The use of mass spectrometry in lipidomics

Jeevan Prasain, PhD
jprasain@uab.edu
6-2612

1

Outlines

- Brief introduction to lipidomics
- Analytical methodology: MS/MS structure elucidation of phospholipids
- Phospholipid analysis in lean and ob/ob mice by mass spectrometry

2

Lipids are very important!!

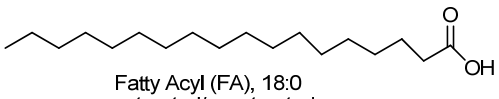
- Structural components of cell membrane
- Nutrition
- Energy source/storage
- Signaling molecules (extra- and intra-cellular)

3

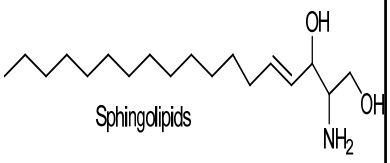
- Lipidomics- A comprehensive analysis of lipid molecules in response to cellular stress and challenges
- Changes in lipid interactions

4

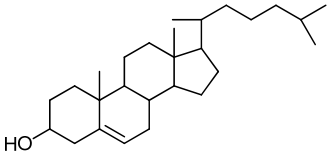
Structures of different lipids classes



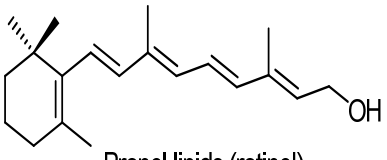
Fatty Acyl (FA), 18:0
saturated/unsaturated



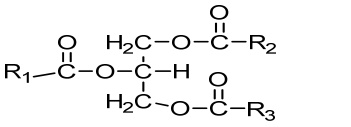
Sphingolipids



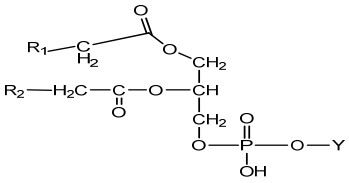
Sterol lipids



Prenol lipids (retinol)



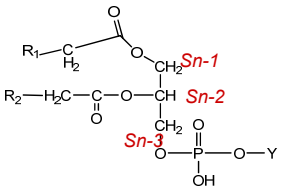
Glycerolipid, R =
saturated/unsaturated FA



Glycerophospholipid

5

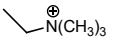
Structures of main phospholipids



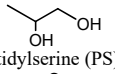
Phosphatidylethanolamine (PE)

Y= 

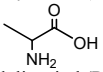
Phosphatidylcholine (PC)

Y= 

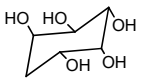
Phosphatidylglycerol (PG)

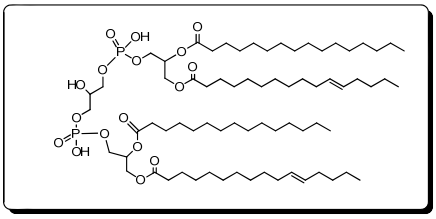
Y= 

Phosphatidylserine (PS)

Y= 

Phosphatidylinositol (PI)

Y= 



Cardiolipin (diphosphatidylglycerol)

6

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 and added 1.0 ml water and 1.25 ml chloroform additionally 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 phase and evaporate to dryness and stored at -20 ^{o}C untill analysis.

7

Shotgun lipidomics: intrasource separation of lipids for quantitative lipidomics

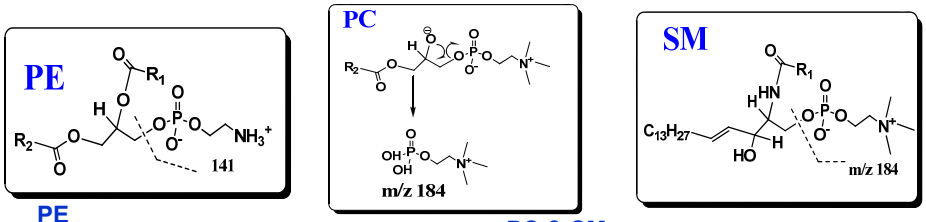
Group	Electrical Propensity	Lipid Classes
Anionic lipids	Carry net negative charge(s) at physiological pH	Cardiolipin, acylCoA, sulfatide, PtdIns (PtdInsP, PtdInsP$_2$, PtdInsP$_3$), PtdGro, PtdSer, PtdH, etc.
Weak anionic lipids	Carry a net negative charge at alkaline pH	PE, lysoPE, ceramide, NEFA, 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, Mass Spec Rev. 2005

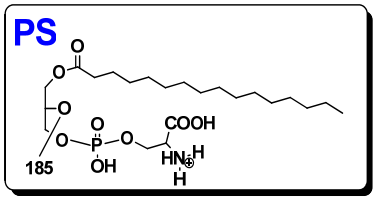
8

Profiling glycerophospholipids in a complex mixture using MS/MS



PE
Neutral Loss scan 141

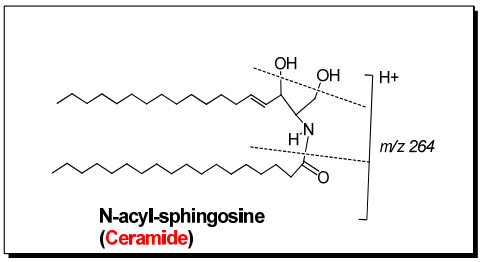
PC & SM
Precursor ion scan 184

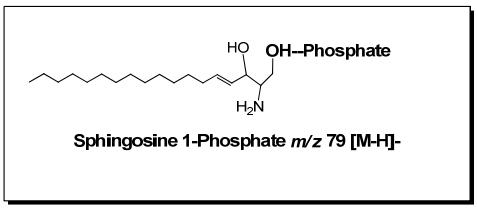


PS
Neutral Loss scan 185

9

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



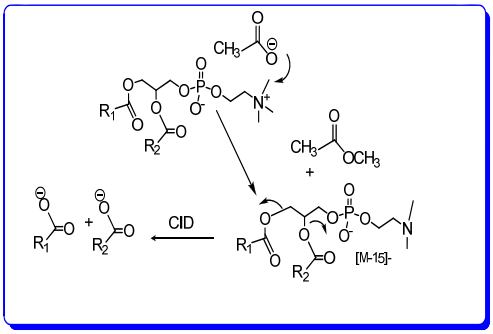


***m/z* 264 is a characteristic ion for all compounds containing a sphingosine backbone in +ve ion mode**

10

Phosphatidylcholine loses a methyl group to form a negatively charged, pseudomolecular ion

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



11

ESI-MS/MS analyses of various lipids

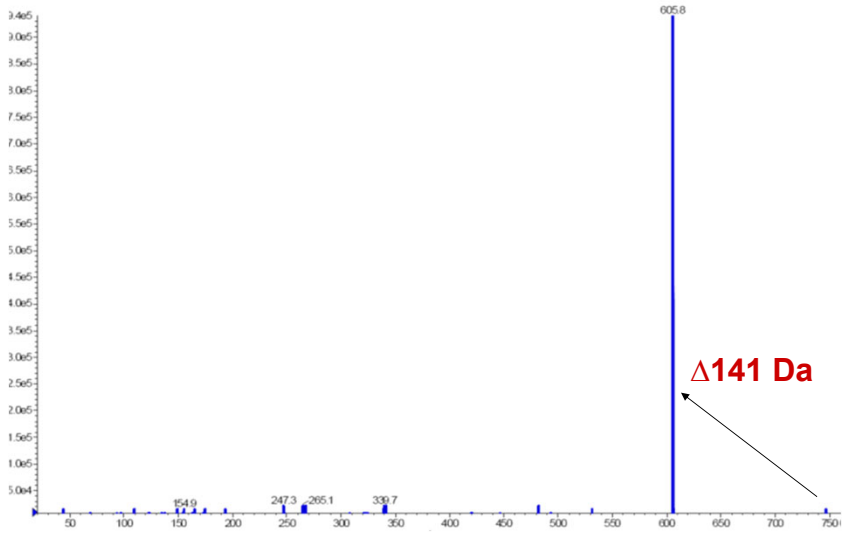
Lipid Class(s)	Precursor Ion	MS/MS Mode & Conditions	Fragment
cardiolipin	$[M-2H]^{2-}$	PI, *m/z* 153.0, 35 eV	glycerol phosphate derivative
PtdGro, PtdH	$[M-H]^-$	PI, *m/z* 153.0, 35 eV, *	glycerol phosphate derivative
PtdIns	$[M-H]^-$	PI, *m/z* 241.1, 45 eV	cyclic Inositol phosphate
		PI, *m/z* 153.0, 35 eV	glycerol phosphate derivative
PtdInsP	$[M-H]^-$	PI, *m/z* 321.1, 53 eV	phosphoinositol phosphate
$PtdInsP_2$	$[M-H]^-$	PI, *m/z* 401.1, 62 eV	diphosphoinositol phosphate
PtdSer	$[M-H]^-$	NL, 87.0 amu, 25 eV, *	serine
		PI, *m/z* 153.0, 35 eV	glycerol phosphate derivative
sulfatide	$[M-H]^-$	PI, *m/z* 97.0, 65 eV	sulfate
acylCoA	$[M-2H]^{2-}$	PI, *m/z* 339.0, 30 eV, *	doubly-charged CoA derivative
PE, lysoPE	$[M-H]^-$	PI, *m/z* 196.0, 50 eV	glycerol phosphoethanolamine derivative
ceramide	$[M-H]^-$	NL, 256.2 amu, 32 eV *	
		NL, 327.3 amu, 32 eV	
		NL, 240.2 amu, 32 eV *	2-*trans*-palmitoyl alcohol
PC, lysoPC, SM	$[M+Li(Na)]^+$	NL, 59.1 amu, -28 eV, *	trimethylamine
	$[M+Li(Na)]^+$	NL, 183.1 amu, -32 eV	phosphocholine
	$[M+Li]^+$	NL, 189.1 amu, -42 eV	lithium cholinephosphate
	$[M+Na]^+$	NL, 205.1 amu, -35 eV	sodium cholinephosphate
	$[M+H]^+$	PI, *m/z* 184.1, -30 eV, *	phosphocholine
	$[M+Cl]^-$	NL, 50.0 amu, 24 eV, *	methylchloride
cerebroside	$[M+Li]^+$	NL, 162.2, -50 eV, *	
	$[M+Cl]^-$	NL, 36.0 amu, 30 eV	hydrogen chloride
MGDG	$[M+Li(Na)]^+$	PI, *m/z* 227(243), -45 eV	Li(Na)+galactose derivative
DGDG	$[M+Li(Na)]^+$	PI, *m/z* 227(243), -66 eV	Li(Na)+galactose derivative
acylcarnitine	$[M+H]^+$	PI, *m/z* 85.1, -20 eV, *	carnitine
chol. ester	$[M+NH_4]^+$	PI, *m/z* 369.3, -50 eV, *	cholestane cation
TAG	$[M+Li]^+$	NL, X amu, -35 eV	a fatty acid

Source: Gross and Han, Mass Spec Rev. 2005

12

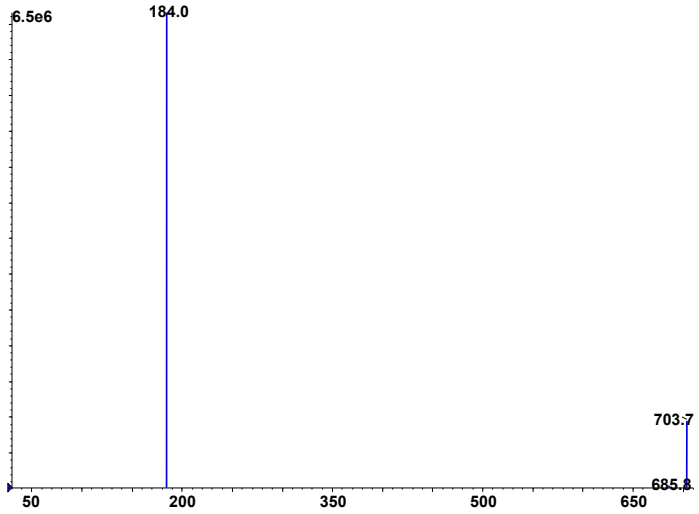
MS/MS of m/z 746.90: PE Std

Neutral loss of 141 is a characteristic for detecting PE



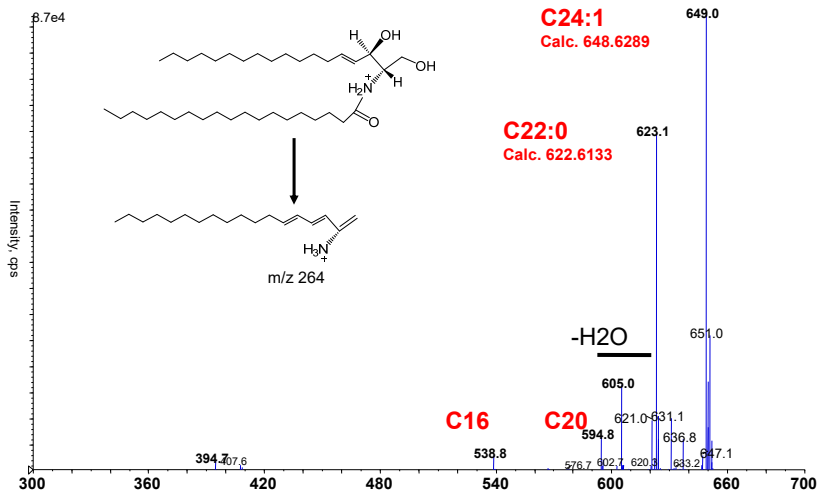
13

MS/MS of sphingomyelin standard (2S,3R,4E)-2-acylaminooctadec-4-ene-3-hydroxy-1-Phosphocholine m/z 703.7



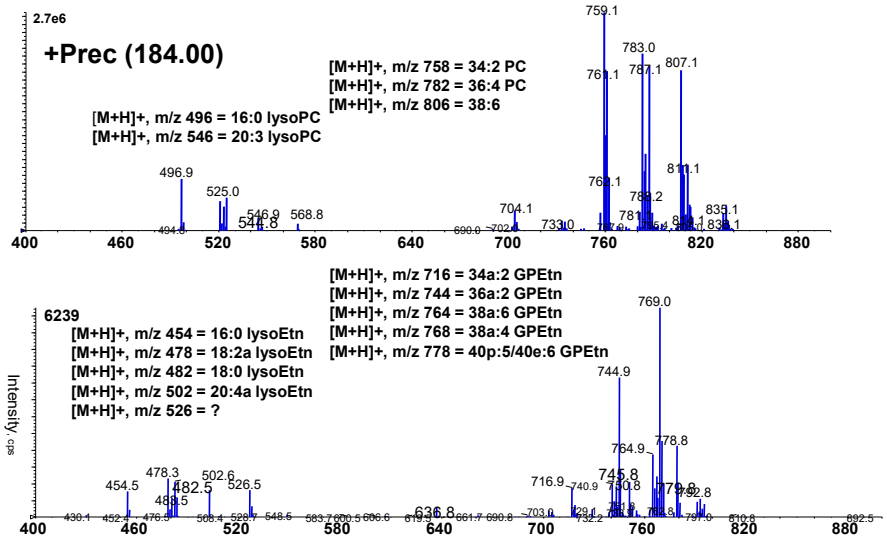
14

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



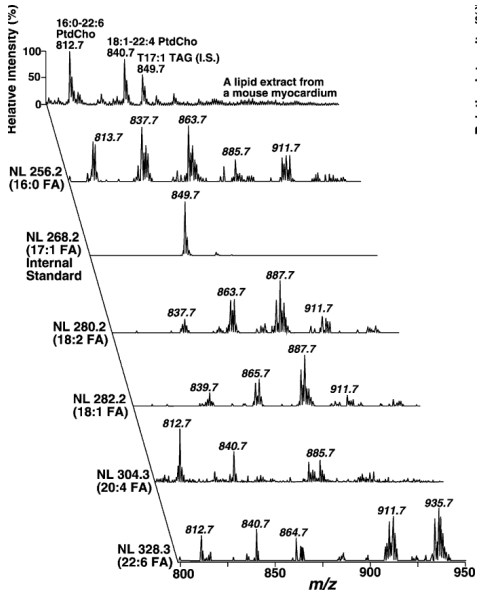
15

Profiling of phospholipids using precursor ion m/z 184 and neutral loss scan 141 for PC, SM and PE



16

Neutral loss scans can be used to profile triacylglycerides (TAG)

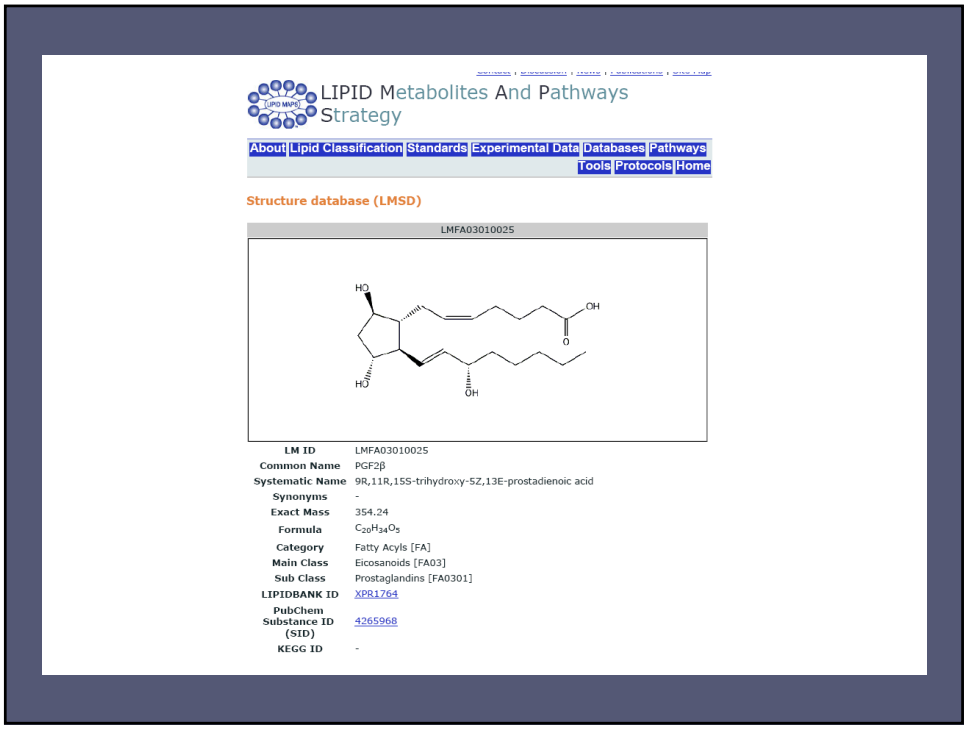


Source: Gross and Han, Mass Spec Rev. 2005

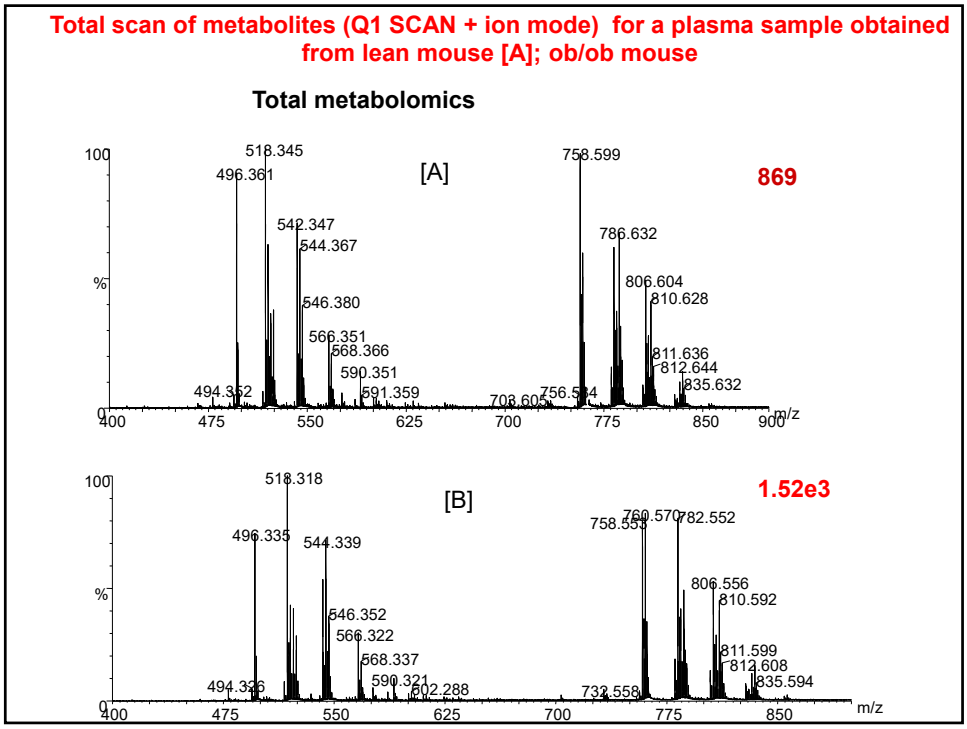
17

Library search for eicosanoid http://www.lipidmaps.org/

18

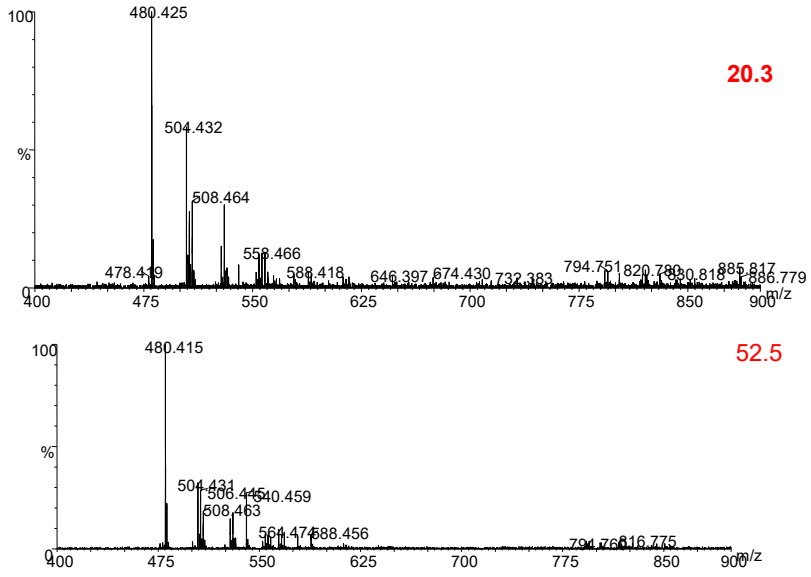

LIPID Metabolites And Pathways Strategy
About Lipid Classification Standards Experimental Data Databases Pathways Tools Protocols Home
Structure database (LMSD)
LMFA03010025
HO
OH
O
HO
OH
LM ID LMFA03010025
Common Name PGF2β
Systematic Name 9R,11R,15S-trihydroxy-5Z,13E-prostadienoic acid
Synonyms -
Exact Mass 354.24
Formula $C_{20}H_{34}O_5$
Category Fatty Acyls [FA]
Main Class Eicosanoids [FA03]
Sub Class Prostaglandins [FA0301]
LIPIDBANK ID XPR1764
PubChem Substance ID (SID) 4265968
KEGG ID -

19


Total scan of metabolites (Q1 SCAN + ion mode) for a plasma sample obtained from lean mouse [A]; ob/ob mouse
Total metabolomics
[A]
869
518.345
496.361
542.347
544.367
546.380
566.351
568.366
590.351
591.359
494.352
758.599
786.632
806.604
810.628
811.636
812.644
835.632
756.584
708.605
[B]
1.52e3
518.318
496.335
544.339
546.352
566.322
568.337
590.321
602.288
494.326
758.555
760.570
782.552
806.556
810.592
811.599
812.608
835.594
732.558
100
%
0
400
475
550
625
700
775
850
900
m/z

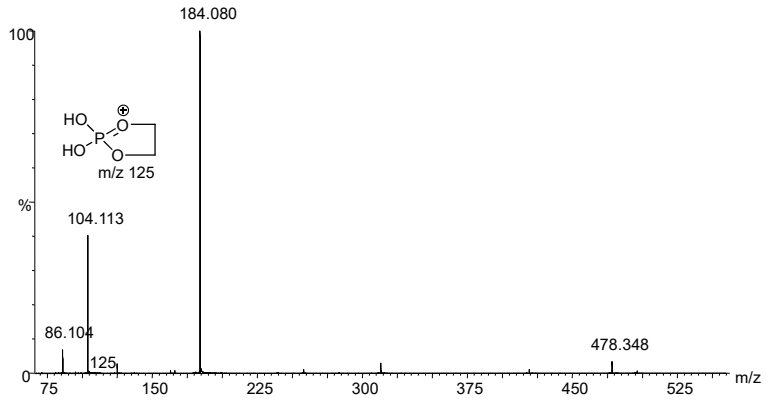
20

Total scan of metabolites (Q1 SCAN -ve ion mode) for a plasma sample obtained from lean mouse [A]; ob/ob mouse



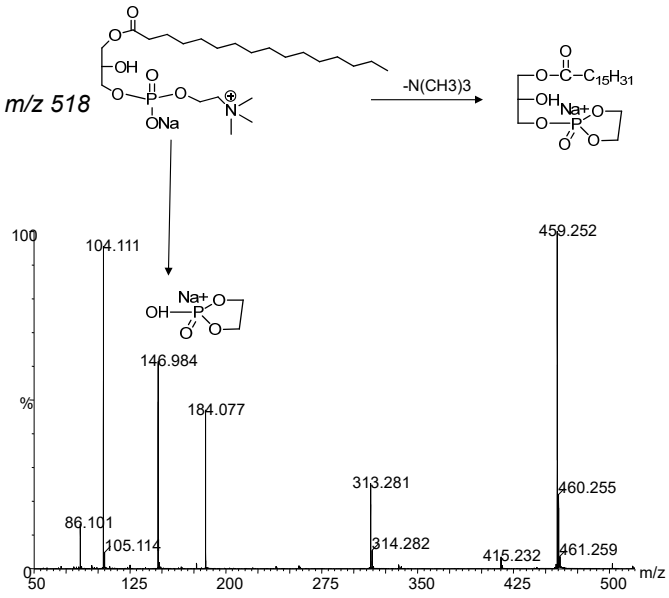
21

MSMS fragmentation of *m/z* 496 [M+H]+ obtained from a plasma sample in positive ion mode



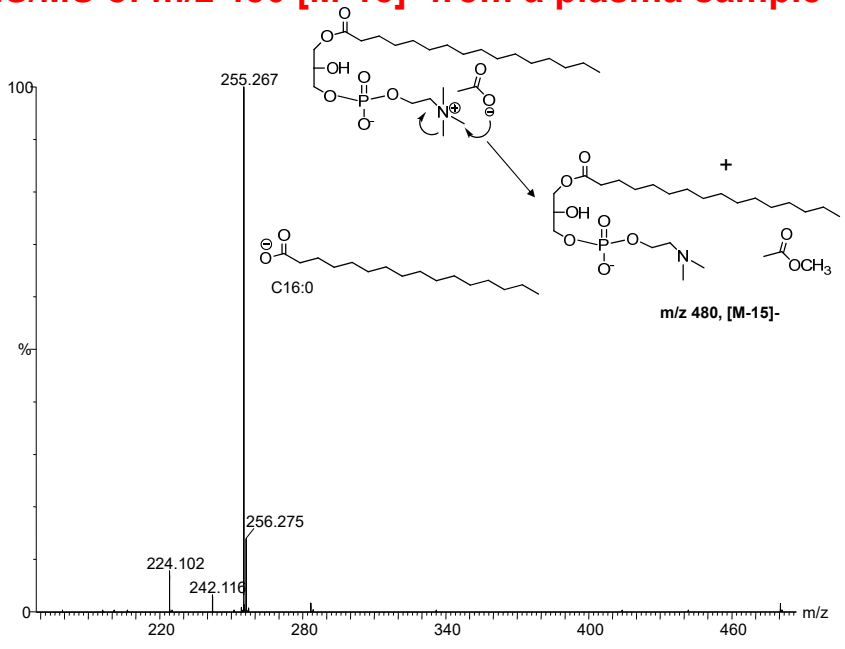
22

MSMS of m/z 518 [M+Na]+ indicates that it may be a sodiated ion of a phospholipid with mol. Wt. 495 Da



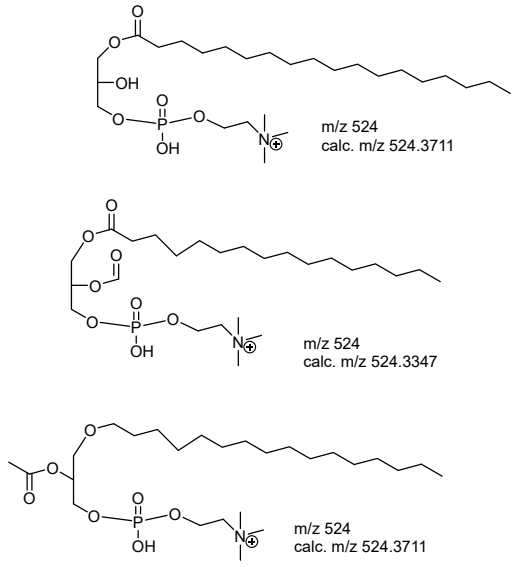
23

MS/MS of m/z 480 [M-15]- from a plasma sample



24

Several isobaric compounds- Identification by high resolution mass spectrometry



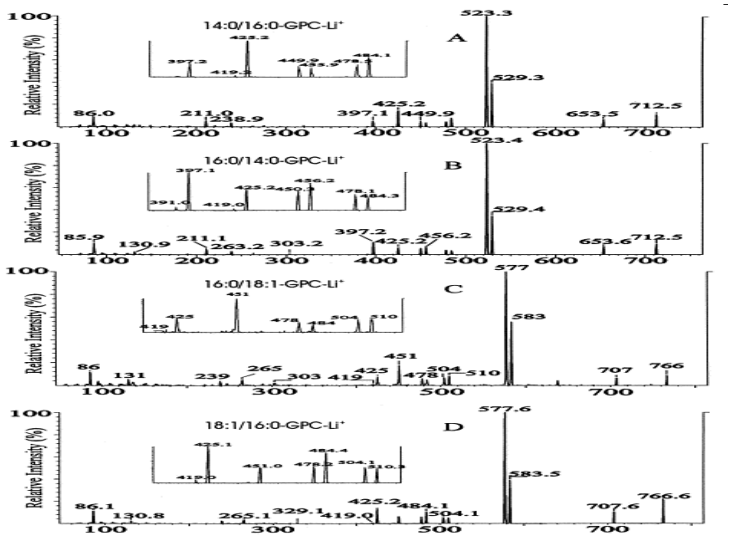
25

Lithiated adducts of phosphocholine provide more structural information in their MS/MS spectra

Source: Hsu et al. J. Am Soc. Mass Spectrom, 1998

26

Relative abundances of product ion can be used to distinguish positional isomers of lithiated phospholipids



Source: Hsu et al. J. Am Soc. Mass Spectrom, 1998

27

Conclusions

- **Shotgun lipidomics approaches are high throughput and applicable to perform profiling 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.**

28