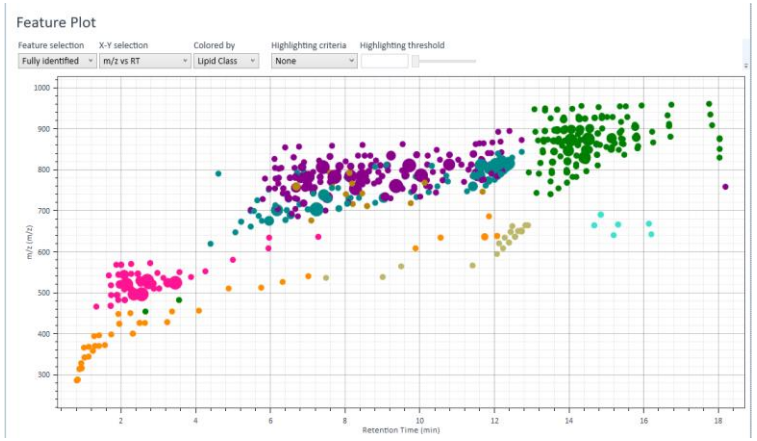
Lipids Greatest Hits

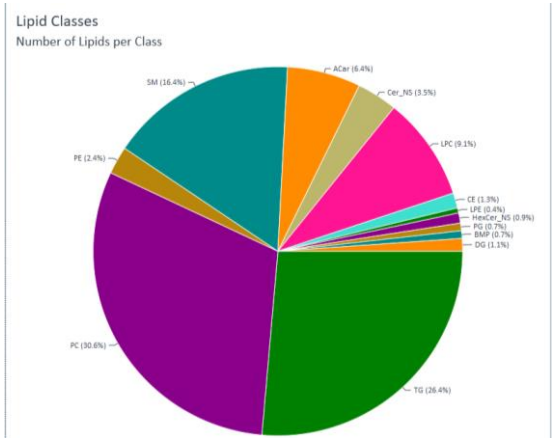
Featuring the Agilent 6546 LC/Q-TOF and Lipid Annotator

Special Breakfast Performance

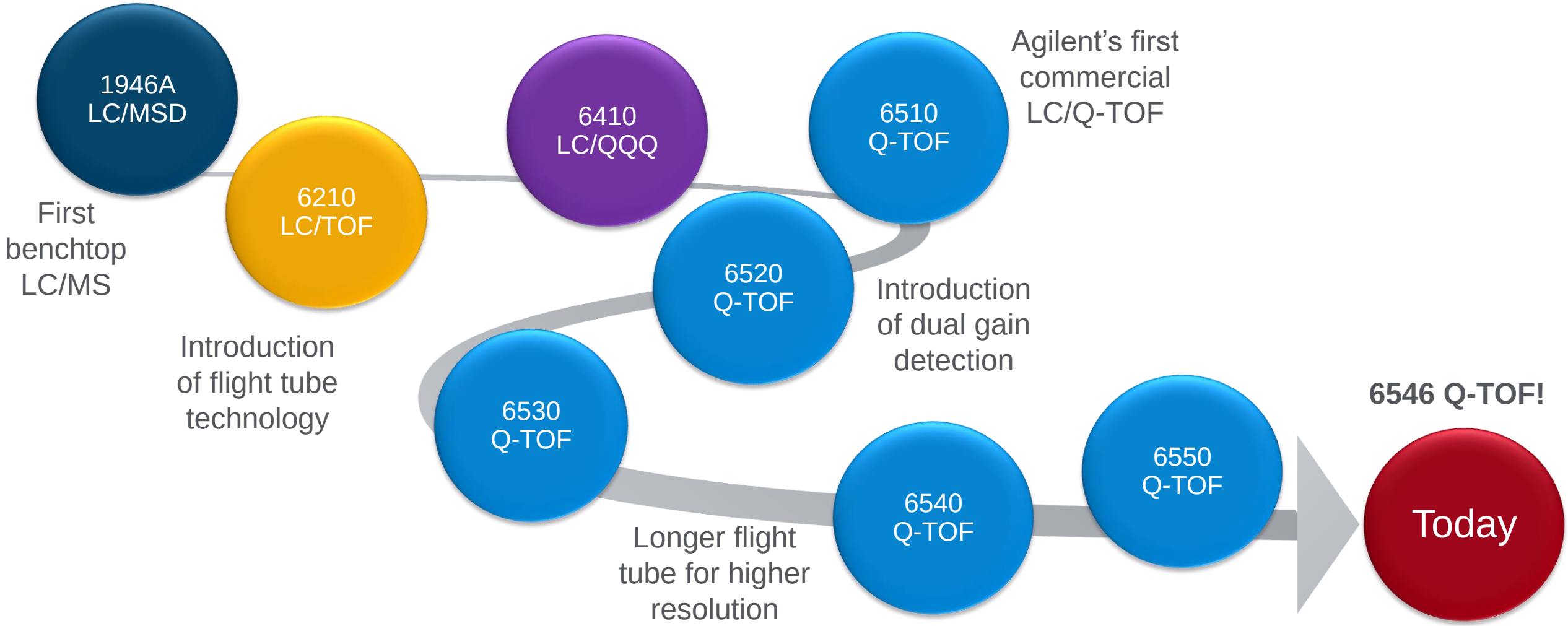
Christian Klein, Ph.D.
Product Manager LC/Q-TOF and IM/Q-TOF

Mark Sartain, Ph.D.
Applications Scientist





History of Agilent's continuous innovation in LC/MS Technology



Agilent 6546 LC/Q-TOF

Evolving Q-TOF performance

High resolution at low *m/z* resolves metabolites

Broad dynamic range covers the wide abundance range

Performance unaffected by acquisition rate

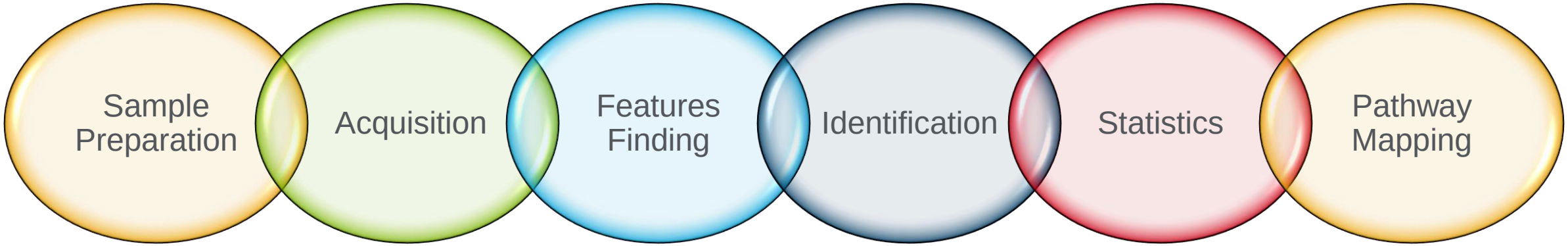
Isotopic fidelity (±5%) aids confident ID and flux analysis

Stable performance for large sample sets

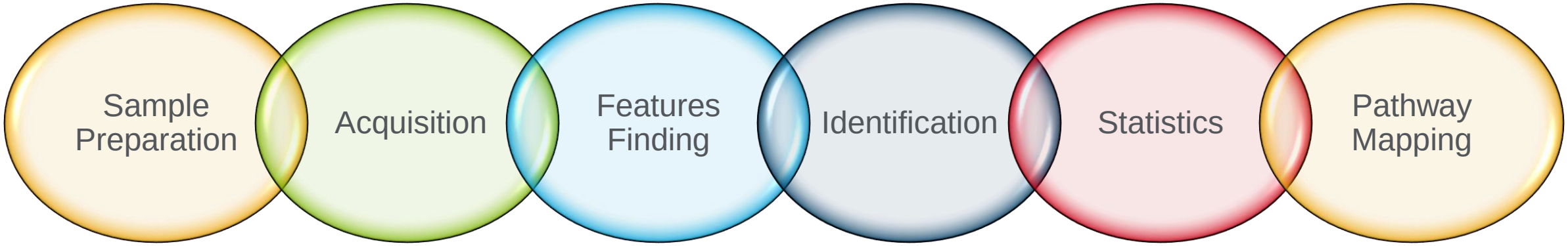
Robustness with easy maintenance for greater up-time

Ideal for metabolomics!!

Improving the Agilent Metabolomics Workflow



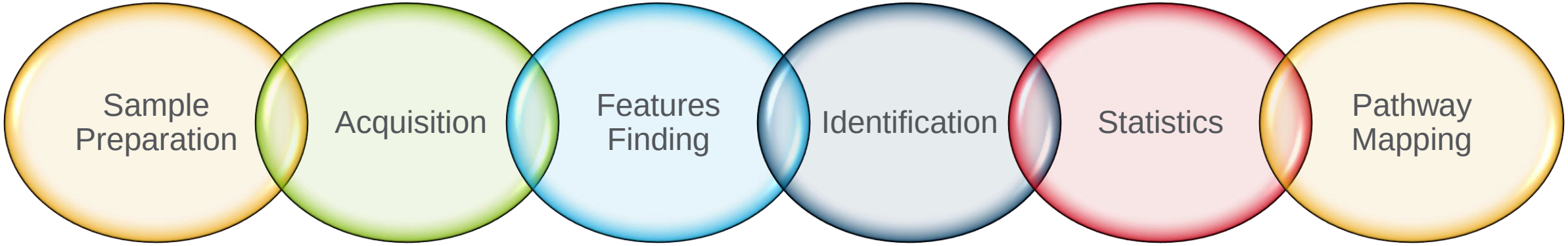
Improving the Agilent Metabolomics Workflow



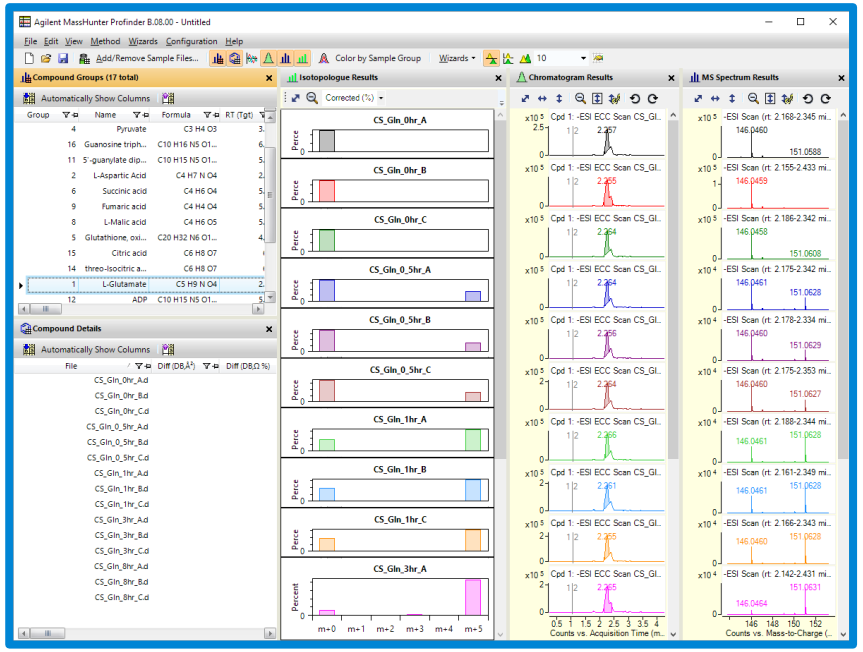
7250 GC/Q-TOF with CI

6546 LC/Q-TOF

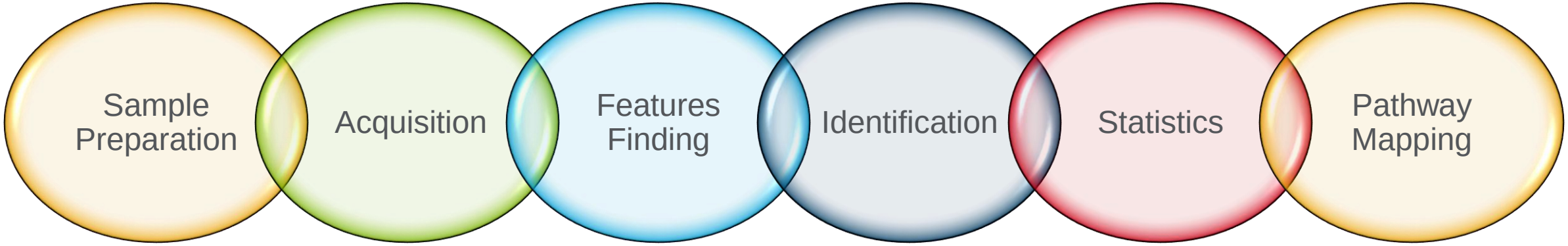
Improving the Agilent Metabolomics Workflow



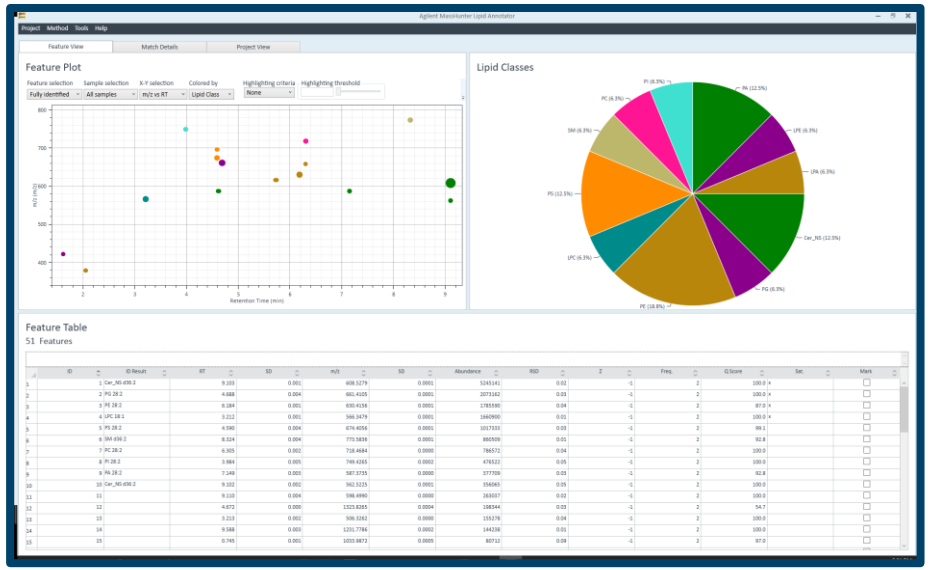
Profinder 10



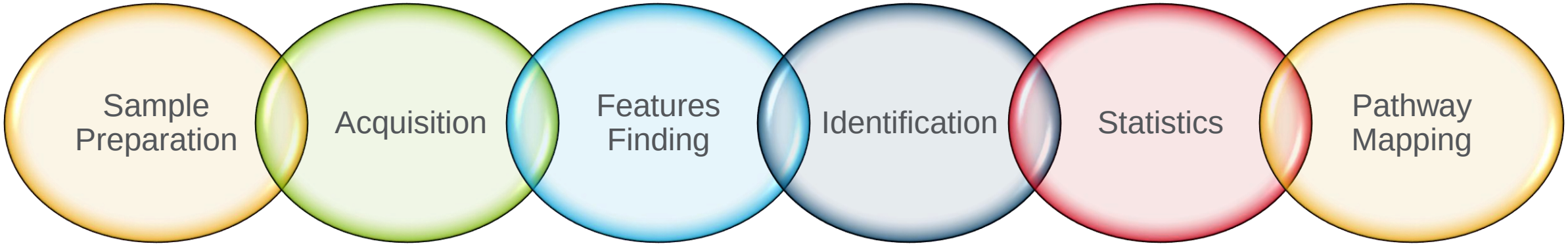
Improving the Agilent Metabolomics Workflow



Lipid Annotator



Improving the Agilent Metabolomics Workflow



Mass Profiler Professional 15

Bravo Metabolomics Platform for better reproducibility in sample preparation

For plasma sample preparation

Simple user interface with optimized methodology

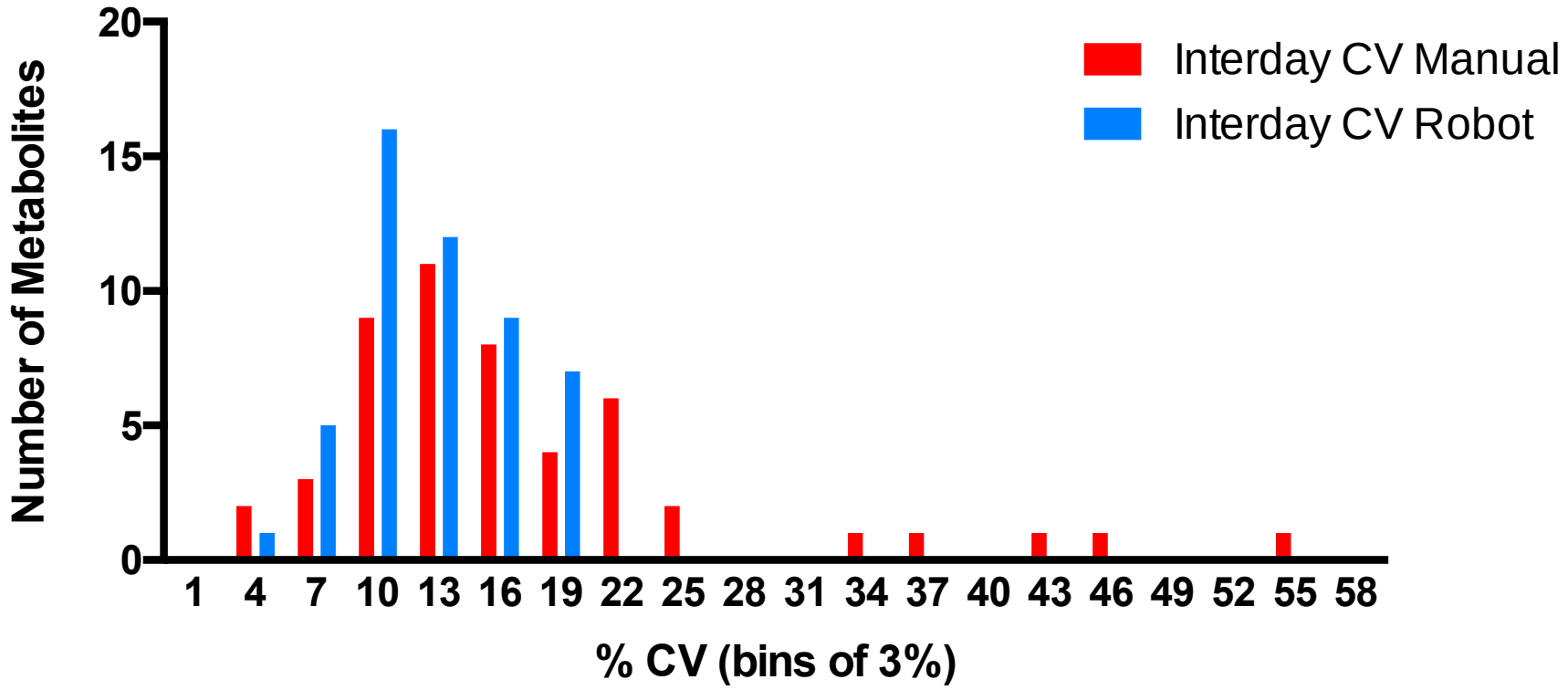
Includes room temperature quench protocol for both on-site and off-site plasma collection

Uses Captiva EMR—Lipid removal system for removing protein precipitate & lipids

Can use as few as 8 wells (1 column) in EMR—Lipid plate

Sample quenching → Protein removal → Lipid removal → Sample drying (off-deck) → Sample reconstitution

Better reproducibility with the Bravo Metabolomics Workbench



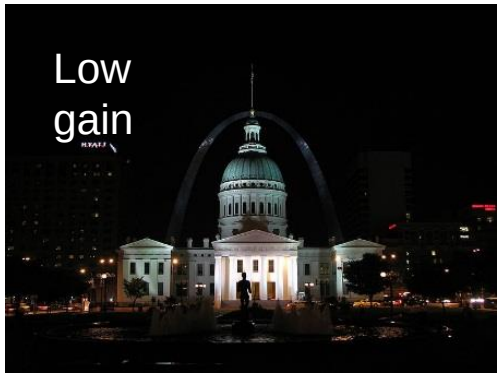
Automated, standardized protocol that is reproducible between projects, staff, and over time.

Data courtesy of Van Eyk Lab, Cedars-Sinai Medical Center
Agilent Application note 5994-0685EN

Time-of-Flight (TOF) MS Design

10 GHz High speed digitization of detector signal delivers high resolution

Dual gain for greater dynamic range



The detector signal must be digitized:

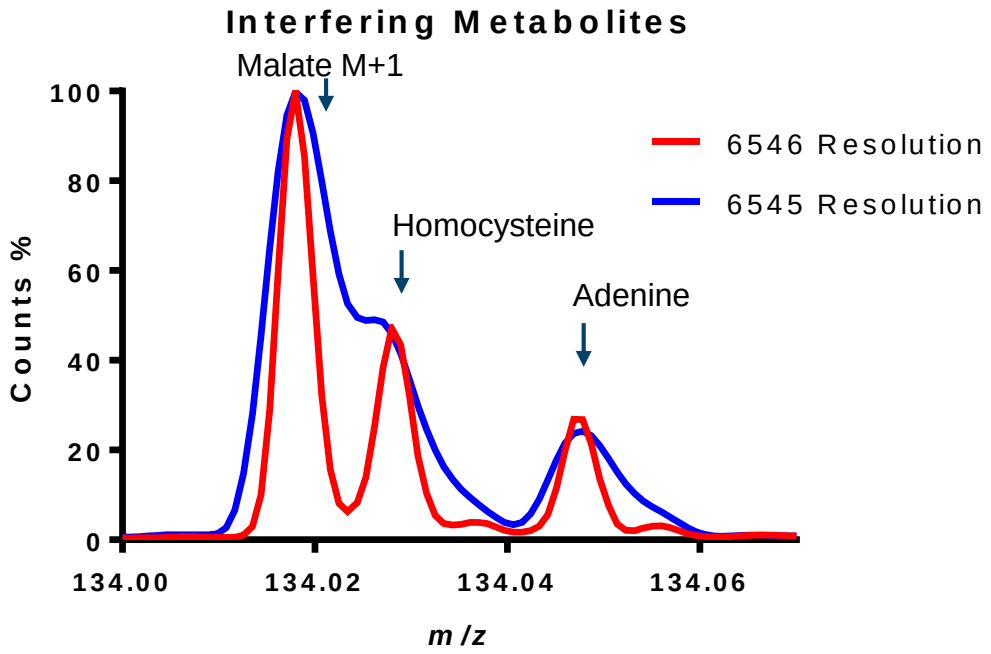
- High speed analog-to-digital converter (ADC) of 80 distinct ADCs
- Multiple low speed ADCs interleaved and synchronized for effective sampling rate
- Narrow detector signal requires high speed digitization for high resolution
- Two 10 GHz channels (high and low gain) result in single Dual Gain spectrum

https://commons.wikimedia.org/wiki/File:StLouisArchMultExpEV%2B4.09.JPG © Kevin McCoy

6546 LC/Q-TOF Performance for Metabolomics

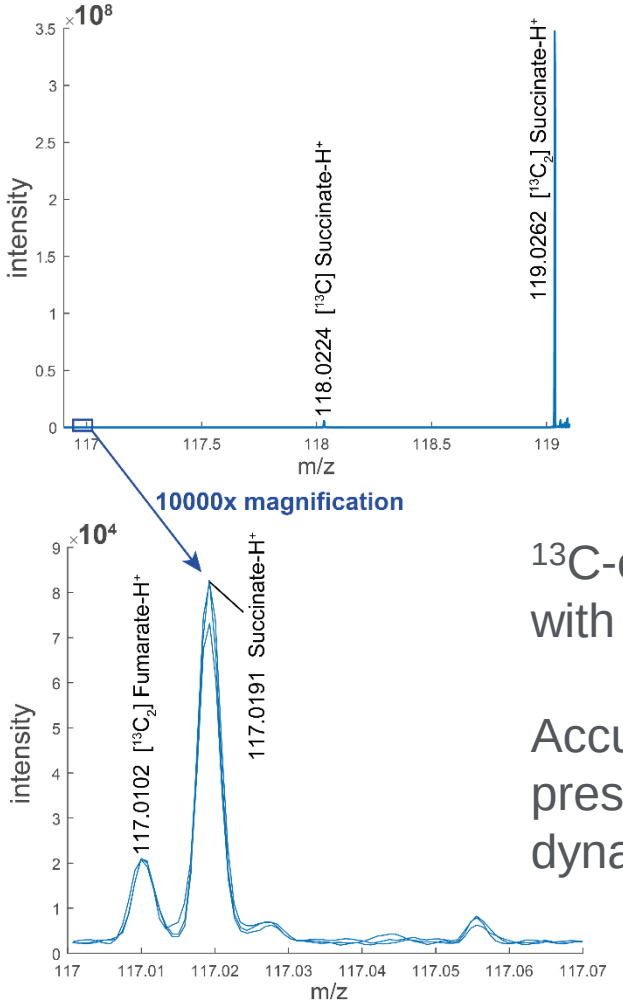
Benefiting from the design innovations

Resolving coeluting metabolites



Malate M+1 $[M-H]^-$ is 134.0176
Homocysteine $[M-H]^-$ is 134.0281
Adenine $[M-H]^-$ is 134.0472

In-spectrum dynamic range: >4 orders

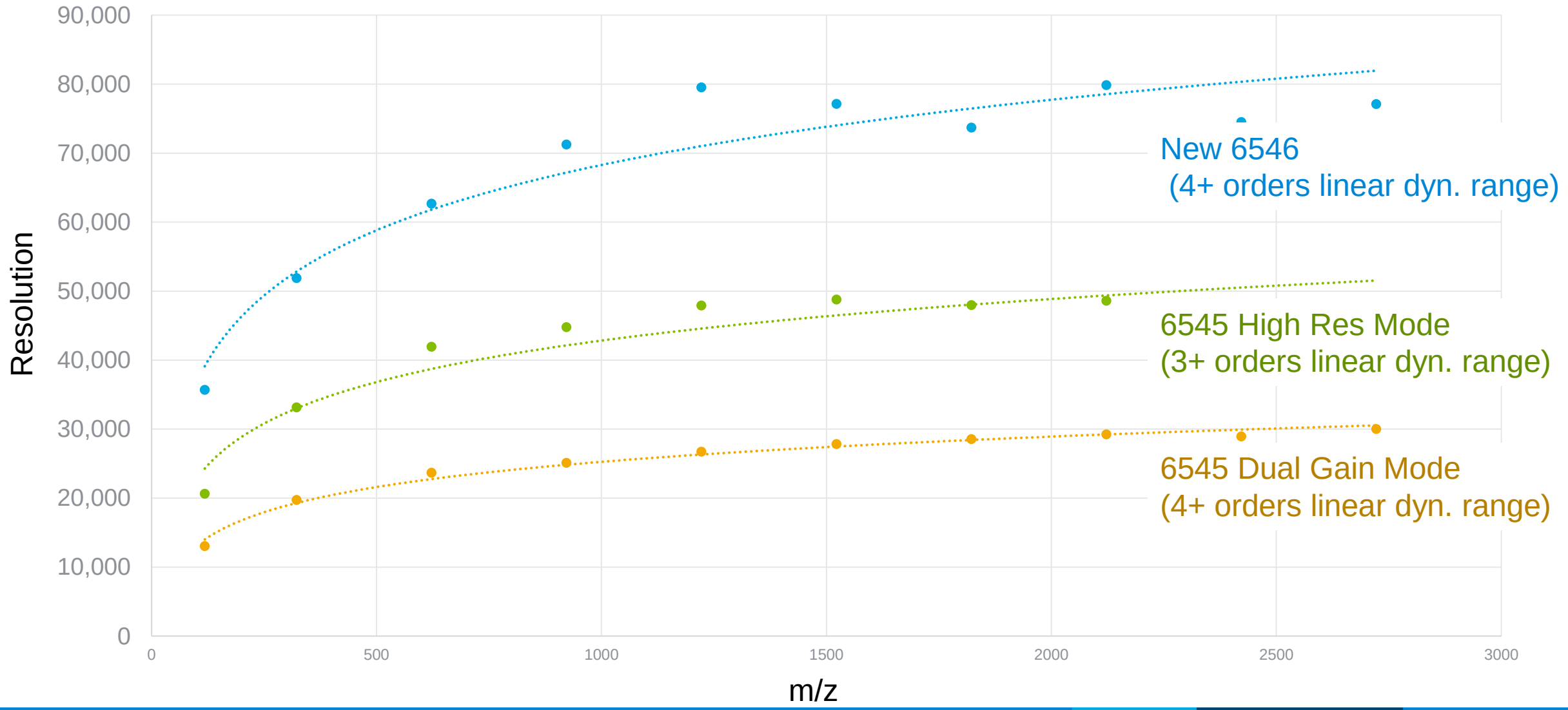


^{13}C-enriched *E. coli* spiked with $^{13}C_2$-succinate (n=3)

Accuracy and resolution preserved across wide dynamic range

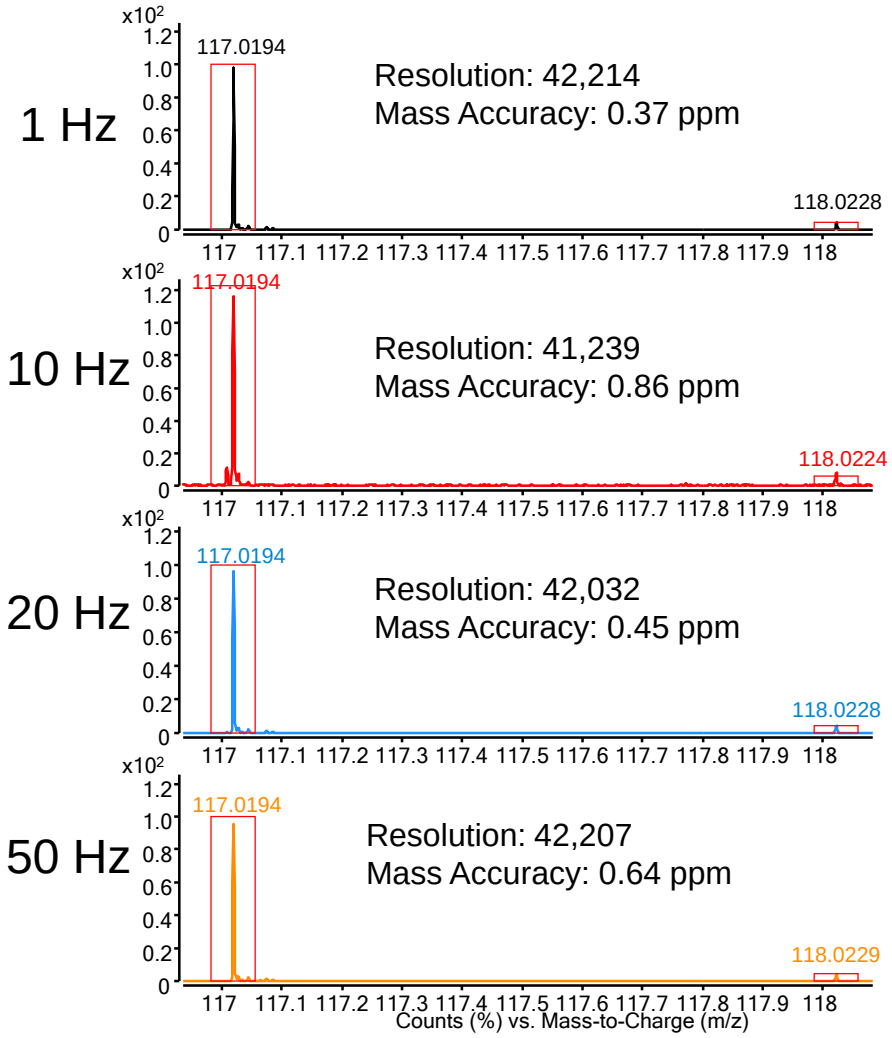
Agilent 6546 LC/Q-TOF: Gaining Higher Confidence and Throughput in Metabolite Analysis. Agilent application note 5994-0724EN

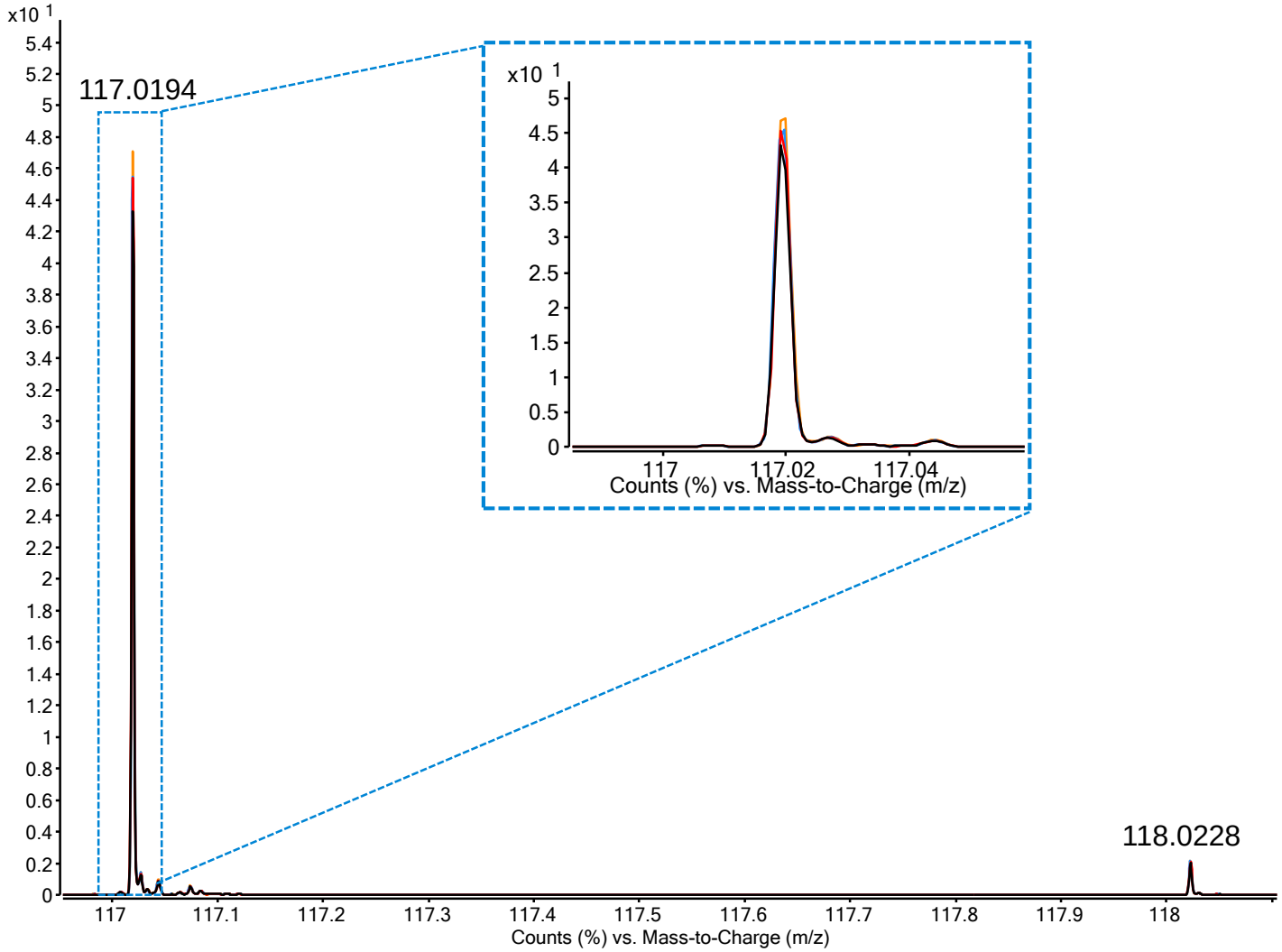
Resolution Increase of the 6546 LC/Q-TOF



6546 LC/Q-TOF Performance for Metabolomics

Resolution independent of acquisition rate for succinic acid





6546 LC/Q-TOF Performance for Metabolomics

Resolution independent of acquisition rate for ATP

1 Hz

Resolution: 70,434
Mass Accuracy: 0.54 ppm

10 Hz

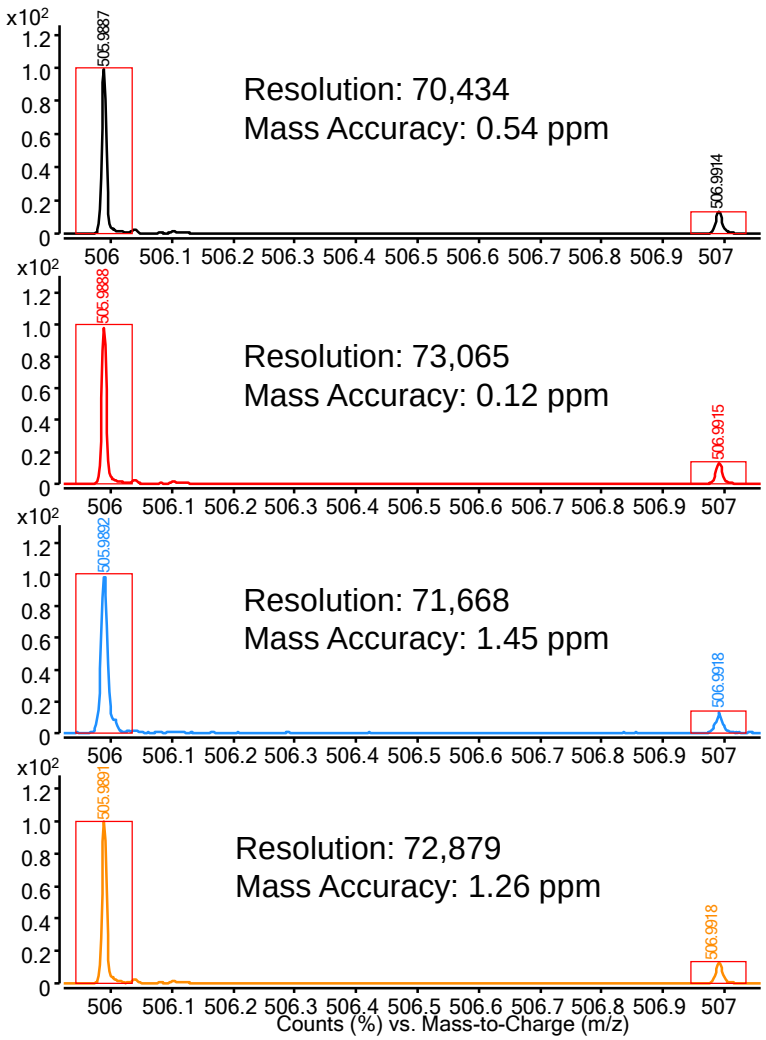
Resolution: 73,065
Mass Accuracy: 0.12 ppm

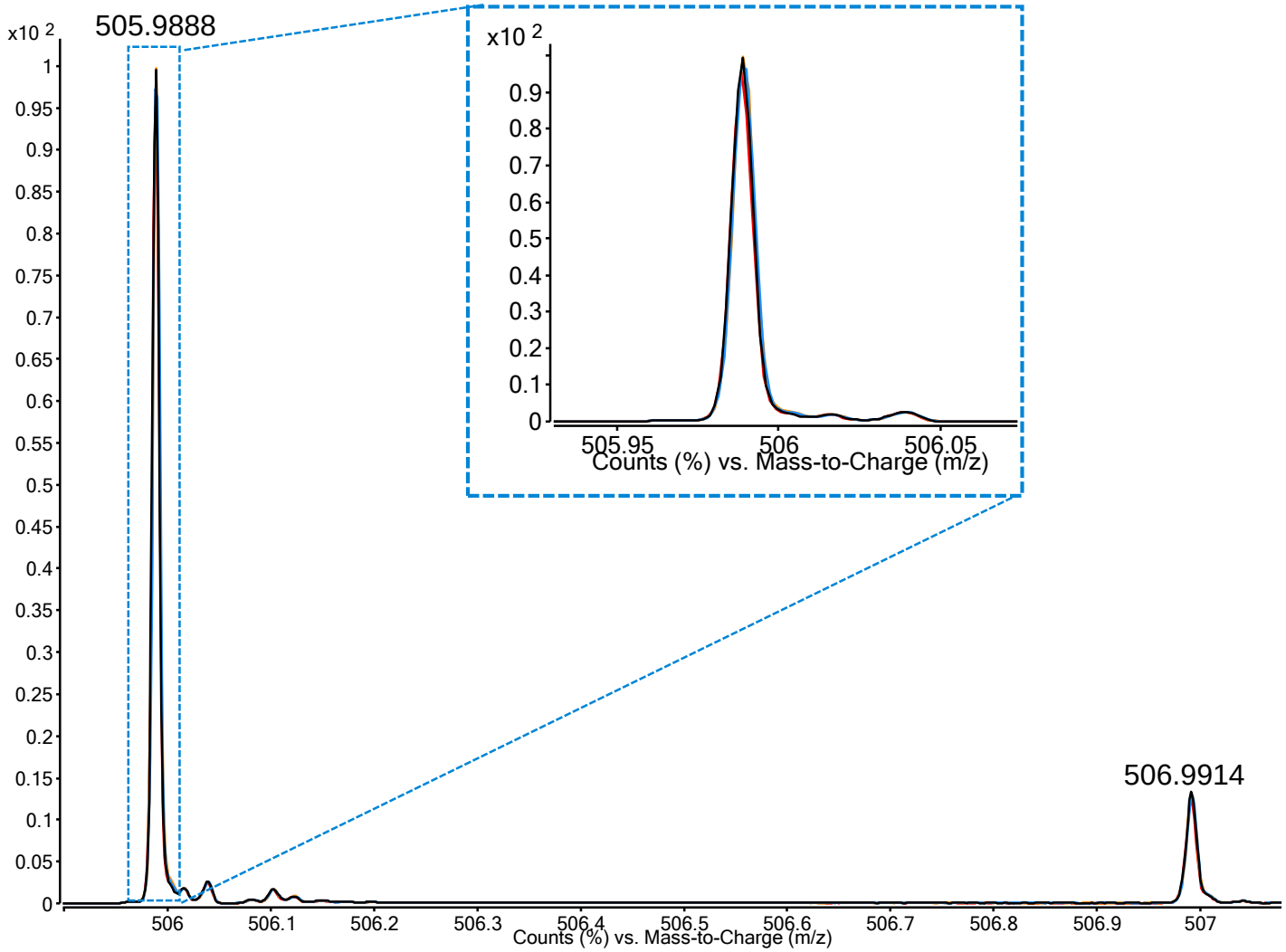
20 Hz

Resolution: 71,668
Mass Accuracy: 1.45 ppm

50 Hz

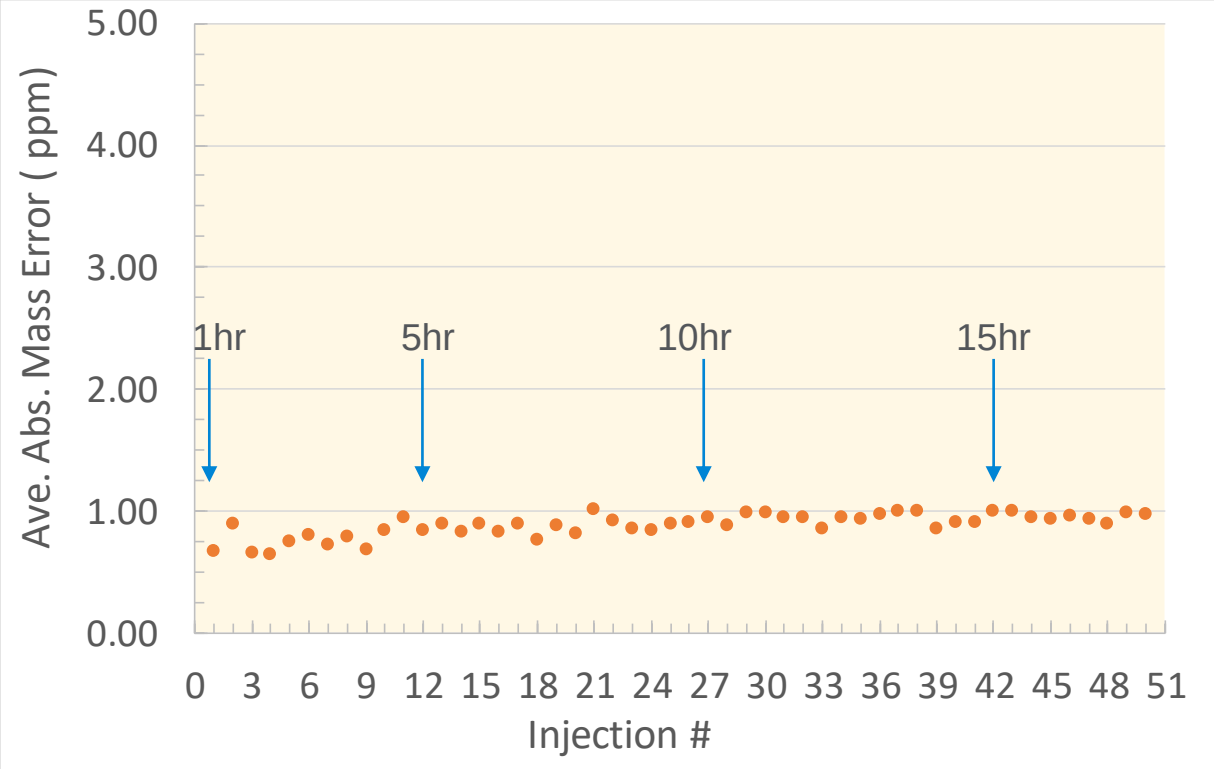
Resolution: 72,879
Mass Accuracy: 1.26 ppm

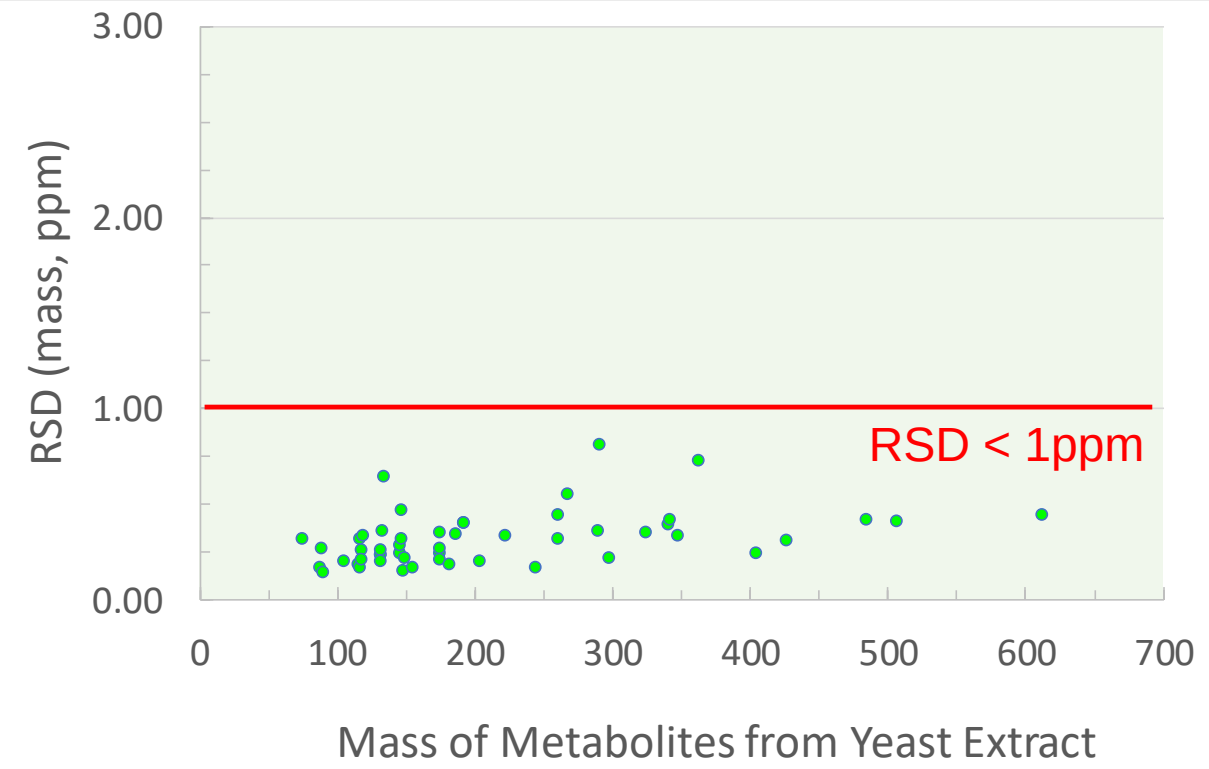




6546 LC/Q-TOF Performance for Metabolomics

Stable mass accuracy over 18 hours for metabolites in yeast extract



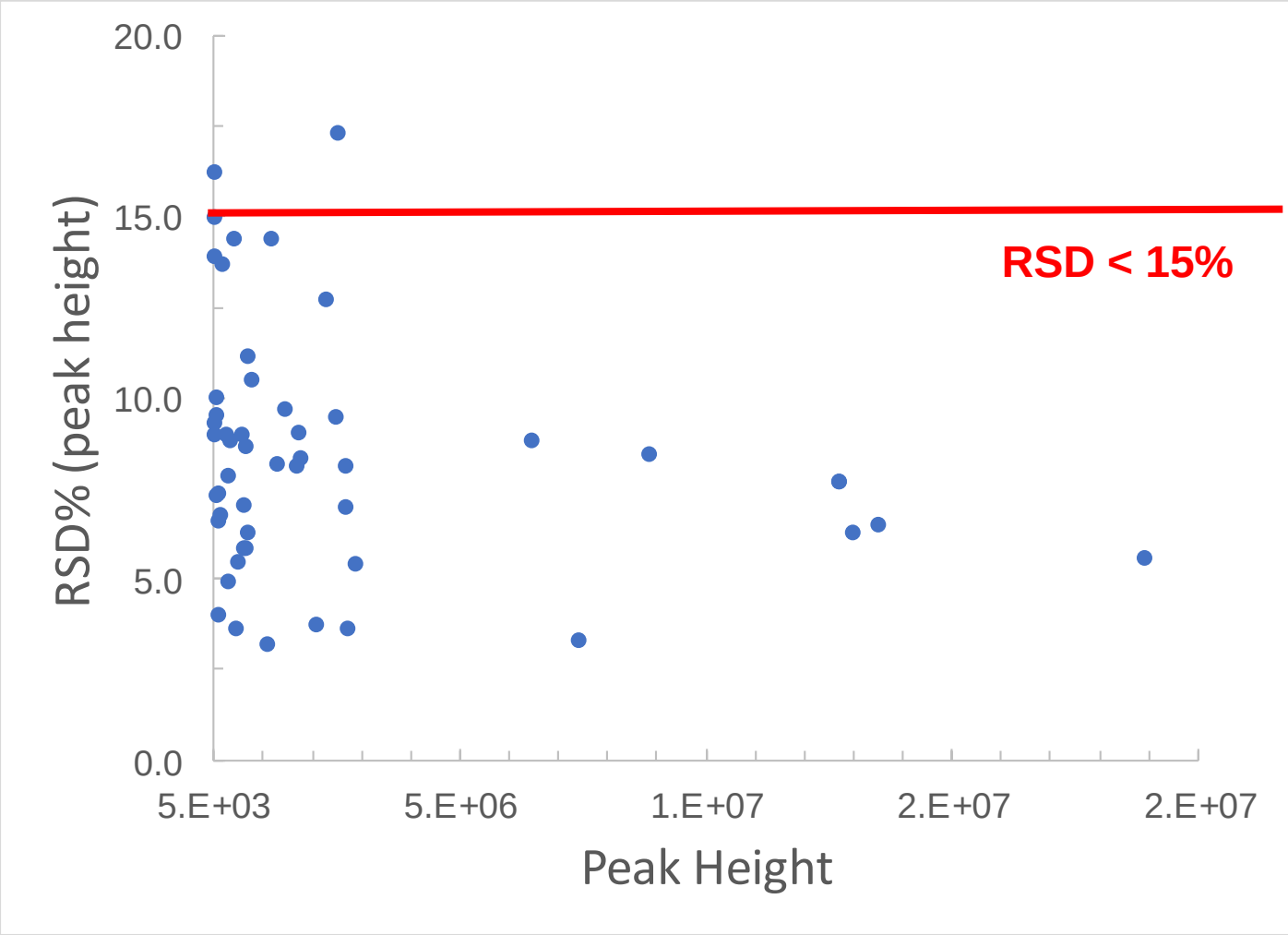


Average absolute mass error ≤ 1 ppm (n=51 metabolites)

RSD of mass error <1 ppm (n=50 injections)

6546 LC/Q-TOF Performance for Metabolomics

Stable signal response over 18 hours



Monitored 51 metabolites in yeast extract

Metabolites intensity over 3.5 orders

Observed stable signal over 18 hours:

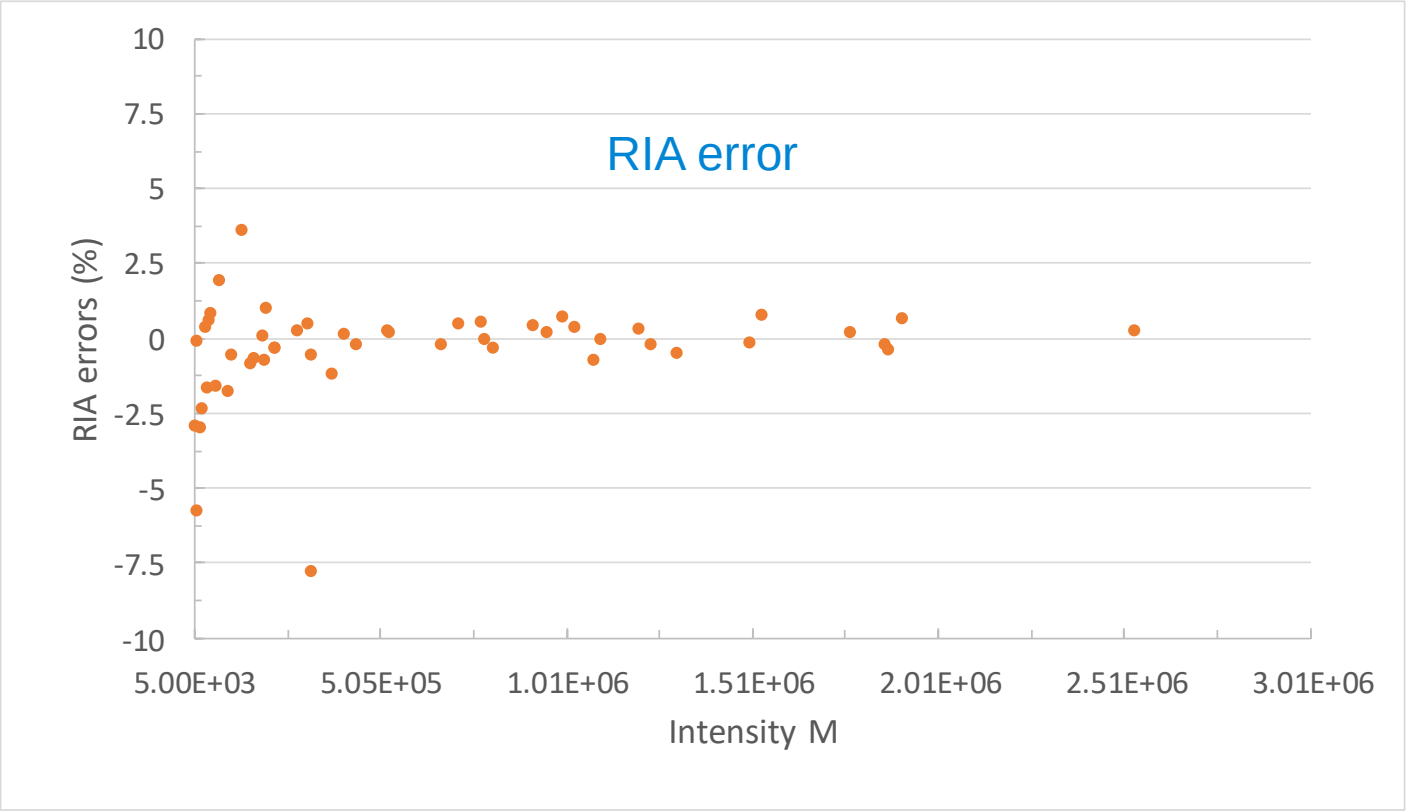
- No metabolite monitored had >20%RSD
- 49 of 51 metabolites have RSD < 15%
- 40 of 51 metabolites have RSD <10%

Signal stability means less instrument variation over large experiments

Less instrument variation = fewer biological replicates needed to power study!

6546 LC/Q-TOF Performance for Metabolomics

Exceptional isotopic fidelity observed

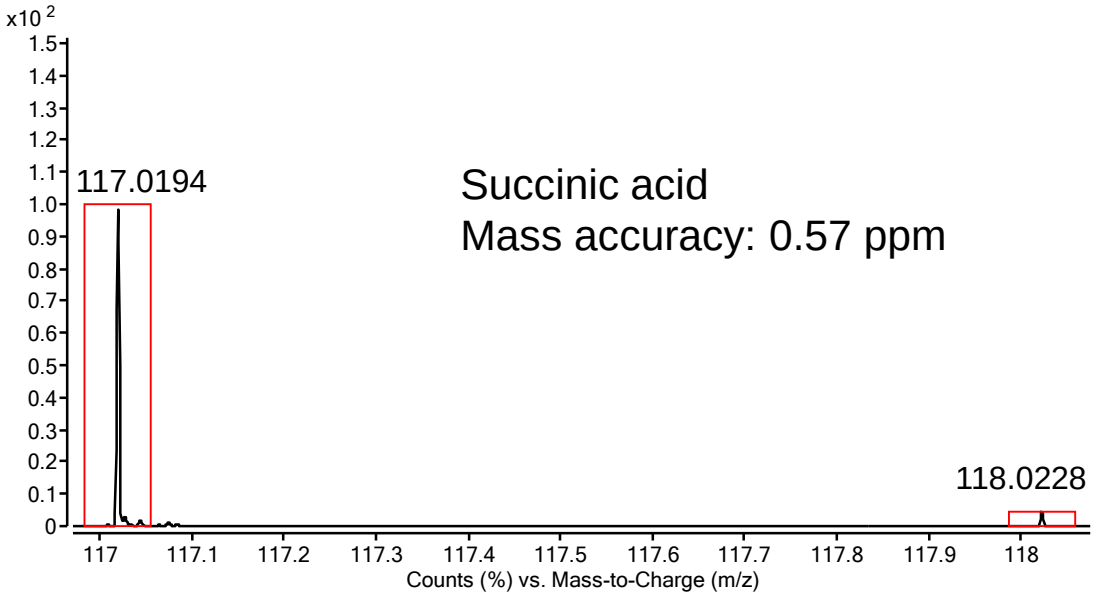


Calculated relative isotopic abundance error for 51 metabolites in yeast extract

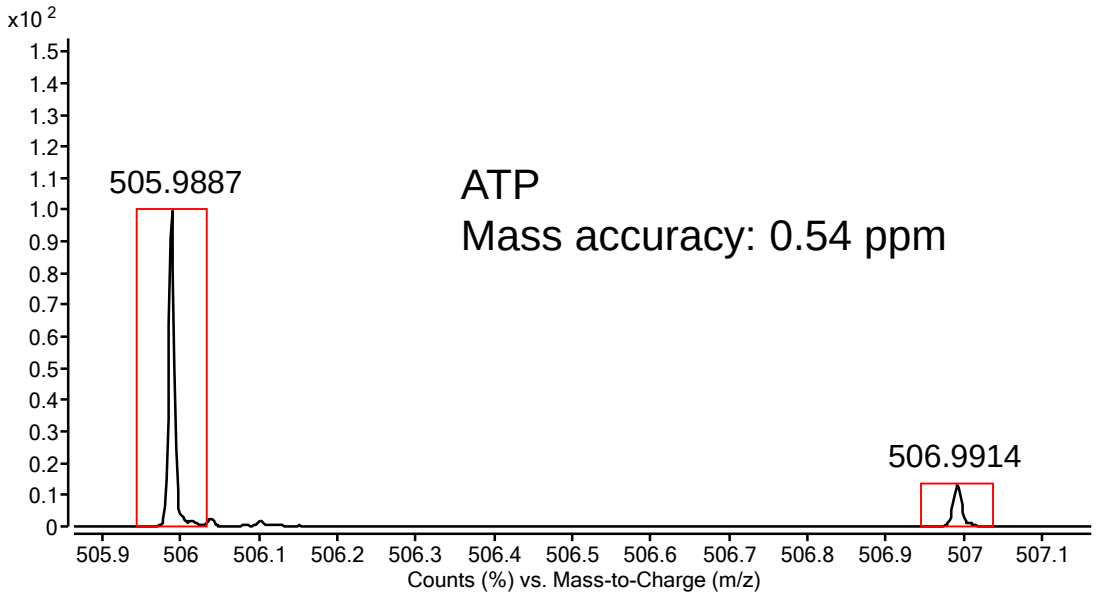
- Very small error even at low ion intensity
- 49 out of 51 metabolites have relative isotope abundance errors ≤ 5%

6546 LC/Q-TOF Performance for Metabolomics

Exceptional isotopic fidelity observed for succinic acid and ATP



Succinic acid
Mass accuracy: 0.57 ppm



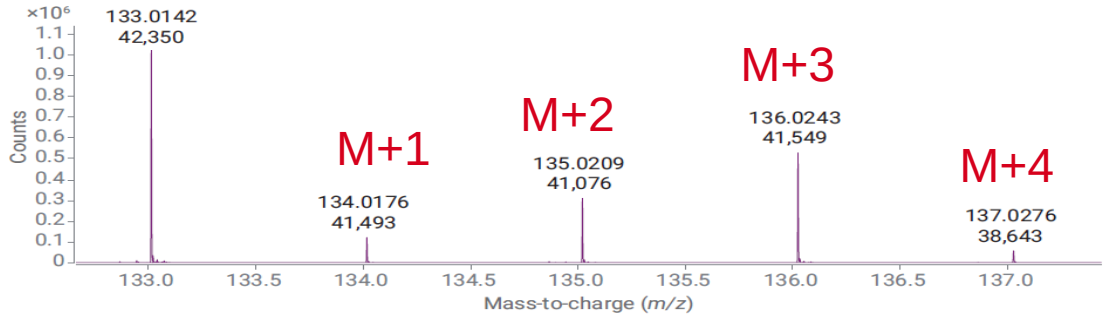
ATP
Mass accuracy: 0.54 ppm

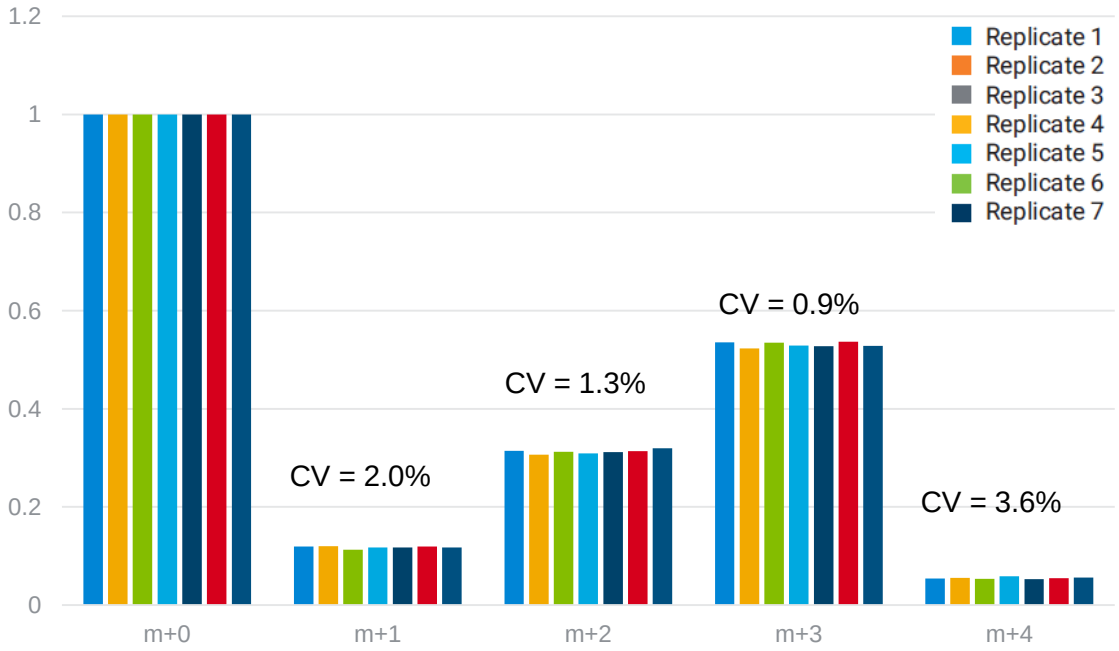
Succinic acid		
m/z	Height %	Height % (Calc)
117.0194	100	100
118.0228	4.67	4.54
119.0237	0.91	0.90
120.0269	0.06	0.04

ATP		
m/z	Height %	Height % (Calc)
505.9887	100	100
506.9914	13.26	13.31
507.9929	3.74	3.50
508.9960	0.41	0.39
509.9979	0.06	0.06

6546 LC/Q-TOF Performance for Metabolomics

Reproducible isotopologue abundances in qualitative flux





Flux experiment using Hep2G cells grown with U-^{13}C glucose

Histogram plot of isotopologue abundances show response CV <4% even for lowest intensity isotopologue

^{13}C Glucose Qualitative Flux Analysis in HepG2 cells
Agilent application note 5994-0713EN

Stable Isotope Tracing for LC/MS

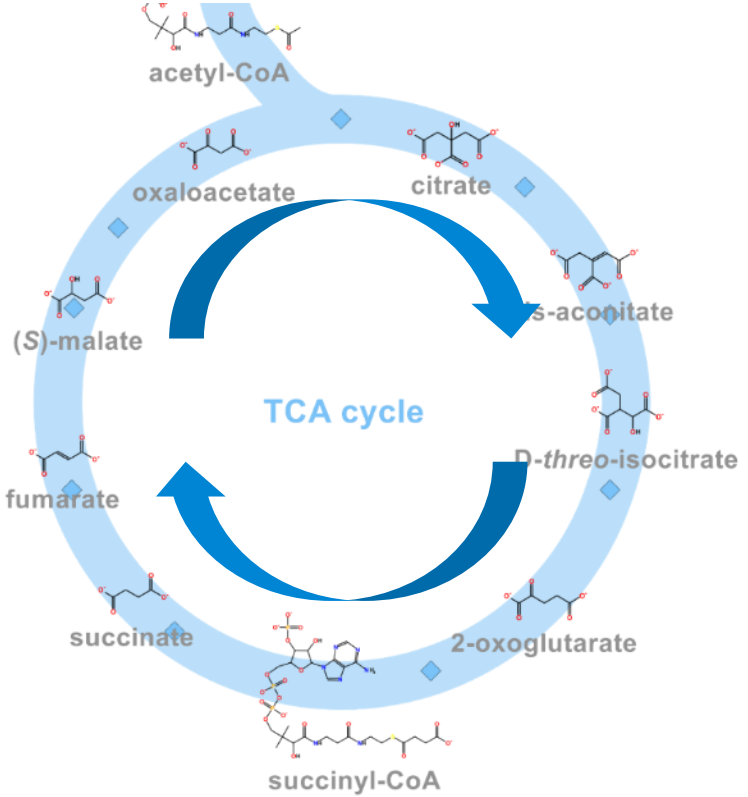
Batch qualitative flux analysis with VistaFlux

Metabolomics provides static information on cellular molecular composition

Qualitative flux analysis reveals *in vivo* pathway activity

Qualitative flux analysis tracks the flow of metabolites through a pathway

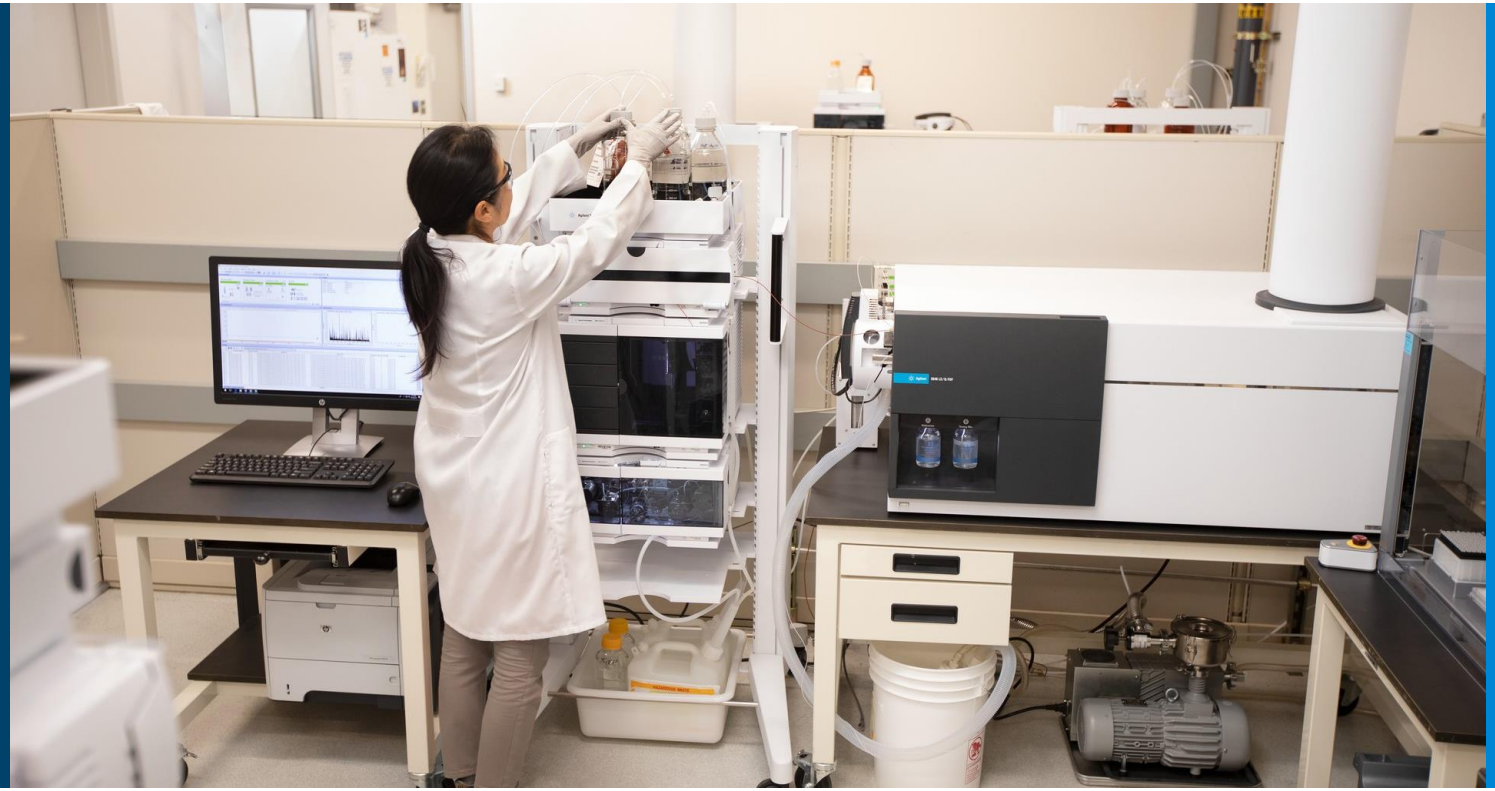
6546 has a isotope ratio spec which is important to this application



Create Target Metabolite List → Acquire TOF Data → Extract Isotopologue Features → Visualize flux on Pathways

Introducing New Lipid Profiling Workflow with Lipid Annotator Software

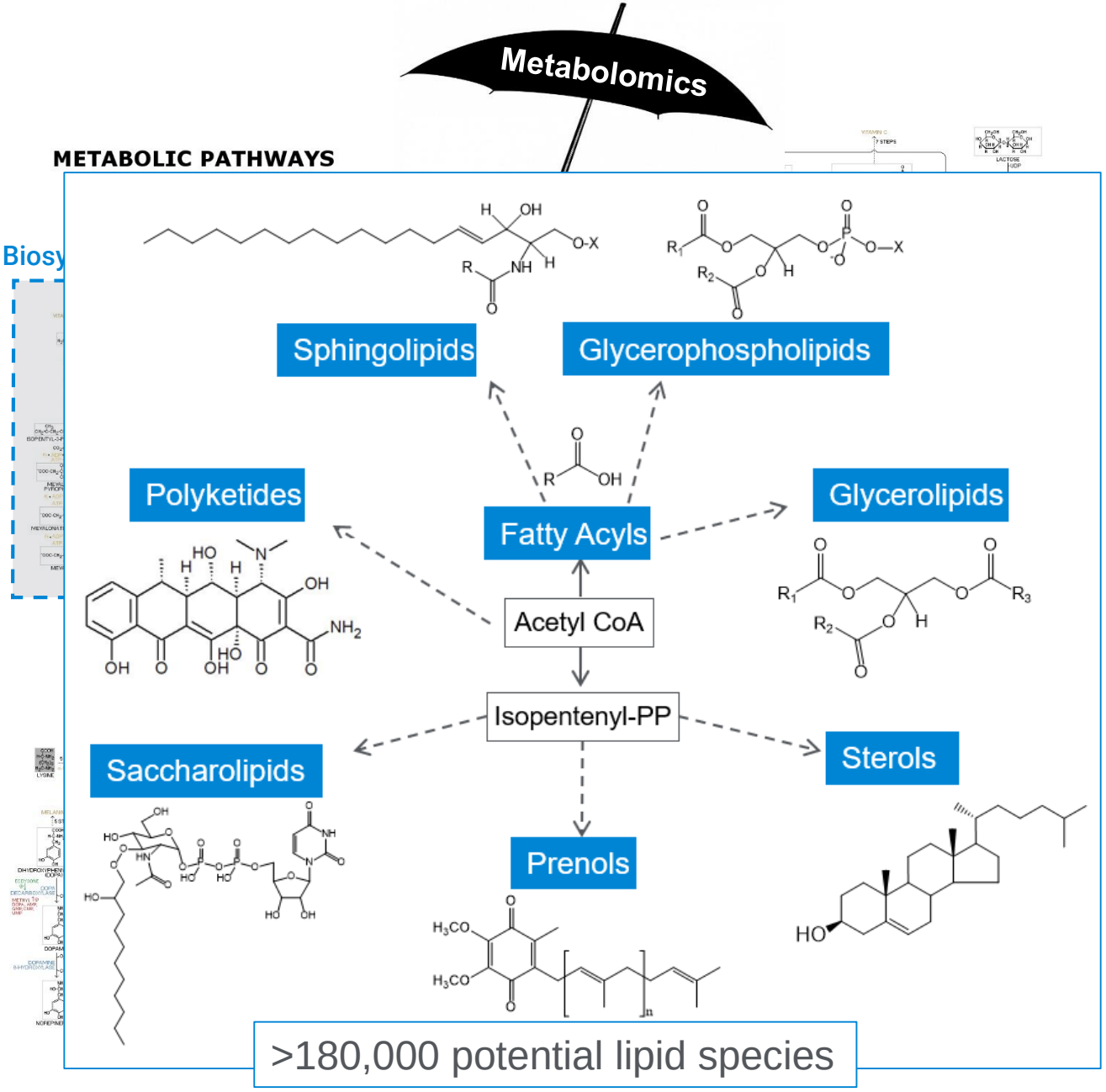
Supported by the 6546 LC/Q-TOF



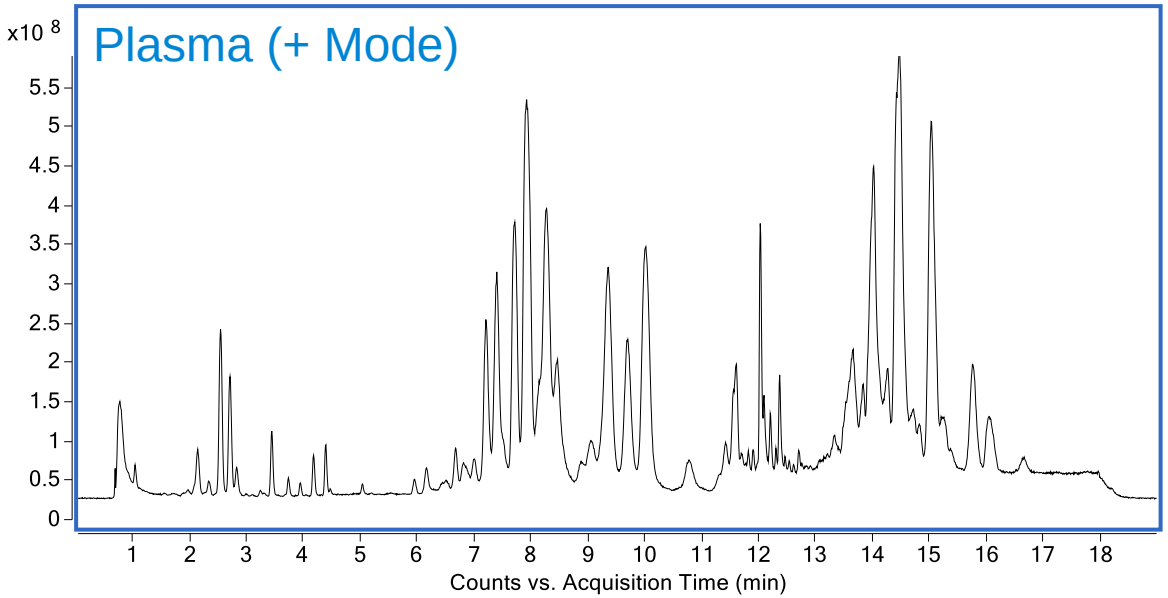
Agilent

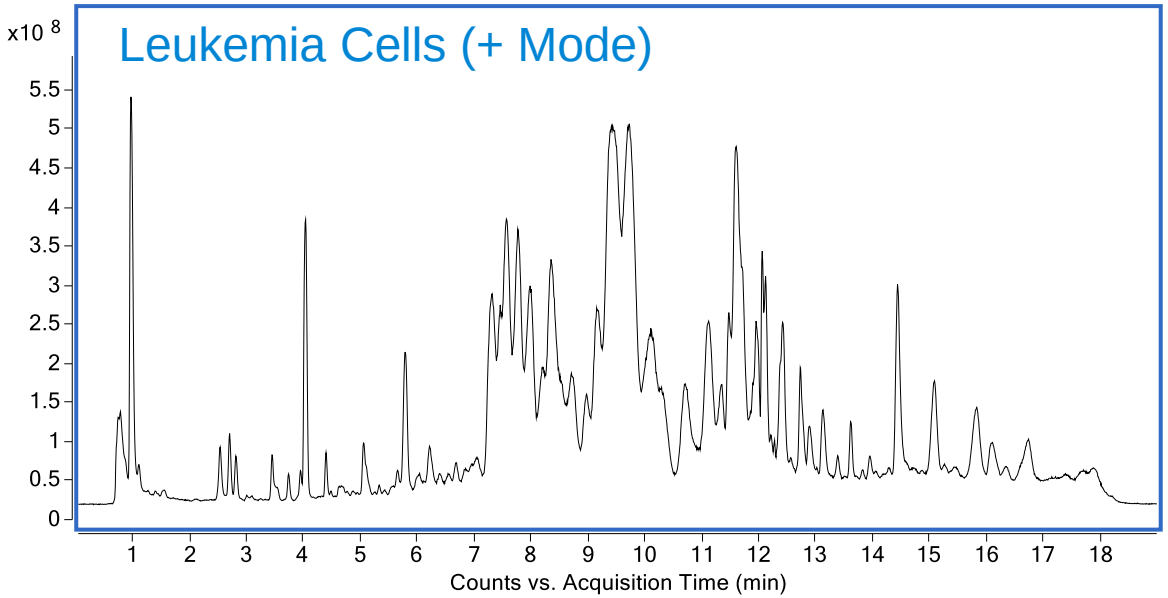
Lipidomics Analysis

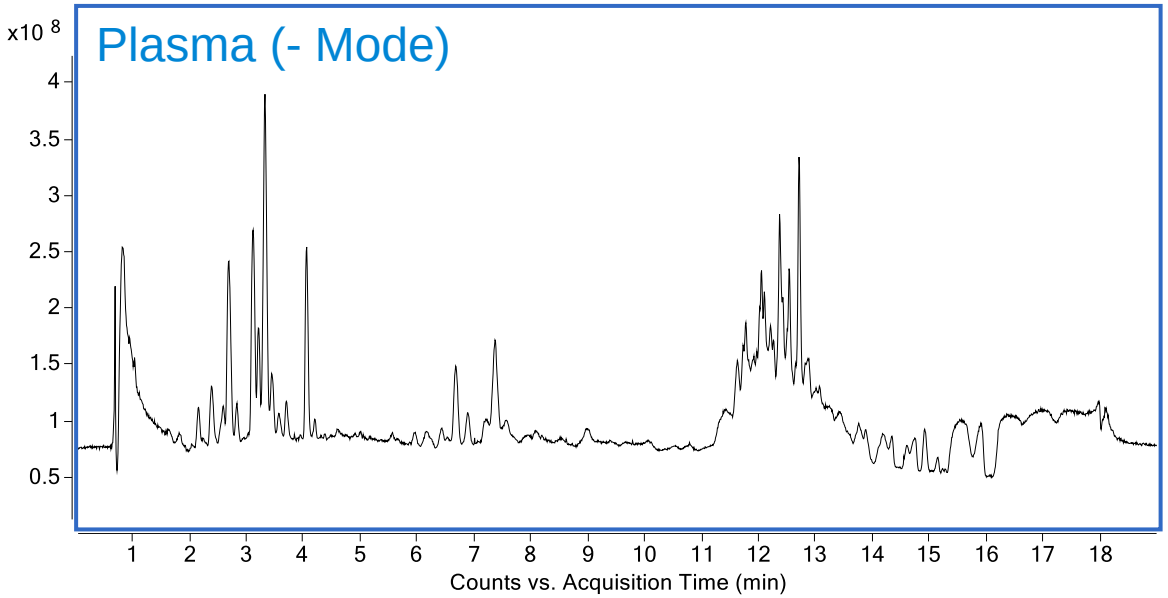
6546 LC/Q-TOF

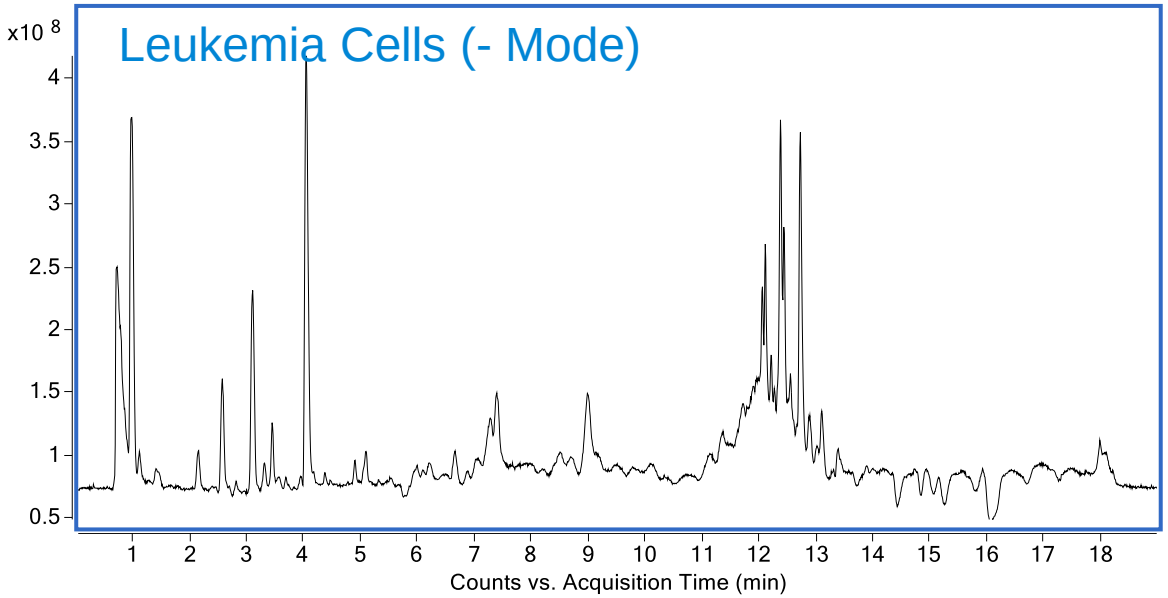


RP-LC/MS – Single LC method

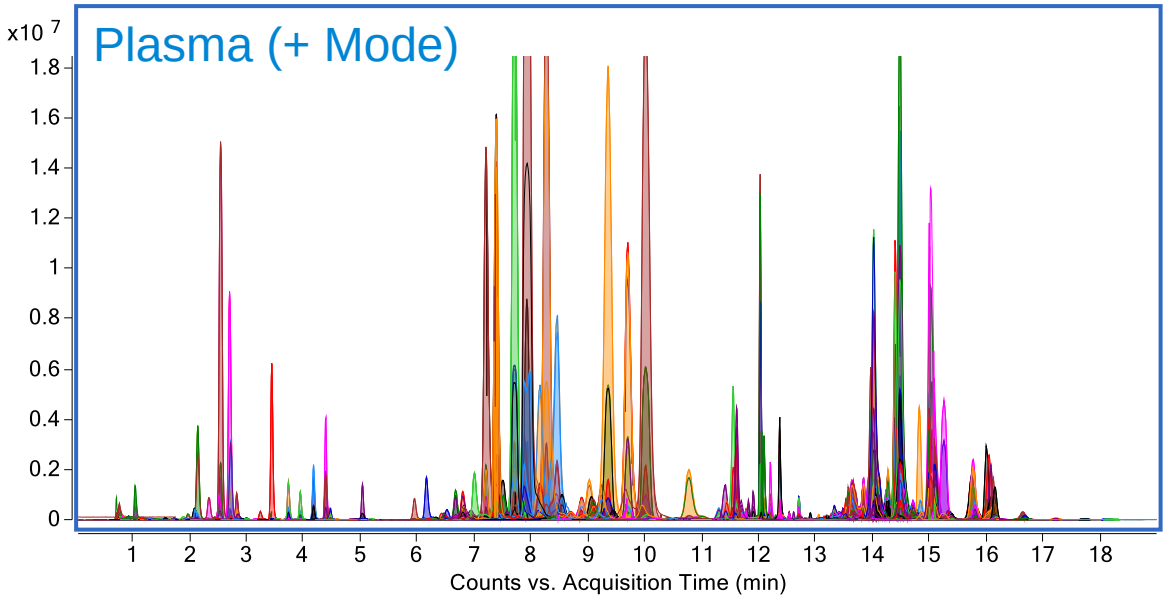


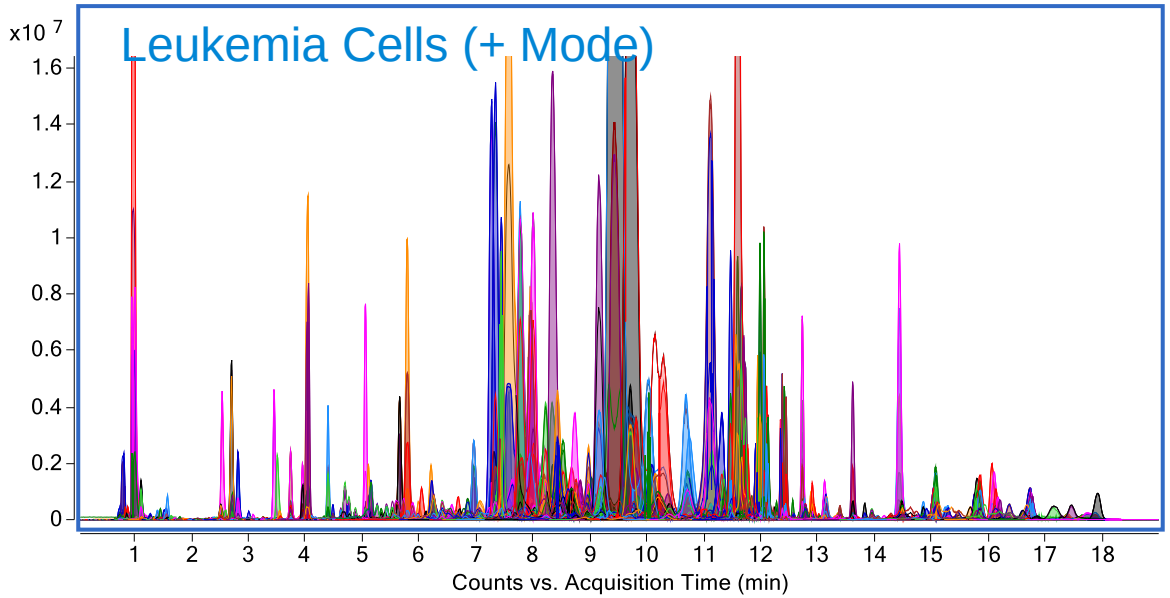


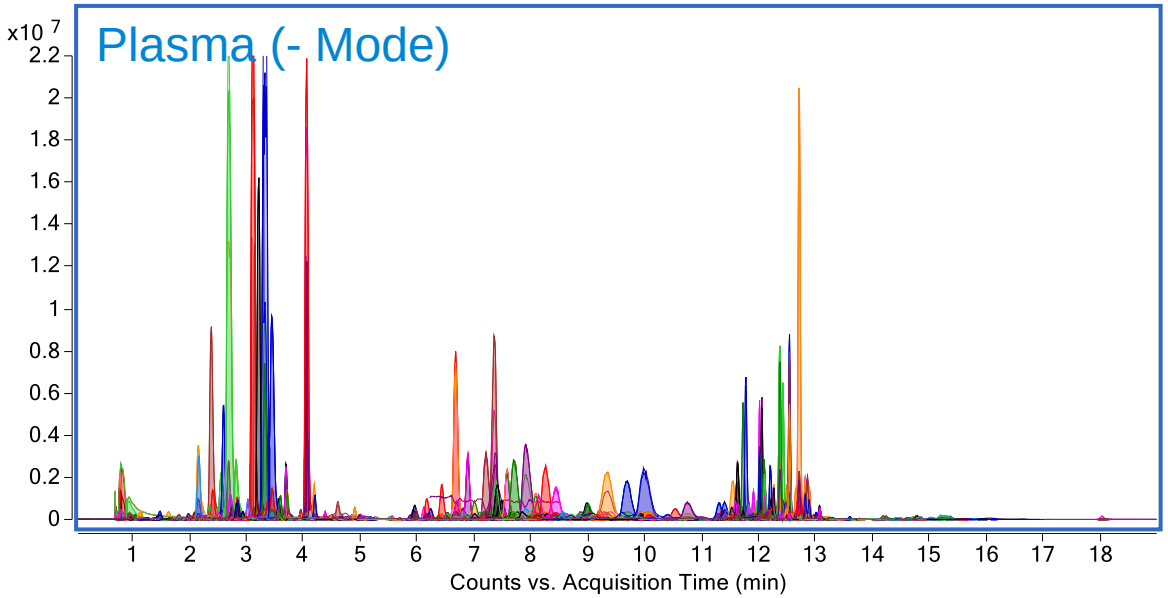


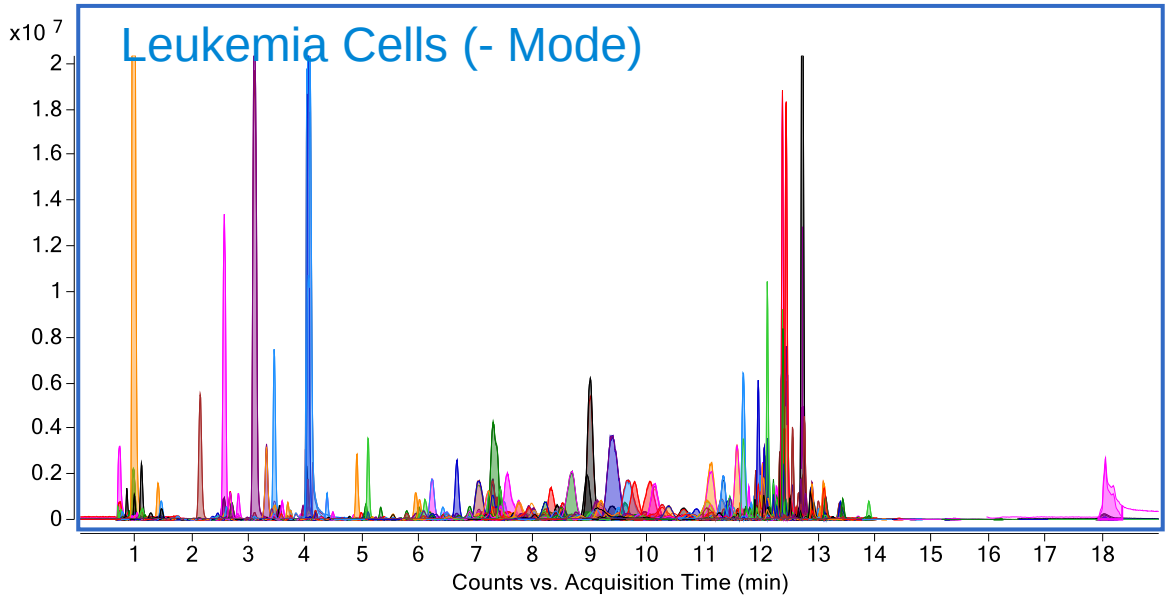


RP-LC/MS – Single LC method



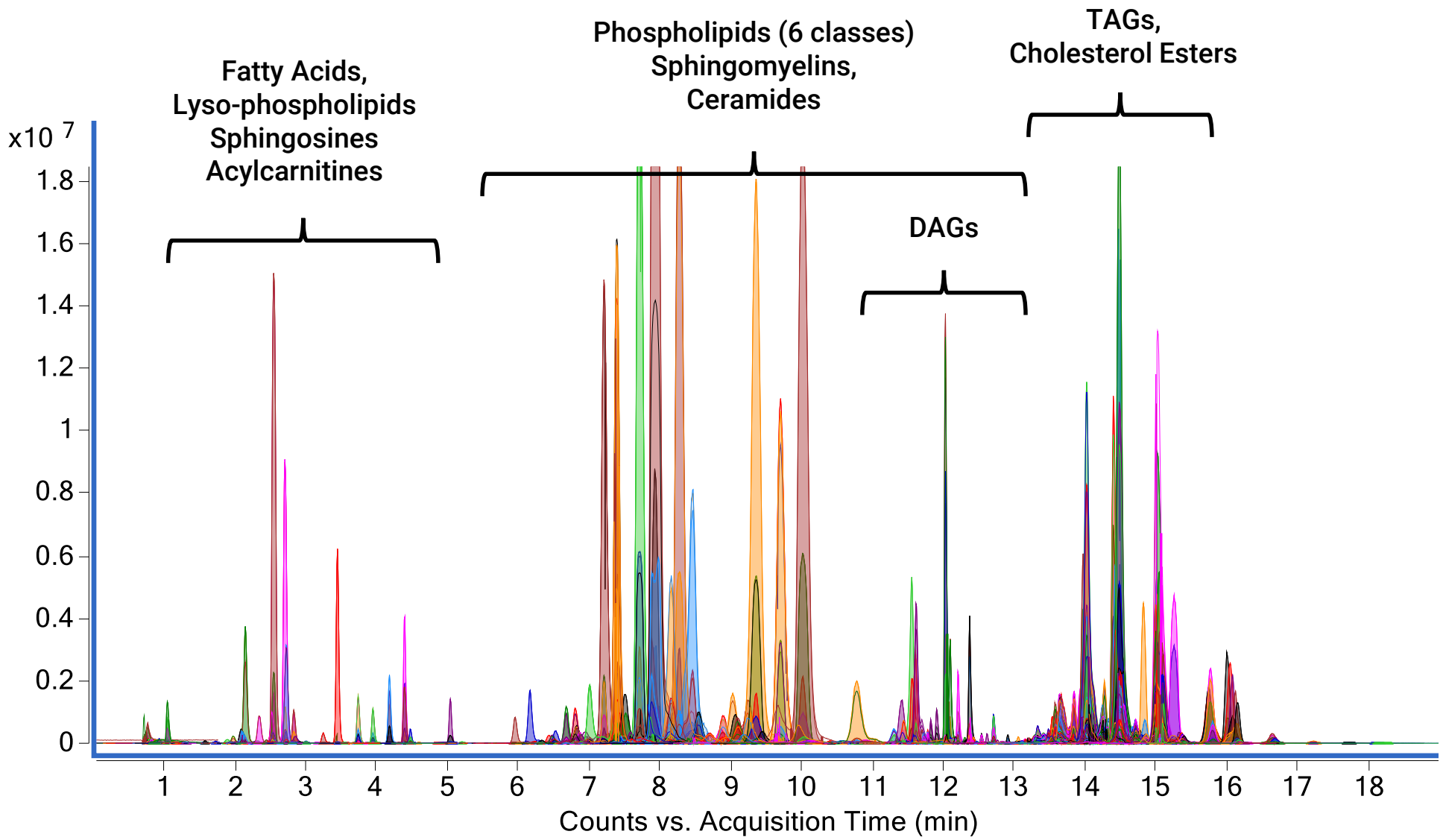






Comprehensive Coverage of Human Plasma Lipids

RP-LC/MS



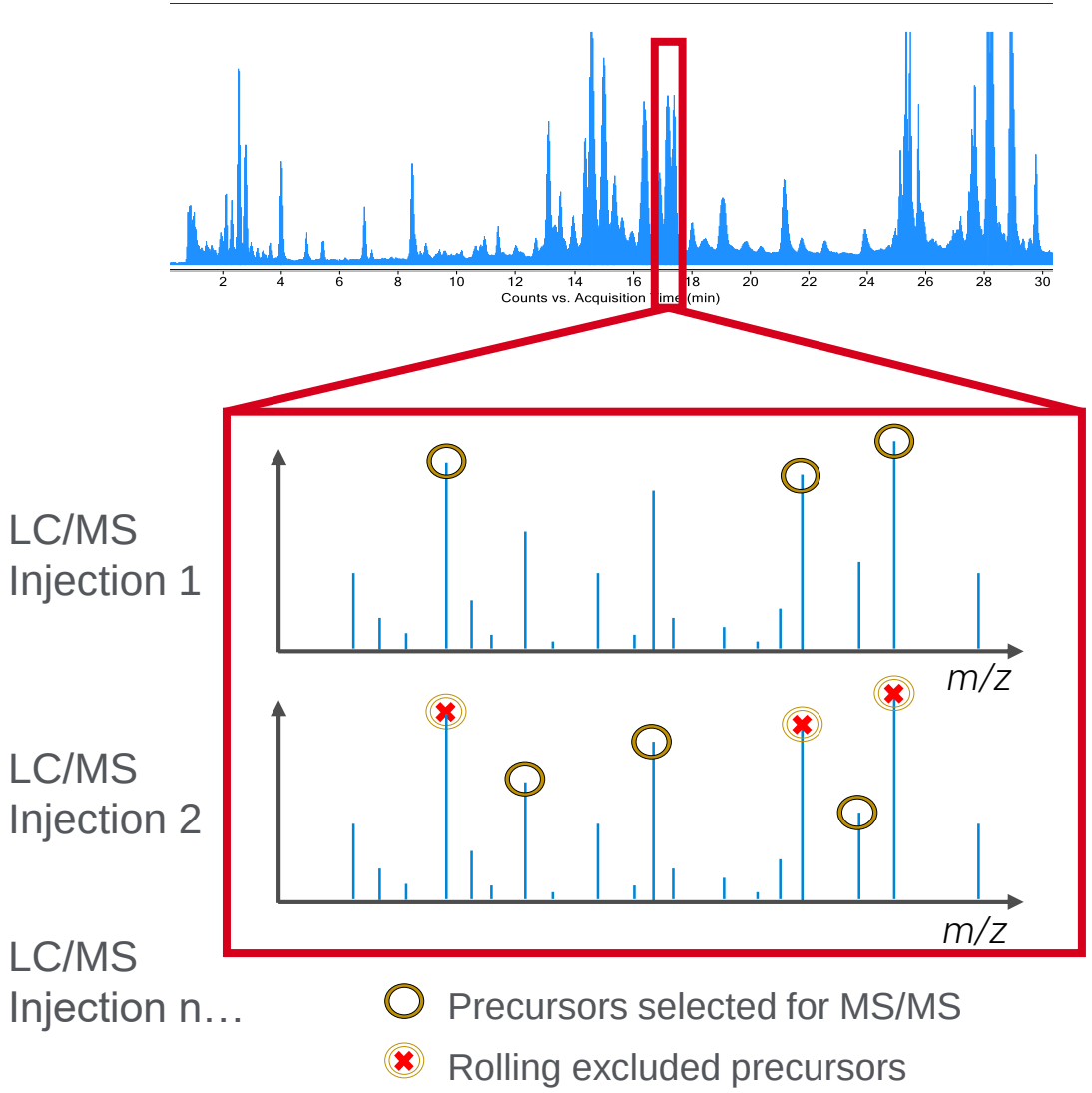
Iterative MS/MS

Comprehensive data-dependent LC-MS/MS analysis provides the highest quality MS/MS.

This mode can only select a limited number of precursors in a given time

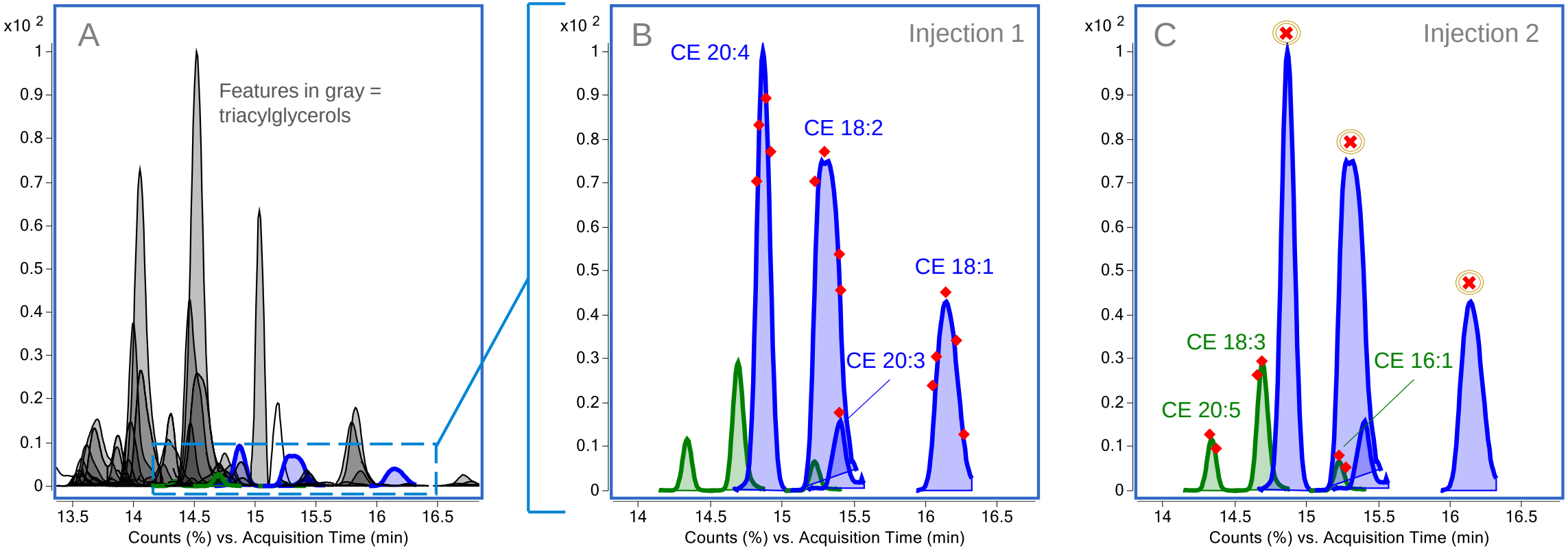
- Fast chromatography has narrow peak widths
- Low abundance compounds may be missed

Iterative MS/MS automatically excludes previously selected precursors on a rolling basis with multiple injections

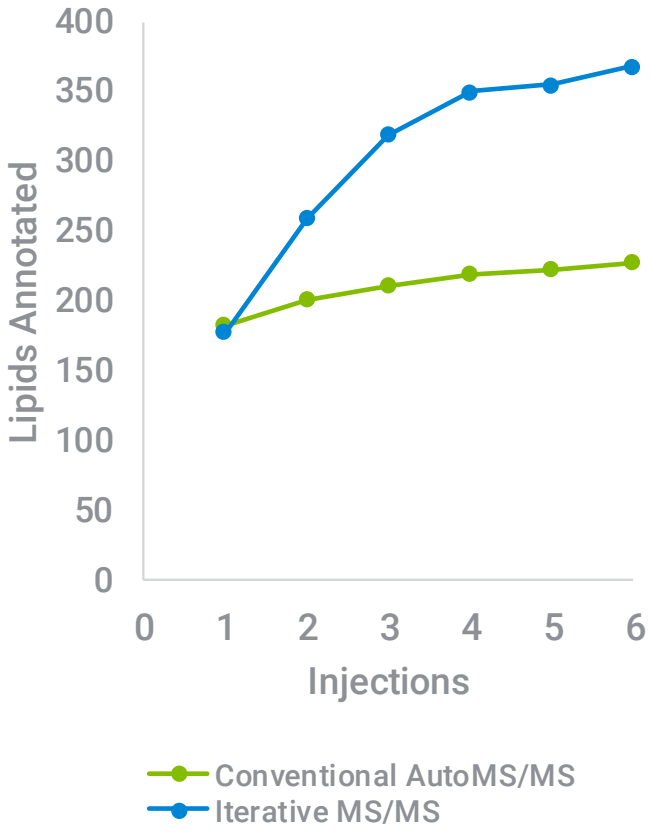


Iterative MS/MS

Example – Selection of Cholesterol Ester Precursors

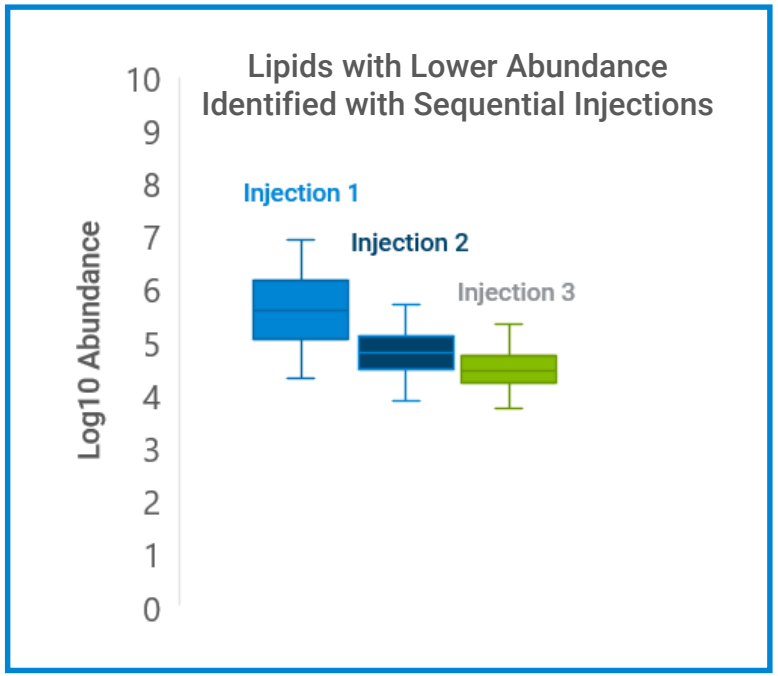


Improved Coverage of Plasma Lipidome with Iterative MS/MS



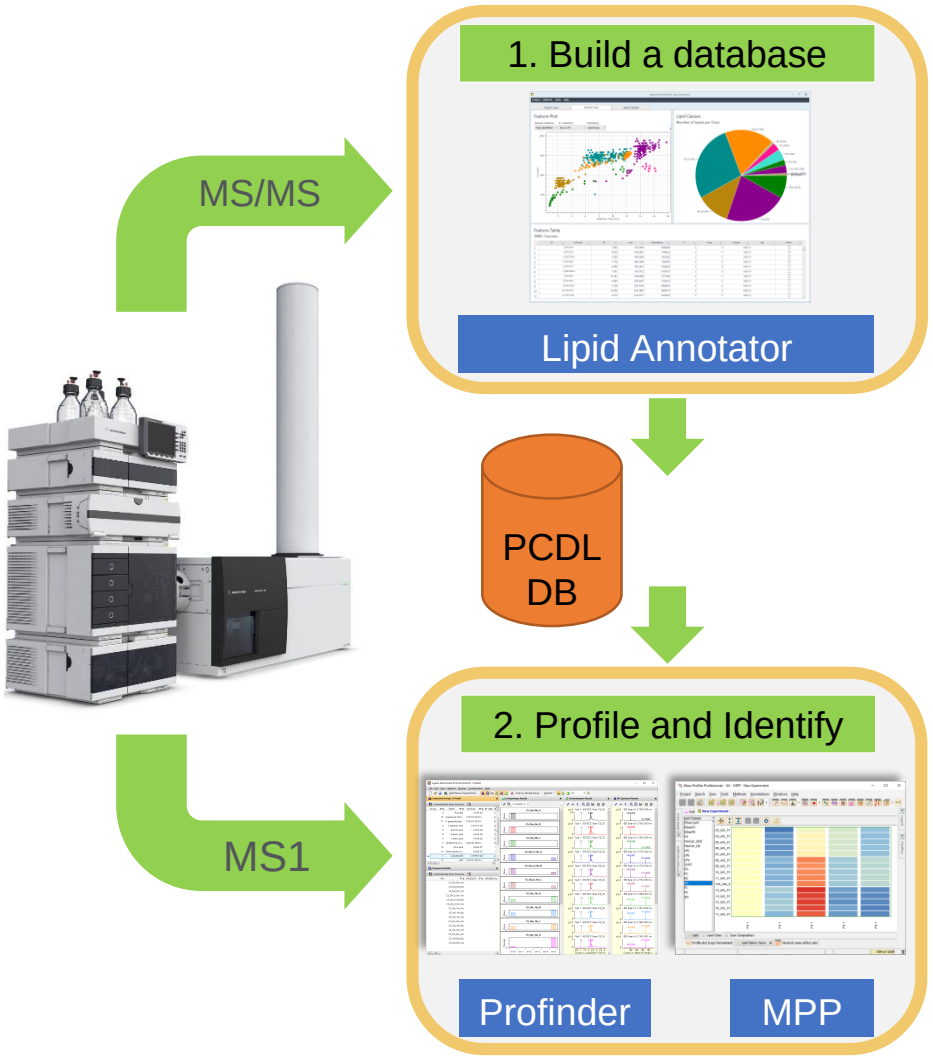
Iterative MS/MS

- Yields more unique lipid annotations compared to repeat Auto MS/MS
- Sequential iterative injections selected lipid ion precursors of lower abundance



Lipid Profiling

Targeted Workflow



Build the library from representative (often pooled) samples

Individual replicates for differential analysis \ lipid profiling

Agilent MassHunter Lipid Annotator: New Lipid Profiling Tool

in silico lipid annotation and personal library creation

Lipid Annotator

Includes an expanded LipidBlast theoretical lipid library

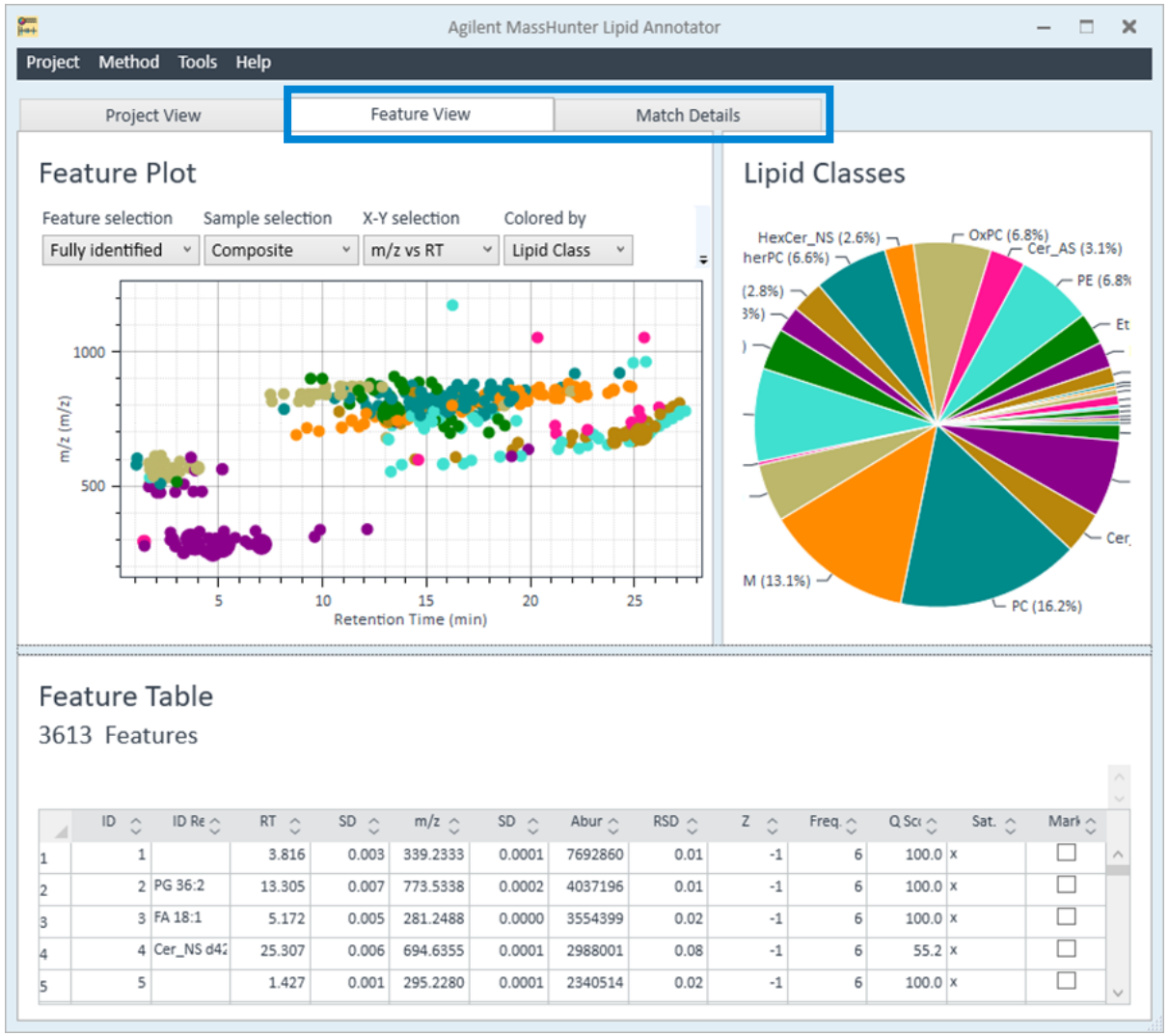
Annotates MS/MS spectra

Exports results to a compound database (PCDL) or .csv for targeted mining

Feature View
- Scatter Plot
- Pie chart
- Feature list

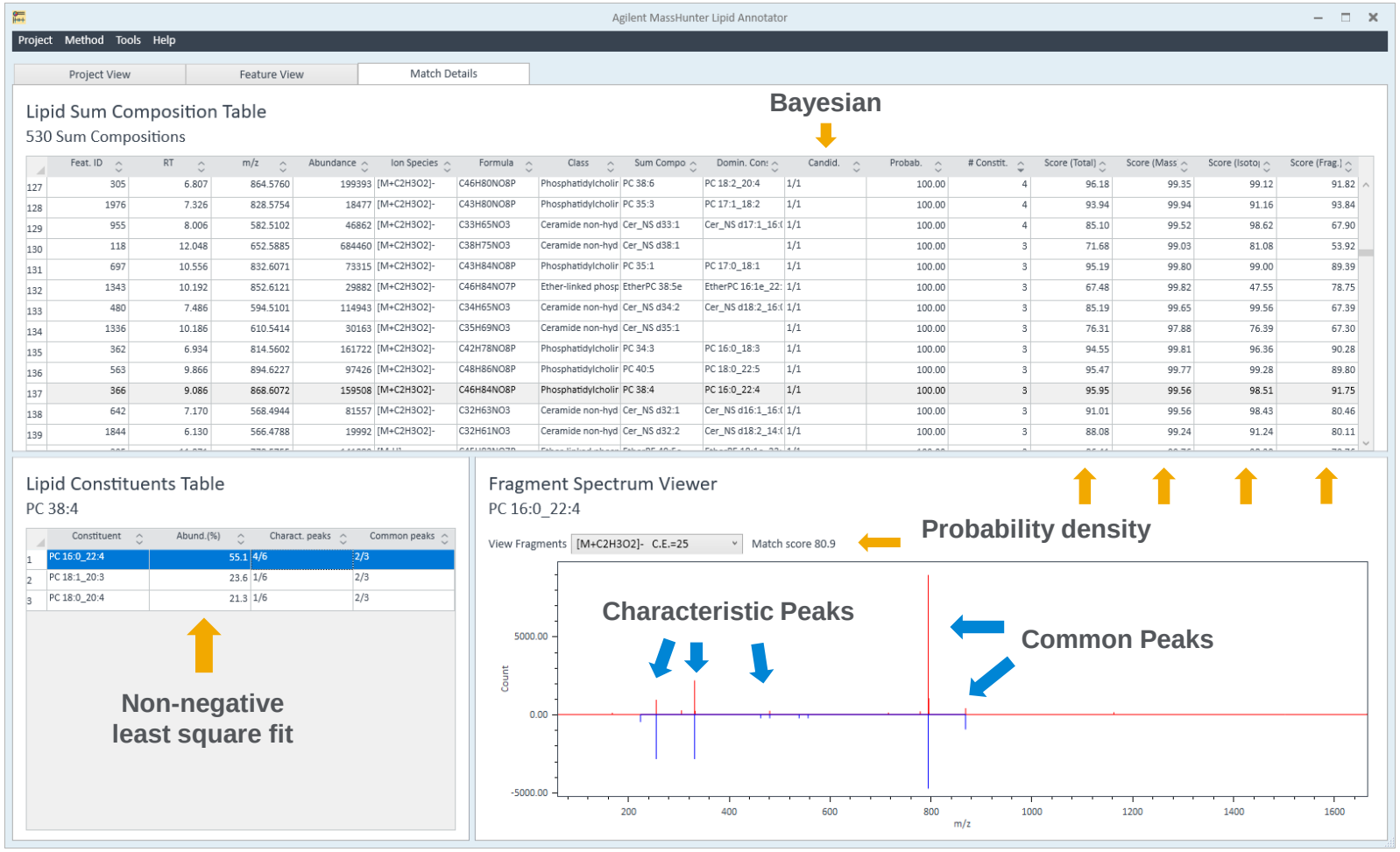
} Colored by lipid class

Match Details View
- Lipid sum composition table
- Lipid constituent tables
- Mirror plot – Observed vs. LipidBlast



Lipid Annotator

Algorithm

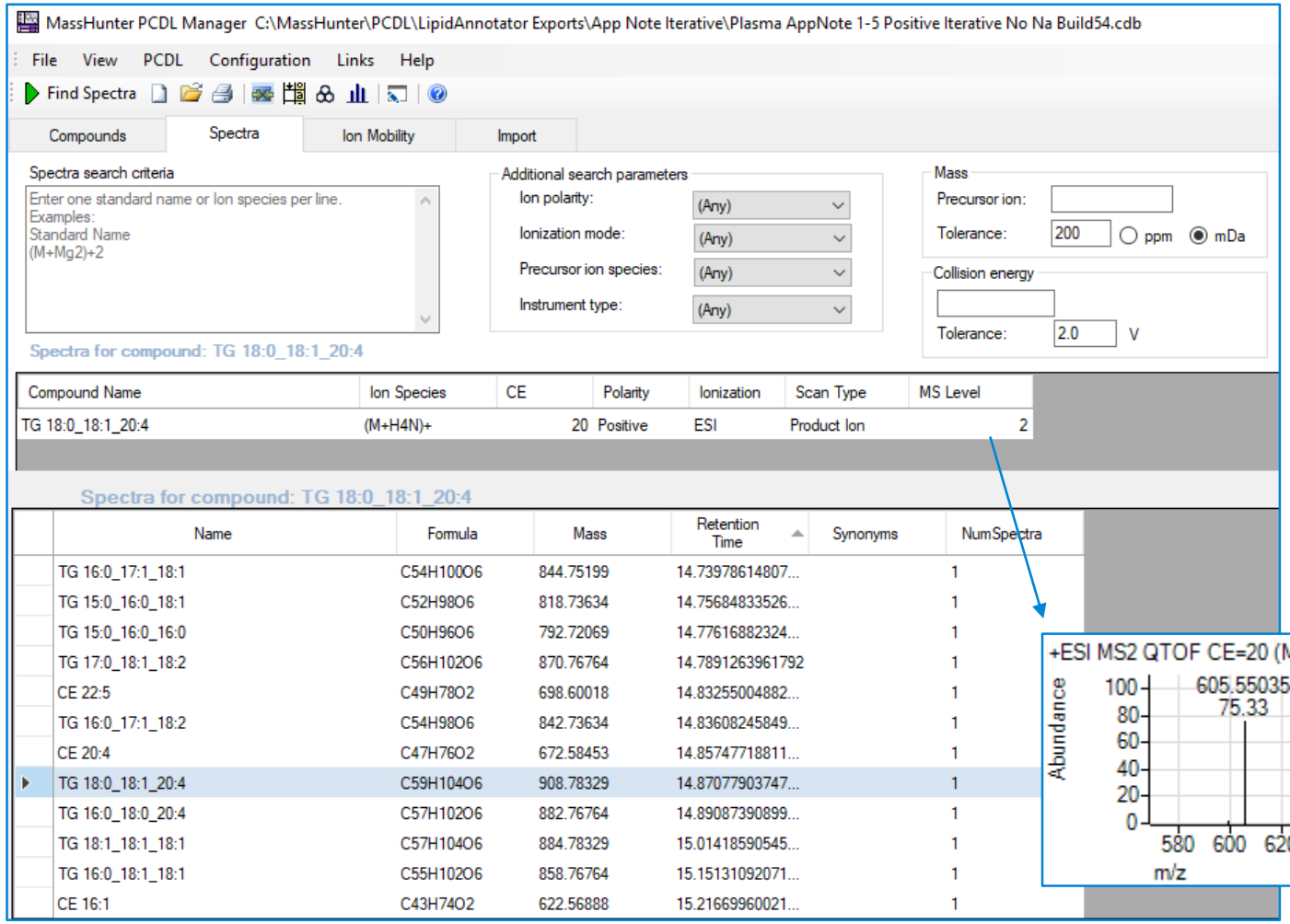


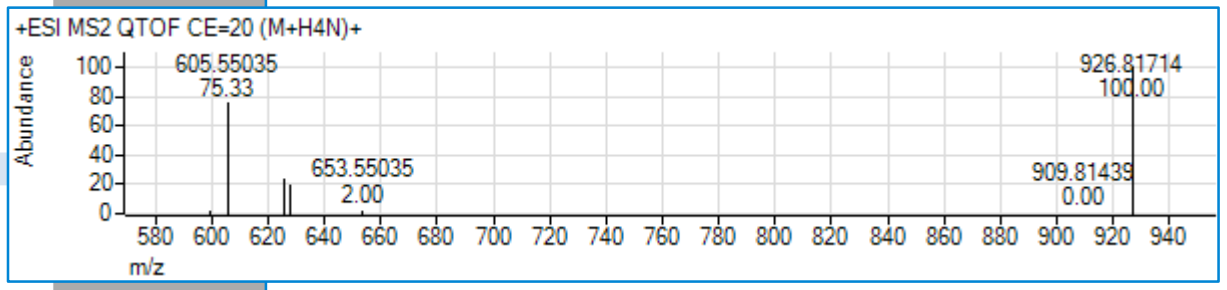
- Algorithm uses a combination of Bayesian probability, probability density, and non-negative least squares fit to make lipid identifications

Lipid Annotator

Automated Export to Database

PCDL Manager





- Lipid annotations can be automatically exported to a database. Both .CSV and Personal Compound Database and Library (**PCDL**) formats are supported.
- PCDL Manager can be used to edit entries and even merge databases.
- The user can also **export the MS/MS spectra** to the PCDL

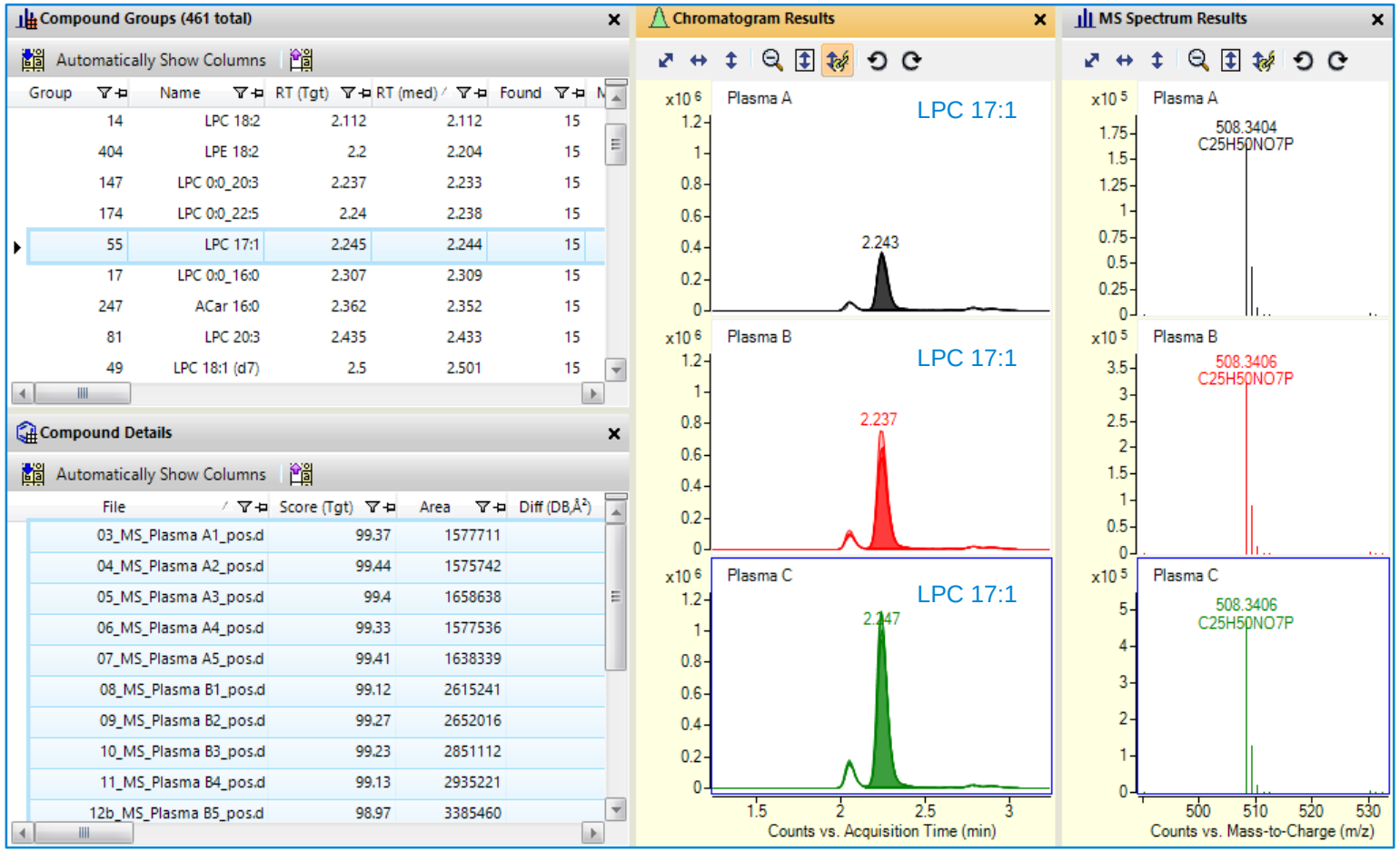
MassHunter Profinder 10.0

Batch Targeted Feature Extraction

Profinder 10 Supporting 6546 LC/Q-TOF

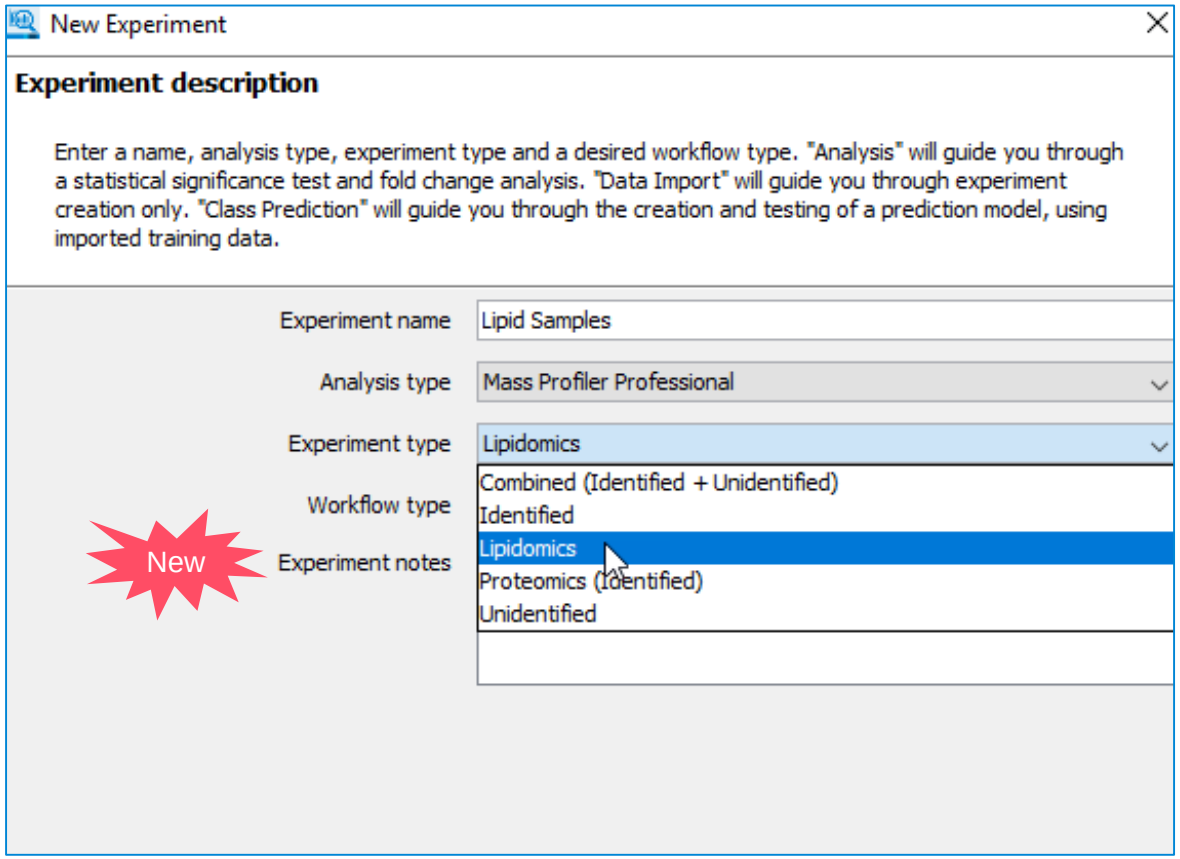
The lipid PCDL is used as the RT and formula source to align features across MS1 datafiles

Sample grouping in Profinder provides initial insight into how certain lipids vary across sample sets. Here LPC 17:1 is present in different concentrations across the 3 sample sets.

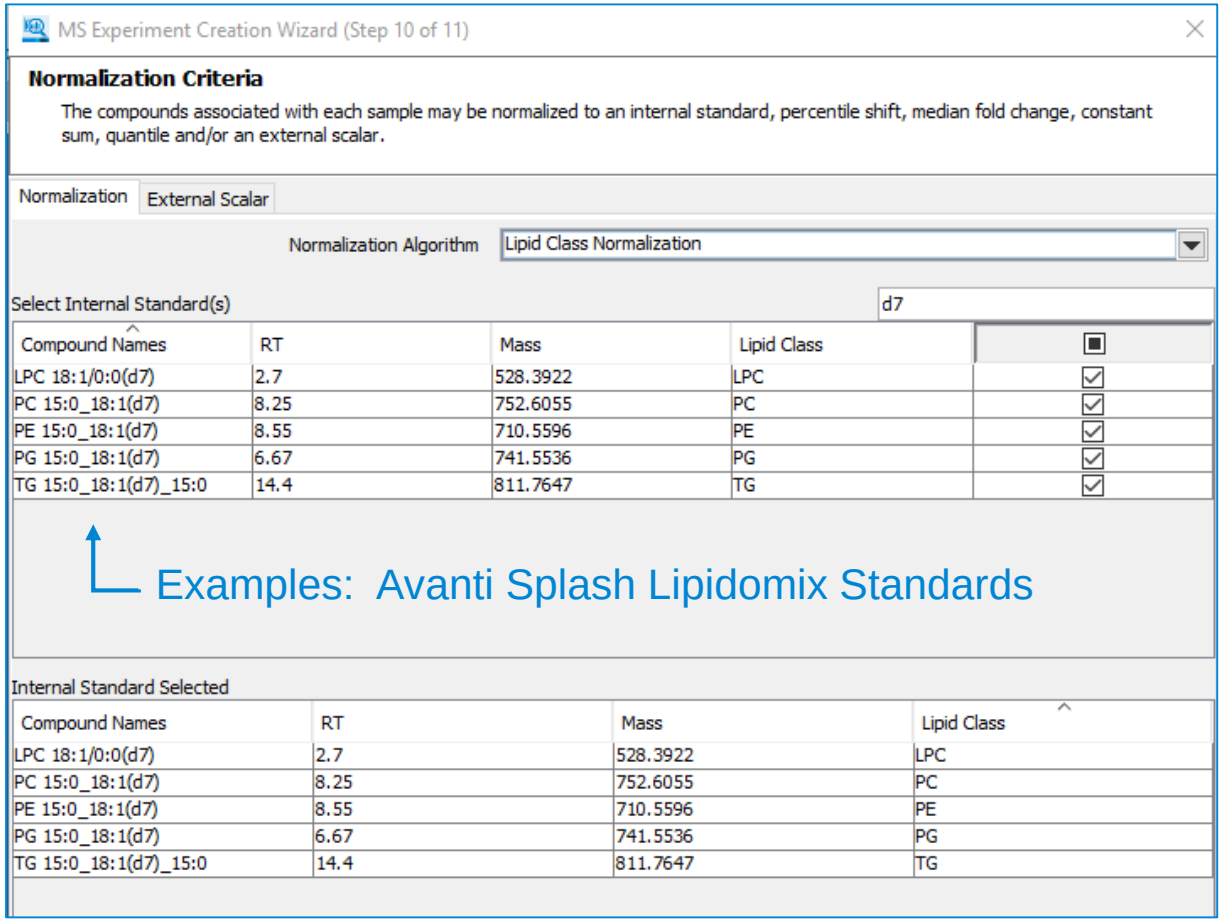


Agilent MassHunter Mass Profiler Professional (MPP) 15.0

New Support for Lipidomics Experiment



Lipidomics Experiment Type



Normalization by Lipid Class

Use internal standards to normalize by lipid class

Background

Cancer Lipidomics

- Acute myelogenous leukemia (AML) is an aggressive blood cancer that responds well to high-grade chemotherapy. However, new treatments are needed for elderly patients that cannot tolerate these cytotoxic treatments.
- Drug redeployment - a combination (BaP) of the lipid-lowering drug bezafibrate (BEZ) and the contraceptive medroxyprogesterone acetate (MPA) has potent *in vitro* anticancer effects against AML.
- A recent study[1] found that
  - BaP treatment slows de novo fatty acid and phospholipid biosynthesis through downregulation of lipogenic enzymes
  - Pronounced lipidome changes were identified with mass spectrometry

Goal: Apply a new lipidomics profiling workflow to identify changes with BaP treatment

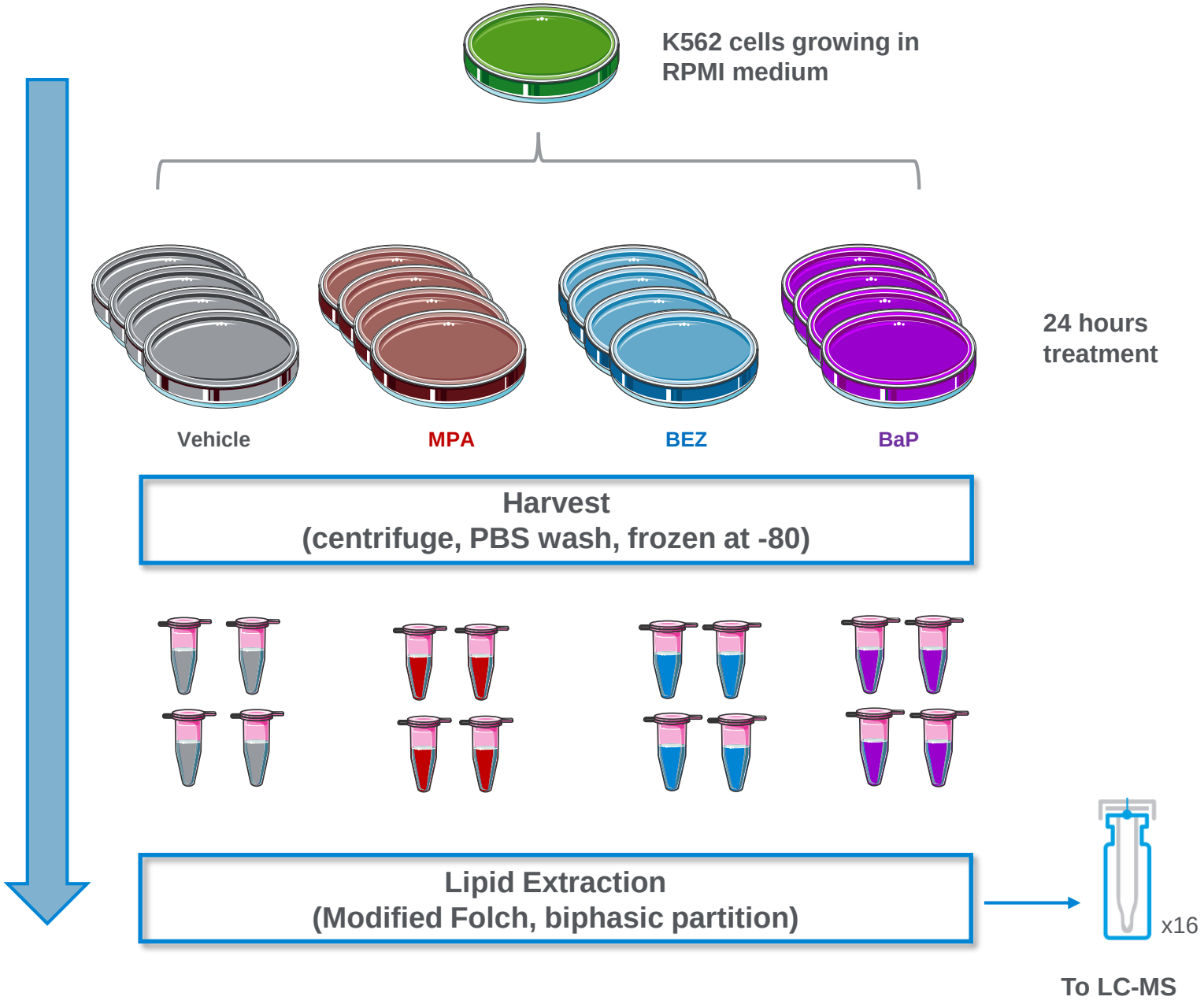
1. Southam, A.D. et al. *Drug Redeployment to Kill Leukemia and Lymphoma Cells by Disrupting SCD1-Mediated Synthesis of Monounsaturated Fatty Acids.* Cancer Res. 2015 June; 75(12): 2530-40

Experimental Design

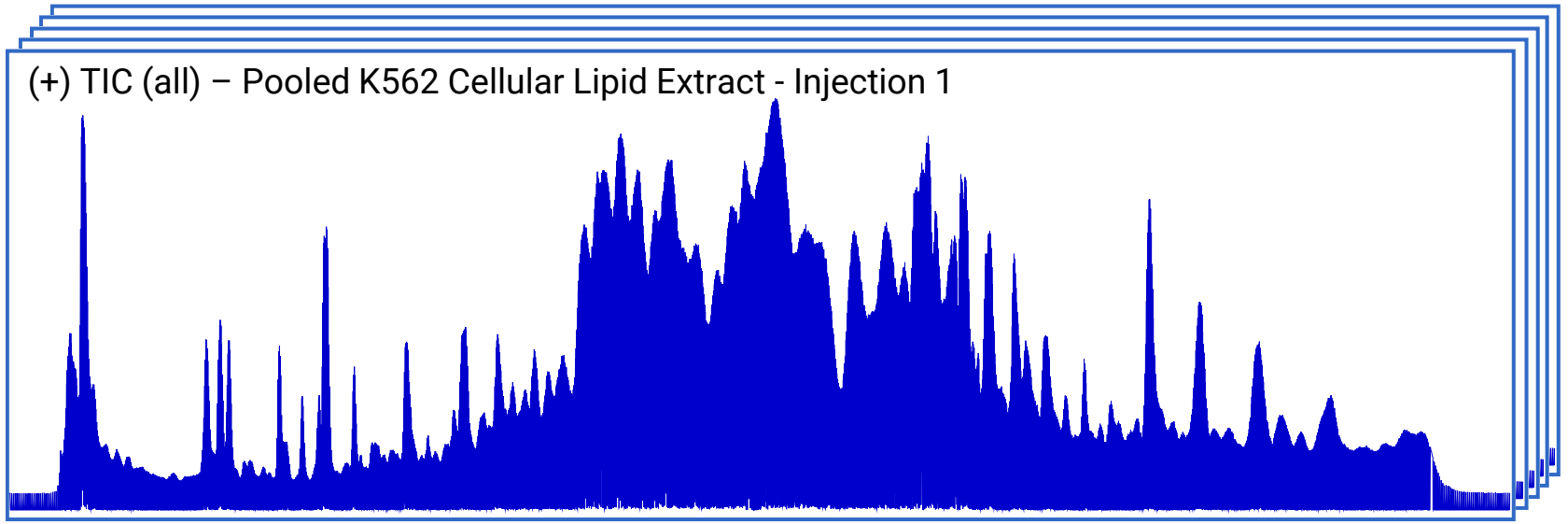
Cells: AML K562

Drugs:

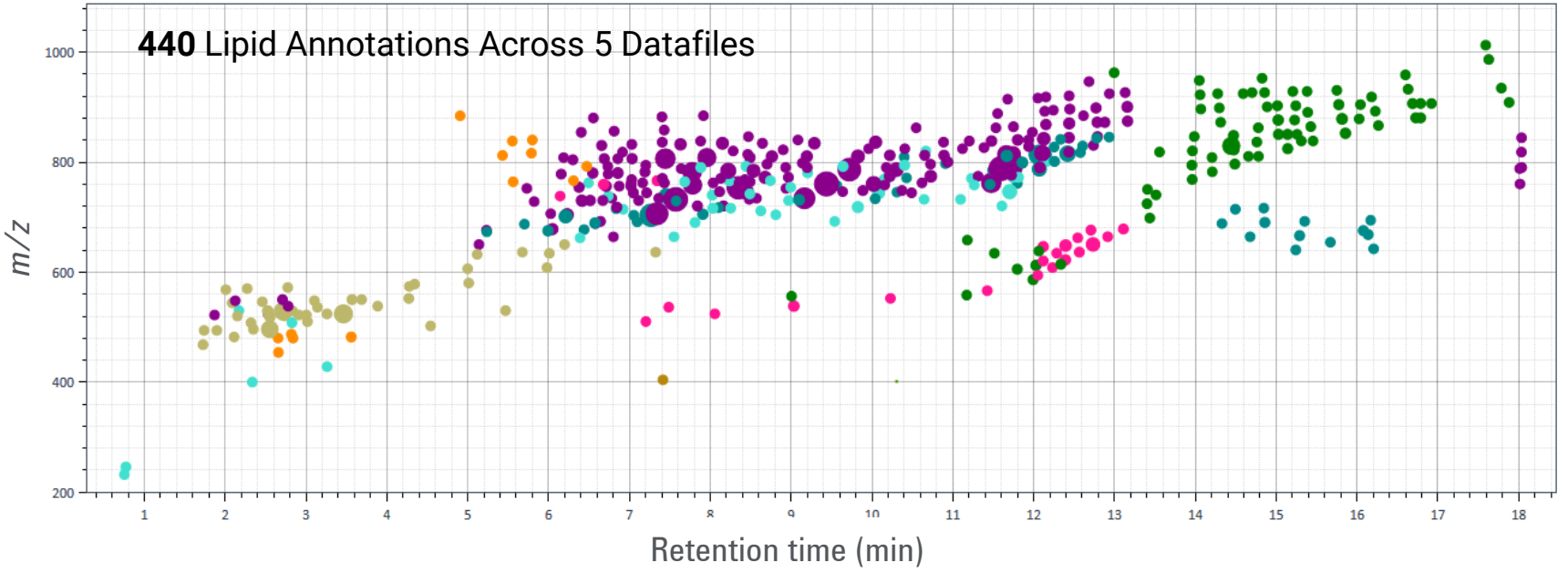
- Vehicle: 1:1 ethanol/DMSO
- MPA: Medroxyprogesterone acetate
- BEZ: Bezafibrate
- BaP: BEZ + MPA

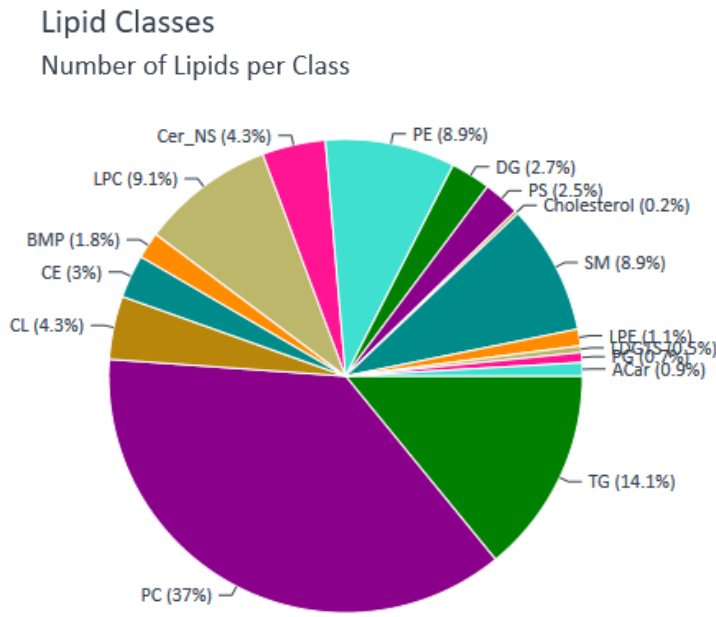


Lipid Annotator - Results

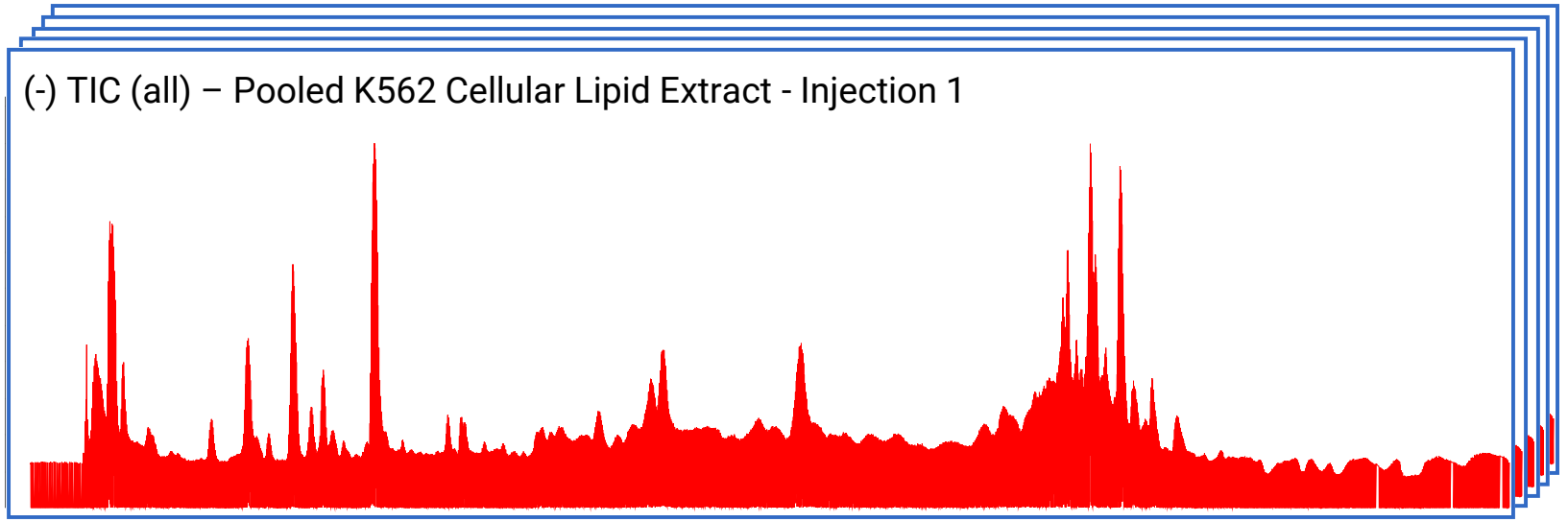


5 Iterative MS/MS Injections

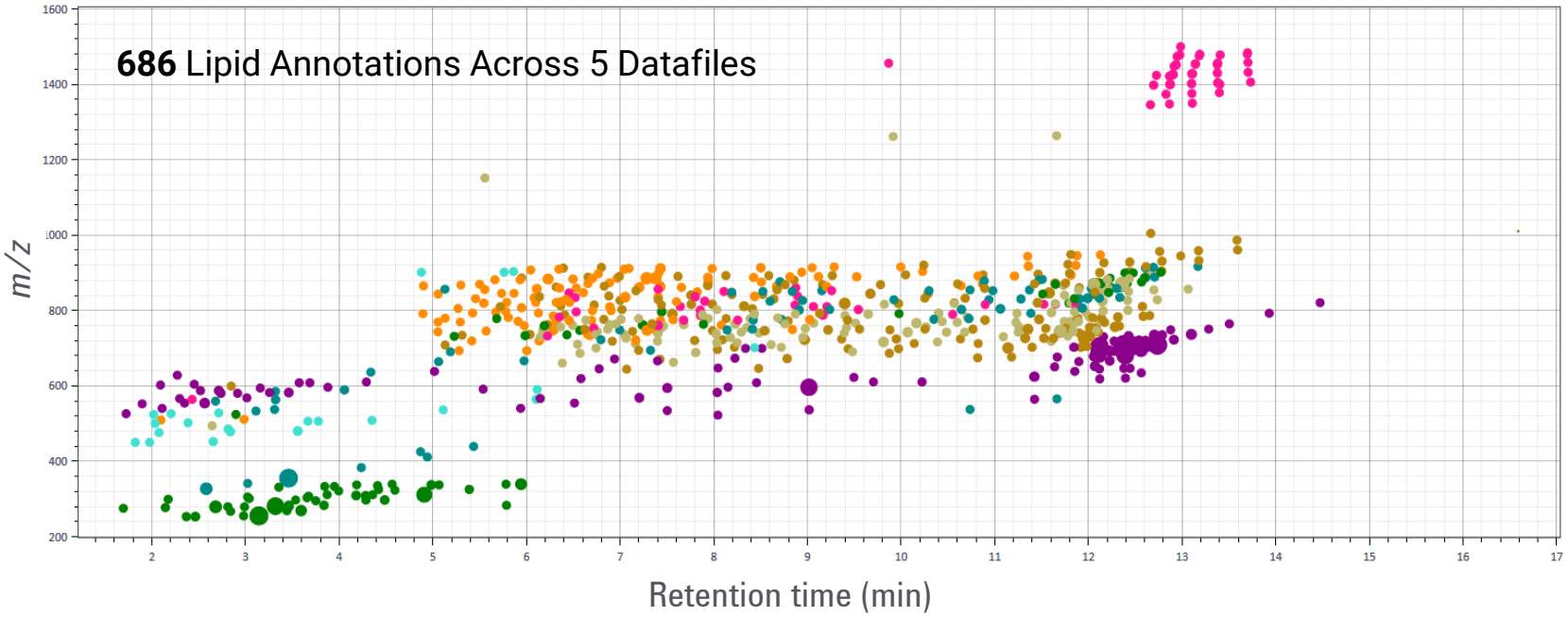


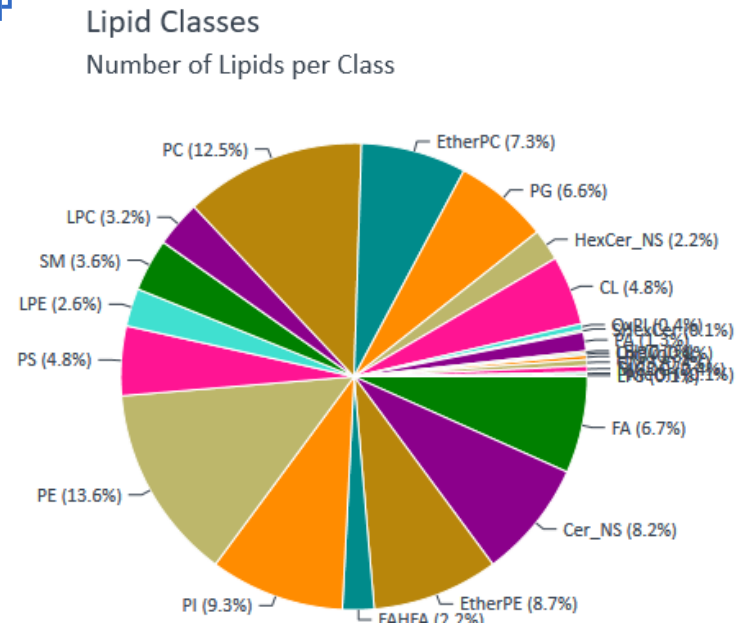


Lipid Annotator - Results



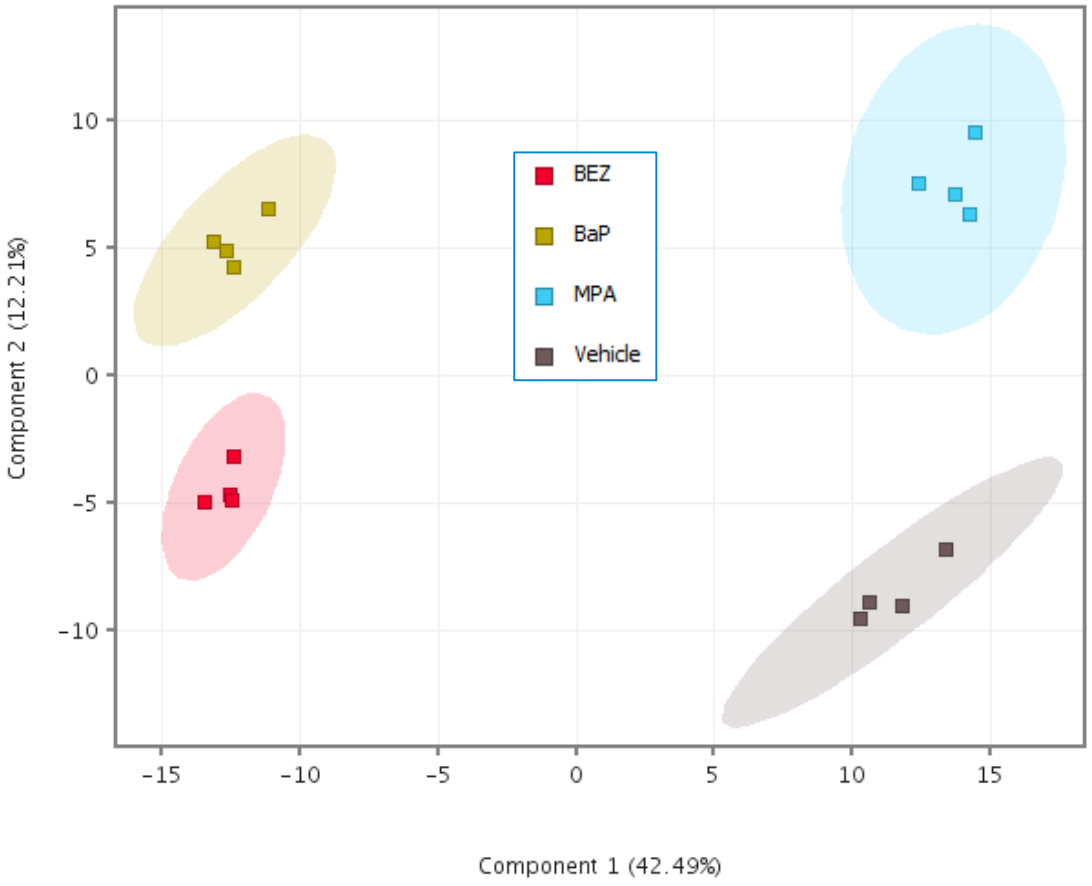
5 Iterative MS/MS Injections

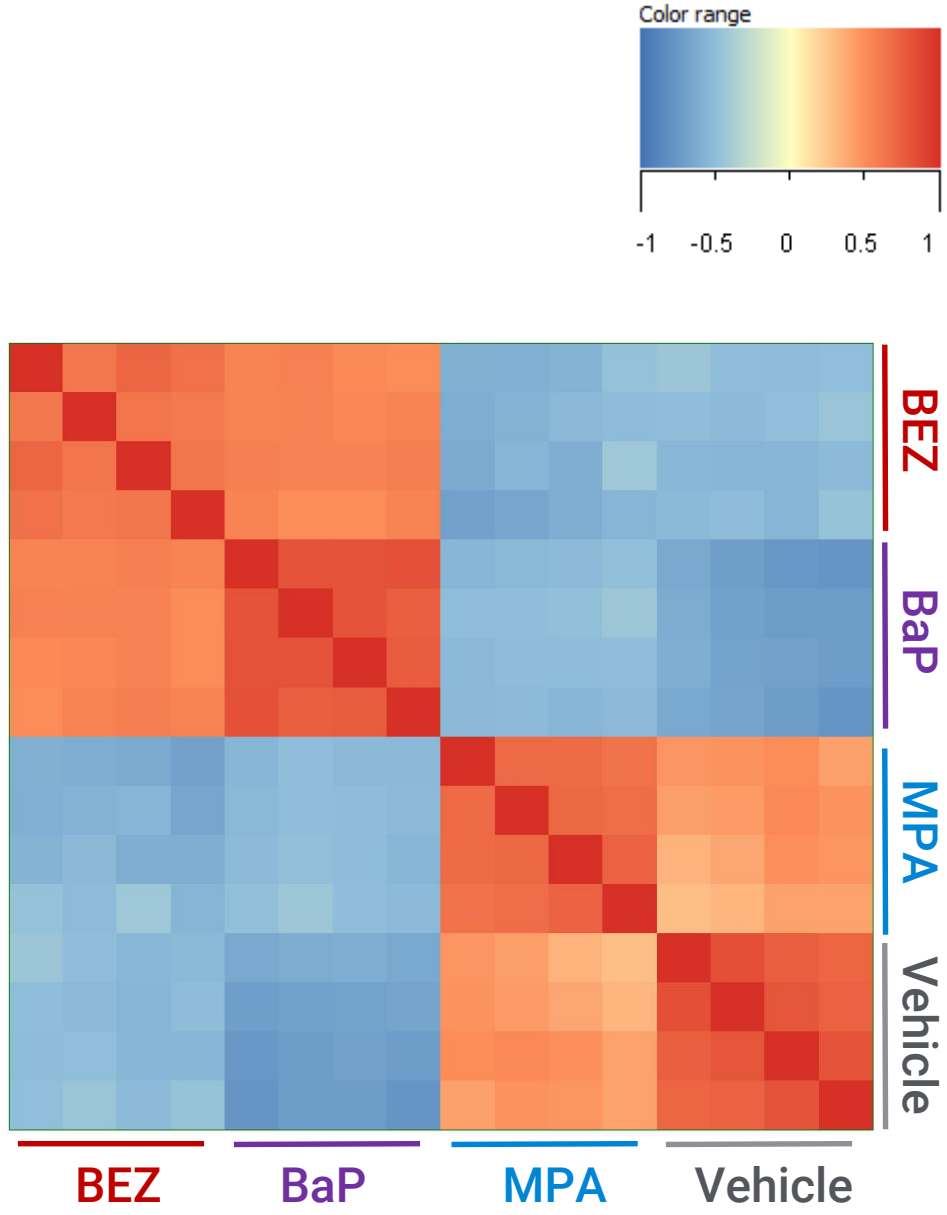




Lipid Profiling - MPP Results

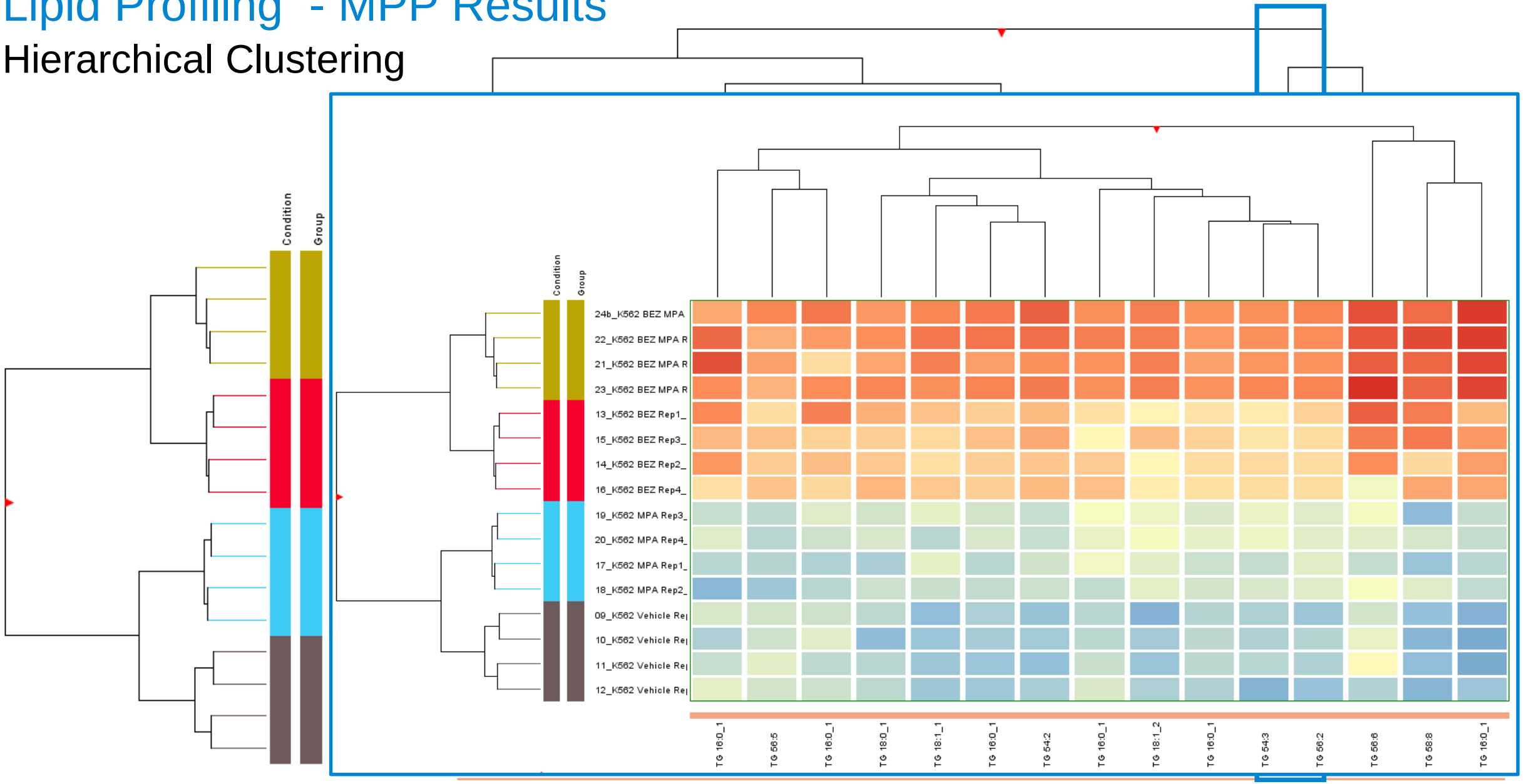
PCA and Sample Correlation





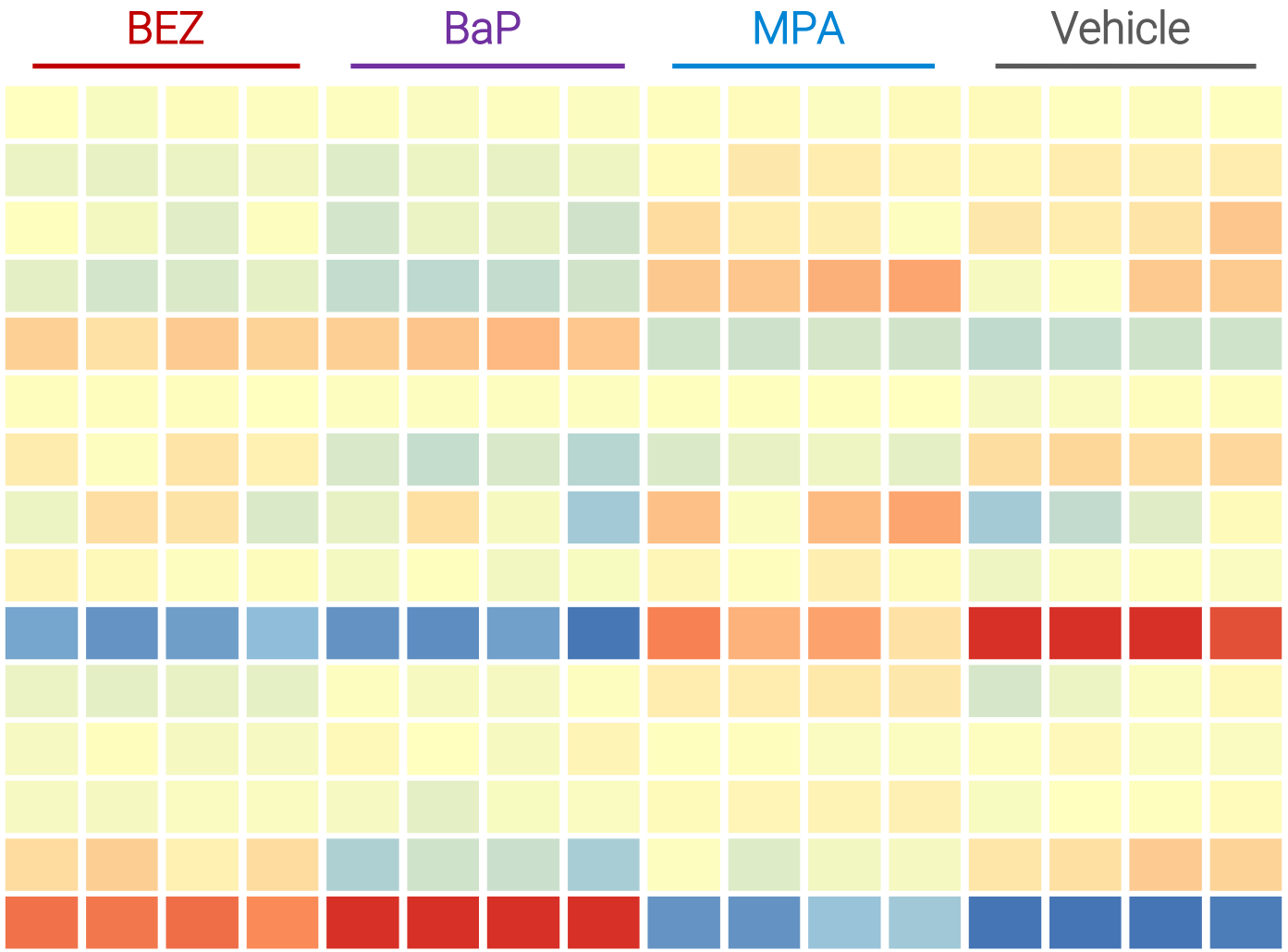
Lipid Profiling - MPP Results

Hierarchical Clustering



Lipid Profiling - MPP Results

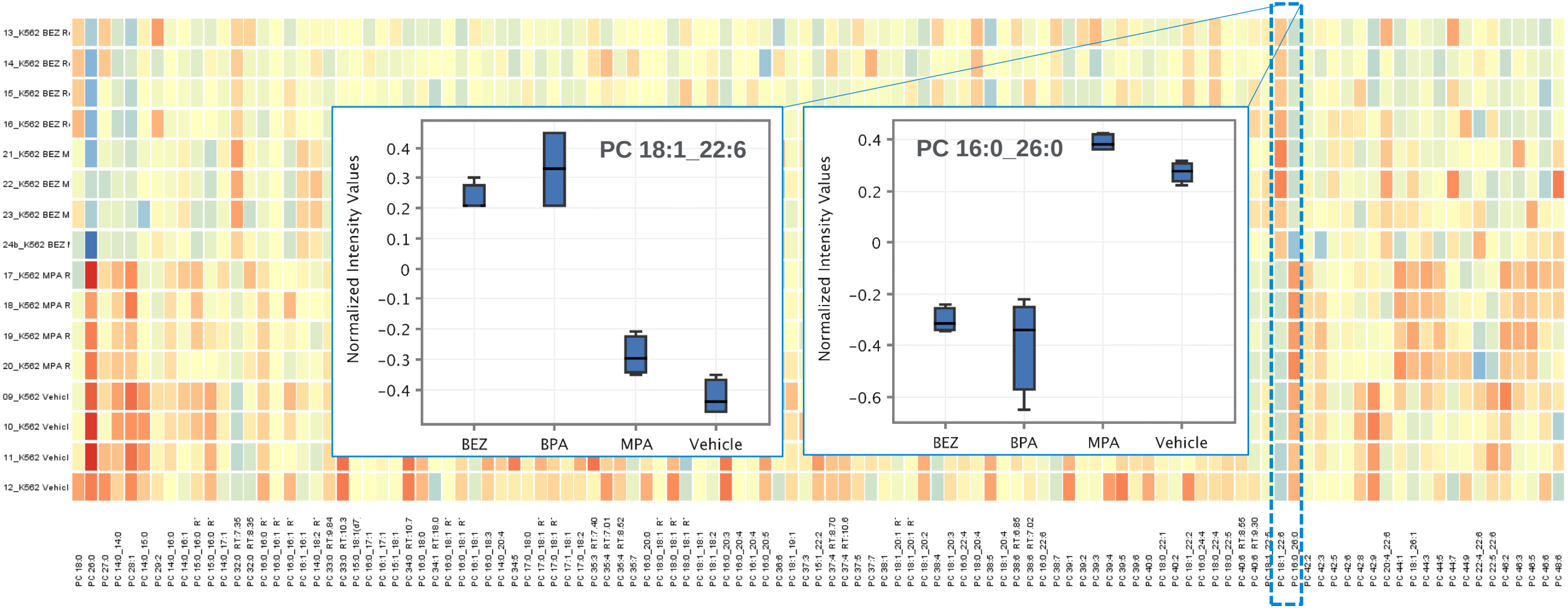
Lipid Matrices (Heat Maps)



Lipid Class	BaP Effect	Notes
ACar	--	
BMP	↓	NEW
CE	↓	NEW
CL	↓	NEW
Cer_NS	↑	NEW
Cholesterol	--	
DG	↓	
LPC	--	
LPE	--	
PC	↓	
PE	--	
PG	--	
PS	--	
SM	↓	NEW
TG	↑	

Lipid Profiling - MPP Results

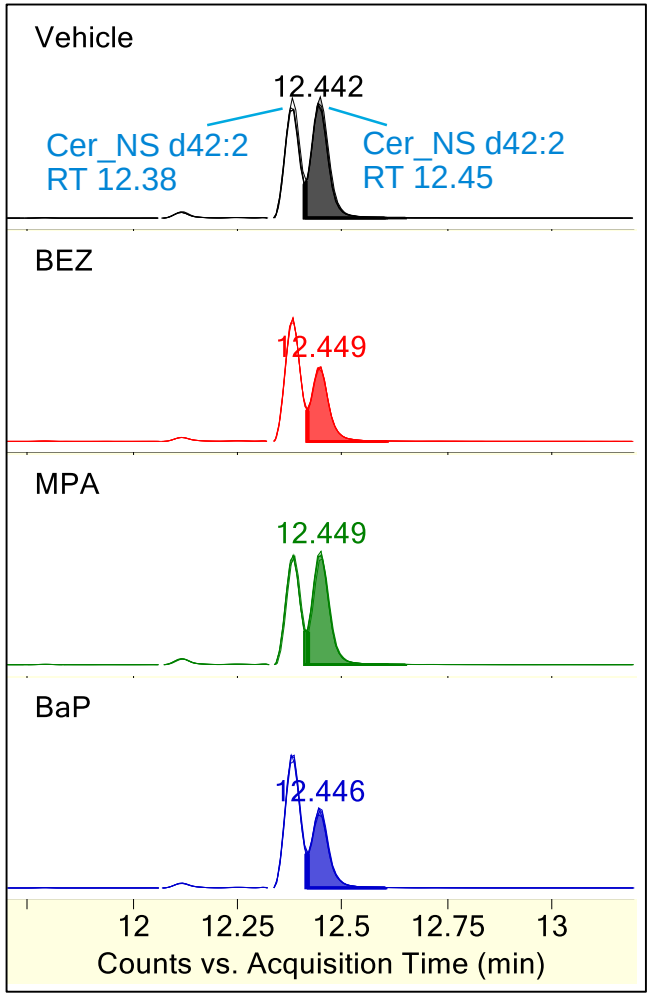
Phosphatidylcholine Features



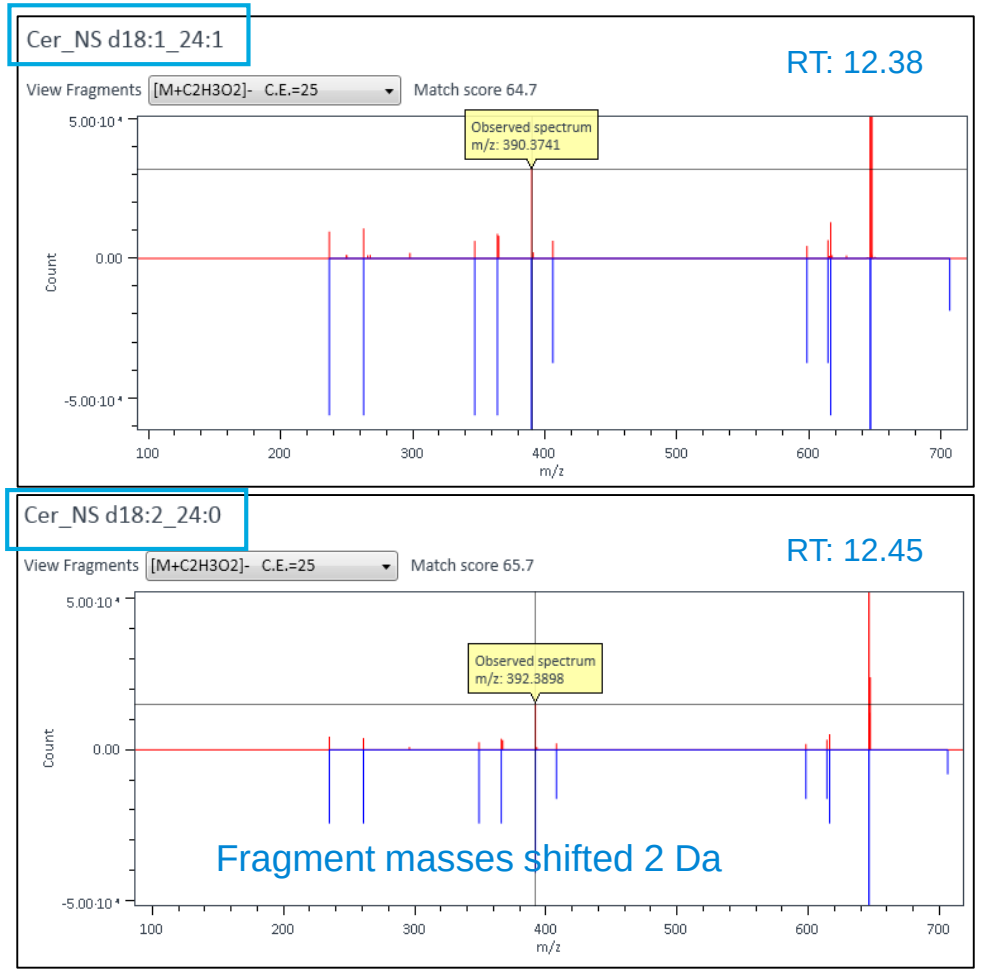
Lipid Profiling

Lipid Isomers

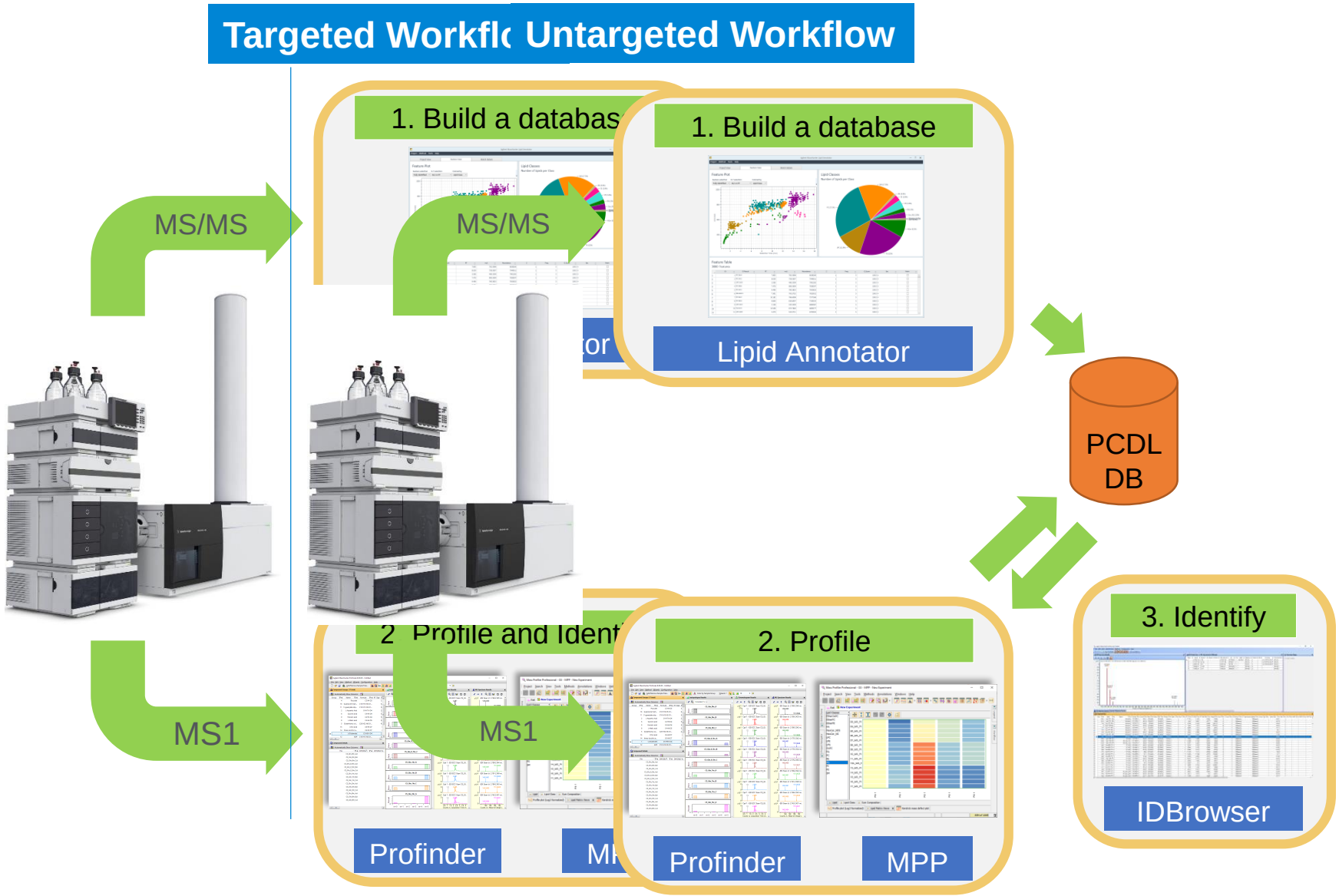
Profinder Results



Lipid Annotator Results



Lipid Profiling Workflows



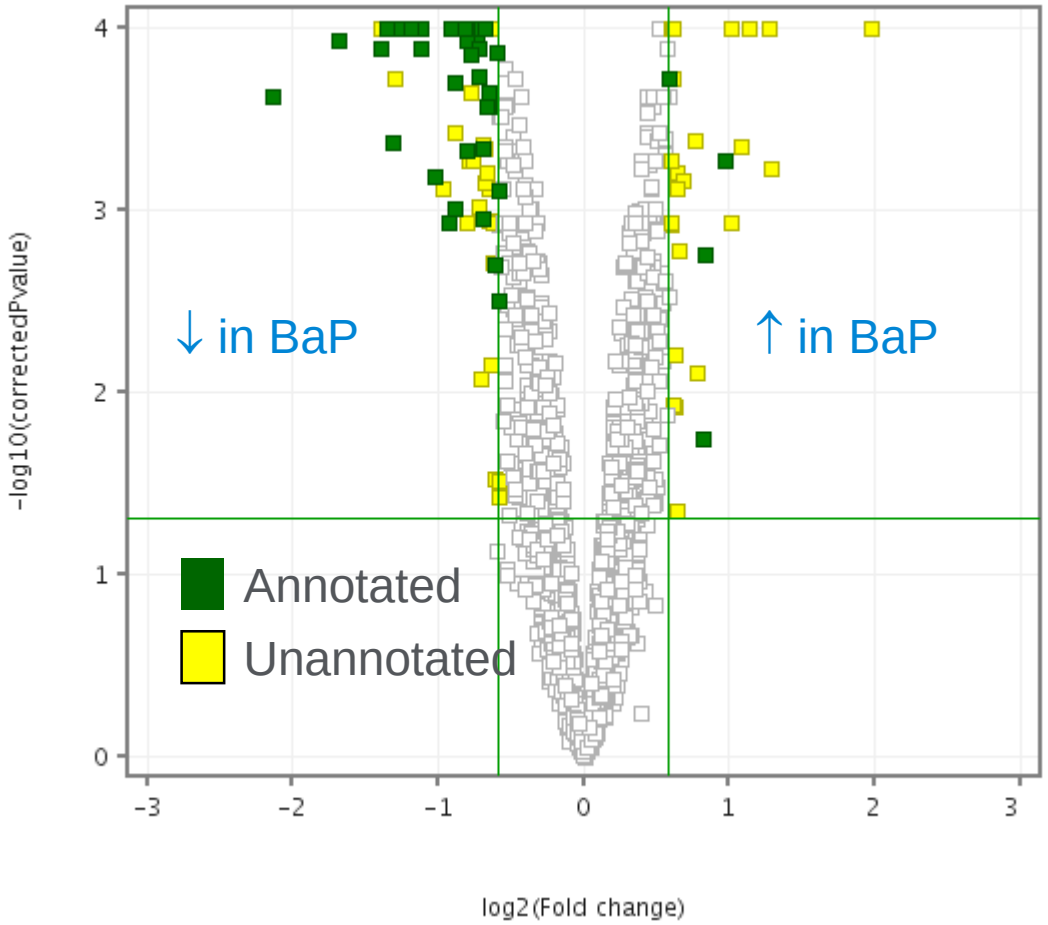
Lipid Profiling - MPP Results

Untargeted Workflow

Volcano Plot

- BaP drug treatment versus vehicle control
- Moderated T-test with Benjamini Hochberg FDR
- Significance cutoffs
  - Fold change 2.0
  - p-value 0.05

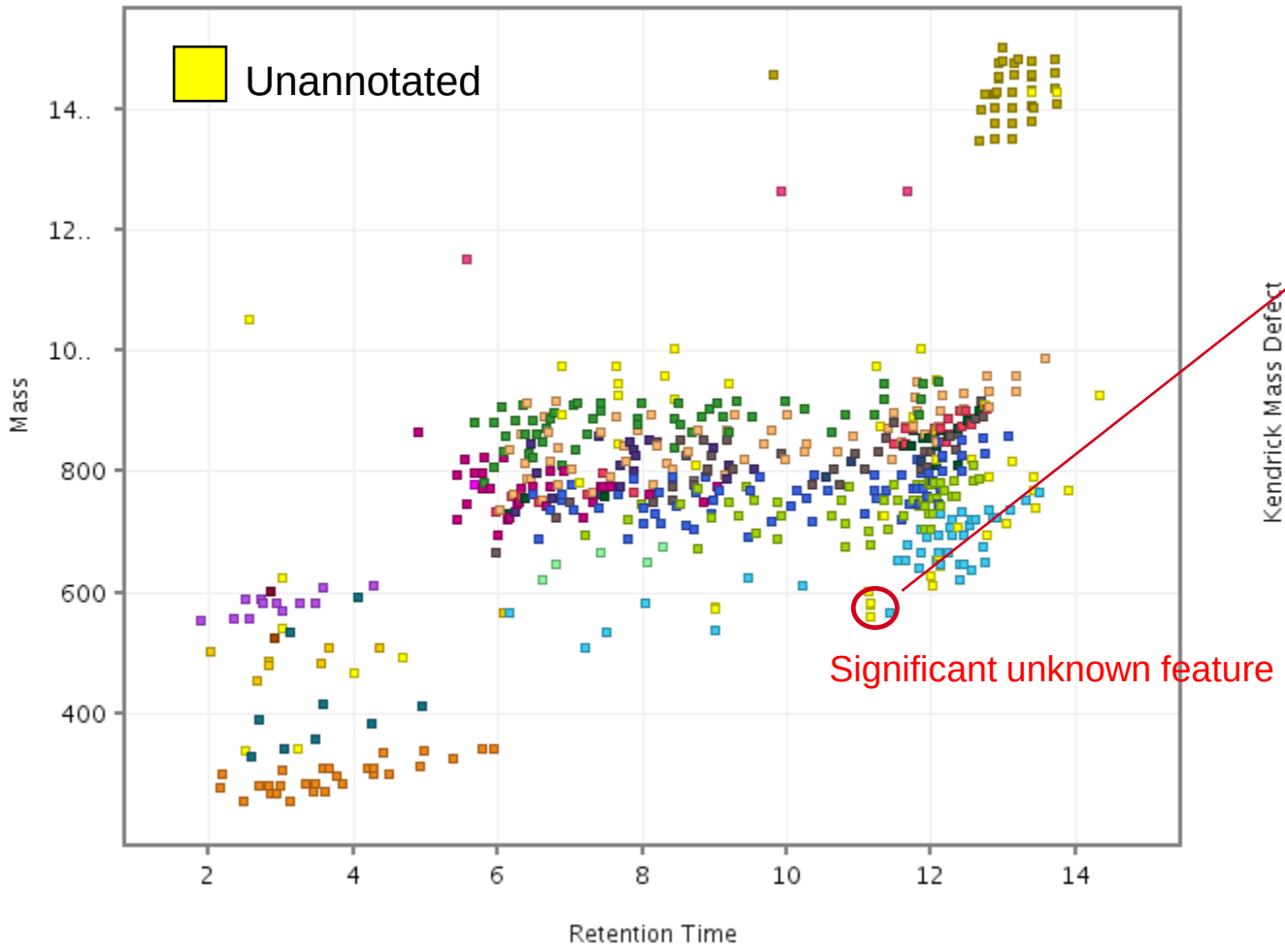
93 Significant Features



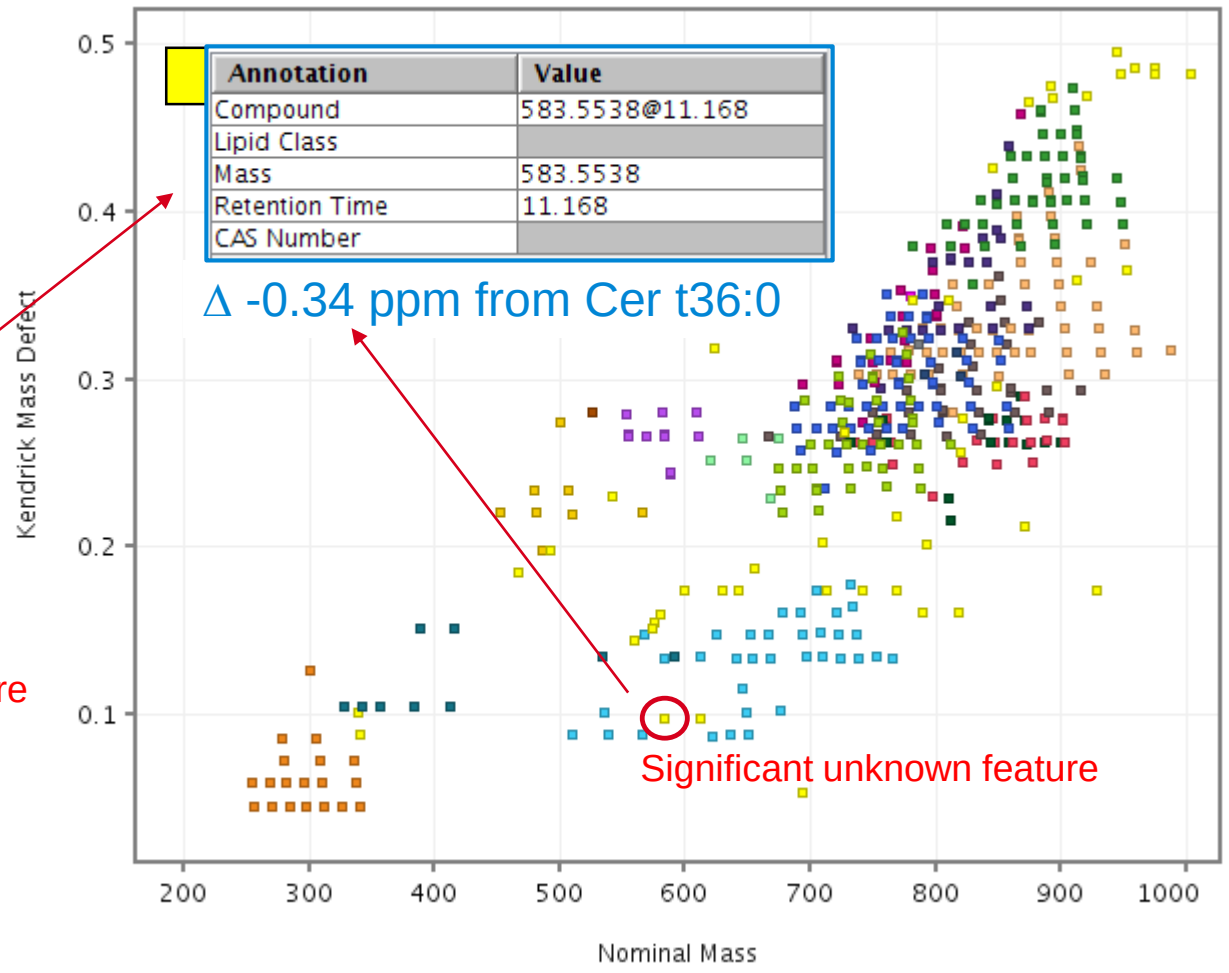
Lipid Profiling - MPP Results

Untargeted Workflow

Mass vs RT Plot



Kendrick Mass Defect Plot



Summary

Enabling larger sample sets and faster analysis than ever

Better reproducibility with the new Bravo Metabolomics Sample Prep Platform

The Agilent 6546 LC/Q-TOF delivers:

- Performance unaffected by acquisition rate
- High resolution at low *m/z*
- Broad dynamic range
- Isotopic fidelity of <±5%

Instrumentation is complemented by new metabolomics software:

- Novel Lipid Annotator software supports targeted and untargeted lipidomics workflows
- More comprehensive lipid annotation and more in-depth lipid profiling achieved

See Printed Handouts for 6546 and Lipidomics Application Notes and ASMS Oral/Poster Presentations