Untargeted Lipidomics

ASMS 2019

Dr. Sheher Banu Mohsin
Agilent Technologies
The Lipidome

CE  TG  SM  DG  PC  FA  PI  S1P  LPA  Acyl CoA

Non-Polar Lipids  Polar Lipids

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²Agilent Technologies Inc, Wood Dale, IL, USA
³Agilent Technologies Inc, Wilmington, DE, USA
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

- Cell (n=3) and tissue (n=3) lysis in 8M urea
- Protein concentration determined via BCA
- Spiked in 10uL of Avanti Splash Mix
- CHC$_3$:MeOH extraction(x3 replicates, 300µg protein equivalent each)
- Vortex for 1min and centrifuge @ 4990 rpm for 20min at 4C
- Separate into fractions
- Metabolites (top phase)
- Lipids (bottom phase)
- Proteins (pellet)
- Remove liquid and dry to ~100uL

MPLEx

- Vortex for 1min and centrifuge @ 4990 rpm for 20min at 4C
- Process with EMR plasma protocol
- Lipids (retained)
- Metabolites (wash)

Captiva EMR—Lipid

Still Some Challenges
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 30°C under nitrogen and stored at -80°C.

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

Dried at 30°C 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

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Lipid Analysis of HeLa Samples: Statistical Analysis

Captiva EMR—Lipid is more reproducible
Lipid Analysis of Cerebella Samples: Chromatography

MPELEX

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

1290 Infinity II UHPLC

Hi-DEF Q-TOF 6500 series

QQQ 6400 Series

TOF 6200 series

7000C GC/QQQ

7200B GC/Q-TOF

5977A GC/MS

Q-TOF 6500 series

1260 SFC

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SFC Fundamentals

<table>
<thead>
<tr>
<th>Substance</th>
<th>Density (g/cm³)</th>
<th>Viscosity (cP)</th>
<th>Diffusivity (cm²·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas</td>
<td>10⁻³</td>
<td>10⁻²</td>
<td>0.2</td>
</tr>
<tr>
<td>Supercritical Fluid</td>
<td>0.5</td>
<td>5x10⁻²</td>
<td>5x10⁻⁴</td>
</tr>
<tr>
<td>Liquid</td>
<td>1</td>
<td>1</td>
<td>10⁻⁵</td>
</tr>
</tbody>
</table>

- Supercritical CO₂ 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 (CO₂) 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?

Solute Families

- Normal Phase HPLC
- Reversed Phase HPLC
- Ion Pairing HPLC
- Ion Chromatography
- HILIC

- SFC w/ pure CO₂
- CO₂ w/ organic modifiers
- CO₂ w/ modifiers + additives + water
- CO₂ w/ modifiers + additives
Agilent SFC/Q-TOF System
New generation SFC/MS: a robust, routine, automated analytical tool
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 Mohsin¹, Alex Apffel¹, Kevin Williams²
¹Agilent Technologies, 5301 Stevens Creek Blvd, Santa Clara, CA, USA
²UCLA, 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
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HILIC-Z + EC-C18
RX-Sil + EC-C18

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Iterative MS/MS for Improved Coverage
Lipidomics Workflow
For Iterative and Auto MSMS

1. Build a database
   - Lipid Annotator
     - Annotate lipid based on MS/MS level information

2. Profile and Identify
   - DB
     - m/z & RT
     - MS/MS spectra (optional)
   - 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

MS/MS Data → MFE → Mass Profiler Professional

.cef file → SimLipid Annotation → Report/MetaboAnalyst

Filter and select significant features annotate, normalize

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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 Mohsin¹; Sanjib Meitei²; Peter Siegel³; Daina Avizonis³; Gaëlle Bridon¹
¹Agilent Technologies Inc, Wood Dale, IL; ²PREMIER Biosoft, Indore, Madhya Pradesh, India; ³Goodman Cancer Research Centre, McGill University, Montreal, Quebec, Canada

Studying lipids in breast cancer metastasis
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, Frédéric Carmont, Christine F. Tam, Anne-Marie Mest-Masson, and Peter M. Siegel
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

<table>
<thead>
<tr>
<th>Column</th>
<th>Analytical column: Agilent InfinityLab Poroshell 120 EC-C18, 3.0x100mm, 2.7µm, P/N 695975-302</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guard</td>
<td>Agilent InfinityLab Poroshell 120 EC-C18, 3.0x5mm, 2.7µm, P/N 823750-911</td>
</tr>
<tr>
<td>Note</td>
<td>System passivation followed by column/guard/nebulizer phosphation can improve PA/PS peak shape</td>
</tr>
<tr>
<td></td>
<td>Passivation and phosphation guidelines can be found in the current G6412-90004 Analysis Guide</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column temperature</th>
<th>50 ºC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume</td>
<td>No more than 5ul, can be dependent on injection solvent strength</td>
</tr>
<tr>
<td>Autosampler temp</td>
<td>4 ºC</td>
</tr>
<tr>
<td>Needle wash</td>
<td>15 seconds in wash port (50:50 methanol/isopropanol)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>A = 10mM ammonium acetate, 0.5mM ammonium fluoride in 90:10 water/methanol</td>
</tr>
<tr>
<td></td>
<td>B = 10mM ammonium acetate, 0.5mM ammonium fluoride in 2:3:5 acetonitrile/methanol/isopropanol</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.6 mL/min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>B (%)</th>
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<tbody>
<tr>
<td>0.00</td>
<td>70</td>
</tr>
<tr>
<td>1.00</td>
<td>70</td>
</tr>
<tr>
<td>3.50</td>
<td>86</td>
</tr>
<tr>
<td>10.00</td>
<td>86</td>
</tr>
<tr>
<td>11.00</td>
<td>100</td>
</tr>
<tr>
<td>17.00</td>
<td>100</td>
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<tr>
<td>17.10</td>
<td>70</td>
</tr>
<tr>
<td>19.00</td>
<td>70</td>
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</table>

<table>
<thead>
<tr>
<th>Stop time</th>
<th>19 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post time</td>
<td>none</td>
</tr>
<tr>
<td>Observed column pressure</td>
<td>170-330 bar</td>
</tr>
</tbody>
</table>

Full LC and MS Method details to be included in upcoming App Note (2019)
Results with MassHunter Lipid Annotator

Feature Plot

Lipid Classes

Feature selection: Fully identified
Sample selection: Composite
X-Y selection: m/z vs RT
Colored by: Lipid Class

- PC (25%)
- TG (27.3%)
- EtherPC (6.5%)
- LPE (1.4%)
- LPC_2 (0.9%)
- BMP (3.3%)
- SM (8.4%)
- Cer_NS (2.6%)
- FE (0.7%)
- PC (0.7%)
- PS (3%)
- ACar (2.1%)
- DG (4.2%)
- Hexanoyl_NS (1.3%)
- PE (4.7%)
Increased Level of BMP in Mutant
BMP and PG

Phosphatidylglycerol and Bis(monoacylglycerol)phosphate (BMP)

PG(18:1/18:1)
MW 774.5
1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phospho-(1’-sn-glycerol)

BMP(18:1/18:1)
MW 774.5
1-(9Z-octadecenoyl)-sn-glycero-3-phospho-(3’-9Z-octadecenoyl)-(1’-sn-glycerol)
Kendrick Mass Defect Plot
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