SCIEX

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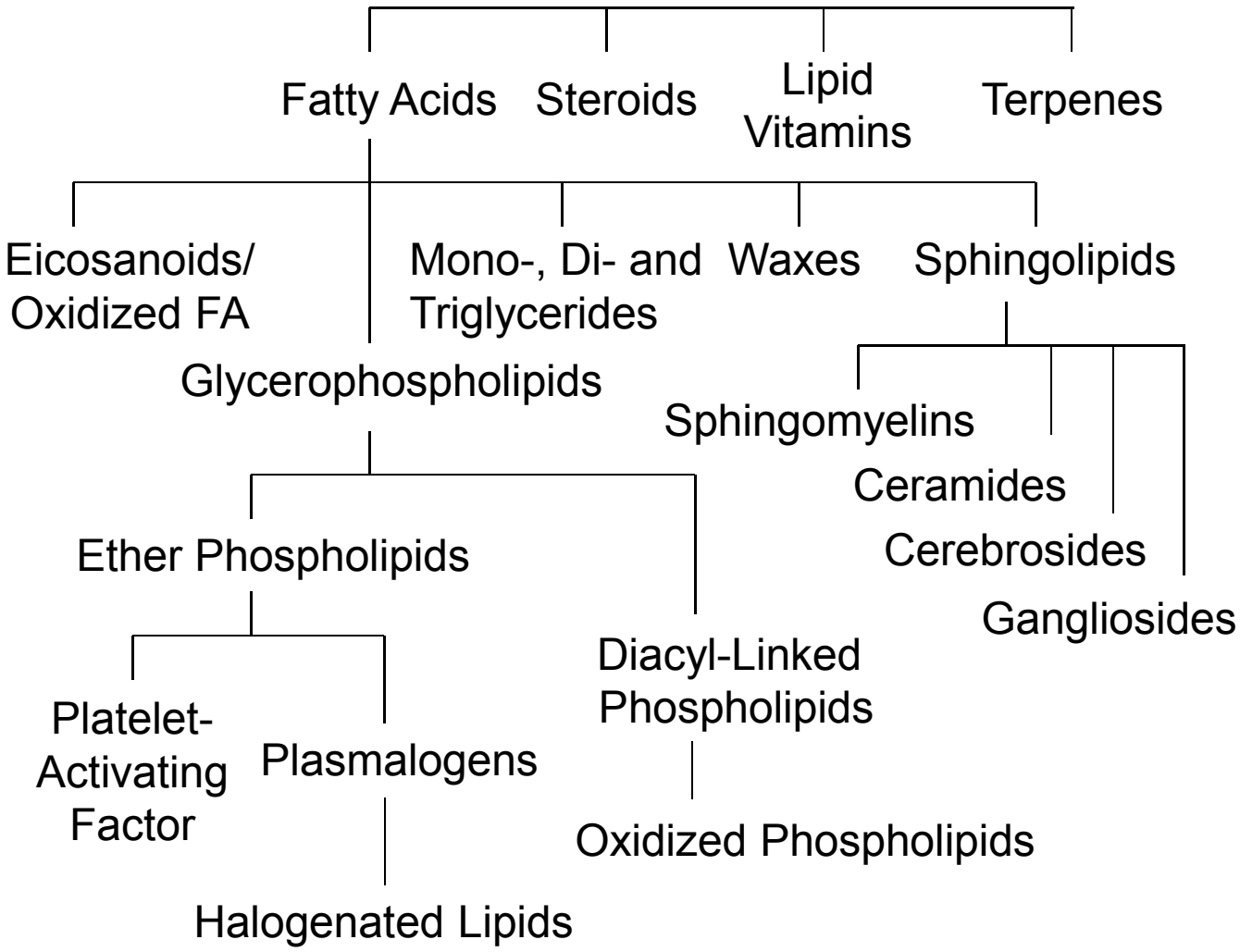
Knowledge for Life.™

The Convergence of Differential Ion Mobility Spectrometry and Mass Spectrometry:
Next Generation Global and Targeted Lipid Analysis

Alexandre Paccou, Sr Manager, Support, EMEA

The Lipidome

Comprised of multiple, structurally distinct lipid classes and sub-classes



Lipids play an essential role in human physiology:

- Metabolic homeostasis
- Cell and organelle structure
- Cell signaling

And disease:

- Inflammation
- Cancer
- Cardiovascular disease
- Diabetes
- Inflammatory bowel disease
- Neurological diseases

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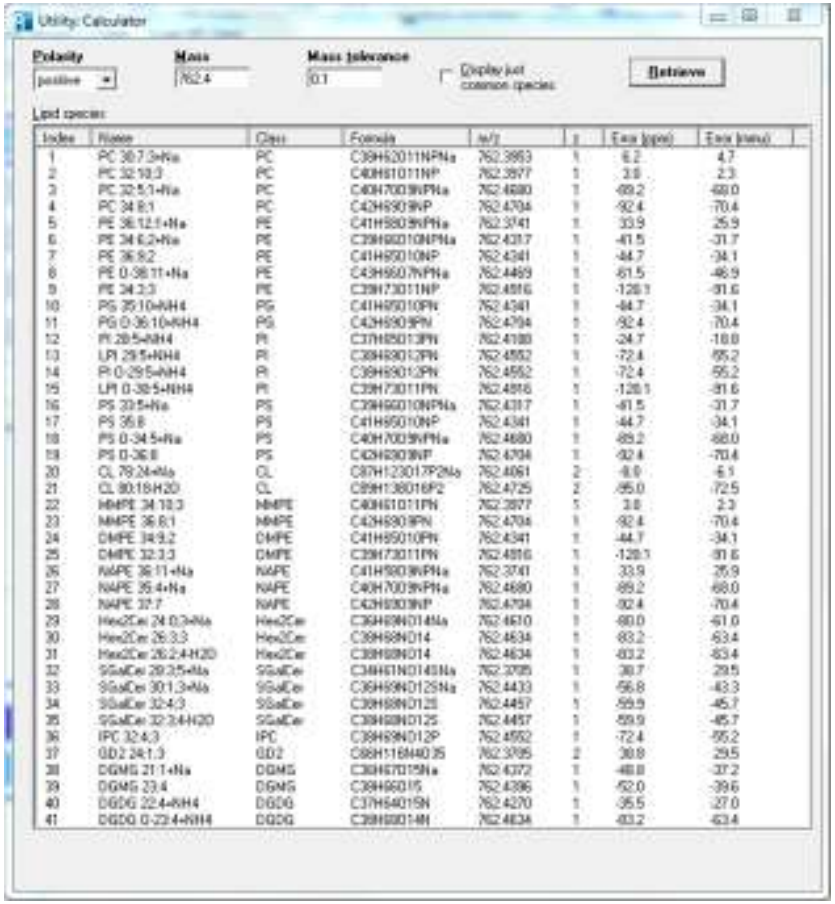
Fundamental Challenge in Lipidomic Analysis: Isobaric Overlap

There are as many as 180,000 different lipid molecular species possible in a narrow mass range of ~700 amu



<u>LipidView™ Software calculator exercise:</u>

Select mass of 762.4 with a tolerance of 0.1 amu → **41 lipids identified, many of which are isoelemental**

Problem: Identification and quantification of lipids based on high resolution MS alone is not possible.

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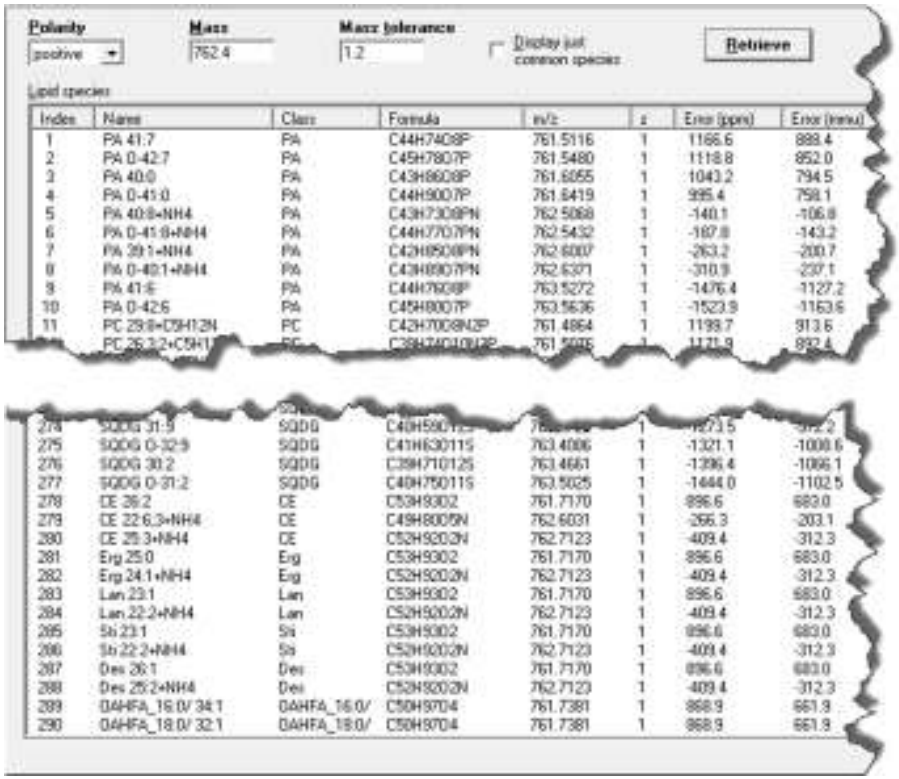
Challenges in Lipidomic Analysis: Isobaric Overlap

There are as many as 180,000 different lipid molecular species that are found in a narrow mass range of ~700 amu; most LC applications require 2D separation or multiple injections



LipidViewTM Software calculator exercise:

Select mass of 762.4 with a tolerance of 1.2 amu → **290 Lipids identified**

Due to the wide Q1 isolation window, isobaric overlap makes unambiguous identification and quantification nearly impossible

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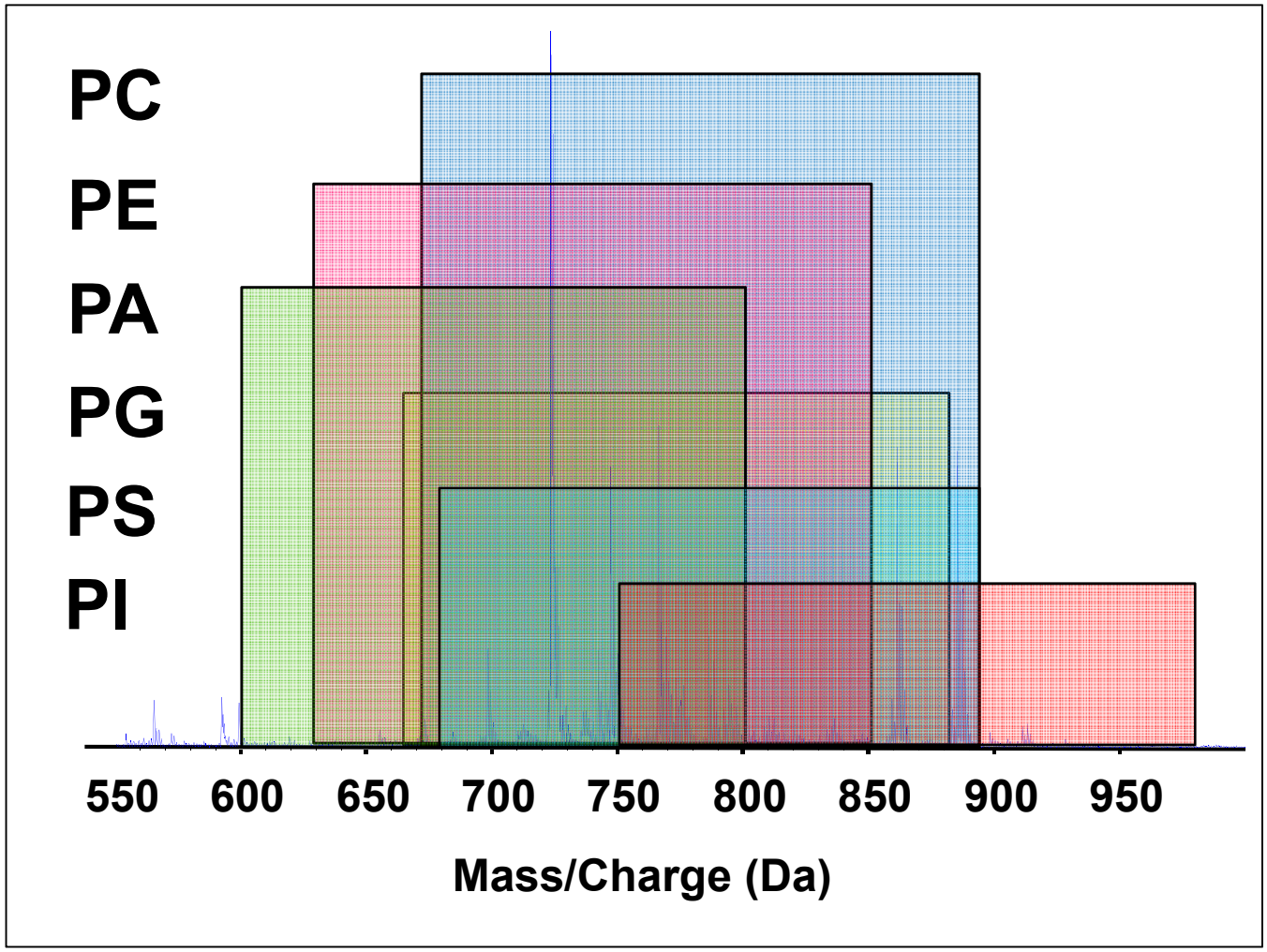
Isobaric Overlap of Phospholipids

Experiment: EMS scan of Bovine Heart Extract (BHE)



- **Lipidomic spectra are incredibly complex**
- **MS/MS spectra generated on precursors in zones of isobaric overlap will contain product ions from other isobaric species**

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Current Strategies in Lipidomics

Separation approaches:

- SPE extraction
- Fractionation
- Chemical derivatization
- Chromatography

Mass spectrometry approaches:

- Dedicated precursor and neutral loss scans (Qtrap® Systems)
- IDA-based methodologies (Triple TOF Instruments® Systems and Qtrap® Systems)
- MS/MS^{ALL} (Triple TOF® Systems Instruments)

New approach: Differential Ion Mobility Spectrometry

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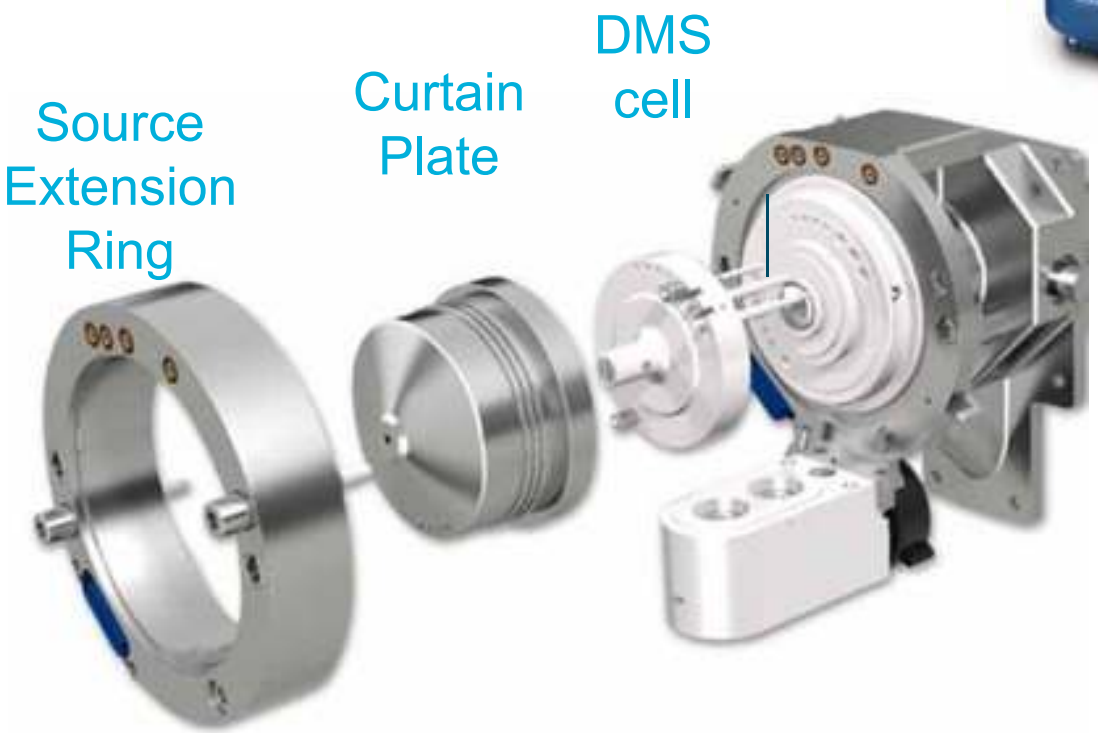
SelexION™ Technology for Lipid Analysis

SelexION™ Hardware Configuration

Compatible with QTRAP® and TTOF technology

- Differential Mobility Spectrometry (DMS)
- Installation / removal of DMS in < 2mins – no tools required



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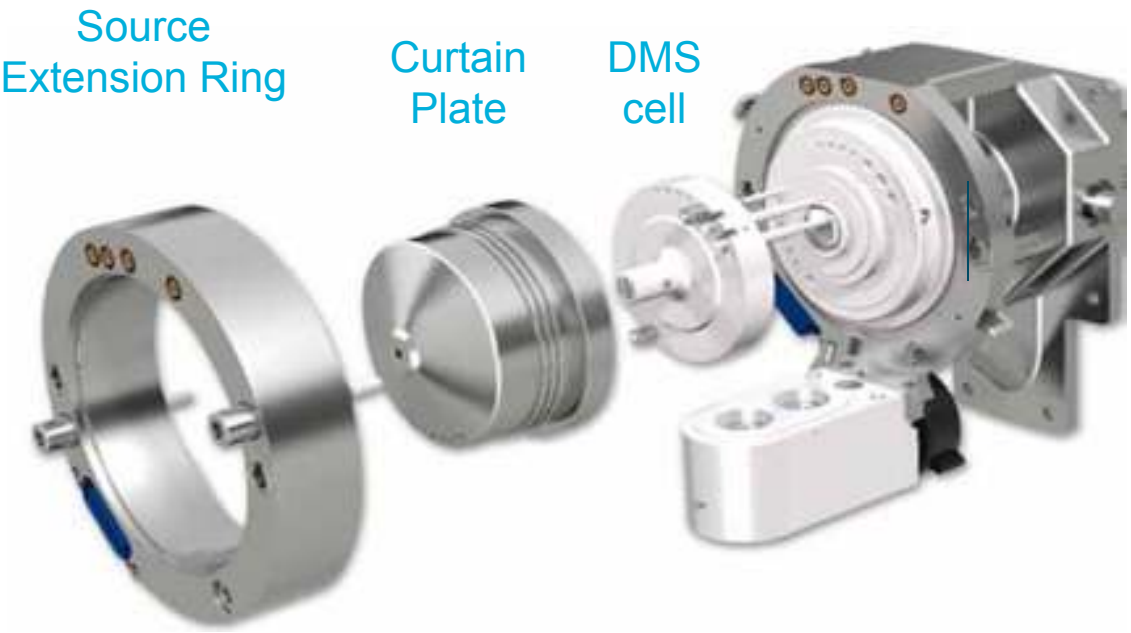
SelexION™ Hardware Configuration

- Compatible with QTRAP® and TTOF technology
- Installation / removal of DMS in < 2mins – no tools required
- Resolves analytes prior to MS analysis



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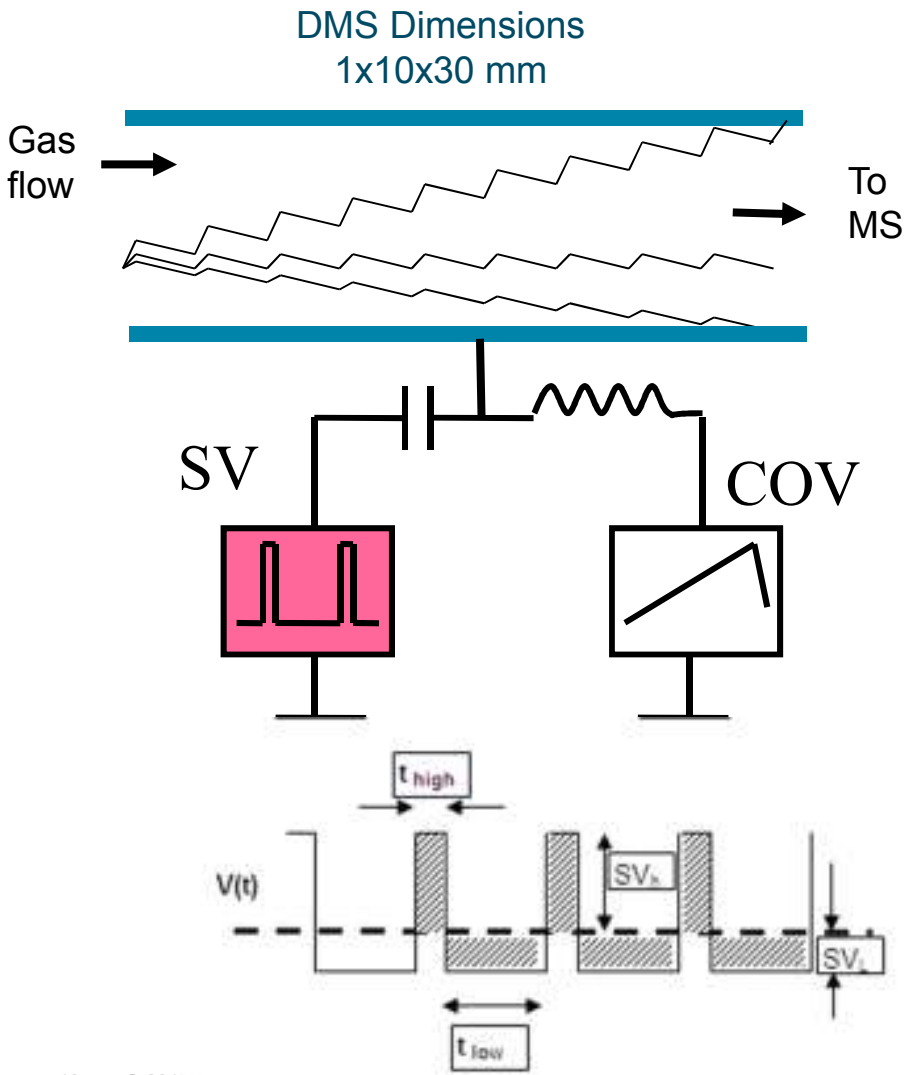
Differential Mobility Spectrometry- DMS

SelexION™ Technology



- Planar geometry
- Gas flow towards MS draws ions (transport gas)
- Asymmetric waveform applied which alternates between high field, K(E) and low field, K(0) – separation voltage (SV)
  - Moves charged ion back and forth between plates
  - Ion will have net drift base on its high and low field mobility
- Compensation voltage (COV) is small DC offset between the plates – filtering voltage

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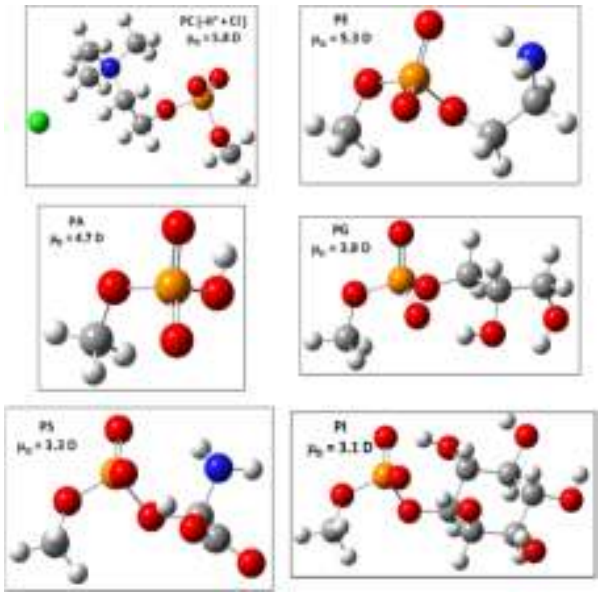
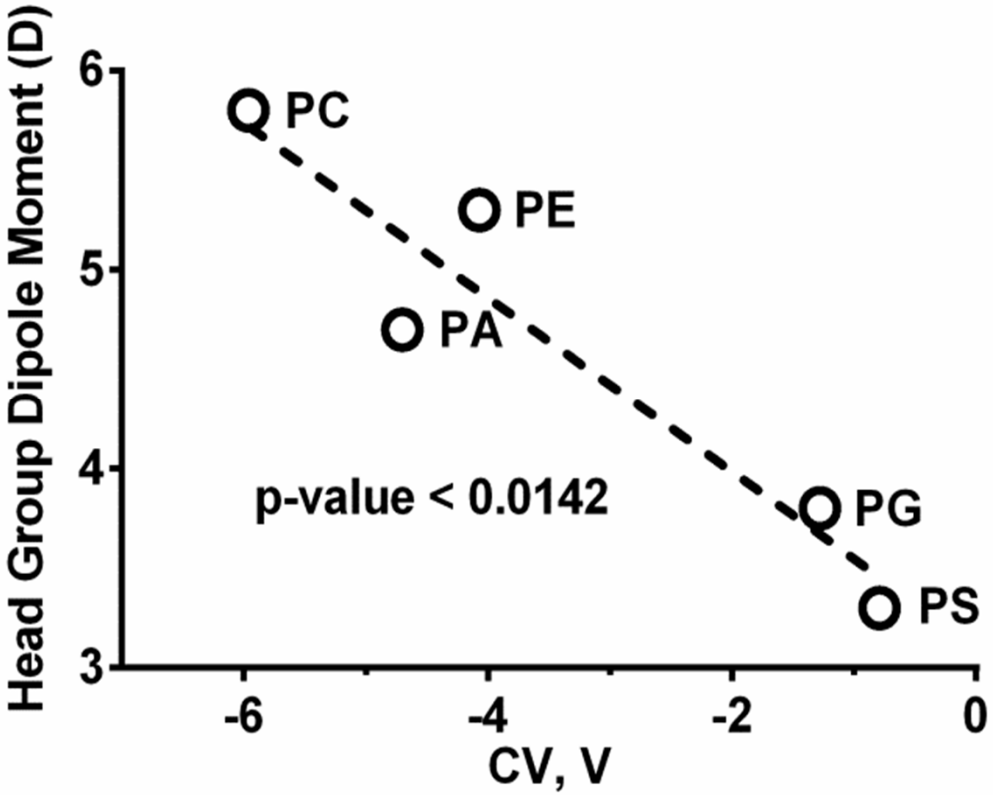
Relationship Between Dipole Moment and CoV



Differential Mobility Spectrometry-Driven Shotgun Lipidomics
Anal. Chem. 2014. 86. 9662-9669
10.1021/ac5021744

Theoretical dipole moments were calculated using isopropanol as a modifying solvent



Molecules that have different dipole moments can be separated by DMS

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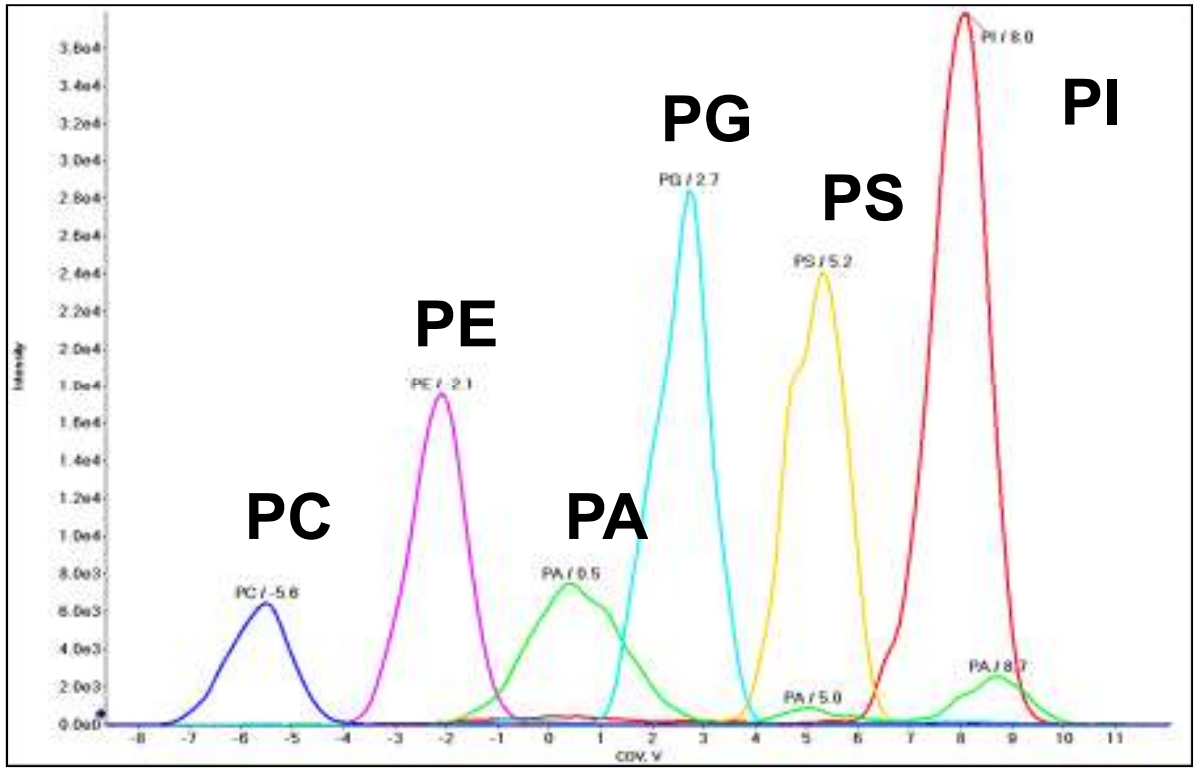
SelexION™ Technology Separates Phospholipid Sub-Classes

Experiment: MRM scan of 6 phospholipid standards with COV ramp



- **Using DMS alone, a mixture of lipids can be separated into it's individual components**
- **Baseline separation can be achieved, which abrogates**
- **isobaric interference**

Proof of concept: DMS separates different lipid classes
Implications: High degree of lipid class specificity without LC

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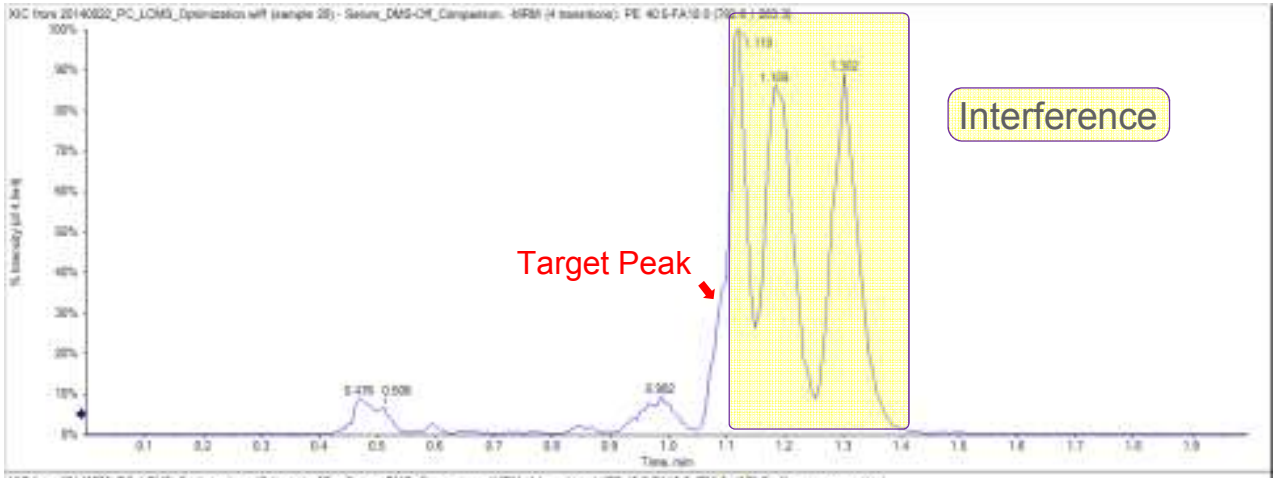
Isobaric Interference Among Different Lipid Molecular Species

Isobaric interference makes ‘unassisted’ MRM analysis by infusion non-specific. SelexION™ Technology makes it possible

Experiment: mrm analysis of CSF (PE 40:5; 792.6/283.2)



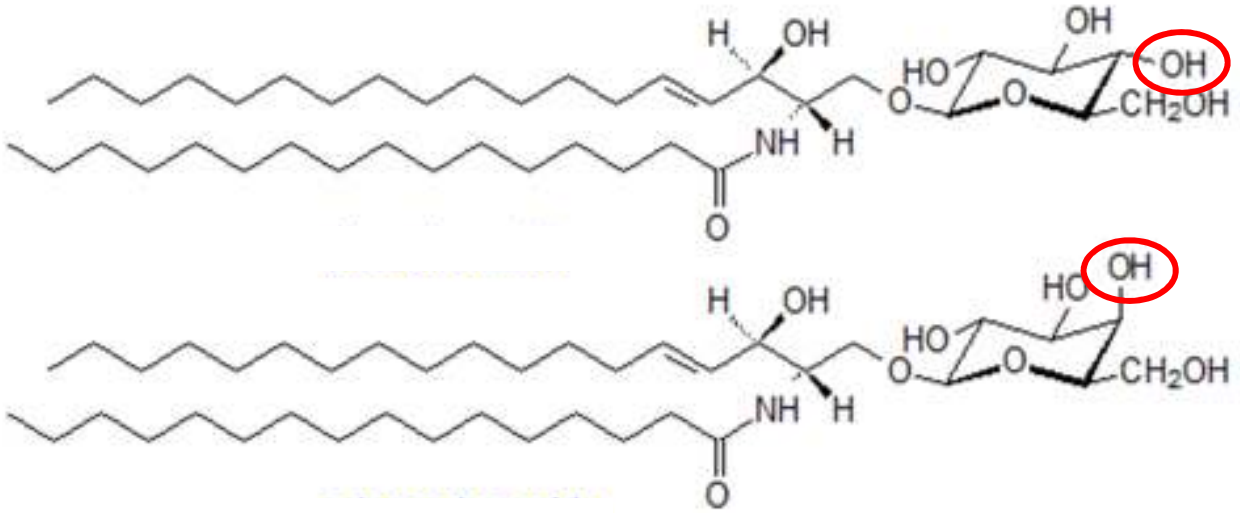
SelexION™ Device Off

Multiple different lipids have the same MRM transition:

Isobaric Interference

Resolution of Glucosylceramides (Cerebrosides)

- Galactosylceramide (Galβ1-1'Cer) is the principal glycosphingolipid in brain tissue and is an essential structural component of myelin
- Glucosylceramide (Glcβ1-1'Cer) is found in animal tissues, and is a major component of skin lipids and neuronal tissue.



Due to identical product ion spectra, mrm transitions and similar physical properties, LC separation and individual characterization/quantitation of these two lipid classes is challenging.

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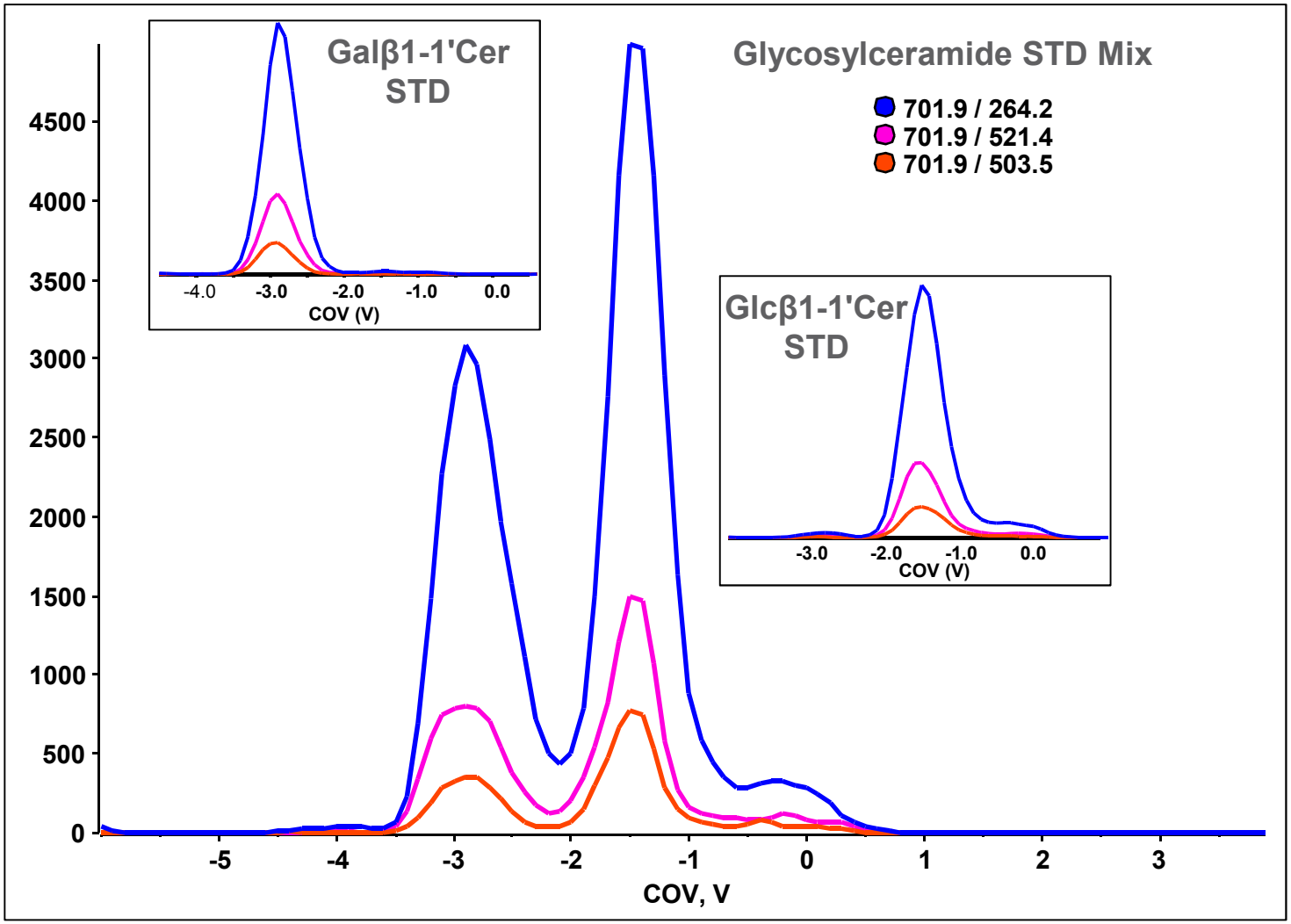
DMS Resolves Glycosylceramide Isomers without Chromatography



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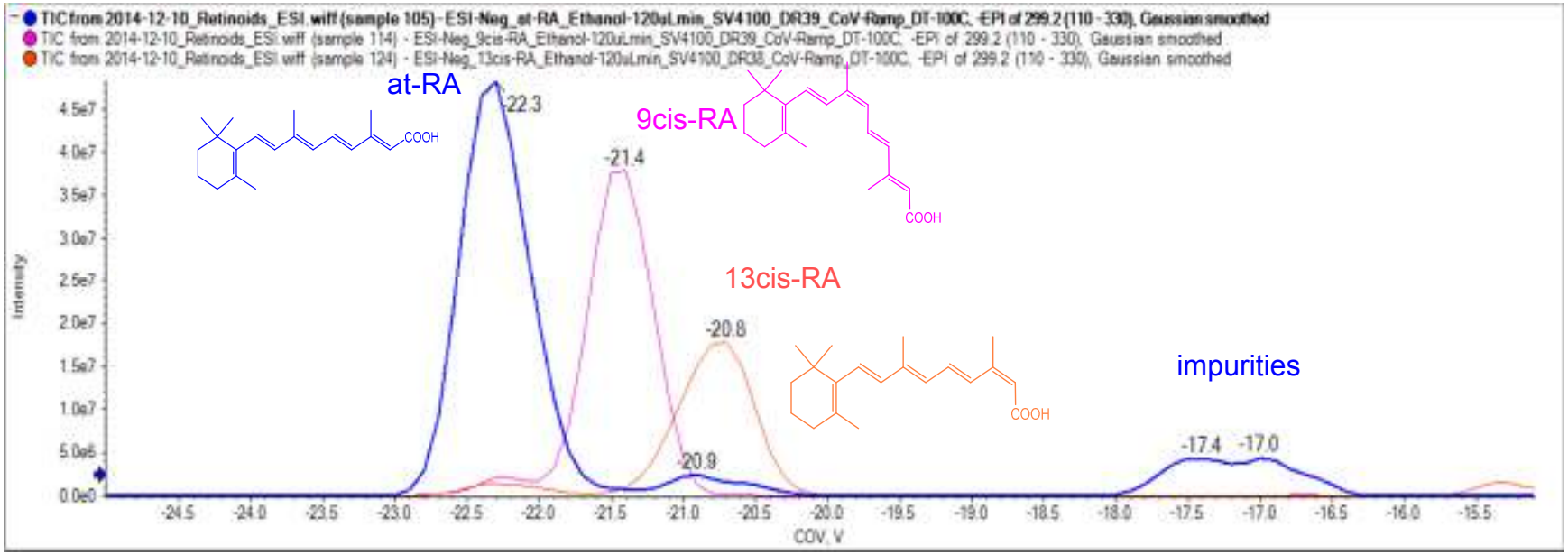
Resolution of Retinoic Acid Isomers by SelexION™ Technology



Complete resolution of RA isomers is possible, but the sensitivity is affected such that it is not a biologically relevant assay.

→Combine LC and DMS to balance need for selectivity and sensitivity

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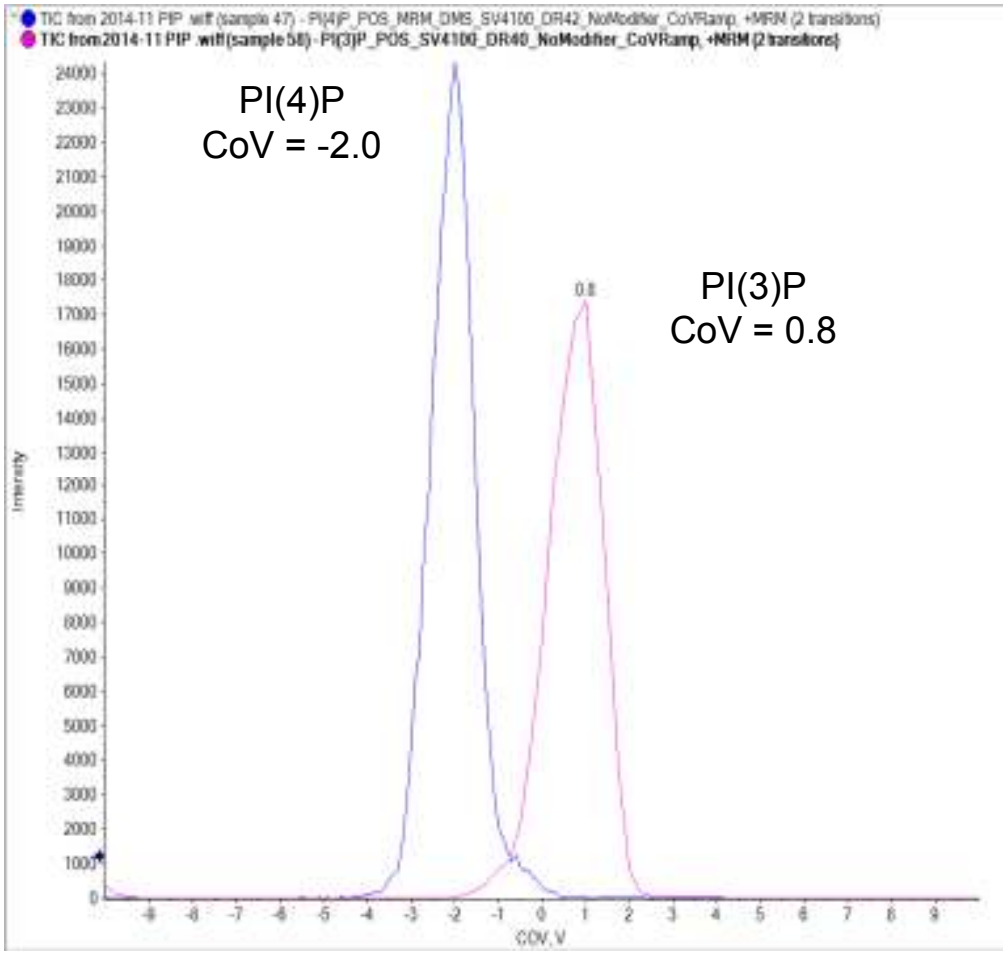
SelexION Separation of PI(3)P and PI(4)P

Baseline separation without chromatography



Experimental Conditions

SelexION Conditions

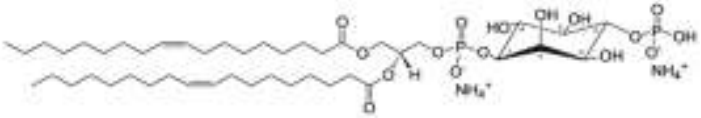
Separation Voltage (SV):	4100V
DMS Resolution Enhancement (DR):	42
Modifier:	None
DMS Temperature (DT):	Low=150 ºC

6500 QTRAP Conditions

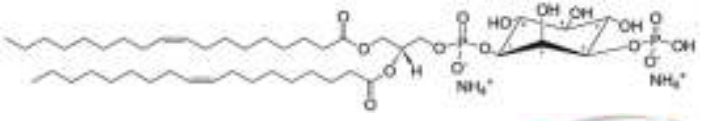
IonSpray Voltage (IS):	4500
Temperature (TEM):	100
Gas 1 (GS1):	30
Gas 2 (GS2):	40
Curtain Gas (CUR):	15
Declustering Potential (DP):	100
Entrance Potential (EP):	10

MRM: 953.5 → 613.4 (CE=30)

PI(4)P



PI(3)P



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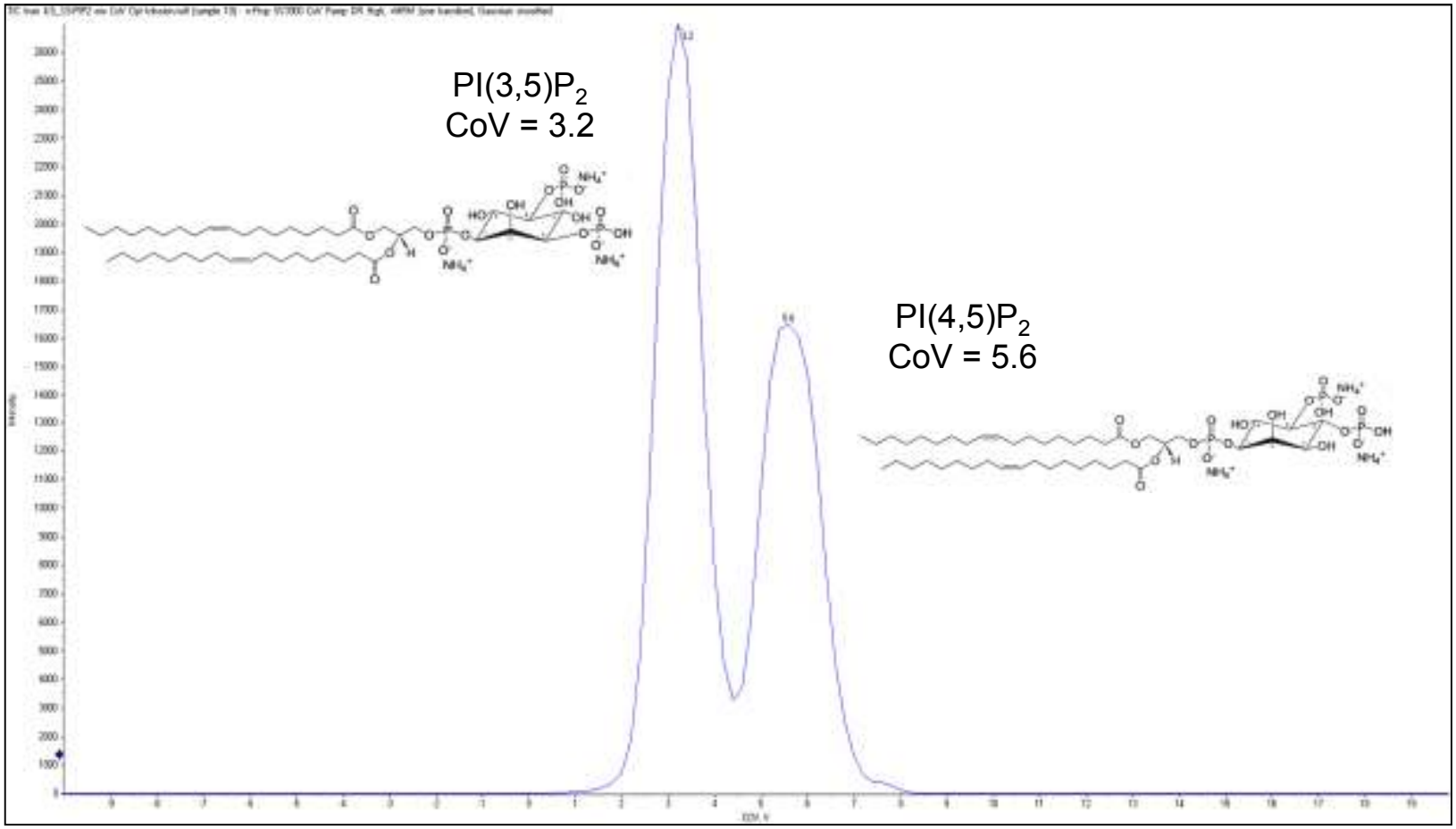
Resolution of $PI(3,5)P_2$ and $PI(4,5)P_2$ by DMS

Traditional methods to separate the PIP_2 molecular species rely on chromatography and derivatization



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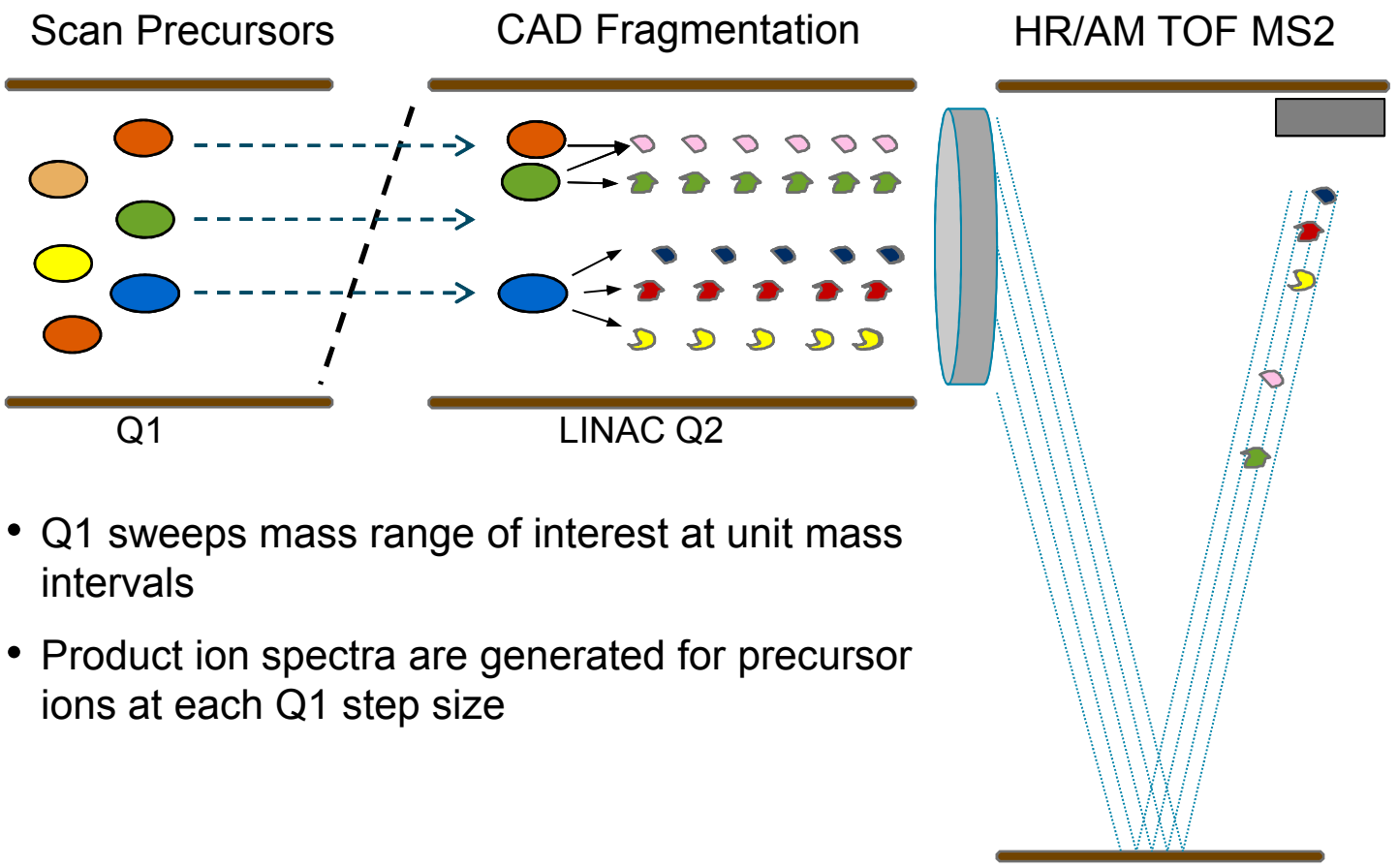
Applying SelexION™ Technology to MS/MS^{ALL} with the TripleTOF® 5600+

MSMS^{ALL} Introduction

Product ion spectrum generated for every precursor ion



- Q1 sweeps mass range of interest at unit mass intervals
- Product ion spectra are generated for precursor ions at each Q1 step size

Ståhlman, M et al. High-throughput shotgun lipidomics by quadrupole time-of-flight mass spectrometry, J Chrom B 2009
Ekroos, K., Methods in Pharmacology and Toxicology: Biomarker Methods in Drug Discovery and Development, Humana Press 2008

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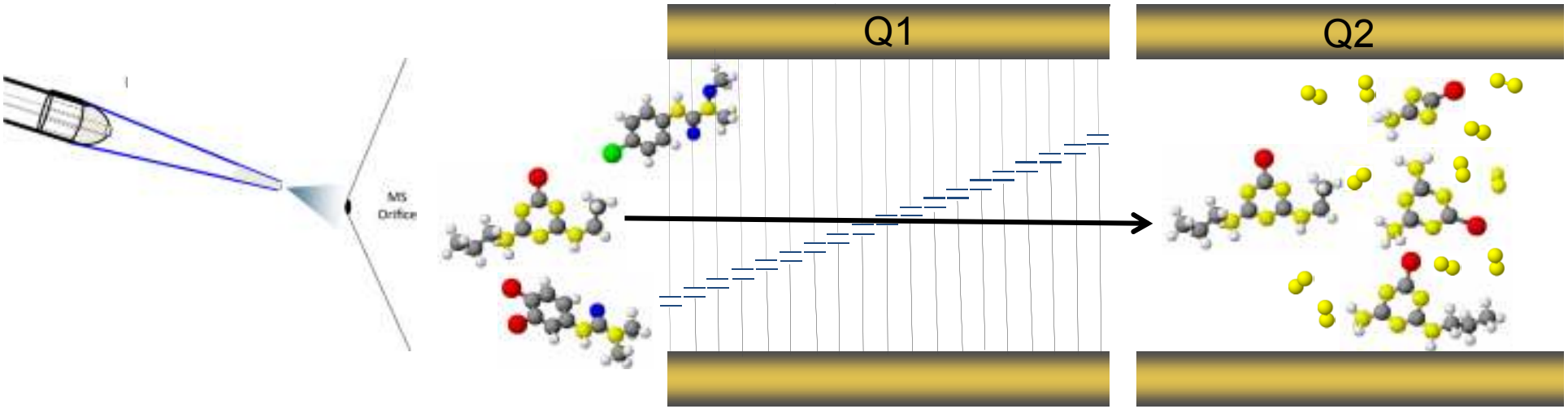
MS/MS^{ALL} Information-Independent Data Collection



Direct infusion, flow injection, and lipid-class targeted LC techniques

Fast Q1 precursor selection step-wise through mass range

CID Fragmentation

Precursor mass range 200-1250; total analysis time 3-5 min

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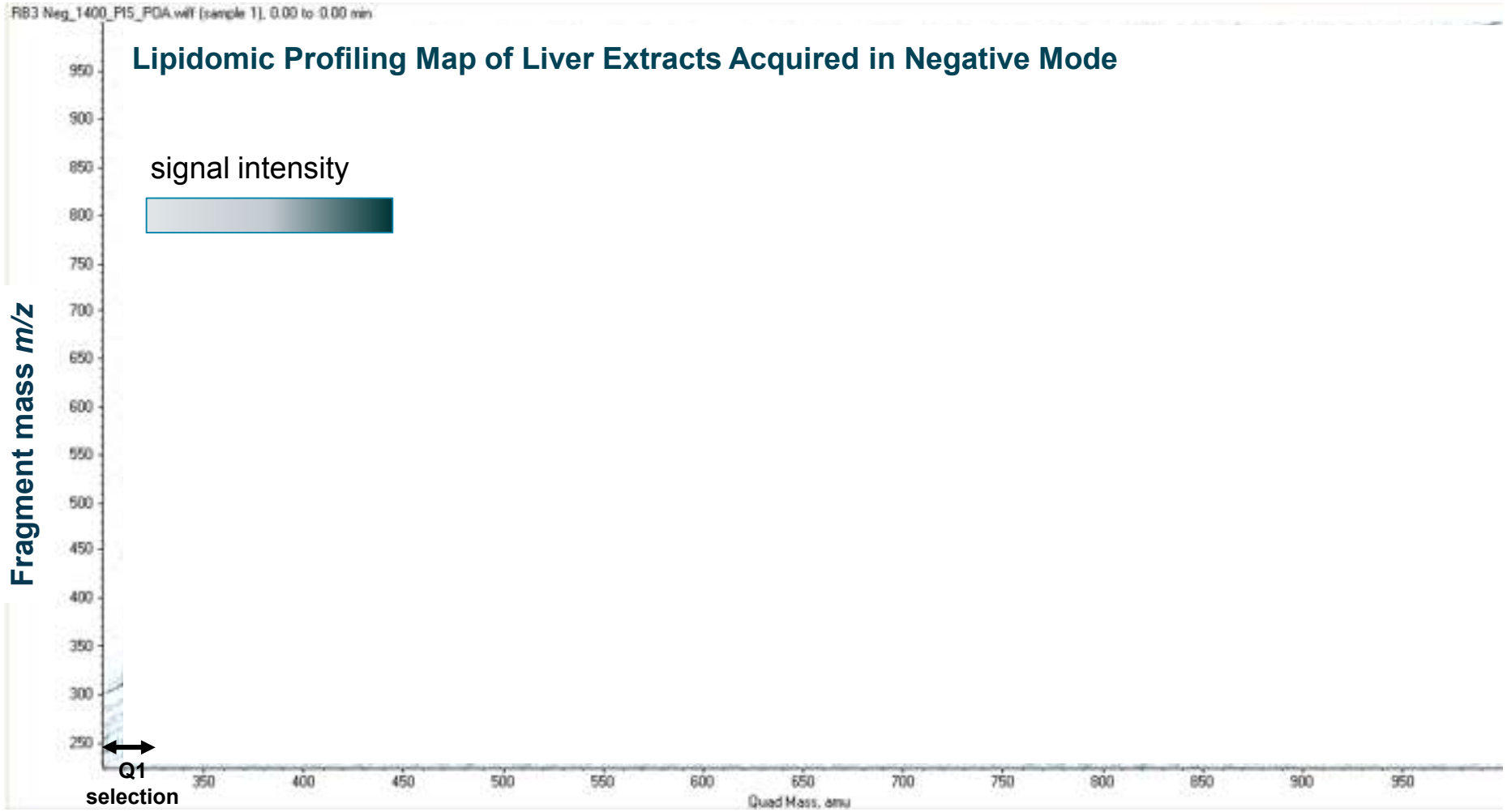
MS/MS^{ALL}



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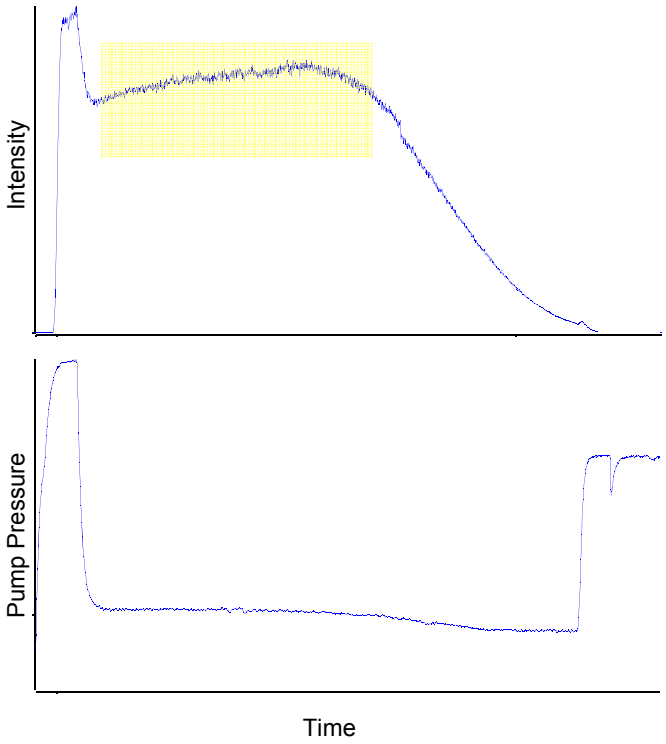
6600 TripleTOF with FIA Configuration

LC Pump and Auto-Sampler

6600 TripleTOF® Mass Spectrometer
With SelexION® Technology



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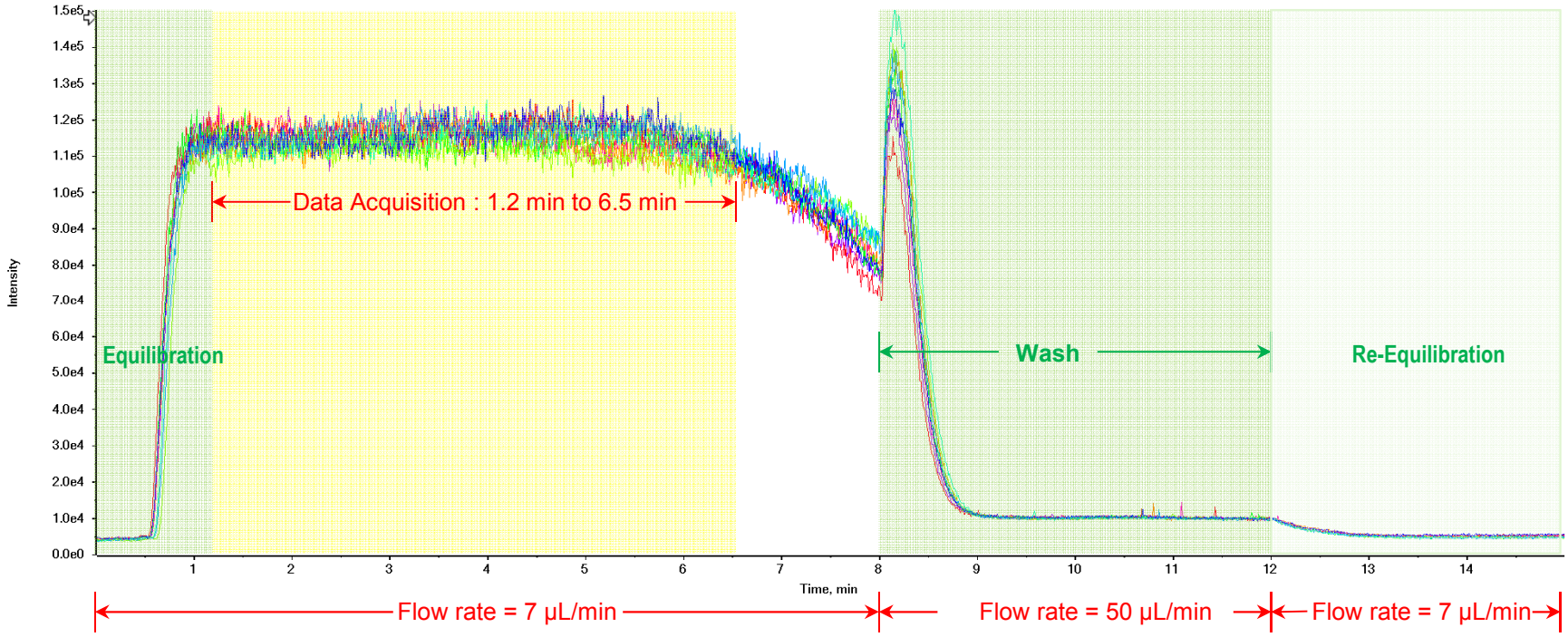
Eluting Profile and Data Acquisition Window



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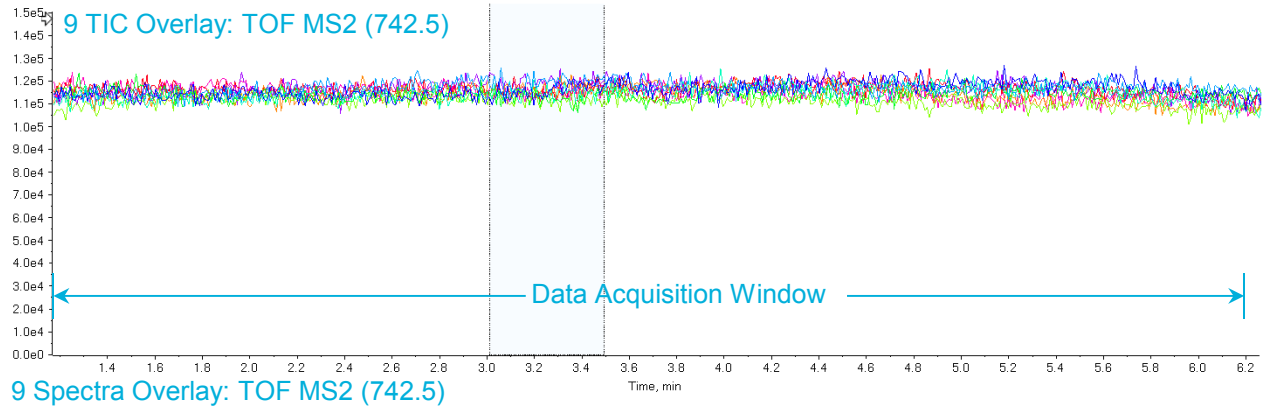
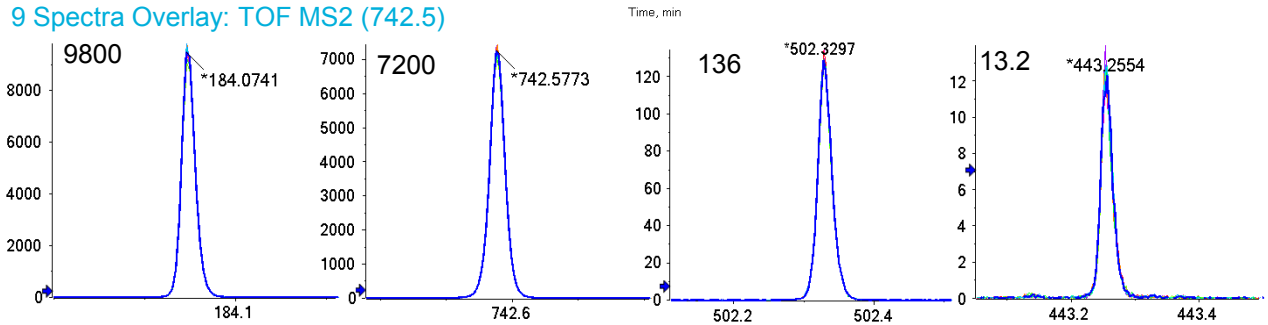
Excellent Reproducibility in Elution Profile and MS Intensity





m/z 184.074
- Peak Height %RSD = 2.67%
- Peak Area %RSD = 2.48%

m/z 742.577
- Peak Height %RSD = 2.10%
- Peak Area %RSD = 1.83%

m/z 502.330
- Peak Height %RSD = 4.27%
- Peak Area %RSD = 2.87%

m/z 443.255
- Peak Height %RSD = 3.50%
- Peak Area %RSD = 3.20%

- Excellent Reproducibility
  - Within-run
  - Run-to-run

- m/z 184.074
  - Peak Height %RSD = 2.67%
  - Peak Area %RSD = 2.48%
- m/z 502.330
  - Peak Height %RSD = 4.27%
  - Peak Area %RSD = 2.87%
- m/z 443.255
  - Peak Height %RSD = 3.50%
  - Peak Area %RSD = 3.2%

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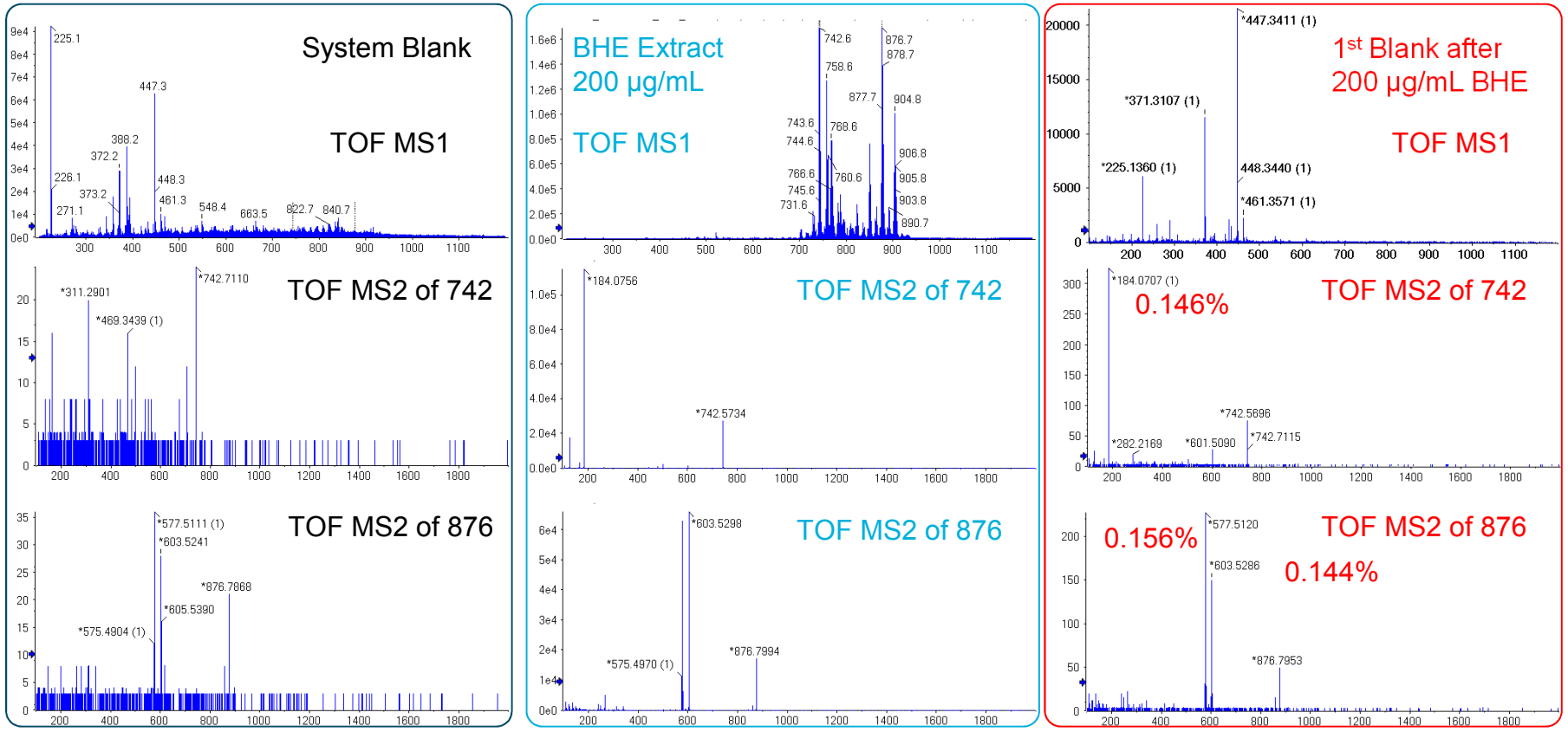
Quantitative Level Carryover



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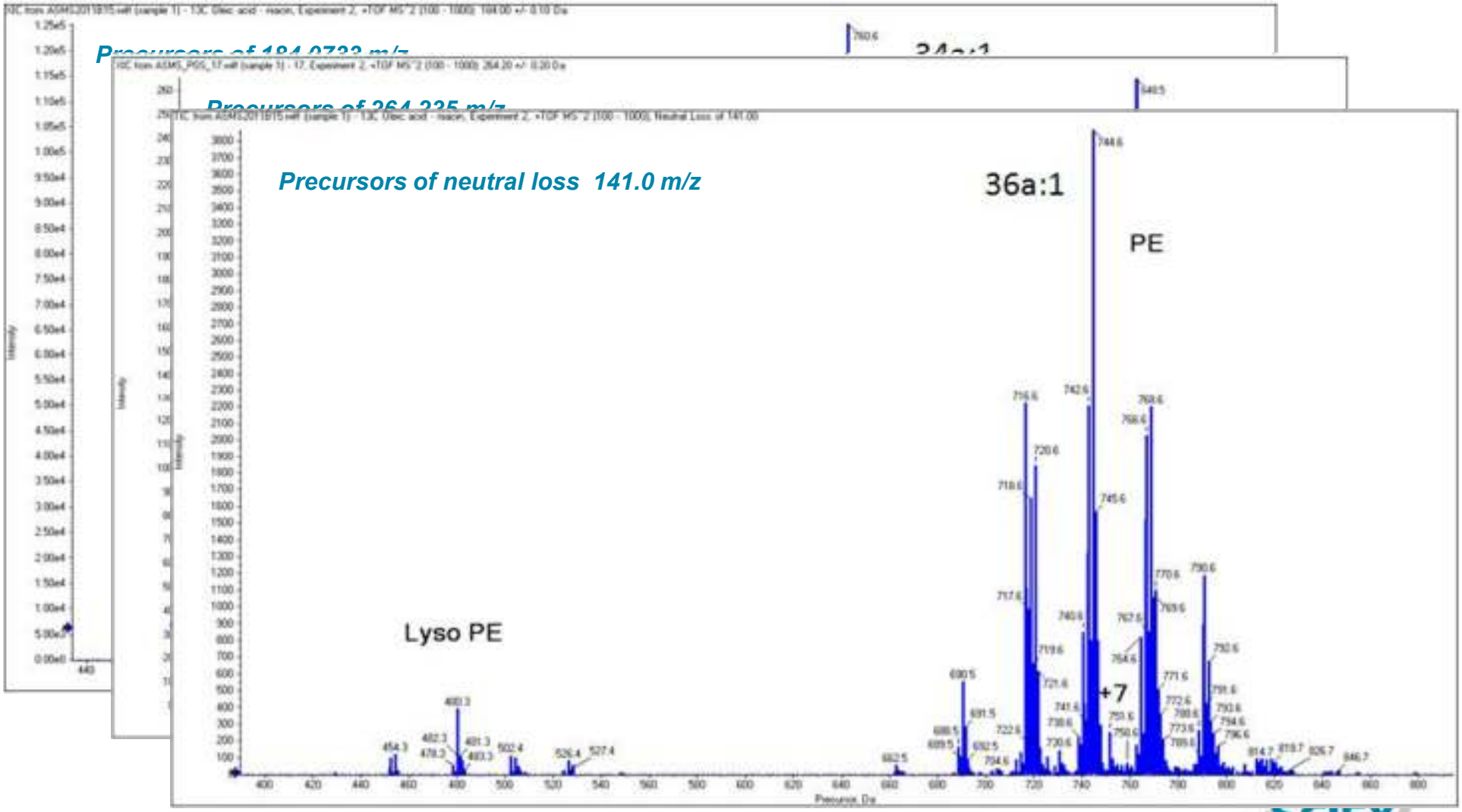
MS/MS^{ALL} – Fragment and Neutral Loss FIlter



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MS/MS^{ALL} with SelexION^{TM}

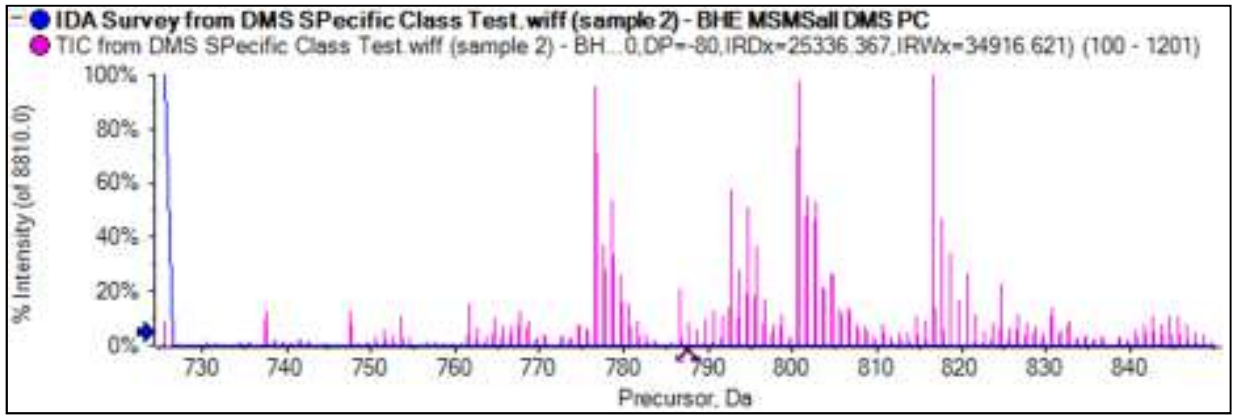
Use the SelexION^{TM} technology to isolate individual lipid classes for analysis

- Identify CoV values for each lipid class of interest
- Focus mass list to analyze only masses that are germane to lipid class of interest (e.g., PC: 675-925)
- Run MS/MS^{All} sequence for all lipid classes

Primary Benefits

- Clean MS/MS data without isobaric contamination
- Reduced background
- Improved ID confidence

PC MS/MS^{ALL} – CoV = -6.5



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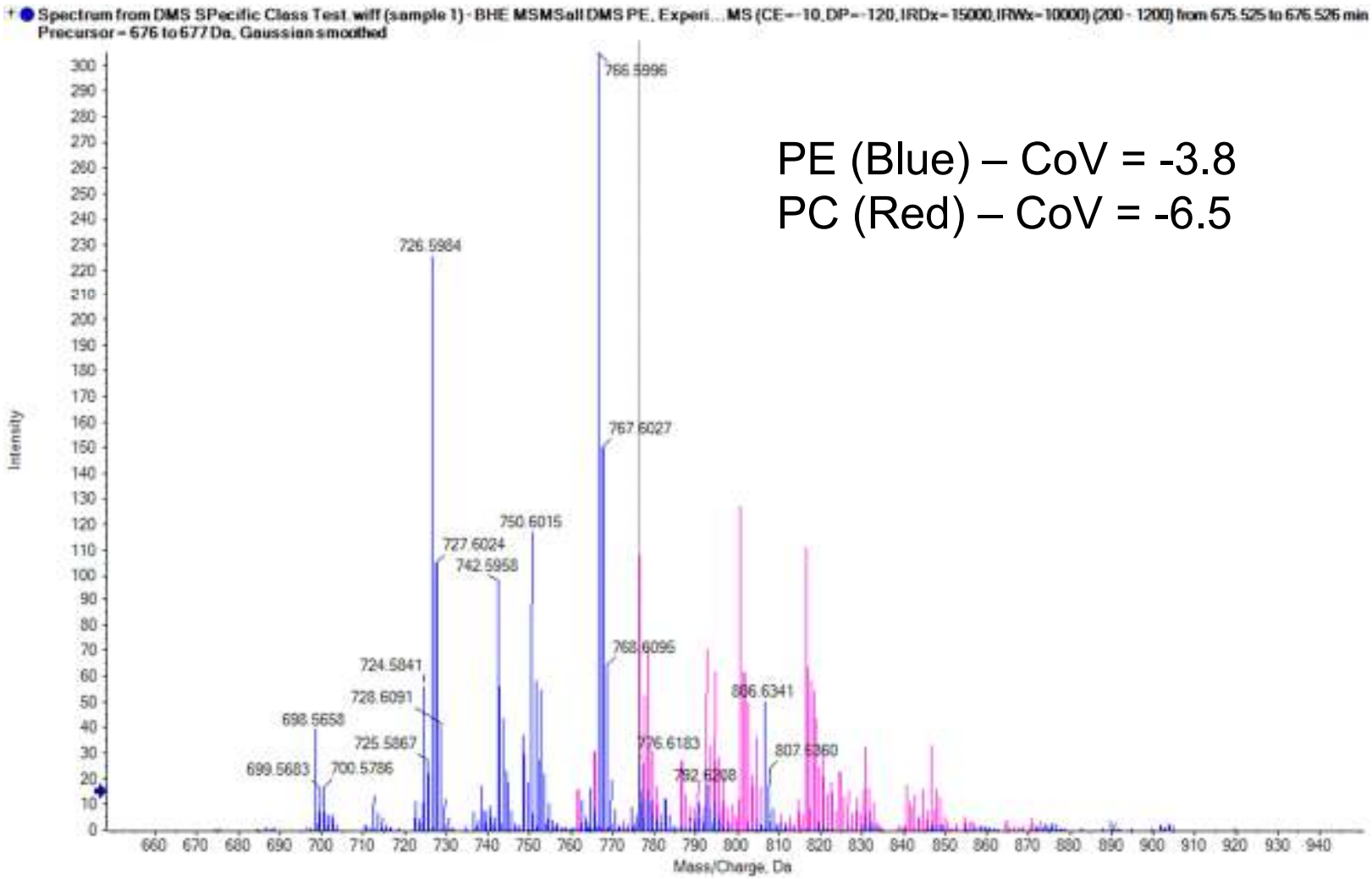
MS/MS^{ALL} with SelexION^{TM}

Use the SelexION^{TM} technology to isolate individual lipid classes for analysis



PE (Blue) – CoV = -3.8
PC (Red) – CoV = -6.5

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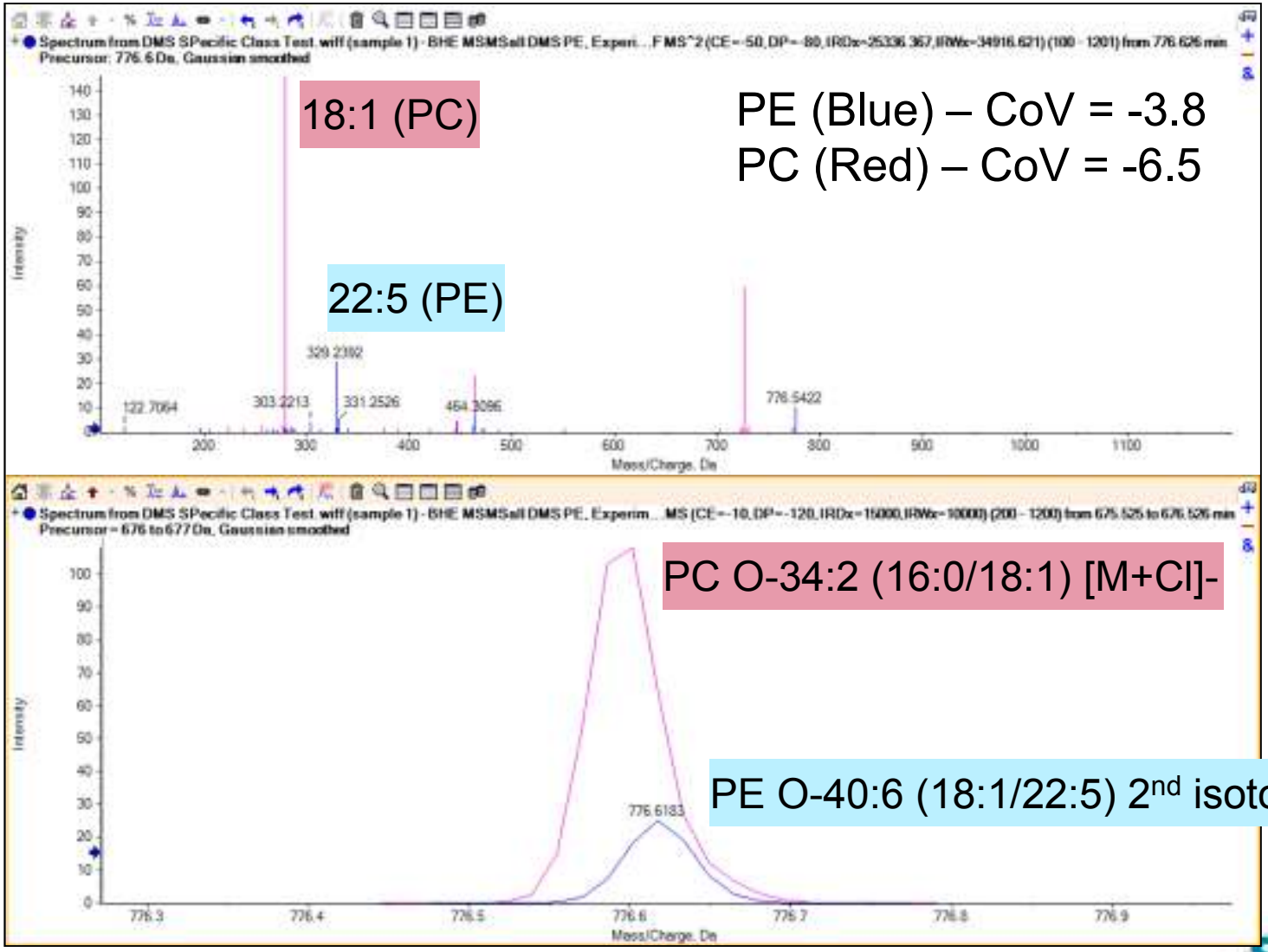
MS/MS^{ALL} with SelexION^{TM}

Use the SelexION^{TM} technology deconvolves complex spectra



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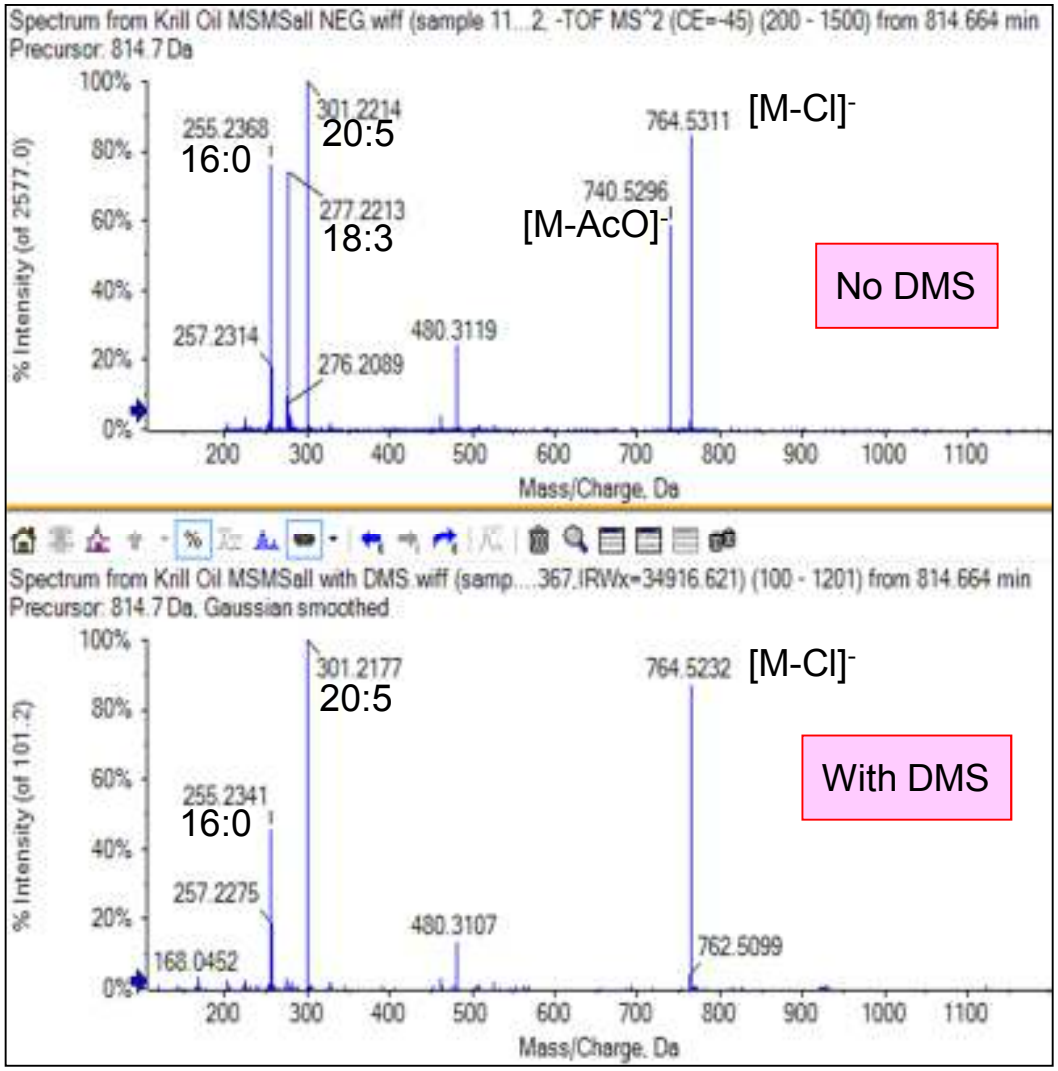
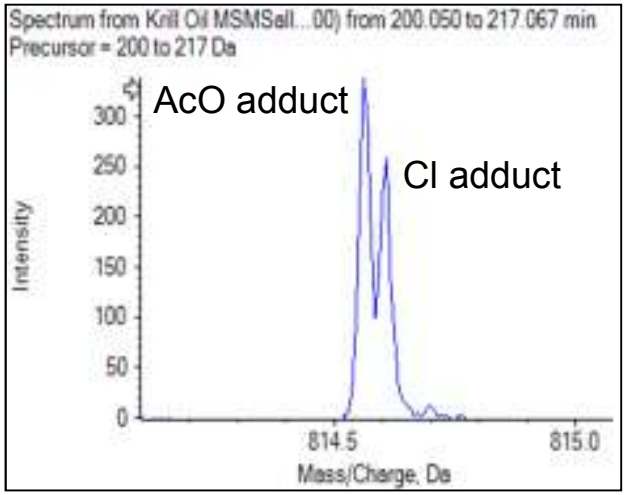
The Power of DMS to Deconvolute Product Ion Spectra

MS/MS of 814.5 (PC 36:5 + Cl and PC 34:3 + AcO)



TOFMS data show 2 peaks at 814.5



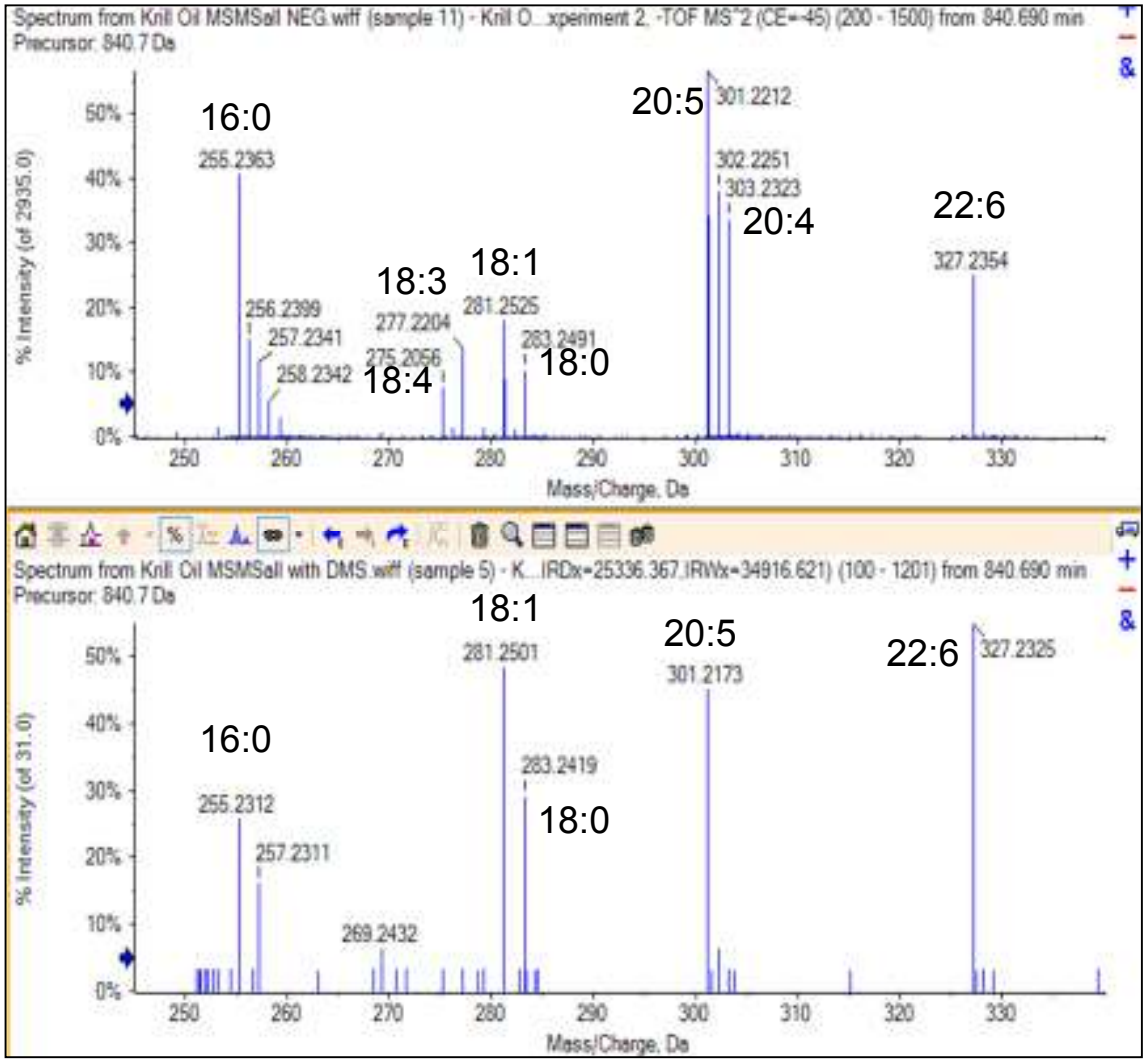
Resolution = ~33,0000

Resolution of these adducts is not possible during precursor ion isolation on ANY instrument unless DMS is used. Extensive chromatography or careful selection of quantitative fragments is required otherwise

Experiment: Krill oil analyzed by MS/MS^{ALL} (negative ion mode) with and without DMS

The Power of DMS to Deconvolve Product Ion Spectra

MS/MS of 840.6 (PC 38:6 + Cl and PC 36:4 + AcO)



No DMS

PC 38:6 (Cl-)

PC (22:6/16:0)
PC (20:4/18:2)
PC (20:5/18:1)

PC 36:4 (AcO-)

PC (20:4/16:0)
PC (18:0/18:4)
PC (18:1/18:3)
PC (18:2/18:2)

With DMS

PC 38:6 (Cl-)

PC (22:6/16:0)
PC (20:5/18:1)

DMS isolates the precursor ion from other isobars, including adducts, to produce a clear product ion spectrum

Experiment: Krill oil analyzed by MS/MS^{ALL} (negative ion mode) with and without DMS

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Acknowledgements

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- Paul Baker
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- Cyrus Papan

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