Lipidomics

Lipidomics- A comprehensive analysis of lipid molecules in response to cellular pathophysiology

Outlines

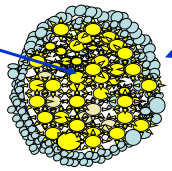
- **Brief introduction to lipidomics**
- **Analytical methodology: MS/MS structure elucidation of phospholipids**
- **Phospholipid analysis by MS/MS**
- **MS/MS analysis of eicosanoids**

Why measure lipids?

Lipids are important- as a membrane bilayer
- provides hydrophobic environment for protein function
- reservoir of energy
- signaling molecules

Storage lipids

Glycerolipids
Cholesteryl esters



Membrane lipids

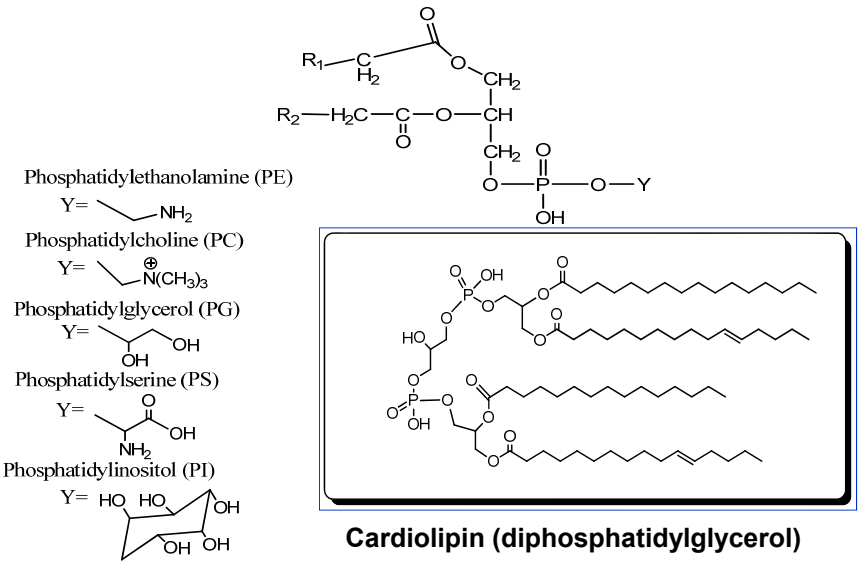
Phospholipids
Sphingolipids
Sterols

Lipidomics can perhaps best be defined as a comprehensive analysis of lipids on the systems-level scale together with their interacting factors

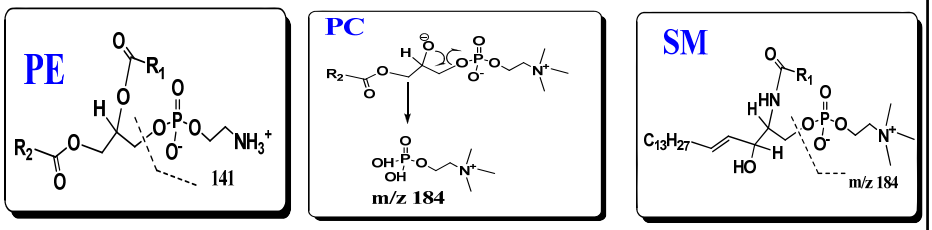
Landmarks in the analysis of lipids

- Early 1800s Fractional distillation
- 1908-1950s Liquid (adsorption) and paper chromatography
- 1930-1950 Fractional crystallization
- 1940-1960 Urea-fatty acid and metal-fatty acid complexes
- 1952 **AT James and AJP Martin invent gas-liquid chromatography for volatile fatty acids (C1-C12)**
- 1958 Fatty acid methyl esters and polar liquid phases extend analysis to very long chain fatty acids C_{34} and unsaturated fatty acids
- 1963 EC Horning applies capillary gas chromatography
- 1980s Thermospray, electrospray and APCI ionization interfaces link LC (HPLC) with mass spectrometry
- 2000s Improvements in mass accuracy and uPLC

Structures of major phospholipids

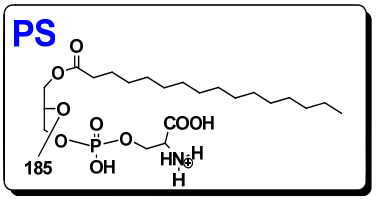


How to profile phospholipidsin a complex mixture using MS/MS?



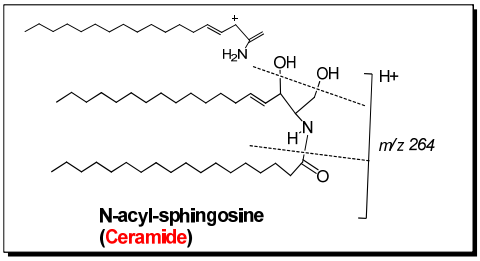
PE
Neutral Loss scan 141

PC & SM
Precursor ion scan 184

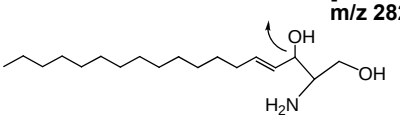


PS
Neutral Loss scan 185

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

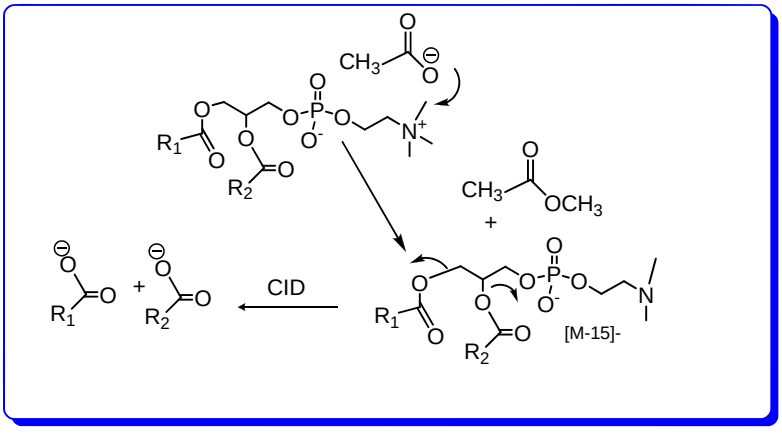


[M+H-H2O]+
m/z 282



***m/z* 264 and 282 are characteristic ions for compounds containing a sphingosine backbone**

Tandem mass spectrometry has the ability to characterize the fatty acyl chain in -ve ion mode



Phospholipids may undergo demethylation and then the loss of the fatty acyl groups from glycerophosphocholine backbone.

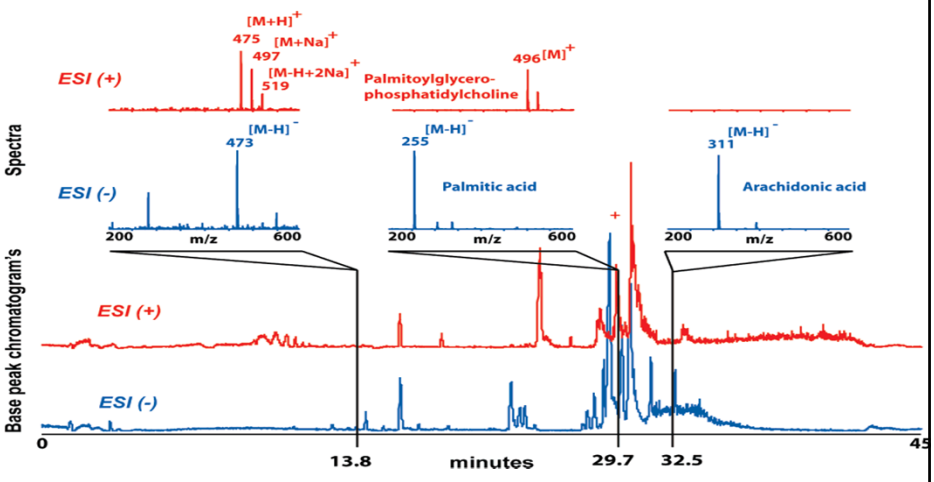
Shotgun lipidomics: intrasource separation of lipids for quantitative lipidomics

Group	Electrical Propensity	Lipid Classes
Anionic lipids	Carry net negative charge(s) at physiological pH	Cardiolipin, acylCoA, sulfatide, PtdIns (PtdInsP, $PtdInsP_2$, $PtdInsP_3$), PtdGro, PtdSer, PtdH, etc.
Weak anionic lipids	Carry a net negative charge at alkaline pH	PE, lysoPE, ceramide, NEFA, eicosanoids, etc.
Neutral polar lipids	Neutral at alkaline pH	PC, lysoPC, SM, glycolipid, TAG, etc.
Special lipids	Vary	Acylcarnitine, sterols, etc.

The ionization efficiency of an analyte greatly depends on the electrical propensity of an individual analyte in its own microenvironment to lose or gain a charge

Source: Gross and Han, 2004

Increasing metabolite coverage using +ve and –ve ion mode



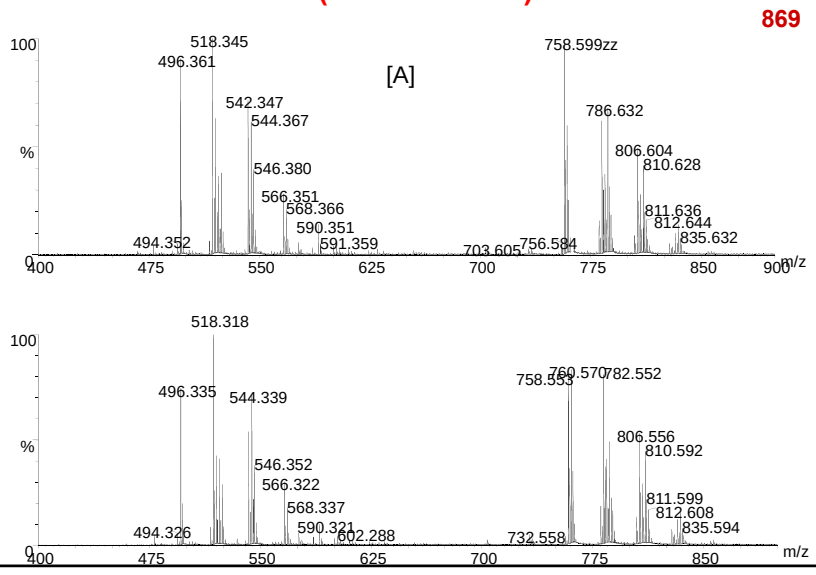
Representative Q1 scans of a methanolic extract of human blood serum

Source: Nordstrom et al. Analytical Chemistry, 2008

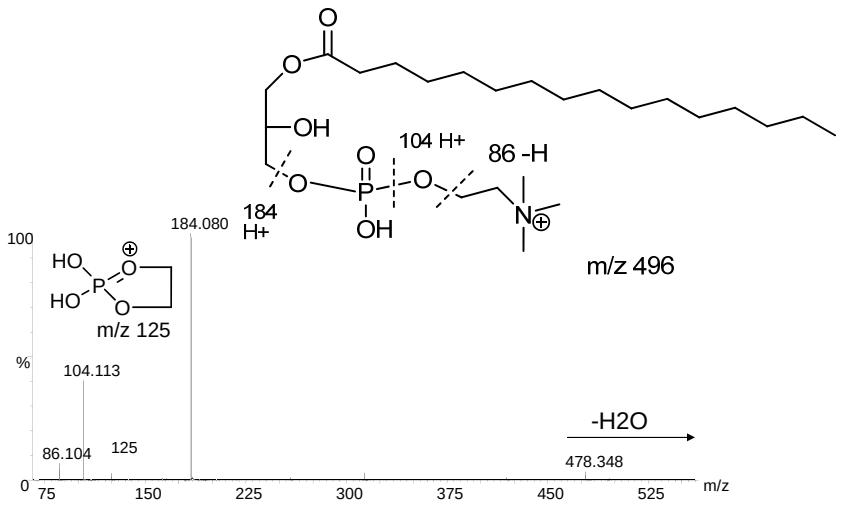
How to extract lipids?
Extraction of lipids by Bligh/Dyer method

- To a homogenized sample (1 ml containing internal standards) add methanol (2.5 ml) and chloroform (1.25 ml), sonicate by 4-5 bursts; extra 1.0 ml water and 1.25 ml chloroform added and vigorously shaken.
- Centrifuge (1,000 x g) for 2 min and separate the chloroform layer (bottom layer) and repeat the process twice.
- Combine the chloroform soluble phases and evaporate to dryness and store at -20^{o}C until analysis.

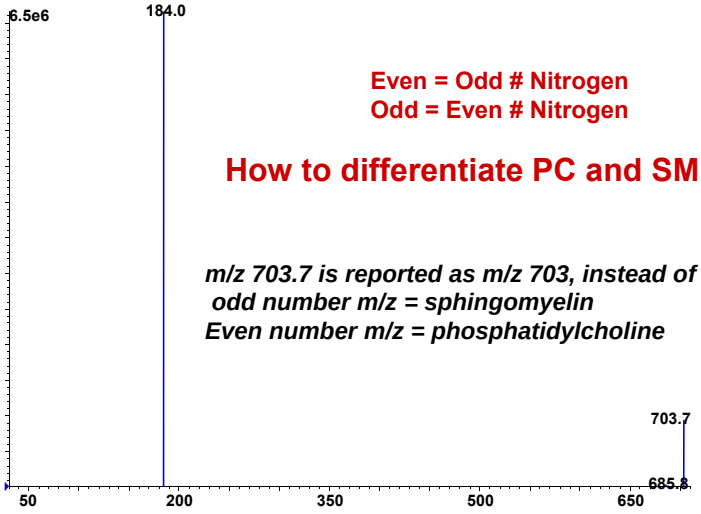
Survey scan of metabolites (+ ion mode) for a plasma sample from lean mouse [A]; ob/ob mouse [B]. Plasma lipidomes of obese mice are higher than lean littermates (1.5e3 vs. 863)



MSMS fragmentation of *m/z* 496 obtained from plasma of obese mouse identified to be LPC 16:0



MS/MS of sphingomyelin standard (2S,3R,4E)-2-acylaminooctadec-4-ene-3-hydroxy-1-Phosphocholine



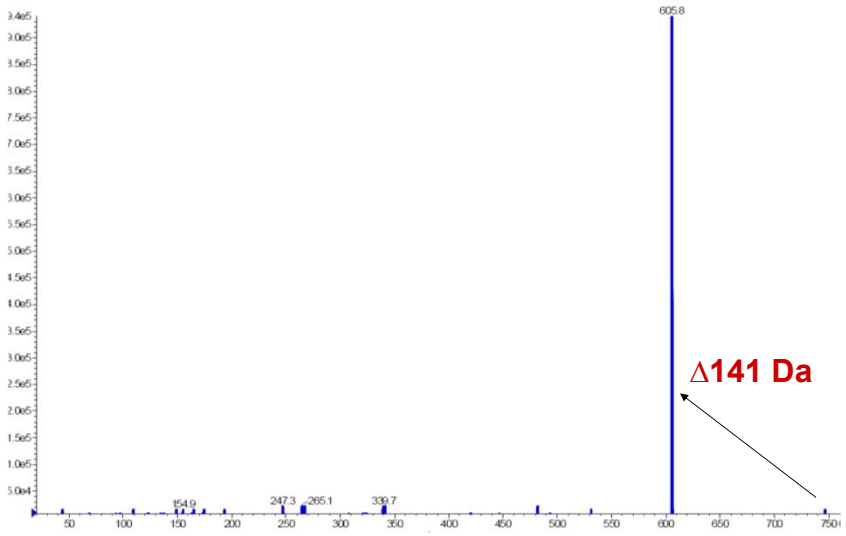
Even = Odd # Nitrogen
Odd = Even # Nitrogen

How to differentiate PC and SM?

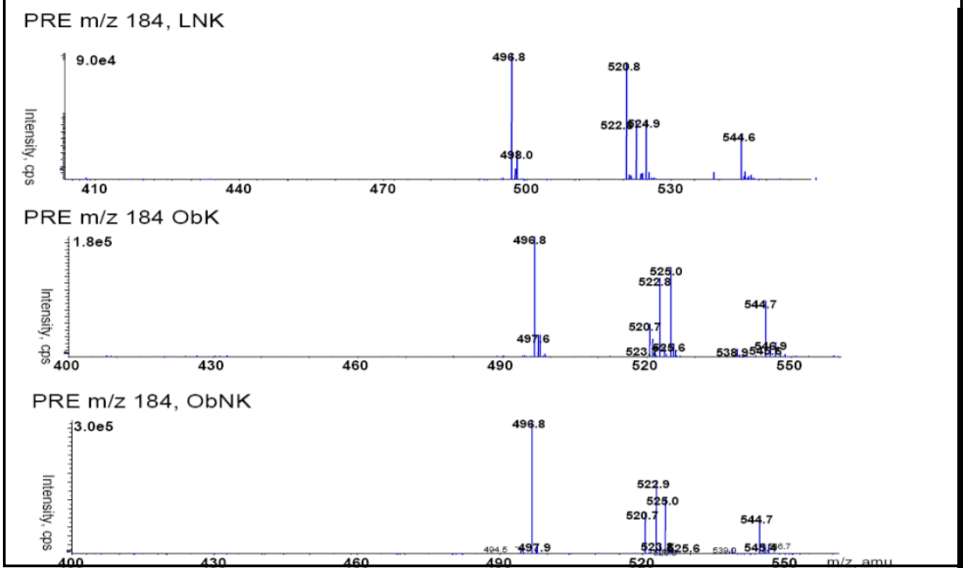
m/z 703.7 is reported as m/z 703, instead of m/z 704
odd number m/z = sphingomyelin
Even number m/z = phosphatidylcholine

MS/MS of m/z 746.90: PE Std

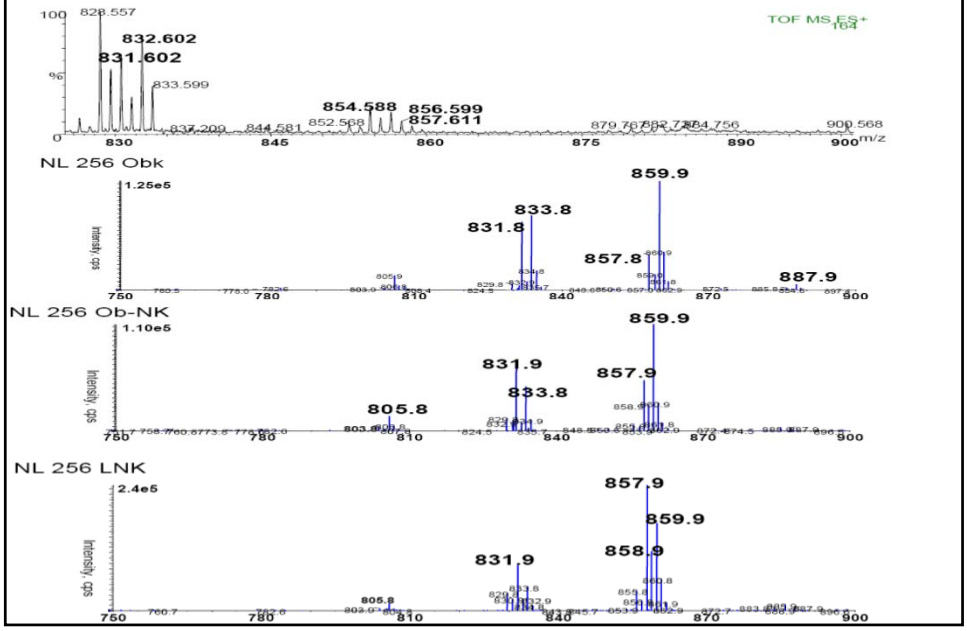
Neutral loss of 141 is a characteristic for detecting PE

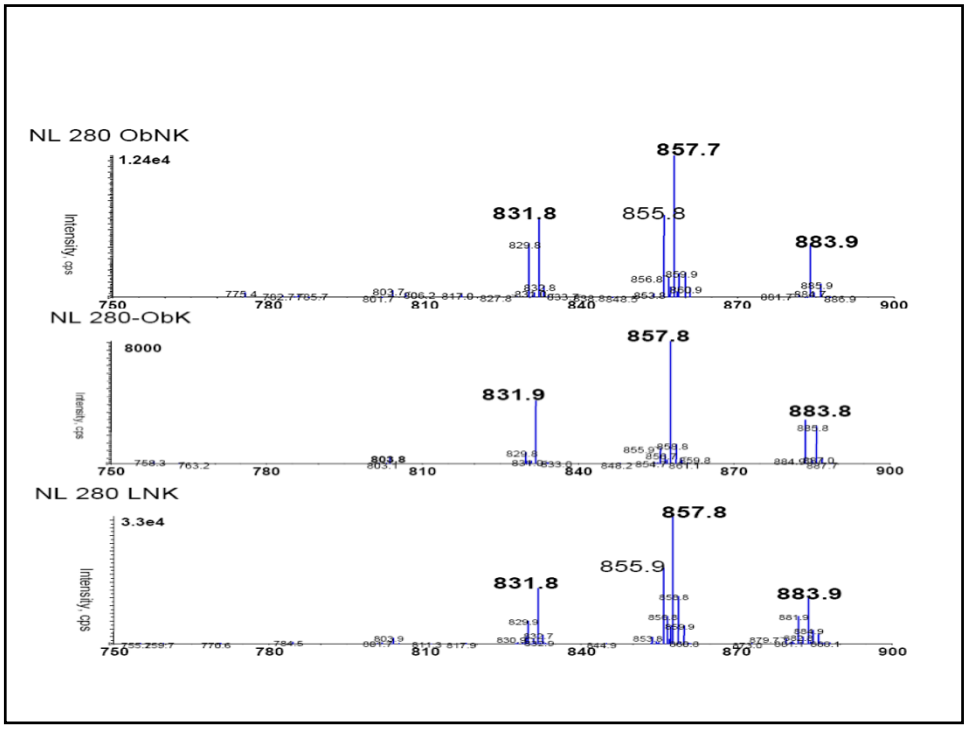


Targeted lipidomics- Precursor ion spectra (PRE *m/z* 184) from LNK, ObK and ObNK hepatocytes.

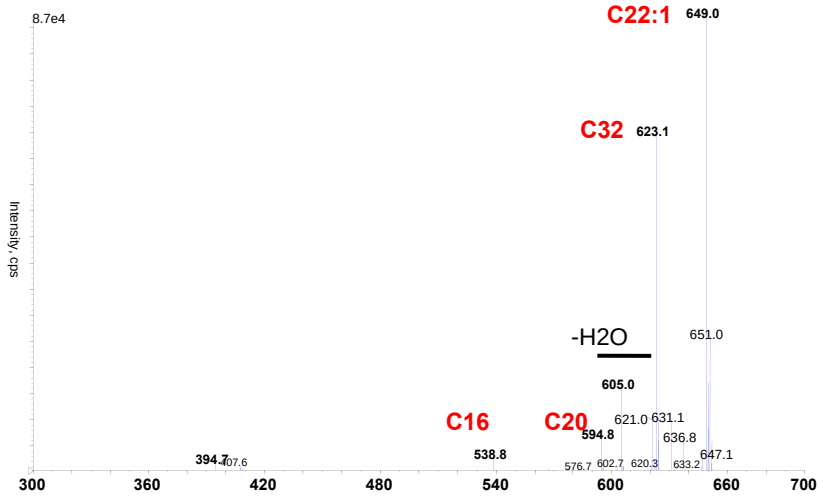


A 2D ESI mass spectrometric finger print for TG molecules

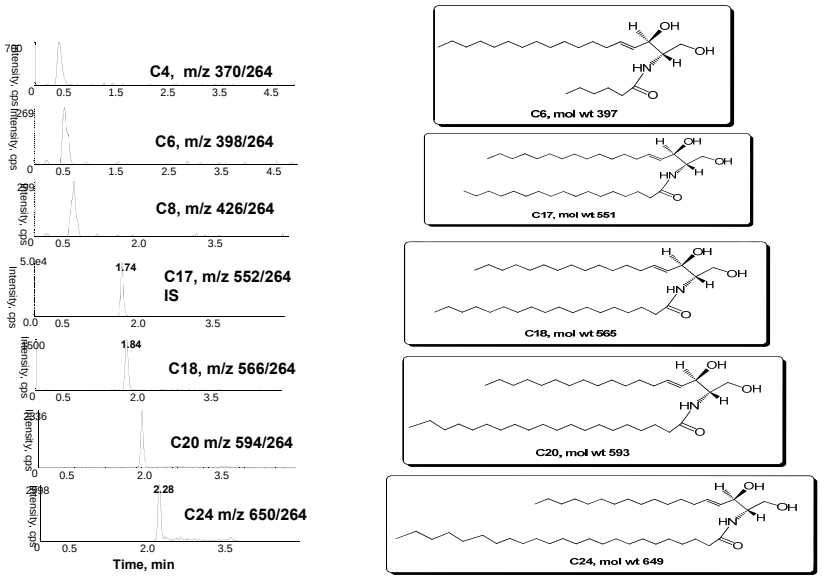




Precursor ion scan m/z 264 in +ve ion mode is specific to identify ceramides in a sample



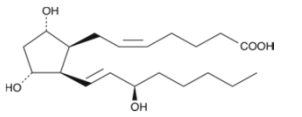
MRM chromatograms showing simultaneous determination of ceramides (C4-C24)



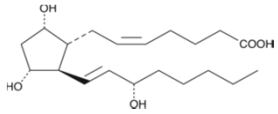
Eicosanoids, meaning 20 derived from a 20-carbon acid, arachidonic acid

-**Important lipid mediators and elicit potent effects in various biological systems mediated through specific protein receptors**

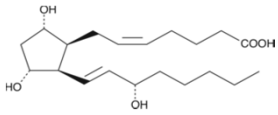
PGs and their diastereoisomer isoprostanes can be distinguished based on retention time in LC-MS



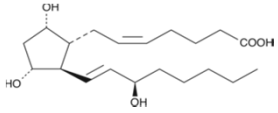
8-iso-15(R)-Prostaglandin $F_{2\alpha}$



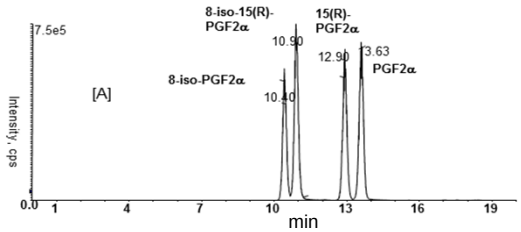
Prostaglandin $F_{2\alpha}$



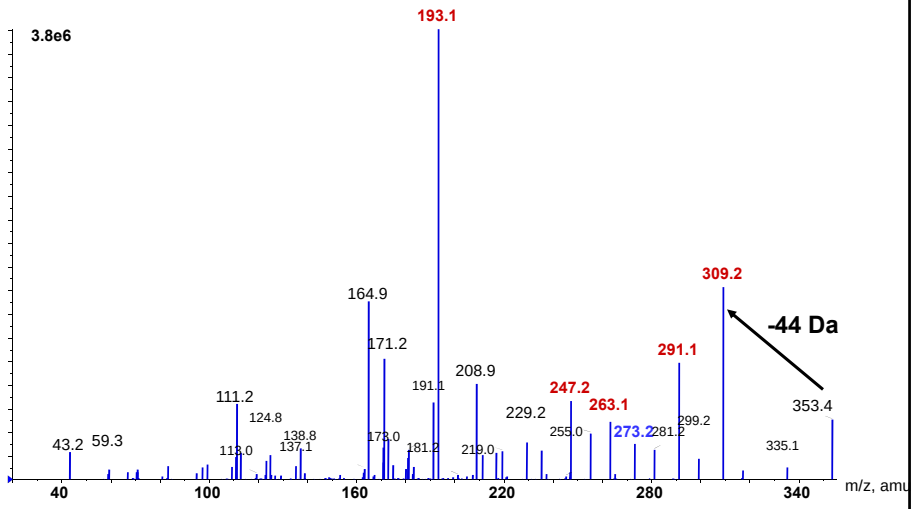
8-iso Prostaglandin $F_{2\alpha}$



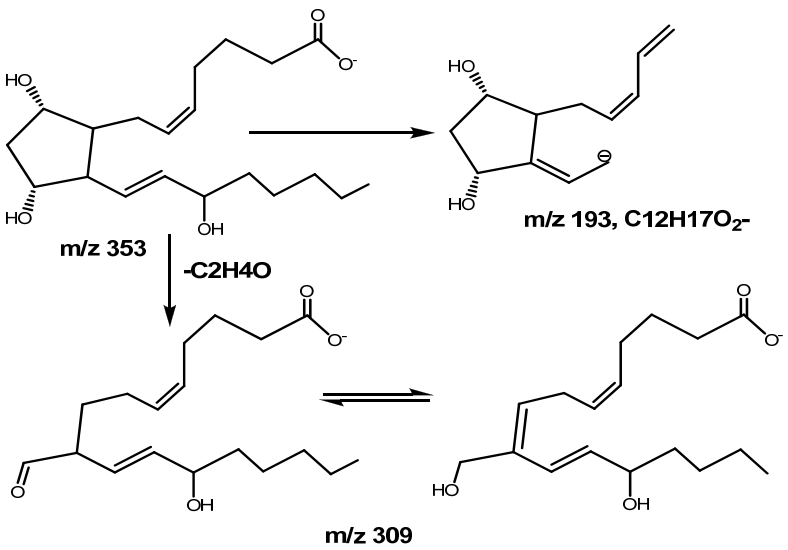
15(R)-Prostaglandin $F_{2\alpha}$



MS/MS of the [M-H]- from PGF2a m/z 353 using a quadrupole mass spectrometer

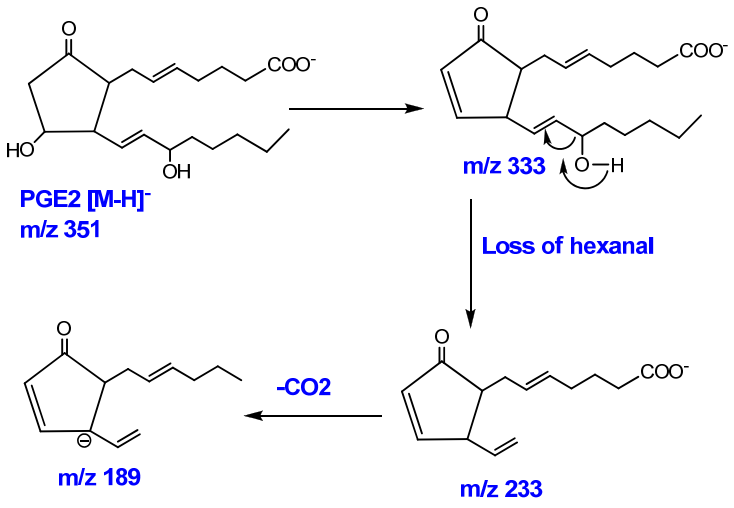


Fragmentation scheme of PGF2α [M-H]⁻ m/z 353



Ions m/z 309, 291, 273 and 193 are indicative of F2-ring

MS/MS fragmentation of PGE2 $[M-H]^-$ m/z 351



The first loss of water, m/z 189 and m/z 233 are characteristics of PGE2/PGD2

Conclusions

- 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.
- Shotgun lipidomics can be used for rapid and reproducible global analysis of lipids in biological samples.
- Identification of metabolites (lipids or any other metabolites) at a molecular level present a great challenge due to their structural diversity (isobars and isomers) and dynamic metabolism.