Distinguishing Lipid Isomers with Advanced Separations, Ion-Molecule Reactions and Fragmentation Approaches to Evaluate Their Role in Biochemical Processes

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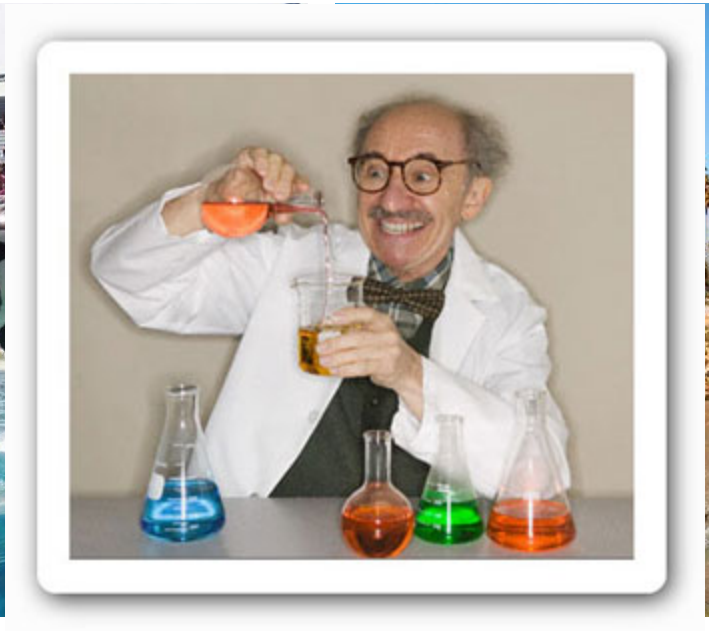
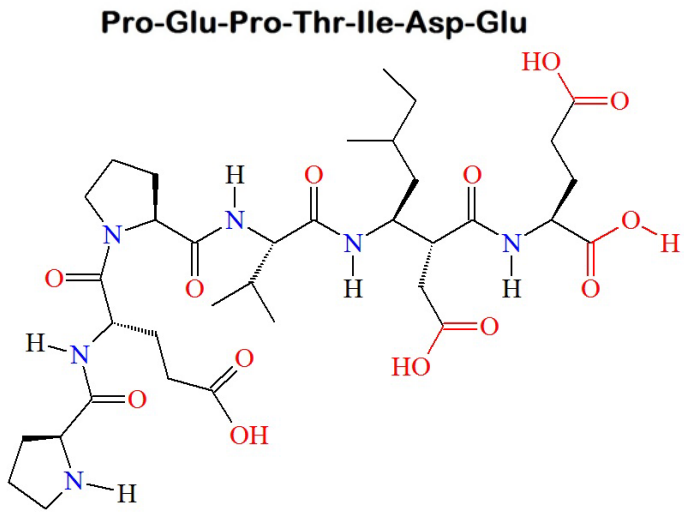
Thank you for coming to the Thursday afternoon orals!!

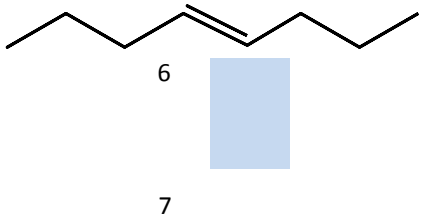


Lipidomic isomers

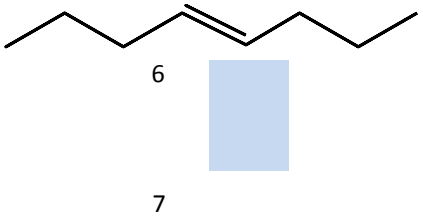
- Unlike the diverse amino acids in proteins, lipid species often differ by changes in similar components



Peptide



18:0(2S-OH) Ceramide



18:0(2R-OH) Ceramide

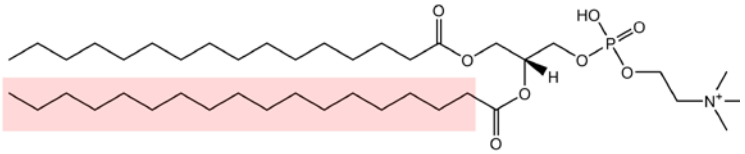
Lipids

Lipidomic isomers

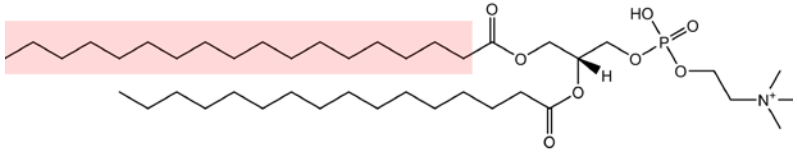
- Fatty acid and double bond positions and orientations results in numerous lipid isomers with functional differences

C16:1, cis 9

C16:1, cis 6



PC(16:0/18:0)



PC(18:0/16:0)

- Advanced separations, ion-molecule reactions and fragmentation approaches are needed to aid in distinguishing the isomers

Methods

- Liquid Chromatography (LC)
- Supercritical Fluid Chromatography (SFC)
- Ion Mobility Spectrometry (IMS)
- Ozone Induced Dissociation (OzID)
- Collision Induced Dissociation (CID)
- Paternò–Büchi (P-B) Reaction
- Higher-Energy Collisional Dissociation (HCD)
- Ultraviolet Photodissocation (UVPD)
- Electron Impact Excitation of Ions from Organics (EIEIO)
- Surface Induced Dissociation (SID)

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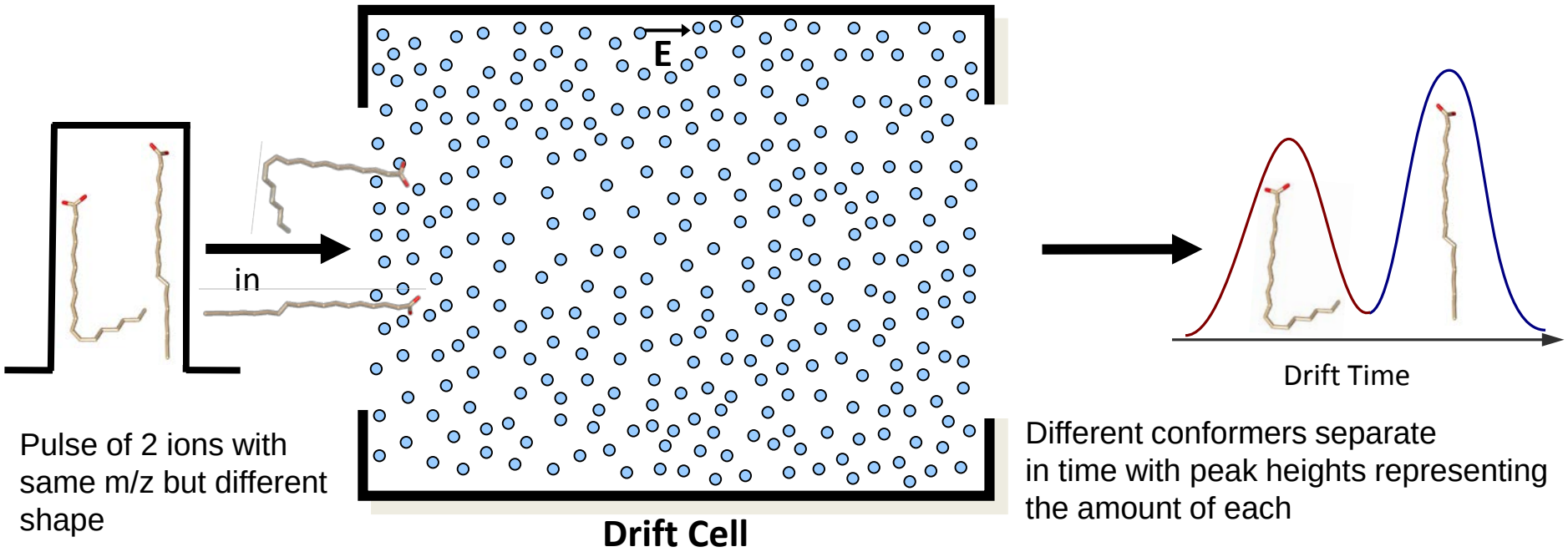
Why use IMS in lipidomic studies?

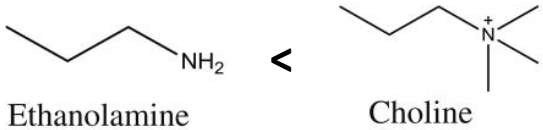
- IMS is a very fast separation technique which provides structural information
- In conjunction with LC, IMS-MS provides the ability resolve many isomers



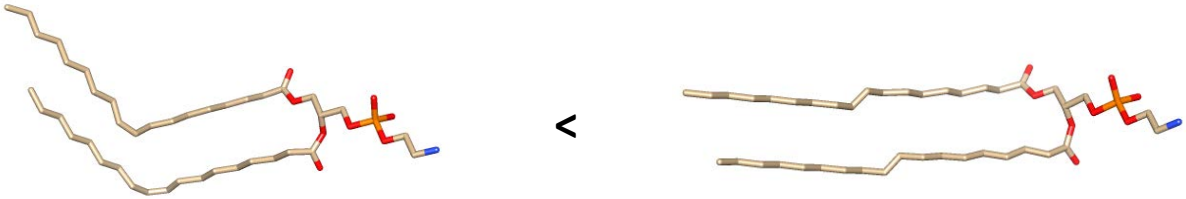
Lipid Isomers

1. Class isomers (PC and PE) and stereoisomers (R versus S)

PE 36:2 < PC 33:2 but has the same elemental composition, $C_{41}H_{78}NO_8P$



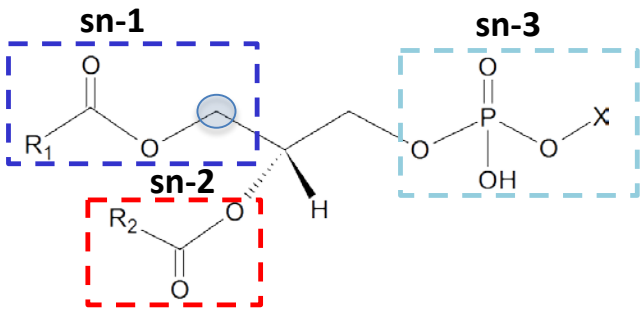
2. cis/trans double bond orientations



PE(18:1/18:1) (cis-Δ^9) **PE(18:1/18:1) (trans-Δ^9)**

3. sn-1/sn-2/sn-3 fatty acyl positions

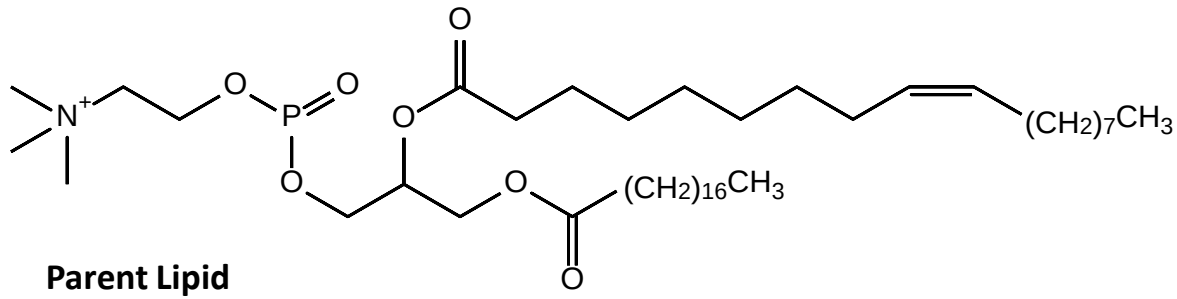
PC(14:0/<u>16:0</u>) < PC(<u>16:0</u>/14:0)

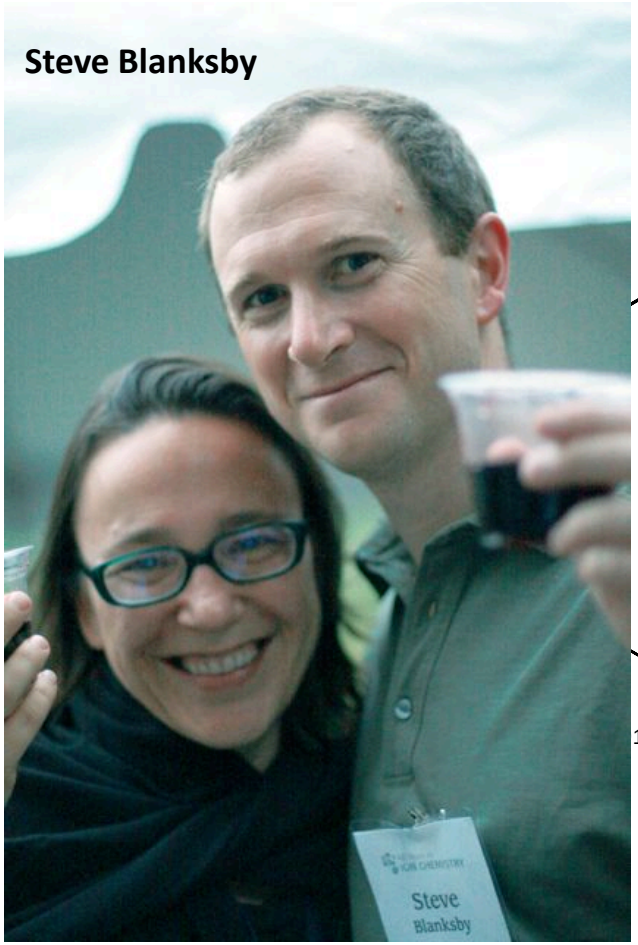


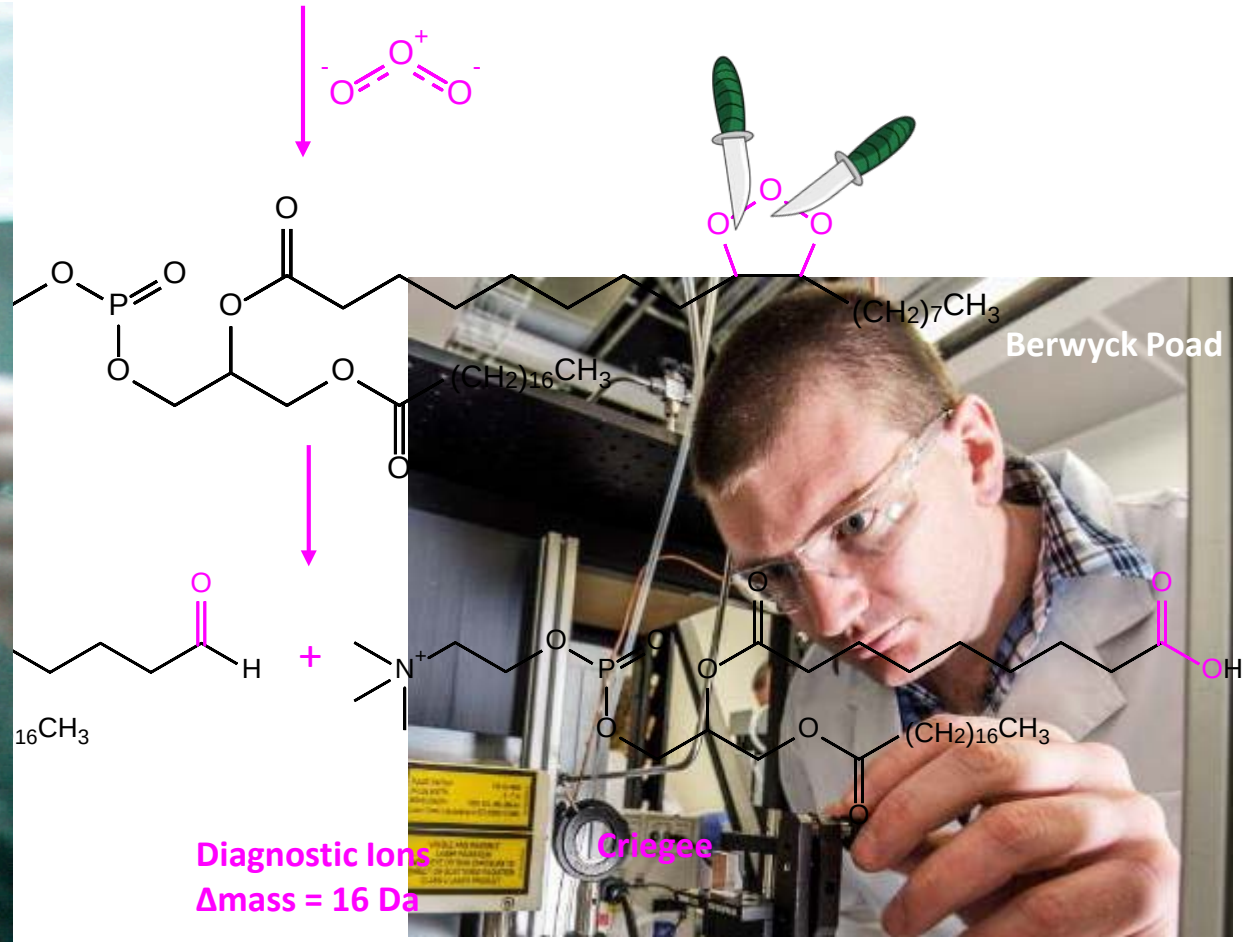
4. double bond locations

cis-Δ9 **cis-Δ6**

Ozonolysis or OzID

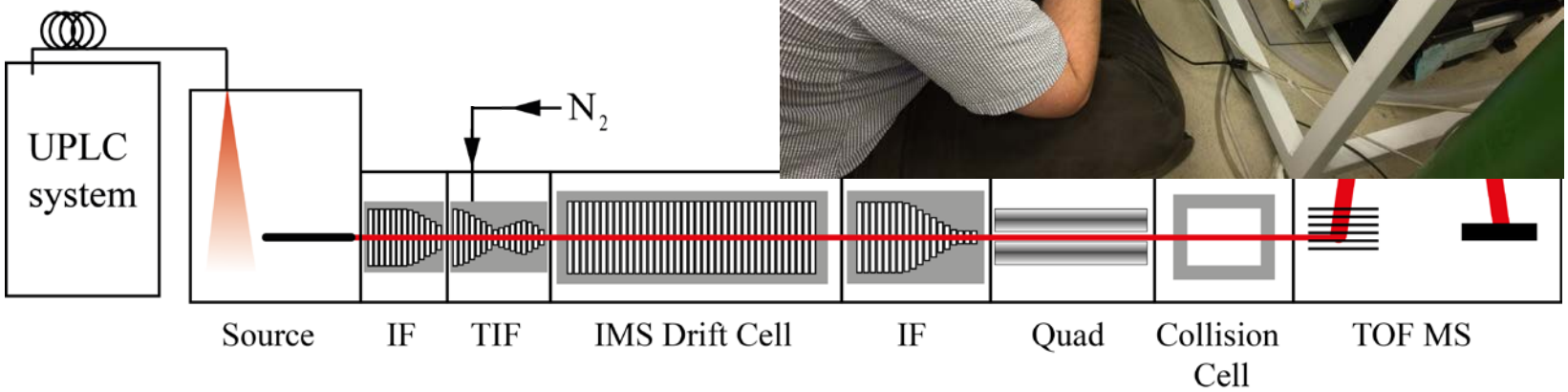




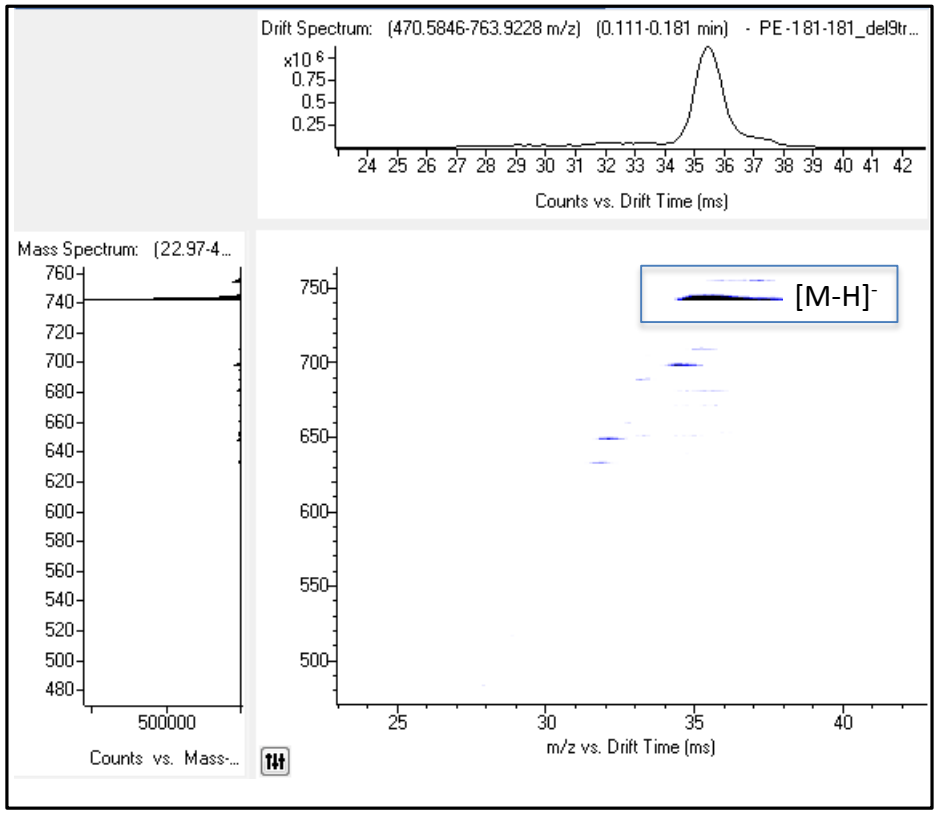


Ozonolysis on IMS instrument

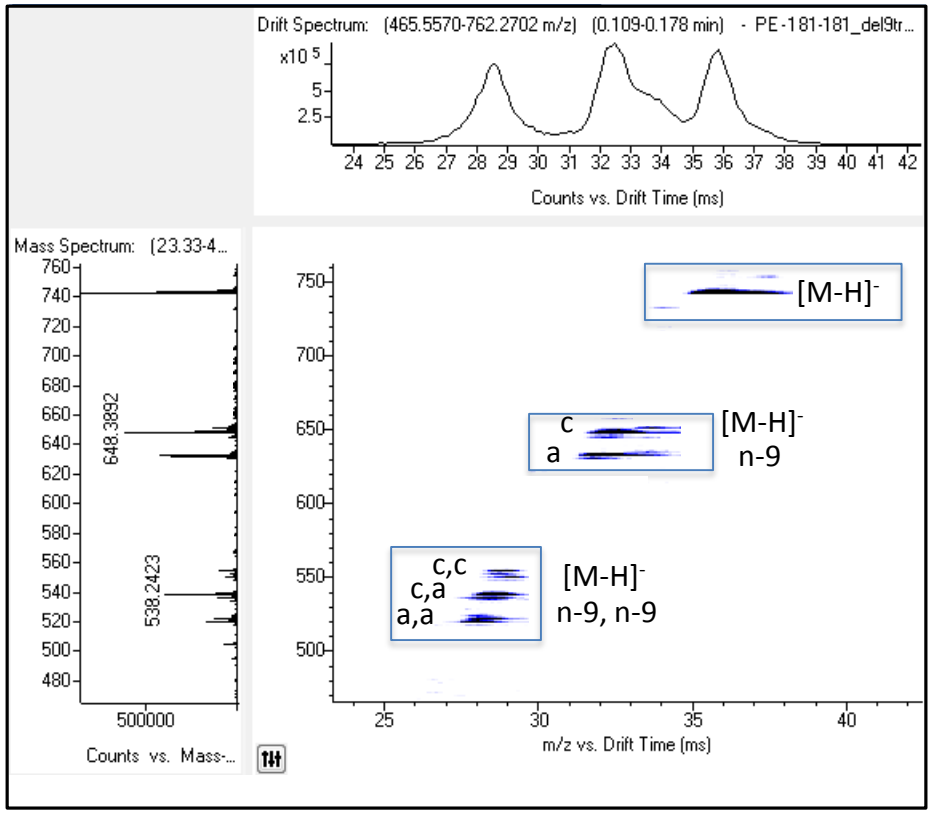
Berwyck Poad



ESI-OzID-IMS-MS of [PE(18:1/18:1)-H]$^-$



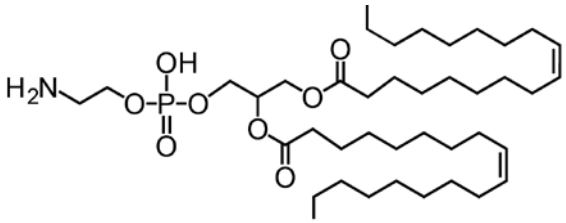
No Ozone



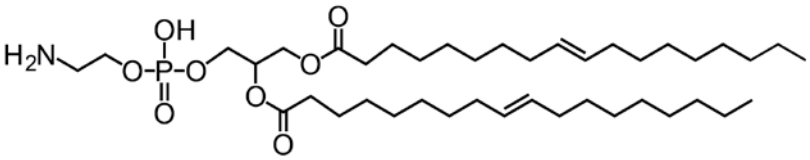
Ozone

Both double bonds trans-Δ9

ESI-OzID-IMS-MS of PE(18:1/18:1)

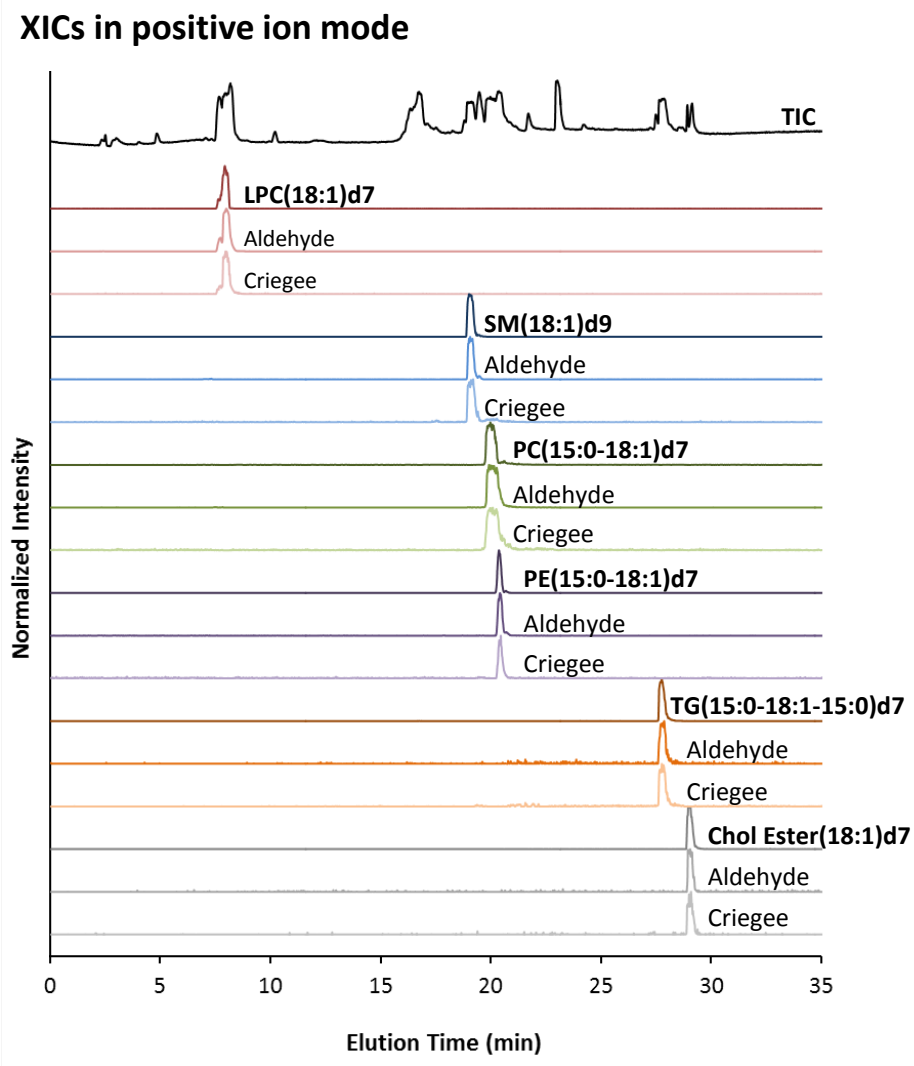


cis, cis

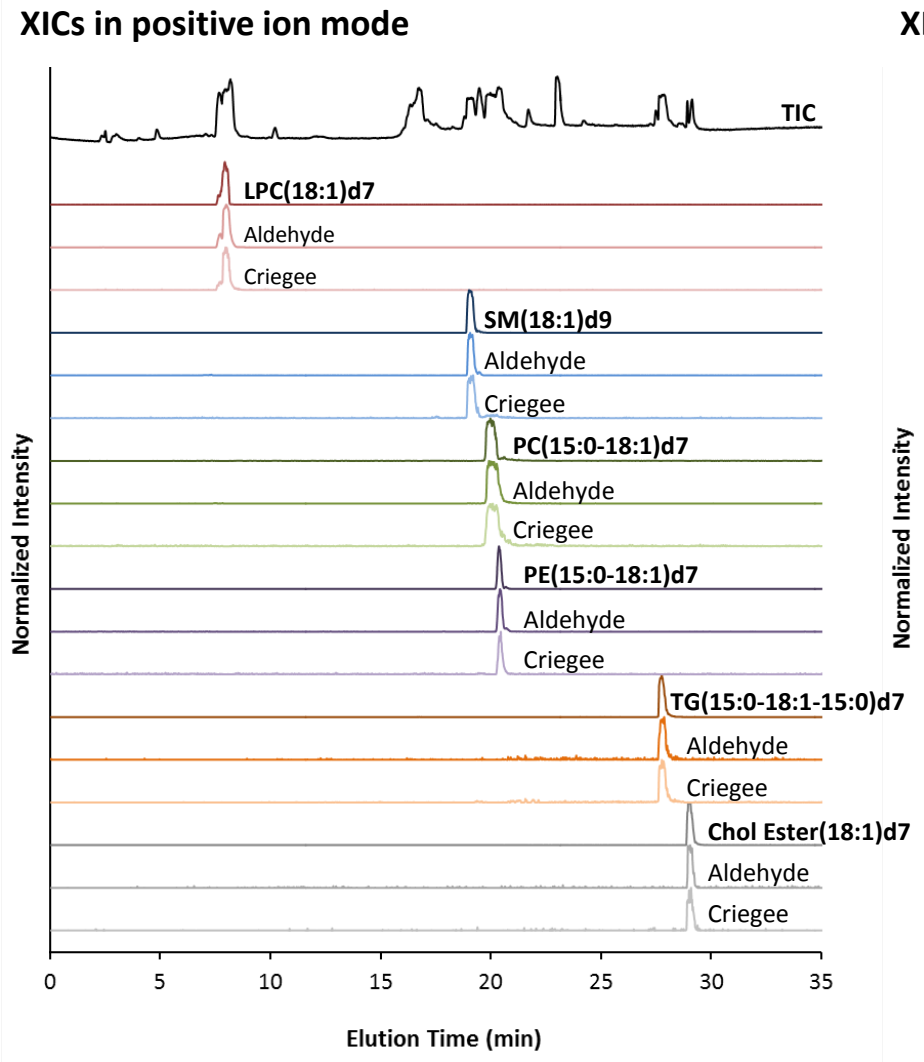


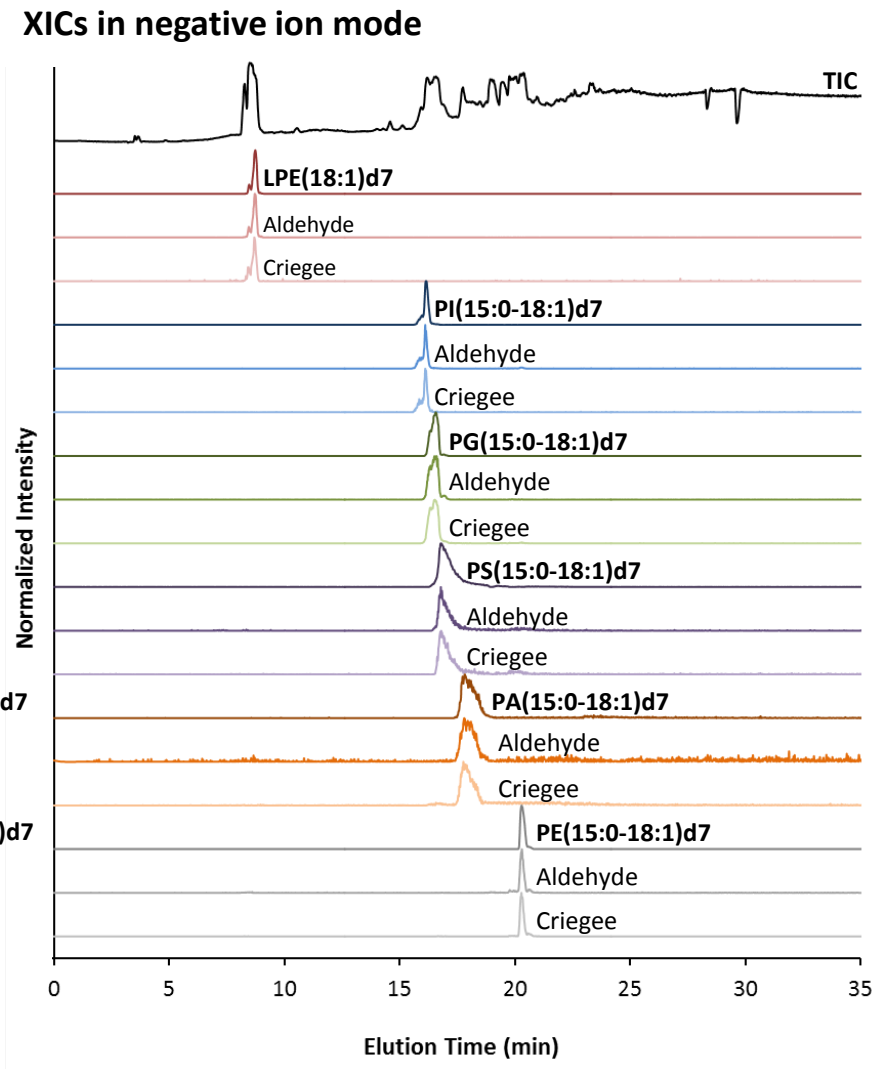
trans, trans

LC-OzID-IMS-MS of Splash mix

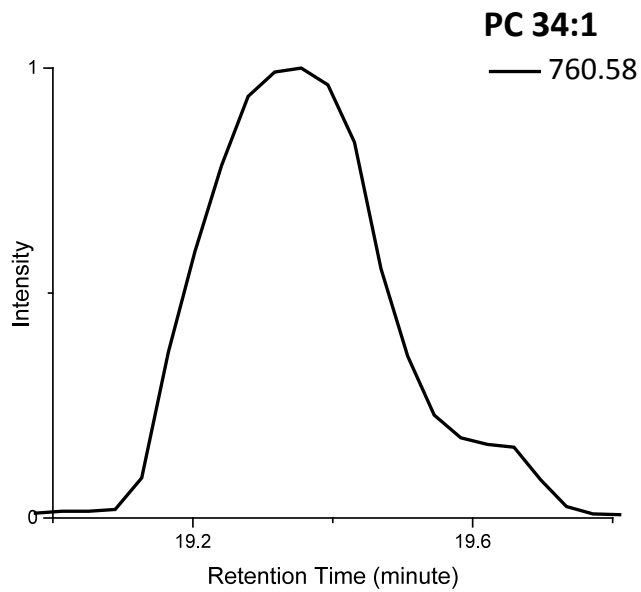


LC-OzID-IMS-MS of Splash mix





LC-OzID-IMS-CID-MS in human plasma



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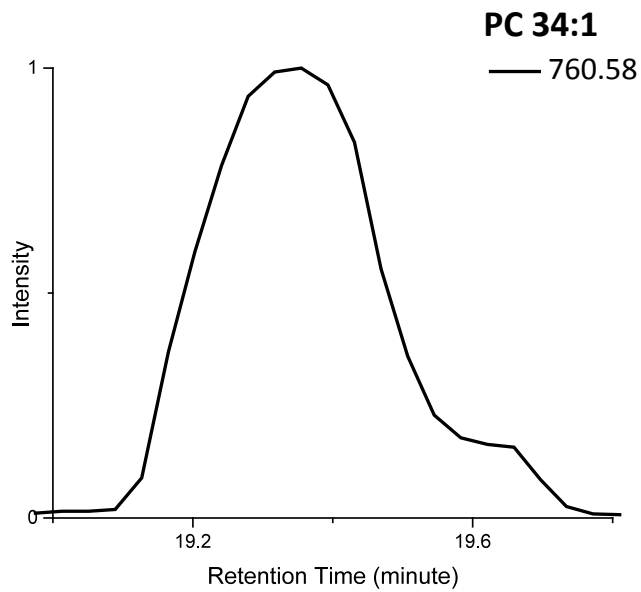

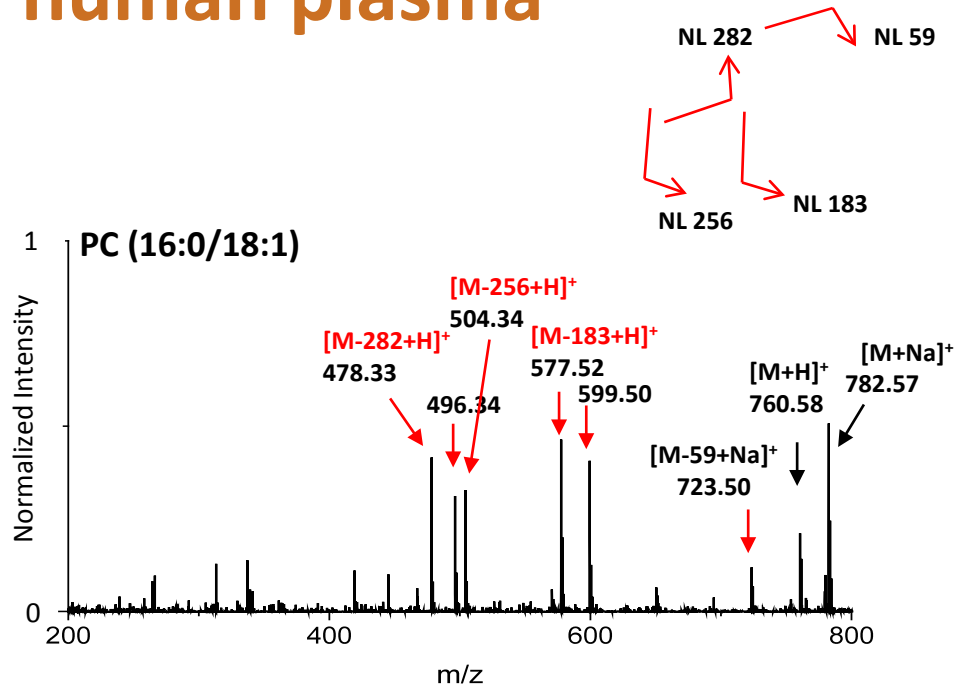

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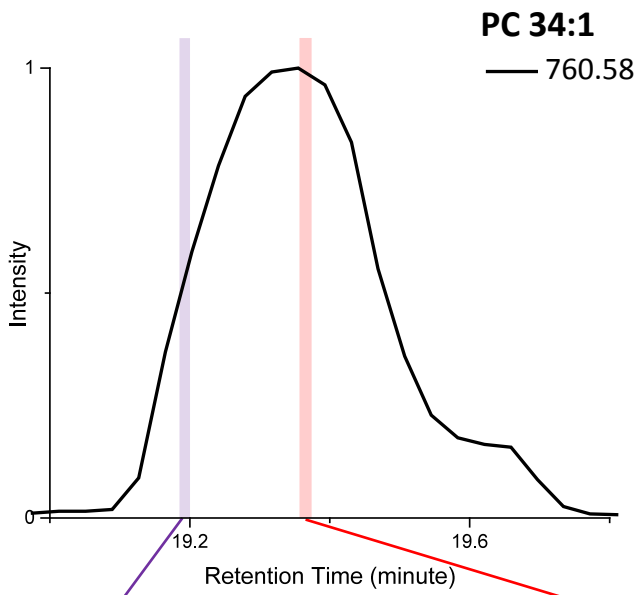
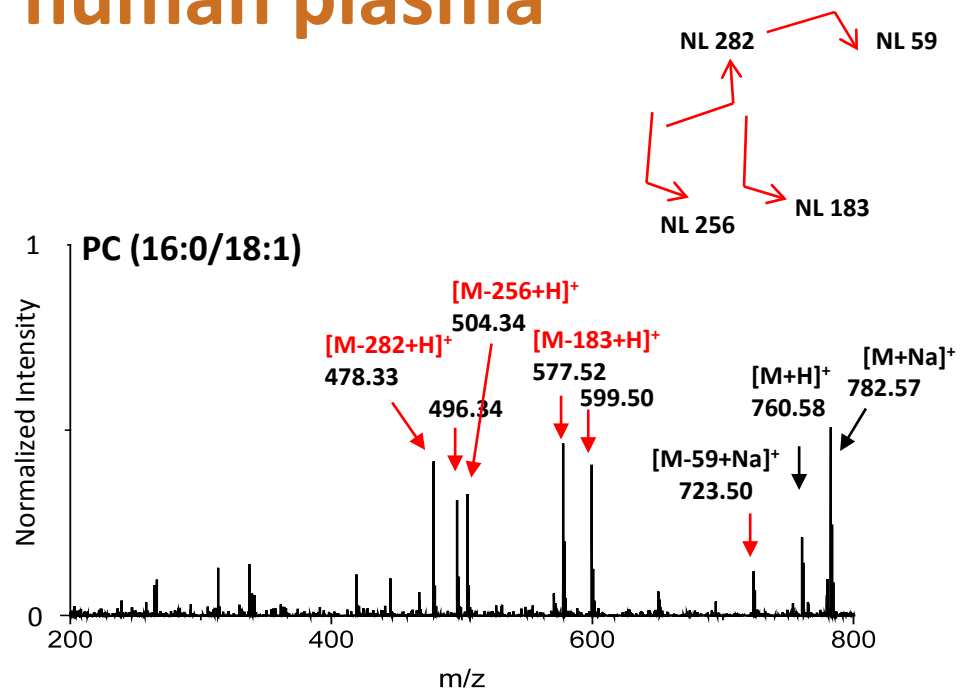
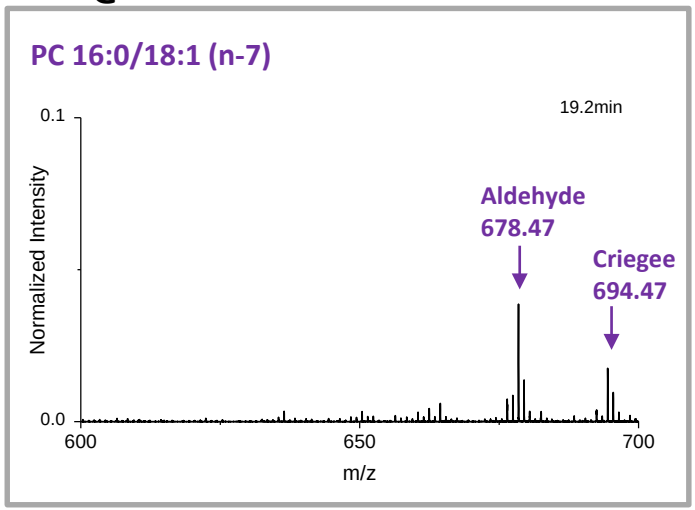
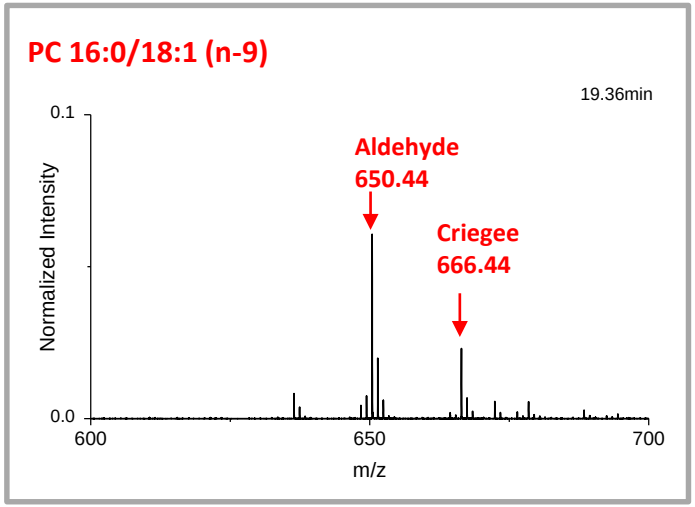




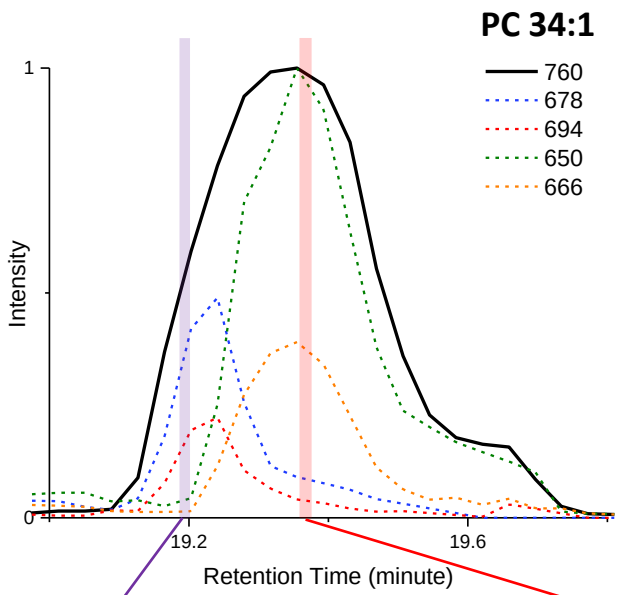
OzID @ RT 19.2 min

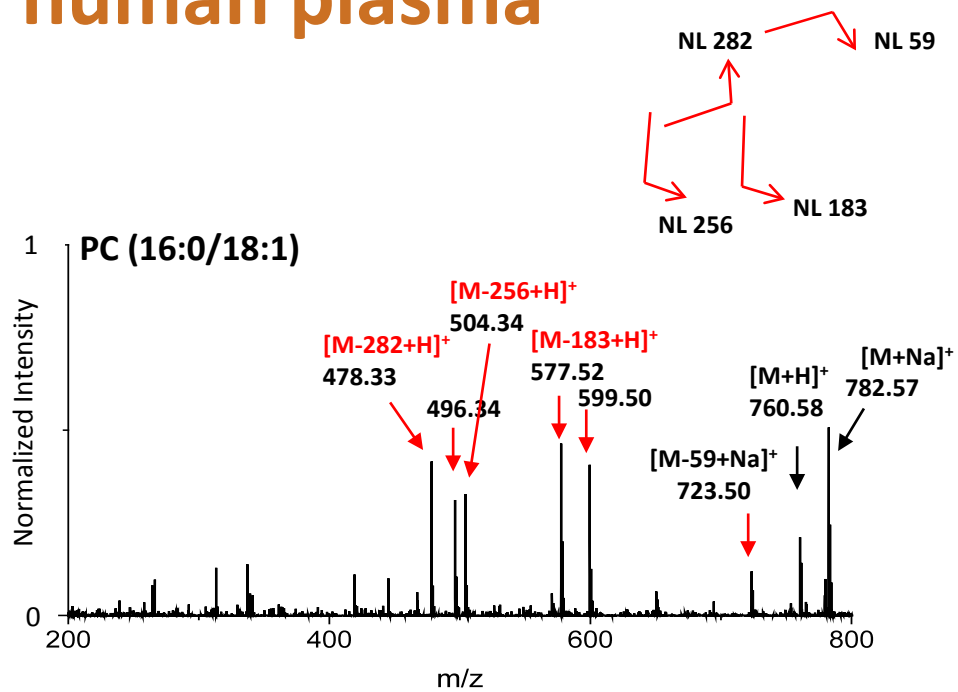


OzID @ RT 19.36 min

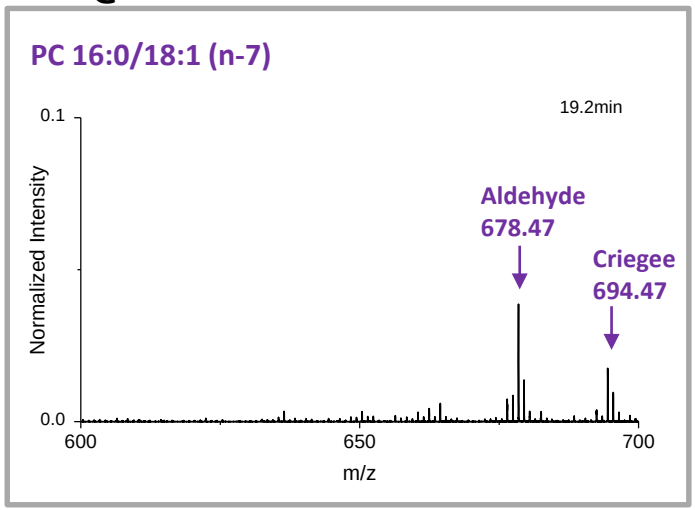


LC-OzID-IMS-CID-MS in human plasma

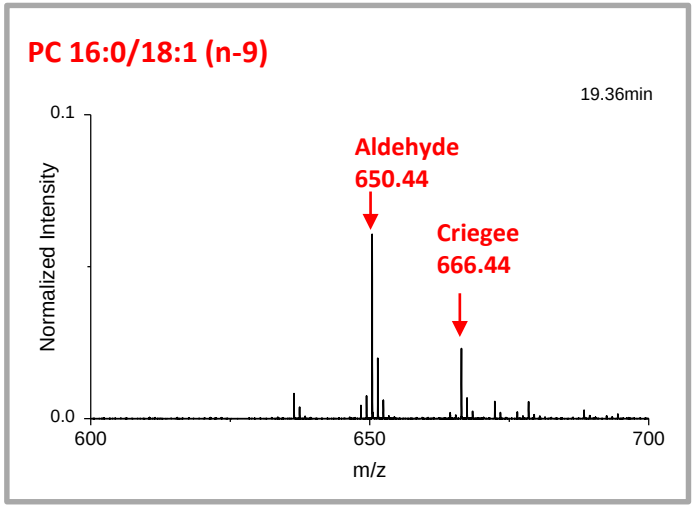




OzID @ RT 19.2 min



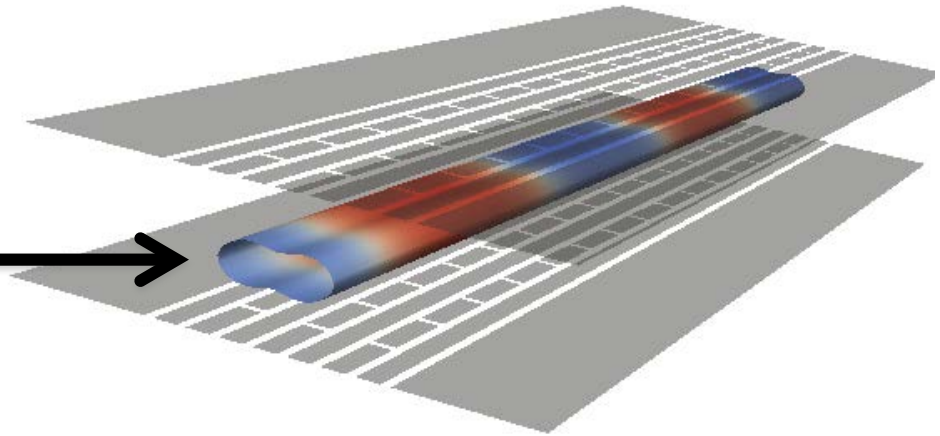
OzID @ RT 19.36 min

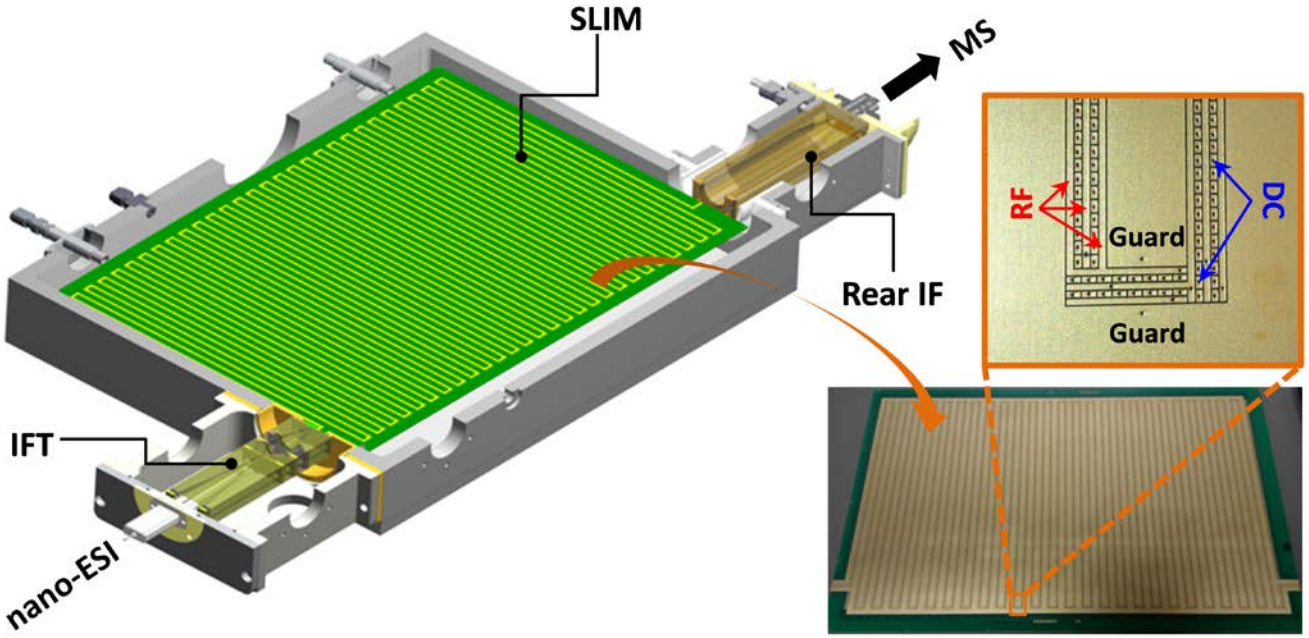


Other isomer separation methods

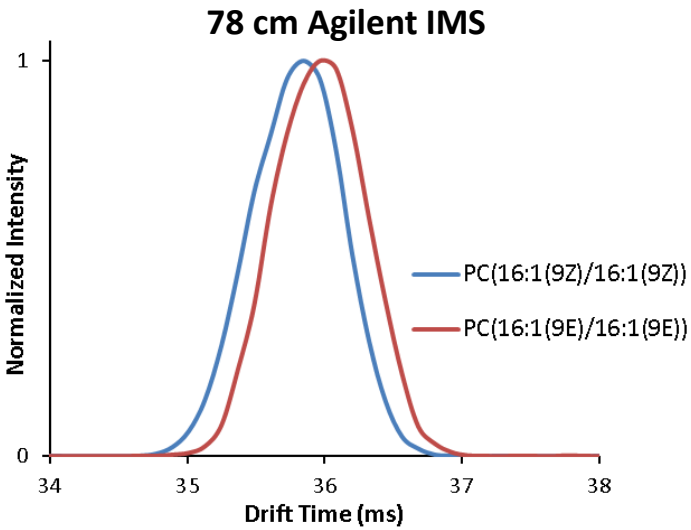
Structures for lossless ion manipulations

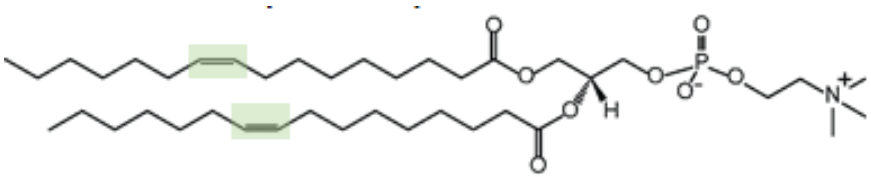
Ion confinement



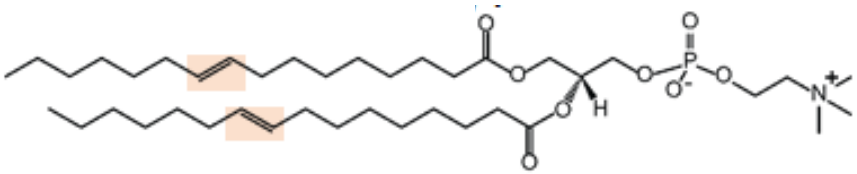


cis/trans double bond analysis

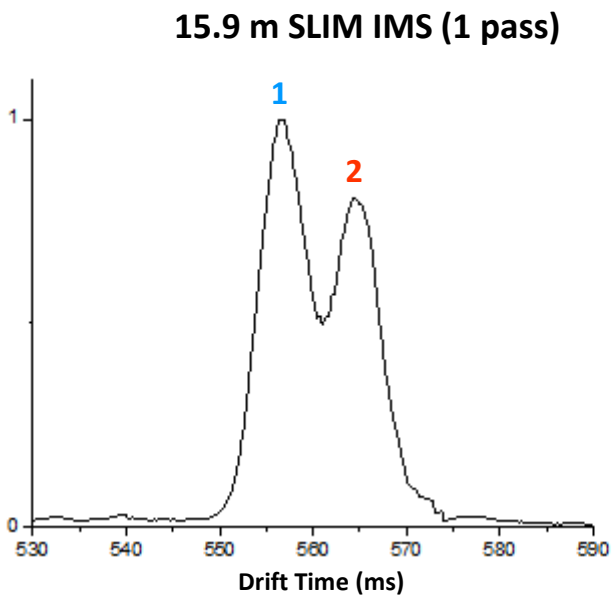


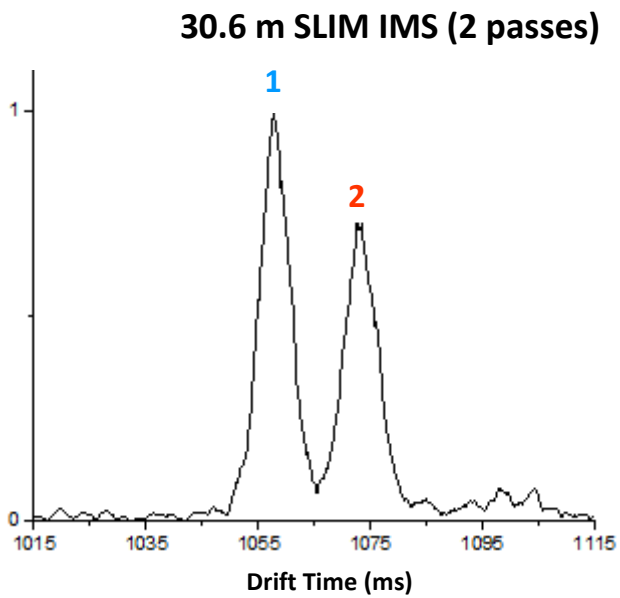


1 = PC(16:1(9Z)/16:1(9Z))



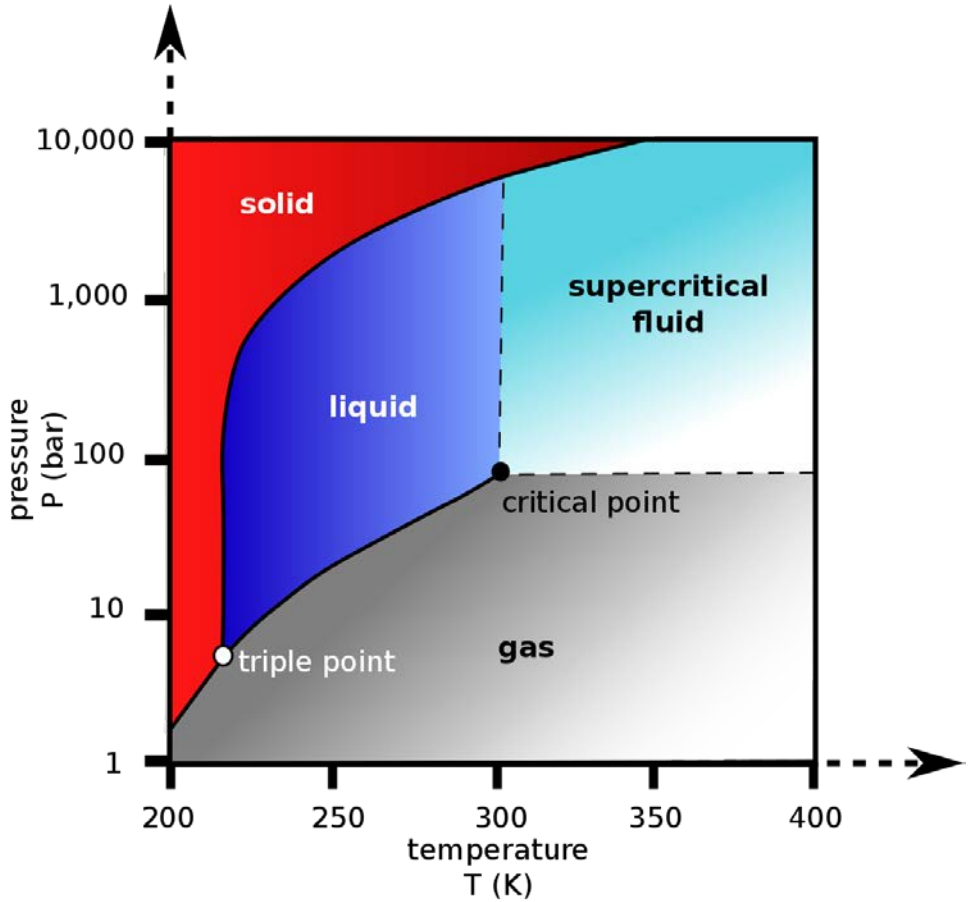
2 = PC(16:1(9E)/16:1(9E))





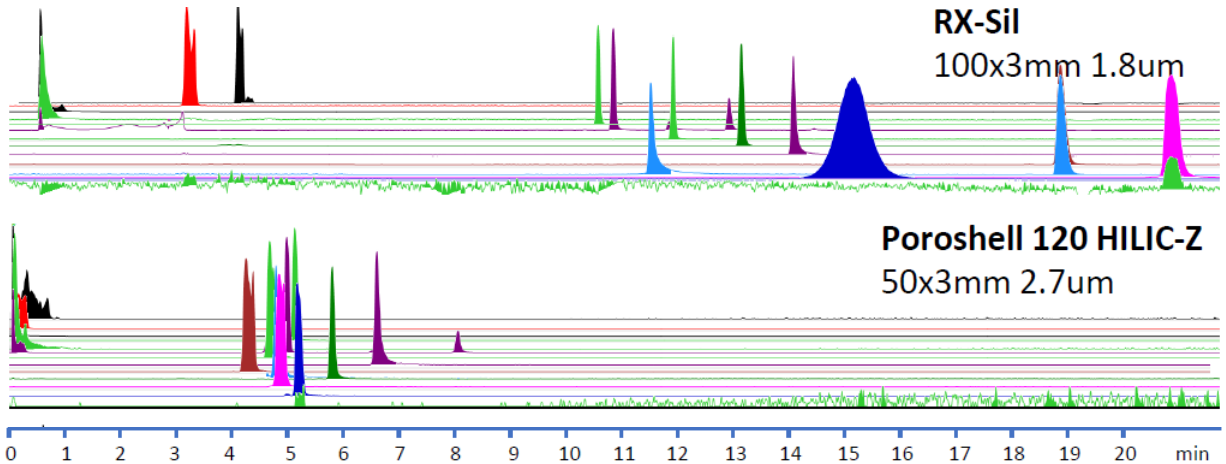
SFC isomer separation

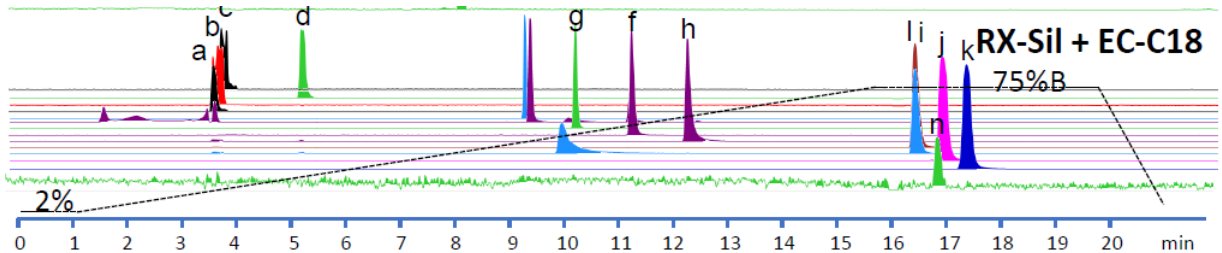
- SFC uses supercritical CO_2 as a mobile phase
  - Viscosity similar to a gas, density and solubilizing power of liquids, and intermediate diffusivity between gases and liquids
  - Green



SFC lipid separation

- Column selection key variable
- No single column offers both adequate selectivity and resolution across all classes of lipids
- Combination of a “normal phase” (Silica) and “reversed phase” (C18) column yielded best results

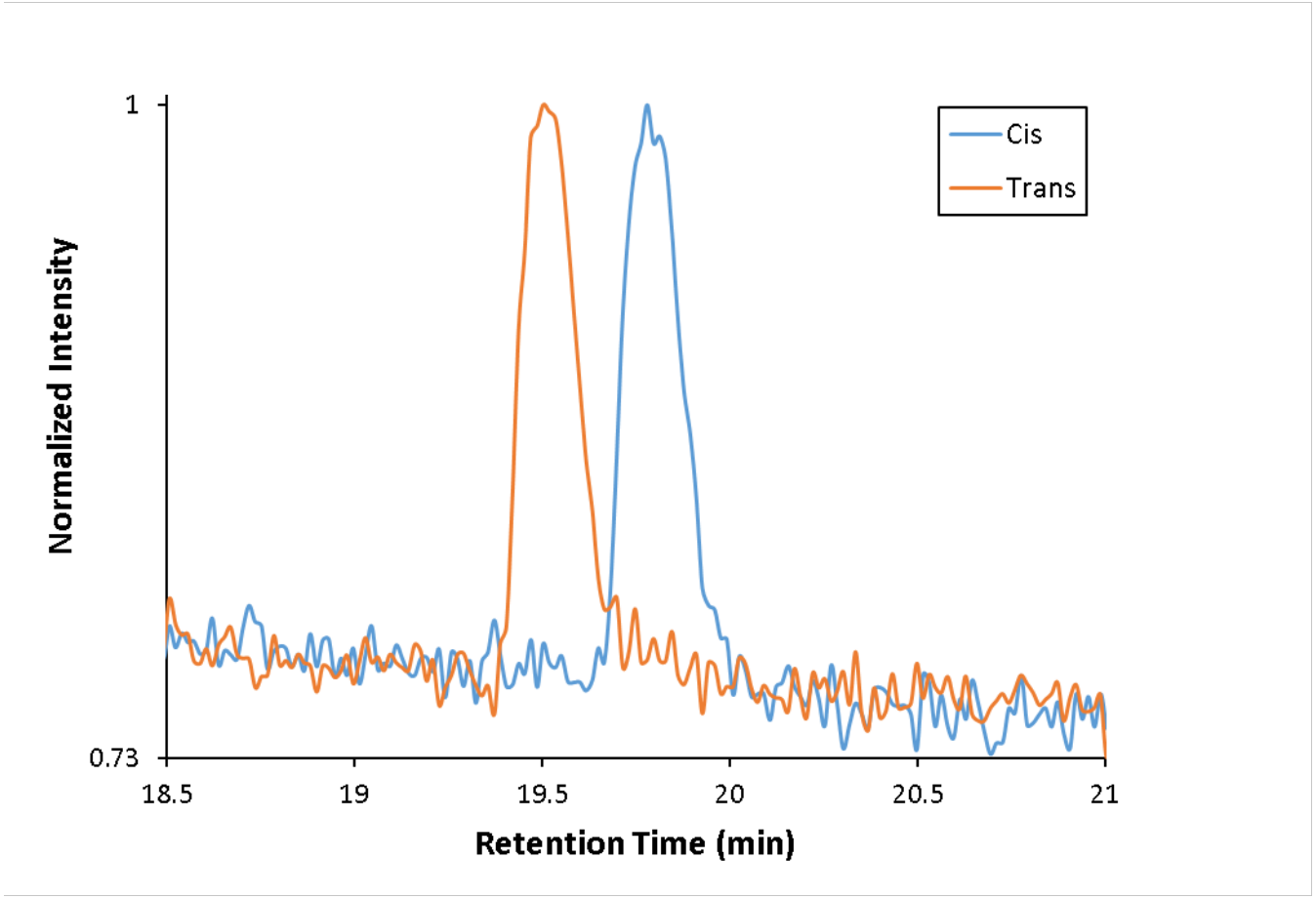




Work by Sheher Mohsin, Alex Apffel, and Kevin Williams
Agilent and UCLA

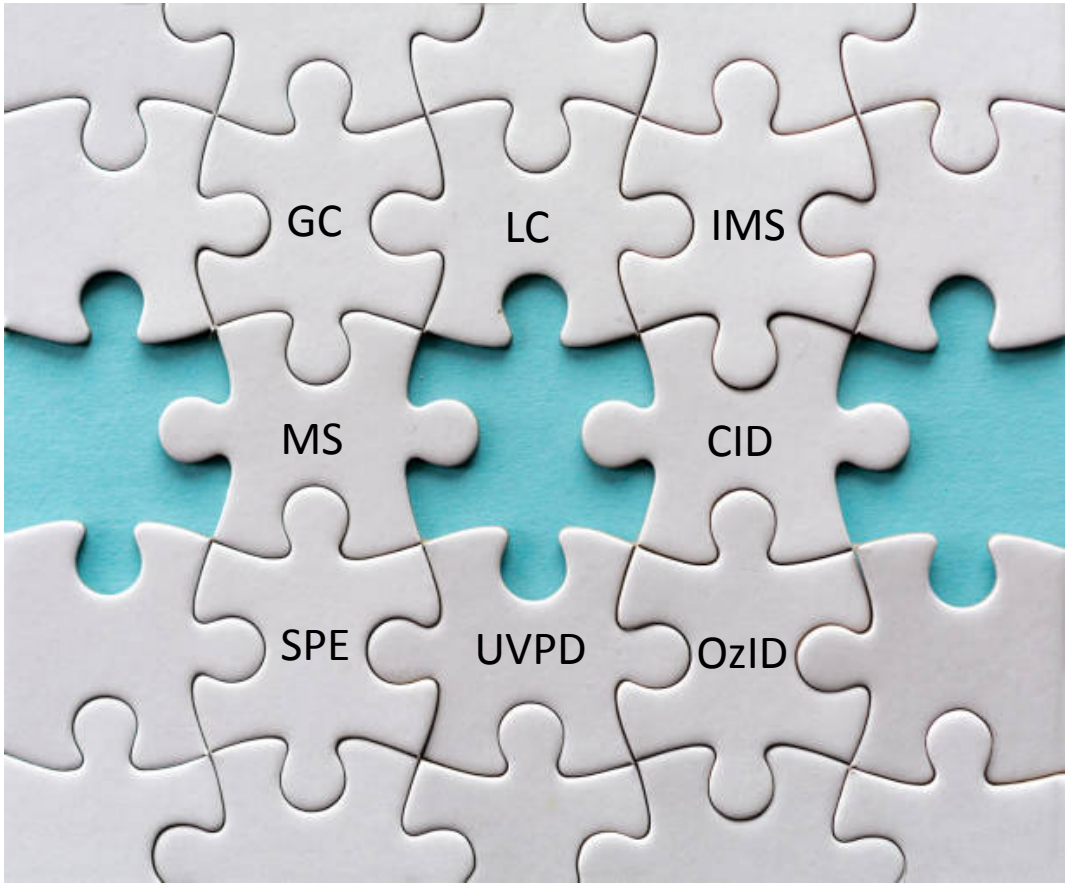
SFC isomer separation

- Utilized inline 2 column setup and InfinityLab Deactivator Additive in methanol and 10 mM ammonium acetate
- Separated PC(16:1(9Z)/16:1(9Z) and PC(16:1(9E)/16:1(9E)



Summary

- Combining multiple separations and methods enables faster and better lipid identifications
  - LC
  - SFC
  - IMS
  - OzID
  - SLIM IMS
  - LC-OzID-IMS-CID-MS
  - SFC-OzID-IMS-CID-MS



Acknowledgements

- Agilent Technologies
- NIEHS R01 ES022190
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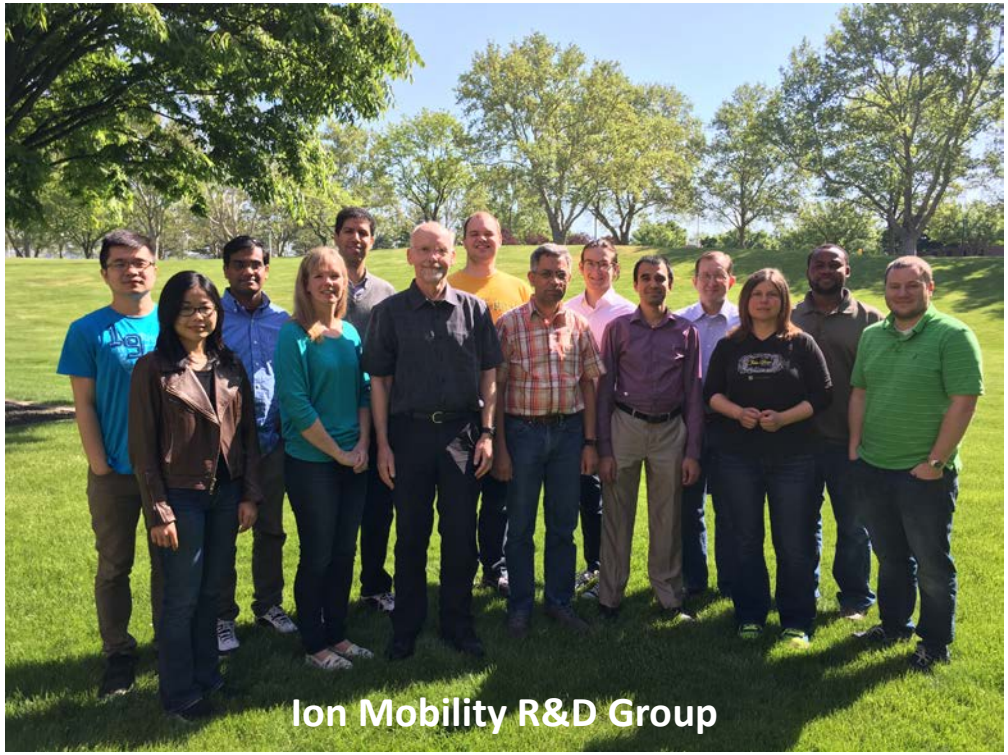
- PNNL Laboratory Directed Research and Development Program
- Environmental Molecular Sciences Laboratory



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