Targeted lipidomics

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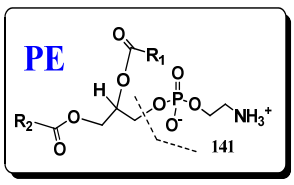
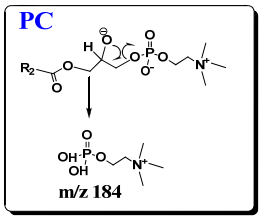
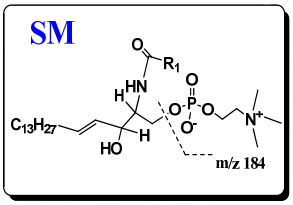
Profiling phospholipids in a complex mixture using MS/MS

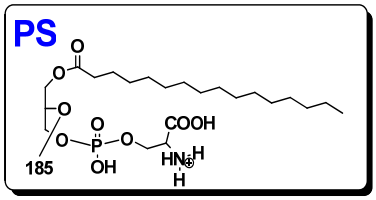


PE
Neutral Loss scan 141



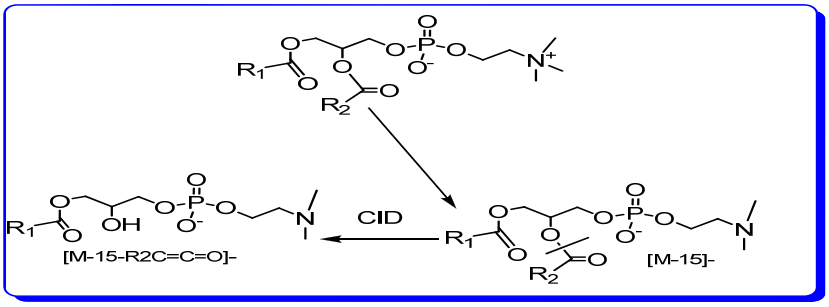


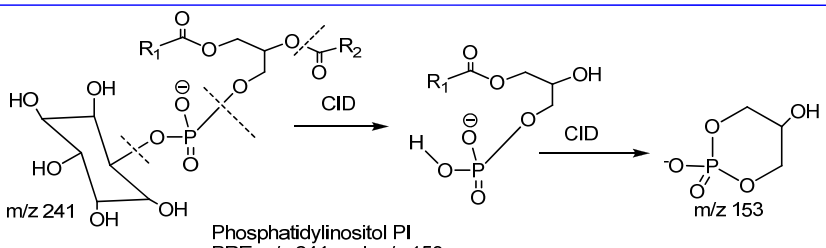
PC & SM
Precursor ion scan 184



PS
Neutral Loss scan 185

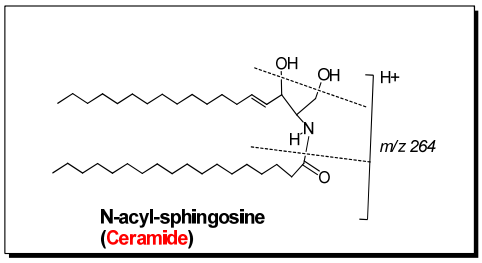
MS/MS in negative ion mode of phospholipids provide structural information more than positive ion mode

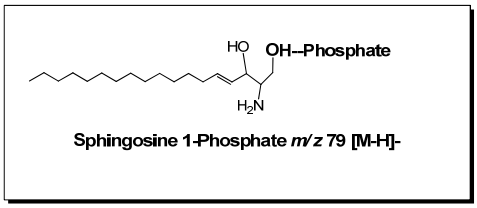




Phosphatidylinositol PI
PRE m/z 241 and m/z 153

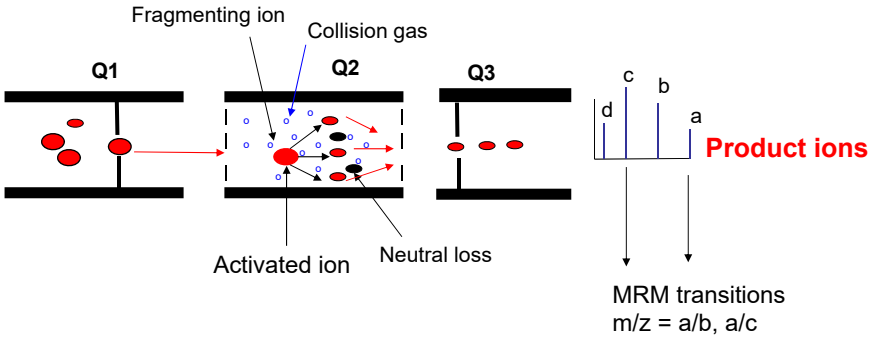
How to profile sphingolipids in a complex mixture using MS/MS?





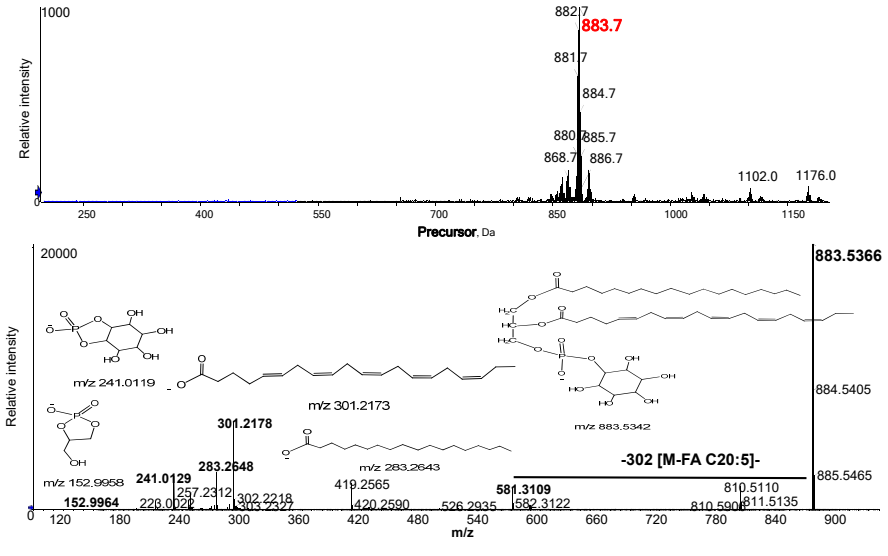
***m/z* 264 is a characteristic ion for all compounds containing a sphingosine backbone in positive ion mode**

Schematic of precursor ion scan, neutral loss and MRM experiments in a triple quadrupole instrument

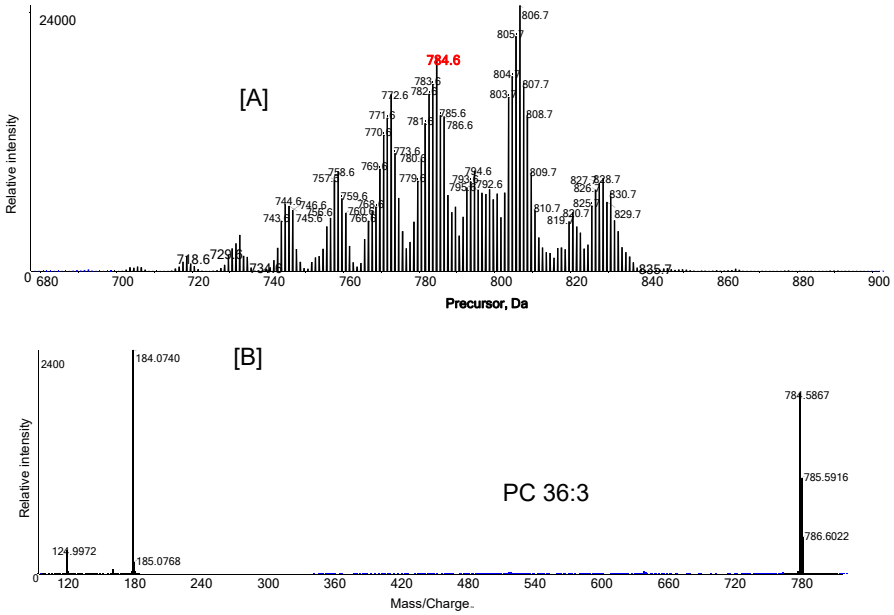


Precursor ion scan PS Q1 (Scan) Q3 (fixed, eg. m/z 184 for PC/SM)
Neutral loss scan NL Q1 (scan) Q3 (scan offset, eg. 141 for PE)

Precursor ion scan m/z 241 for PI in a C. elegans lipid extract [A]; MS/MS of the precursor ion m/z 883[B]

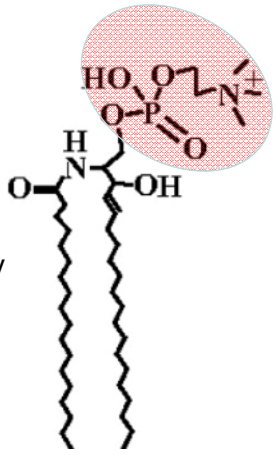


Precursor ion scan m/z 184.073 for PC/SM in a C. elegans lipid extract [A]; MS/MS of the precursor ion m/z 784 [B]

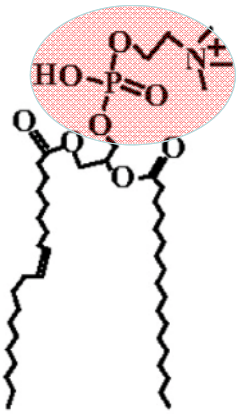


The problem of analyzing lipids

- 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



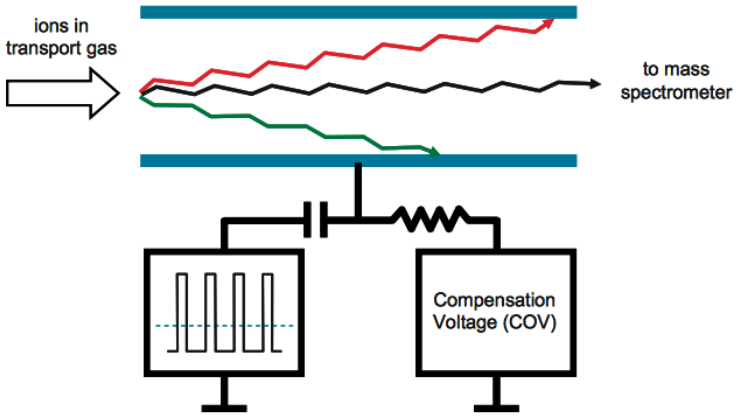
Sphingolipid



Phosphocholine

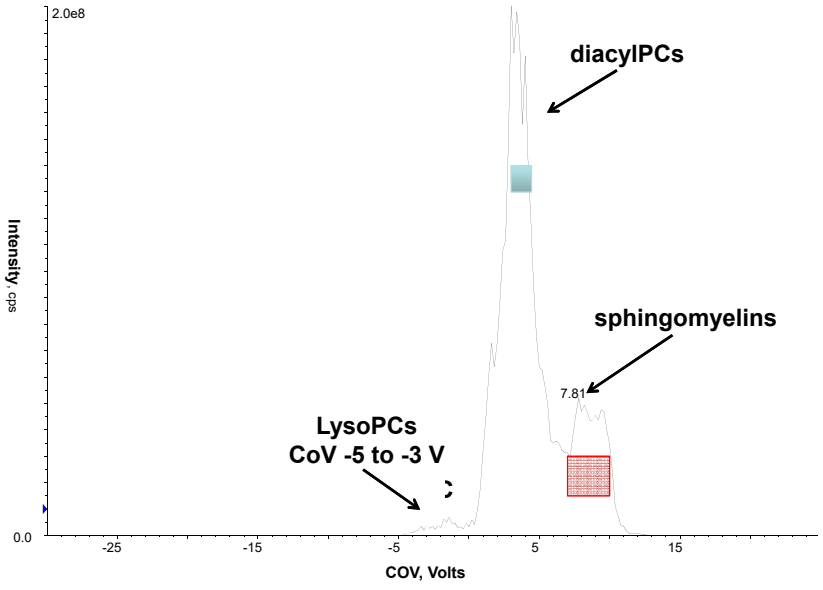
Differential mobility MS is an answer

Innovative Planar Design; SelexION™ Ion Mobility Cell.

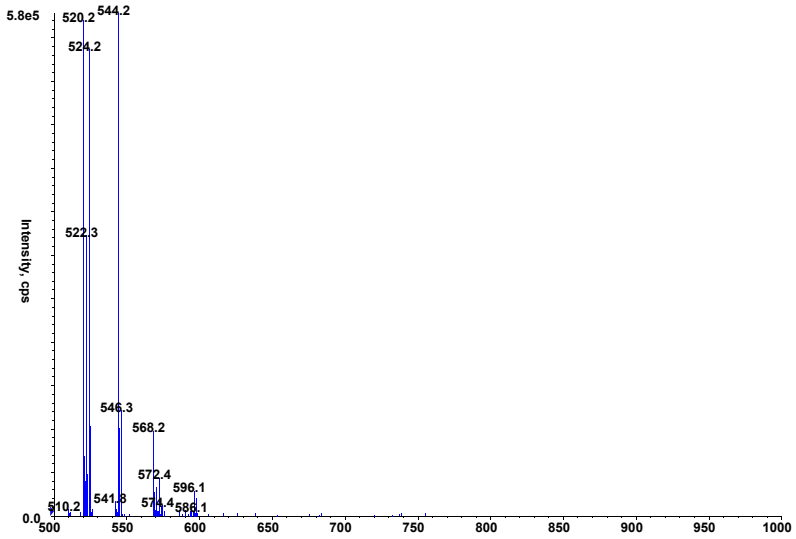


Source: http://www.absciex-korea.com/Documents/Downloads/Literature/SelexION-Forensic-Tech-Note-3460111-01.pdf

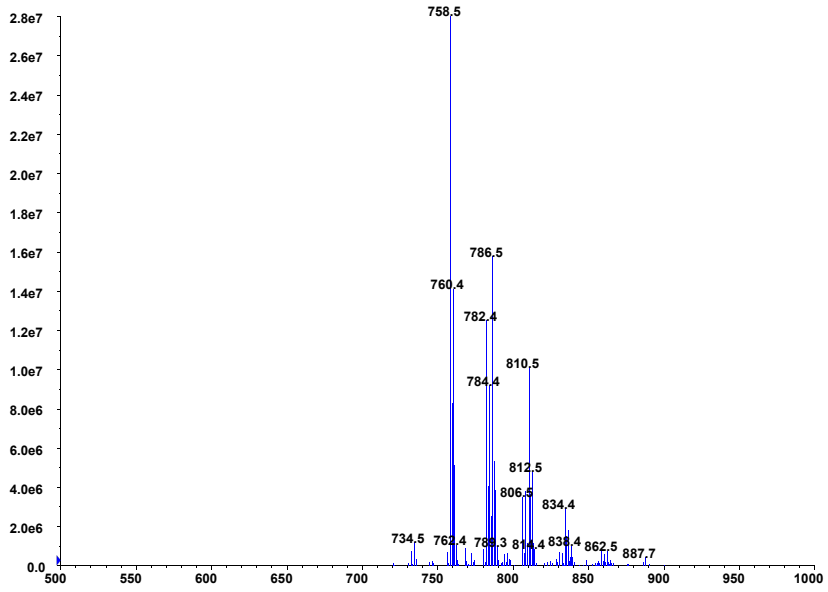
Total ion current of precursors of *m/z* 184.0



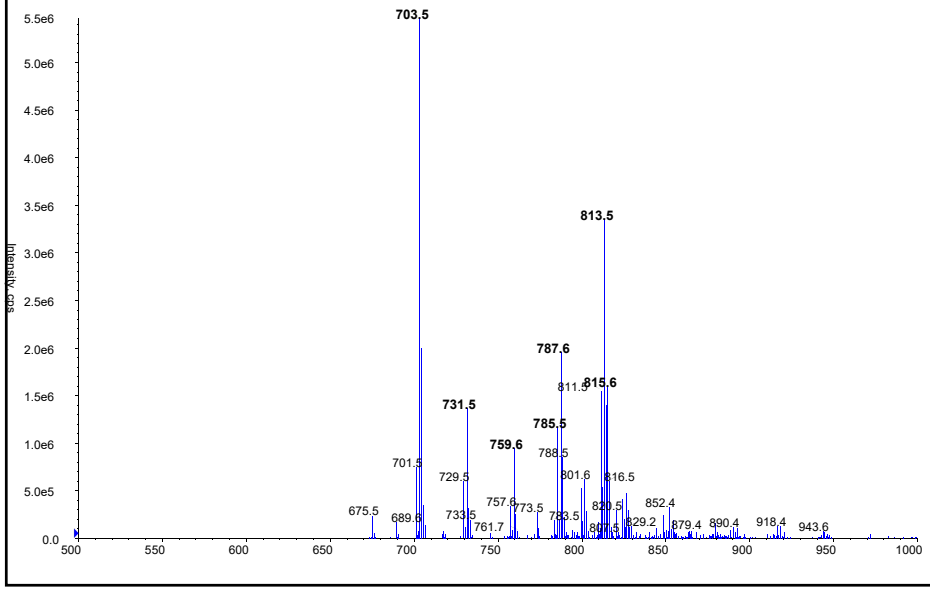
Precursors of *m/z* 184.0 (CoV -3.6 to -0.4 V) Lysophospholipids



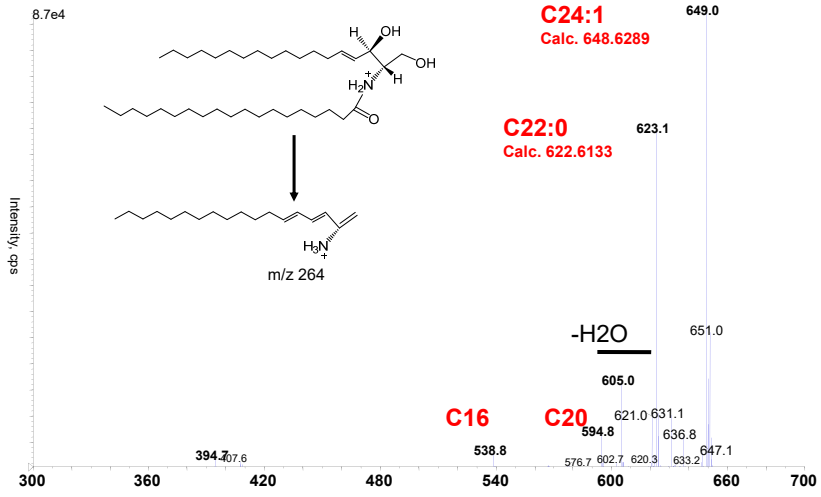
Precursors of *m/z* 184.0 (CoV from 3.0 to 3.6 V)



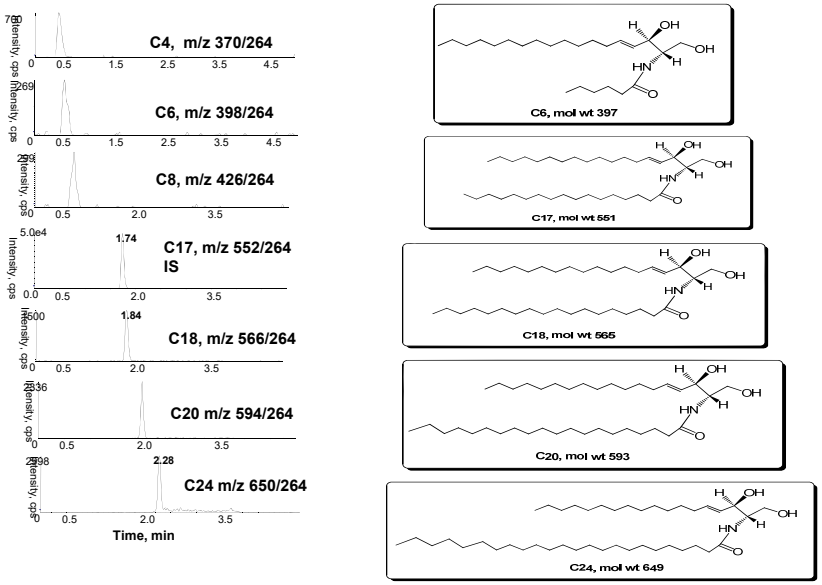
Precursors of *m/z* 184.0 (CoV from 7.8 to 10.0 V)
Sphingomyelins are well separated from PCs



Precursor ion scan m/z 264 in +ve ion mode is specific to identify ceramides in a sample



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



Conclusions

- Shotgun lipidomics approaches are high throughput and applicable to perform qualitative as well as quantitative analysis of various lipids in biological samples.
- Tandem mass spectrometry analysis of phospholipids in +ve ion mode characterizes phospholipid polar head groups, whereas –ve ion mode provide fatty acid chain structural information
- Identification of phospholipids at a molecular level present a great challenge due to their structural diversity and dynamic metabolism.
- Differential mobility mass spectrometry (DMS) is an important new tool in the study of lipids
  - It overcomes many of the problems associated with isobaric peaks and contaminants.