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

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Data acquisition in mass spectrometry-

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
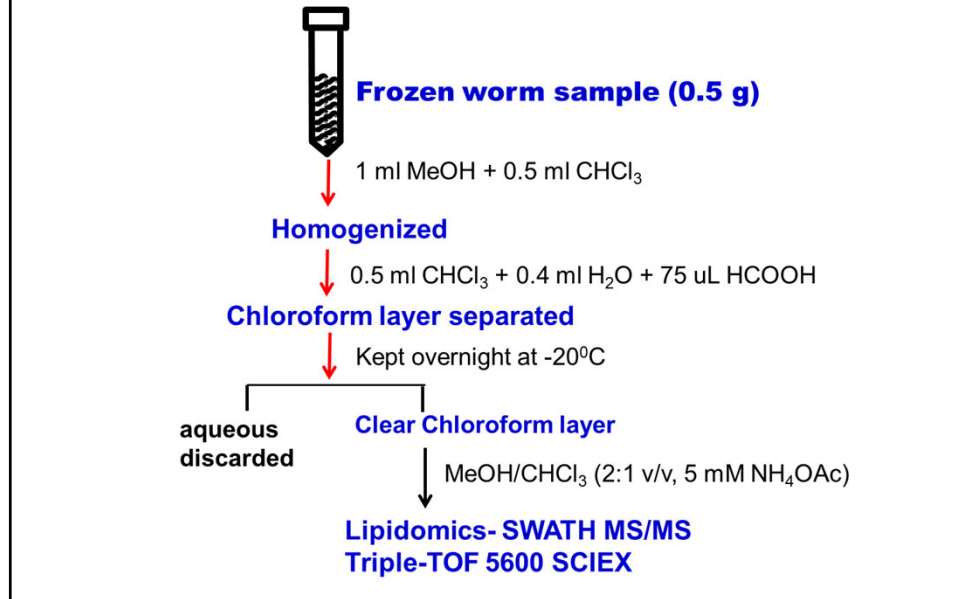
Sciex 5600 Triple-TOF

- **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***
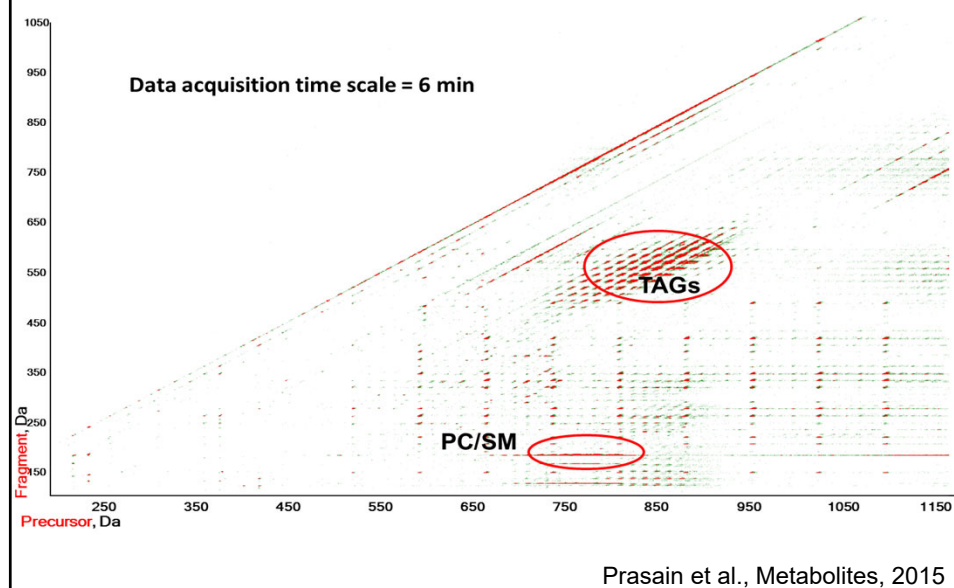

AB Sciex Triple TOF 5600
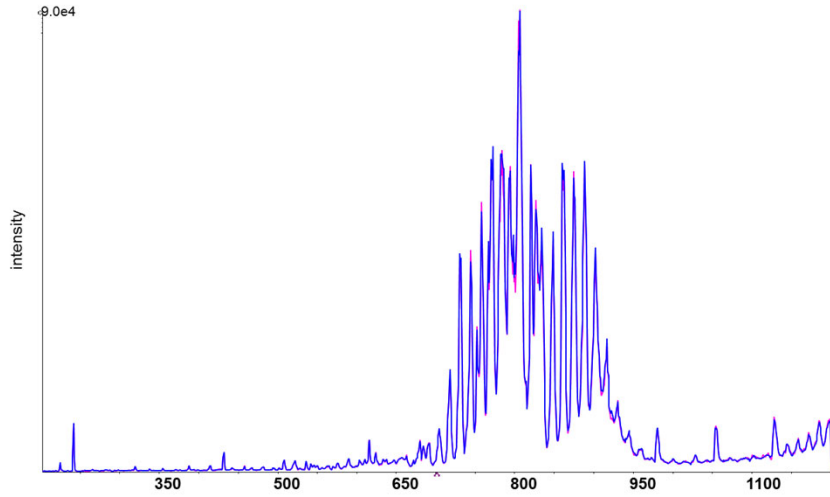
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Extraction of lipids and analysis

Frozen worm sample (0.5 g)

1 ml MeOH + 0.5 ml CHCl$_3$

Homogenized

0.5 ml CHCl$_3$ + 0.4 ml H$_2$O + 75 uL HCOOH

Chloroform layer separated

Kept overnight at -20^{0}C

aqueous discarded

Clear Chloroform layer

MeOH/CHCl$_3$ (2:1 v/v, 5 mM NH$_4$OAc)

Lipidomics- SWATH MS/MS
Triple-TOF 5600 SCIEX

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

Data acquisition time scale = 6 min

TAGs

PC/SM

Fragment, Da

Precursor, Da
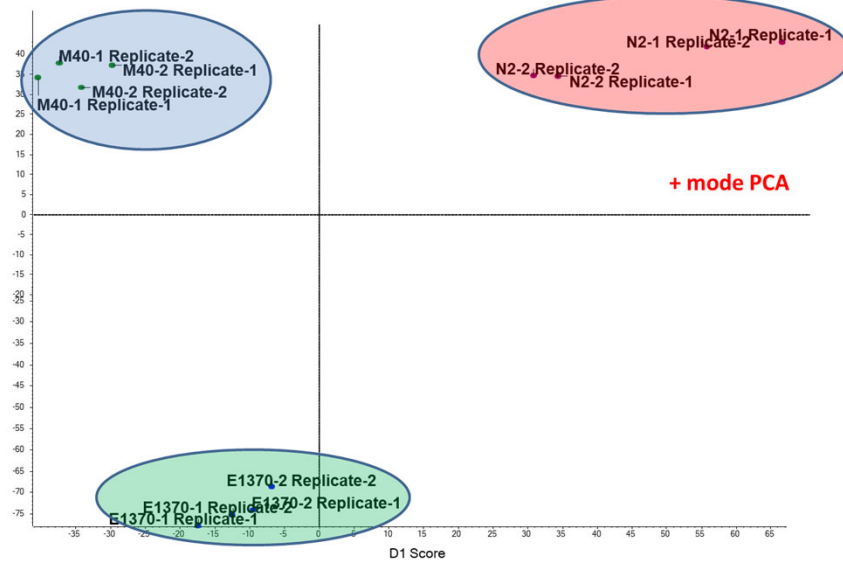
Prasain et al., Metabolites, 2015

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

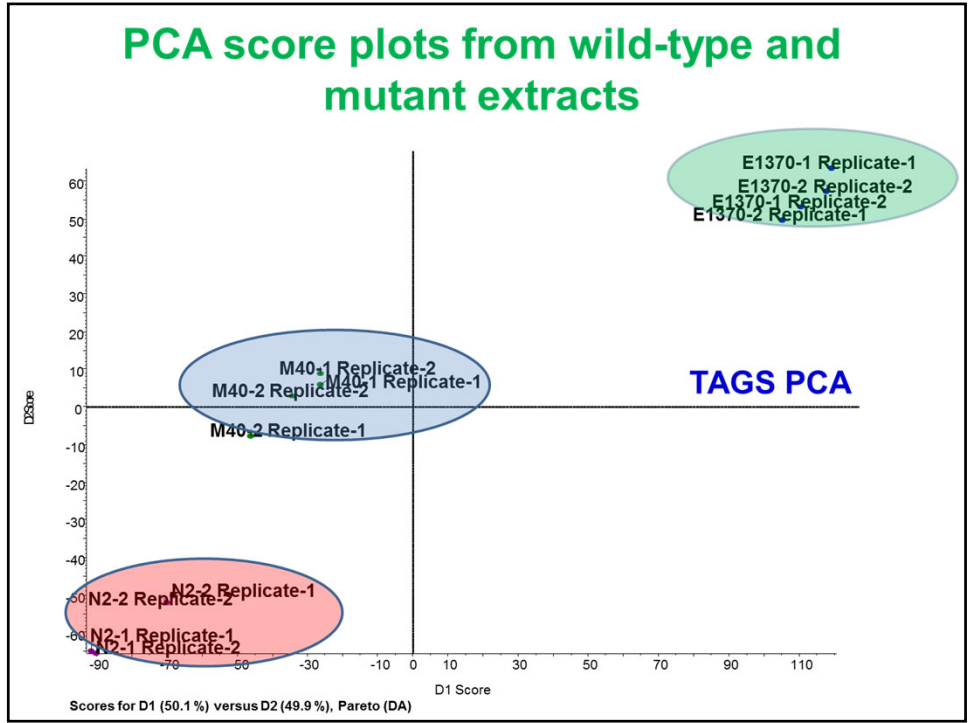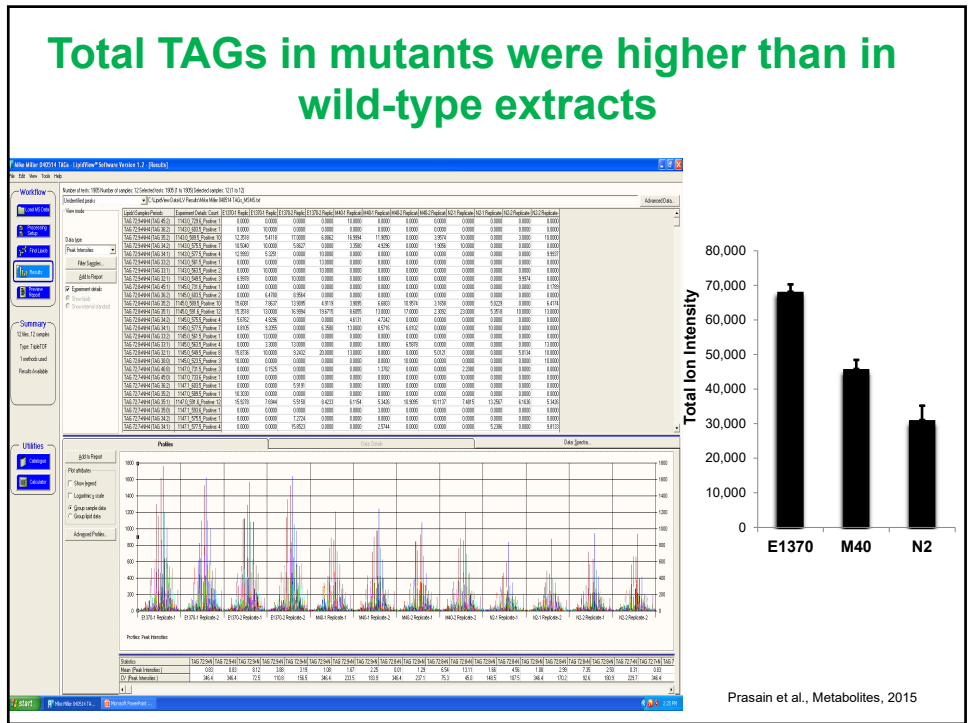*Prasain et al. Metabolites, 2015*

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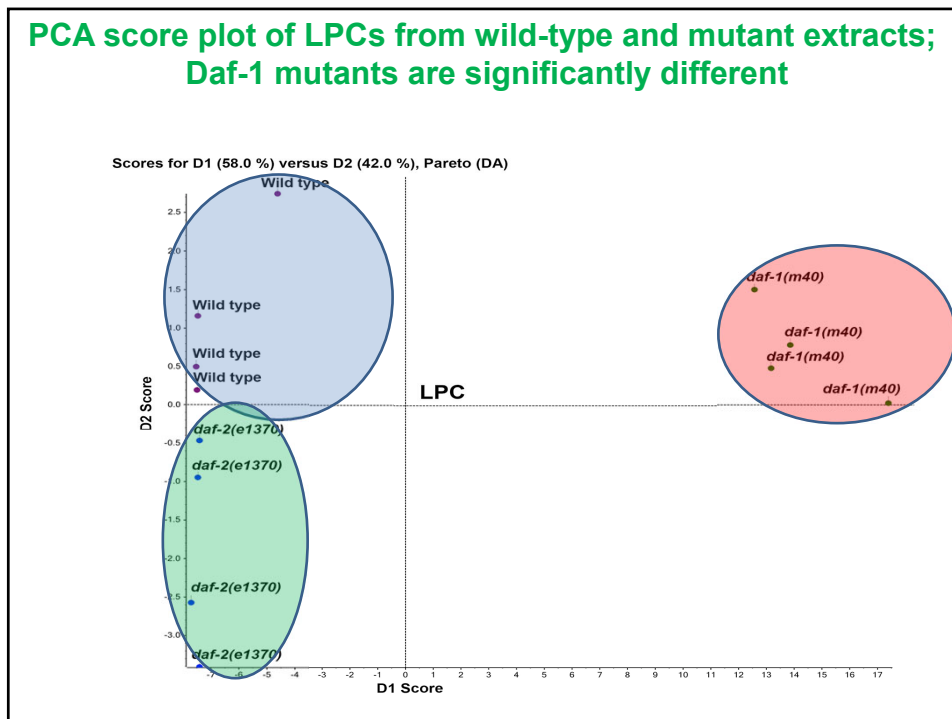# Good separation of groups and reproducibility within groups

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PCA score plots from wild-type and mutant extracts

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

Prasain et al., Metabolites, 2015

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

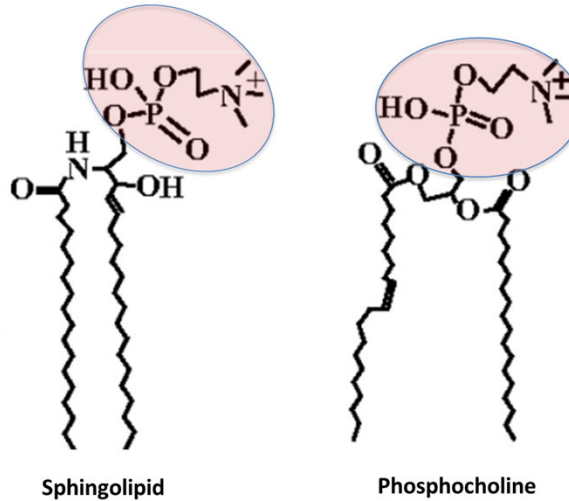Scores for D1 (58.0 %) versus D2 (42.0 %), Pareto (DA)

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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
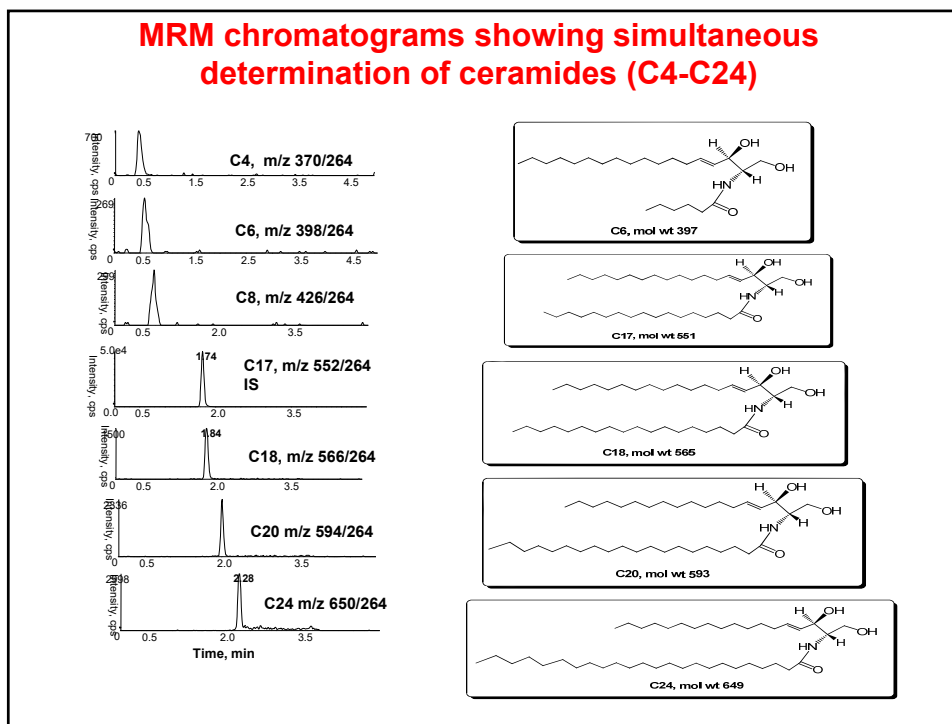 - Head groups are the same

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

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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

(C24 650.5 / 264.0): "Quadratic" Regression ("1 / x" weighting):
$y = -7.9\text{e-}006\, x^2 + 0.0349\, x + 0.035\ (r = 0.9995)$

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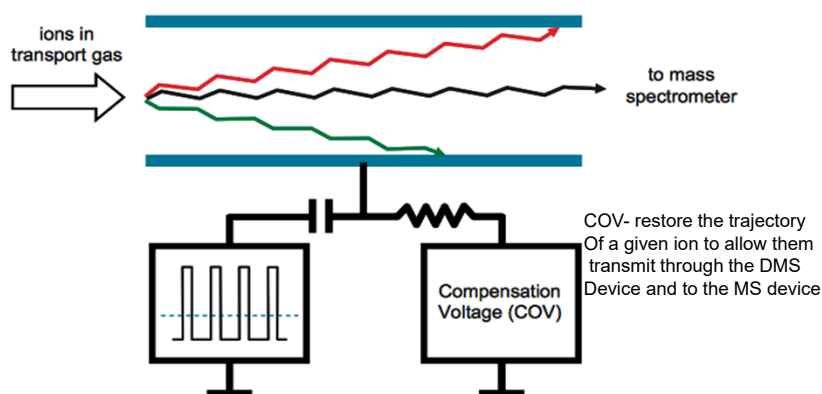
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Differential mobility mass spectrometry

- 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

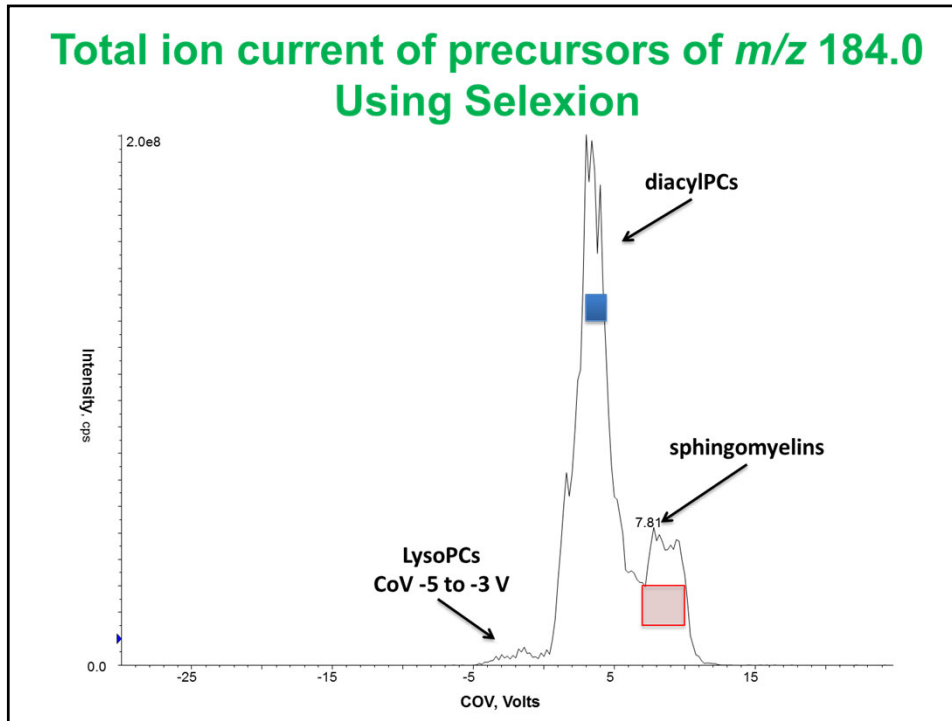**Innovative Planar Design; SelexION™ Ion Mobility Cell.**

ions in transport gas

to mass spectrometer

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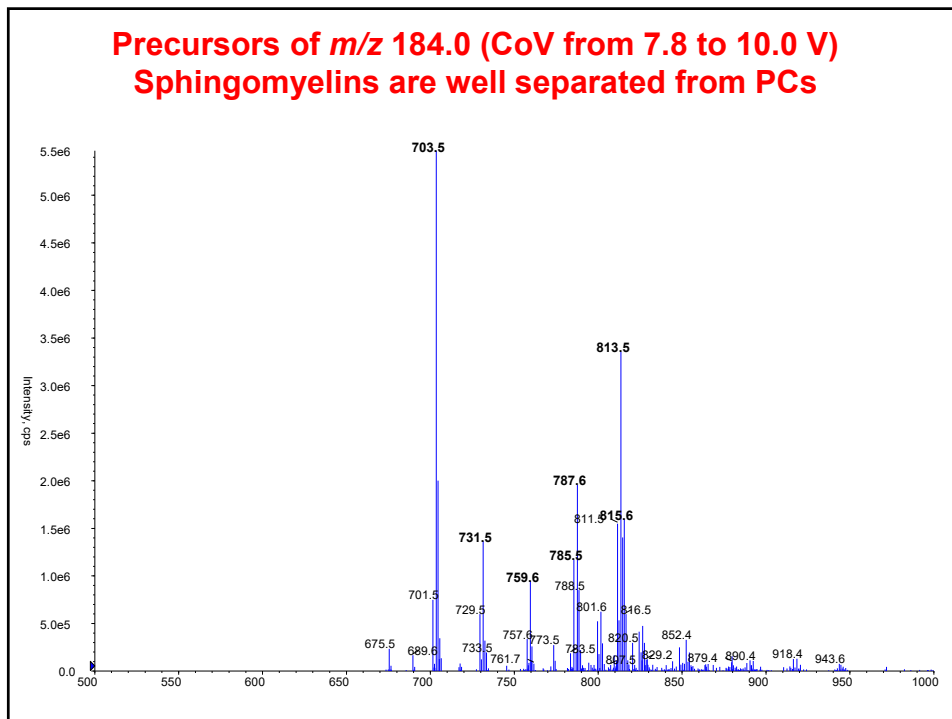
Compensation Voltage (COV)

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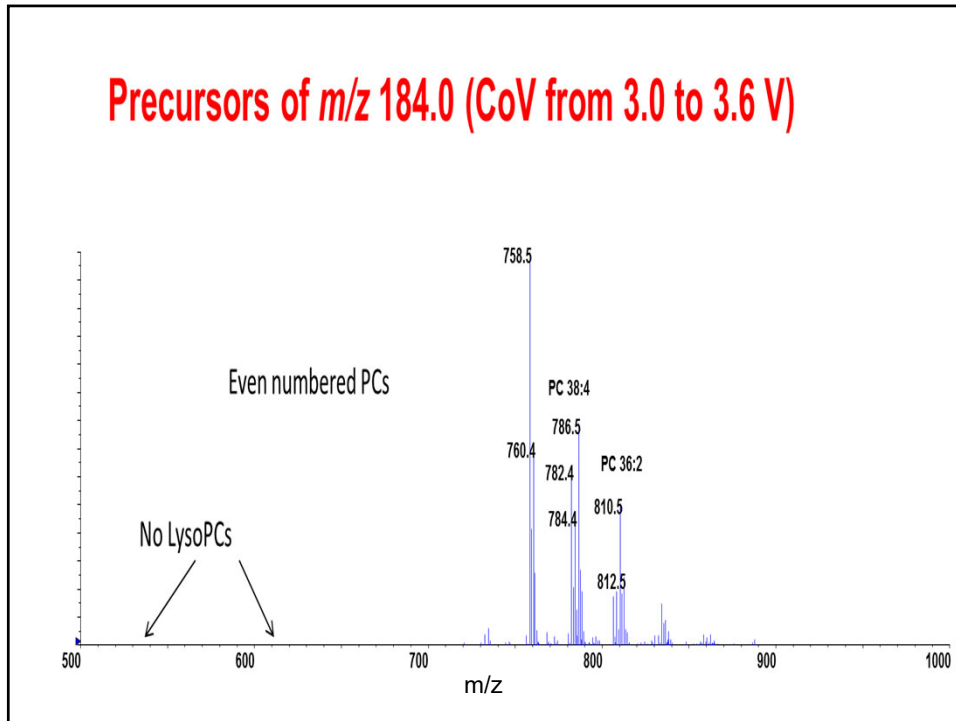
AB SCIEX

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

diacylPCs

sphingomyelins

7.81

LysoPCs
CoV -5 to -3 V

Intensity, cps

2.0e8

0.0

-25   -15   -5   5   15

COV, Volts

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

703.5

813.5

787.6

811.5 815.6

785.5

731.5

759.6

701.5

788.5

729.5

801.6 816.5

675.5 689.6 733.5 757.8 773.5 783.5 820.5 852.4
761.7   801.5 829.2 879.4 890.4 918.4 943.6

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

Even numbered PCs

No LysoPCs

758.5
760.4
782.4
784.4
786.5
PC 38:4
PC 36:2
810.5
812.5

m/z

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

9.5e5
-2.04
-9.73
1.31
7.42
9.90
-12.43
0.0

Intensity, cps

COV, Volts

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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

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Precursors of *m/z* 283.0 – CoV from -11.8 to -8.6 V
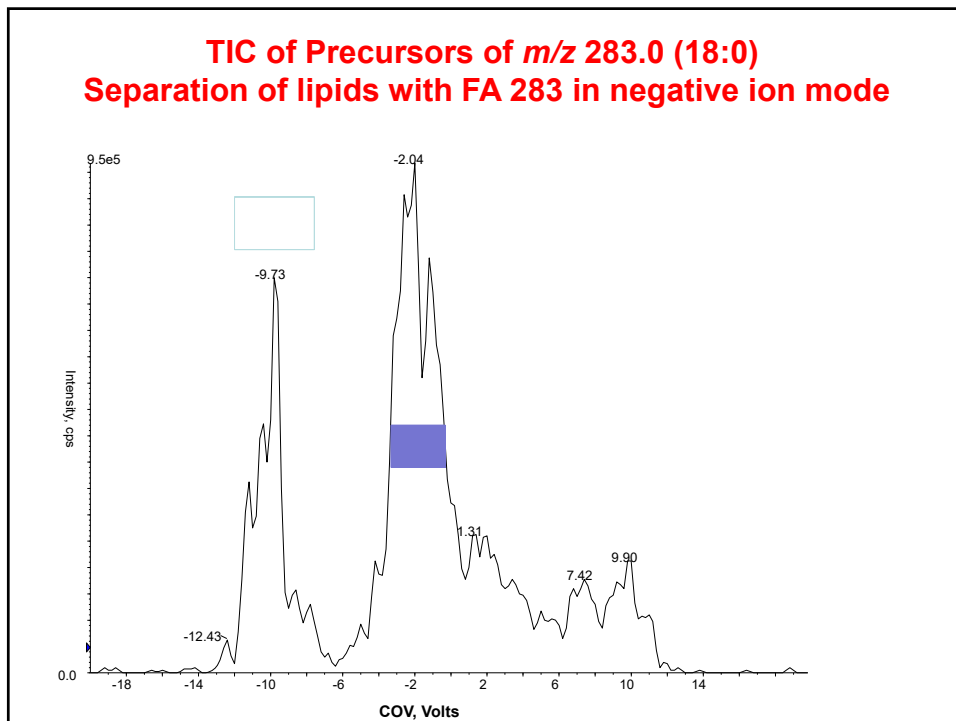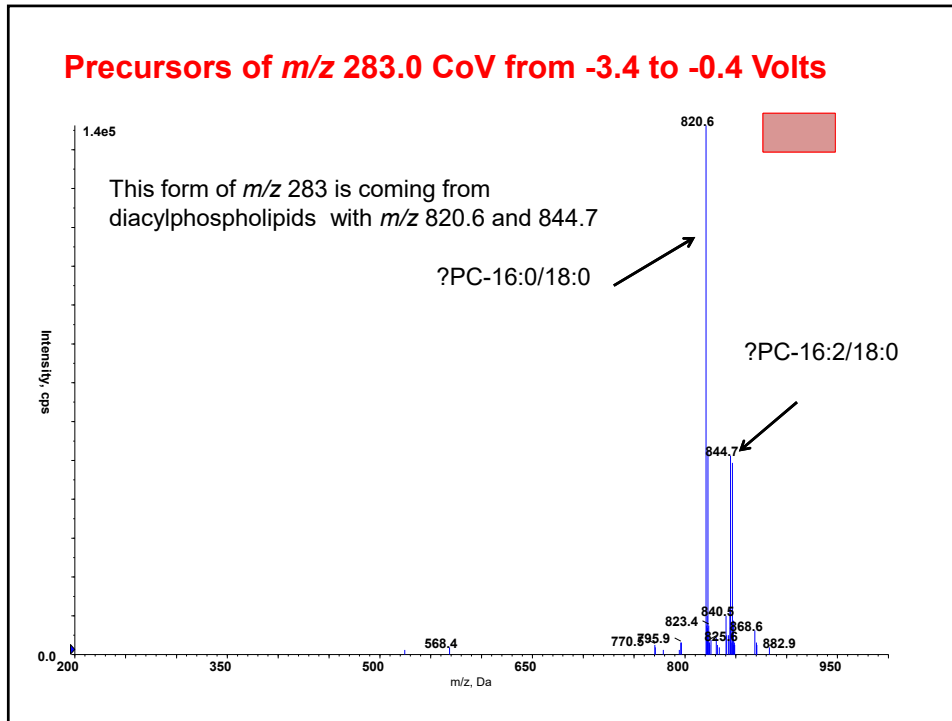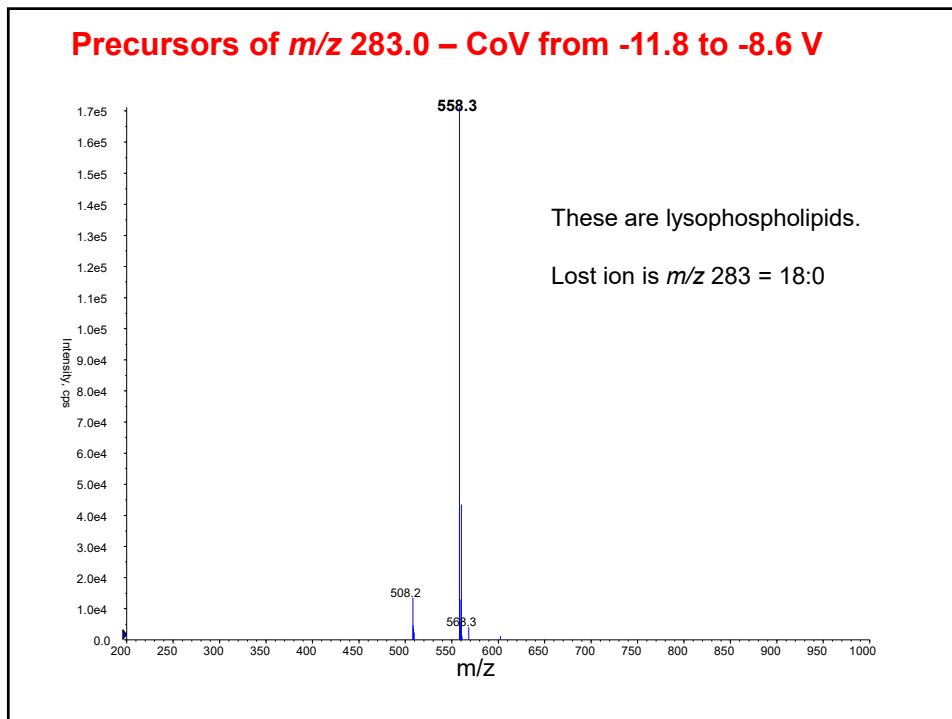
These are lysophospholipids.

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

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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.**

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