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

Jeevan Prasain
jprasain@uab.edu
6-2612

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

MSMS^{ALL}- 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)

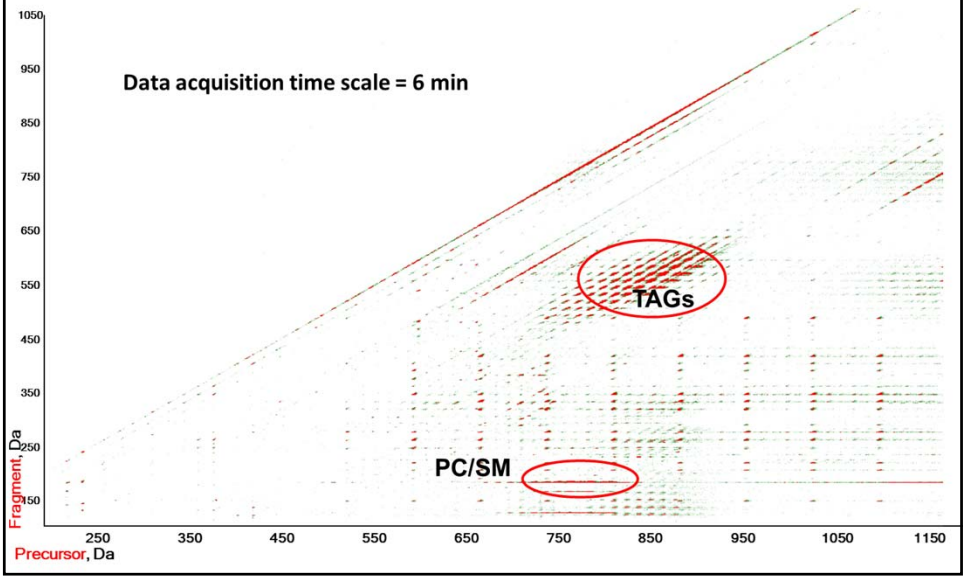
High speed, high resolution, sensitive detection and accuracy are crucial for lipid analysis

Sciex 5600 Triple-TOF

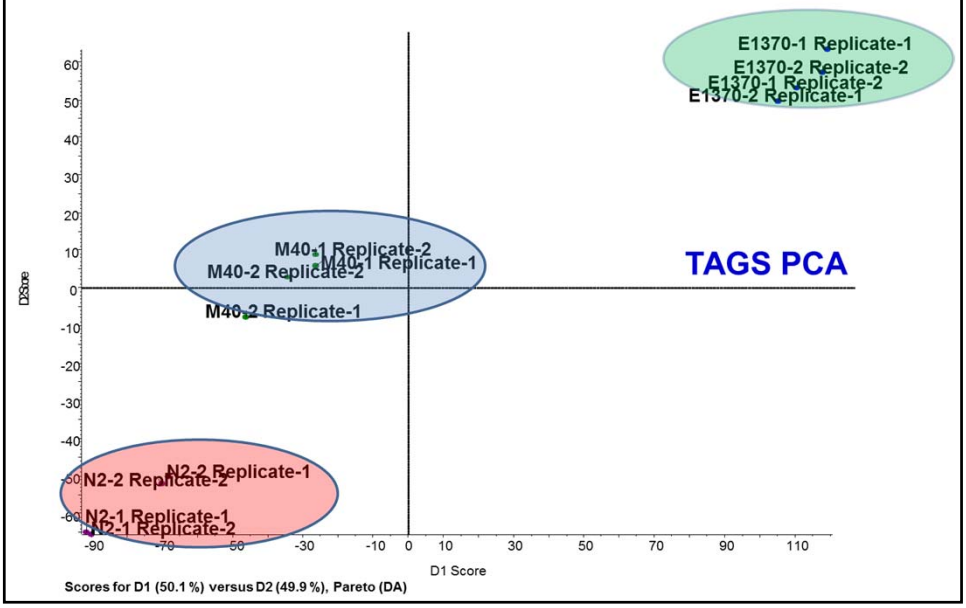
- **Over 30,000 mass resolution**
- **<5 ppm mass accuracy**
- **Very fast acquisition of MSMS spectra (10 ms)**
- **Precursor and neutral loss analyses are possible performed *post hoc***

AB Sciex Triple TOF 5600

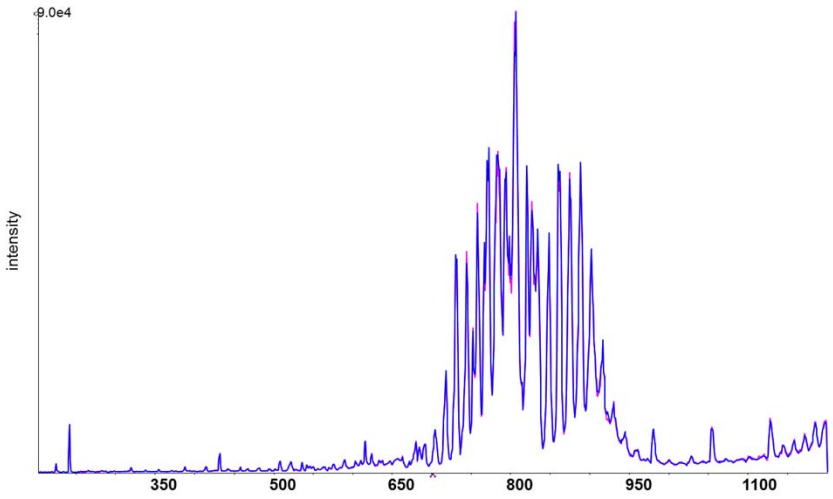
Snapshot of lipidome composition of *C. elegans* by Triple-TOF MS(+ve ion mode)



PCA score plots from wild-type and mutant extracts

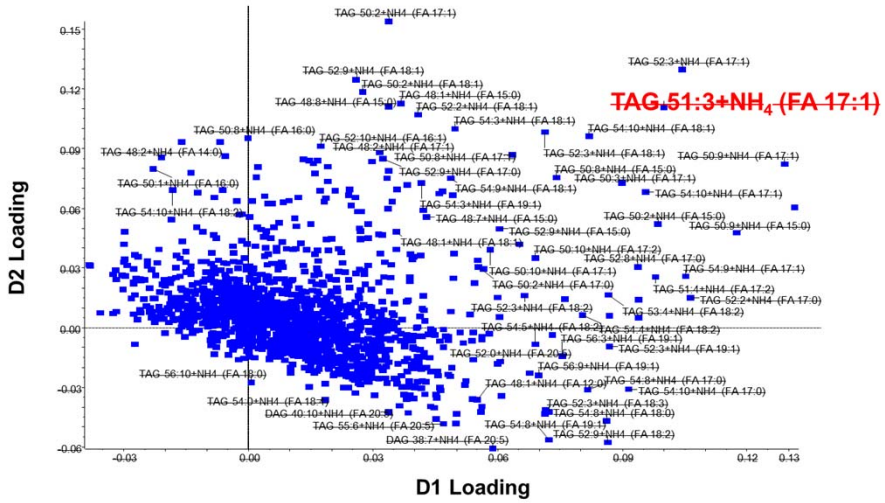


Reproducibility of two replicates of wild type worm extracts in terms of TIC



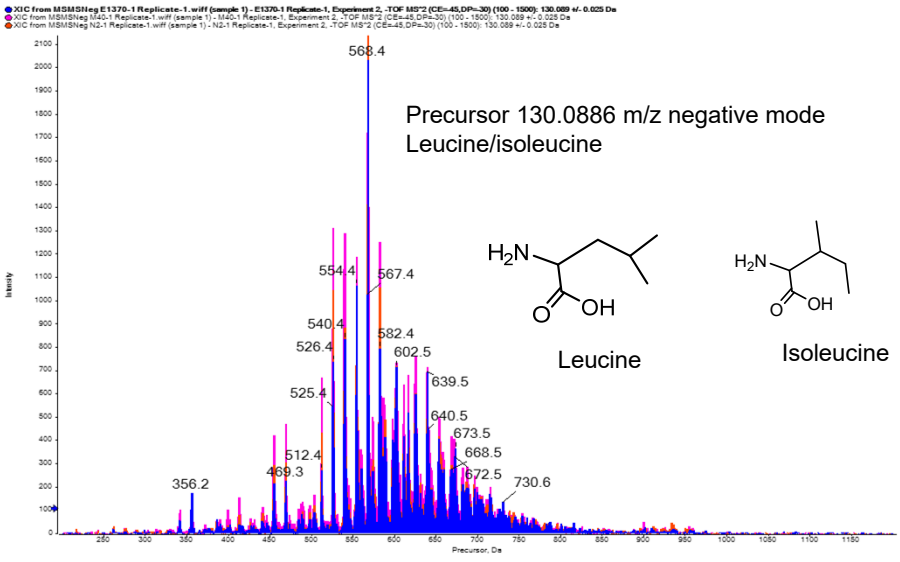
Prasain et al. Metabolites, 2015

TAGs for the separation of mutants and wild-type *C. elegans*

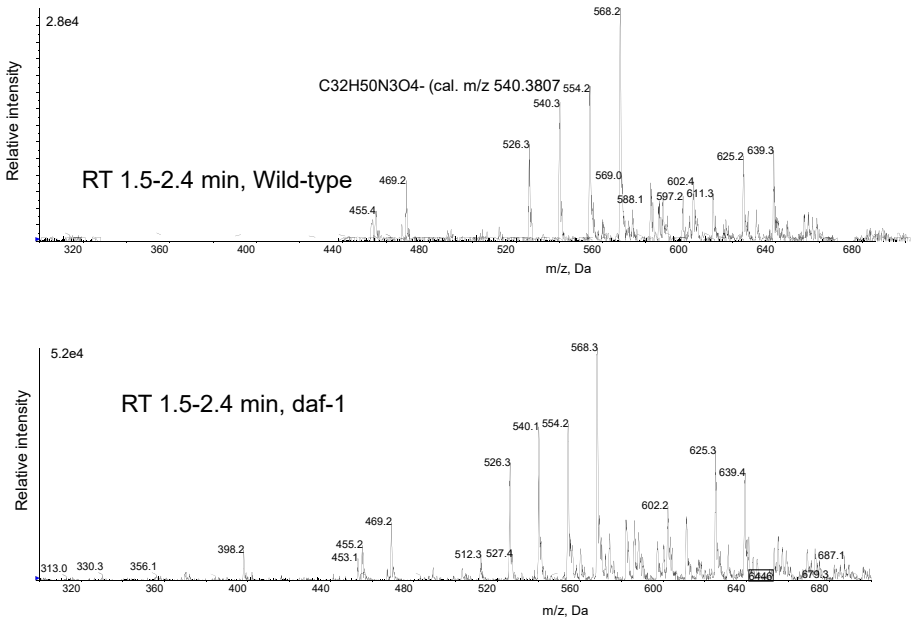


Loadings for D1 (50.0%) versus D2 (50.0%), Pareto (DA)

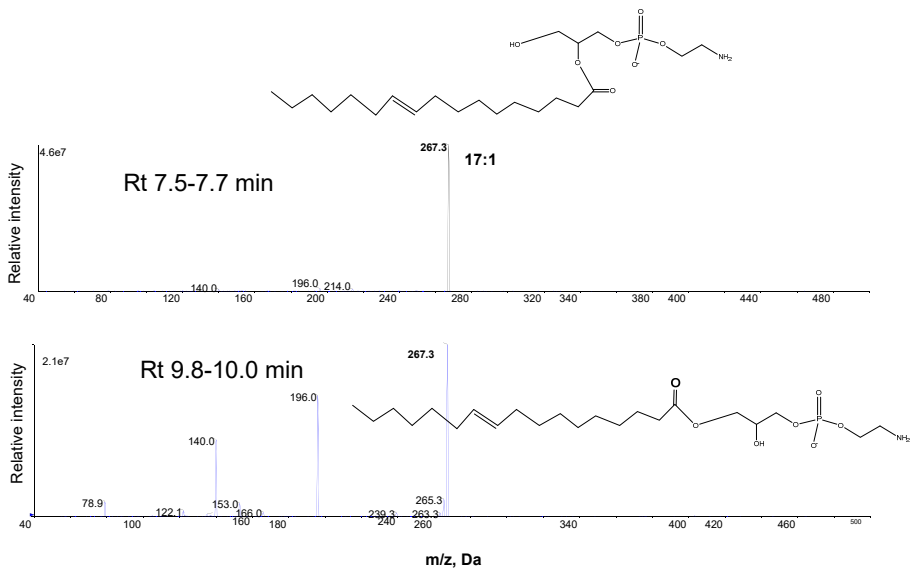
Overlapping isobaric peaks- direct infusion lipidomics



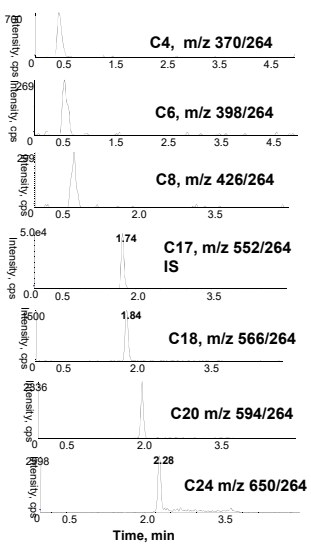
Extracted ion chromatograms for lipids containing Ile/Leu in wild-type and daf-1

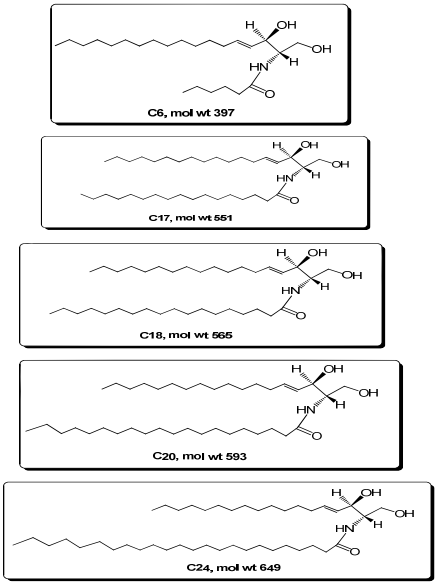


Regioisomeric LPEs m/z 464, distinguished by MS/MS and RT



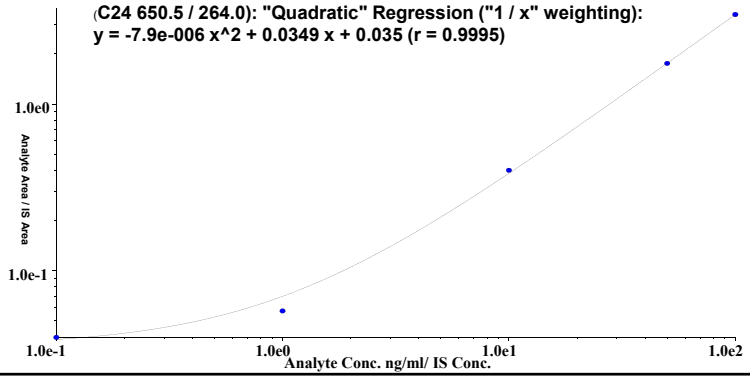
MRM chromatograms showing simultaneous determination of ceramides (C4-C24)



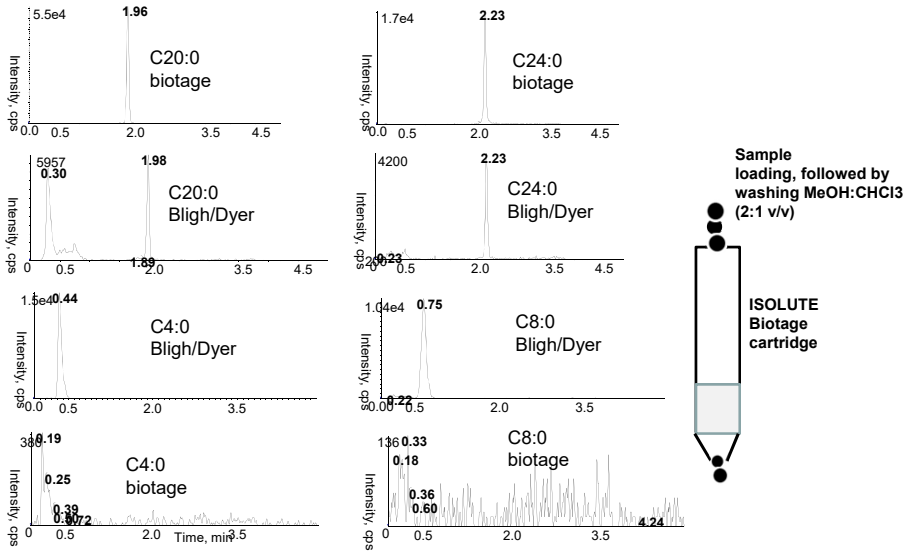


A linear response for Cer C24:0 was observed over a range of 0.1-100 ng/ml with correlation coefficient greater than 0.99

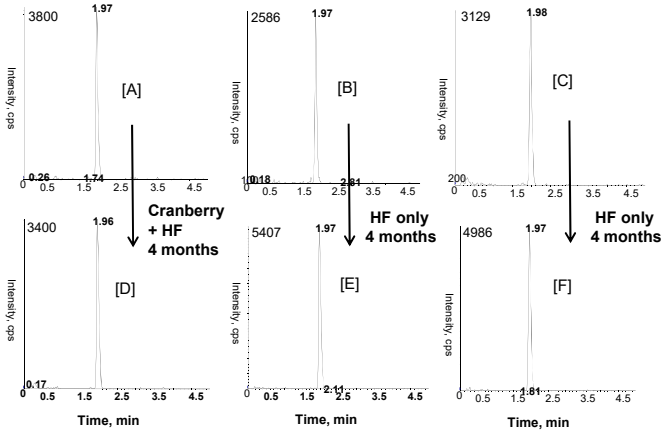
Sample Name	Analyte Peak Name	Calculated Concentration (ng/mL)	Accuracy (%)
Ceramide Standard 100 ng/ml	C24 650.5 / 264.0	100	100
Ceramide Standard 50 ng/ml	C24 650.5 / 264.0	49.8	99.6
Ceramide Standard 10 ng/ml	C24 650.5 / 264.0	10.5	105
Ceramide Standard 1 ng/ml	C24 650.5 / 264.0	0.634	63.4
Ceramide Standard 0.1 ng/ml	C24 650.5 / 264.0	0.132	132



Sample preparation of ceramides in plasma; Poor recoveries of non-polar ceramides Biotage (supported liquid extraction), but better for polar ceramides



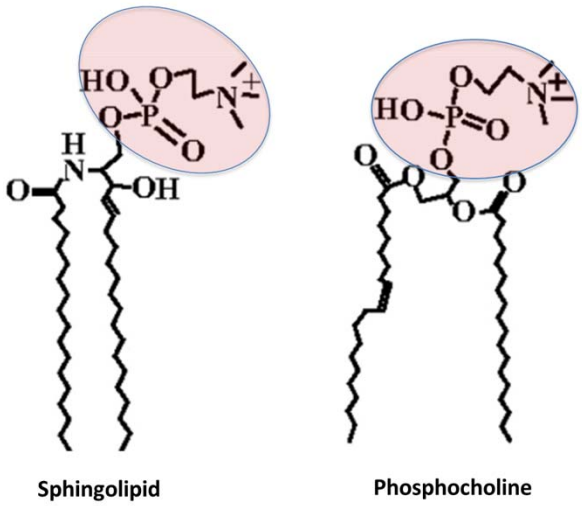
Cranberry fruit powder treatment reduced the HF induced increased levels of Ceramide C20 in rats



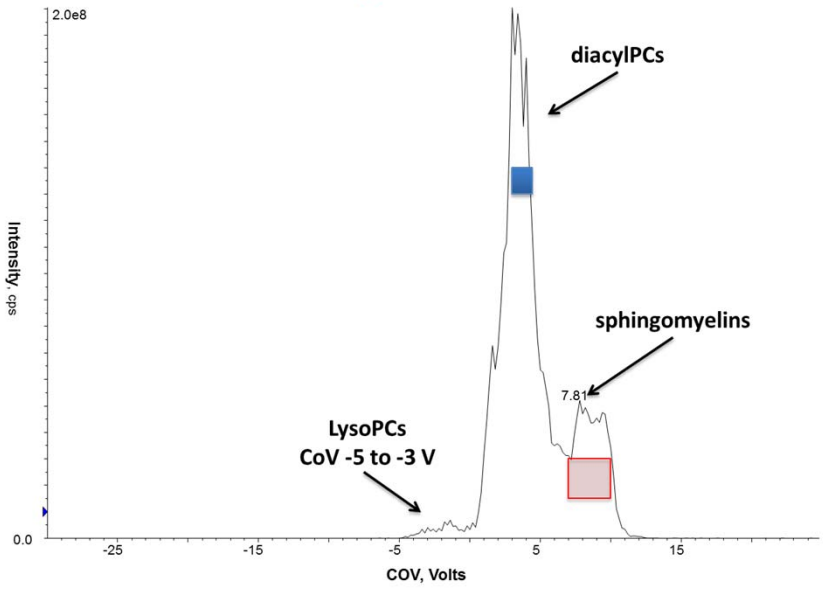
[A]-[C] represent base line plasma ceramide C20 (594/264) from three animals
[D] after 4 months treatment with cranberry (1 g/kg b. w. and high fat diet
[E] & [F] after 4 months treatment with high fat diet only

The problem of analyzing lipids

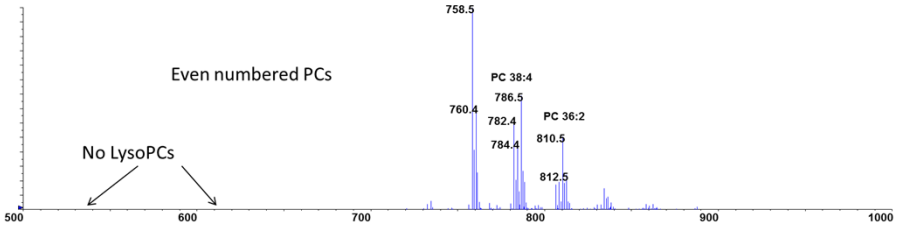
- Despite the sheer number of lipids, the *units* comprising them are closely related and therefore they have similar masses
- Sphingolipids may only be different in mass by 1 Da from their PC analog
  - ^{13}C-Isotope profiles overlap
  - Head groups are the same



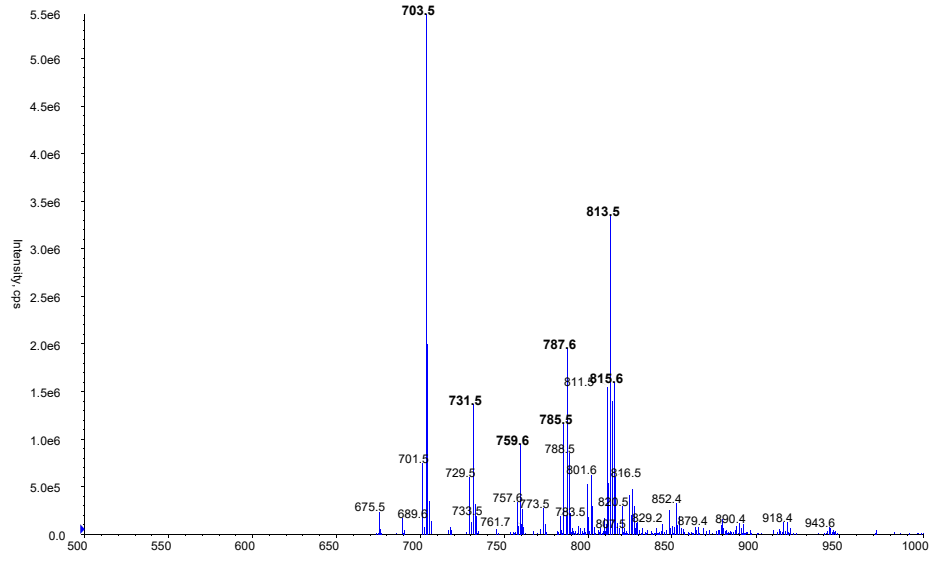
Total ion current of precursors of *m/z* 184.0 Using Selexion



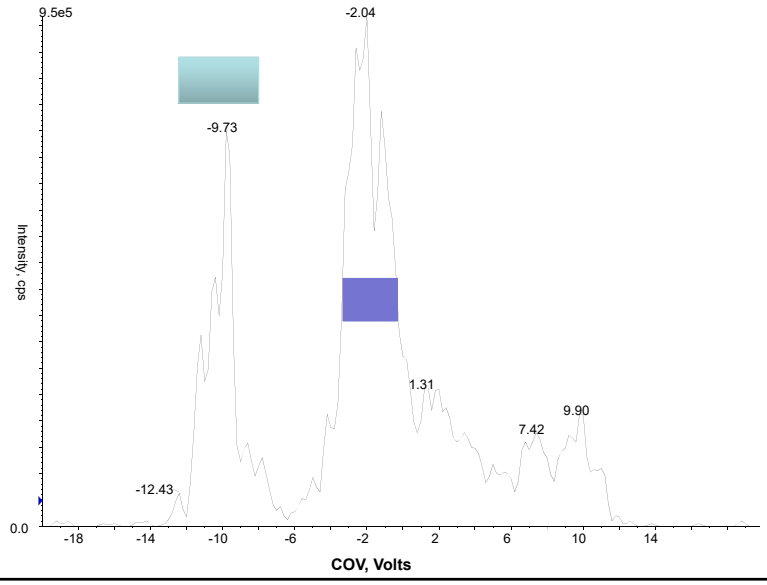
Precursors of *m/z* 184.0 (CoV from 3.0 to 3.6 V)



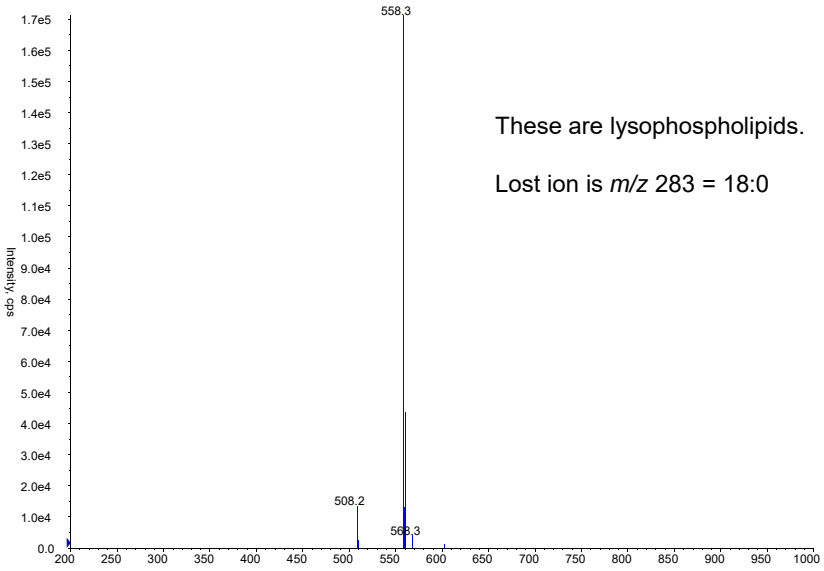
Precursors of *m/z* 184.0 (CoV from 7.8 to 10.0 V)
Sphingomyelins are well separated from PCs



TIC of Precursors of *m/z* 283.0 (18:0)
Separation of lipids with FA 283 in negative ion mode



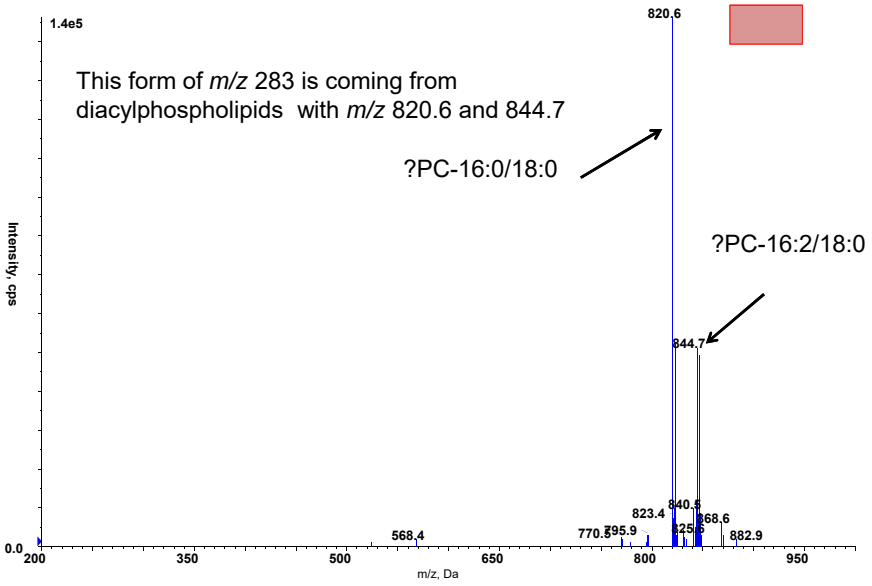
Precursors of *m/z* 283.0 – CoV from -11.8 to -8.6 V



These are lysophospholipids.

Lost ion is *m/z* 283 = 18:0

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



Conclusions

- **Shotgun lipidomics approaches are high throughput and applicable to perform profiling as well as quantitative analysis of various lipids in biological samples.**
- **Identification of phospholipids at a molecular level present a great challenge due to their structural diversity and dynamic metabolism.**
- **Differential ion mobility is useful for reducing or separating isobaric interferences**
- **A rapid five minute liquid chromatography tandem mass spectrometry (LC-MS/MS) method operating in multiple reaction ion monitoring mode (MRM) was developed for identification and simultaneous quantification of six ceramides.**