

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

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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

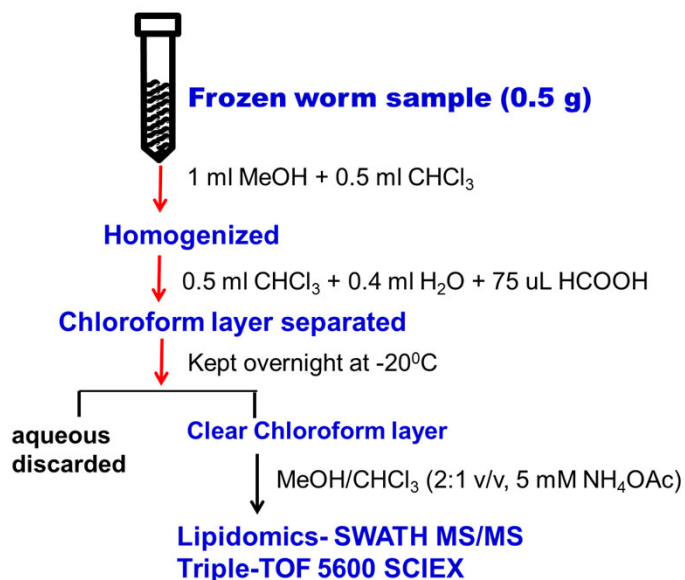
Sciex 5600 Triple-TOF

- 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*

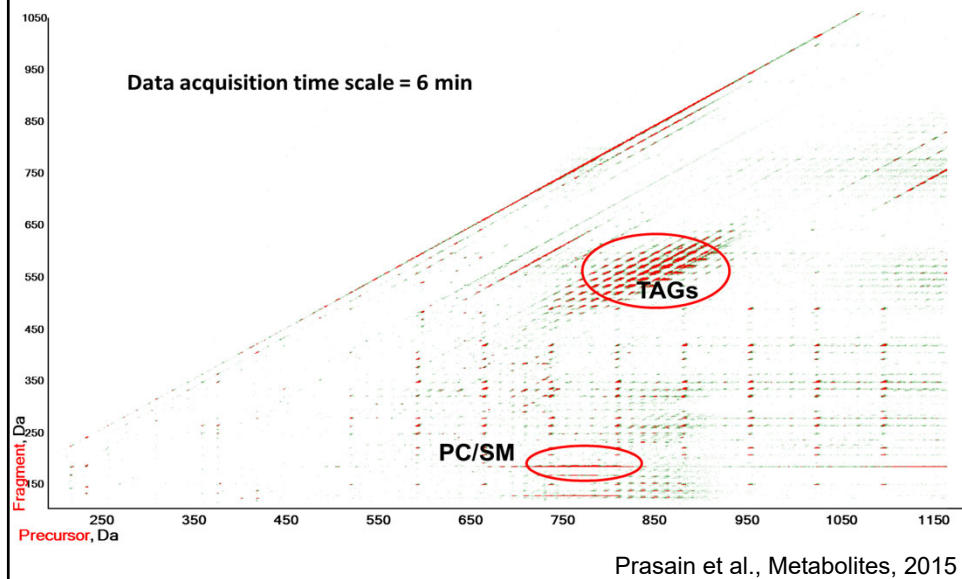


AB Sciex Triple TOF 5600

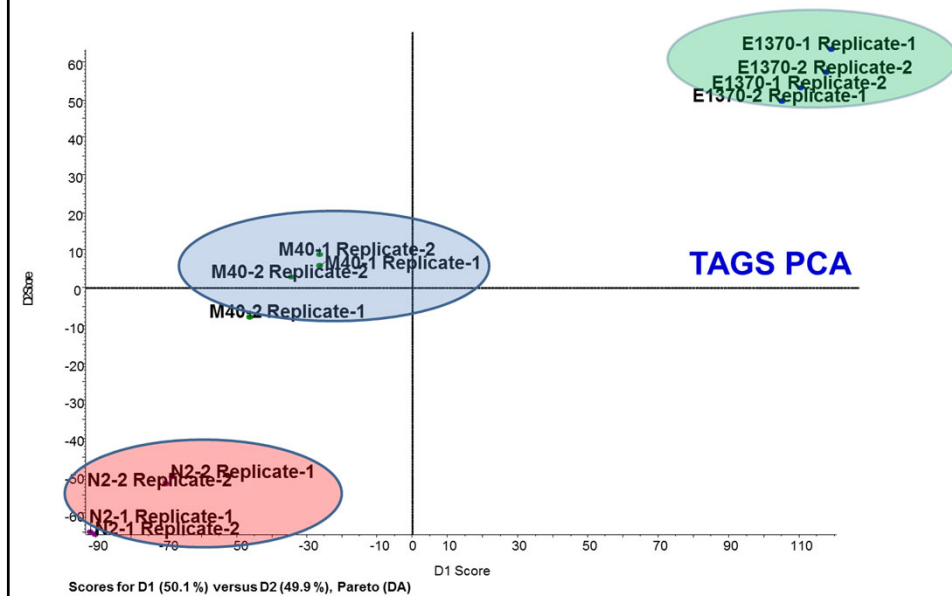
Extraction of lipids and analysis



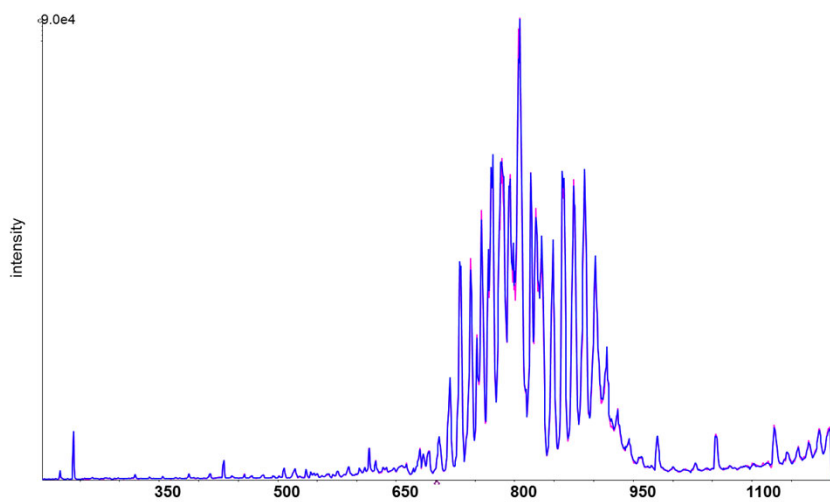
Snapshot of lipidome composition of *C. elegans* by Triple-TOF MS(+ve ion mode)



PCA score plots from wild-type and mutant extracts

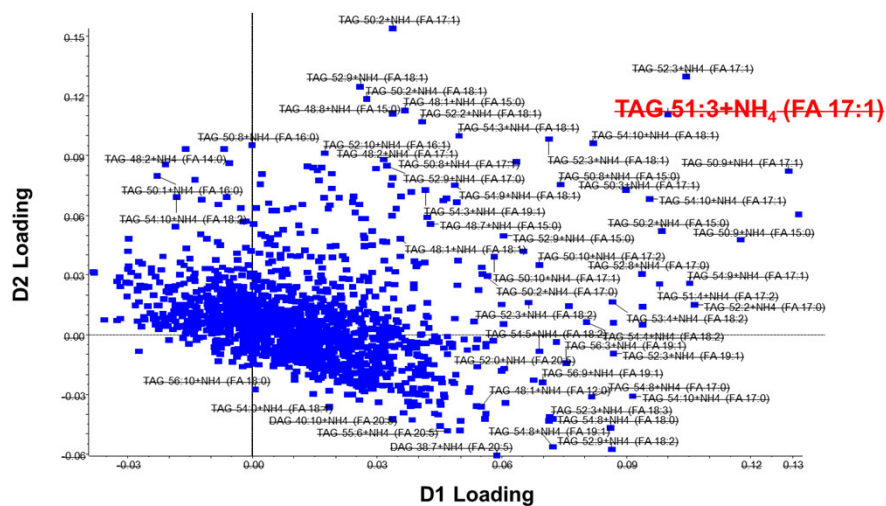


Reproducibility of two replicates of wild type worm extracts in terms of TIC



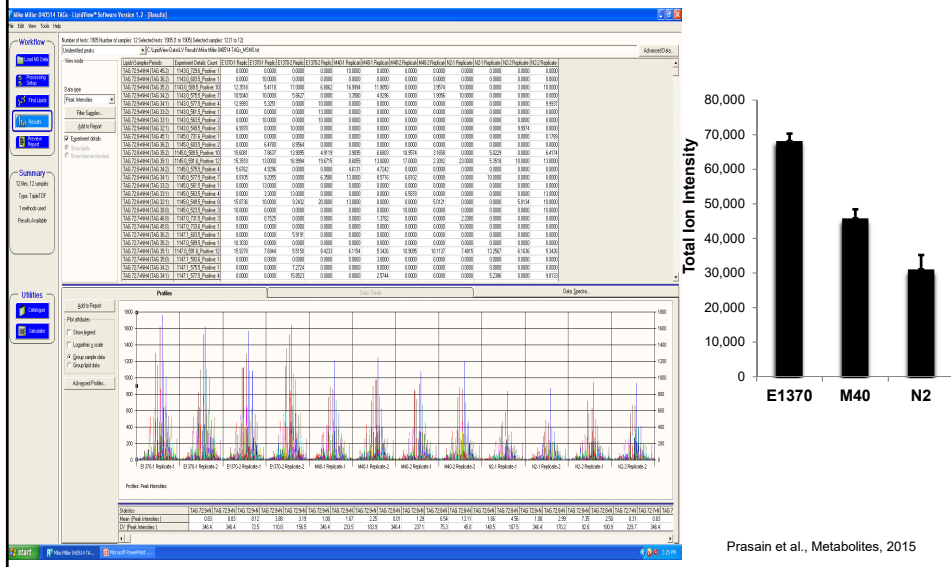
Prasain et al. *Metabolites*, 2015

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



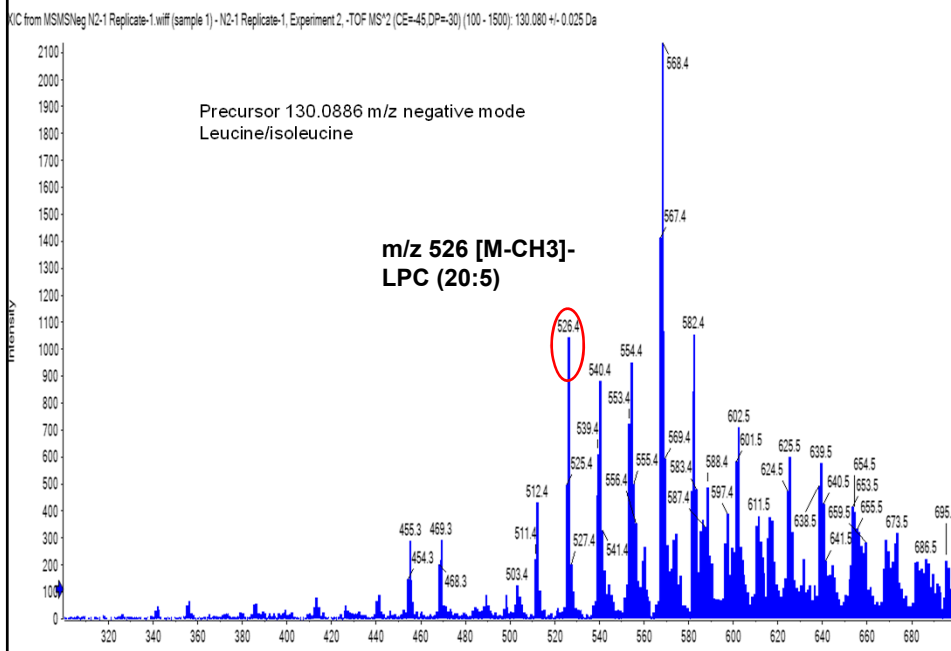
Loadings for D1 (50.0%) versus D2 (50.0%), Pareto (DA)

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

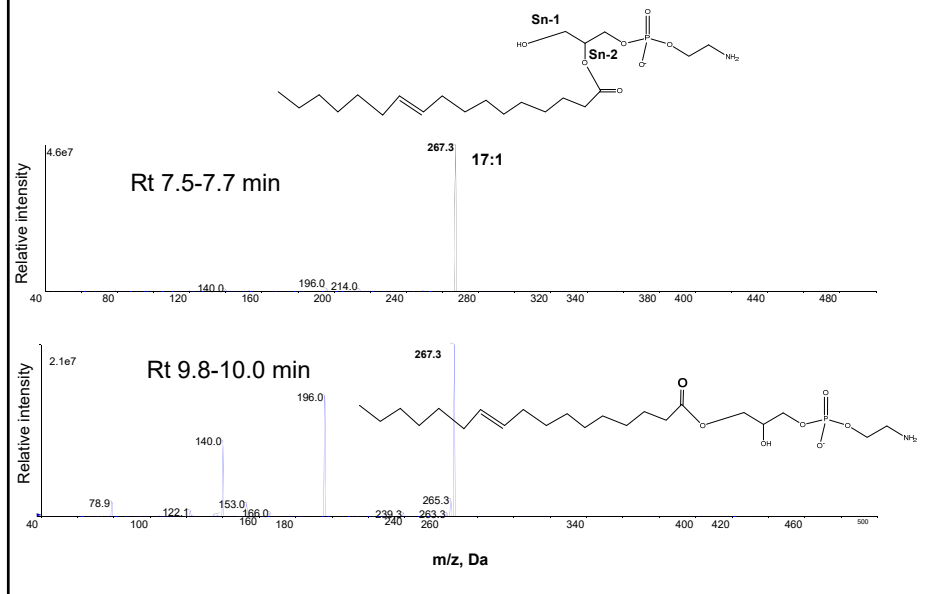


Prasain et al., Metabolites, 2015

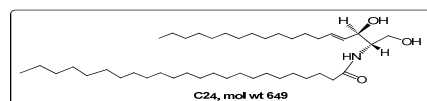
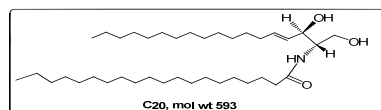
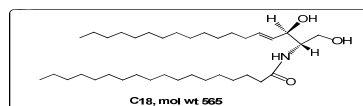
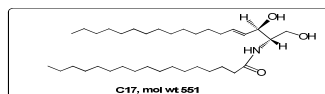
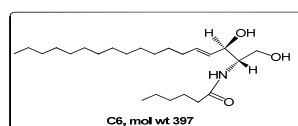
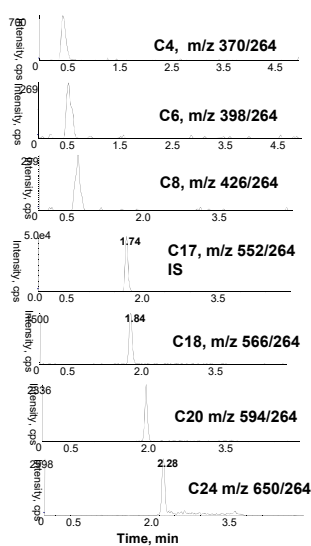
Overlapping isobaric peaks- direct infusion lipidomics



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

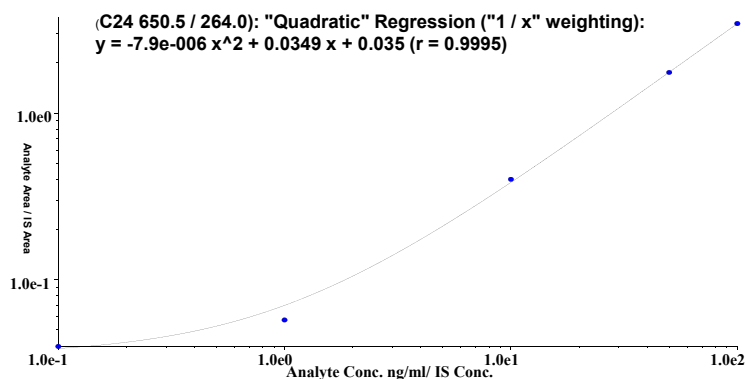


MRM chromatograms showing simultaneous determination of ceramides (C4-C24)

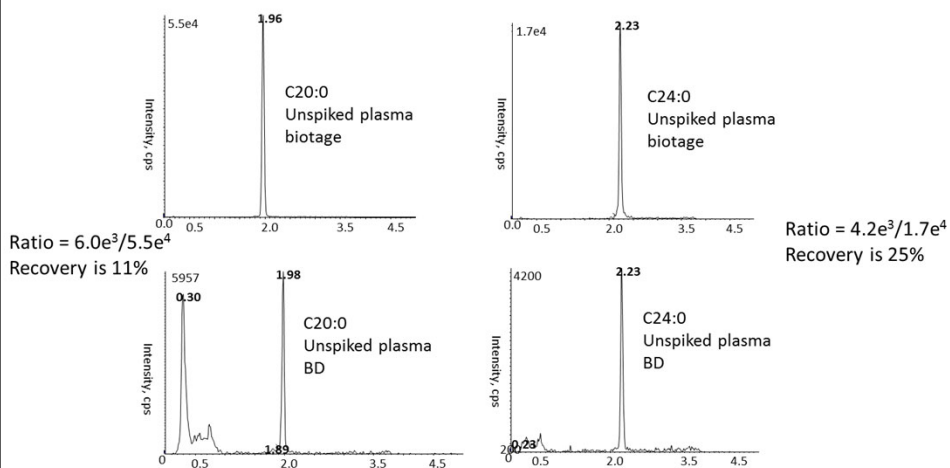


A linear response for Cer C24:0 was observed over a range of 0.1-100 ng/ml with correlation coefficient greater than 0.99

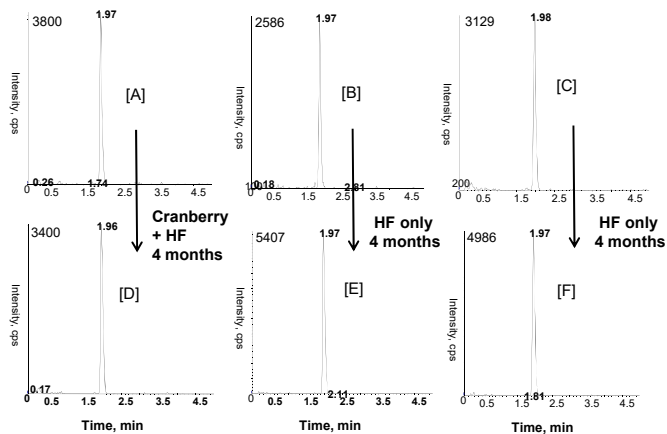
Sample Name	Analyte Peak Name	Calculated Concentration (ng/mL)	Accuracy (%)
Ceramide Standard 100 ng/ml	C24 650.5 / 264.0	100	100
Ceramide Standard 50 ng/ml	C24 650.5 / 264.0	49.8	99.6
Ceramide Standard 10 ng/ml	C24 650.5 / 264.0	10.5	105
Ceramide Standard 1 ng/ml	C24 650.5 / 264.0	0.634	63.4
Ceramide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



**Sample preparation is a crucial step in quantitative analysis of ceramides;
 Poor recoveries of non-polar ceramides in Bligh-Dyer (BD) liquid-liquid
 extraction compared to Biotage (supported liquid extraction)**



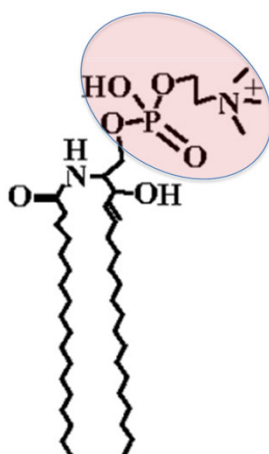
Cranberry fruit powder treatment reduced the HF induced increased levels of Ceramide C20 in rats



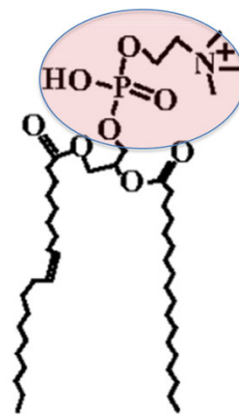
[A]-[C] represent base line plasma ceramide C20 (594/264) from three animals
 [D] after 4 months treatment with cranberry (1 g/kg b. w. and high fat diet
 [E] & [F] after 4 months treatment with high fat diet only

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
 - ¹³C-Isotope profiles overlap
 - Head groups are the same

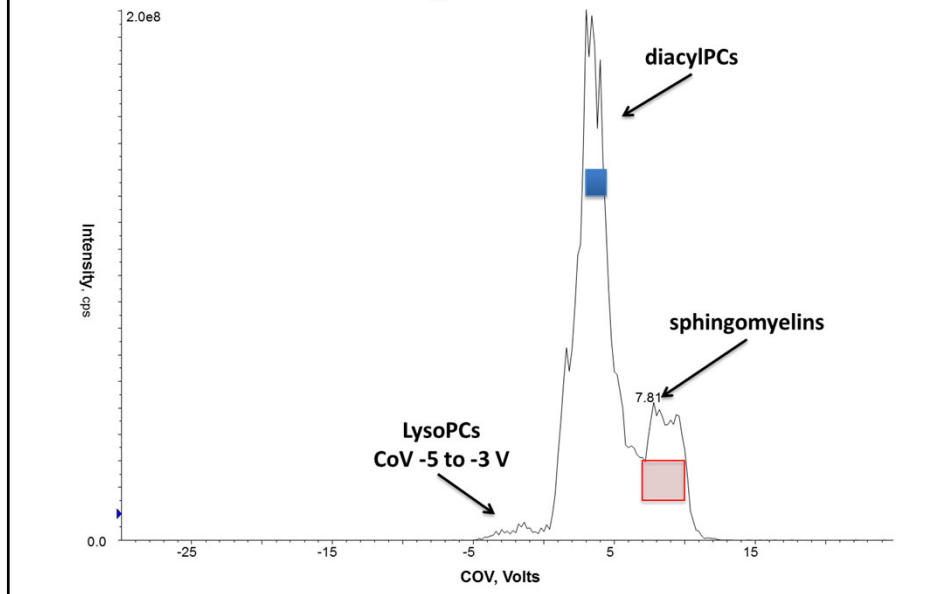


Sphingolipid

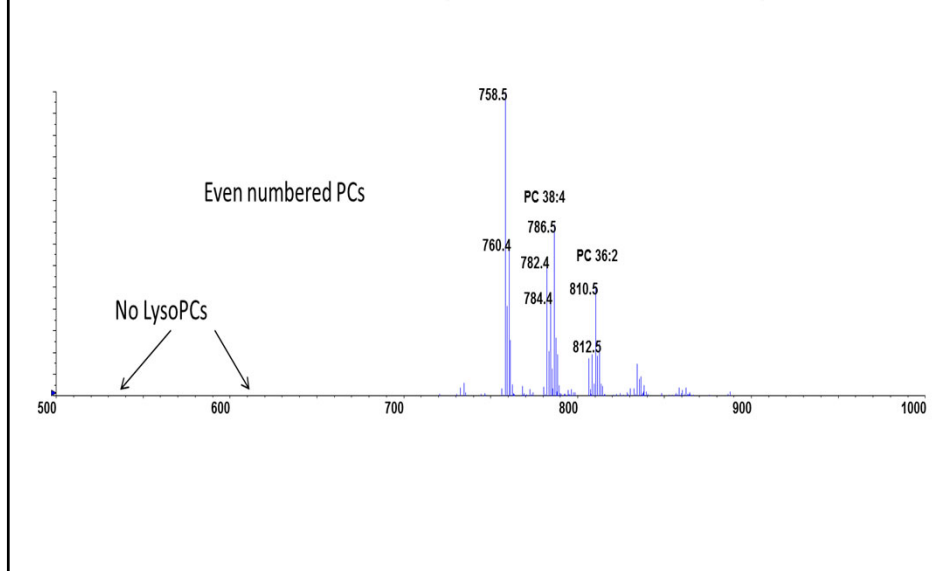


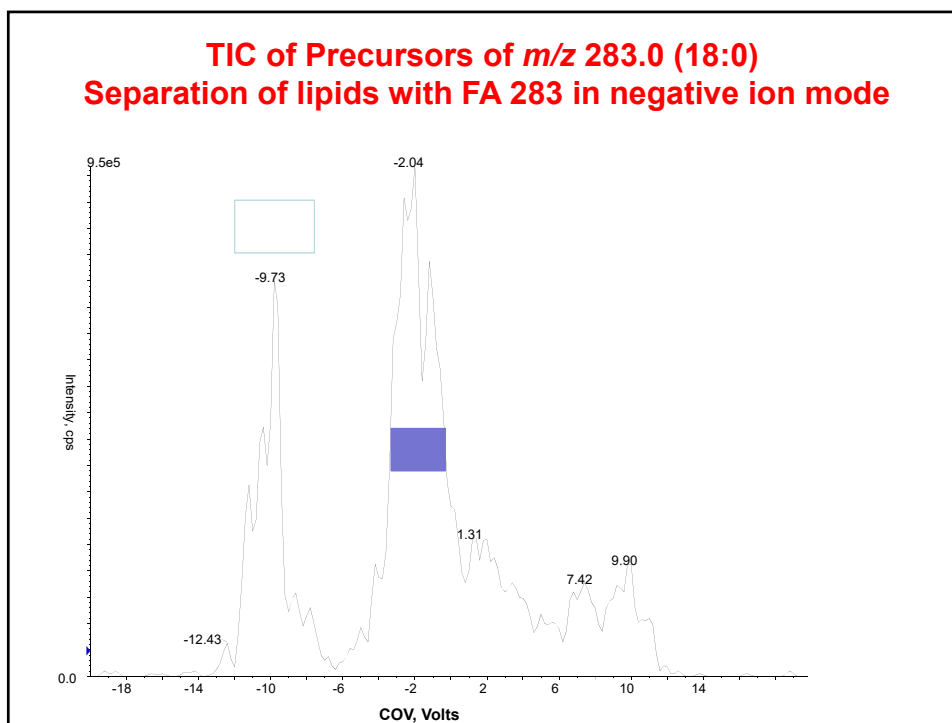
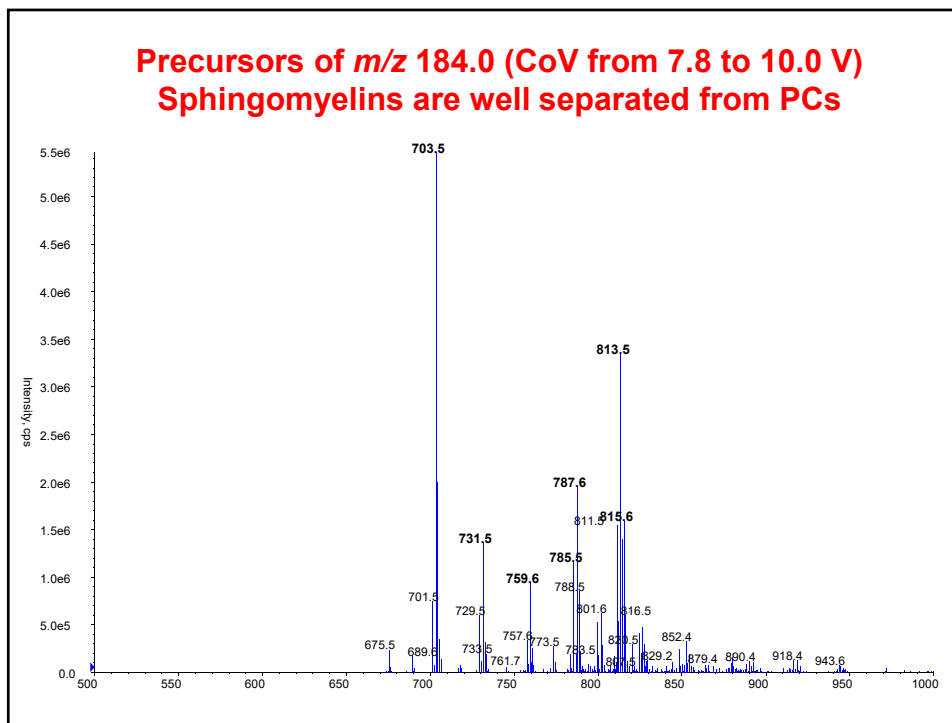
Phosphocholine

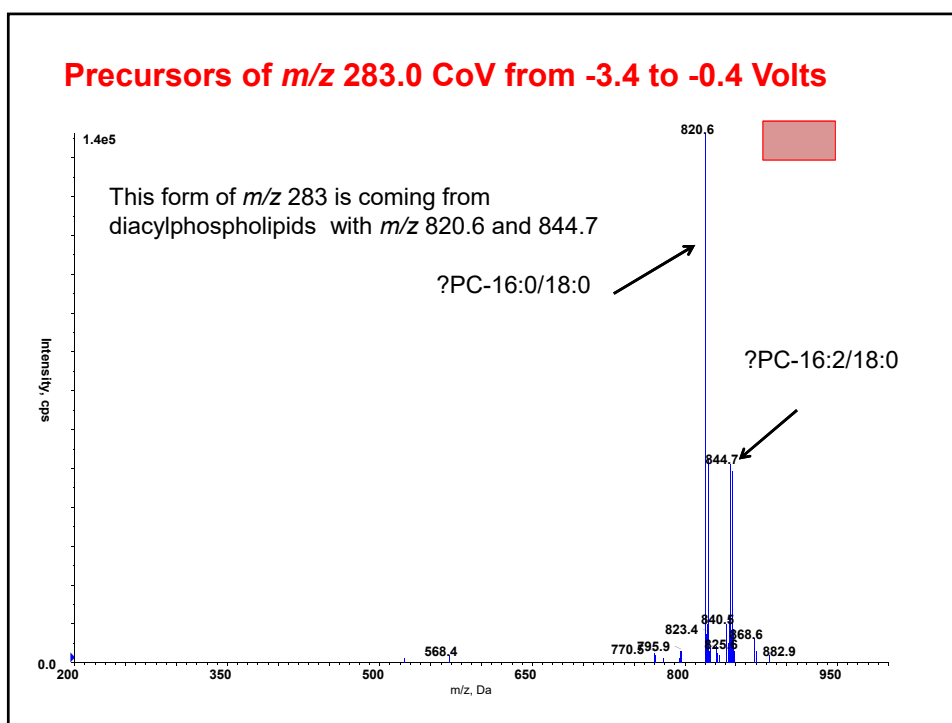
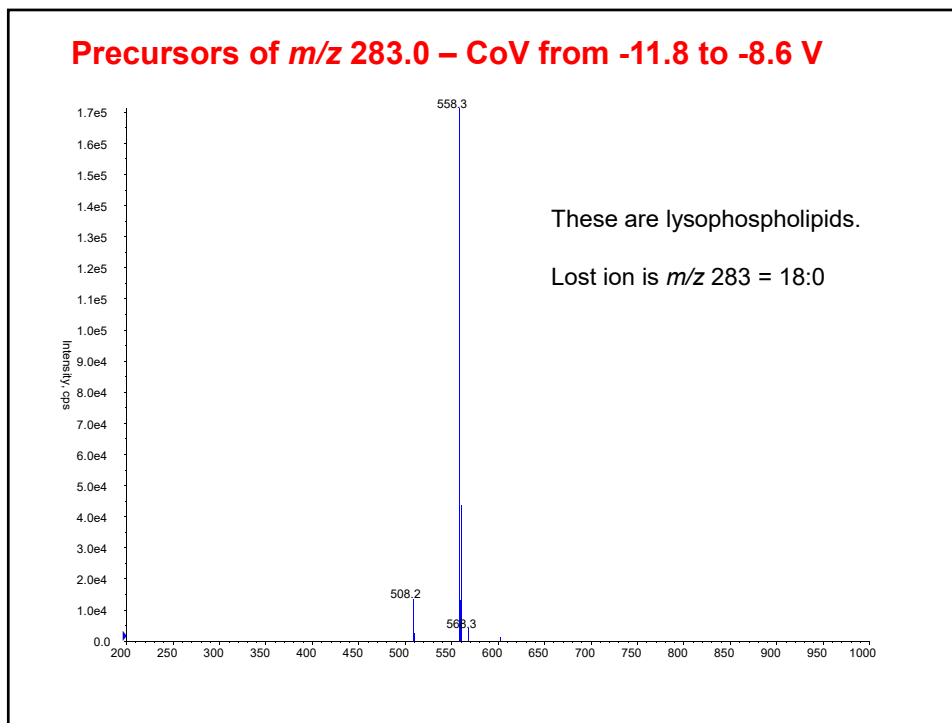
Total ion current of precursors of m/z 184.0 Using Selecion



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







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.**