Separating Lipid Isomers with LC-IMS-MS Measurements to Understand Their Role in Biochemical Processes

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Why use IMS in lipidomic studies?

- Lipids are composed of the same simple double bond and fatty acid components, resulting in numerous isomers.
- IMS is a very fast separation technique which provides structural information.
- In conjunction with LC, IMS-MS provides the capability to resolve many more isomers and reduce false discovery rates.

Pulse of 2 ions with same m/z but different shape

Different conformers separate in time with peak heights representing the amount of each
Importance of fatty acids

Fatty acids are key components of lipids, giving them their hydrophobic properties

Fatty acid analysis with IMS

16:1

cis 9

Normalized Intensity

Drift Time (ms)
Fatty acid analysis with IMS

Lowest energy structures from AMBER molecular dynamics simulations
Both LC and IMS are able to separate different lipids types, but each gave distinct separations


Phospholipid separations with IMS

IMS was able to further separate the lipid subgroups

Compactness
PE < PE(P) = PS = PG < PC

cis/trans double bond analysis

PE(18:1/18:1) (cis-Δ⁹)

PE(18:1/18:1) (trans-Δ⁹)

PE(18:0/18:0)
sn-1/sn-2 positional analysis

Size Comparison (Relative cross sections)

PC(14:0/16:0) < PC(16:0/14:0)

PC(16:0/18:0) < PC(18:0/16:0)

PC(18:1/16:0) < PC(16:0/18:1)
Biological sample complexity

90-min LC run for mouse tissue sample (>400 identifications)

Peaks shown for 1-s acquisition from m/z range 682-882
Biological sample complexity

90-min LC run for mouse tissue sample (>400 identifications)

2 Co-eluting Lipids (0.036 Da difference)
- PC(P-18:0/20:4)
- PE(18:1/20:4)

3 Co-eluting Isomers (same mass)
- PE(P-16:1/18:0)
- PE(P-18:0/16:1)
- PE(P-16:0/18:1)
- PE(P-18:1/16:0)

Possibilities
- PE(P-16:1/18:0)
- PE(P-18:0/16:1)
- PE(P-16:0/18:1)
- PE(P-18:1/16:0)
LysoPCs as disease biomarkers

Different lysoPCs have been linked to diseases so we started looking at lysoPC16:0 in different samples.
LC isomer analyses in biological samples

PC16:0 in Human Embryonic Epithelial Kidney Cell Line
m/z = 496.3403

PC16:1 in Human Embryonic Epithelial Kidney Cell Line
m/z = 494.3246

Cis forms are dominant, trans forms are <10% & immediately follow in elution time (denoted with ⚫)

PC16:1 in Huh7.5.1 Human Hepatoma Cell Line

Collaboration with Steve Polyak at University of Washington on silymarin-treated Huh 7.5.1 cells
LC-IMS-MS isomer analyses in biological samples

PC16:0 in Human Embryonic Epithelial Kidney Cell Line
m/z = 496.3403

PC16:1 in Huh7.5.1 Human Hepatoma Cell Line
m/z = 494.3246
1. Observed separation of sn-1 and sn-2 lysoPCs by LC and IMS

2. Two different double bond positions (P1 and P2) in the Hepatoma Cell Line

3. From IMS size, we hypothesize P1 is in the middle and P2 is near the head group (since it is larger)

4. Cis forms are dominant, trans forms are <10% and immediately follow in elution time (denoted with •)
LC-IMS-MS isomer analyses in biological samples

PC16:0 in Human Embryonic Epithelial Kidney Cell Line
m/z = 496.3403

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PC16:1 in Huh7.5.1 Human Hepatoma Cell Line 24-hr Treatment
Good correlation with phospholipids observed in MALDI imaging
MALDI imaging only identified ~30 lipids, LC-IMS-MS identified ~400 lipids
IMS lipid isomer analysis
Summary

LC-IMS-MS:

• Separates fatty acid and complex lipid isomers

• Use of 3-dimensional LC-IMS-MS information increases the confidence of lipid identifications

• Allows quantification of potentially important isomeric changes in disease states
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