Non-targeted Lipidomic Analysis by Direct Infusion Mass Spectrometry

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Lipidome: A subset of Metabolome

https://en.wikipedia.org/wiki/Lipidomics
Eight Categories of Lipids

- **Fatty Acyls**
- **Glycerophospholipids**
- **Glycerolipids**
- **Sphingolipids**
- **Sterol lipids**
- **Prenol lipids**
- **Saccharolipids**
- **Polyketides**

_Fahy E. et. al., J. Lipid Res. 2005, 839-861_
# Mass Spectrometric Analysis of Lipids

<table>
<thead>
<tr>
<th>Targeted</th>
<th>Lipids of Interest</th>
<th>Mass Spectrometric Analysis</th>
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<tbody>
<tr>
<td></td>
<td>one or several specific lipid species</td>
<td>multiple reaction monitoring (MRM)</td>
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|          | one specific lipid class/subclass | • precursor ion scan  
|          |                                   |   • Neutral loss scan |
| Non-targeted | all lipid classes | • SWATH (Sequential Window Acquisition of all Theoretical fragment-ion spectra)  
|          |                                   |   • MS/MS (Identification) combined with high resolution MS (Quantification) |
Non-targeted Lipidomic Analysis

• Advantages
  • Comprehensive
  • Rapid
  • Big picture

• Challenges
  • Relatively low sensitivity
    • Full scan, neutral lipids, more severe interference peaks
  • Complexed data analysis
    • More severe interference peaks, multiple adduct forms, peak overlapping
Interference Peaks in Mass Spectrometric Analysis

• Contaminants from plastics (additive, polymer)
• Multiple adducts formation: H⁺, NH₄⁺, Na⁺, K⁺
• Non-covalent adduct formation
  • homo/hetero lipid dimers;
  • between lipid and impurity
• In-source dissociation
• Solvent degradation: CHCl₃ → HCl
• Carryover: previous runs, glassware, calibrants
Common Contaminants in Samples

- Plasticizers:
  - phthalates: esters of aliphatic dicarboxylic acids

- Polymers:
  - PEG: 44.03 Da
  - PPG: 58.04 Da
  - Silicone rubber: 74.02 Da

- Detergents:
  - Triton X-100: 44.03 Da

- Ingredients in cosmetics and hair conditioners:
  - Distearoyldimethylammonium chloride: $m/z$ 550.63
Contamination from Detergent

Detergent Triton

44.03 Da
Contamination from Silicone Rubber in LC/MS

74.02 Da
Approaches for Non-targeted Lipidomic Analysis

• Minimize interference from common contaminants
  • Do not use plastics
  • Rinse thoroughly
• Tuning instruments to maximize signal of lipids of interest
  • Electrospray probe position
  • Desolvation temperature
  • Flow rate
  • MS profile
  • Other parameters
    • heating gas, nebulization gas, cone voltage/decluster voltage
• Convert multiple adduct forms to one dominant form
Multiple adduct formation

![Image of mass spectra showing adduct formation with different additives: (a) ammonium acetate, (b) lithium chloride, and (c) sodium iodide. The spectra were recorded with a cone voltage of 15 V for (a) and 50 V for (b) and (c).]

Figure 2. Electrospray ionization mass spectra of 11 equimolar WE and CE standards (100 nM each, 1.1 μM total) using 100 μM of the following additives: (a) ammonium acetate, (b) lithium chloride, and (c) sodium iodide. The sample solution was in a mixture of chloroform and methanol (1:14, vol/vol). The flow rate was 40 μL/min, the desolvation temperature was 250°C, and the acquisition time was 1 minute. For clarity, only the peaks in (c) were labeled.

Non-targeted Mass Spectrometry Analysis of Lipids

Before optimization

- Bis(2-ethylhexyl) adipate
- Dibutyl phthalate

101 detergent

Analyte peak

After optimization

- 101 detergent
- Non-targeted Mass Spectrometry Analysis

+ 100 μM NaI
Cone 50 V

m/z
**MS/MS**^all^ Analysis of All Lipid Peaks in a Sample

- Direct infusion, flow injection, and lipid-class targeted LC techniques
- Fast Q1 precursor selection step-wise through mass range
- CID Fragmentation
- Collection of High resolution MS/MS

[Link to UAB Proteomics/Metabolomics Symposium](https://www.uab.edu/proteomics/metabolomics/symposium/symposium_dec2012.php)
SWATH: Full MS/MS Archive of Every Compound in a Sample

MS 250ms

TIC MS/MS all

Mass/Charge, Da

Intensity

% Intensity (of 6.7e4)

Precursor, Da

Intensities

Mass/Charge, Da

Intensities
Pseudo Precursor Ion Scanning
Extracted from MS/MS$^\text{all}$ Analysis
Pseudo Precursor Ion Scanning
Extracted from MS/MS<sup>all</sup> Analysis
Summary

• Minimizing contaminants/interference peaks is particularly important for non-targeted lipidomic analysis.

• It is important to confirm the identity of lipids by MS/MS before quantifying lipids by MS.

• SWATH appears to be a promising method for non-targeted lipidomic analysis.
References on Contaminants


4. [http://www.abrf.org/index.cfm/list.msg/66994](http://www.abrf.org/index.cfm/list.msg/66994)

Useful websites for lipid analysis


