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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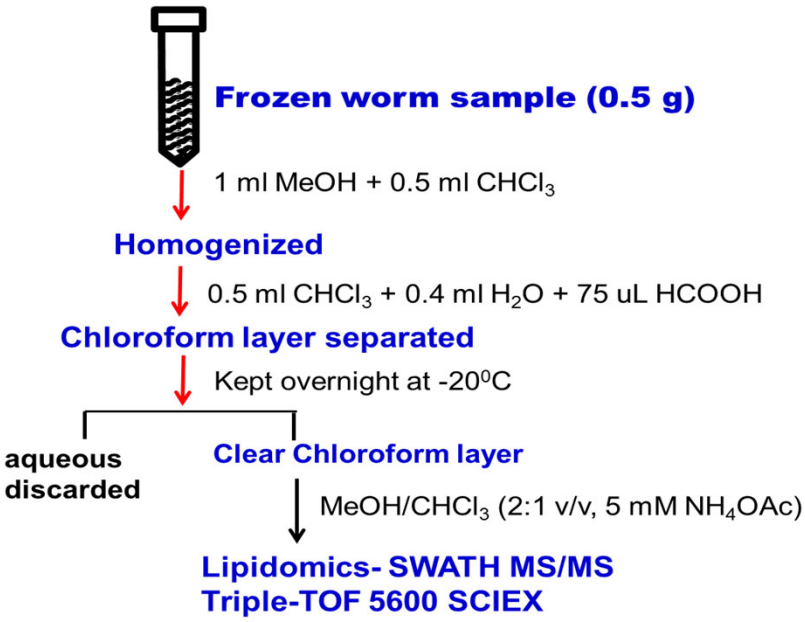
Extraction of lipids and analysis



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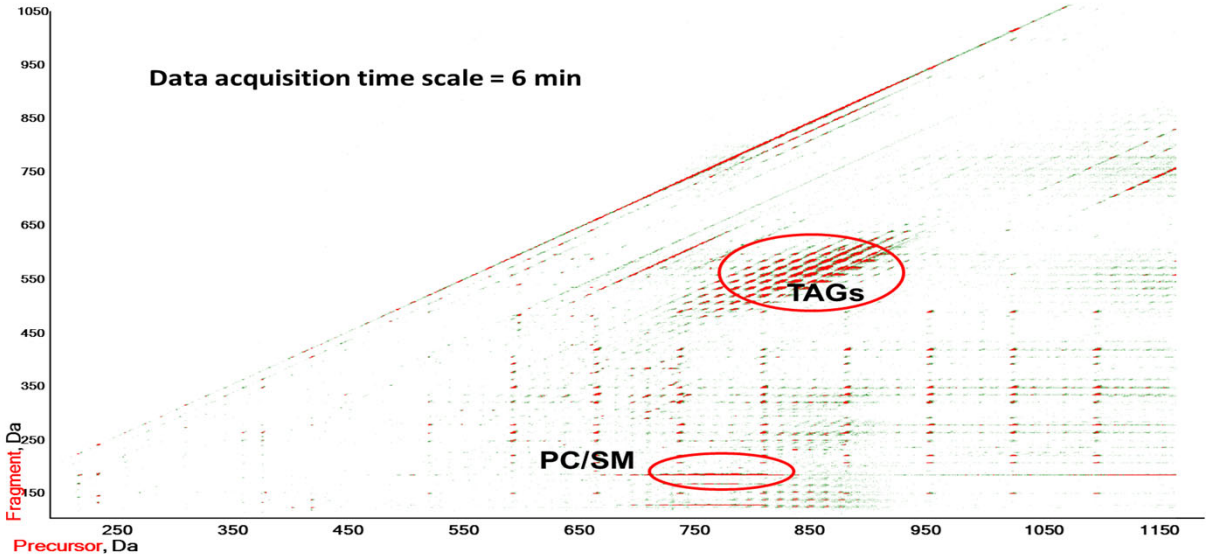
Snapshot of lipidome composition of *C. elegans* by Triple-TOF MS(+ve ion mode)



Prasain et al., Metabolites, 2015

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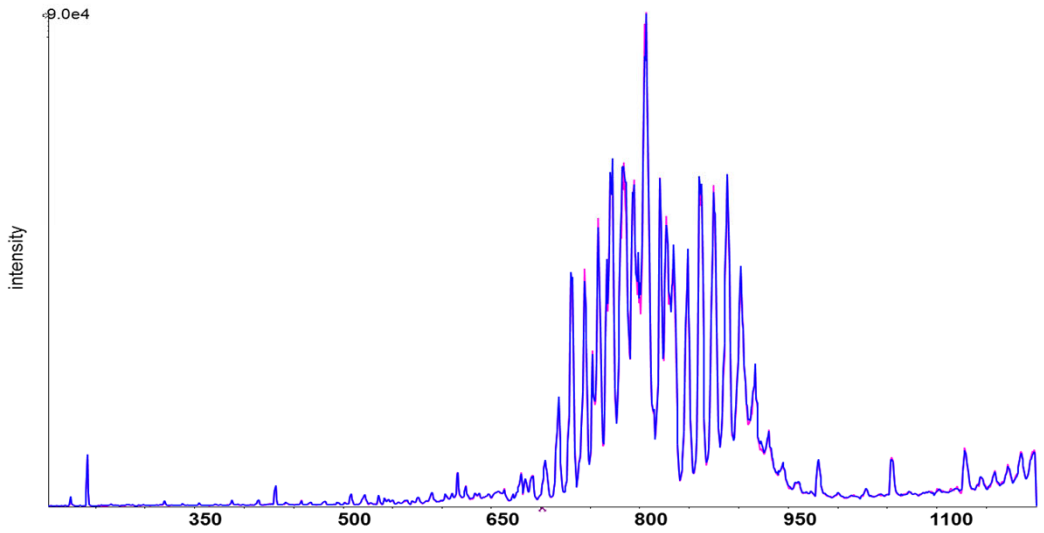
Reproducibility of two replicates of wild type worm extracts in terms of TIC



Prasain et al. Metabolites, 2015

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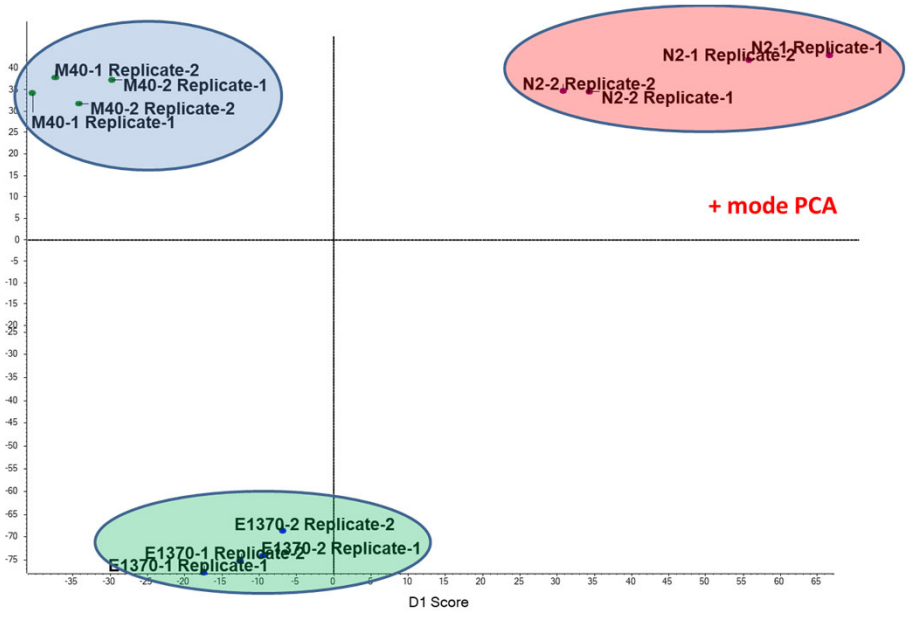
Good separation of groups and reproducibility within groups



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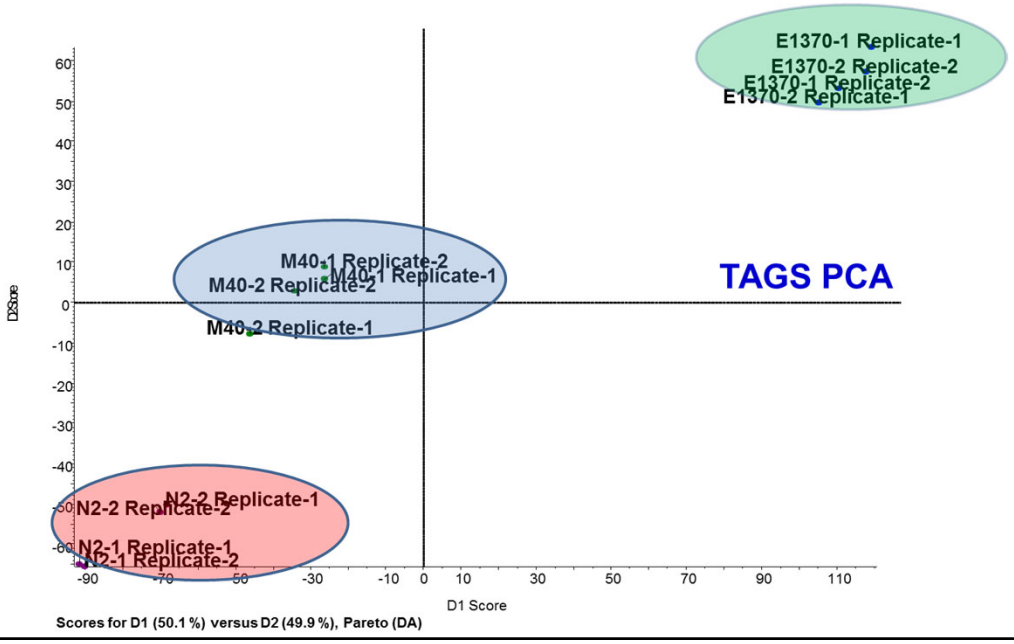
PCA score plots from wild-type and mutant extracts



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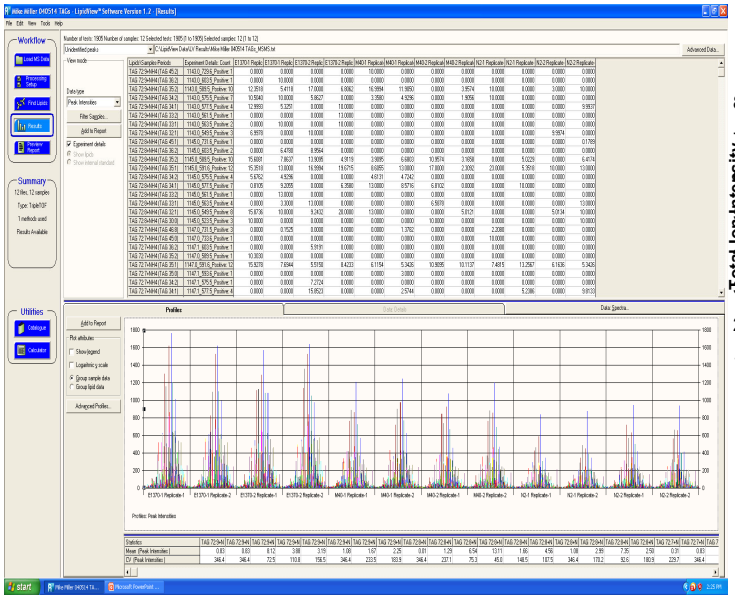
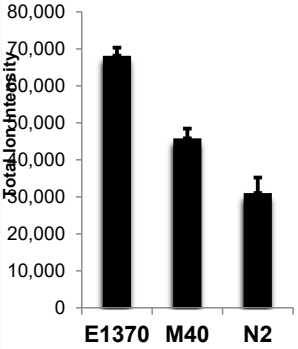
Total TAGs in mutants were higher than in wild-type extracts





Prasain et al., Metabolites, 2015

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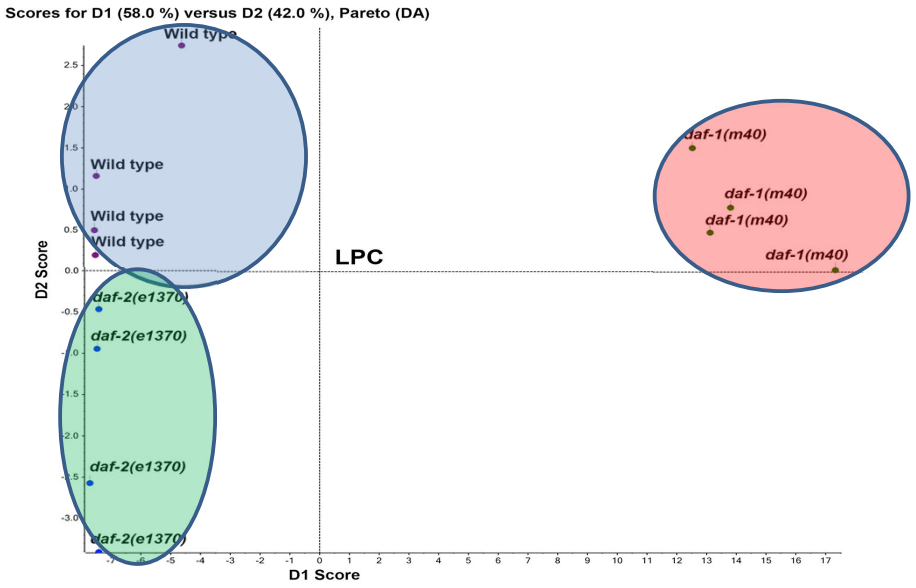
PCA score plot of LPCs from wild-type and mutant extracts; Daf-1 mutants are significantly different



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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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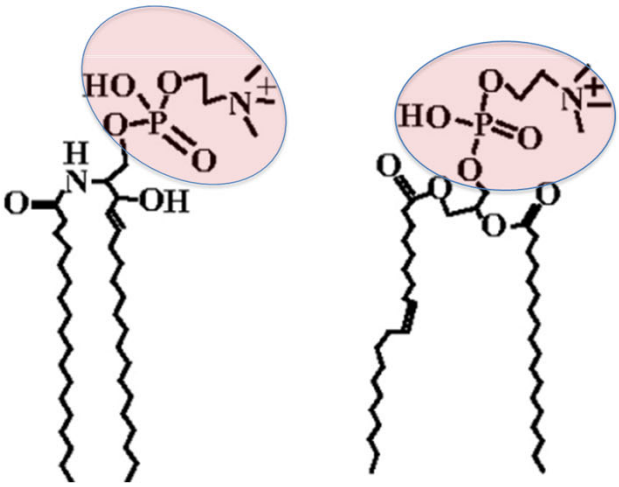
The problem of analyzing lipids

- 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



Overlap may exist between ^{12}C and ^{13}C monoisotopic peaks of PC and SM

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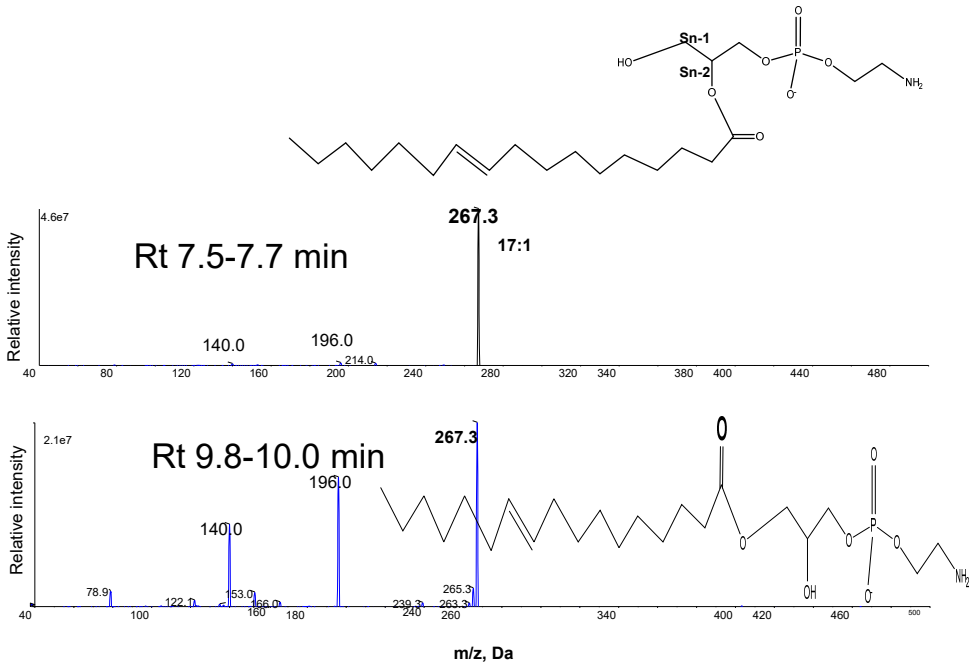
Regioisomeric LPEs *m/z* 464, separated by LC-MS/MS



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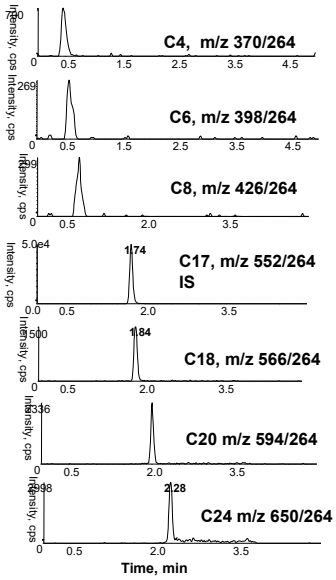
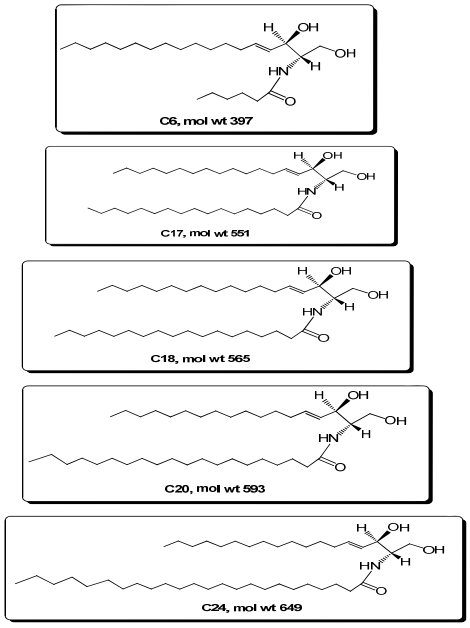
MRM chromatograms showing simultaneous determination of ceramides (C_4-C_{24})





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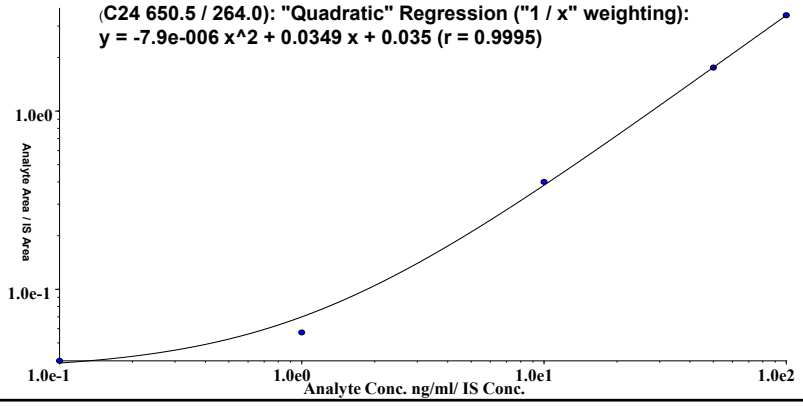
A linear response for Cer C24:0 was observed over a range of 0.1-100 ng/ml with correlation coefficient greater than 0.99

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



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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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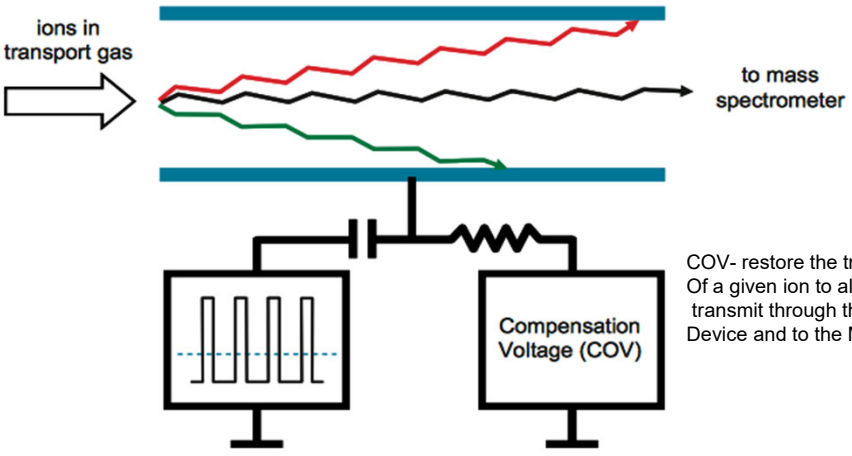
Differential mobility MS is an answer

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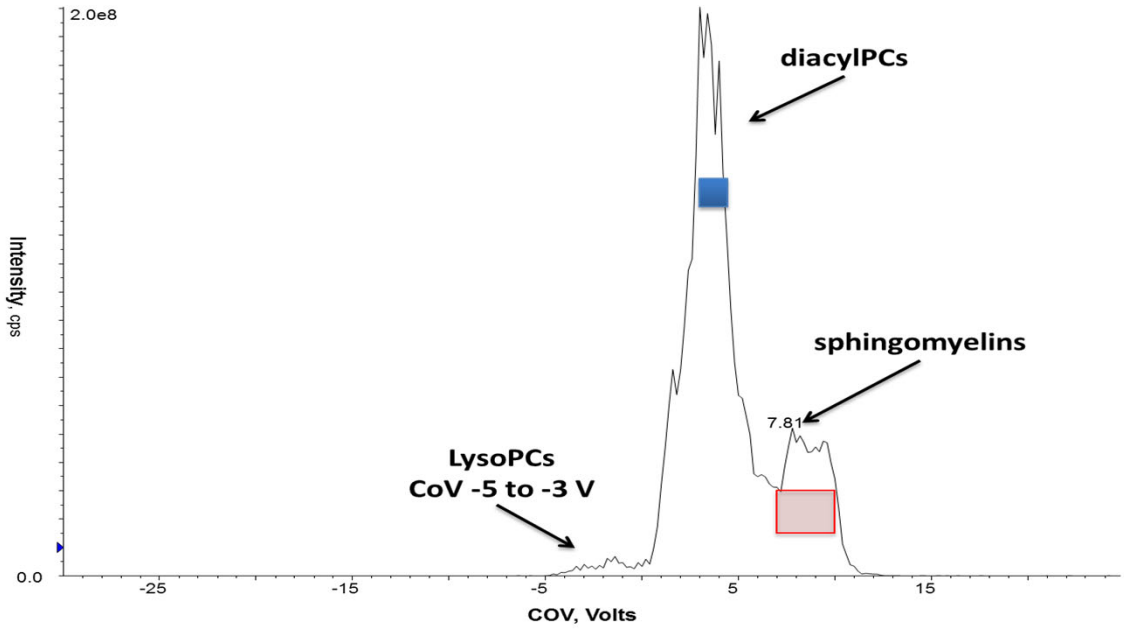
Total ion current of precursors of *m/z* 184.0 Using Selexion



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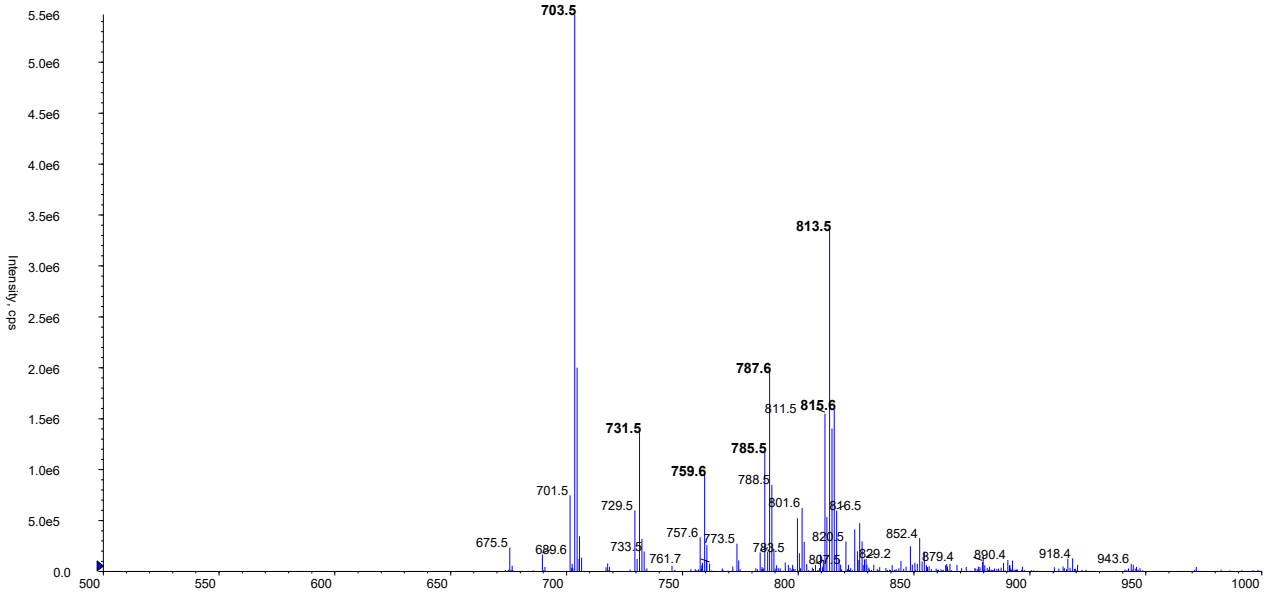
Precursors of *m/z* 184.0 (CoV from 7.8 to 10.0 V) Sphingomyelins are well separated from PCs



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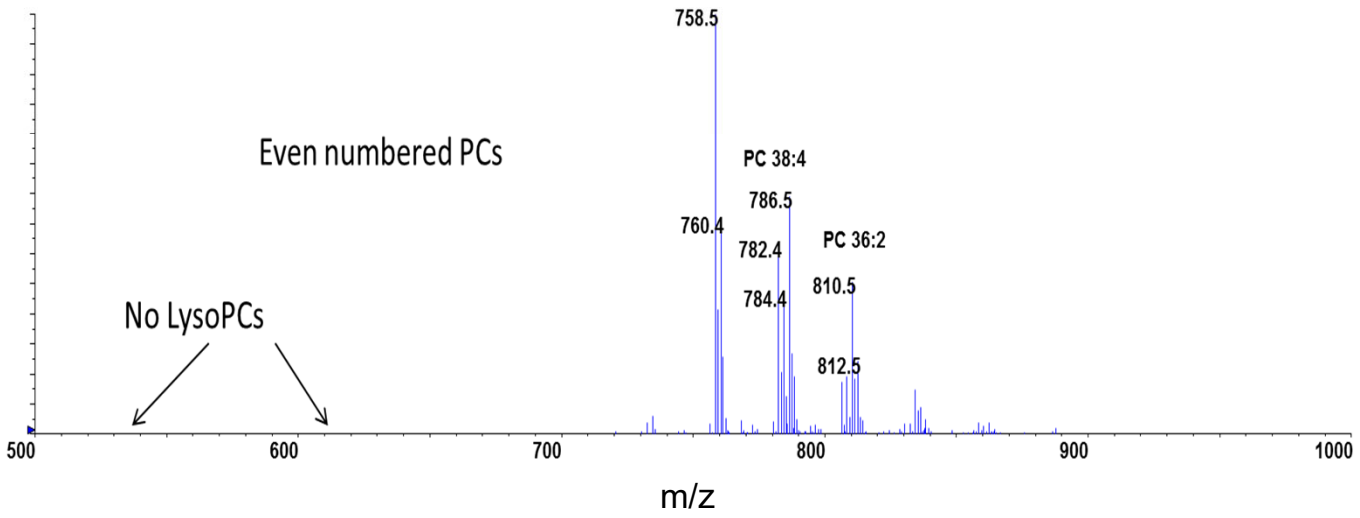
Precursors of *m/z* 184.0 (CoV from 3.0 to 3.6 V)



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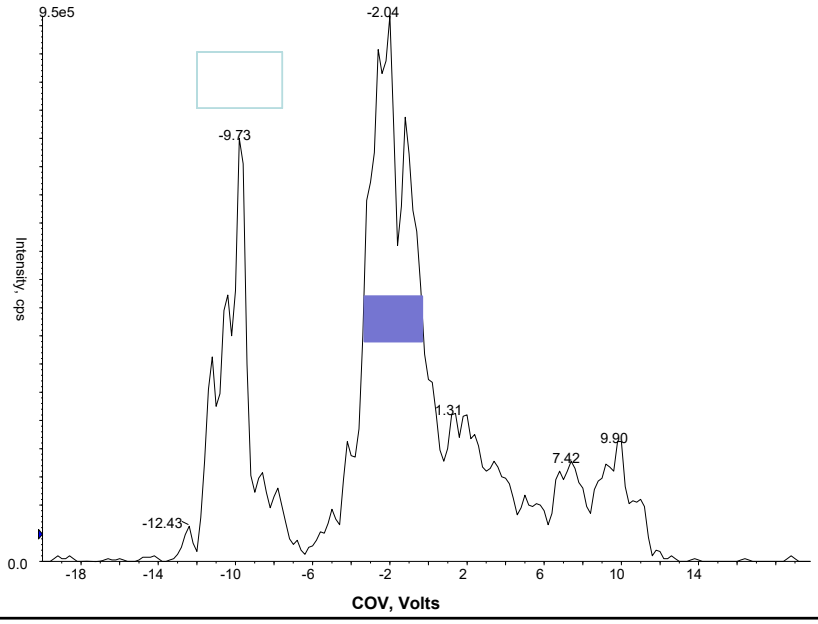
TIC of Precursors of *m/z* 283.0 (18:0)
Separation of lipids with FA 283 in negative ion mode



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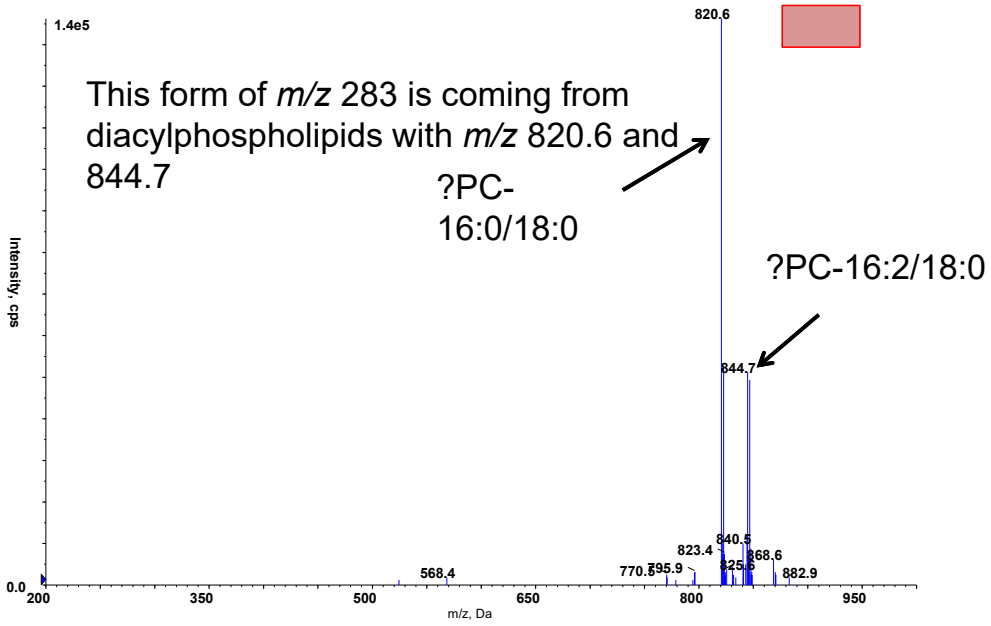
Precursors of *m/z* 283.0 CoV from -3.4 to -0.4 Volts



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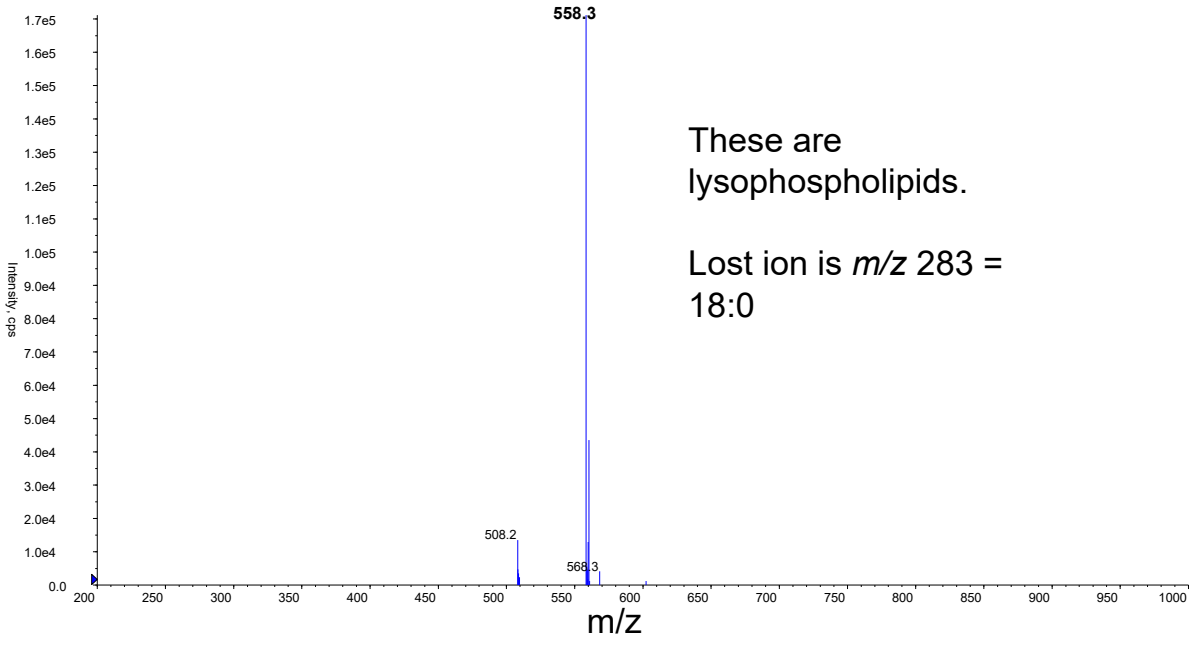
Precursors of *m/z* 283.0 – CoV from -11.8 to -8.6 V



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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……**

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Applying SWATH to LC-MS

Stephen Barnes, PhD, FASN

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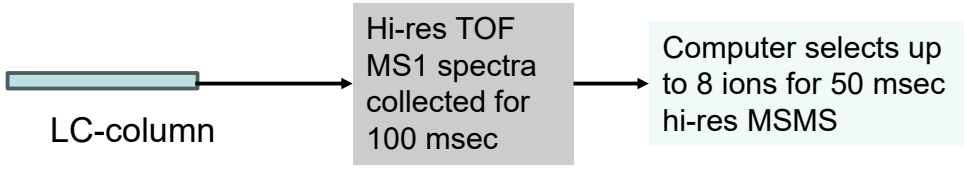
DDA versus DIA (SWATH)

- **Current Data-Dependent-Acquisition method of collecting data**
  - 0-6 min linear gradient of acetonitrile (2-98%) in 0.1% formic acid, followed by 1 min at 99.9% acetonitrile-0.1% formic acid
  - For most samples, hi-res TOF-MS data are collected at 4 Hz (every 250 msec)
    - i.e., 1,680 TOF spectra
    - Peaks are ~3 sec wide, so there will be 12 datapoints per peak – good!!
  - Collecting MSMS data is different – uses a 500 msec duty cycle
    - How many data points per peak?



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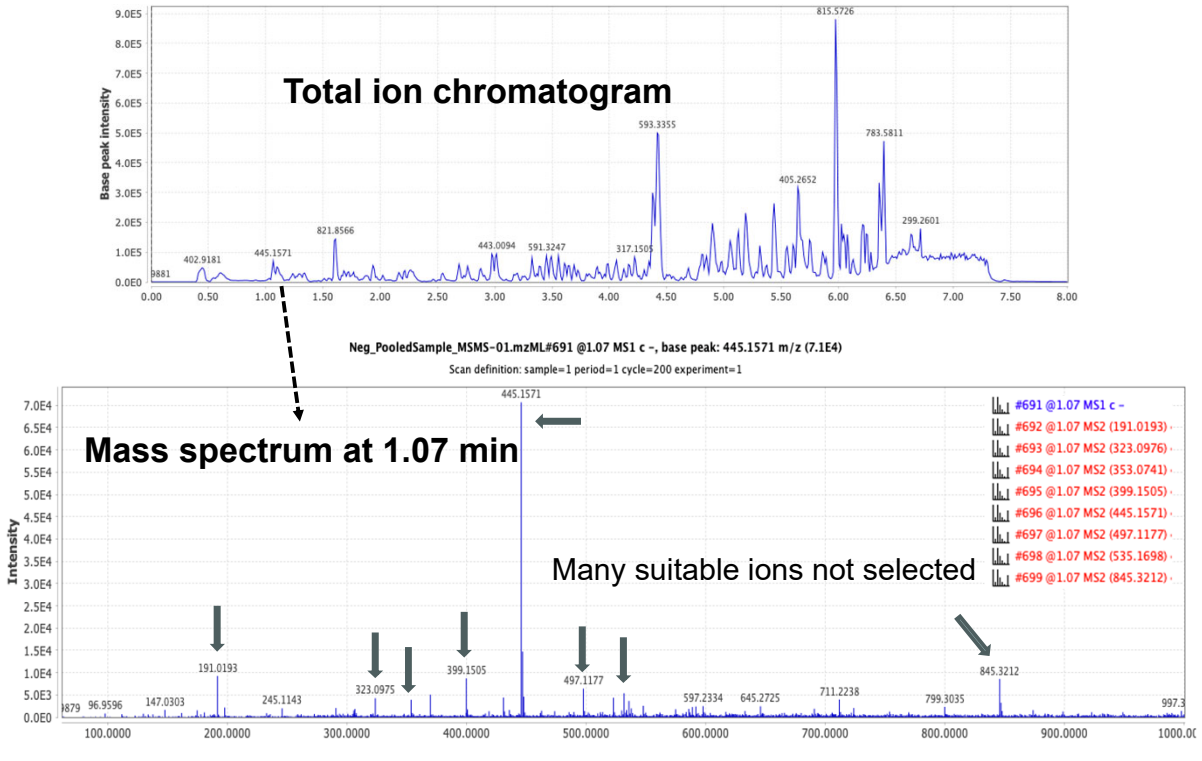

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Applying SWATH-MS to LC-MS

- **The SWATH approach uses wide mass filters and fragments everything in the mass window**
- **For the range *m/z* 50-1000, let's use 50 *m/z* SWATHs**
  - How many SWATHs per cycle?
  - 19
- **Assuming we are using a 500 msec duty cycle with say a 200 msec hi-res MS full scan, how long is an individual SWATH?**
  - =(500-200)/19 -> ~16 msec
- **How does this affect the chromatography?**
  - To have 12 data points per peak, the peak must be 6 sec wide with this cycle
  - So, either slow the gradient of MeCN, or the flow rate

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Instrument Conditions

- **Exion UHPLC + 5600 QToF**
  - ESI - Positive mode only
  - 10, 5, and 2 µl conditions tested
  - Variable SWATH windows (see next page for conditions)
- **HPLC conditions**
  - Phenomenex Luna Omega Polar C18 2.6 µM 100 x 2.1 mm @ 50°C
  - Mobile phase A) 0.1% FA B) MeCN 0.1% FA
  - Gradient 5 – 100%
  - 10 min/injection

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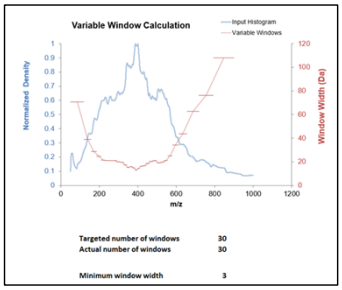
Variable Windows

- Generated using DDA MS1 density information plus SCIEX Excel Calculator
- 30 targeted windows
- 50 – 900 *m/z*
- 1 Da overlap
- 3 Da window width
- 15 Collision Energy Spread

SWATH Variable Window Assay Controls

Target number of windows:	30	(actual # may be less depending on min window width setting)
Lower m/z limit:	50	
Upper m/z limit:	900	(max 1250 for TripleTOF 5600+, max 2250 for TripleTOF 6600)
Round bin edges to x figures:	1	(1 figure past decimal recommended)
Window overlap (Da)	1.0	(1 Da overlap recommended)
Minimum window width (Da)	3	
CES	15	



49.5	120.3
119.3	158.3
157.3	185.9
184.9	209.6
208.6	230
229	250
249	269.9
268.9	289.9
288.9	308.9
307.9	327.4
326.4	343.6
342.6	357.8
356.8	372.6
371.6	386.3
385.3	398.2
397.2	411.5
410.5	426.7
425.7	442.4
441.4	460
459	479.4
478.4	499.4
498.4	517.4
516.4	536
535	555.9
554.9	580.1
579.1	613.4
612.4	656.1
655.1	717.9
716.9	792.9
791.9	899.8

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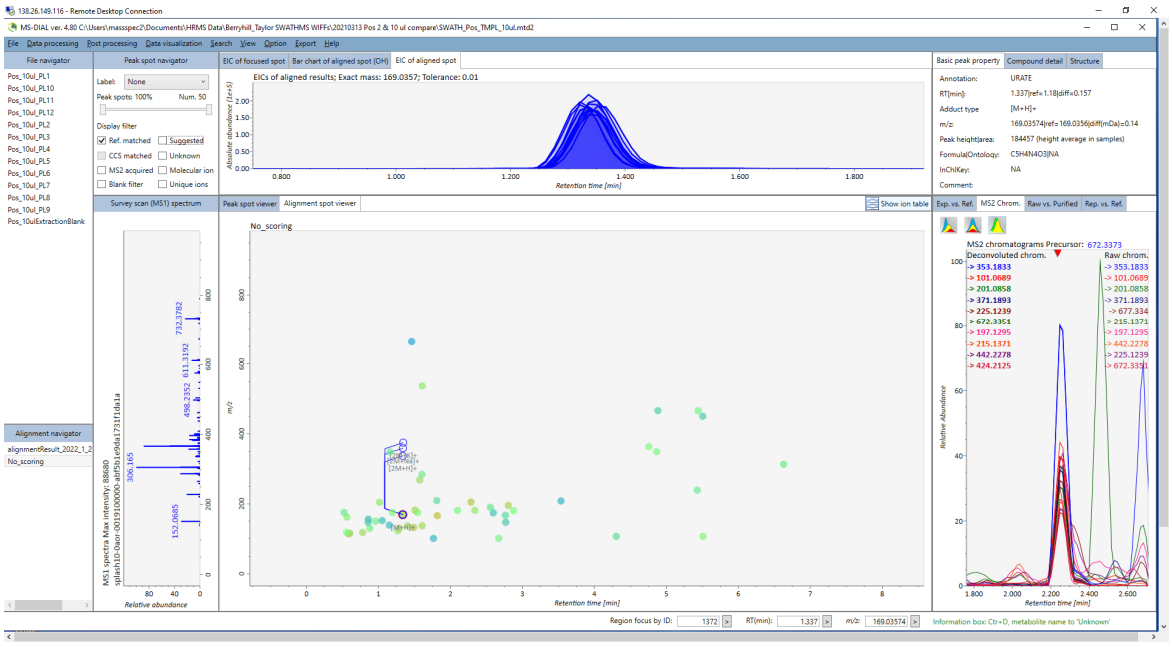
vSWATH – TMPL Lib – ref matched



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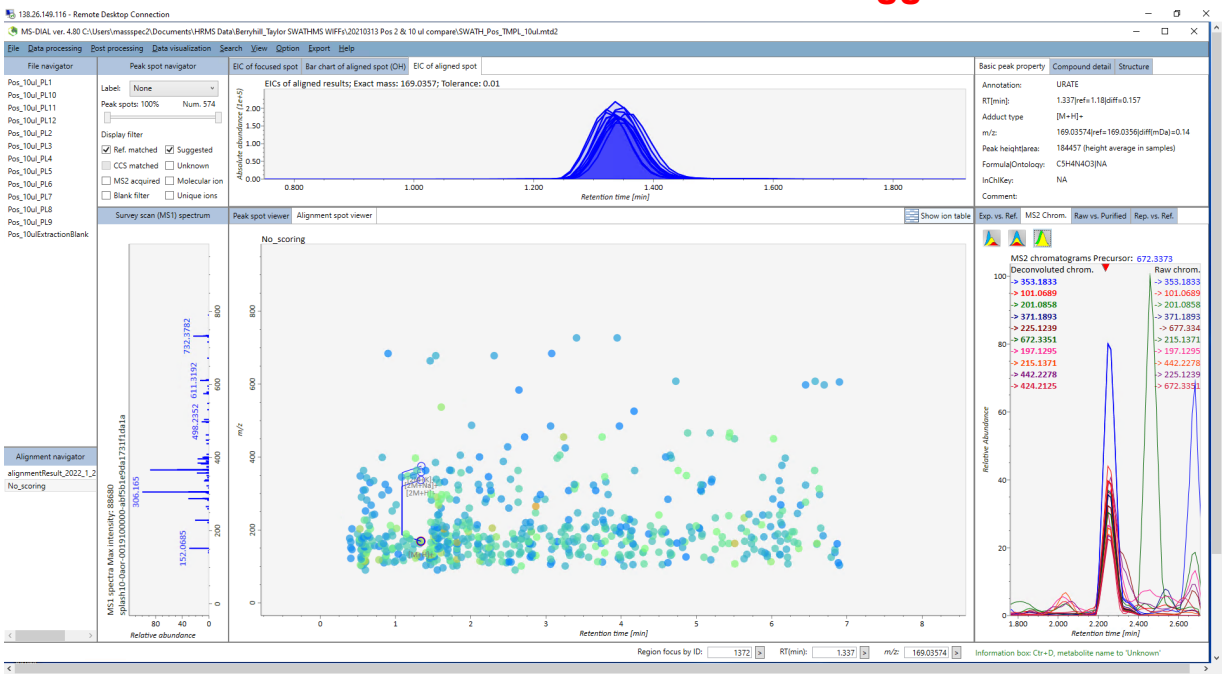
vSWATH – TMPL Lib – ref matched + suggested



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