



MOBILion
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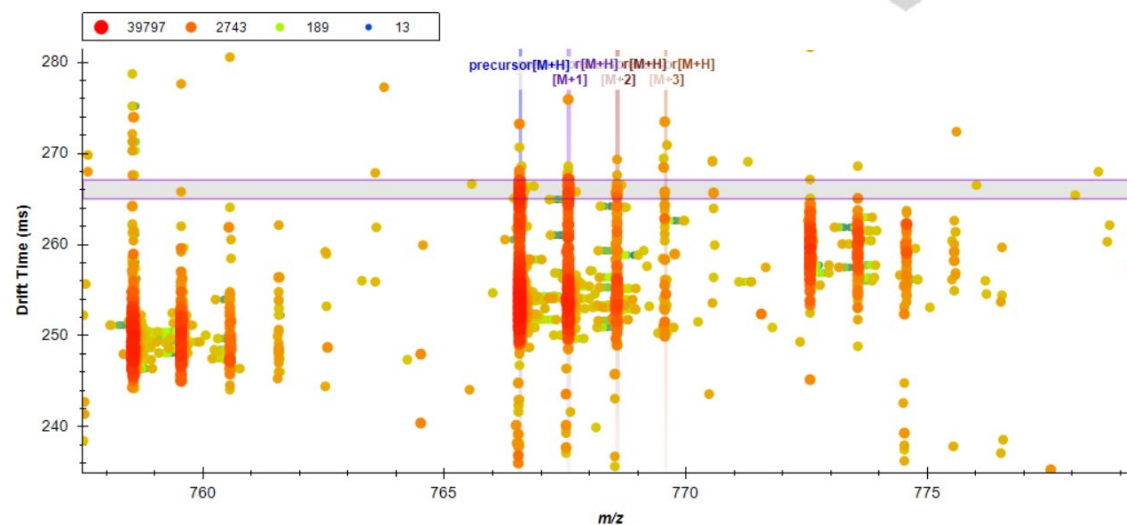
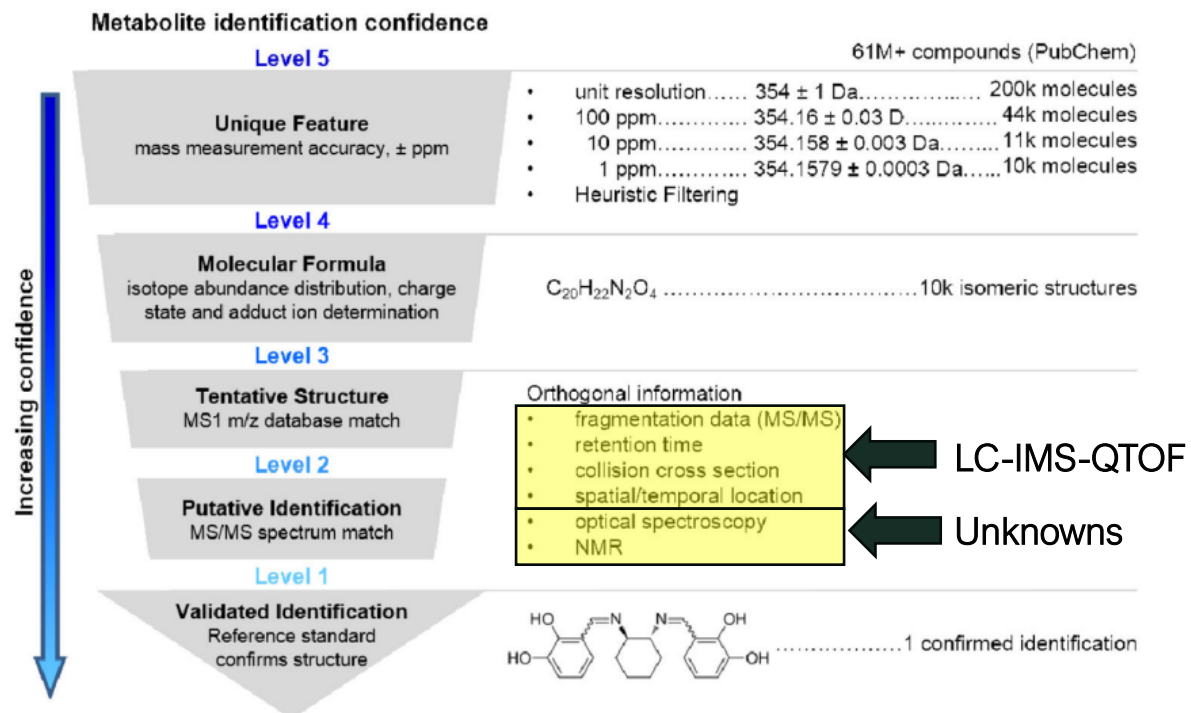
Revealing What Others Leave Unseen

Leveraging CCS and Mobility Aligned Fragmentation for Ion
Mobility Enhanced Analysis of Lipids

Lauren Royer, 2024 User Group Meeting at ASMS

Workflow Goals

- Lipidomics requires detailed confirmation
 - Fragmentation alone is not sufficient for some isomeric species
- Skyline LC-IMS-MS processing
 - Additional confidence w/ CCS



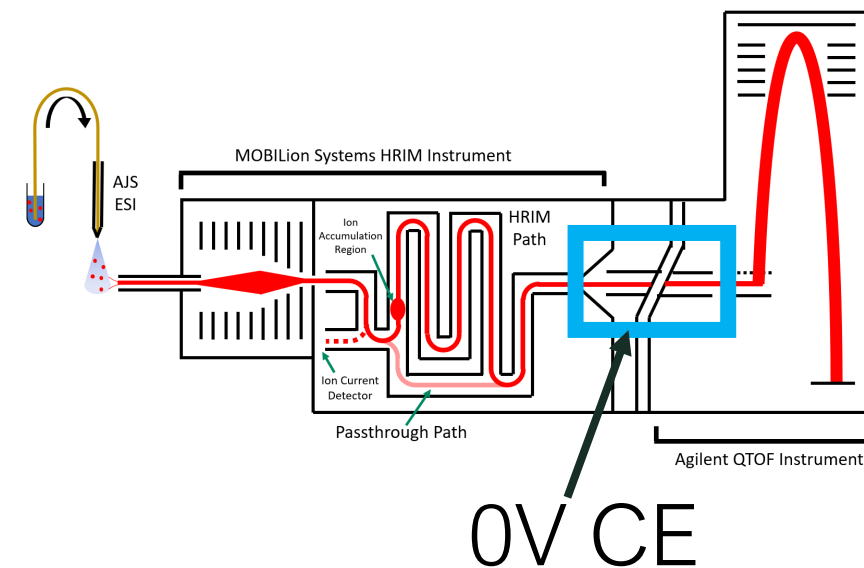
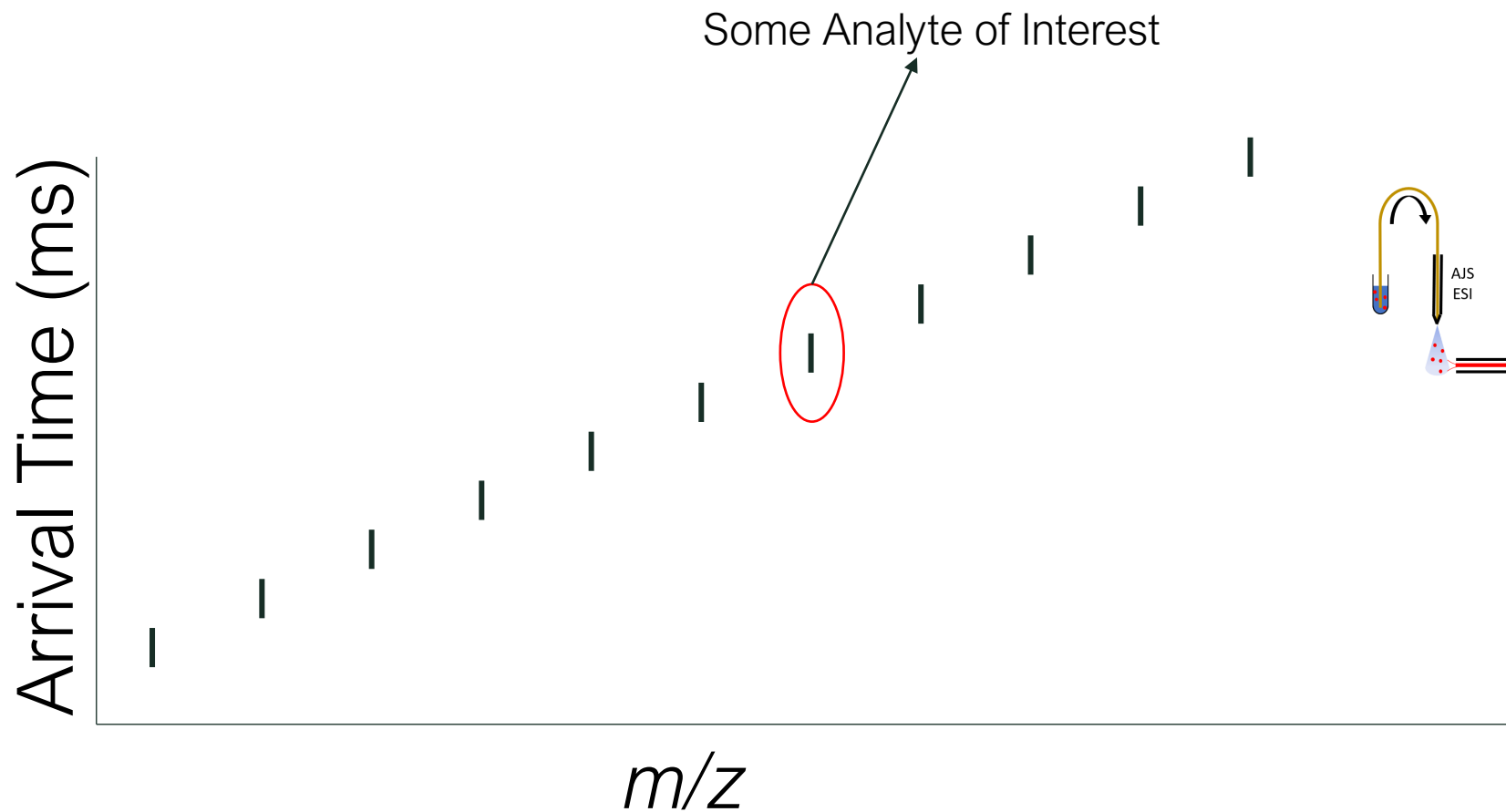
Schrimpe-Rutledge *et al.* Untargeted Metabolomics Strategies – Challenges and Emerging Directions. *J Am. Soc. Mass. Spectrom.* 2016 ; 27(12) pp 1897-1905

Low Energy IM-MS

Exploit the separation of ions in the IM dimension, without needing to mass select

Precursor Ions

Fragment Ions



<https://doi.org/10.1021/jasms.0c00434>

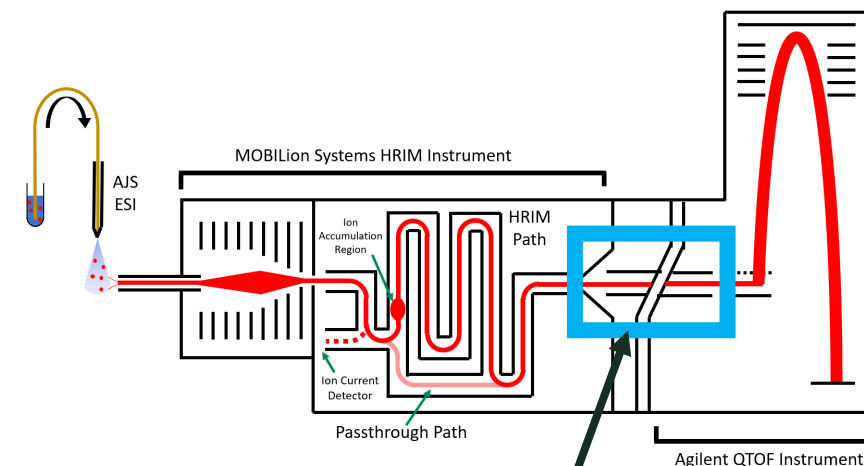
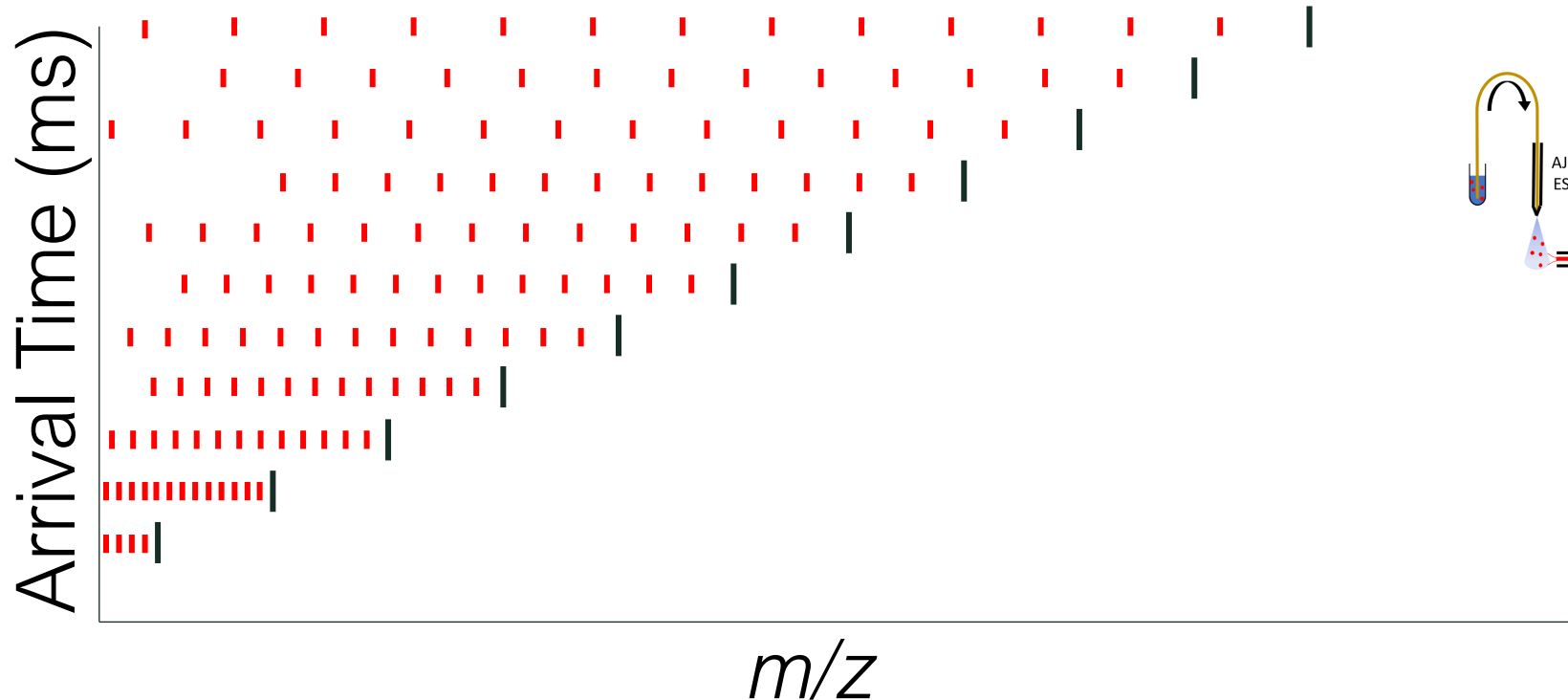
High Energy IM-MS

MS1 Scan provides:

- 1) Arrival Time for precursor alignment
- 2) precursor CCS value

Precursor Ions

Fragment Ions



CE

AFTERSLIM

<https://doi.org/10.1021/jasms.0c00434>

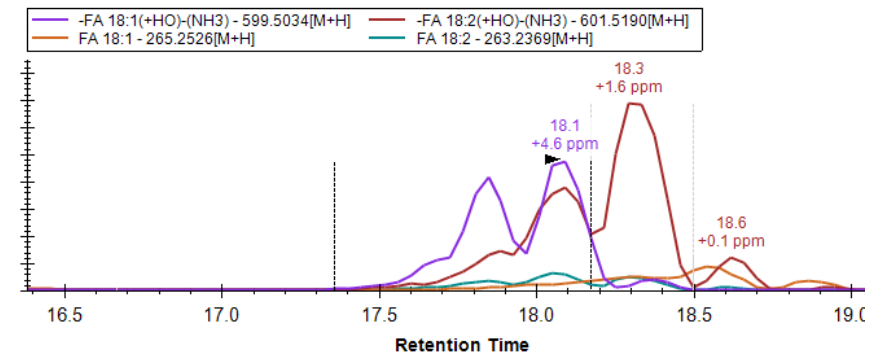
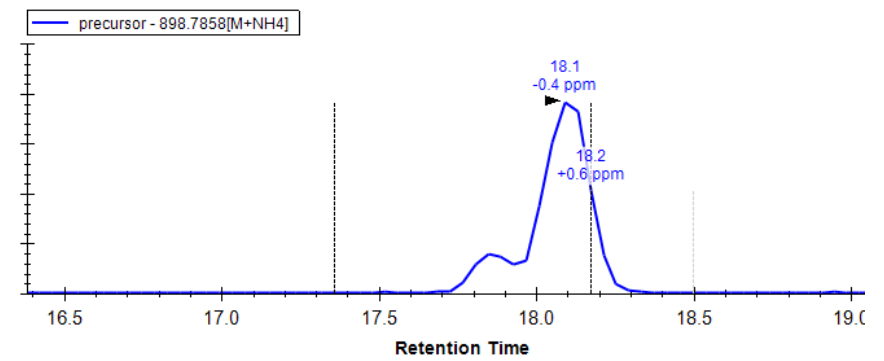
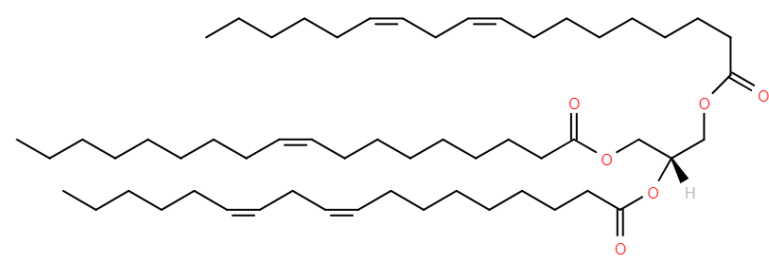
These fragment ions can then be used to determine the lipid composition

Import w/ LipidCreator defaults

- 319 Predefined Human Lipid Plasma entries
 - Precursor + Fragment Info
 - No mobility data yet
- Default entry for Triacylglycerol (54:5)



✓ TAG 18:1-18:2-18:2
✓ 898.7858[M+NH4]
✓ precursor - 898.7858[M+NH4] (i rank 1) [i 1]
✓ -FA 18:1(+HO)-(NH3) - 599.5034[M+H][1]
✓ -FA 18:2(+HO)-(NH3) - 601.5190[M+H][2]
✓ FA 18:1 - 265.2526[M+H][4]
✓ FA 18:2 - 263.2369[M+H][3]



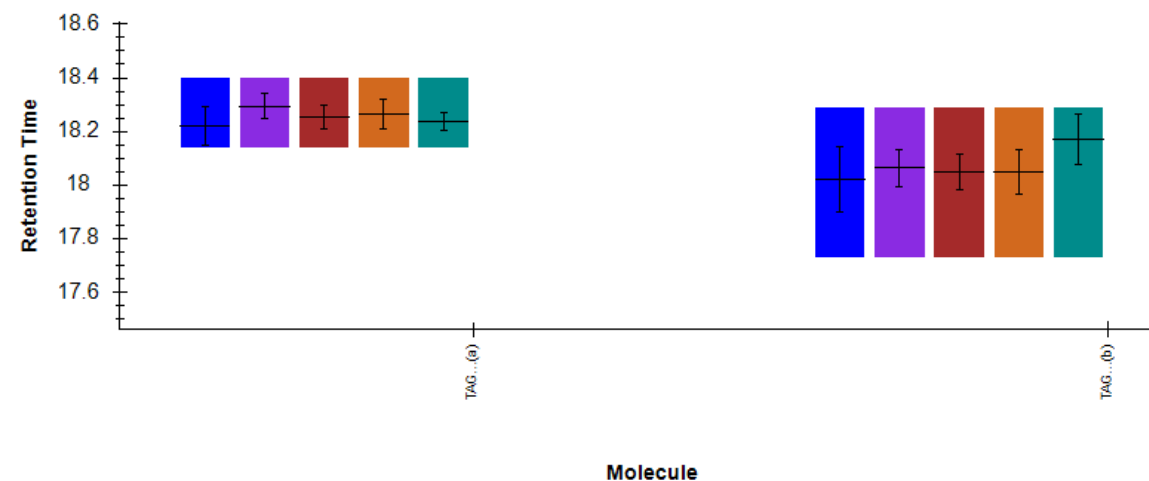
Without Ion Mobility Filtering

Review TG (54:5)

- Multiple distributions for same precursor and fragment m/z across Arrival Times

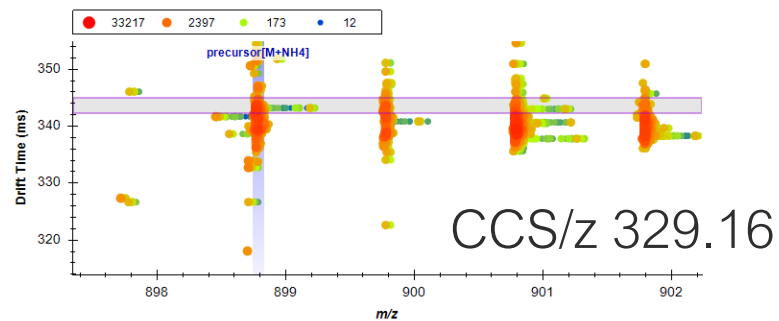
Overlapping
RT, requires
CCS for ID

Retention Times - Molecule Comparison

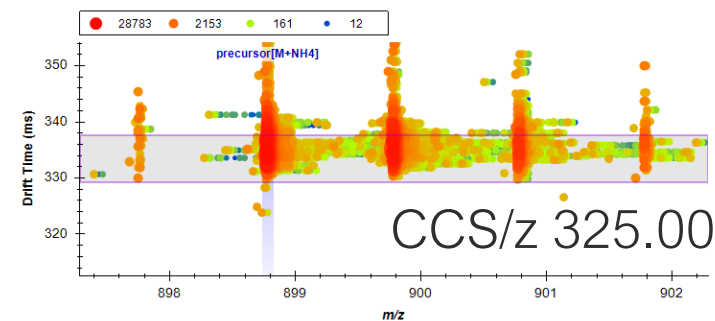


CCS
Filtered
Precursors

2023-08-31 18.50.31-B_250-0023_MIFF0_0[Lo]combined_data[0,20][Hi][Lo]{file2}[Hi].d (18.22 min)

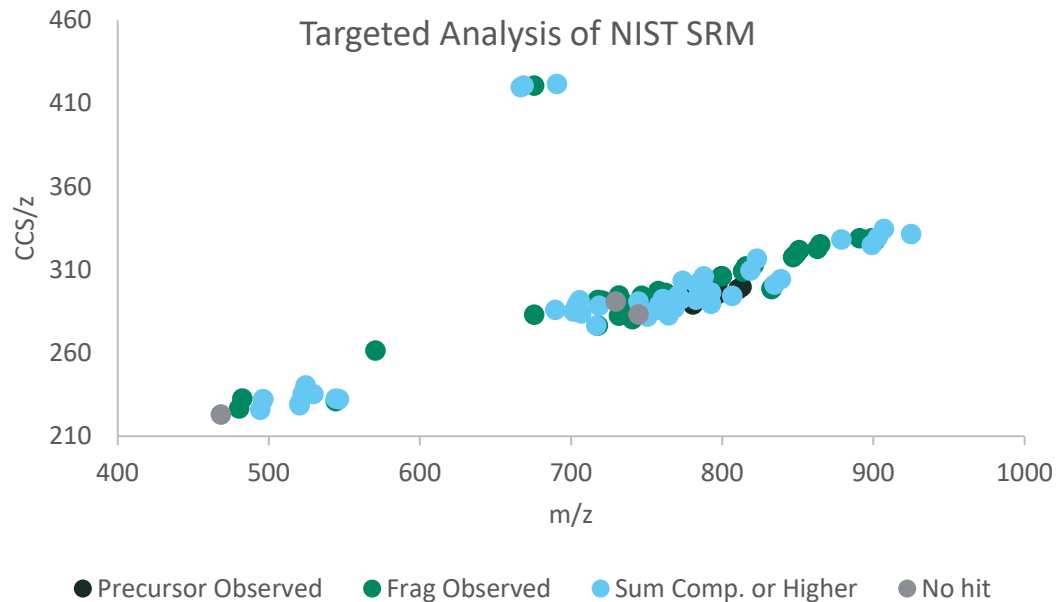


2023-08-31 18.50.31-B_250-0023_MIFF0_0[Lo]combined_data[0,20][Hi][Lo]{file2}[Hi].d (18.10 min)

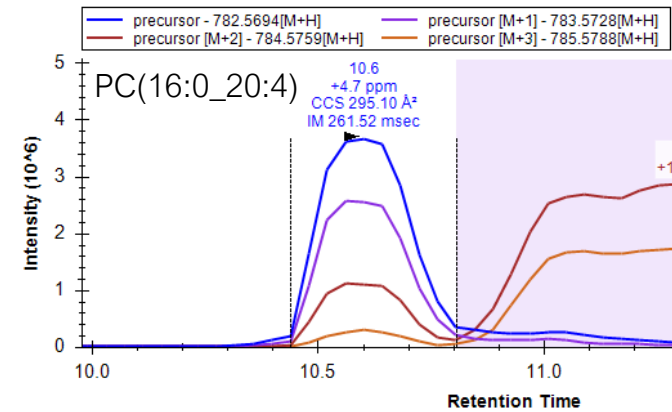


Plasma Lipid CCS library

- SLIM mobility separation to associate fragments with precursor ions
- Precursor ion has unique CCS
 - Reproducible across days + instruments



Positive Mode			
Total entries			129
Isobar + Isomers			63
NIST SRM in Skyline	Precursor Observed	97%	●
	Frag Observed	84%	●
	Sum Comp. or Higher	55%	●

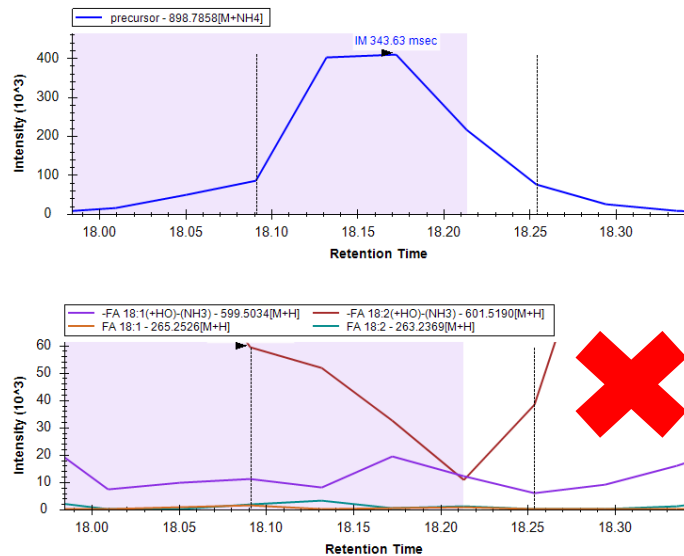


Harris R.. *et al.*, An Interlaboratory Evaluation of Collision Cross Section Measurements from a Plasma Lipid Extract on a Commercial SLIM Ion Mobility Platform. **ASMS 2024: TOB PM 04:10**

Aligned Fragment Data Enables Lipid ID of TG(54:5) [NL-18:1]

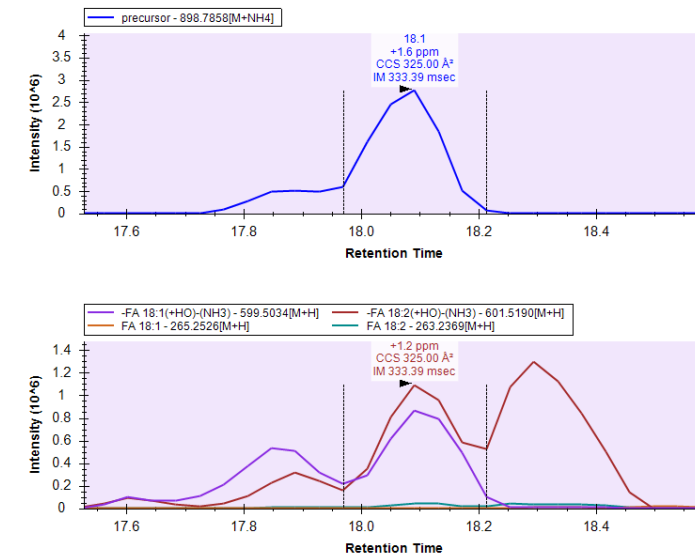
Isomer (a): CCS/z 329.16

- Cannot use same product ions to specify this level of ID
- Saved from over-annotation



Isomer (b): CCS/z 325.00

- Fragments met S/N + intensity requirements after mobility filtering



Thank you!

MOBILion Team

Skyline Team

Interlab DB Contributors

2024 Skyline User Group Meeting

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Thank You