

SWATH-MS, Ion Mobility and LC-MS for lipidomics

- No stone unturned

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MS2 Data acquisition strategy (bottom-up approach)

1. Data Dependent Acquisition (DDA)
2. Data Independent Acquisition (DIA)

MSMS^{ALL}- No stone unturned

Data-independent workflow with a capability of acquiring high resolution MS/MS data for all detectable ions (m/z 200-1200) in a single run (6 min)

SWATH-MS
(Sequential Window Acquisition of all Theoretical-Mass Spectra
(in Triple-TOF system))

Simons et al. Metabolites, 2012

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Strength of MS/MS^{ALL}

- Comprehensive, no requirement of a *priori* assumptions
- Combination of top-down and bottom-up methods
- Digital records of MS and MS/MS (high mass resolution)
- Data can be searched retrospectively
- Comparison (statistical) lipid changes between/among groups

Prasain et al. Metabolites, 2015

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High speed, high resolution, sensitive detection and accuracy are crucial for lipid analysis

Sciex 5600 Triple-TOF

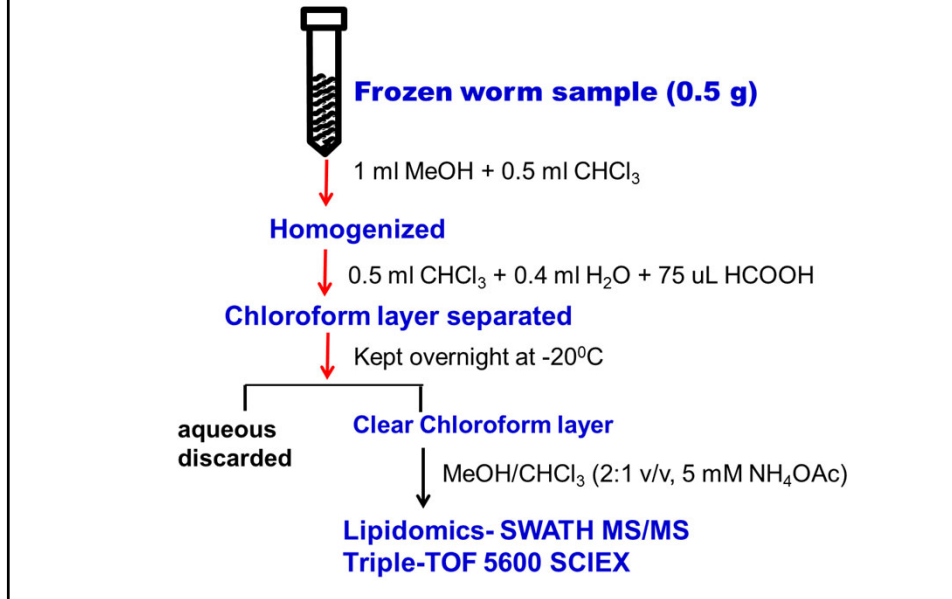
- Over 30,000 mass resolution
- <5 ppm mass accuracy
- Very fast acquisition of MSMS spectra (10 ms)
- Precursor and neutral loss analyses are possible performed *post hoc*



AB Sciex Triple TOF 5600

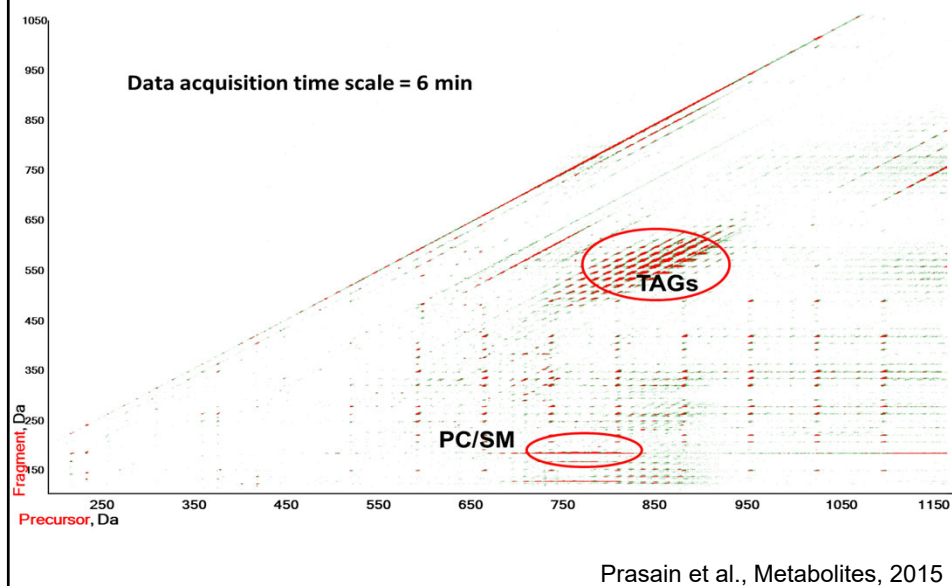
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Extraction of lipids and analysis



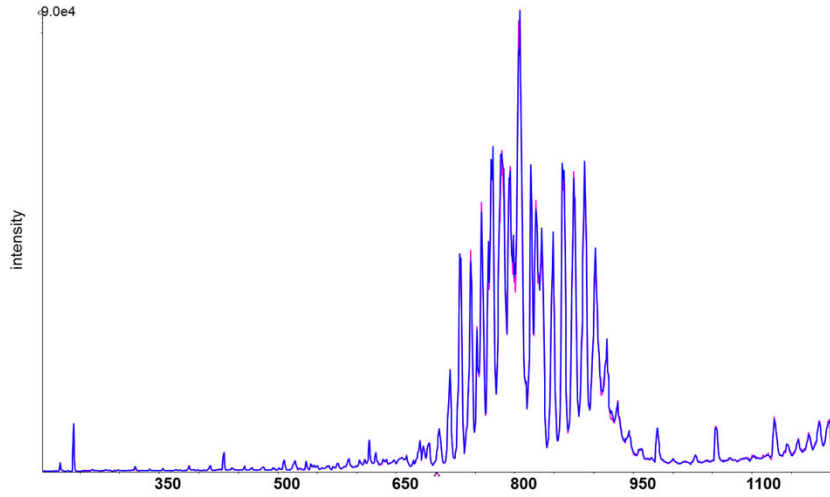
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Snapshot of lipidome composition of *C. elegans* by Triple-TOF MS(+ve ion mode)



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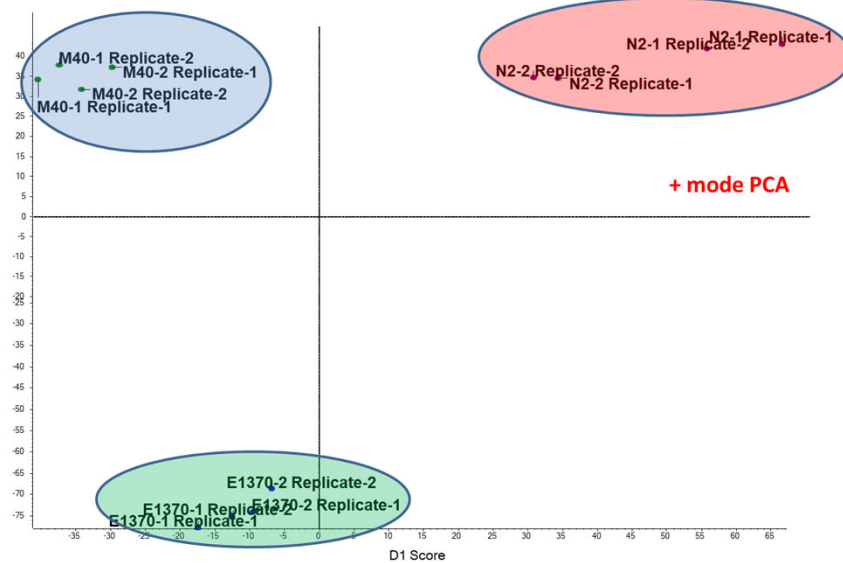
Reproducibility of two replicates of wild type worm extracts in terms of TIC



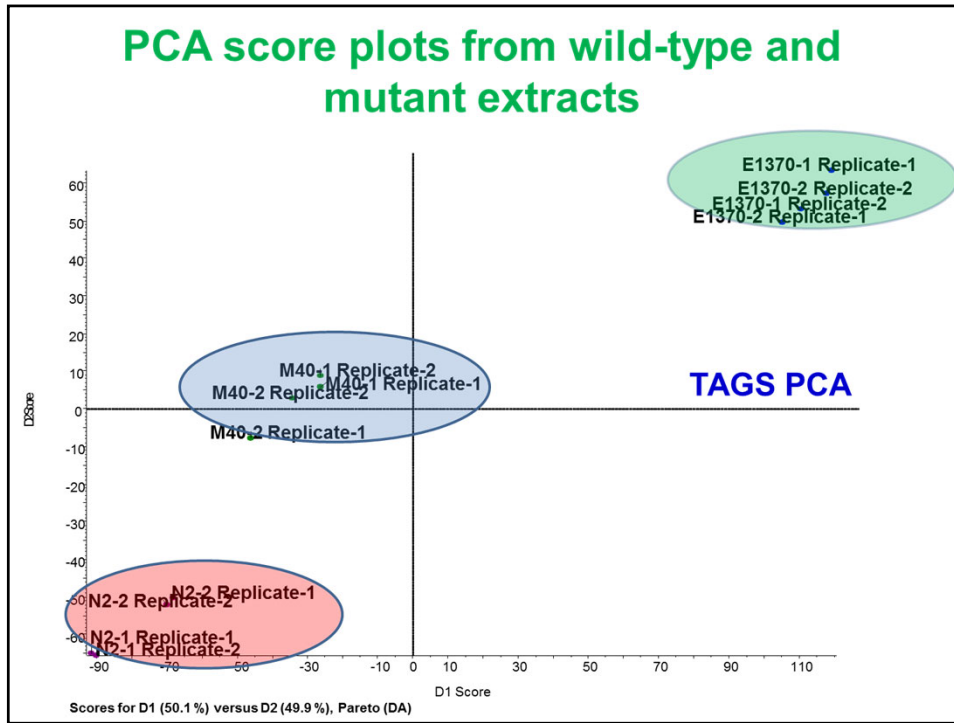
Prasain et al. Metabolites, 2015

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Good separation of groups and reproducibility within groups

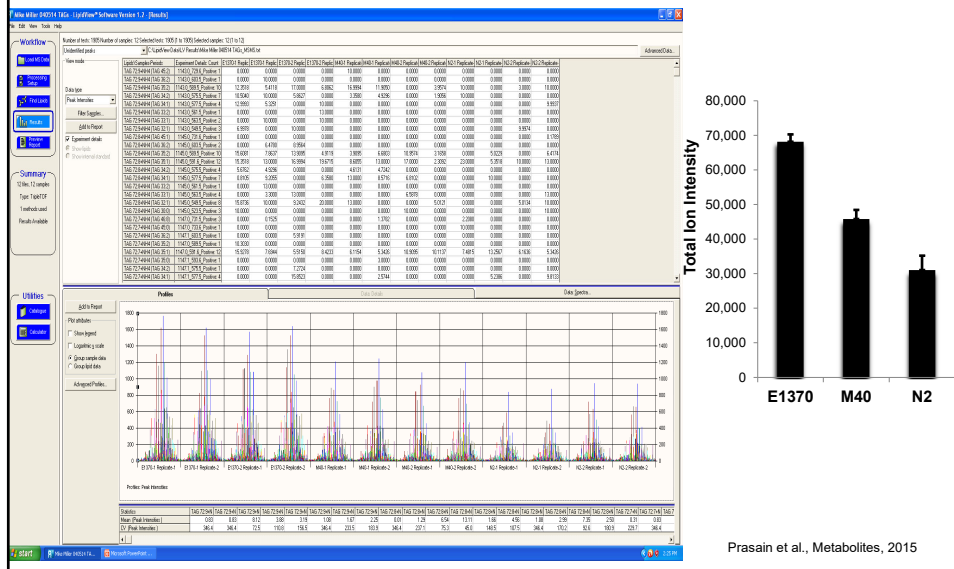


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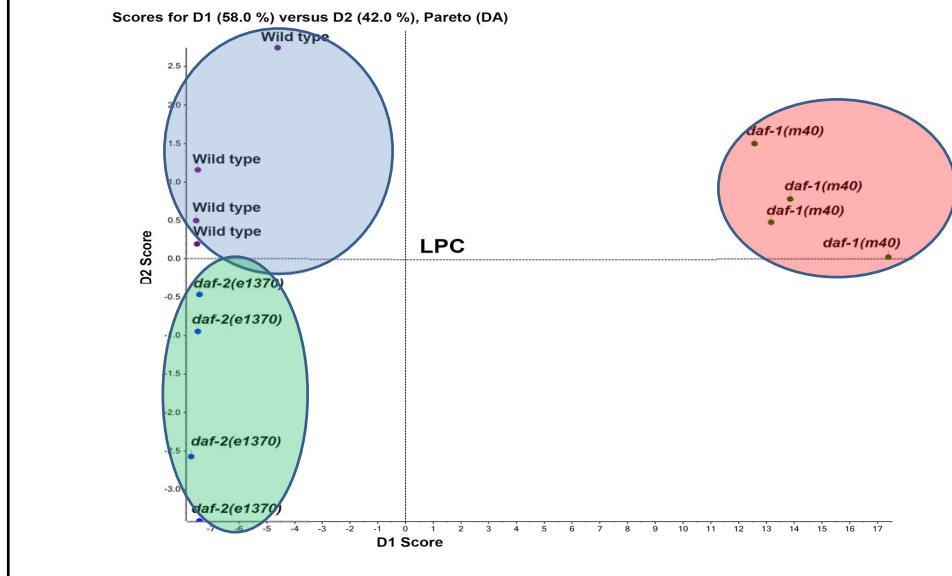
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Total TAGs in mutants were higher than in wild-type extracts



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PCA score plot of LPCs from wild-type and mutant extracts; Daf-1 mutants are significantly different



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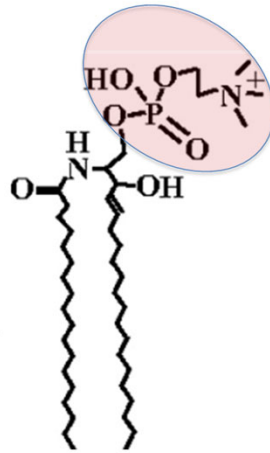
MS/MS^{ALL} limitations

- Poor selectivity
- MS/MS fragment ions may contain fragment ions from other precursor ions such as isobaric species to precursor ion of interest
- Limited to infusion or flow injection acquisition

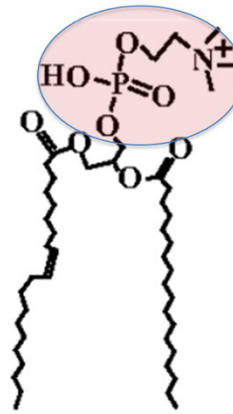
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The problem of analyzing lipids

- Despite the sheer number of lipids, the *units* comprising them are closely related and therefore they have similar masses
- Sphingolipids may only be different in mass by 1 Da from their PC analog
 - ^{13}C -Isotope profiles overlap
 - Head groups are the same



Sphingolipid

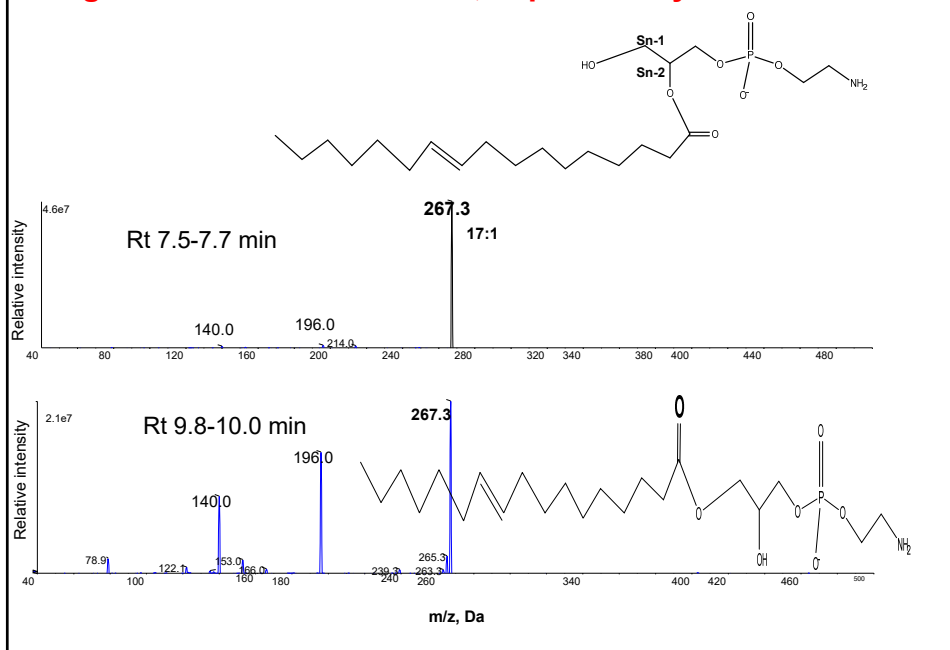


Phosphocholine

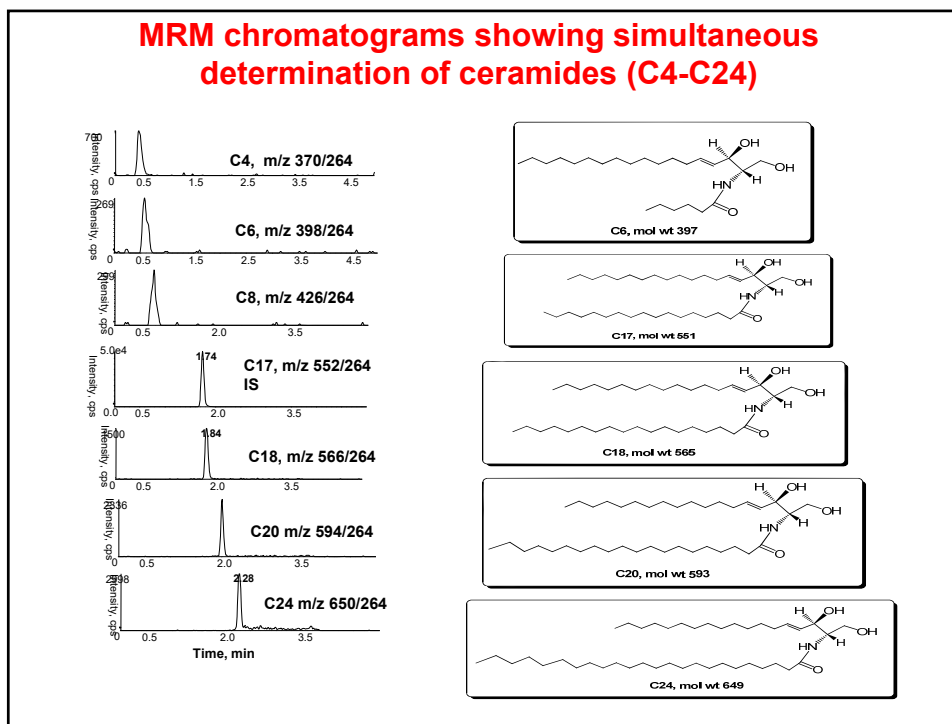
Overlap may exist between C12 and C13 monoisotopic peaks of PC and SM

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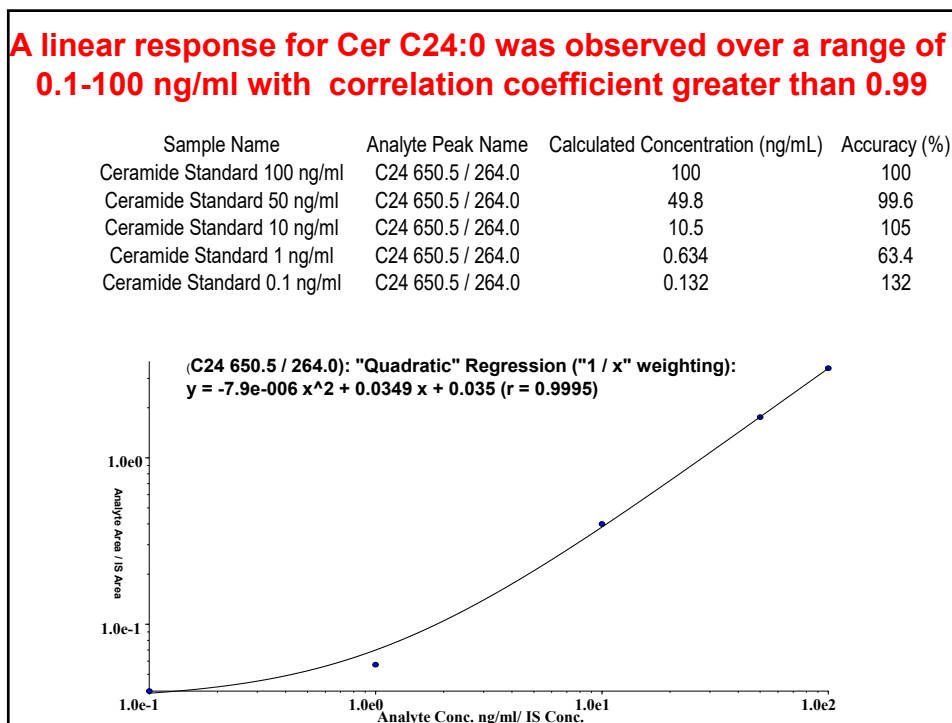
Regioisomeric LPEs m/z 464, separated by LC-MS/MS



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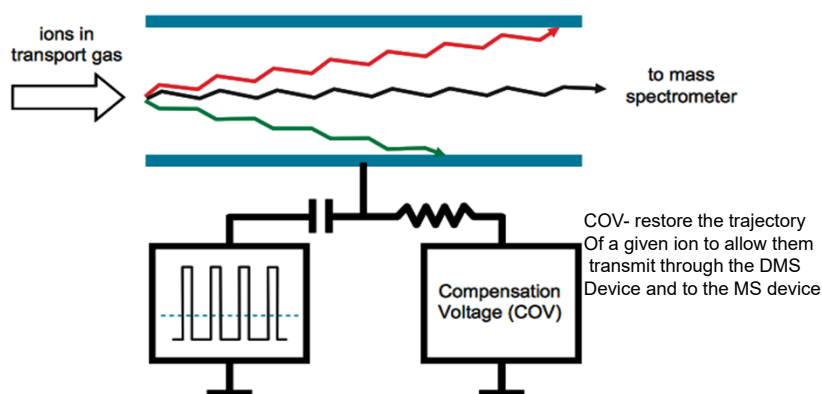
Differential mobility mass spectrometry

- When a fragment ion may have multiple precursor ions, the precursor ions may be separable by DMS before they enter the mass spectrometer
- By scanning with the compensating voltage (CoV), the precursor ions enter the mass spectrometer
- at different CoVs
- (Note: Further separation is possible using resolving agents, e.g., isopropanol)

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Differential mobility MS is an answer

Innovative Planar Design; SelexION™ Ion Mobility Cell.

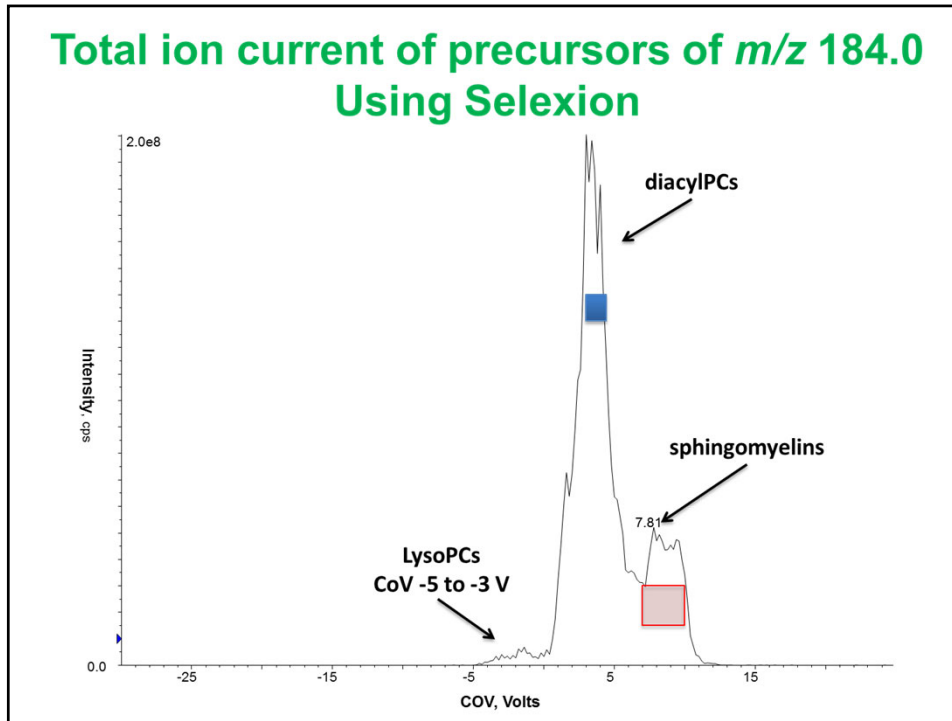


COV- restore the trajectory Of a given ion to allow them transmit through the DMS Device and to the MS device

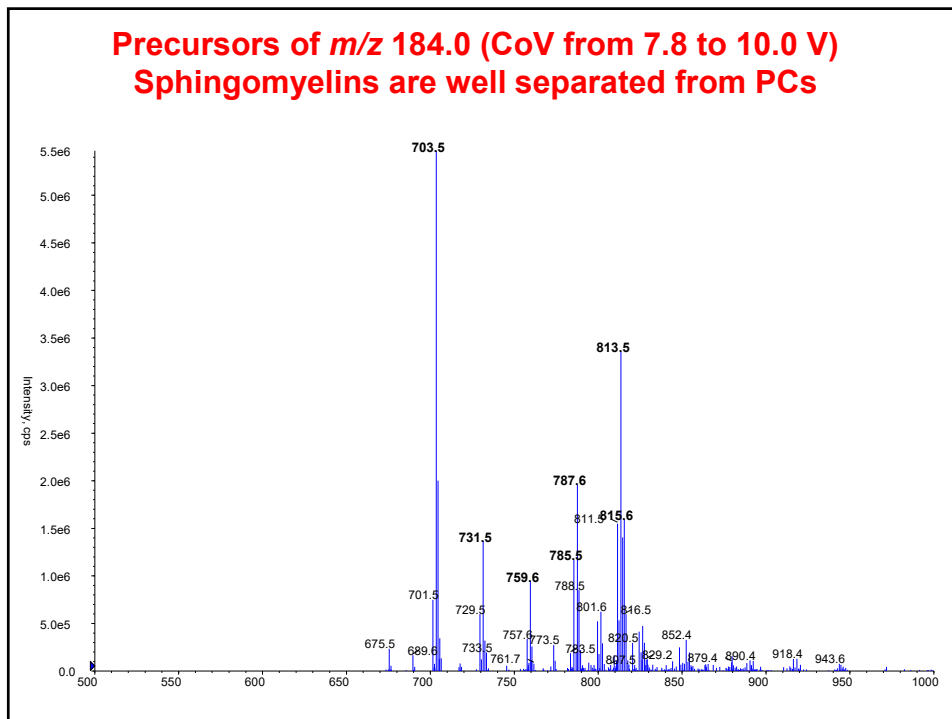
Separation waveform (SV)
Displaces ions from the one or other electrodes
Depending upon high/low field mobility characters

AB SCIEX

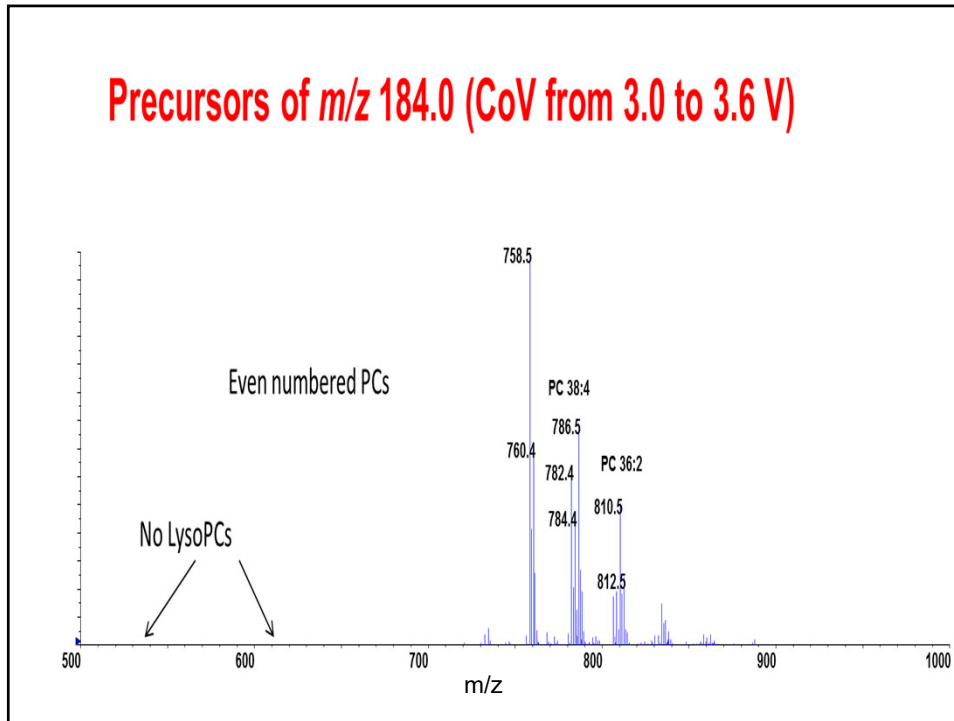
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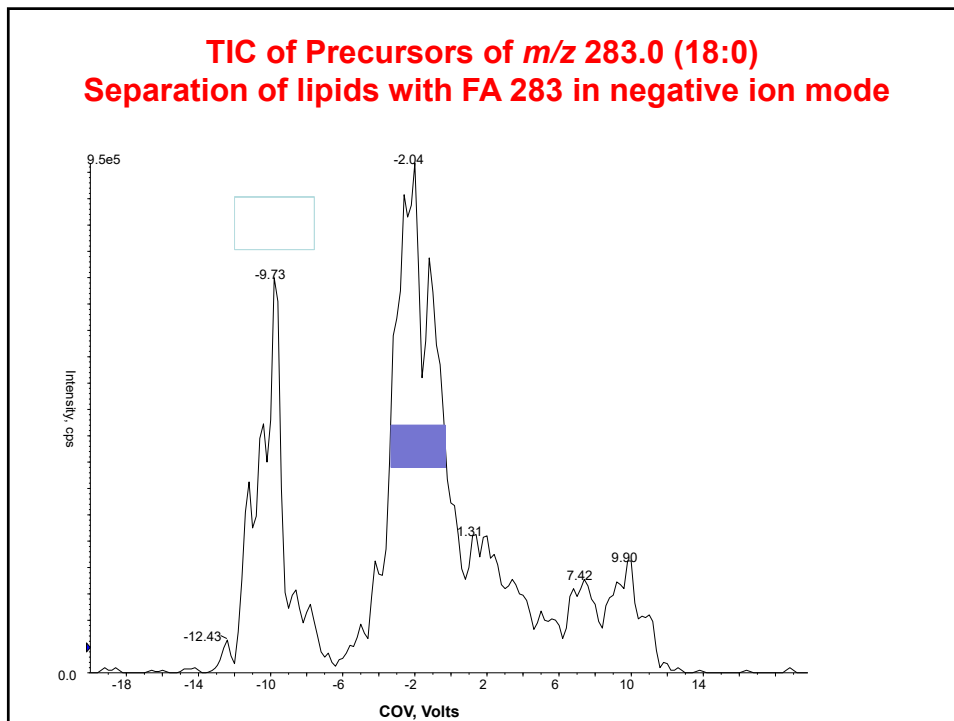
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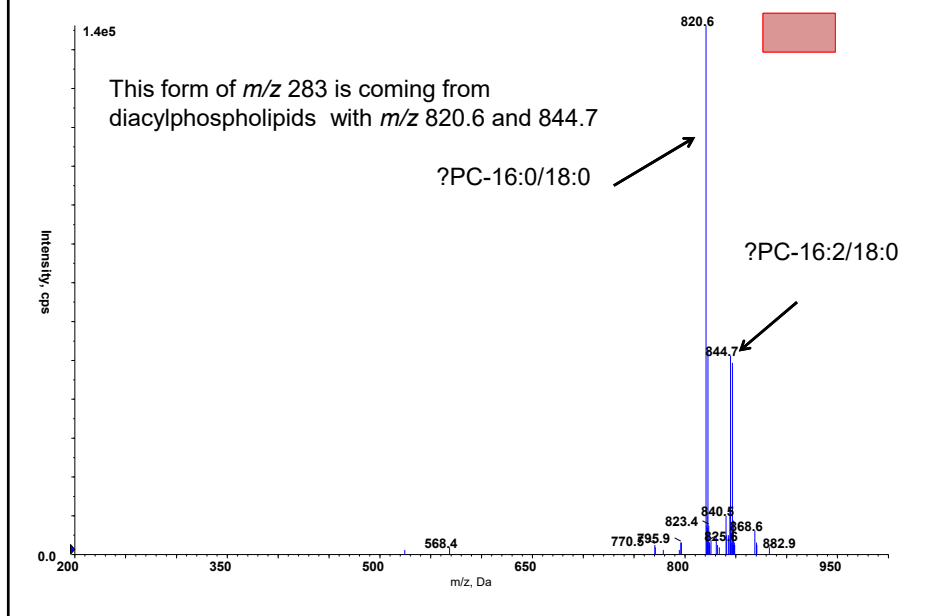


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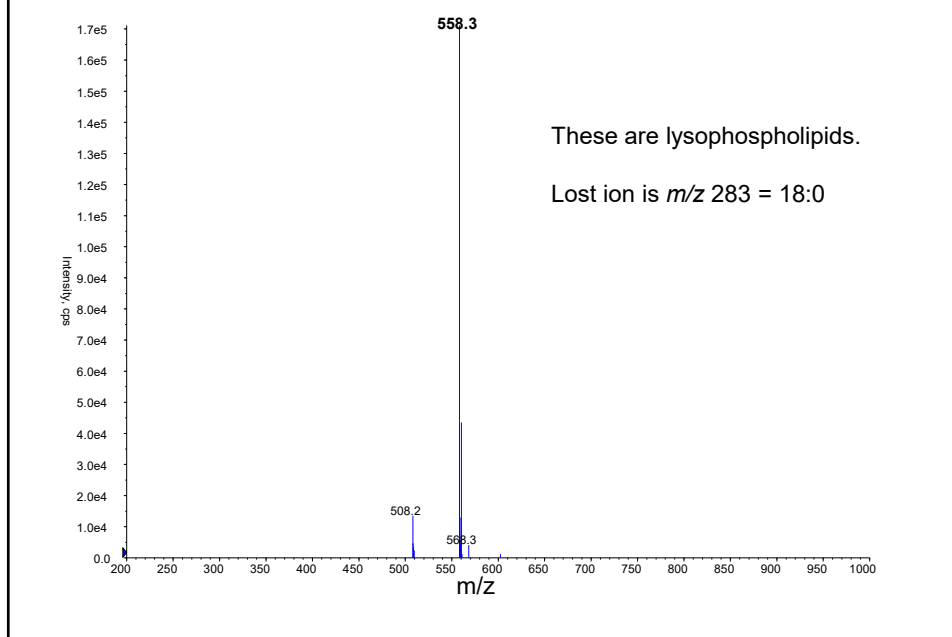
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Precursors of m/z 283.0 CoV from -3.4 to -0.4 Volts



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Precursors of m/z 283.0 – CoV from -11.8 to -8.6 V



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Conclusions

- **Shotgun lipidomics approaches are high throughput and applicable to perform profiling as well as quantitative analysis of various lipids in biological samples.**
- **Differential ion mobility is useful for reducing or separating isobaric interferences**
- **LC-MS/MS method operating in multiple reaction ion monitoring mode (MRM) can be used for identification and simultaneous quantification of lipids.**