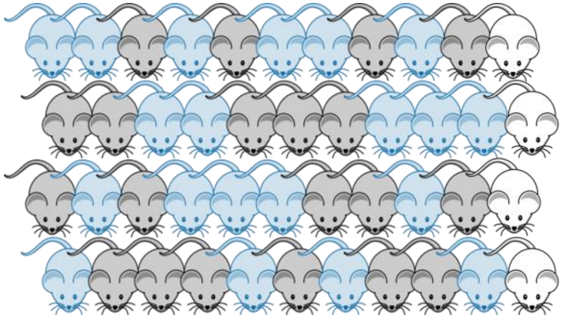
Precursors not re-fragmented

Fragment more unique precursors of lower abundance

A Universal Metabolomics Workflow | Lipid Species

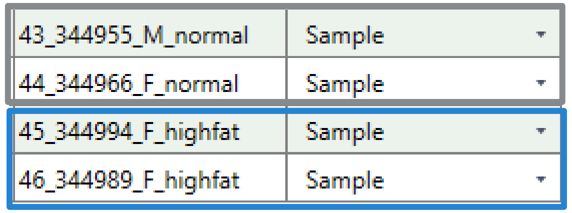
Sample Collection

Mouse plasma samples



Pooled plasma sample

Data Acquisition



MS OT

43_344955_M_normal	Sample
44_344966_F_normal	Sample
45_344994_F_highfat	Sample
46_344989_F_highfat	Sample

MS OT

Intensity

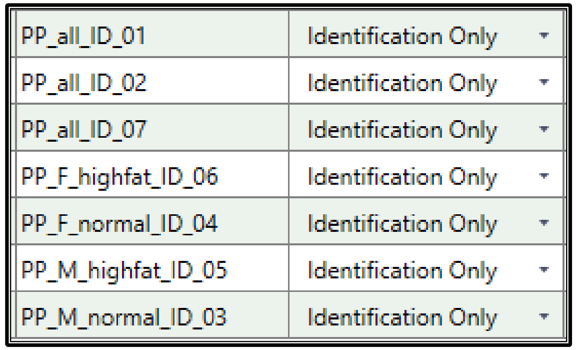
Dynamic Exclusion

Targeted Mass

Targeted Mass Exclusion

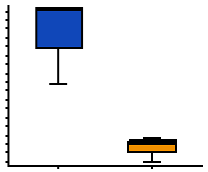
ddMS² OT HCD

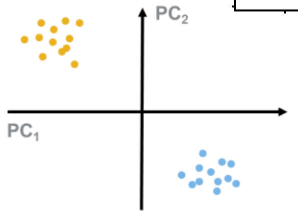
AcquireX Iterative Precursor Workflow
on Pooled Samples

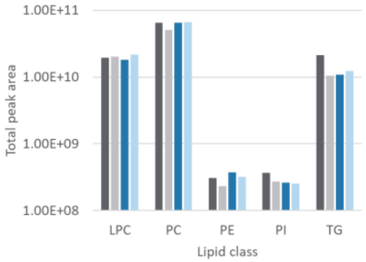


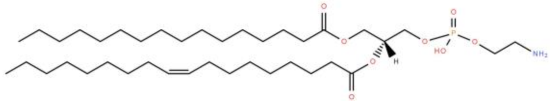
PP_all_ID_01	Identification Only
PP_all_ID_02	Identification Only
PP_all_ID_07	Identification Only
PP_F_highfat_ID_06	Identification Only
PP_F_normal_ID_04	Identification Only
PP_M_highfat_ID_05	Identification Only
PP_M_normal_ID_03	Identification Only

Data Analysis

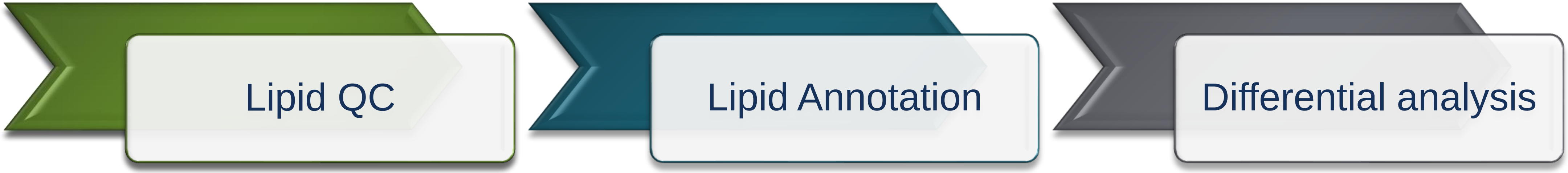








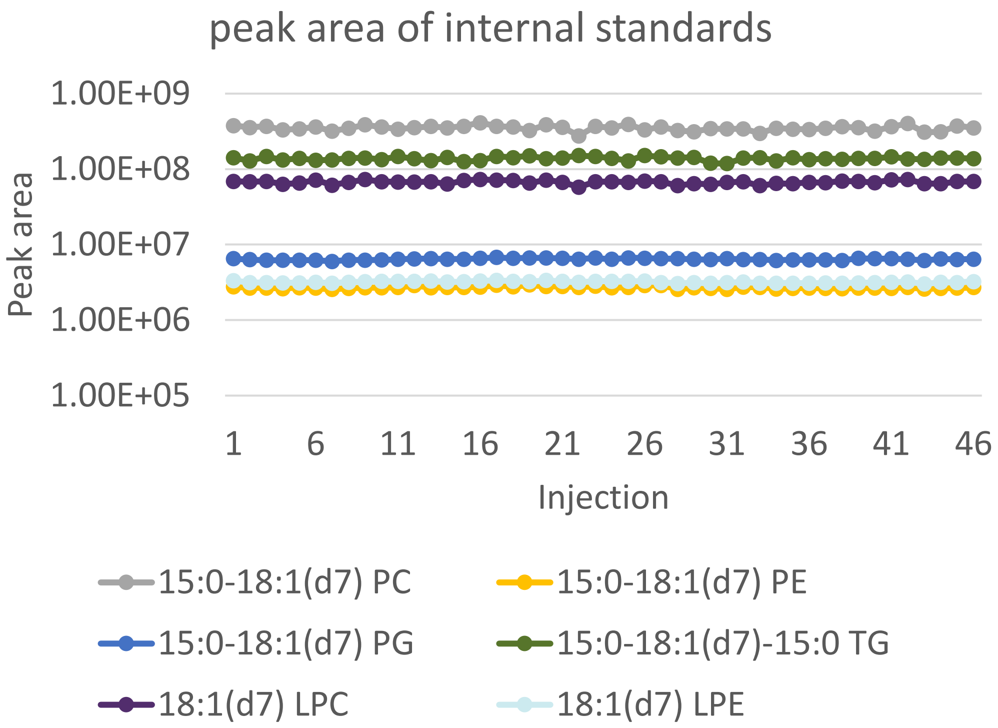
Lipidomics Data Analysis



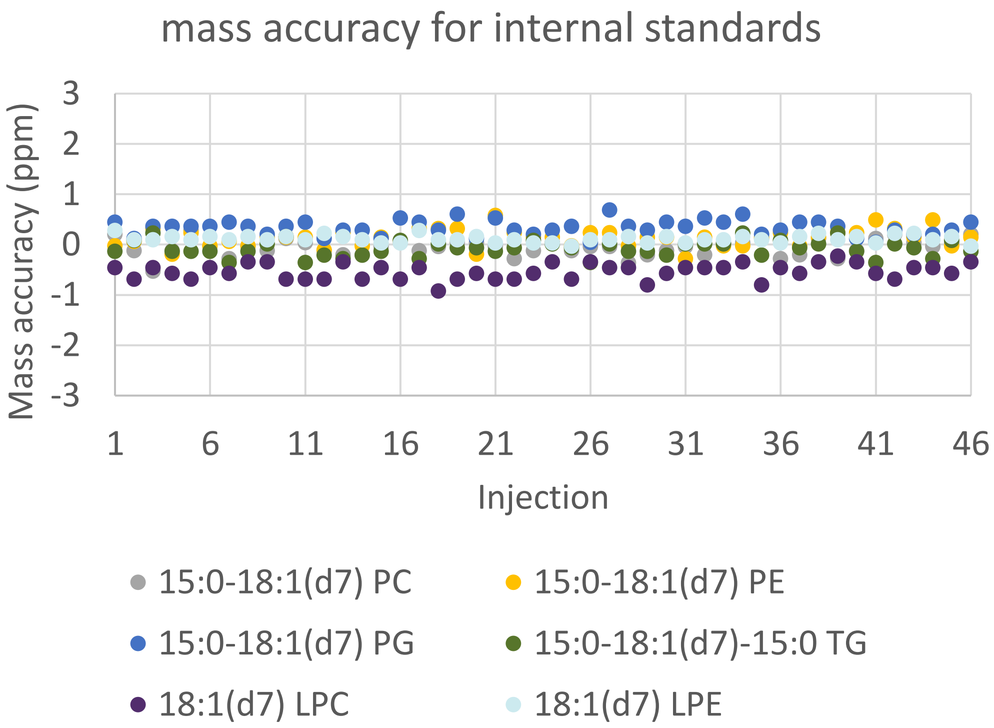
Lipid QC

Lipid Annotation

Differential analysis



peak area of internal standards



mass accuracy for internal standards

Reproducible Instrument Performance from Injection to Injection

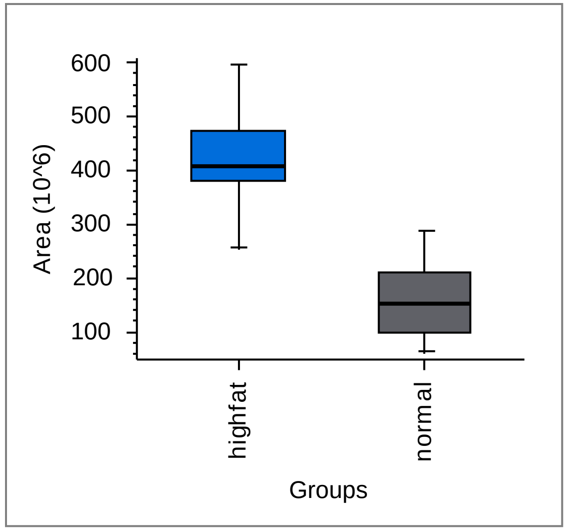
Lipidomics Data Analysis

Lipid QC

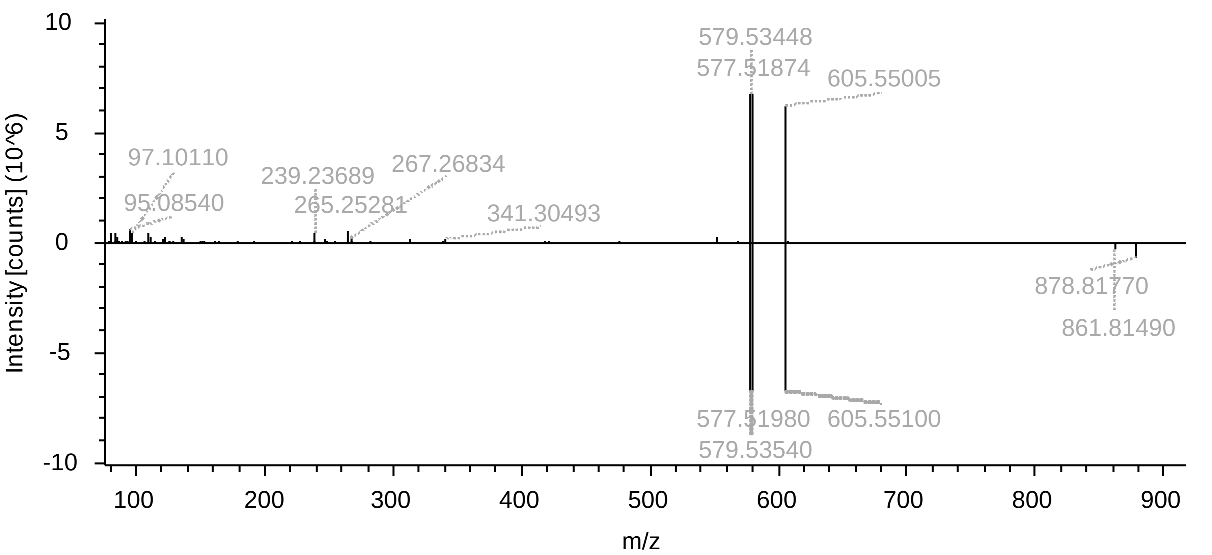
Lipid Annotation

Differential analysis

m/z 878.8177, 21.35 min



TAG 52:1 [M+NH$_4$]$^+$

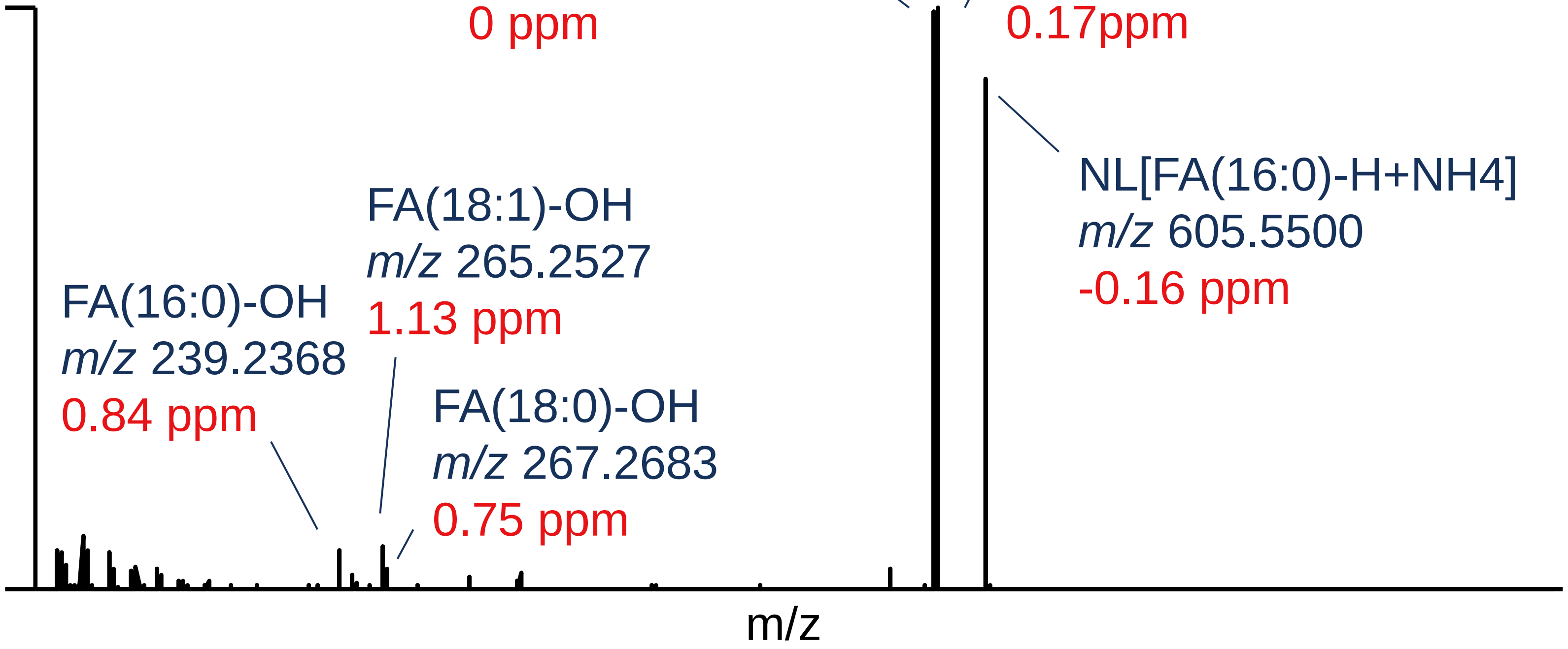


Search against LipidBlast in silico tandem MS database (Fiehn Lab, UC Davis)

Lipidomics Data Analysis | High Quality Spectra for High Quality IDs

Lipid Annotation

1396 total lipids
249 TAGs (total)
170 TAGs (high quality ID)



TAG (18:0_18:1_16:0)
m/z 878.8177
-0.23 ppm

NL[FA(18:0)-H+NH4]
m/z 577.5187
0 ppm

NL[FA(18:1)-H+NH4]
m/z 579.5345
0.17ppm

NL[FA(16:0)-H+NH4]
m/z 605.5500
-0.16 ppm

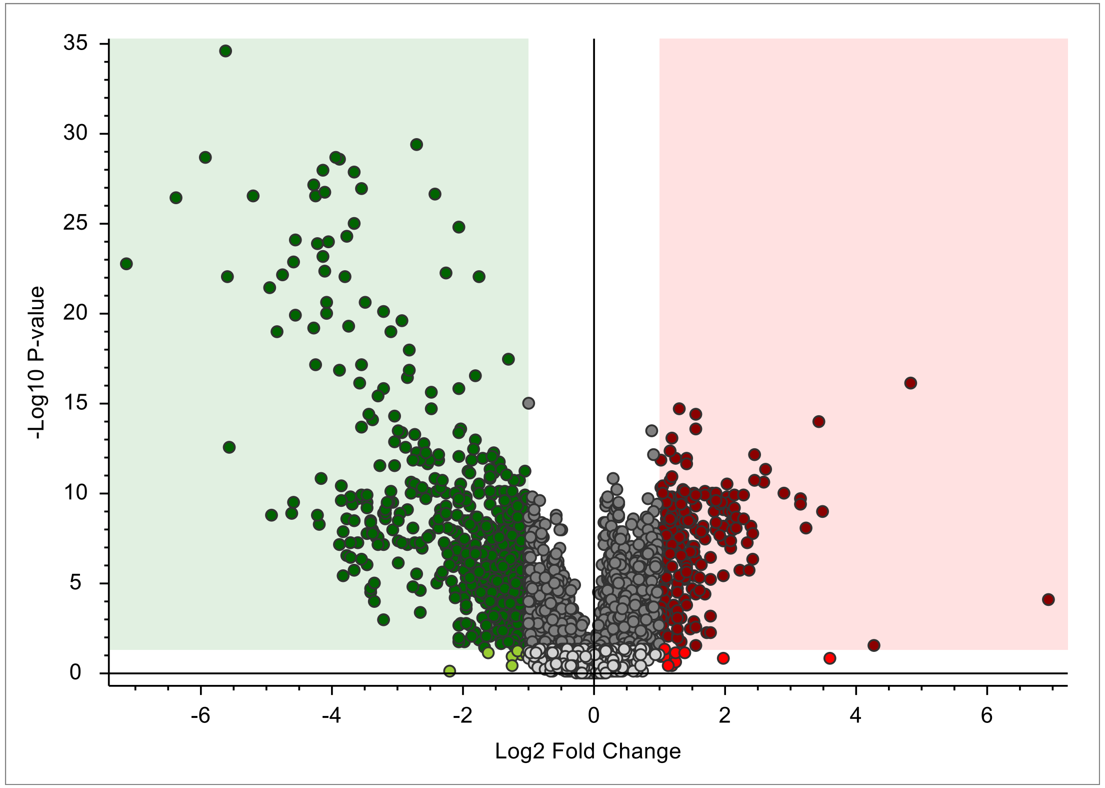
FA(18:1)-OH
m/z 265.2527
1.13 ppm

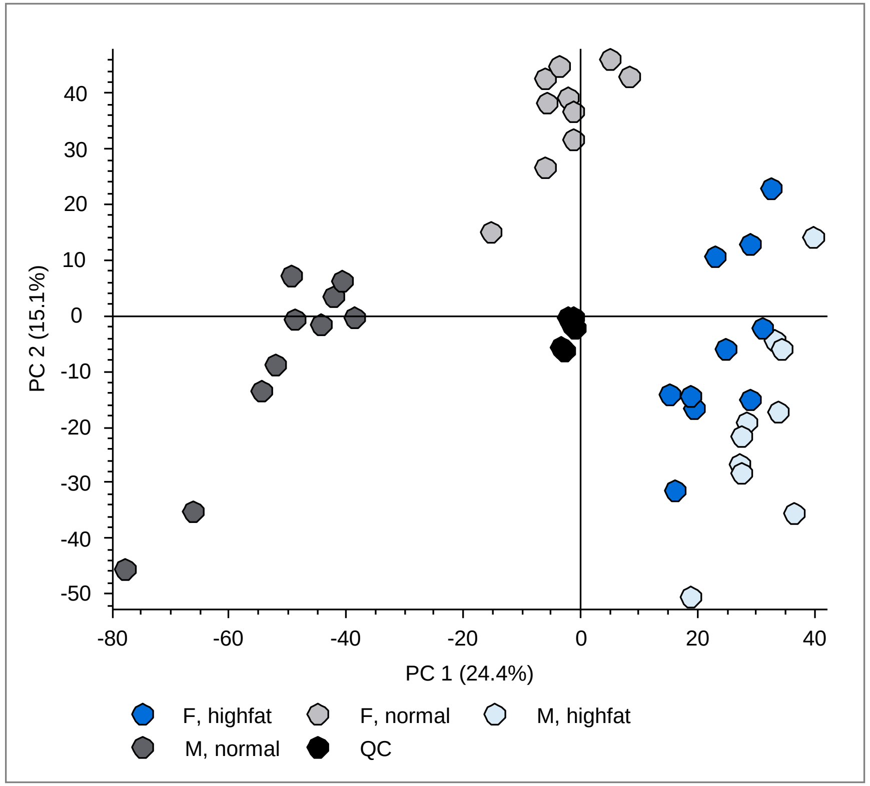
FA(16:0)-OH
m/z 239.2368
0.84 ppm

FA(18:0)-OH
m/z 267.2683
0.75 ppm

Lipidomics Data Analysis

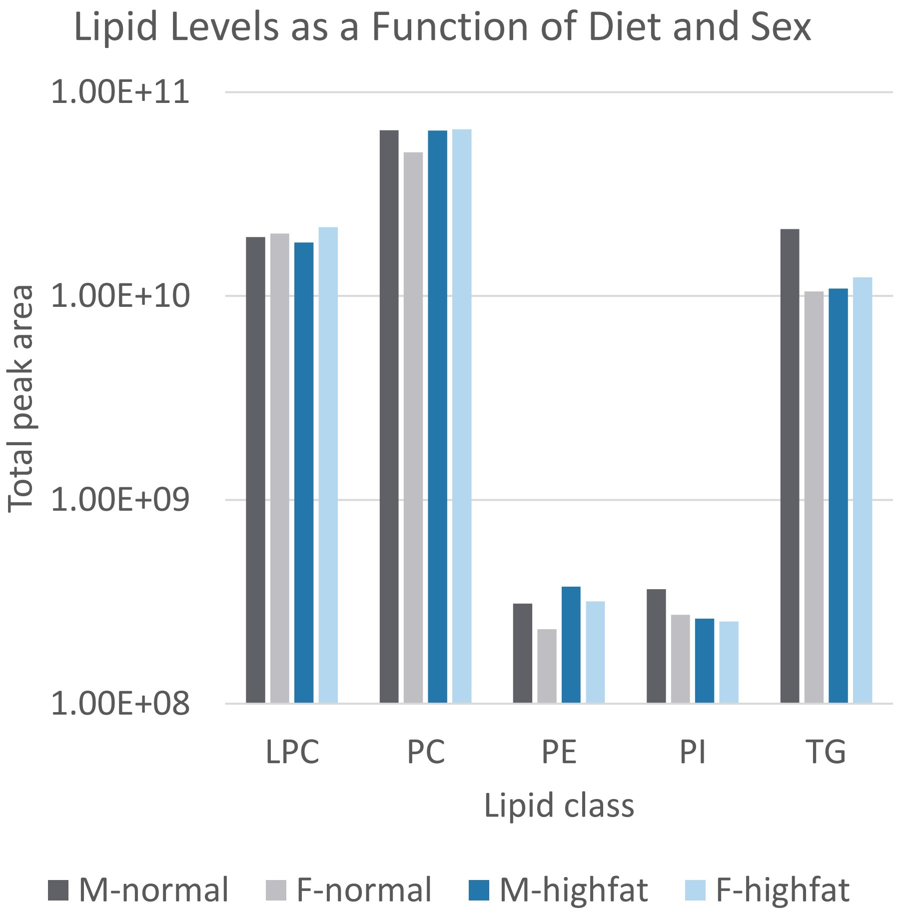
- Lipid QC
- Lipid Annotation
- Differential analysis

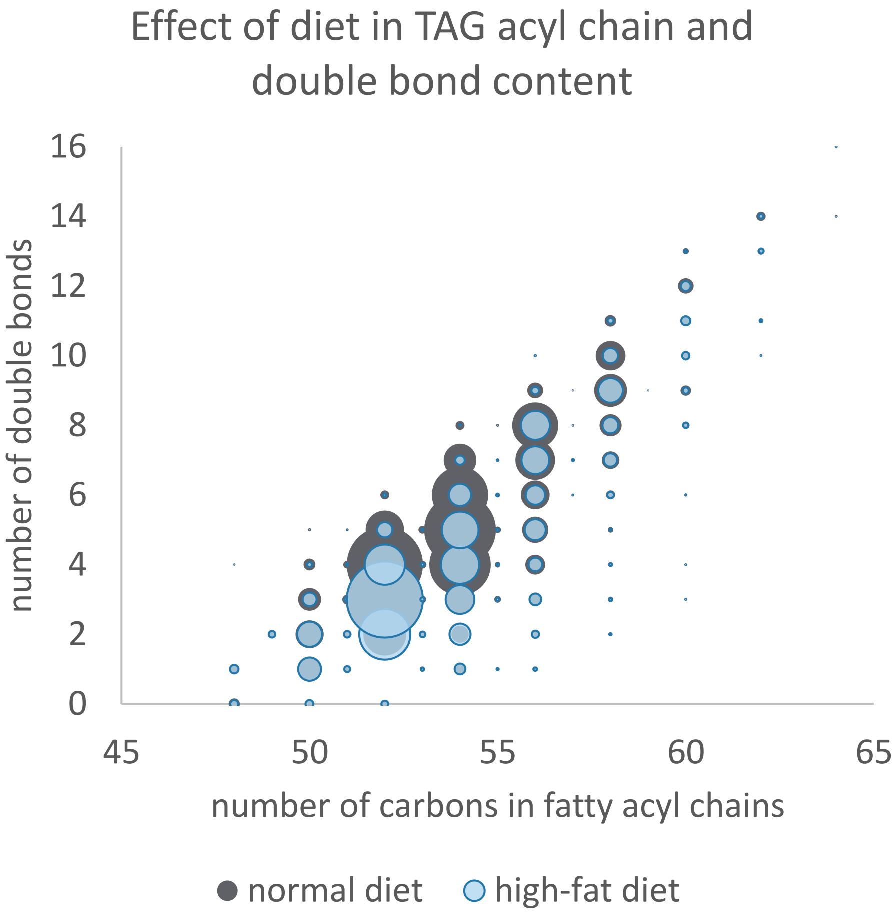




Lipidomics Data Analysis

High-fat Diet Increases Levels of Saturated Triglycerides





Known and Unknown Markers of High-Fat Diet Induced Metabolic Changes

- Changes in lipids and polar metabolites were observed in mice fed high-fat diet
- Metabolomics workflows must facilitate detection of lipids and polar metabolites
- Semi-targeted metabolomics workflow enabled
  - detection of known metabolic differentiators
  - discovery of previously unidentified biomarkers

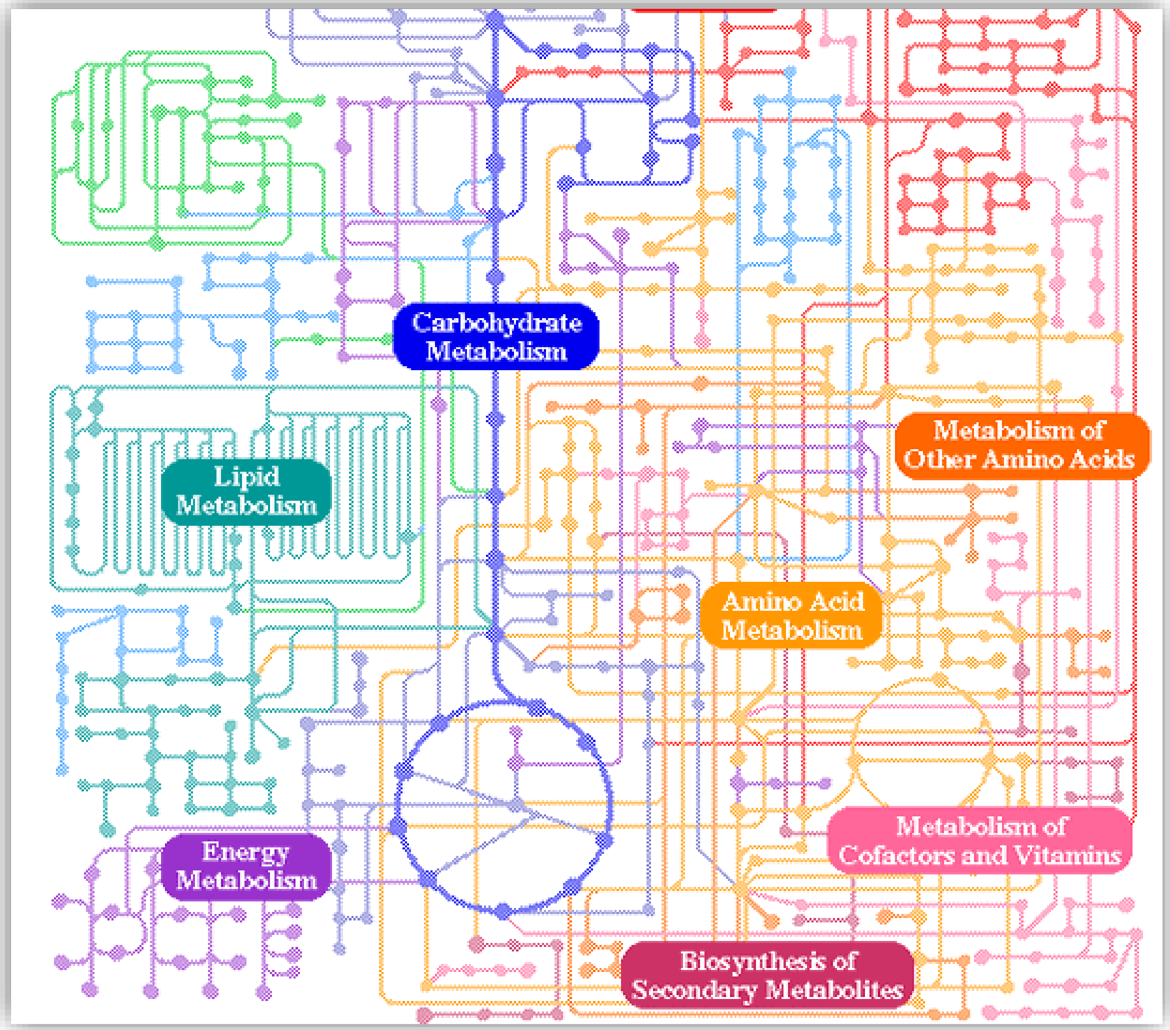


image from KEGG

Thermo Scientific Orbitrap Exploris 240 MS | A New Standard in Metabolomics

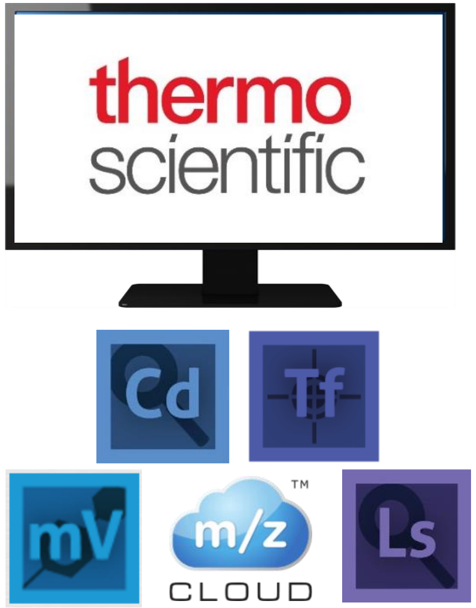
Flexible capabilities to support all metabolomic workflows

- Untargeted
- Semi-targeted
- Stable Isotope Labeling
- Targeted

Outstanding MS delivering robust high-quality data

Orbitrap Exploris 240 MS

Powerful processing software for small molecule analysis



Thermo Scientific Small Molecule Software Suite

Acknowledgements

70,000 Thermo Fisher employees in 50+ countries supporting the global business of science

Bremen

- Alexander Harder
- Maciej Bromirski
- Markus Kellmann
- Julia Kraegenbring
- Tabiwang Arrey
- Alexander Makarov
- Christian Klaas
- Siegrun Mohring
- Catharina Crone
- Kerstin Strupat
- Angela Criscuolo
- Thomas Moehring
- Kai Scheffler
- Eugen Damoc
- Olaf Scheibner
- Marc Guender
- Elizabeth Crawford

Cambridge

- Michelle Dubuke
- Sitara Chauhan
- Sara Trusiak
- Emily Chen
- Krisztina Radi
- David Sarracino

Runcorn

- Kyle d’Silva
- Jon Bardsley
- Simon Cubbon

San Jose

- Andreas Huhmer
- Ken Miller
- Sally Webb
- Lisa Thomas
- Amanda Souza
- Ioanna Ntai
- Mark Schroeder
- Daniel Lopez Ferrer
- Aaron Gajadhar
- Khatereh Motamedchaboki
- Yang Liu
- Aman Makaju
- Shannon Eliuk
- Ed George
- Xiaolei Xie
- Debadeep Bhattacharyya
- Kristine Van Natta
- Stephanie Samra
- Yu Zhou
- Chaitanya Kaza

Thank You!