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

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1

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)

2

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

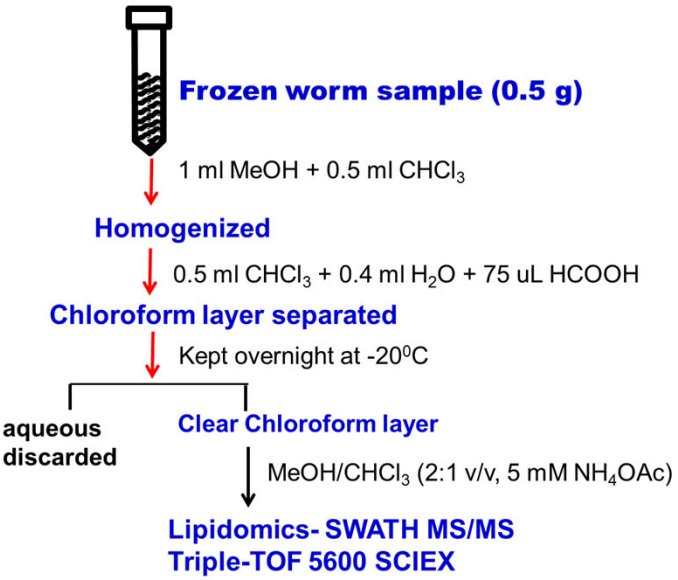
Sciex 5600 Triple-TOF

- **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***

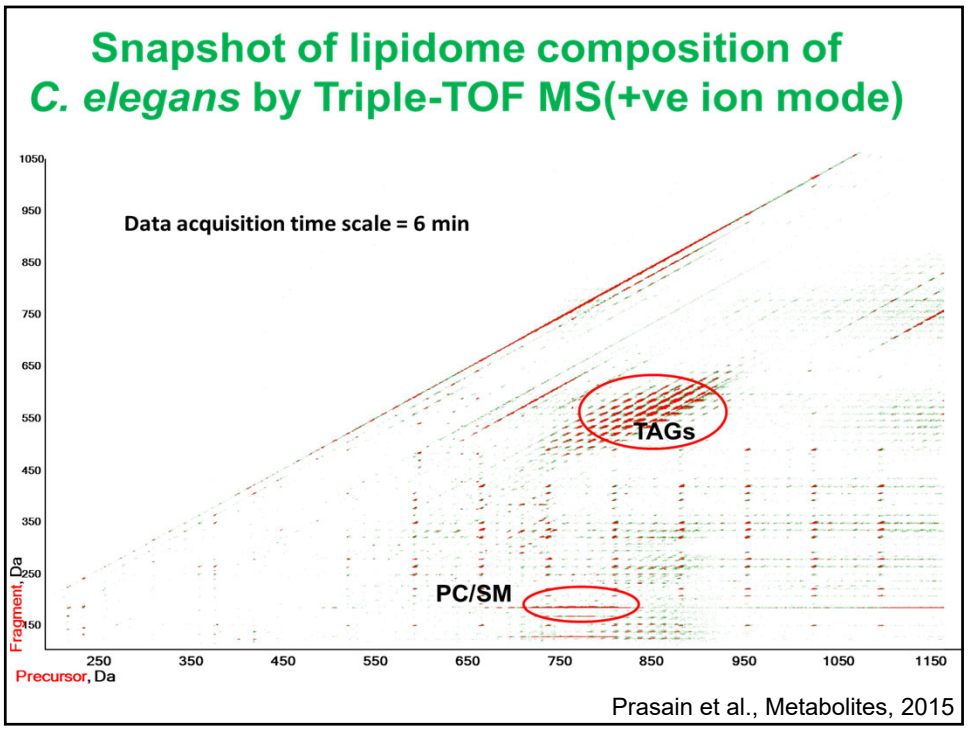
AB Sciex Triple TOF 5600

3

Extraction of lipids and analysis

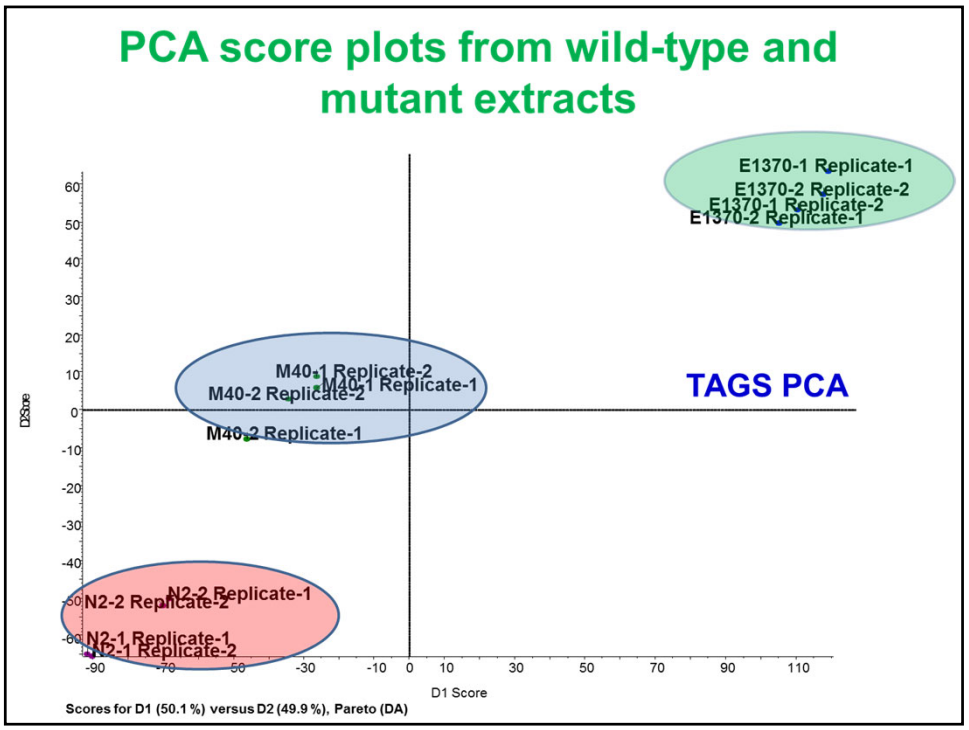


4



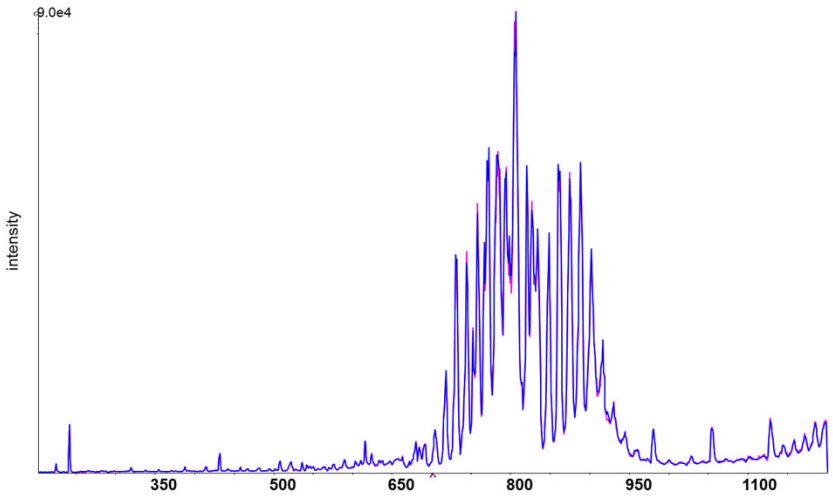
Prasain et al., Metabolites, 2015

5



6

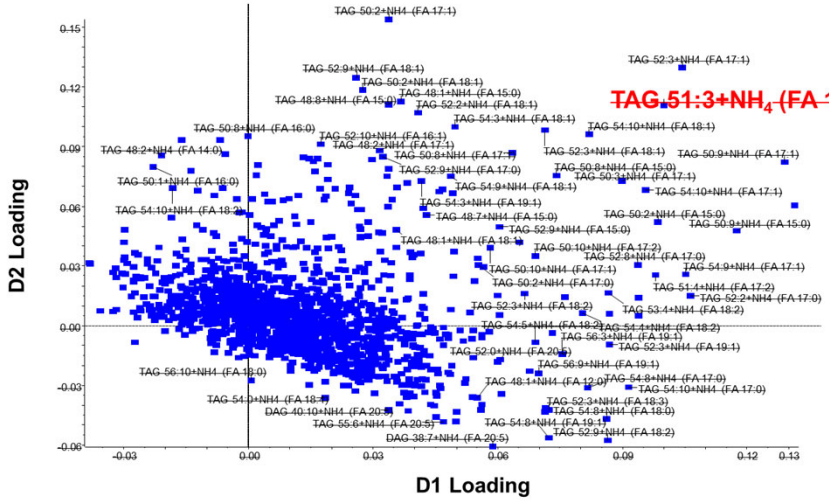
Reproducibility of two replicates of wild type worm extracts in terms of TIC



Prasain et al. Metabolites, 2015

7

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

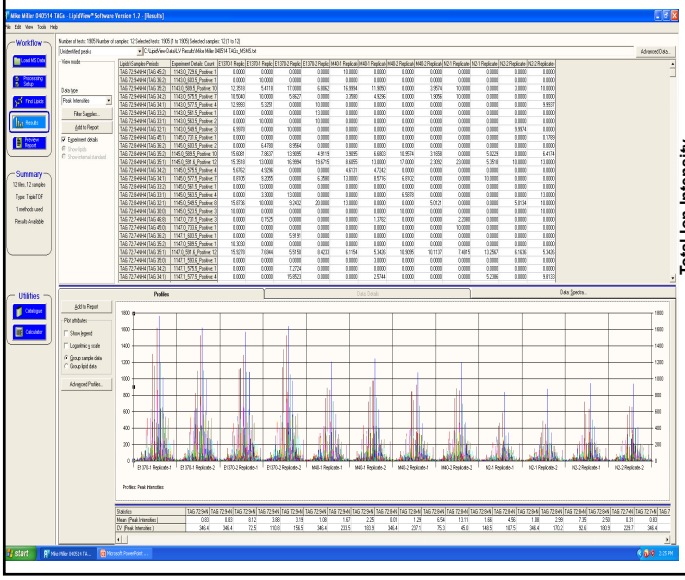


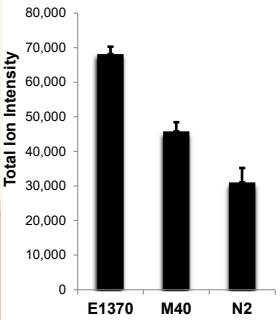
TAG 51:3+NH_4 (FA 17:1)

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

8

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

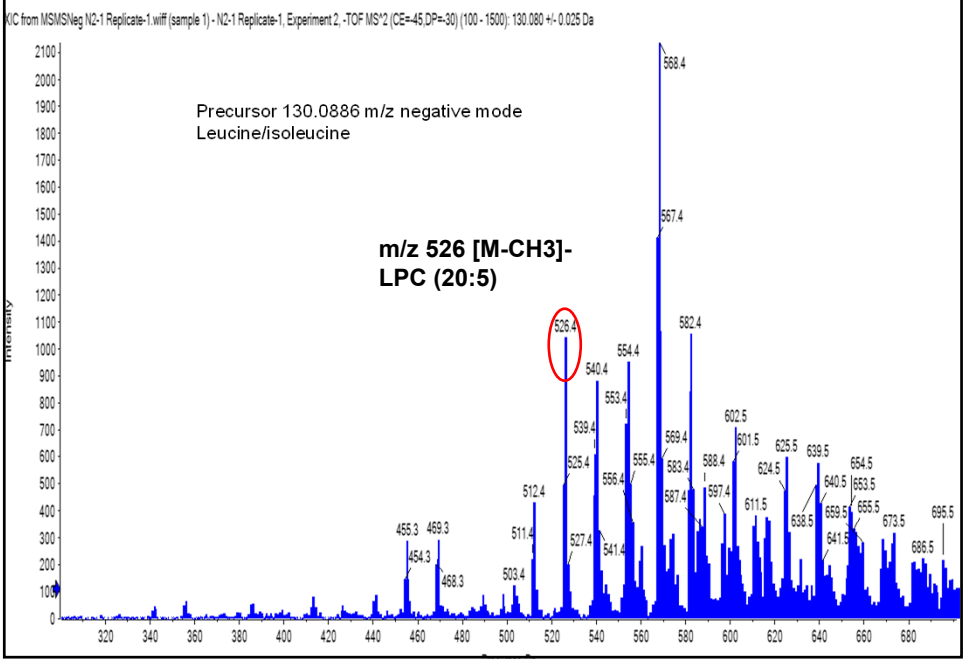




Prasain et al., Metabolites, 2015

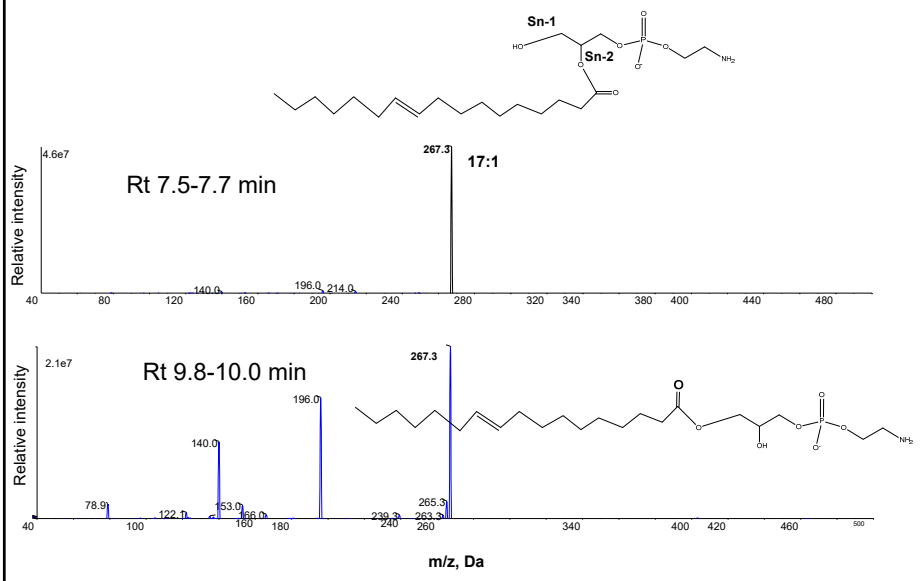
9

Overlapping isobaric peaks- direct infusion lipidomics



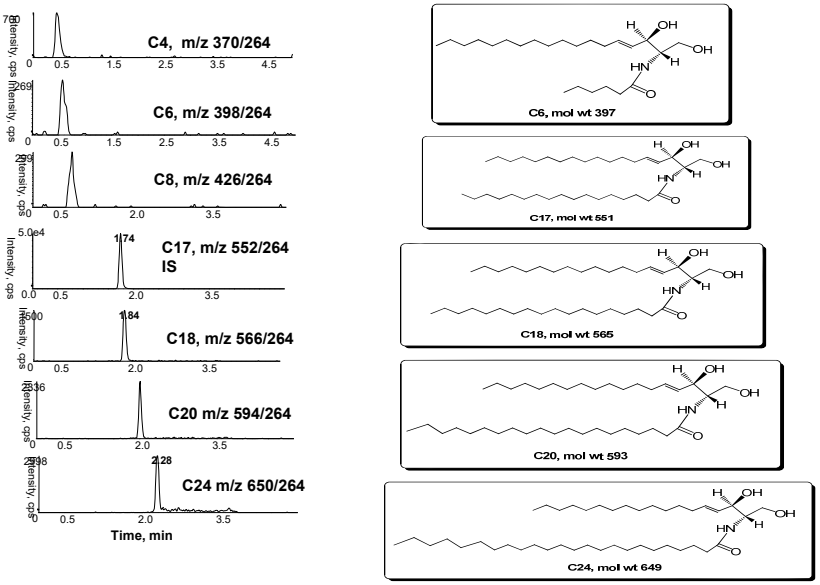
10

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



11

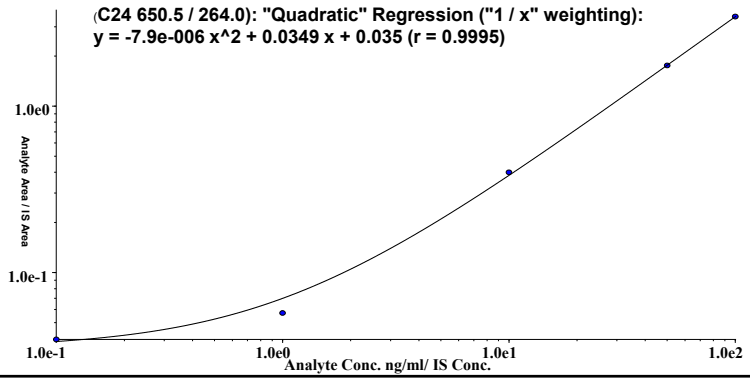
MRM chromatograms showing simultaneous determination of ceramides (C4-C24)



12

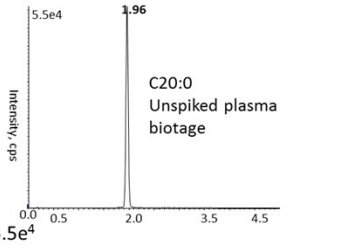
A linear response for Cer C24:0 was observed over a range of 0.1-100 ng/ml with correlation coefficient greater than 0.99

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

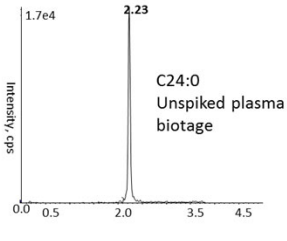


13

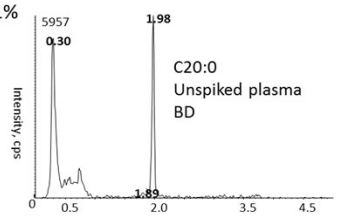
Sample preparation is a crucial step in quantitative analysis of ceramides; Poor recoveries of non-polar ceramides in Bligh-Dyer (BD) liquid-liquid extraction compared to Biotage (supported liquid extraction)

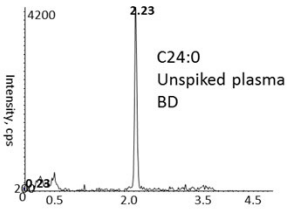


Ratio = $6.0e^3/5.5e^4$
Recovery is 11%



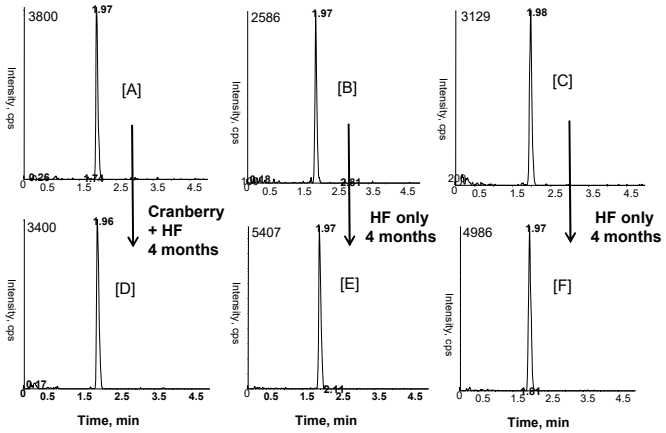
Ratio = $4.2e^3/1.7e^4$
Recovery is 25%





14

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

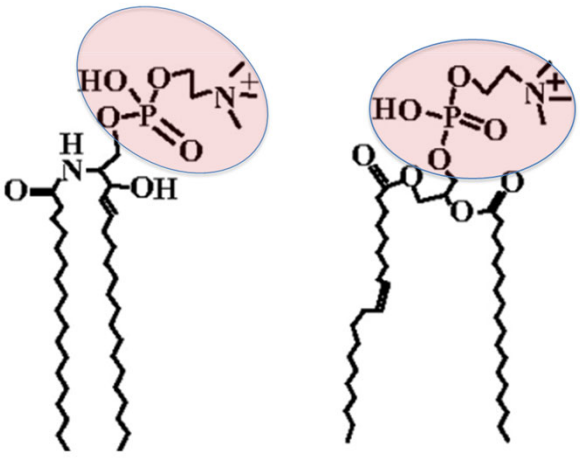


[A]-[C] represent base line plasma ceramide C20 (594/264) from three animals
[D] after 4 months treatment with cranberry (1 g/kg b. w. and high fat diet
[E] & [F] after 4 months treatment with high fat diet only

15

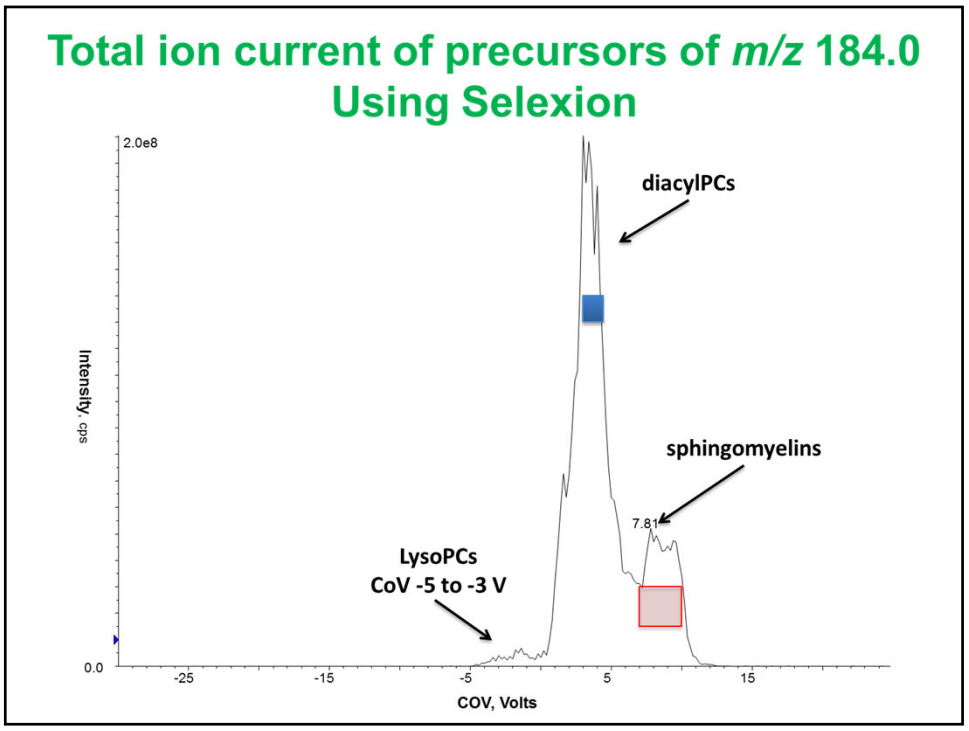
The problem of analyzing lipids

- 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

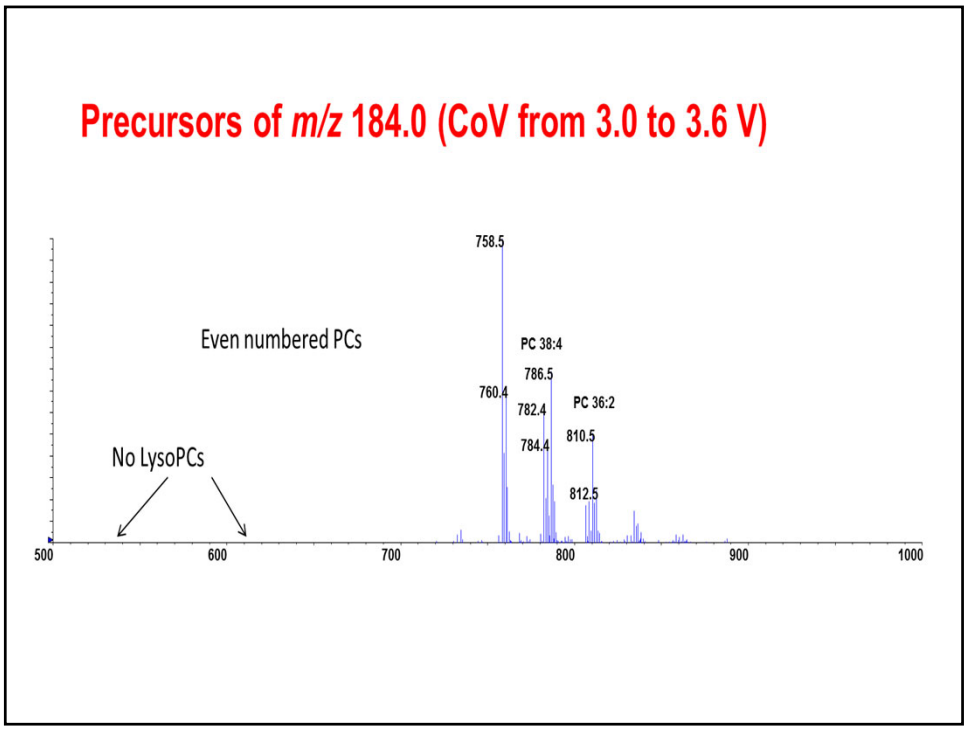


Sphingolipid Phosphocholine

16

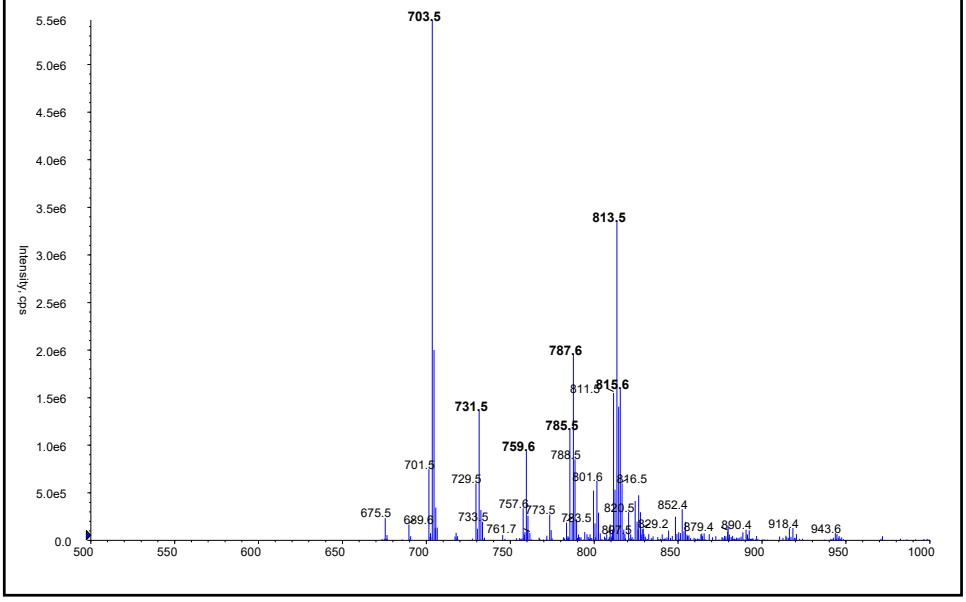

Total ion current of precursors of m/z 184.0
Using Selexion
2.0e8
Intensity, cps
0.0
diacylPCs
sphingomyelins
7.81
LysoPCs
CoV -5 to -3 V
-25
-15
-5
5
15
COV, Volts

17


Precursors of m/z 184.0 (CoV from 3.0 to 3.6 V)
758.5
Even numbered PCs
PC 38:4
786.5
760.4
782.4
PC 36:2
784.4
810.5
No LysoPCs
812.5
500
600
700
800
900
1000

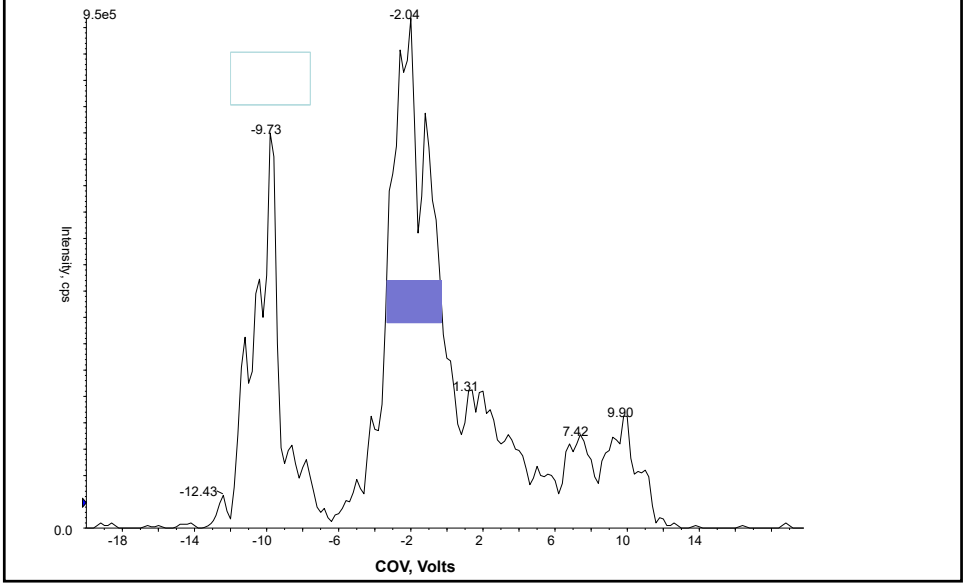
18

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



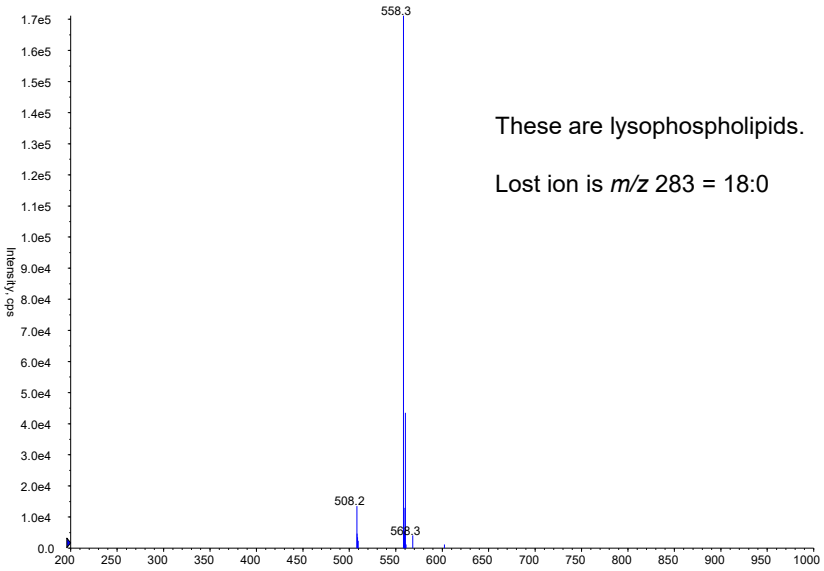
19

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



20

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

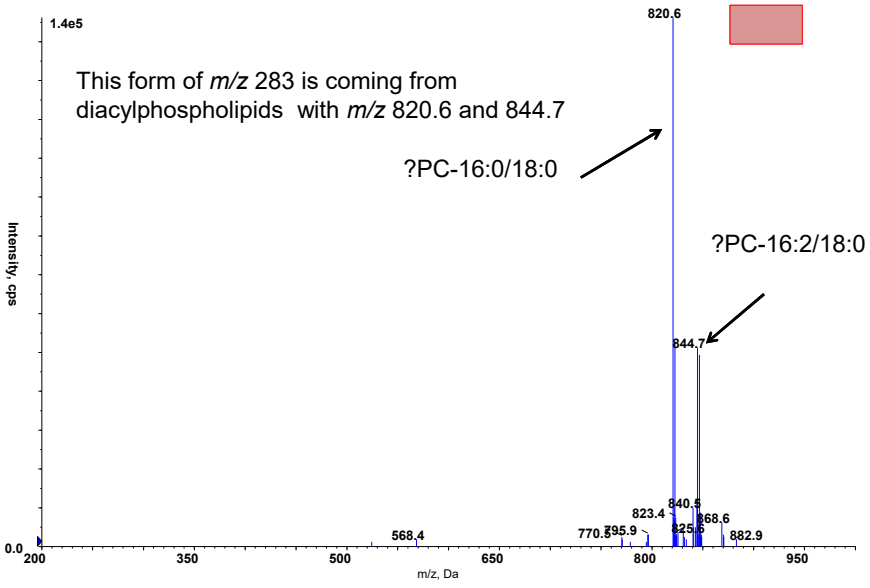


These are lysophospholipids.

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

21

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



This form of *m/z* 283 is coming from diacylphospholipids with *m/z* 820.6 and 844.7

?PC-16:0/18:0

?PC-16:2/18:0

22

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.**

23