Untargeted Lipidomics

ASMS 2019

Dr. Sheher Banu Mohsin
Agilent Technologies

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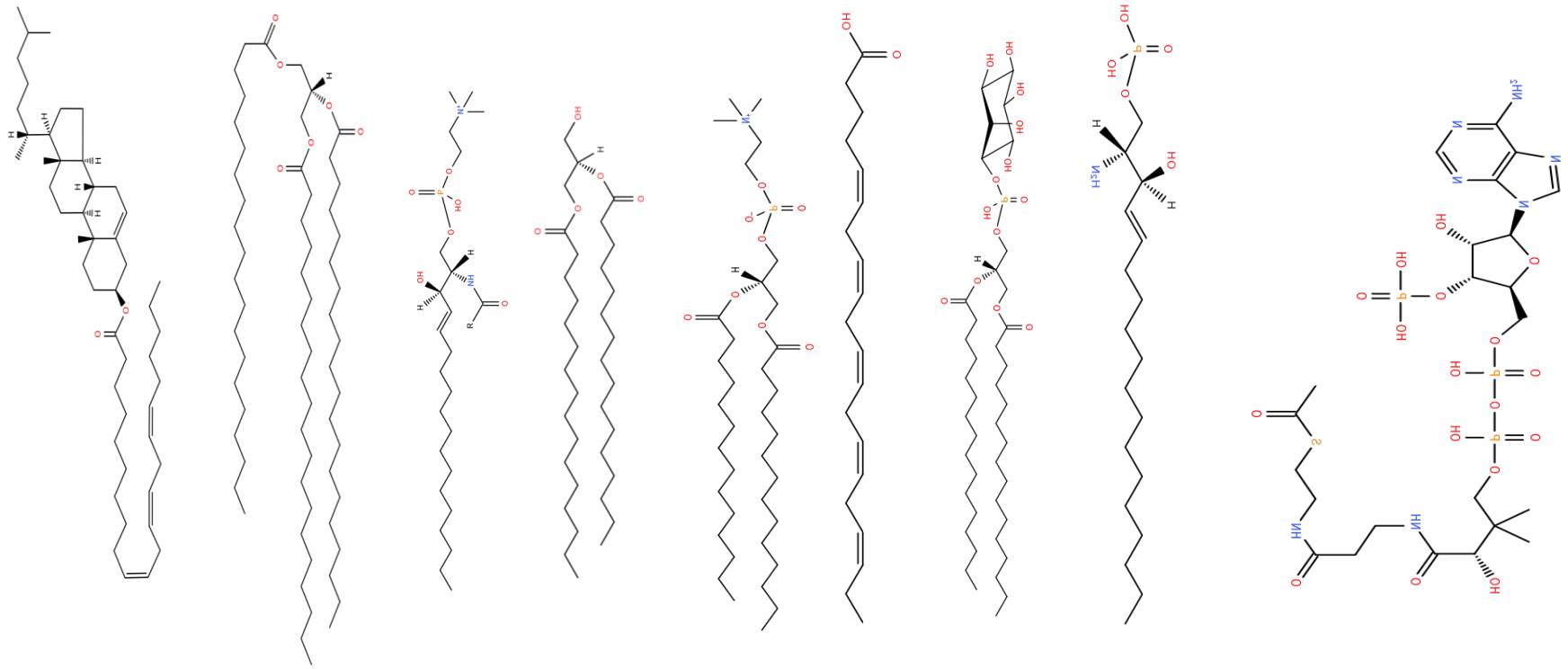
Trusted Answers

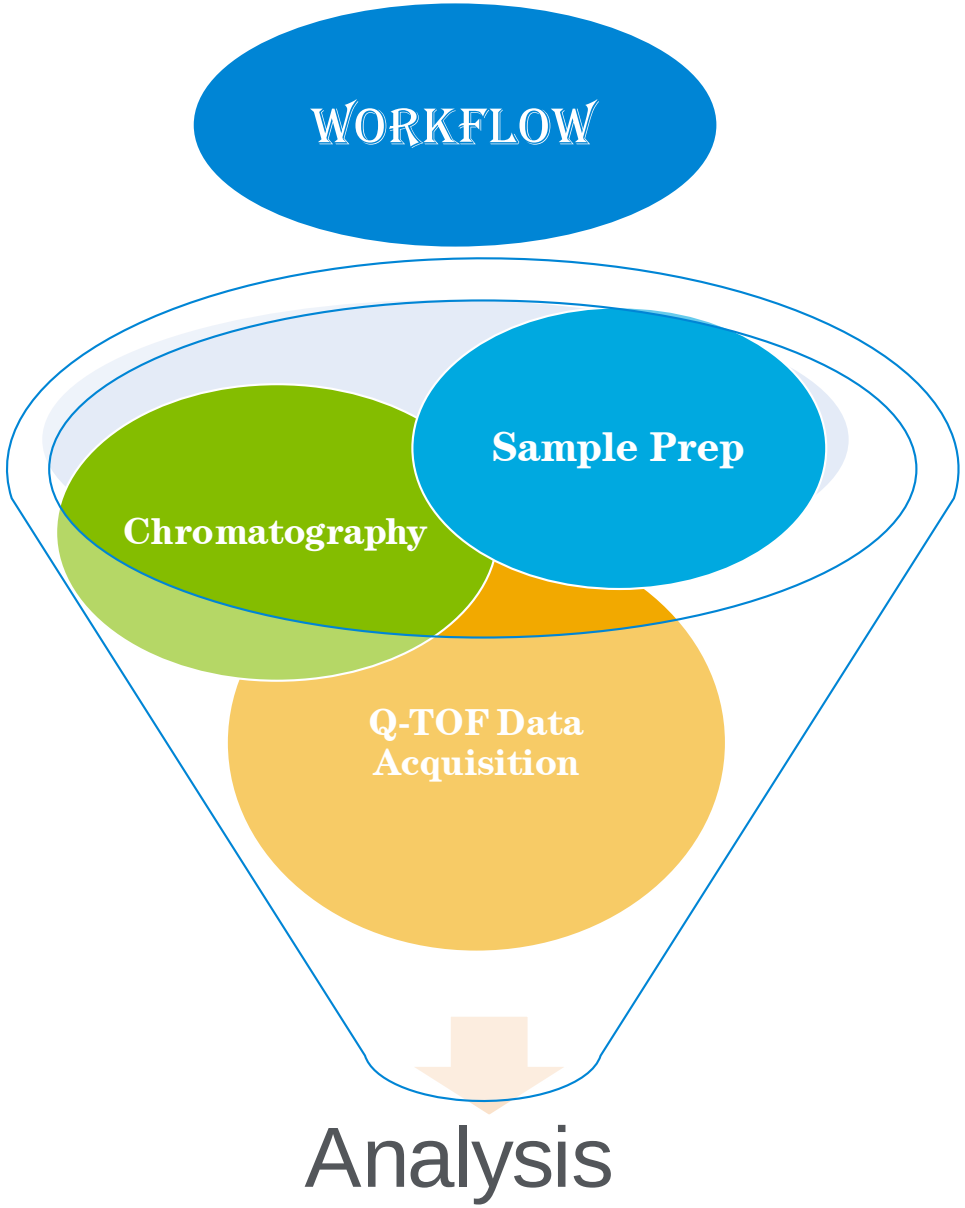
The Lipidome



CE TG SM DG PC FA PI S1P LPA Acyl CoA

Non-Polar Lipids

Polar Lipids



Reference: A comparative analysis of two sample preparation methods for the multi-omic analysis of proteins, lipids, and metabolites

ASMS 2019
WP-519

Melissa R. Pergande[1,2], Sheher Banu Mohsin[2], Limian Zhao[3], and Stephanie M. Cologna[1]

[1]University of Illinois at Chicago, Department of Chemistry, Chicago, IL, USA

[2]Agilent Technologies Inc, Wood Dale, IL, USA

[3]Agilent Technologies Inc, Wilmington, DE, USA

Agilent

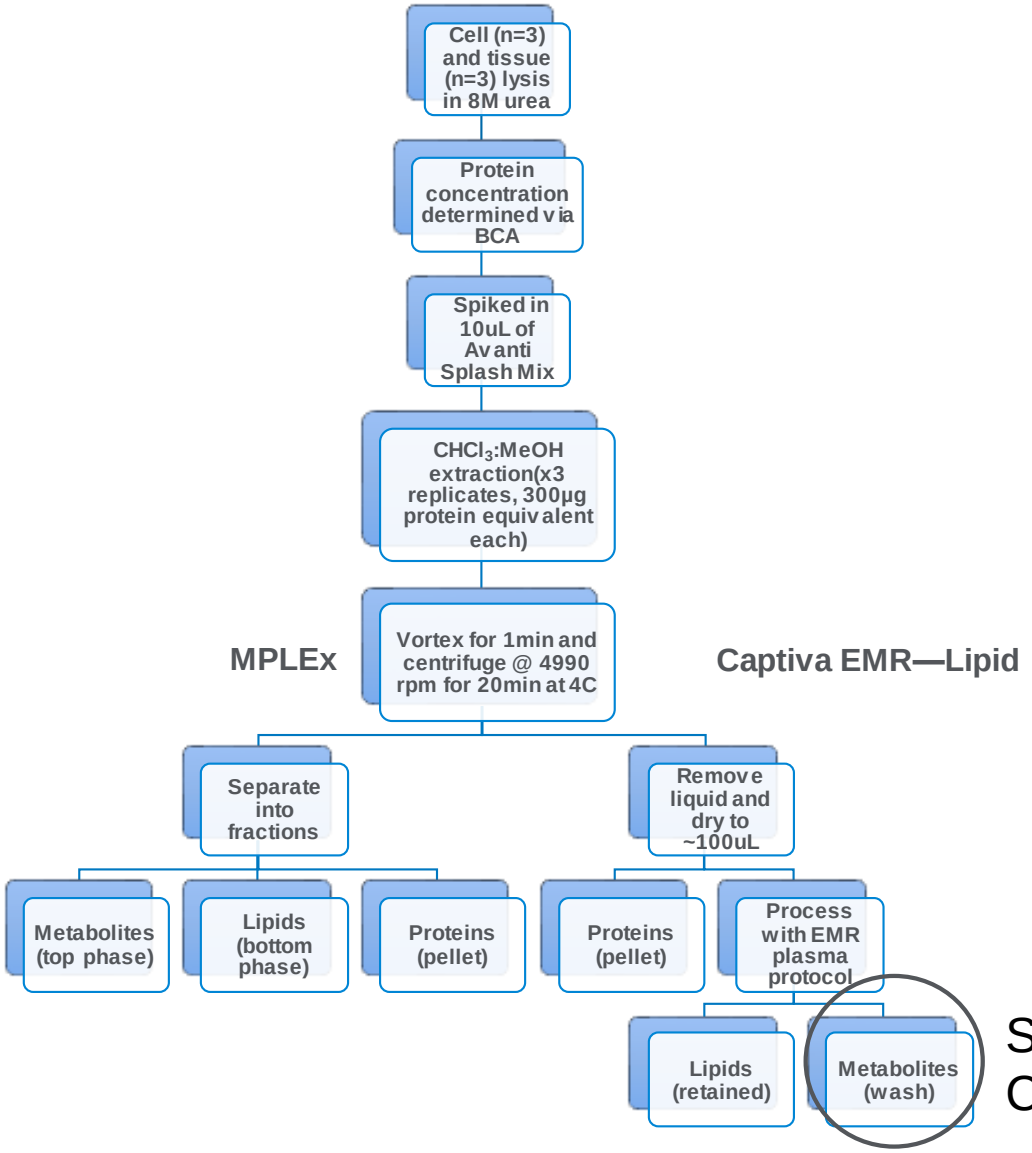
Experimental Design

Goals: To separate proteins, lipids and metabolites reproducibly for multi-omic profiling of complex samples via mass spectrometry

Method development using Avanti Splash Mix, HeLa cell and murine cerebellar tissue lysates cerebellar tissue

- Modified MPLEx
- Captiva EMR—Lipid

Experimental workflow



Still Some Challenges

Captiva EMR—Lipid Plasma Protocol Used in Experiments

Developed by Limian Zhao and Alex Apffel

900 µL of 1% methanol in acetonitrile was added to the extract (100 µL), vortexed for 10 seconds, and sonicated for 10 min

The mixture was added to the EMR—Lipid cartridge and loaded at 4-5 drops per min

Washed x2 with 1 mL of 1:9 water:acetonitrile

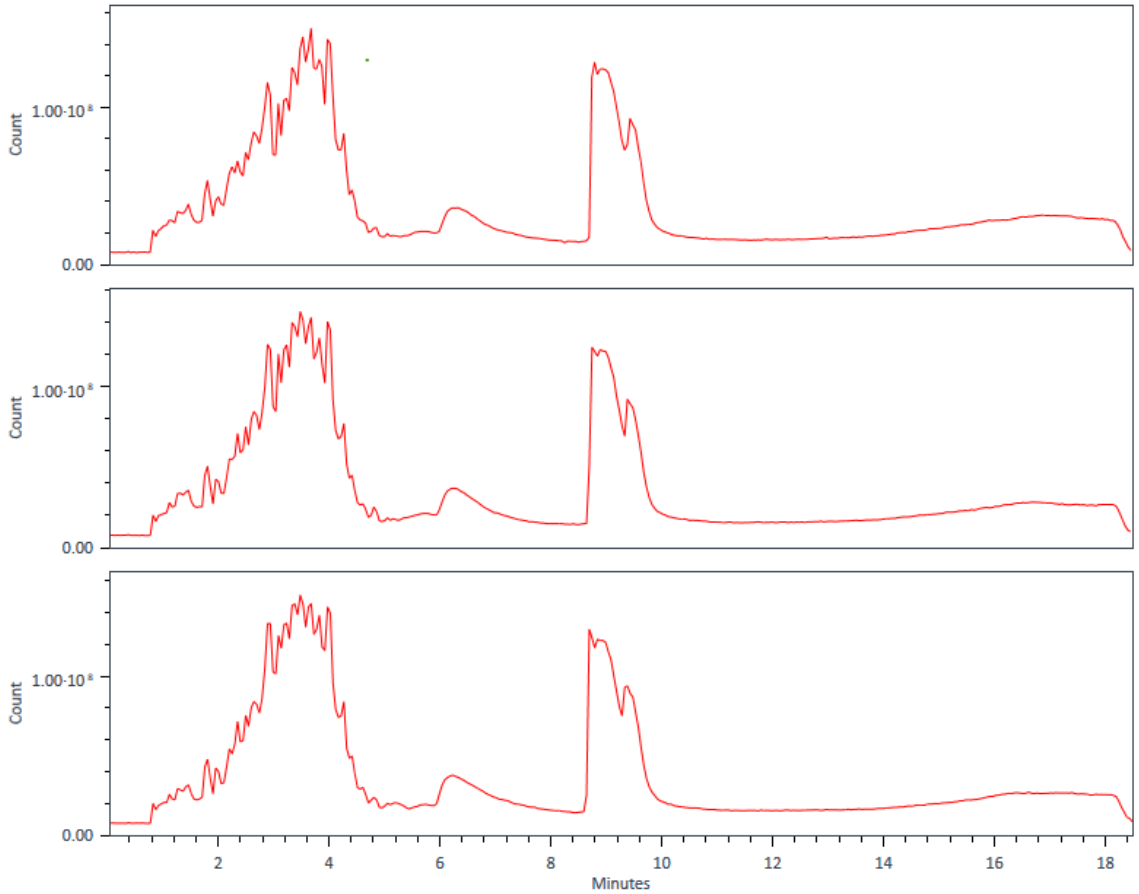
Flow through/wash dried at 30oC under nitrogen and stored at -80oC

Eluted with 1mL of 1:1 chloroform:methanol twice.

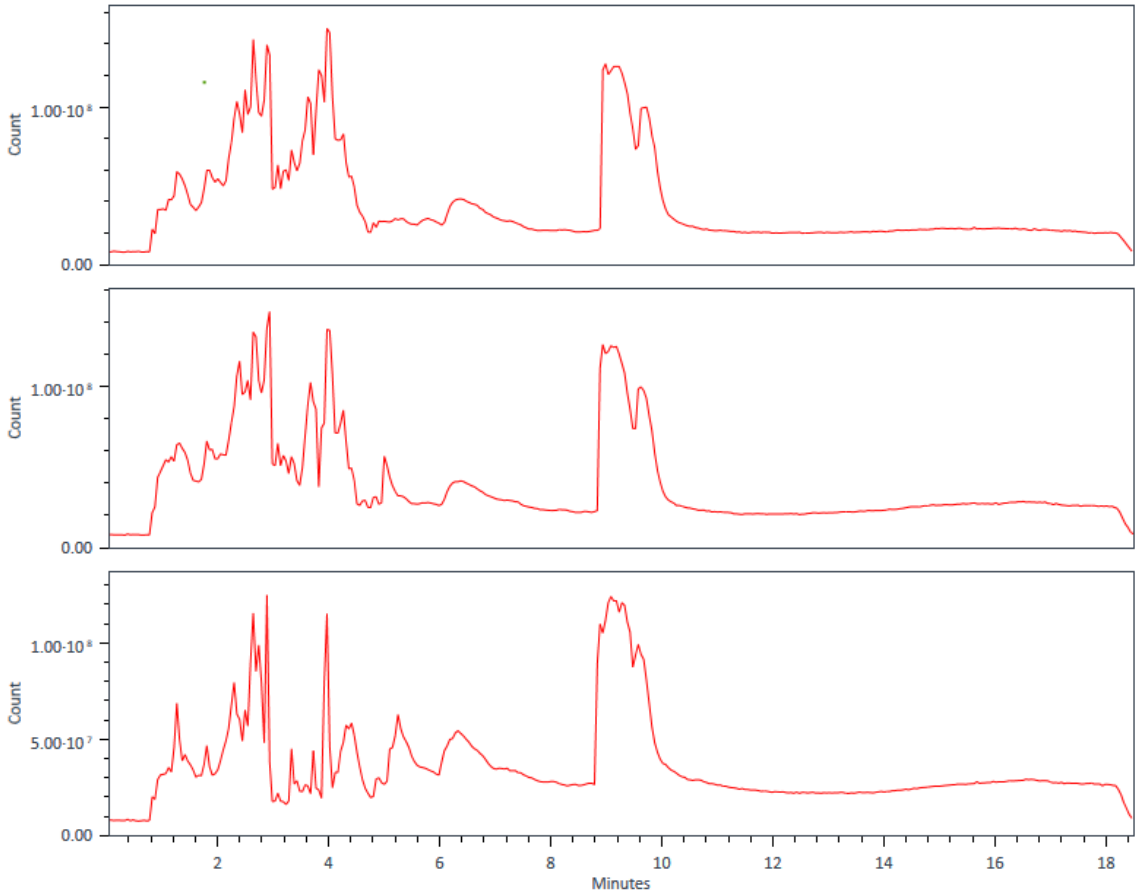
Dried at 30oC under nitrogen and resuspended in 100 µL 9:1 methanol:chloroform prior to analysis via SFC-MS on an Agilent 6545 QTOF mass spectrometer

Lipid Analysis of HeLa Samples: Chromatography

MPLEx

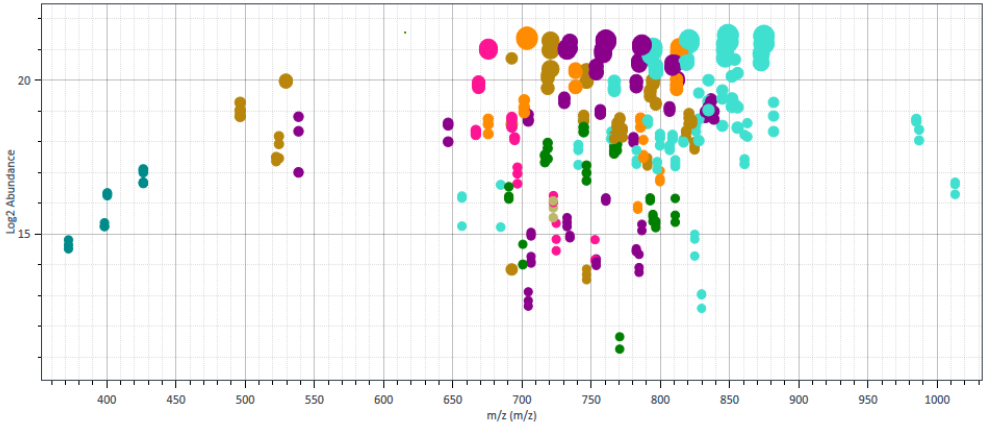


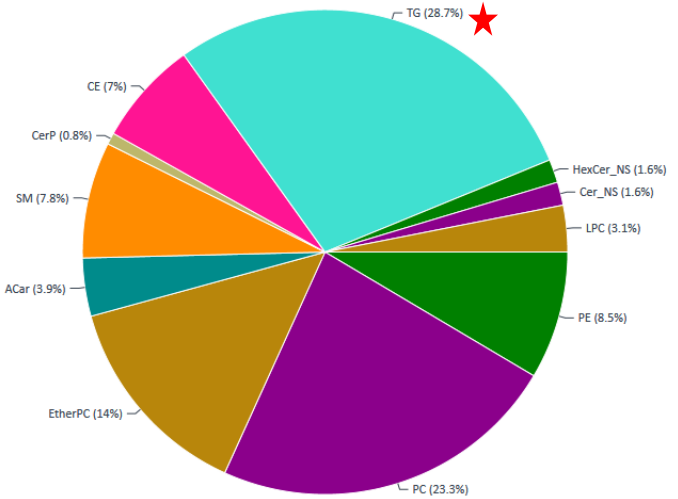
Captiva EMR—Lipid



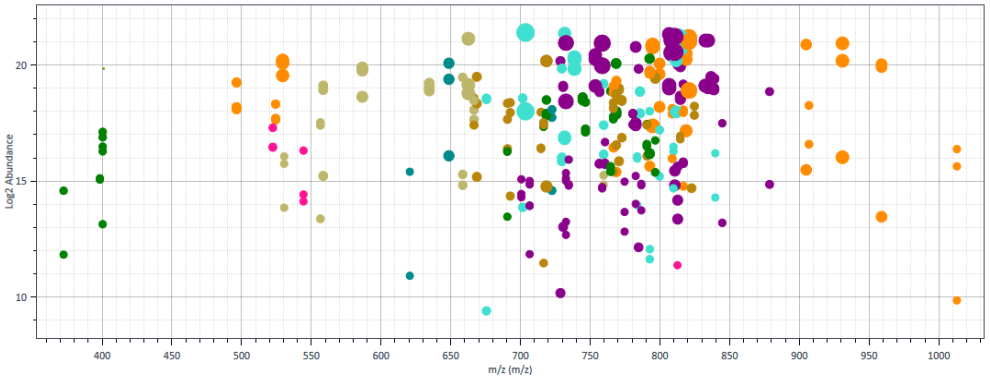
Lipid Analysis of HeLa Samples: Lipid Annotation

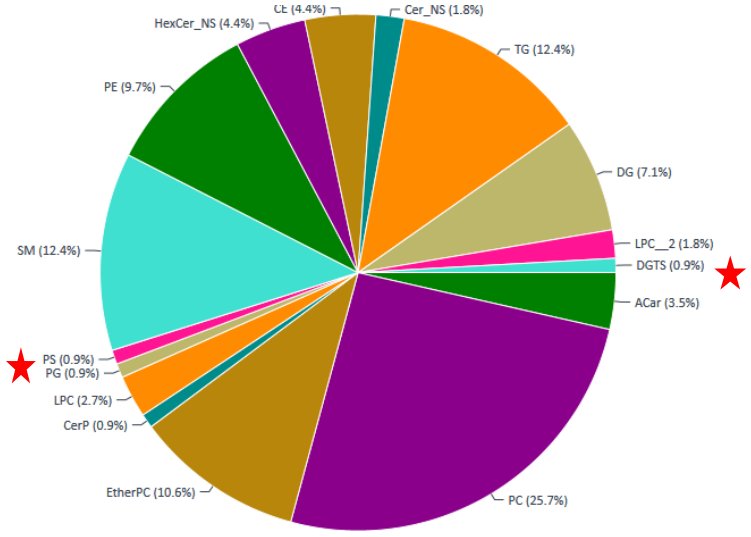
EMR—Lipid



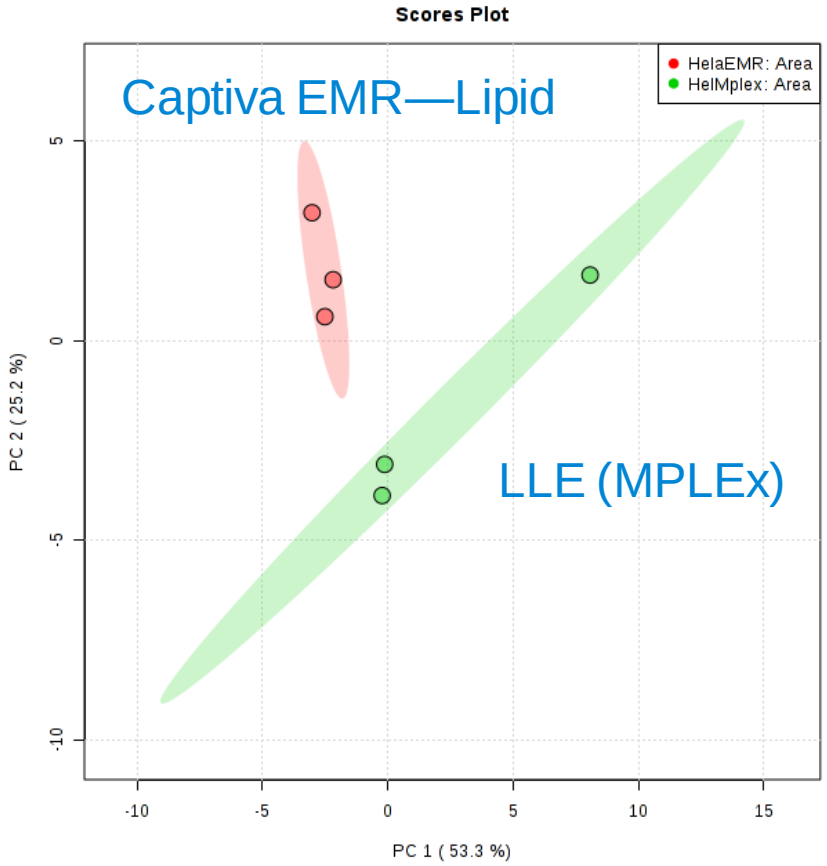


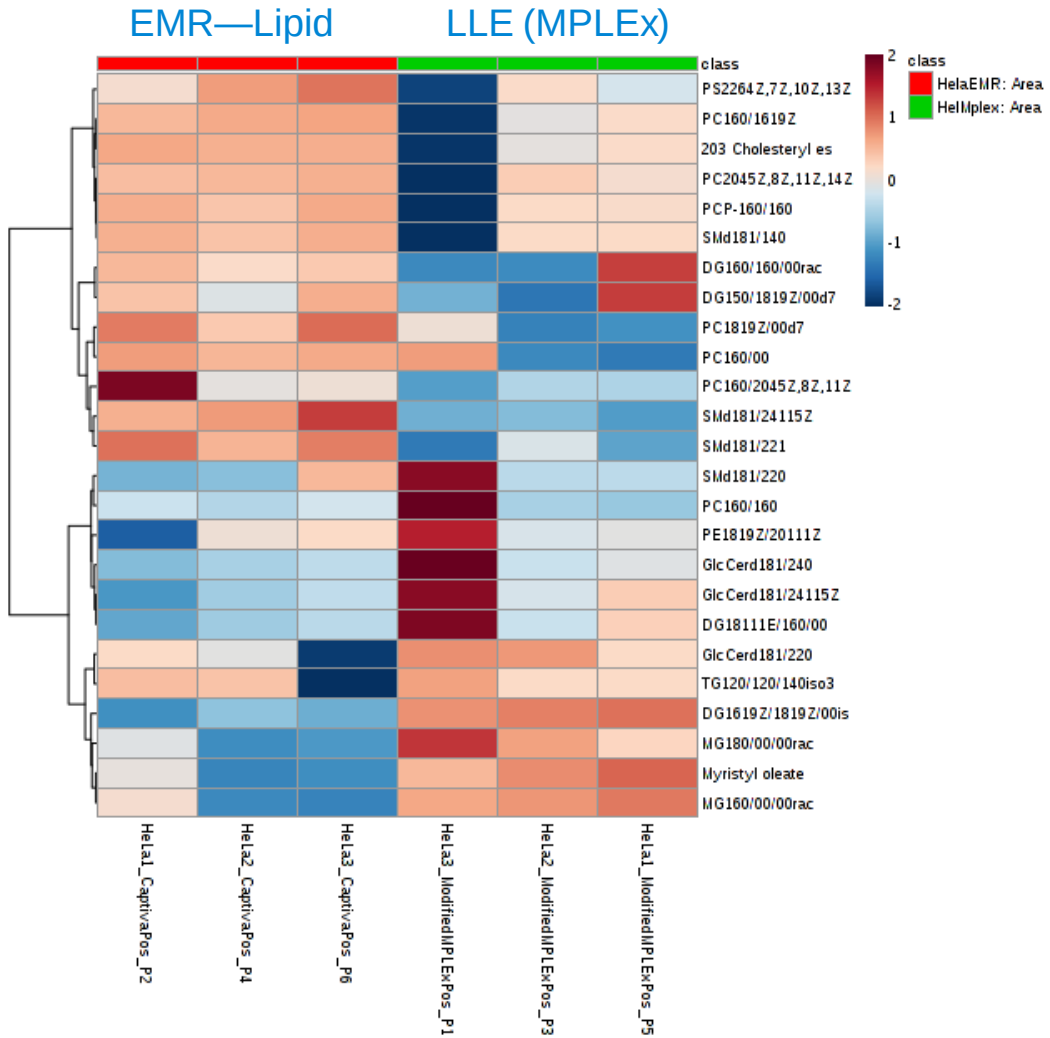
MPLEx





Lipid Analysis of HeLa Samples: Statistical Analysis

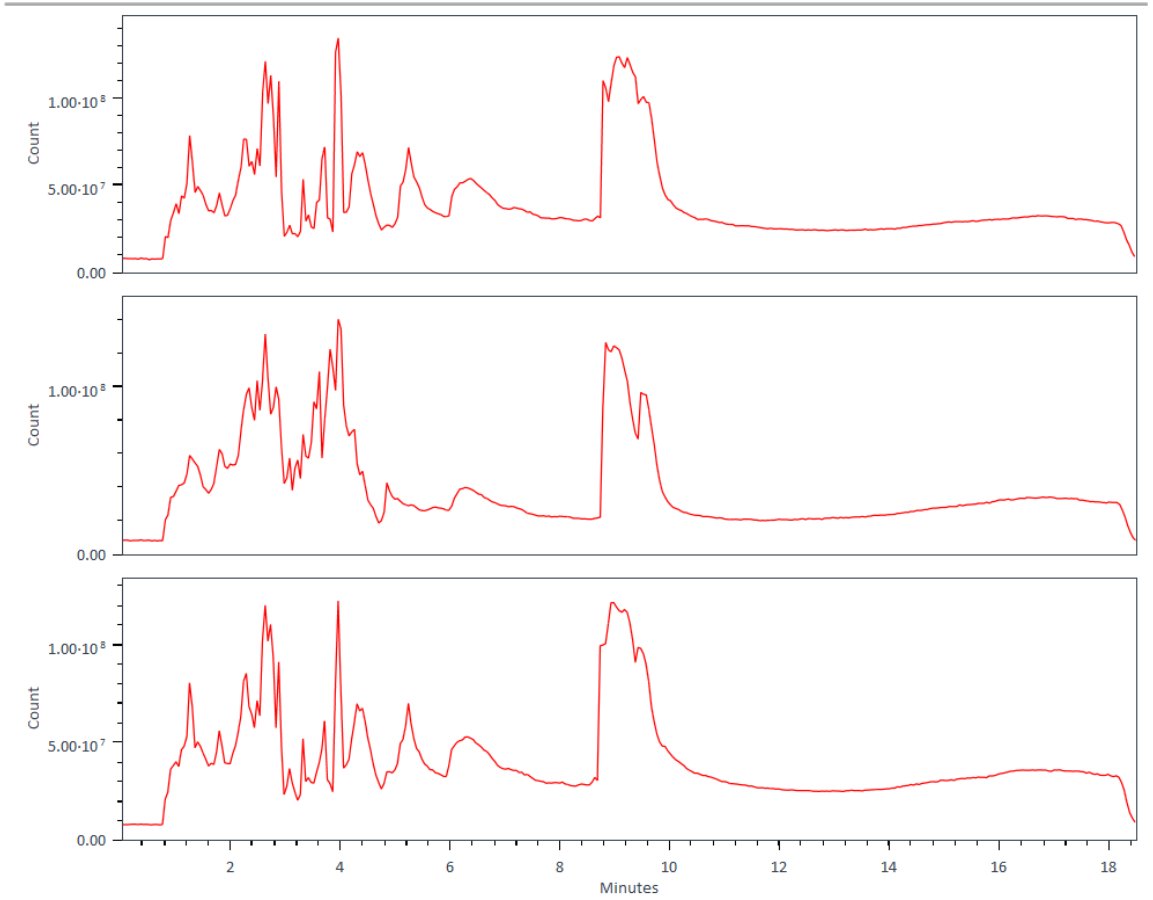




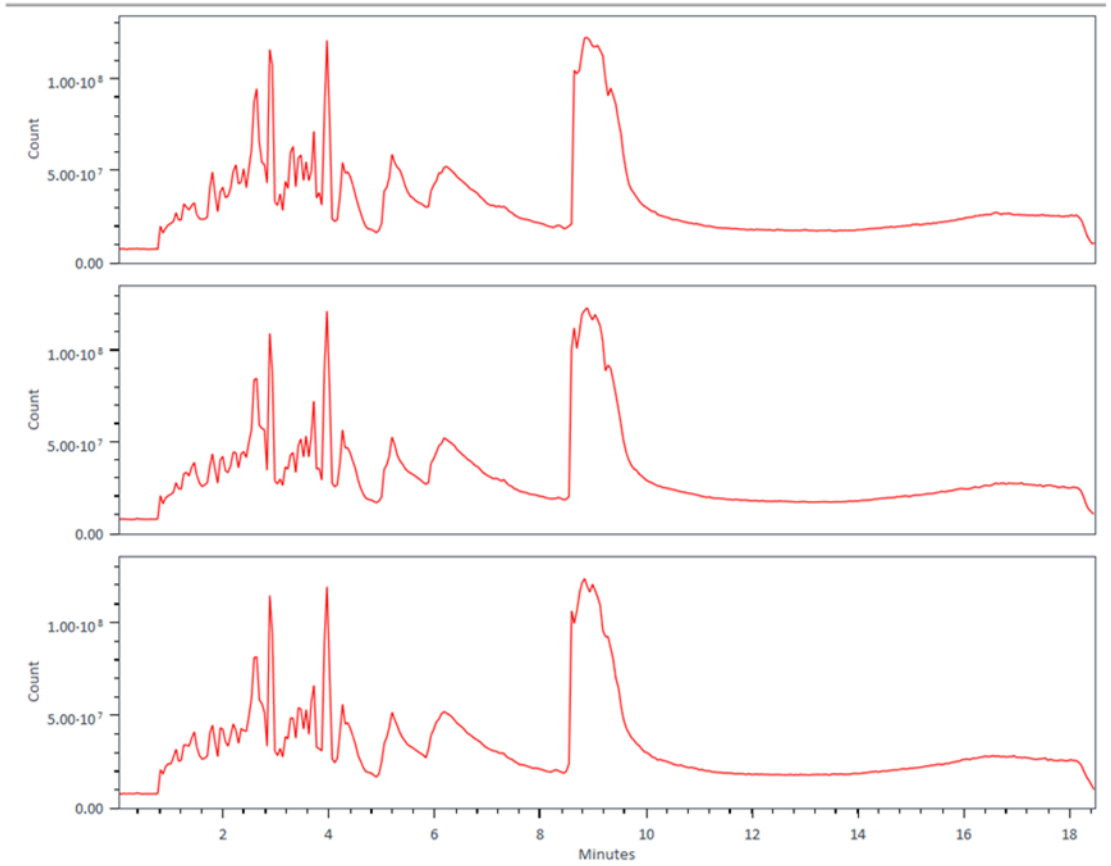
Captiva EMR—Lipid is more reproducible

Lipid Analysis of Cerebella Samples: Chromatography

MPLEx

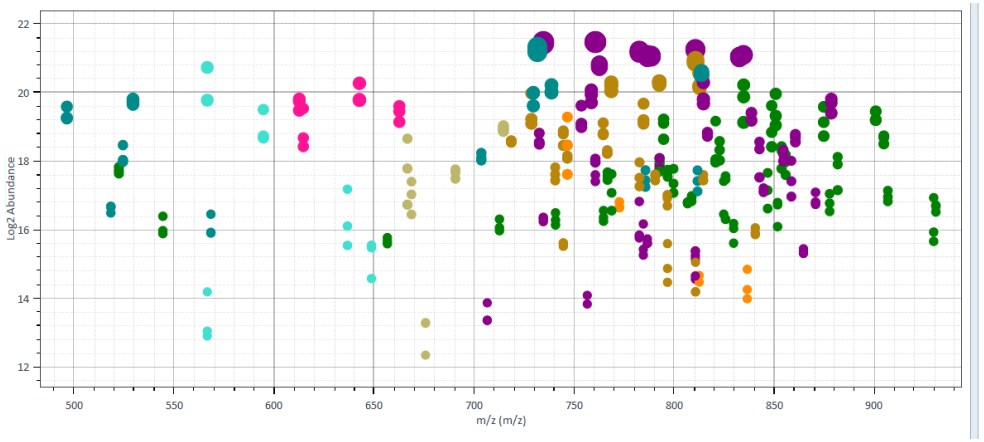


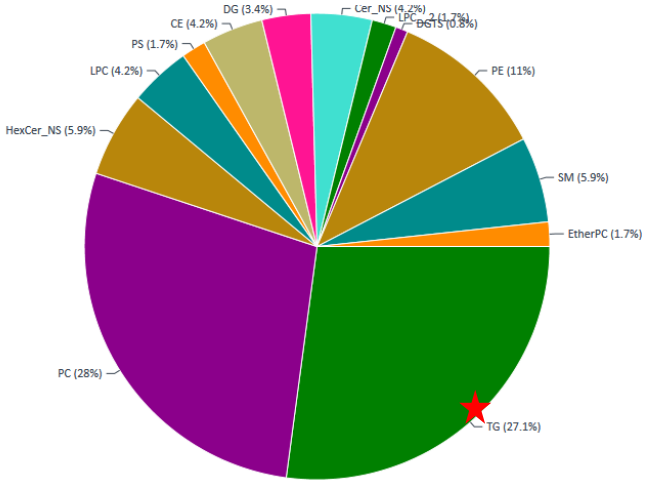
EMR—Lipid



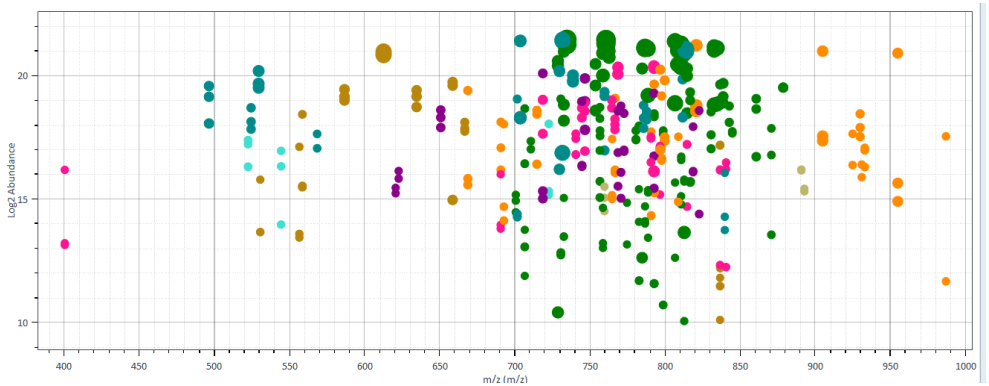
Lipid Analysis of Cerebella Samples: Lipid Annotation

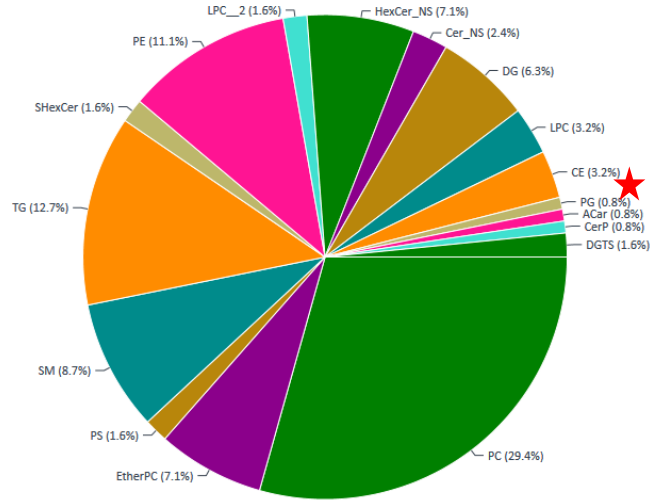
EMR—Lipid



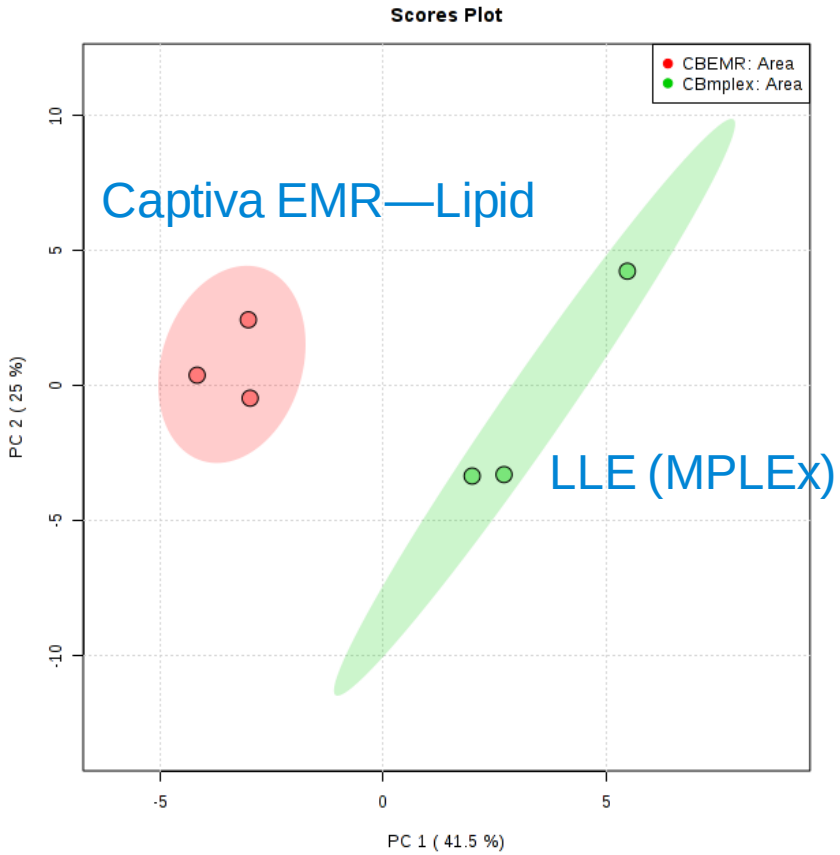


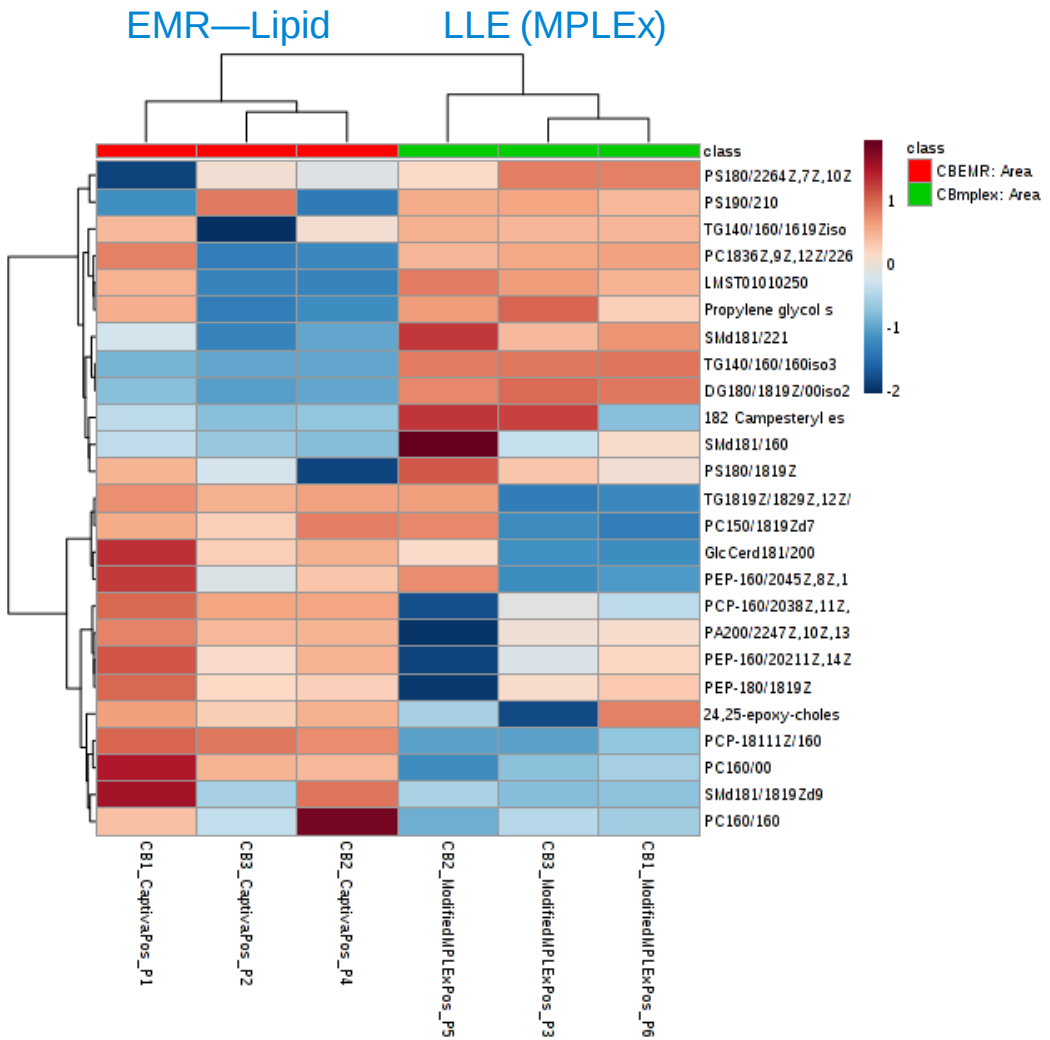
MPLEx





Lipid Analysis of Cerebella Samples: Statistical Analysis





Captiva EMR—Lipid is more reproducible

Proteomics via AJS-ESI (10 µg on-column)

Protein pellets were resuspended in 5% SDS and processed using the S-trap method

MPLEx Approach

5384 + 176 peptides and 1866 + 93 proteins HeLa cells

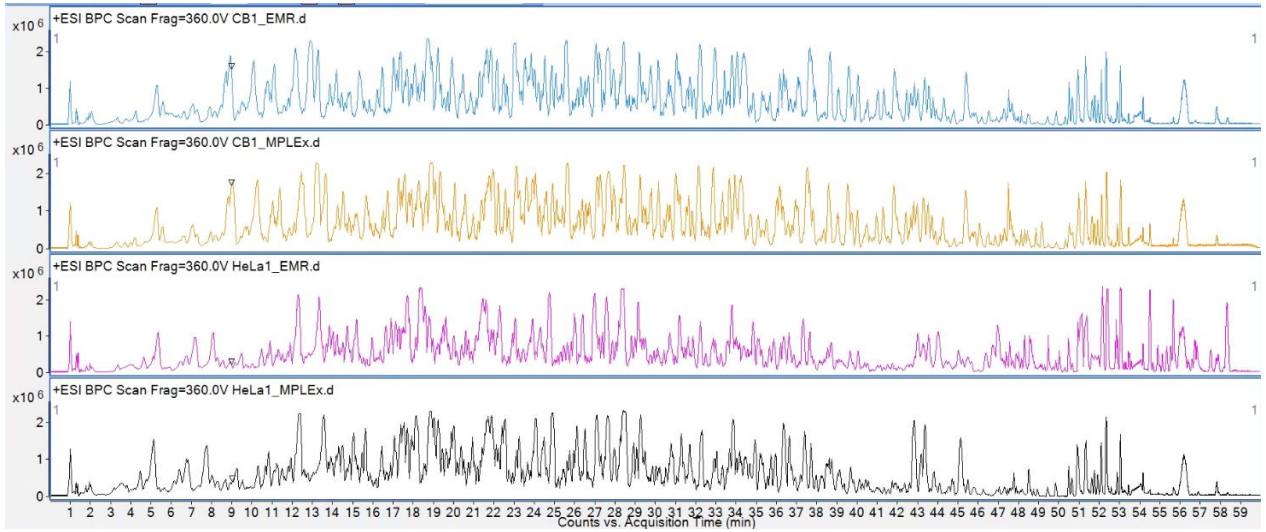
5111 + 146 peptides and 1798 + 51 proteins cerebellar tissue

Captiva EMR—Lipid Approach

5322 + 110 peptides and 1922 + 22 proteins HeLa cells

5378 + 138 peptides and 1978 + 54 proteins cerebellar tissue

Representative BPCs



Cerebella EMR—Lipid

Cerebella MPLEx

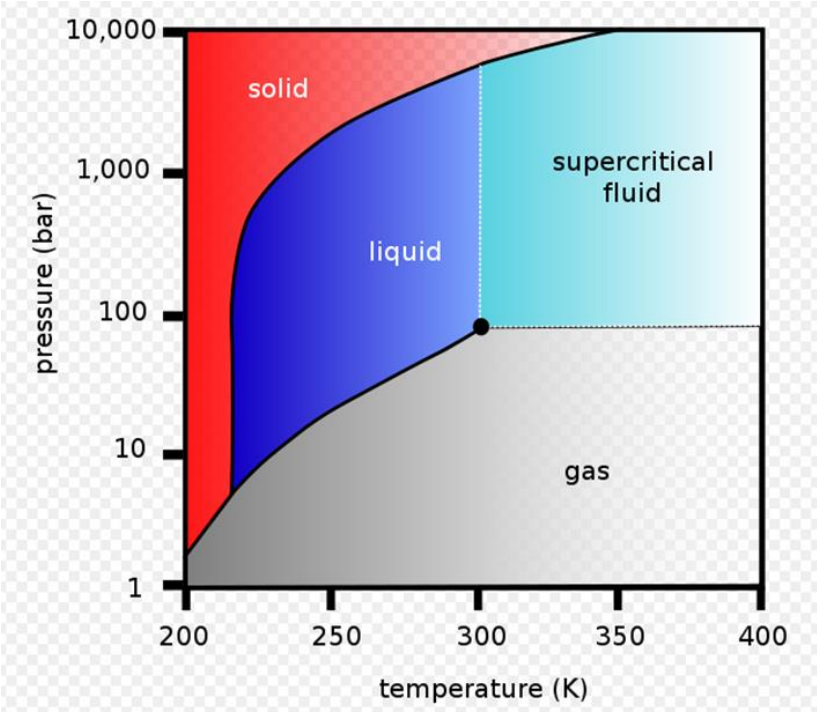
HeLa EMR—Lipid

HeLa MPLEx

Agilent Instrumentation For Lipidomics

SFC Fundamentals

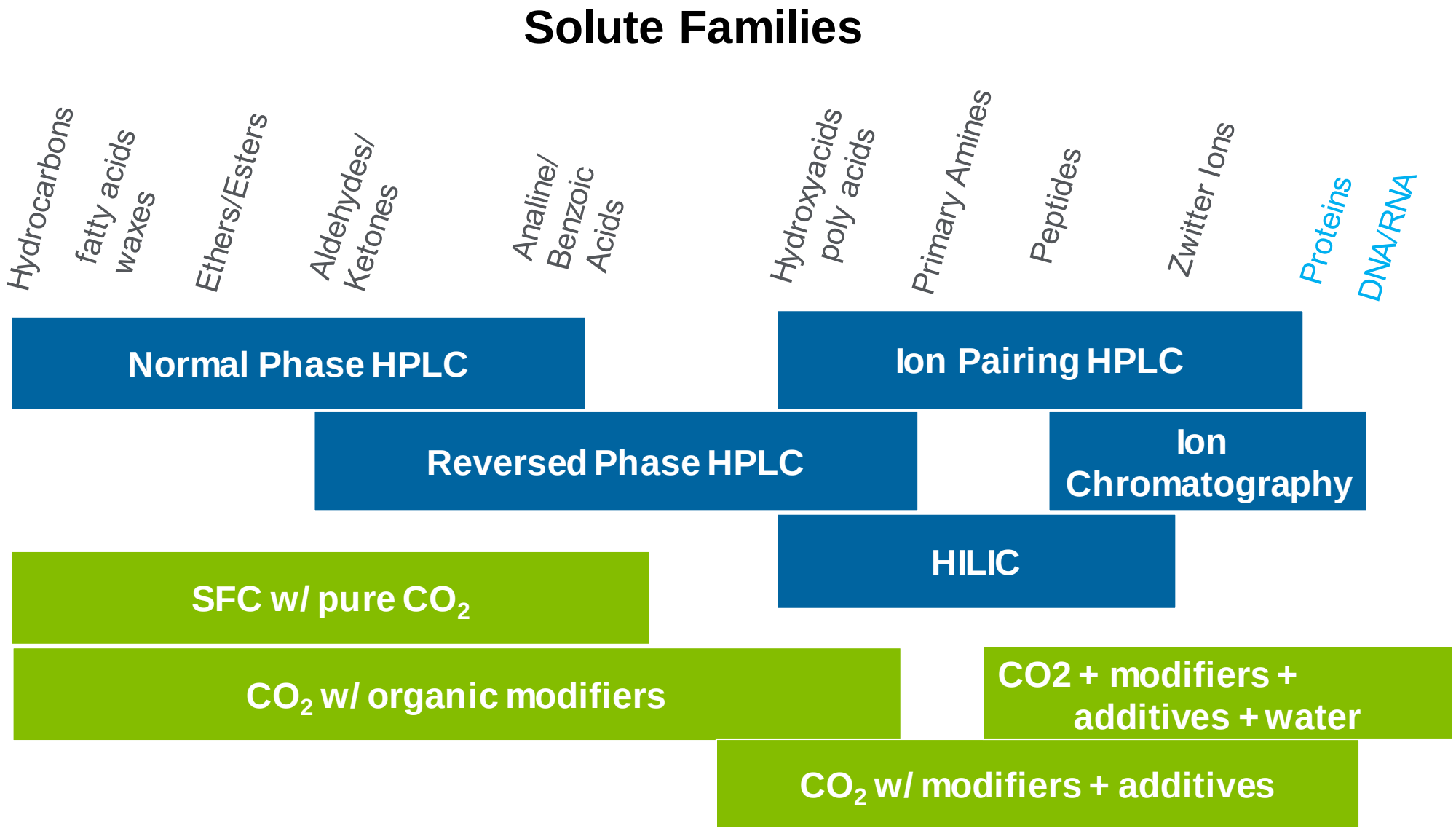
Substance	Density (g/cm^3)	Viscosity (cP)	Diffusivity (cm^{-2}-s^{-1})
Gas	10^{-3}	10^{-2}	0.2
Supercritical Fluid	0.5	5x10-2	$5x10^{-4}$
Liquid	1	1	10^{-5}



- Supercritical CO_2 is the mobile phase
- Viscosity is similar to a gas
- Density and solubilizing power of liquids
- Intermediate diffusivity between gases and liquids.

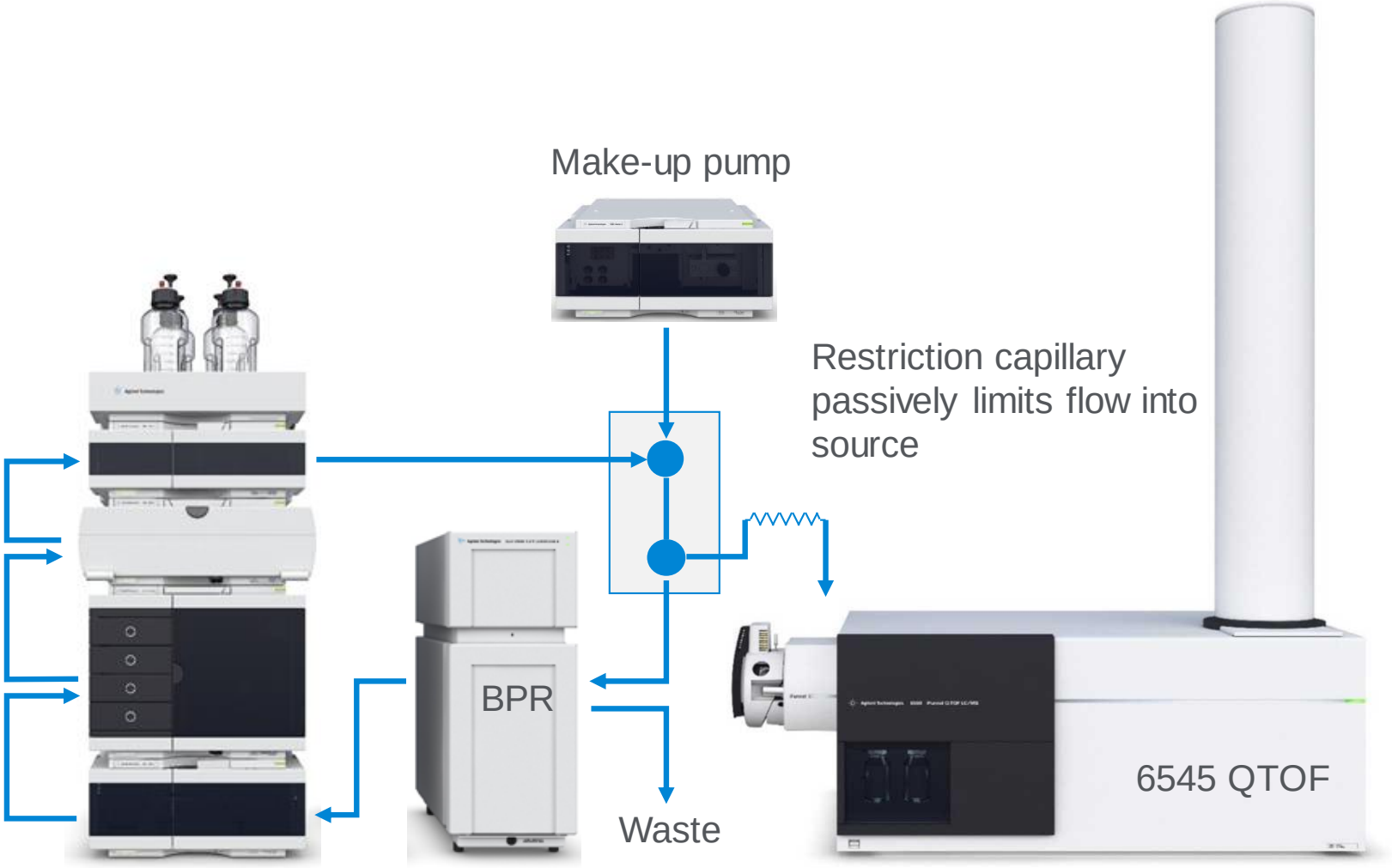
SFC operation requires the mobile phase (CO_2) be maintained in a supercritical state above a critical temperature (T_c: 31°C) and critical pressure (P_c: 74bar)

Where Does SFC Fit Relative to HPLC?



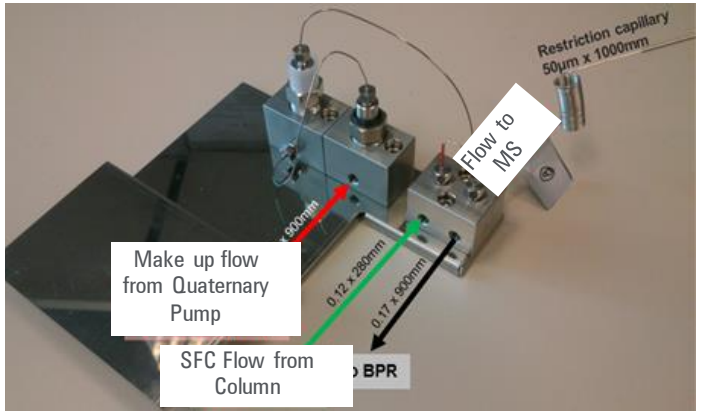
Agilent SFC/Q-TOF System

New generation SFC/MS: a robust, routine, automated analytical tool



Infinity II SFC System

6545 QTOF



Splitter kit

Global Lipid Separation

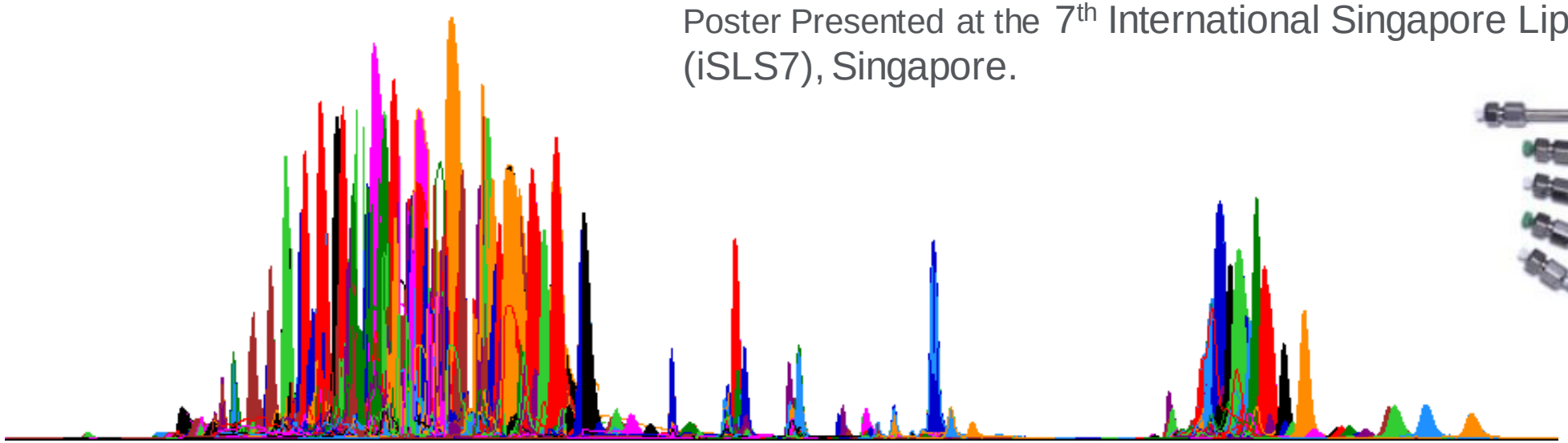
- Column selection is a key variable for SFC separations
- For the SPLASH Lipidomix® used for optimization, no single column offered both adequate selectivity and resolution across all classes of lipids
- Instead a combination of a “normal phase” (silica) and a reversed phase (C18) column yielded the best results

Reference :

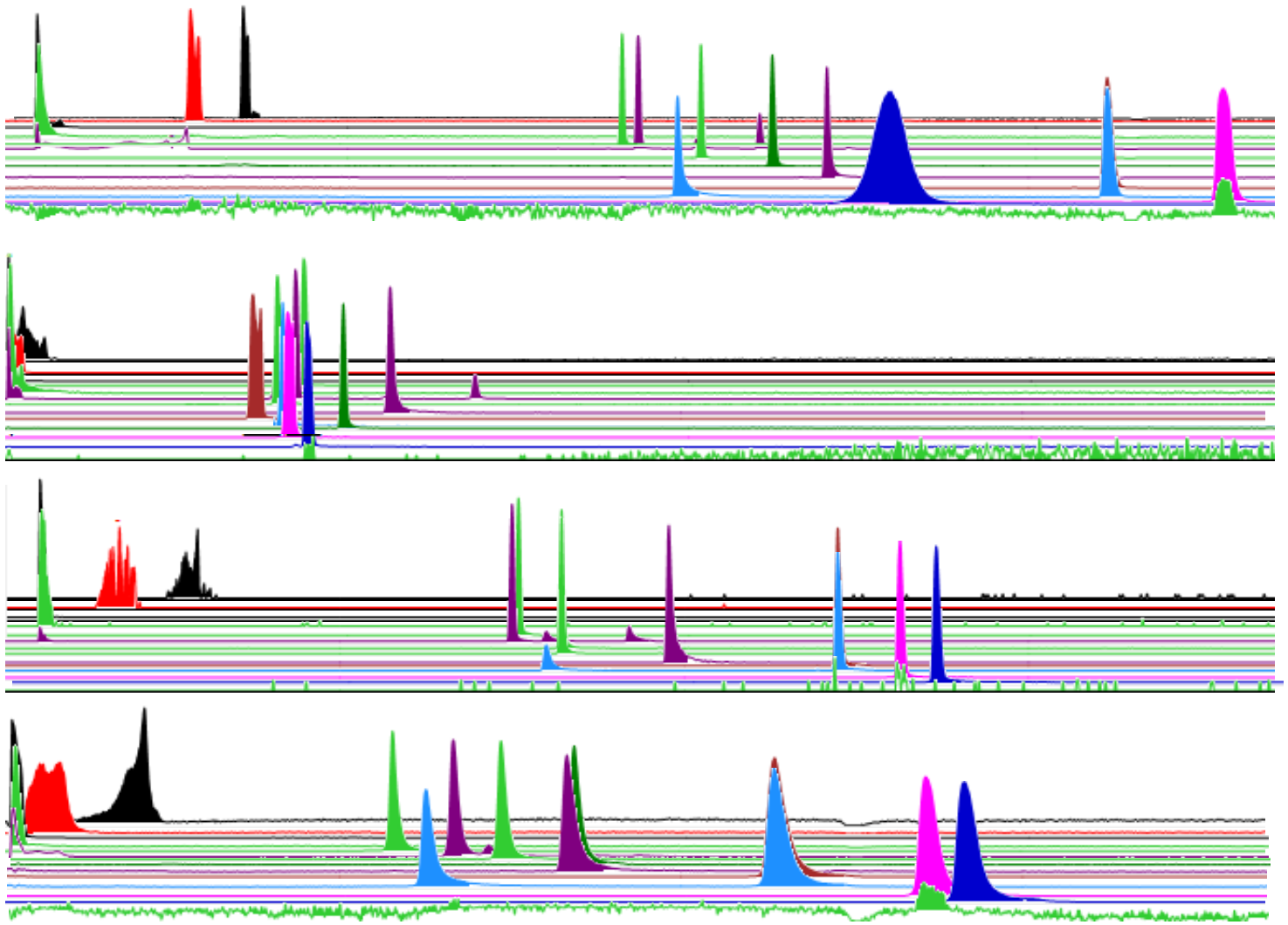
Rapid, efficient intra/inter class lipid separation based on SFC-MS

Sheher Mohsin[1], Alex Apffel[1], Kevin Williams[2]
[1]Agilent Technologies, 5301 Stevens Creek Blvd, Santa Clara, CA, USA
[2]UCLA, Los Angeles, CA, USA

Poster Presented at the 7th International Singapore Lipid Symposium (iSLS7), Singapore.



Global Lipid Separation: Normal Phase/HILIC



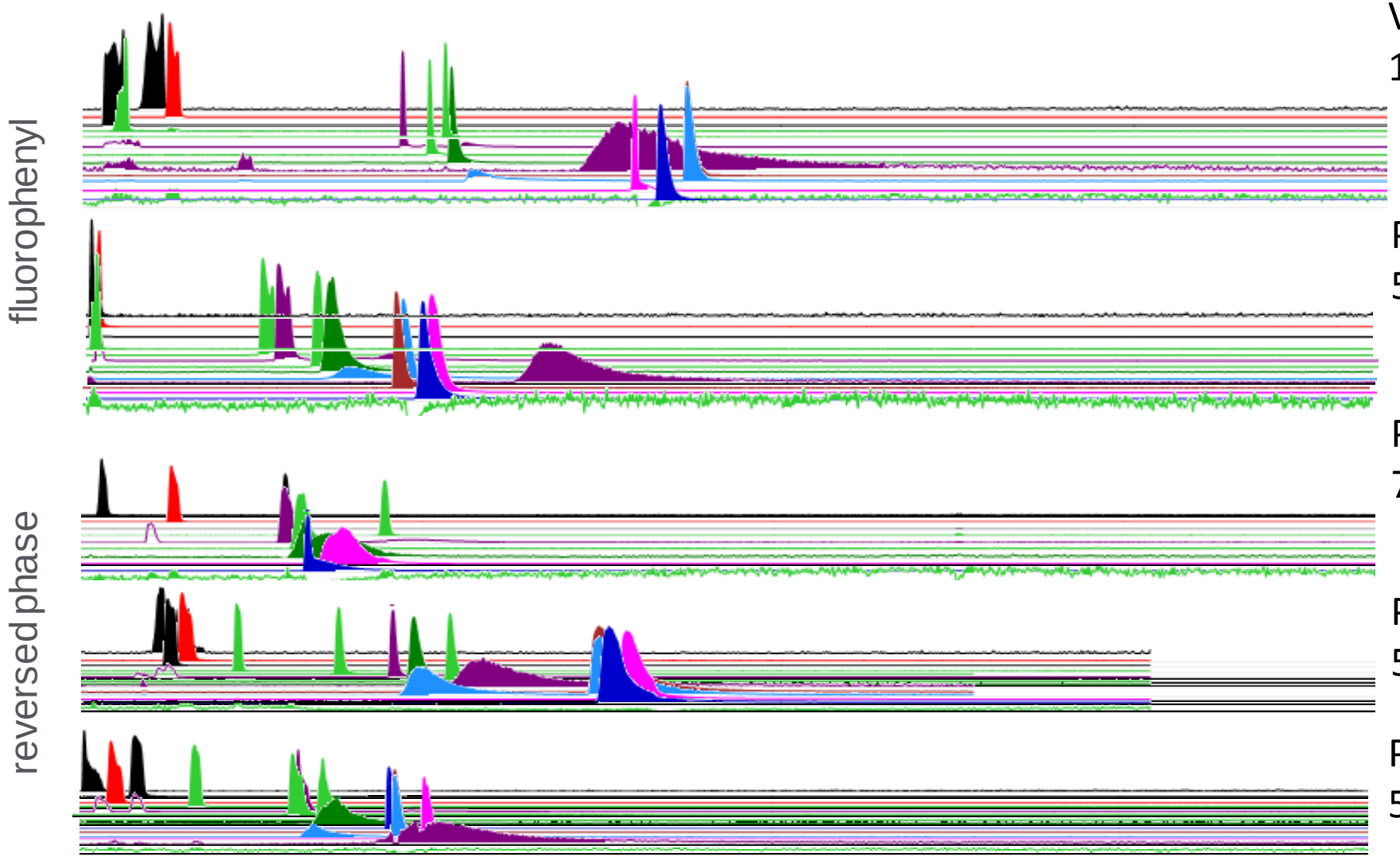
RX-Sil
100x3mm 1.8um

Poroshell 120 HILIC-Z
50x3mm 2.7um

Viridis BEH
100x3mm 1.7um

Cogent Diamond Hydride
50x2.1 2.2um

Global Lipid Separation: Fluorophenyl and Reversed Phase



Viridis BEH CSH Fluorophenyl
100x3 mm 1.7 µm

Poroshell 120 PFP
50x2.1 mm 2.7 µm

Poroshell 120 EC-C18
75x3 mm 2.7 µm

Poroshell 120 SB-AQ
50x2.1 mm 2.7 µm

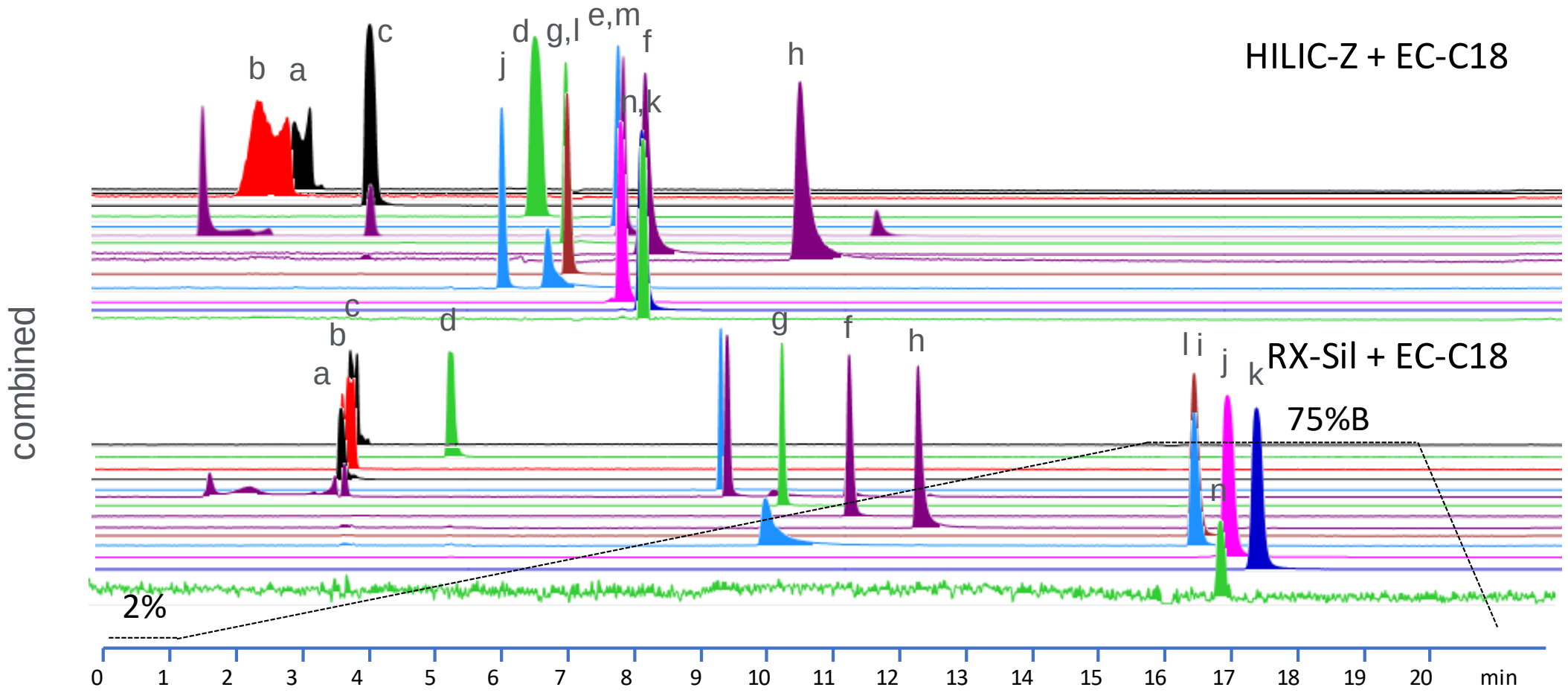
Poroshell 120 HPH-C18
50x3 mm 2.7 µm

Global Lipid Separation: Combined Columns

Rapid, efficient intra/inter class lipid separation based on SFC-MS

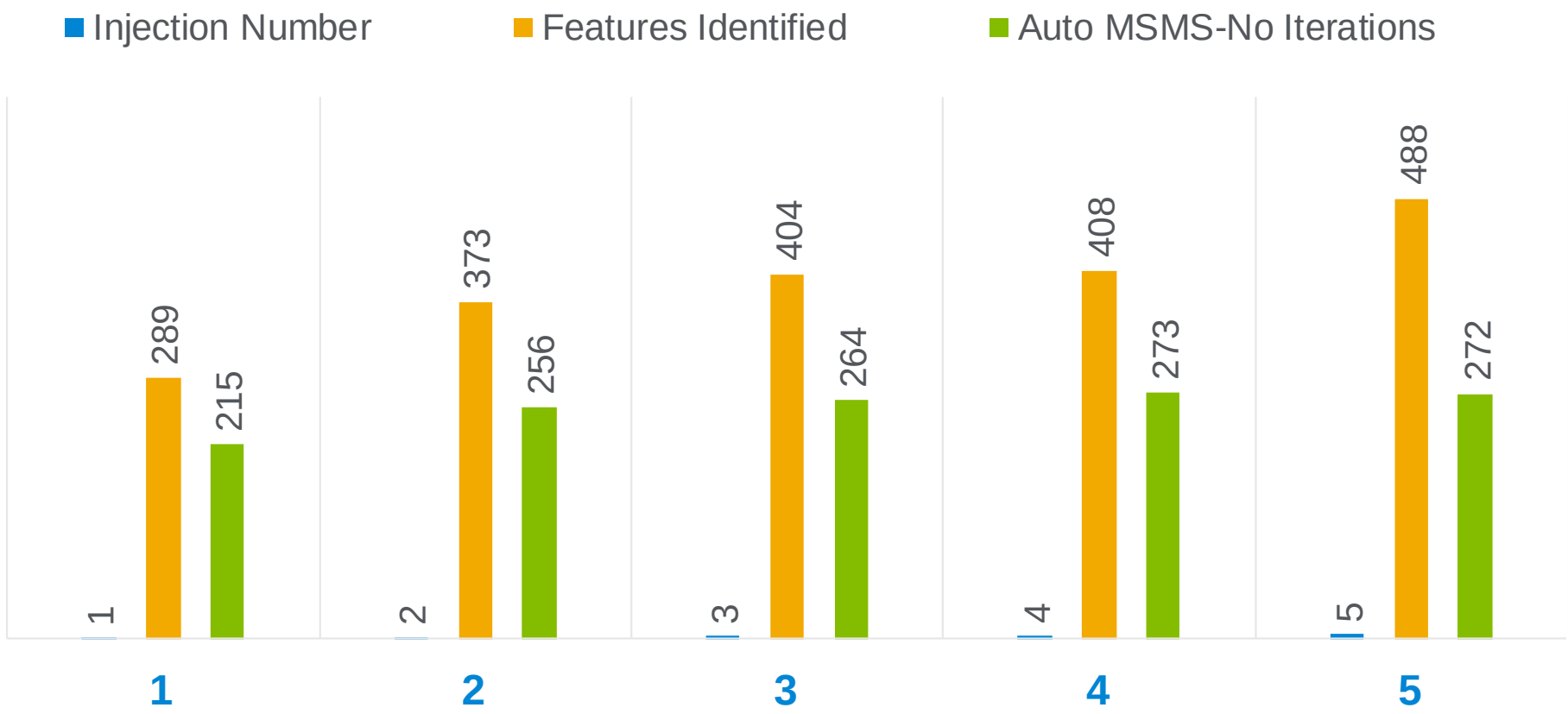
Sheher Mohsin[1], Alex Apffel[1], Kevin Williams[2]
[1]Agilent Technologies, 5301 Stevens Creek Blvd, Santa Clara, CA, USA
[2]UCLA, Los Angeles, CA, USA

Poster Presented at the 7th International Singapore Lipid Symposium (iSLS7), Singapore.



Agilent

Iterative MS/MS for Improved Coverage



Lipidomics Workflow

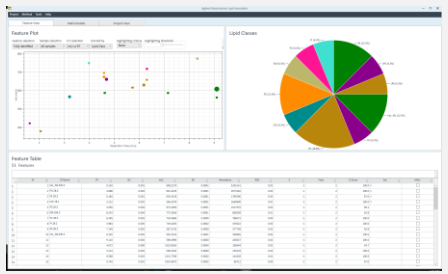
For Iterative and Auto MSMS

LC/Q-TOF

MS/MS

MS1

1. Build a database



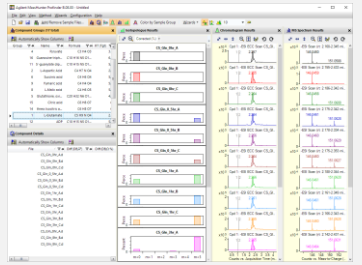
Lipid Annotator

Annotate lipid based on MS/MS level information

DB

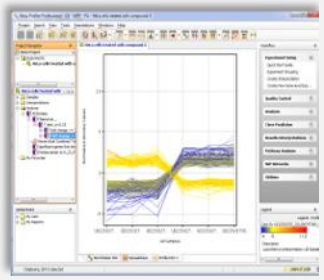
- *m/z* & RT
- MS/MS spectra (optional)

2. Profile and Identify



Profinder

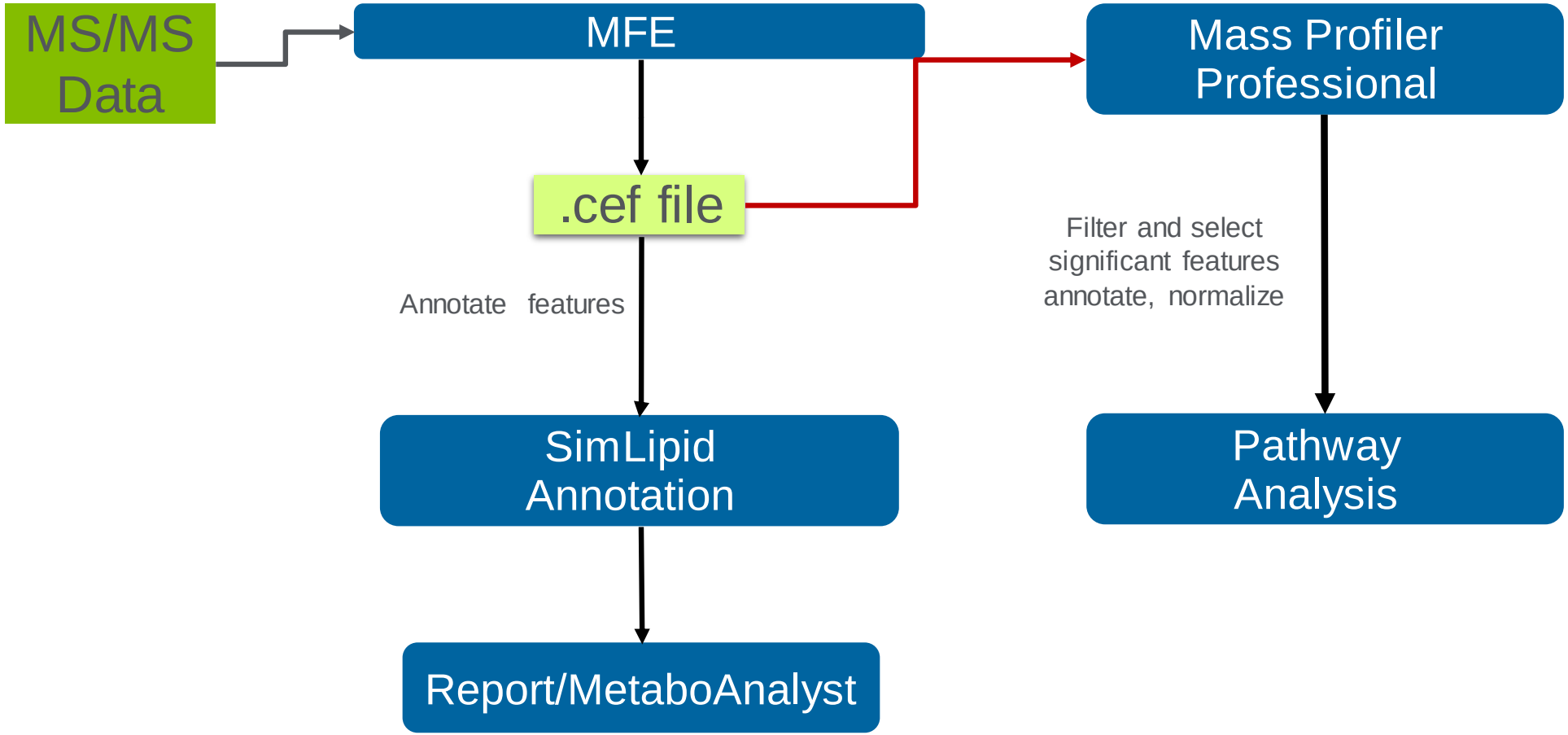
Align annotated lipids with MS1 data based on *m/z* and RT



MPP

Normalize and statistically evaluate annotated data and

Informatics and Annotation with SimLipid



Case Study 1 – McGill University
Study conducted in collaboration with Dr. Peter Siegel and Dr. Daina Avizonis

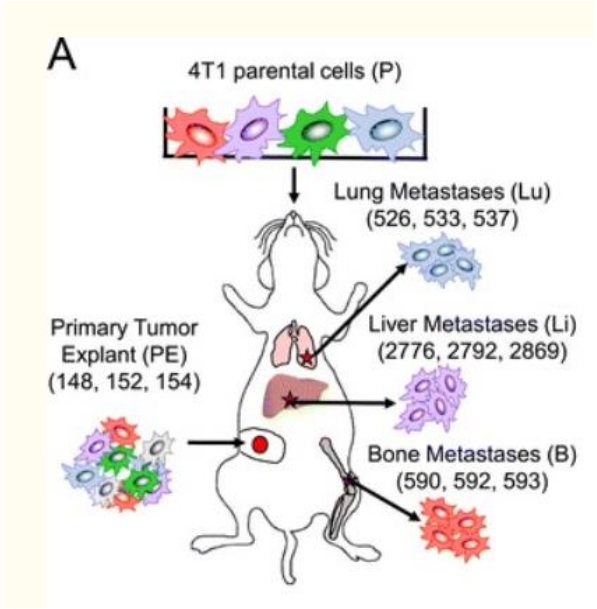
Reference:

Supercritical Fluid Chromatography Separation and Shotgun Lipidomics with High Resolution Mass Spectrometry for the Study of Breast Cancer Metastasis

Sheher Mohsin[1]; Sanjib Meitei[2], Peter Siegel[3]; Daina Avizonis[3]; Gaelle Bridon[1]

[1]Agilent Technologies Inc, Wood Dale, IL; [2]PREMIER Biosoft, Indore, Madhya Pradesh, India ; [3]Goodman Cancer Research Centre, McGill University, Montreal, Quebec, Canada

Studying lipids in breast cancer metastasis



Breast Cancer Metastasis Research Group

Peter Siegel Ph.D., Principal Investigator

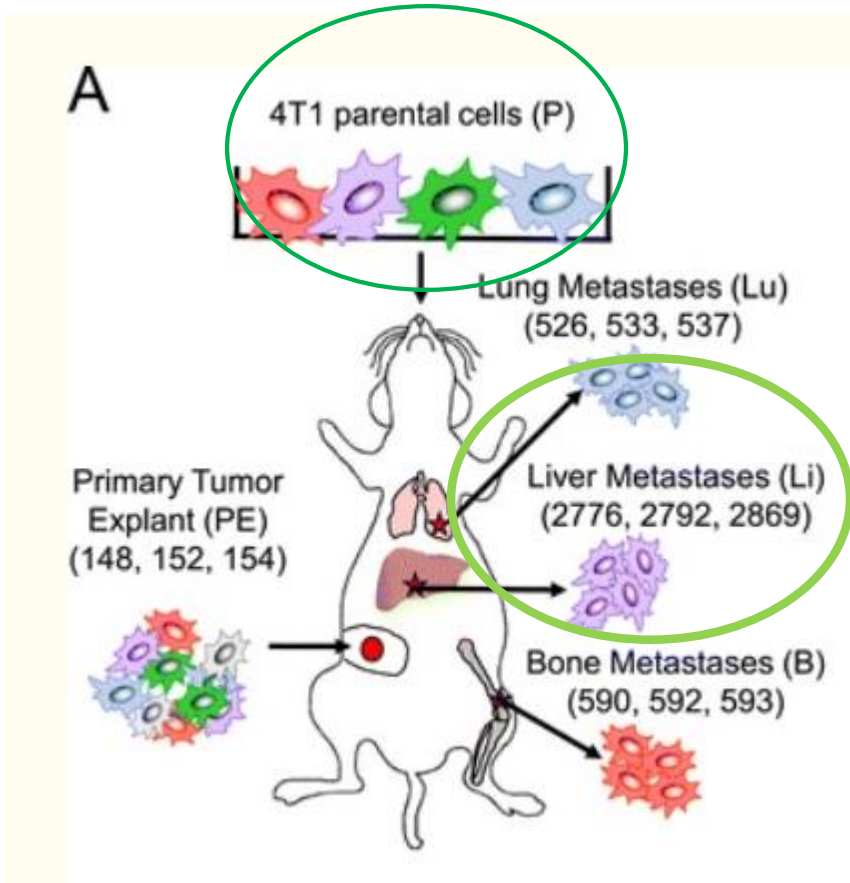
McGill
The Rosalind & Morris Goodman Cancer Research Centre
Centre de recherche sur le cancer Rosalind et Morris Goodman

Breast Cancer Res. 2015; 17(1): 45.
Published online 2015 Mar 27. doi: [10.1186/s13058-015-0558-3]
PMCID: PMC4413545
PMID: 25882816

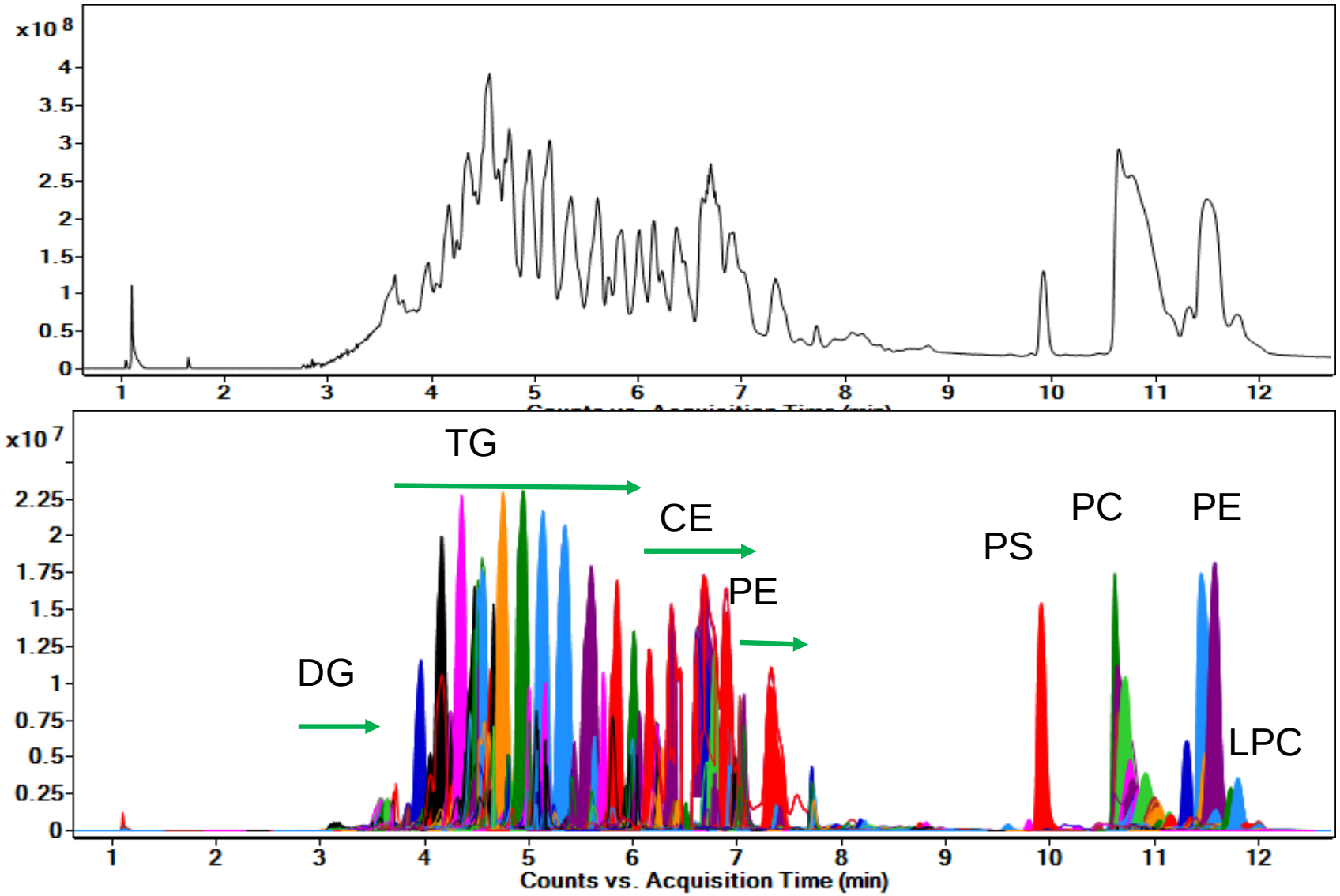
Granulocytic immune infiltrates are essential for the efficient formation of breast cancer liver metastases

Sébastien Tabariès, Véronique Ouellet, Brian E Hsu, Matthew G Annis, April AN Rose, Liliane Meunier, Euridice Carmona, Christine E Tam, Anne-Marie Mes-Masson, and Peter M Siegel

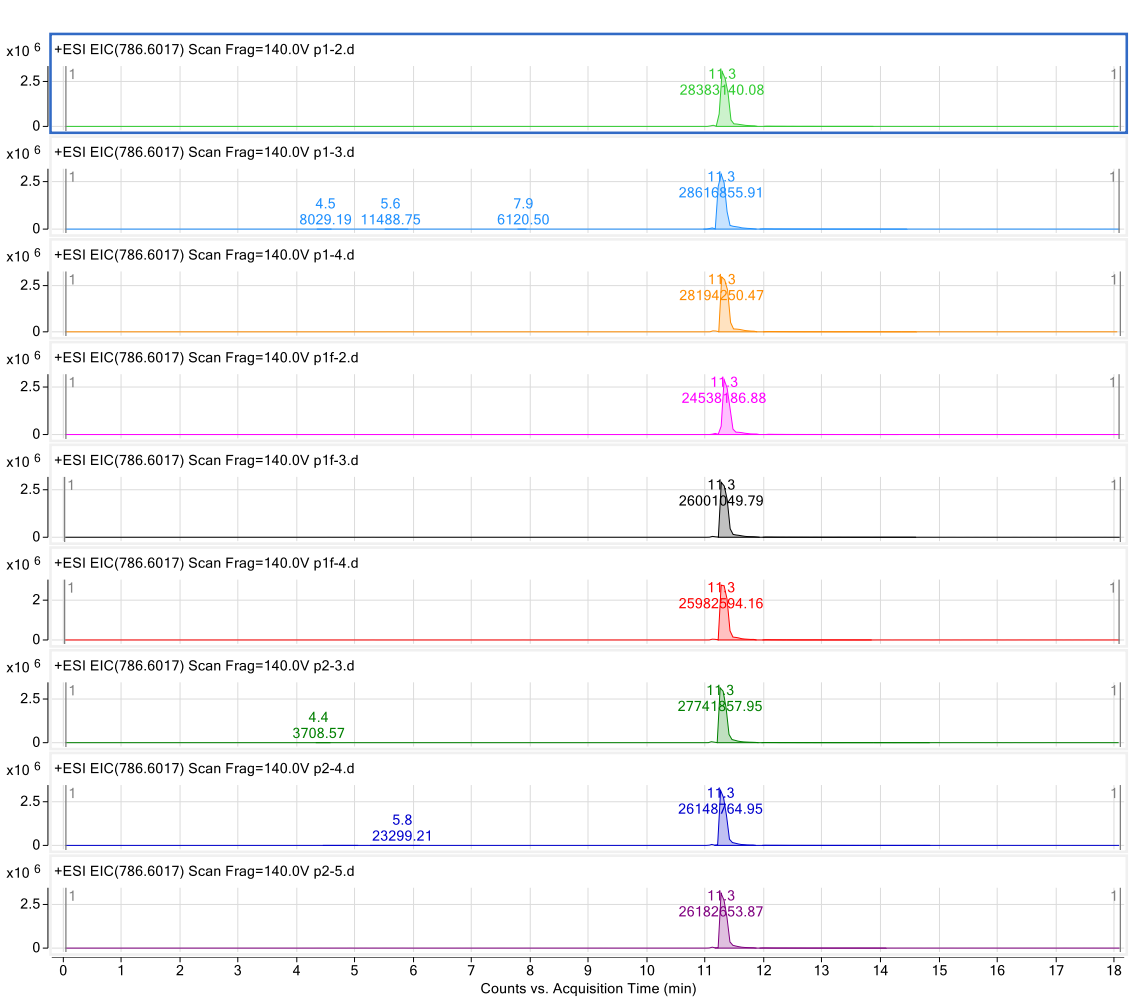
Dr. Daina Avizonis - Facility Manager / Sr. Research associate

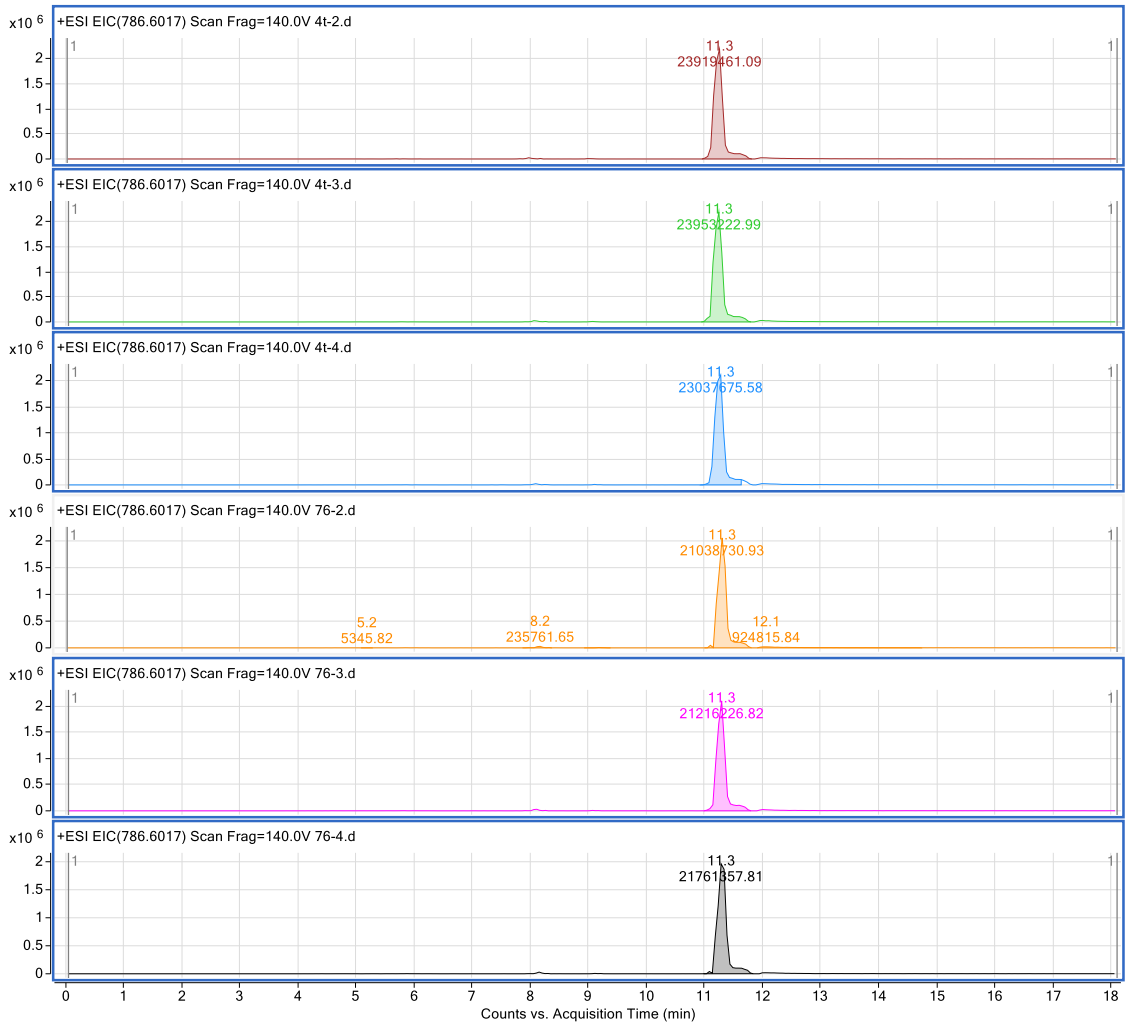


Lipids in Liver Metastasized 2776 Cells Separated by SFC



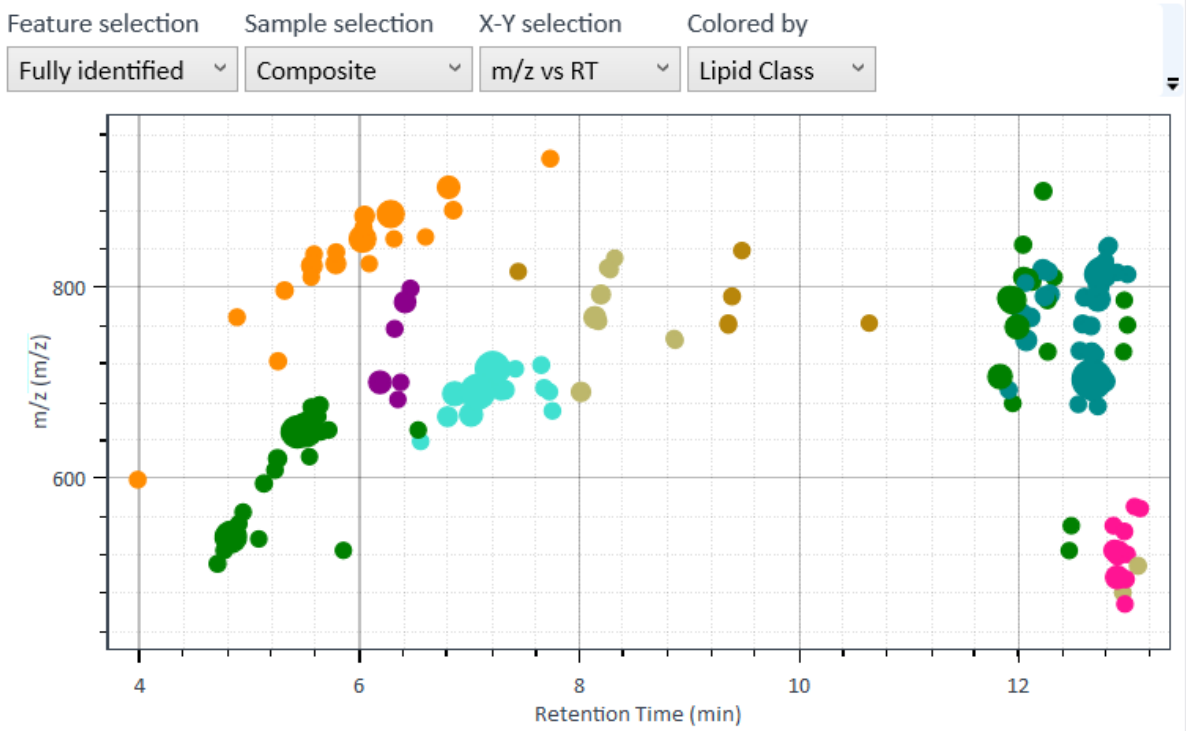
Internal Standard Response Across Samples with SFC



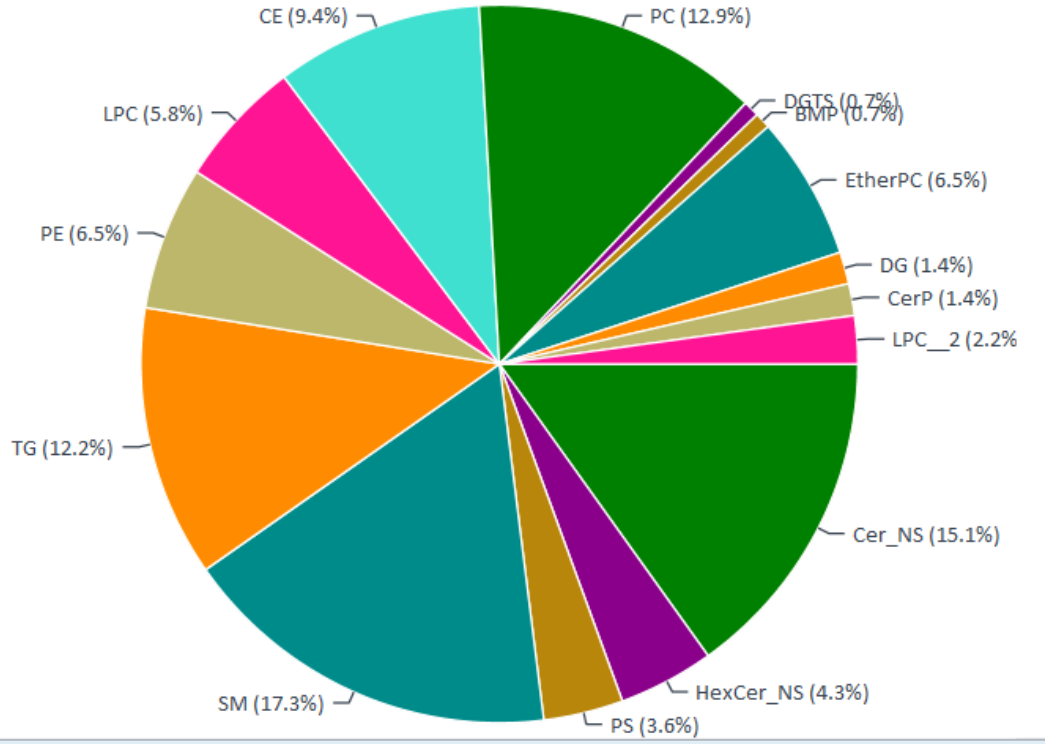


Lipid Features

Feature Plot



Lipid Classes



Feature Table

1004 Features

Lipid Matrix Plot in Mass Profiler Professional: PC-Ether in 2776 and 4T1 Cells

Case Study 2 – UIC
Melissa Pergande (Ph.D Thesis) and Stephanie Cologna

Studying lysosomal storage disorder

Comparing the liver extract of mutant and wildtype mice

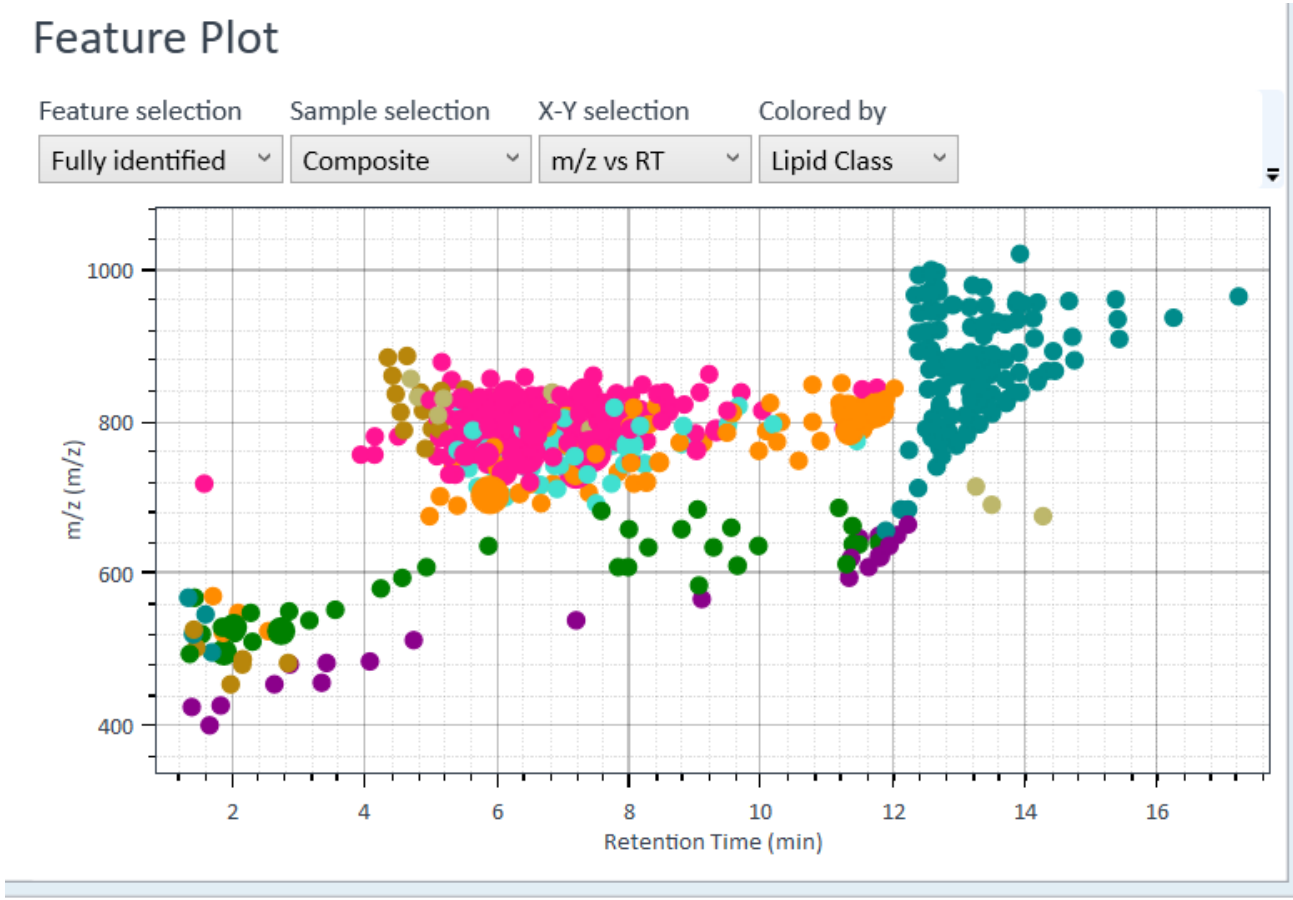
Reversed-phase LC Method

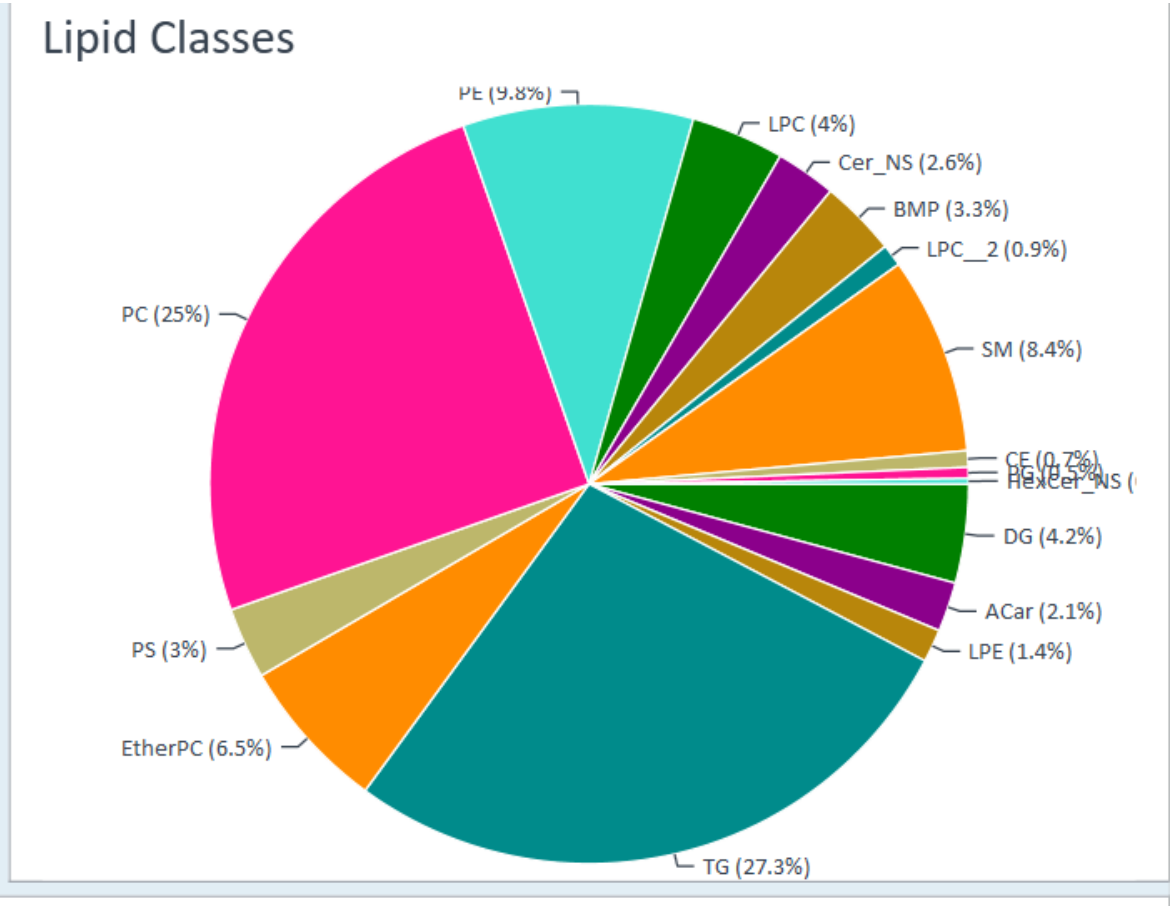
UHPLC: Agilent 1290 Infinity II System: HiSpeed pump, VialSampler, Multicolumn thermostat

LC Conditions	
Column	**Analytical column**: Agilent InfinityLab Poroshell 120 EC-C18, 3.0x100mm, 2.7µm, P/N 695975-302 **Guard**: Agilent InfinityLab Poroshell 120 EC-C18, 3.0x5mm, 2.7µm, P/N 823750-911 *Note: System passivation followed by column/guard/nebulizer phosphation can improve PA/PS peak shape* *Passivation and phosphation guidelines can be found in the current G6412-60004 Analysis Guide*
Column temperature	50 °C
Injection volume	No more than 5ul, can be dependent on injection solvent strength
Autosampler temp	4 °C
Needle wash	15 seconds in wash port (50:50 methanol/isopropanol)
Mobile phase	A = 10mM ammonium acetate, 0.5mM ammonium fluoride in 90:10 water/methanol B = 10mM ammonium acetate, 0.5mM ammonium fluoride in 2:3:5 acetonitrile/methanol/isopropanol
Flow rate	0.6 mL/min
Gradient program	Time / B (%) 0.00 / 70 1.00 / 70 3.50 / 86 10.00 / 86 11.00 / 100 17.00 / 100 17.10 / 70 19.00 / 70
Stop time	19 min
Post time	none
Observed column pressure	170-330 bar

Full LC and MS Method details to be included in upcoming App Note (2019)

Results with MassHunter Lipid Annotator

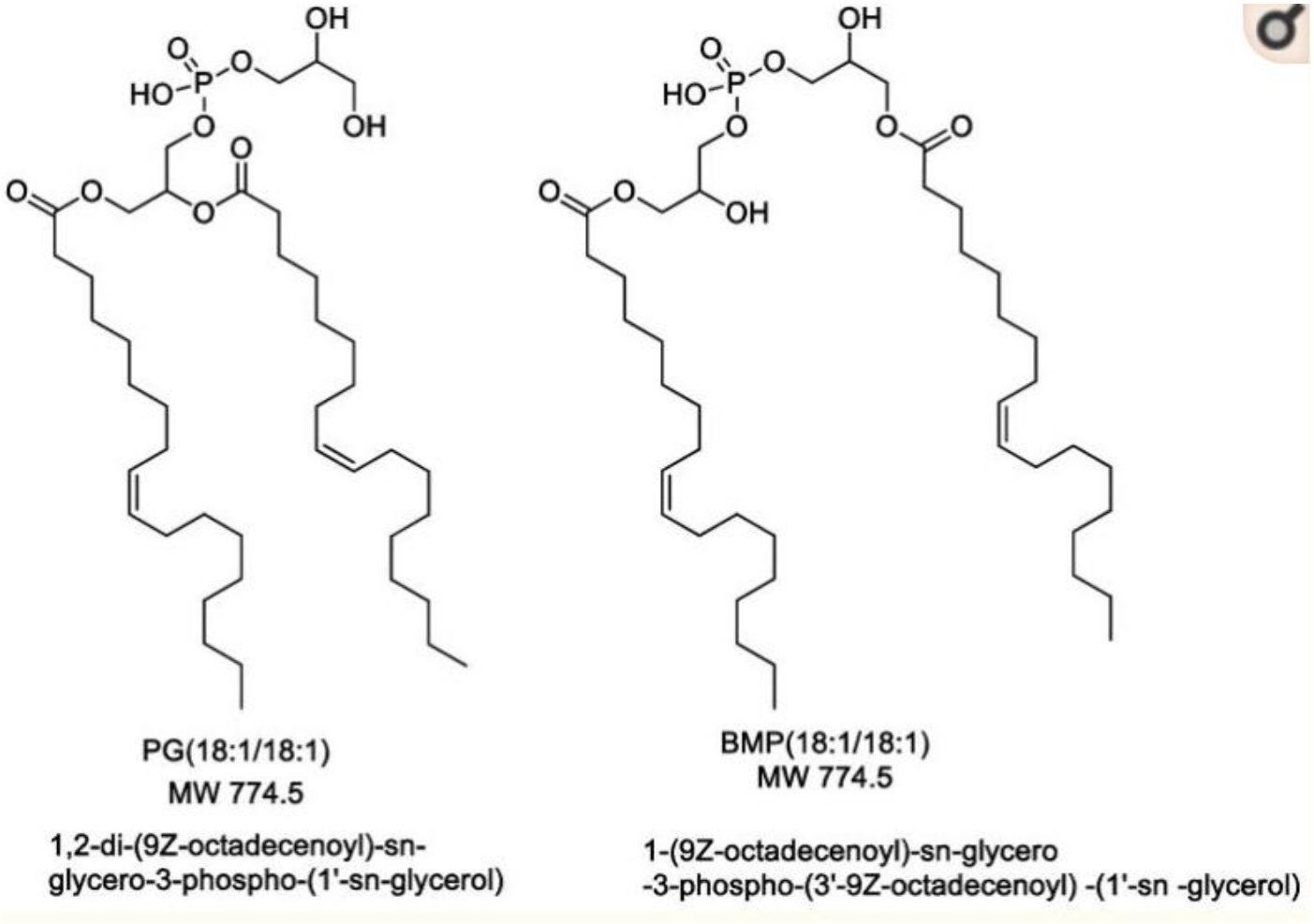




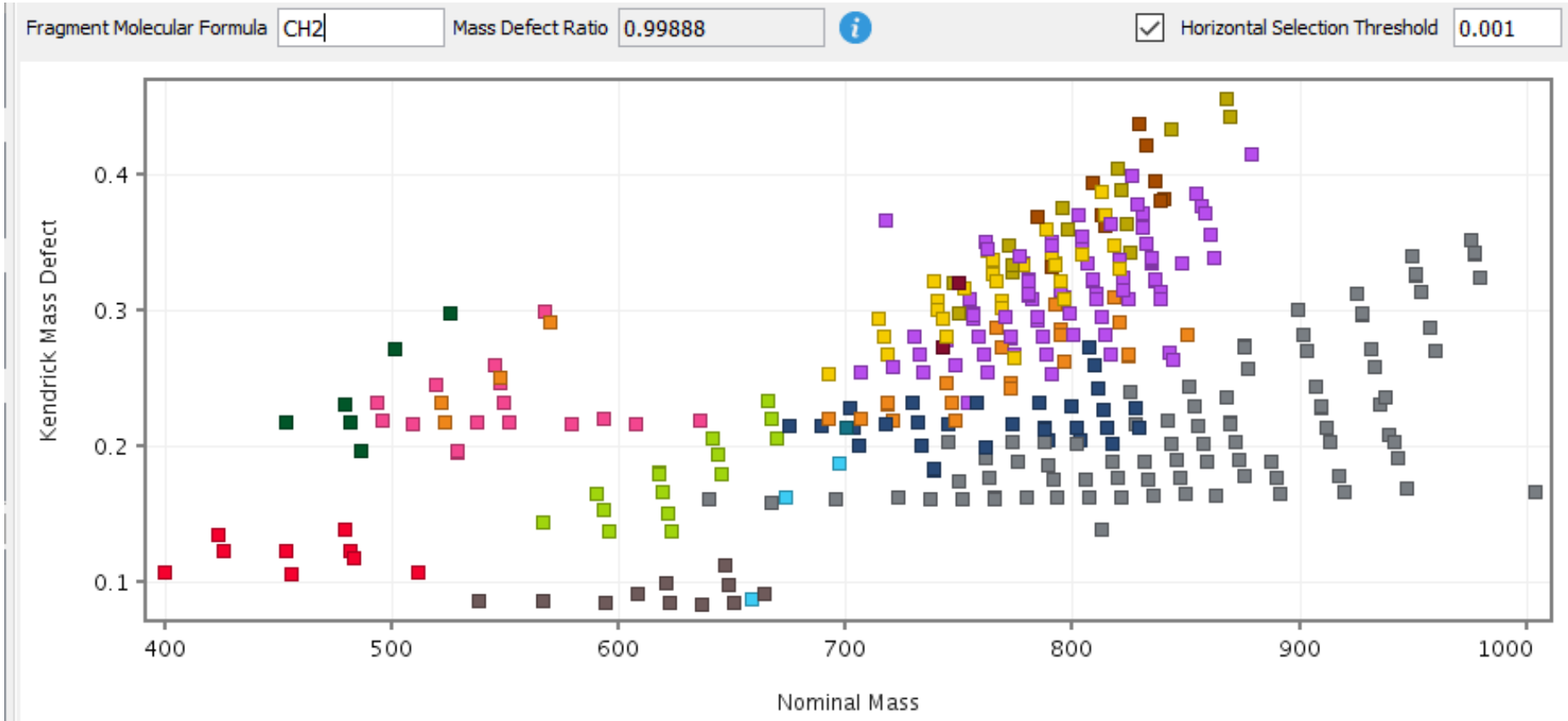
Increased Level of BMP in Mutant

BMP and PG

Phosphatidylglycerol and
Bis(monoacylglycerol)phosphate (BMP)



Kendrick Mass Defect Plot



Compound	Nominal ...	Kendri...	Nominal ...	Kendrick ...	Mass	Lipid Class	Compoun...	Retention...	Formula	Frequency
Cer_NS d...	636.0	0.08514	635.0	634.9149	635.6246	Cer_NS	Cer_NS d...	11.935	C41 H81 ...	6
Cer_NS d...	622.0	0.08539	621.0	620.9146	621.6087	Cer_NS	Cer_NS d...	11.794	C40 H79 ...	6
Cer_NS d...	650.0	0.08612	649.0	648.9139	649.6392	Cer_NS	Cer_NS d...	12.070	C42 H83 ...	6
Cer_NS d...	594.0	0.08618	593.0	592.9138	593.5766	Cer_NS	Cer_NS d...	11.323	C38 H75 ...	6
Cer_NS d...	538.0	0.08673	537.0	536.9133	537.5134	Cer_NS	Cer_NS d...	7.187	C34 H67 ...	6

Summary

We have discussed the Lipidomics Workflow from separation to differential analysis using the latest Agilent tools for lipidomics

Two case studies were presented

- Breast cancer metastasis and lipid changes
- Lysosomal lipid storage disorder