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

SM

PS Neutral Loss scan 185
MS/MS in negative ion mode of phospholipids provide structural information more than positive ion mode.

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

PC 36:3
Differential mobility MS is an answer

Innovative Planar Design; SelexION™ Ion Mobility Cell.


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
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.