Mass Spectrometry based metabolomics

Metabolomics- A realm of small molecules (<1000 Da)

Jeevan Prasain, PhD

What is metabolomics?

- **Identification and quantification of the complete set of metabolites in a biological system**
- **Quantitative global analysis of metabolites from cells, tissues and fluids**
- **Quantitative measurement of the dynamic metabolite response of living systems to pathophysiological stimuli or genetic modification**

Workflow for metabolome analysis

Sample collection

- Treatment or Diseased group
- Control group

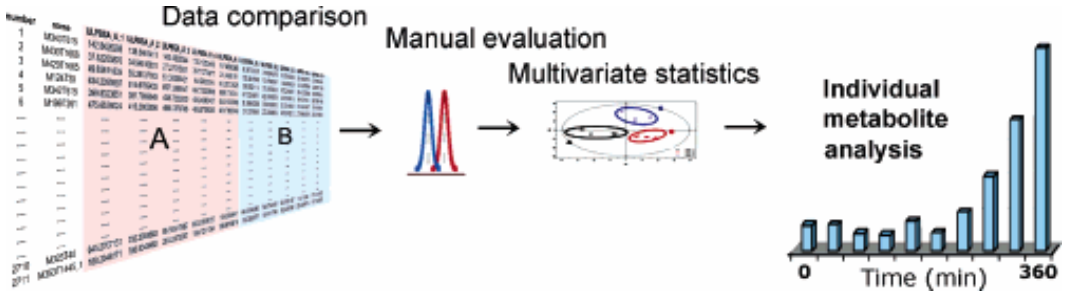
Urine, plasma, tissue etc.

Sample preparation → Internal standard spiking for quantitative analysis
Extraction (liquid-liquid, ppt with or without hydrolysis)

Sample analysis → LC-MS and LC-MS/MS analysis

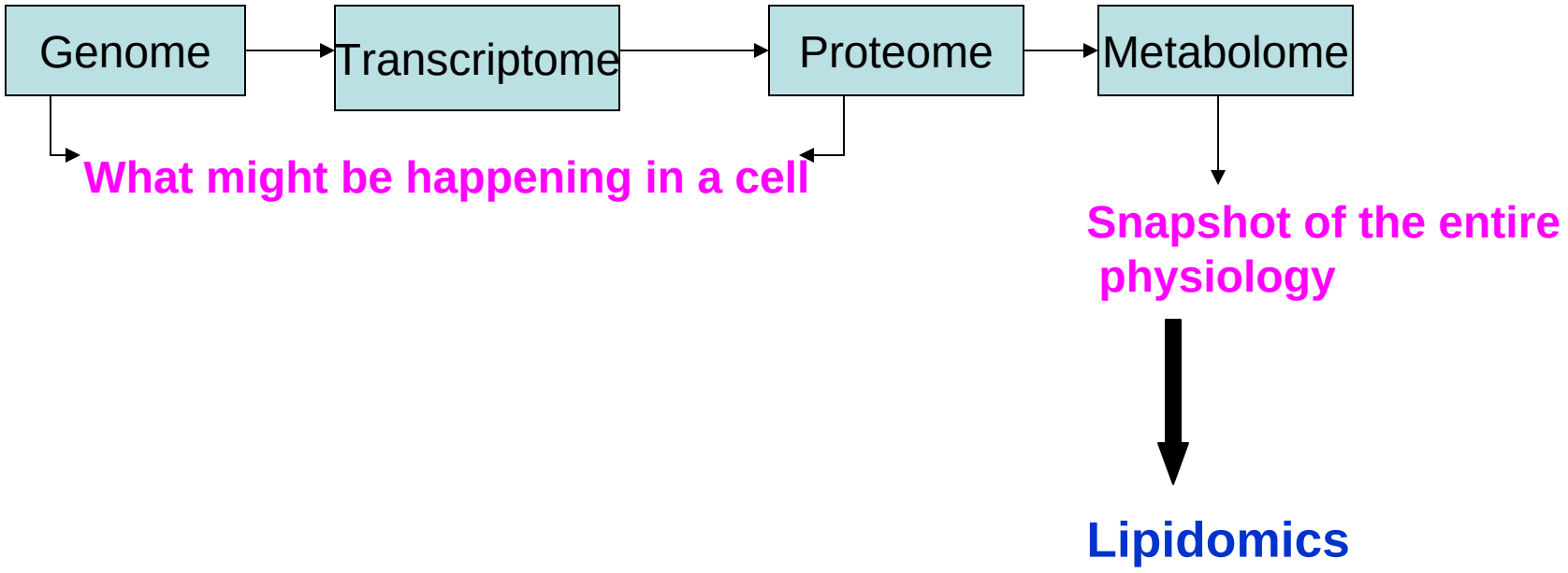
- Q1 scan using HPLC or UPLC/-TOFMS
- +ve/-ve ion mode- accurate mass measurement
- MS/MS experiments using a hybrid instrument Q-Trap

Data export → **Data analysis**
Multivariat analysis e.g. PCA



Marker identification

Metabolomics in the context of other omics



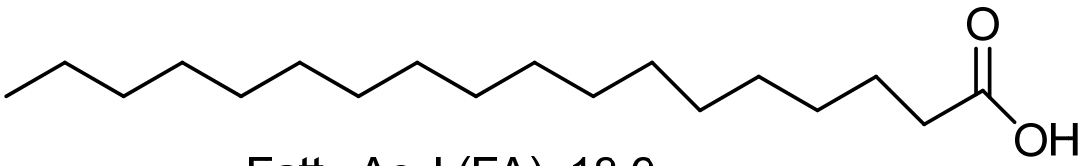
Lipids are important- as a membrane bilayer
- provides hydrophobic environment for protein function
- reservoir of energy
- signaling molecules

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

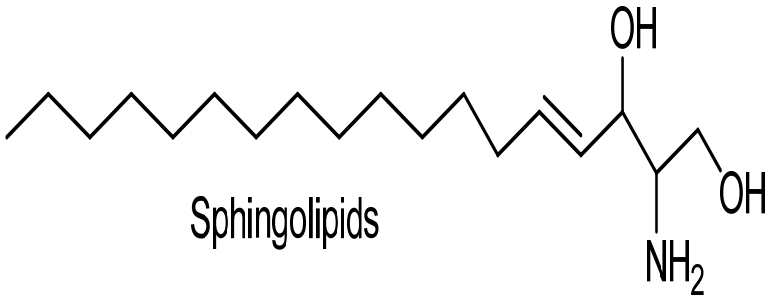
Outlines

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

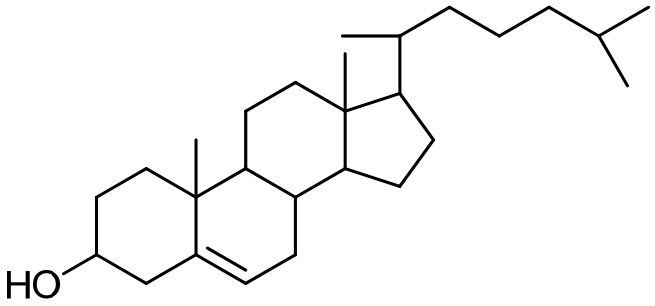
Structures of different lipids classes



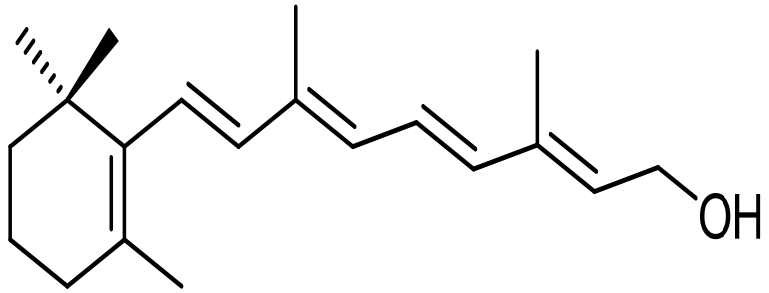
Fatty Acyl (FA), 18:0
saturated/unsaturated



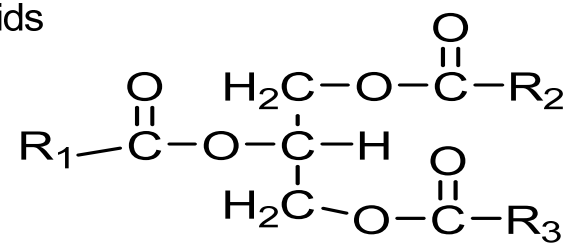
Sphingolipids



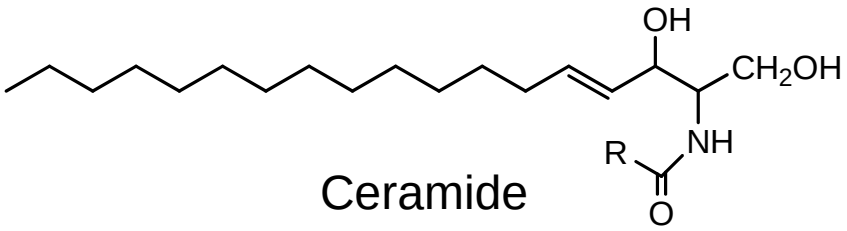
Sterol lipids



Prenol lipids (retinol)

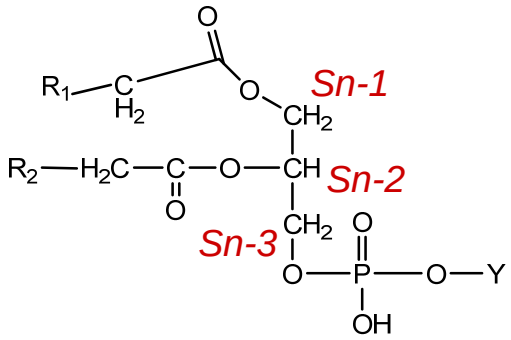


Glycerolipid, R =
saturated/unsaturated FA

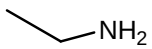


Ceramide

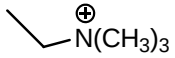
Structures of main phospholipids



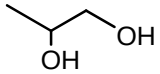
Phosphatidylethanolamine (PE)

Y= 

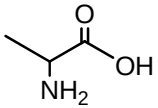
Phosphatidylcholine (PC)

Y= 

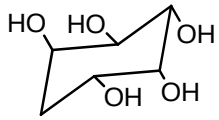
Phosphatidylglycerol (PG)

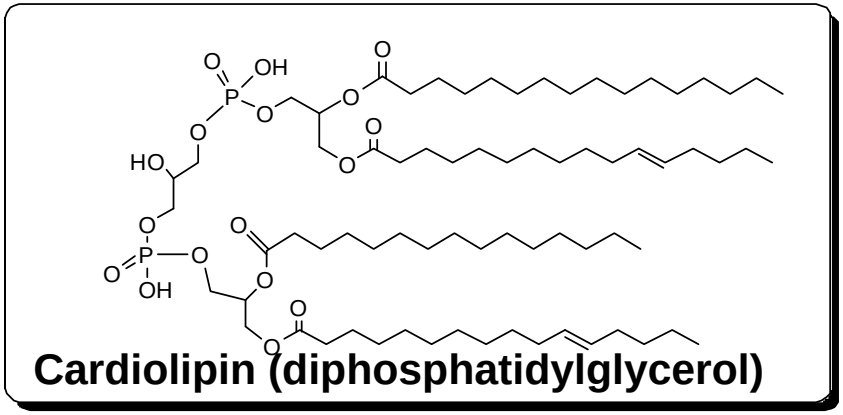
Y= 

Phosphatidylserine (PS)

Y= 

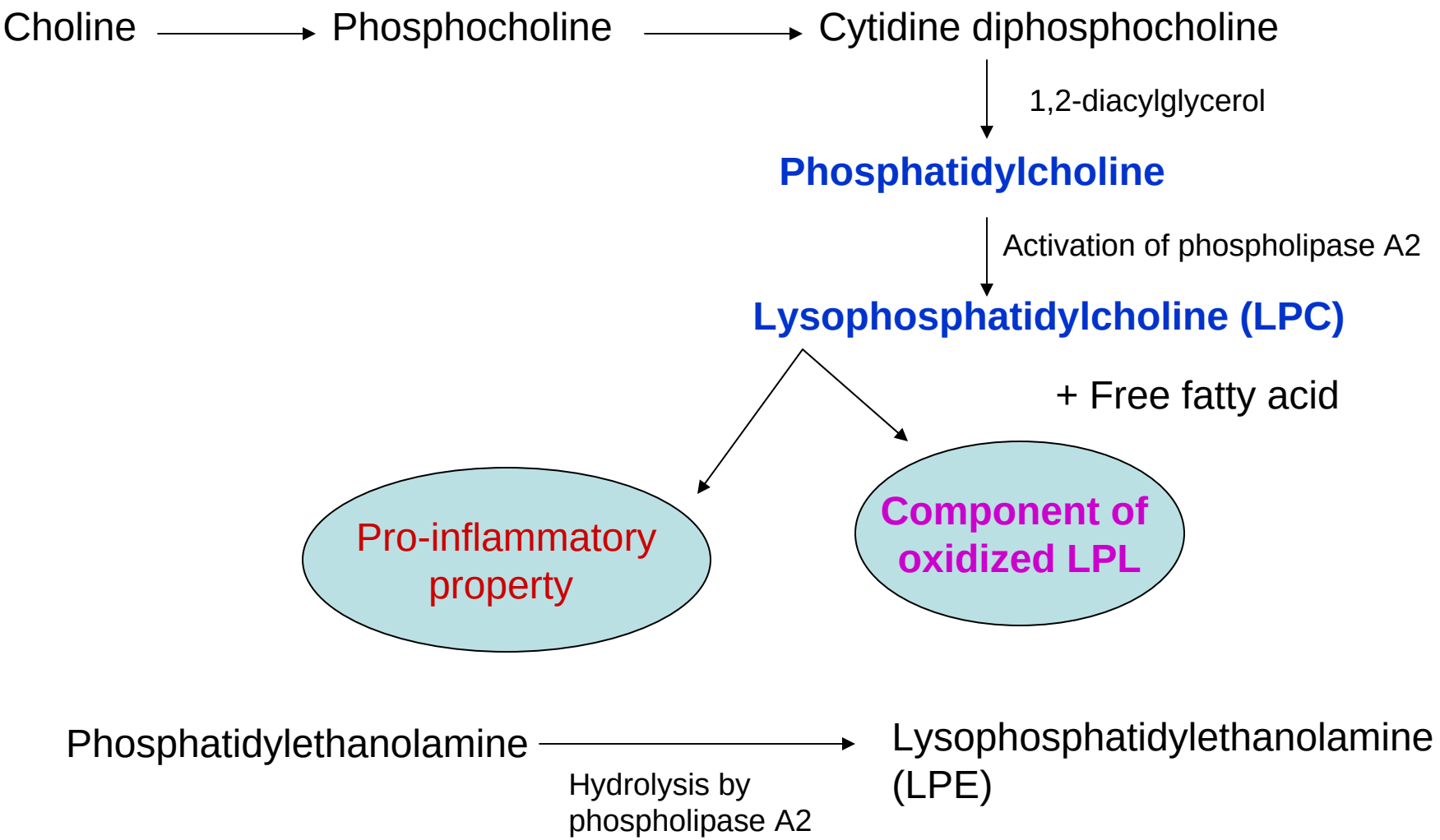
Phosphatidylinositol (PI)

Y= 



Cardiolipin (diphosphatidylglycerol)

How phospholipids are synthesized?



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.**

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

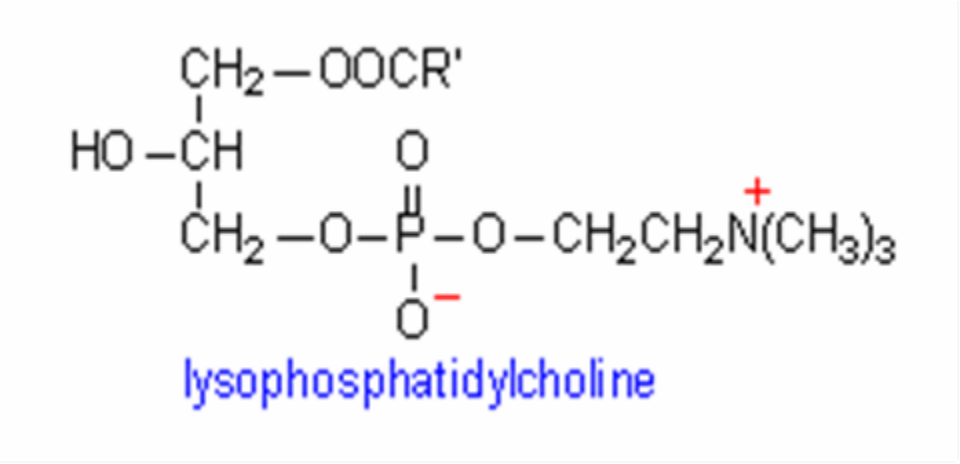
Which ionization mode for which phospholipids?

Positive ion mode

PC
LPC
PE
LPE
SM
PS

Negative ion mode

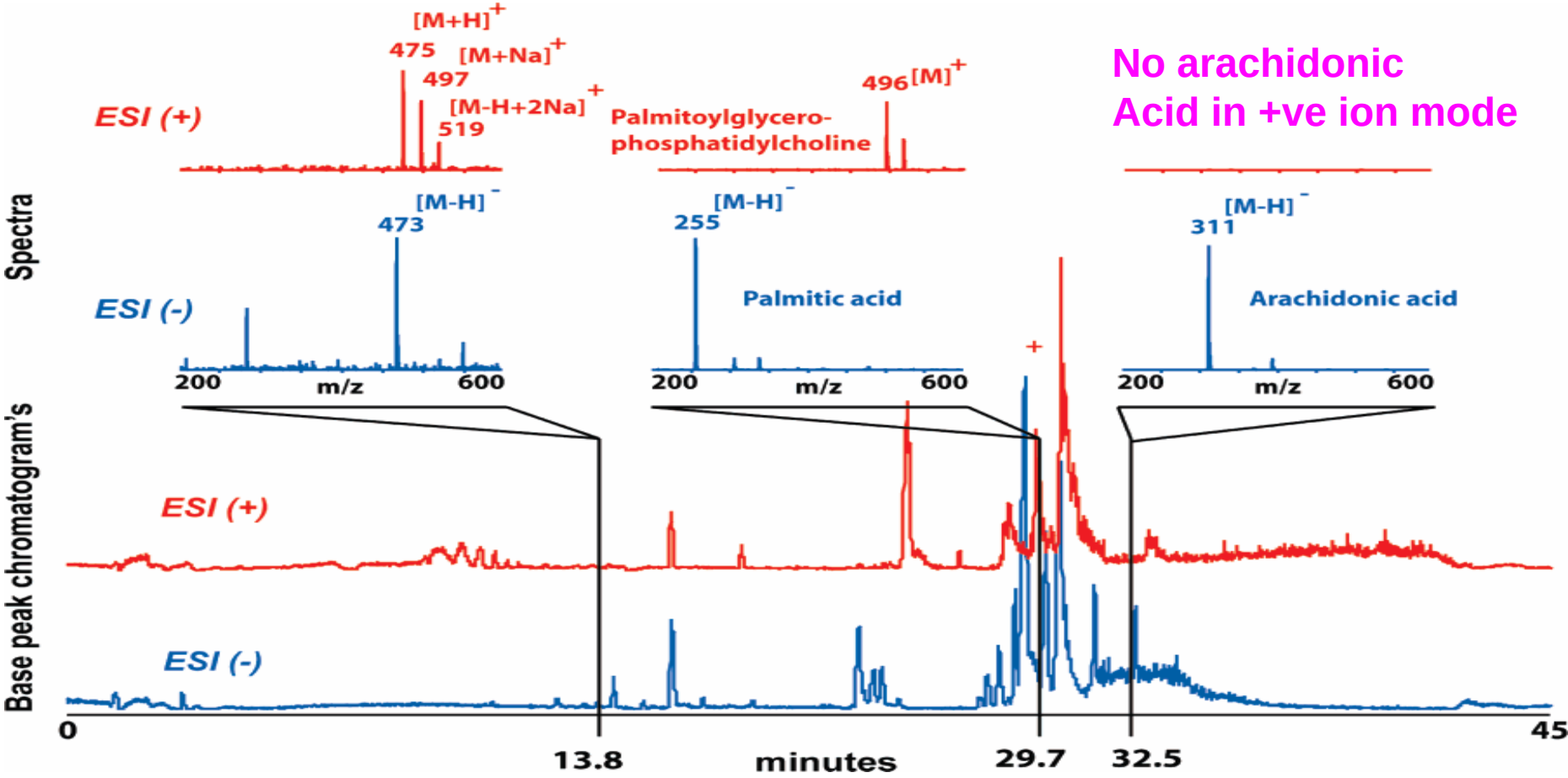
PE
PA
PI
PI
PG
PIPs



lysophosphatidylcholine

PC = phosphatidylcholine
PA = phosphatic acid
PE = phosphatidylethanolamine
PS = phosphatidylserine
PG = phosphatidylglycerol
PI = phosphatidylinositol
PIP = PI monophosphate
SM = sphingomyelin
LPE = lysoPE

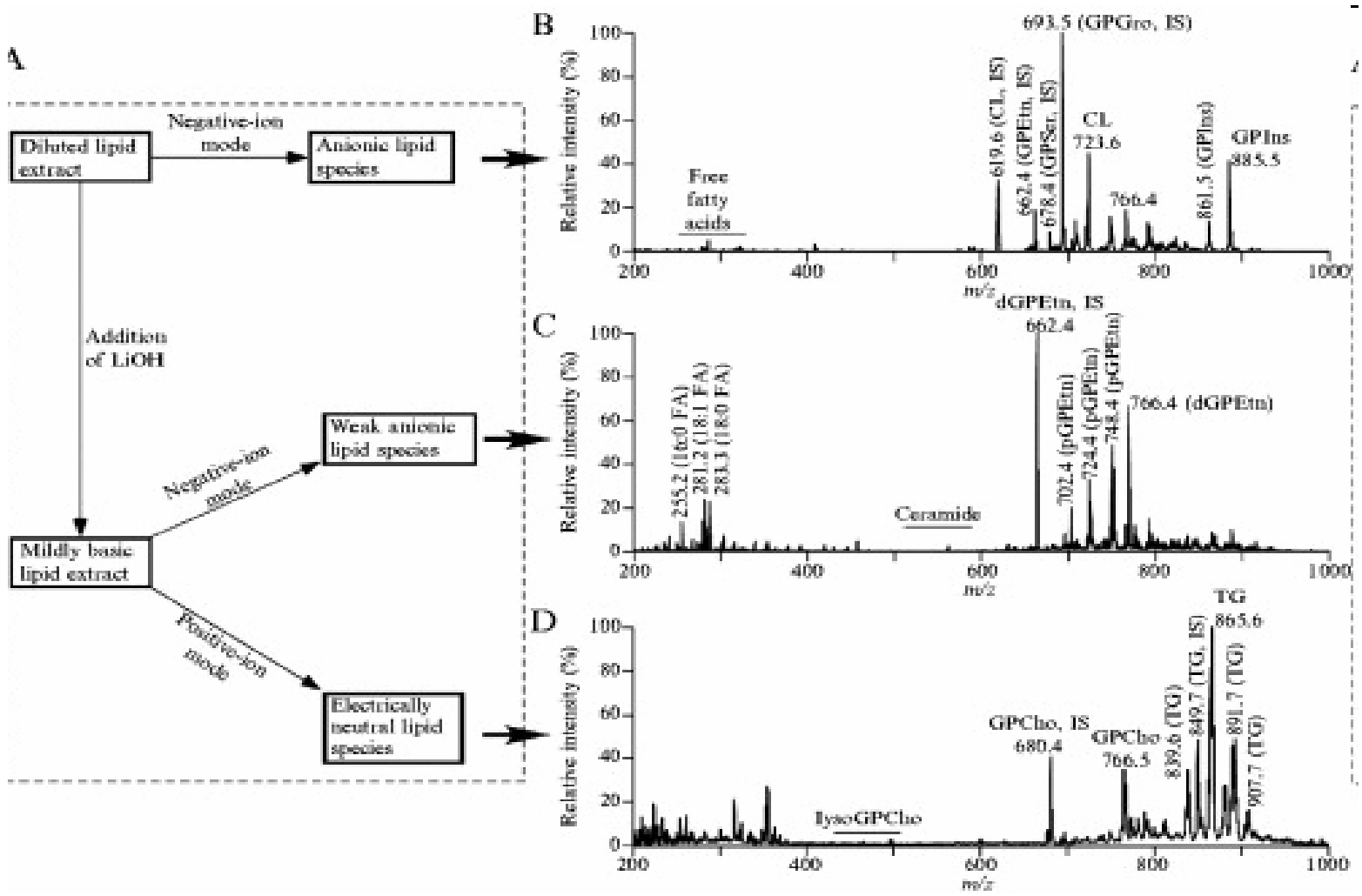
Increasing metabolite coverage using +ve and –ve ion mode



Representative Q1 scans of a methanolic extract of human blood serum

Source: Nordstrom et al. Analytical Chemistry, 2007

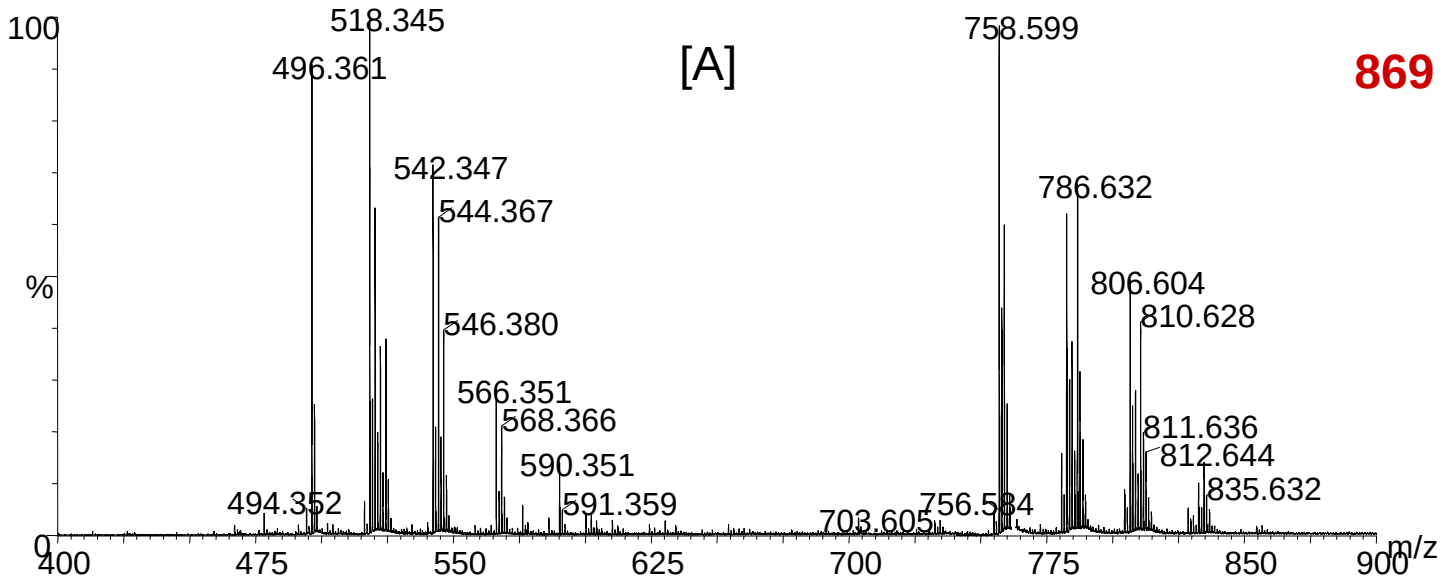
Application of shotgun lipidomics: intra-source separation of lipids

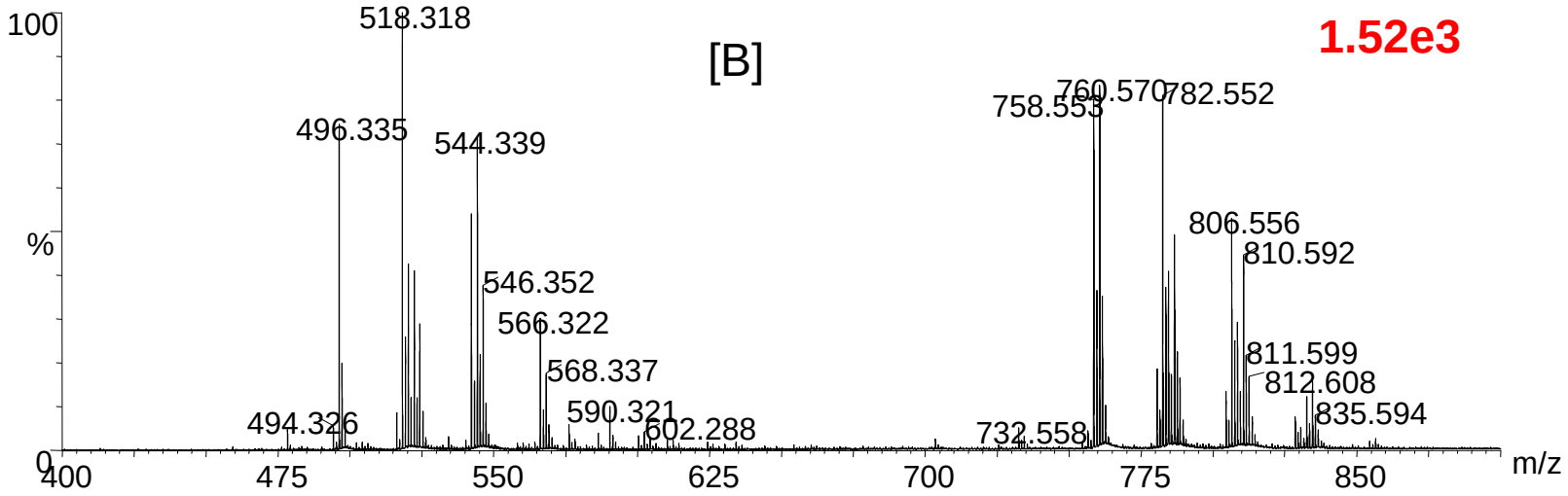


Source: Gross and Han, methods in Enzymology, 2007

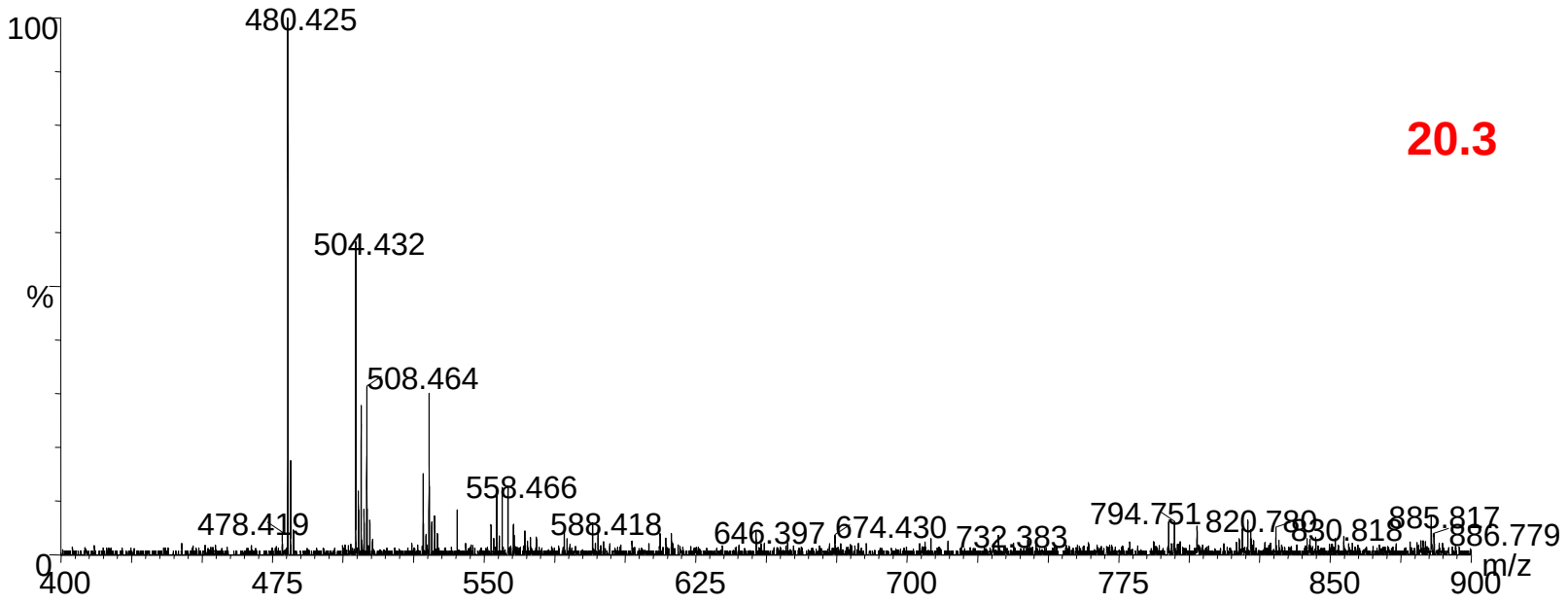
Total scan of metabolites (Q1 SCAN + ion mode) for a plasma sample obtained from lean mouse [A]; ob/ob mouse

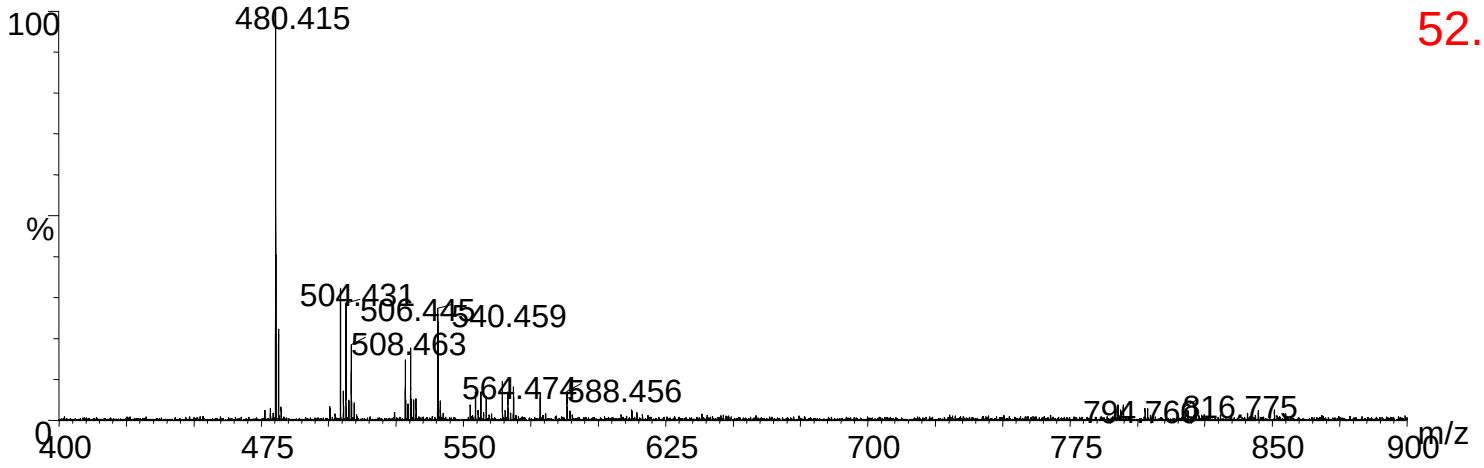
Total metabolomics



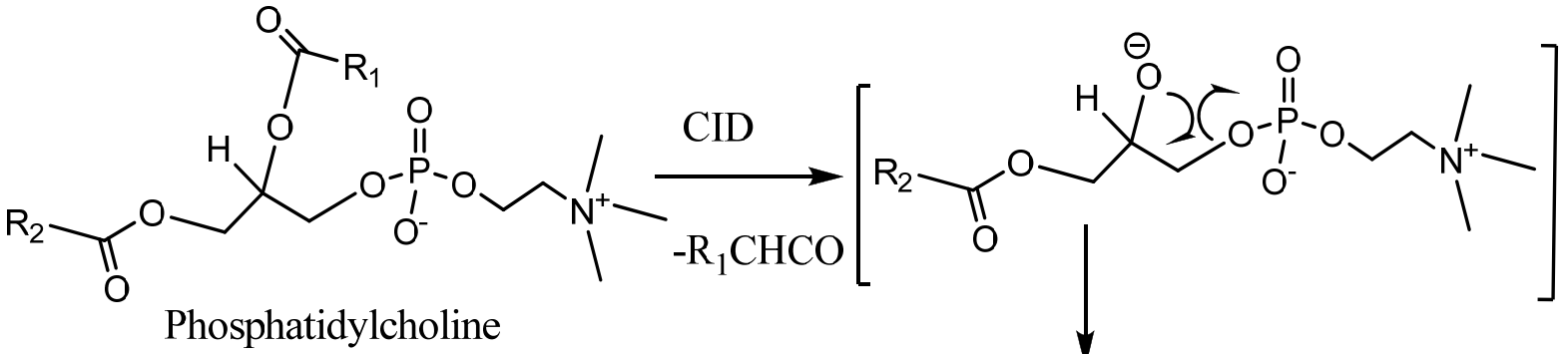


Total scan of metabolites (Q1 SCAN -ve ion mode) for a plasma sample obtained from lean mouse [A]; ob/ob mouse

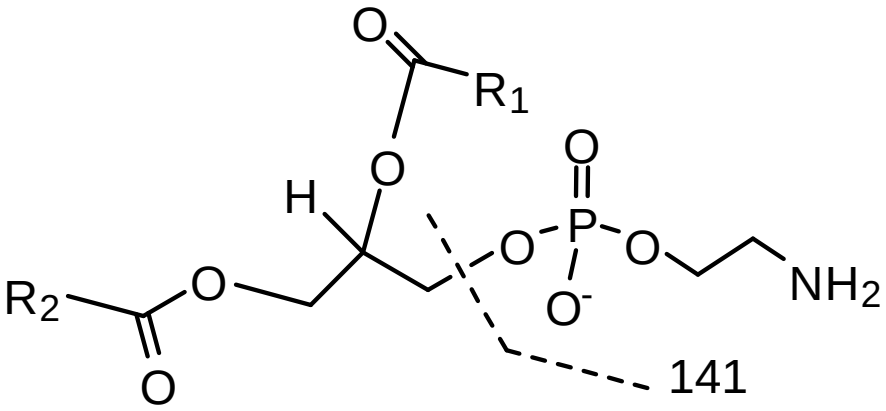




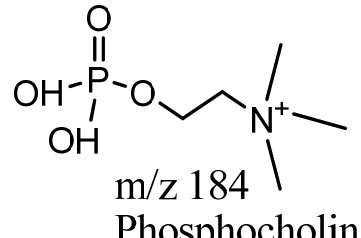
Tandem mass spectrometry (MS/MS) of phospholipids



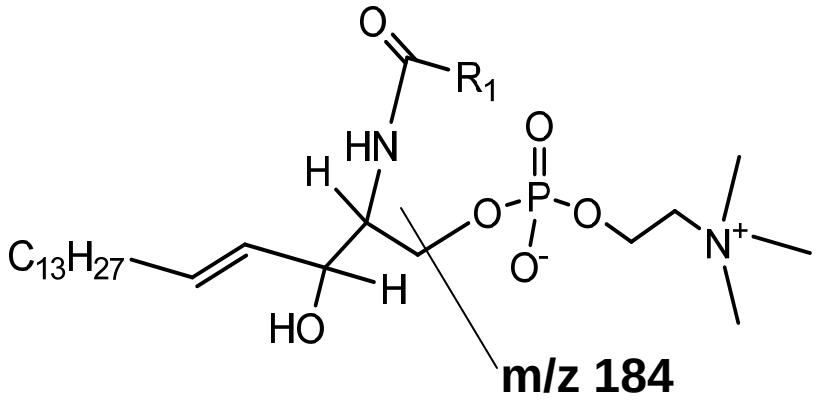
Phosphatidylcholine



Phosphatidylethanolamine

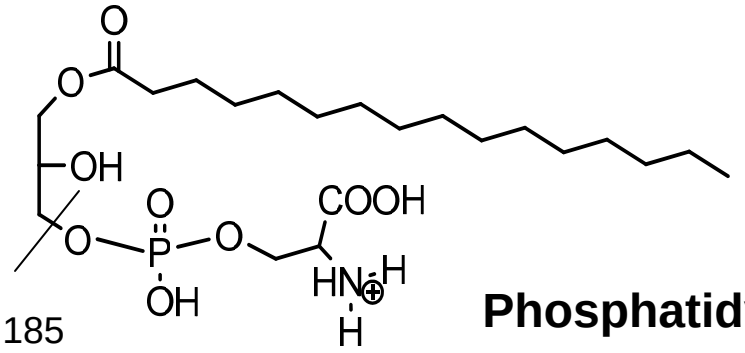


m/z 184
Phosphocholine



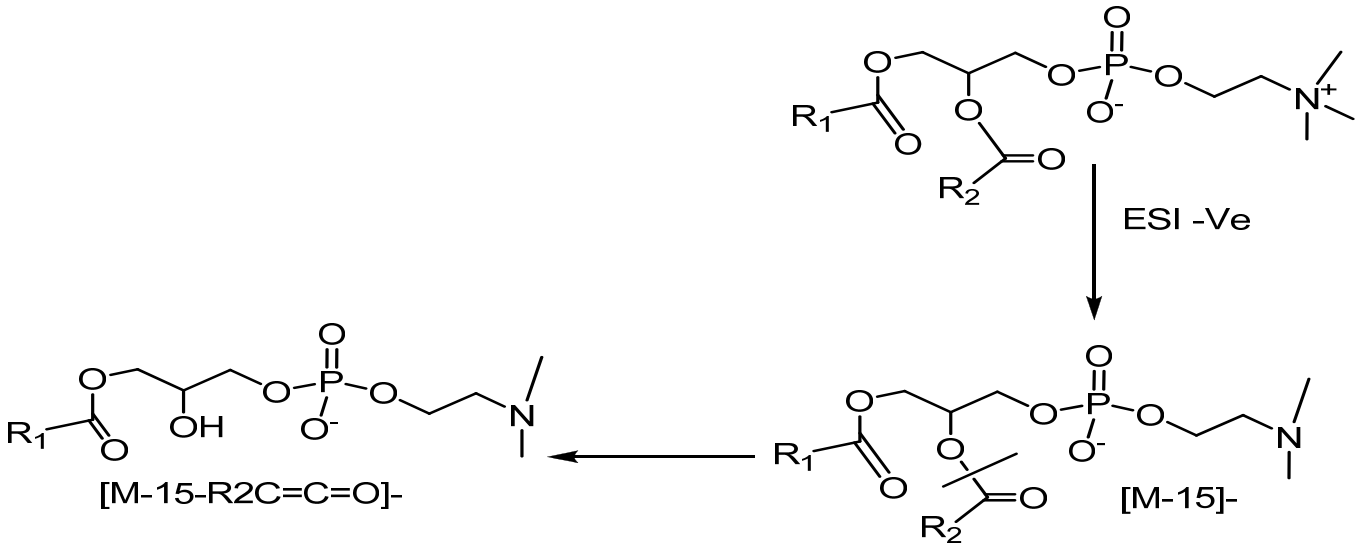
m/z 184

Sphingomyelin



Phosphatidylserine

MS/MS in negative ion mode of phospholipids provide information about the fatty acyl chain



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,, 2004

Focused lipidomics

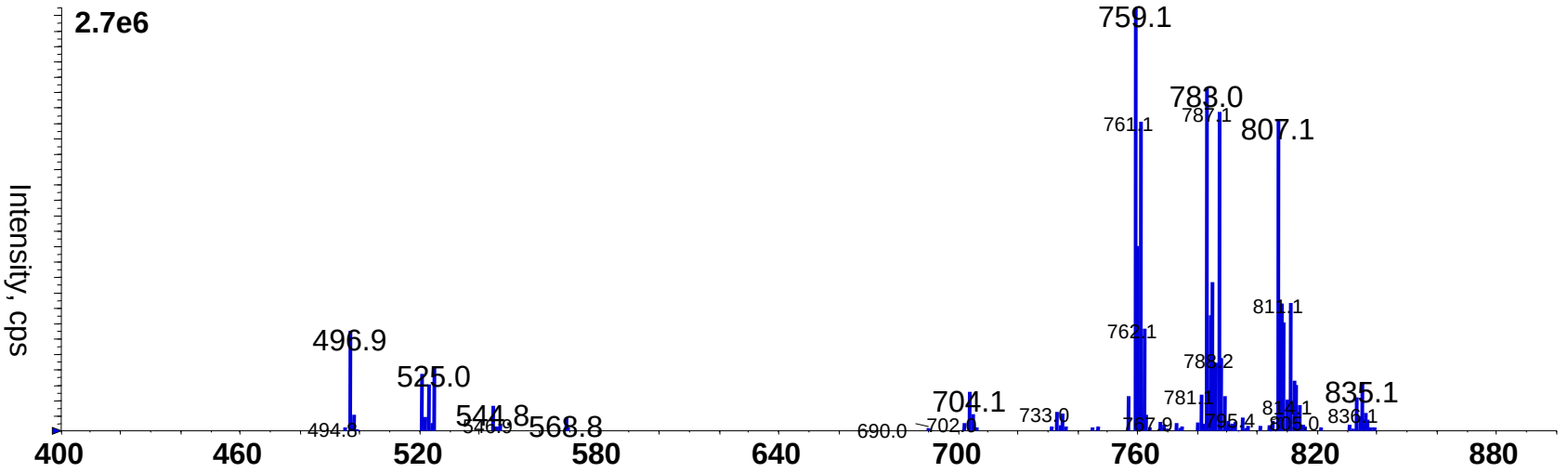
A. Flow injection (ESI-MS/MS)

-Precursor ion scanning at *m/z* 184-choline-containing phospholipids +ve ion mode

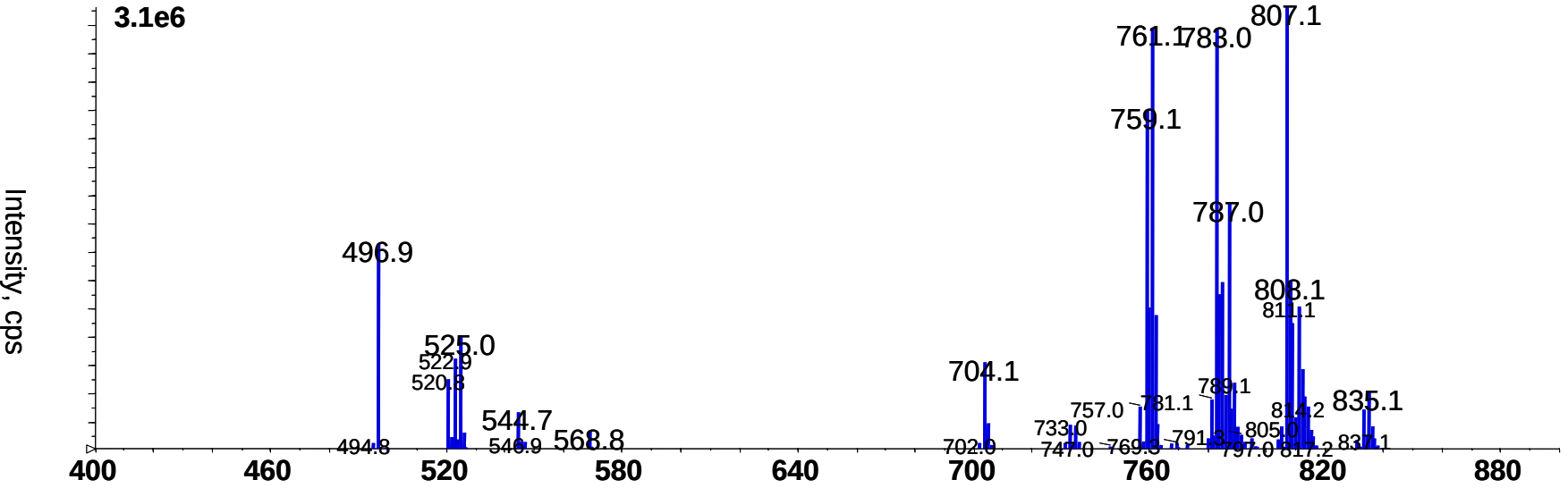
- Neutral scanning of 141, 185, 189, and 277 u used for PE, PS, phosphatidylglycerol (PG), and phosphatidylinositol (PI), respectively

- precursor ion scanning at *m/z* 153 and 241 in –ve ion mode-glycerol-containing phospholipids and inositol-containing phospholipids, respectively

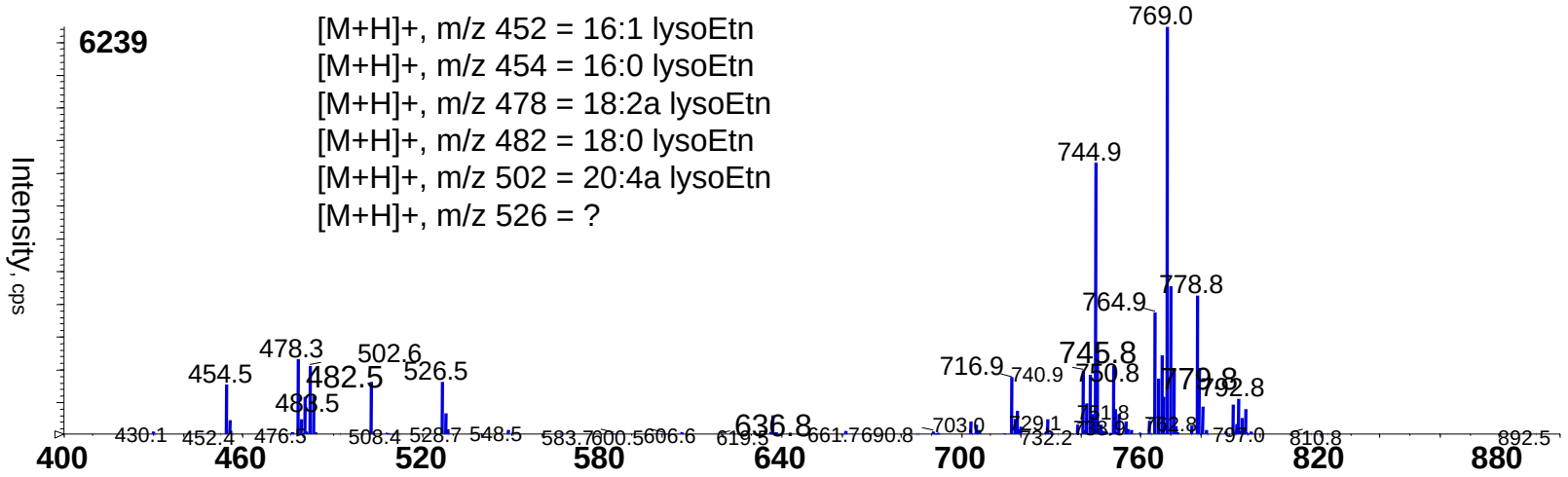
+Prec (184.00): Exp 1, 2.764 to 30.604 min from Sample 11 (Lean 53 NK) of ZhangSET1.wiff (Turbo Spray), Centroided



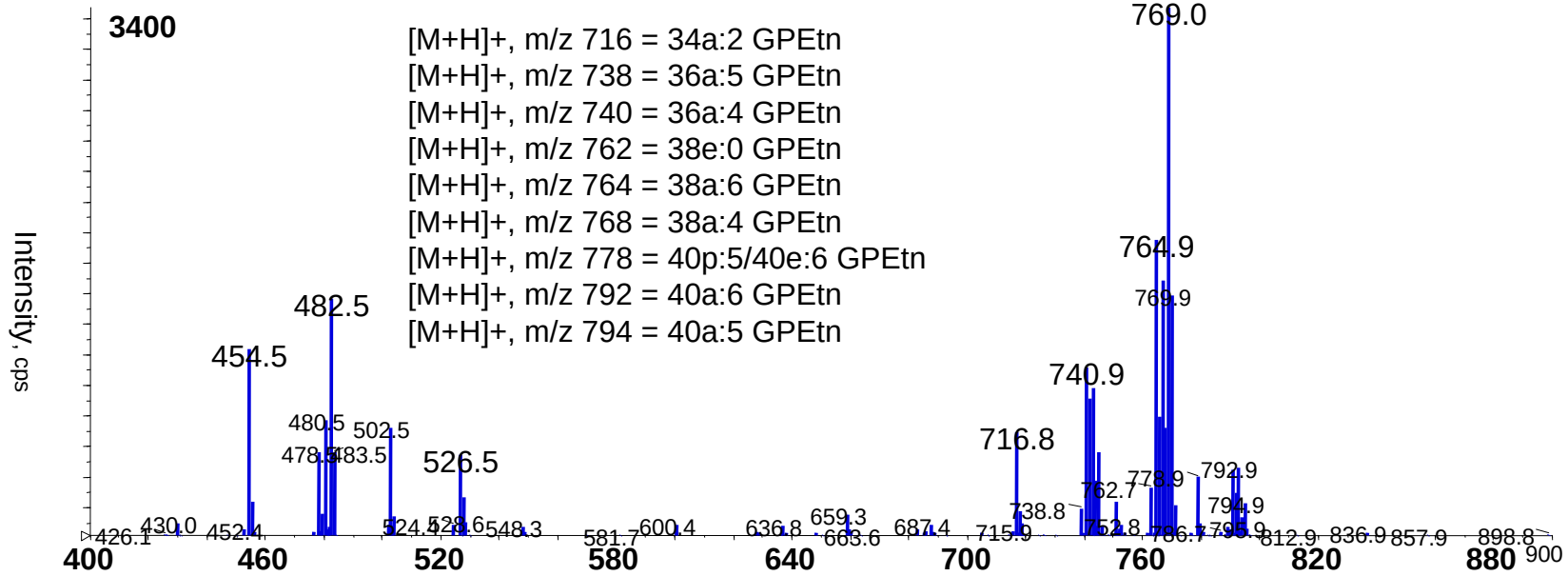
+Prec (184.00): Exp 1, 2.764 to 30.603 min from Sample 16 (Ob 27 NK) of ZhangSET1.wiff (Turbo Spray), Centroided



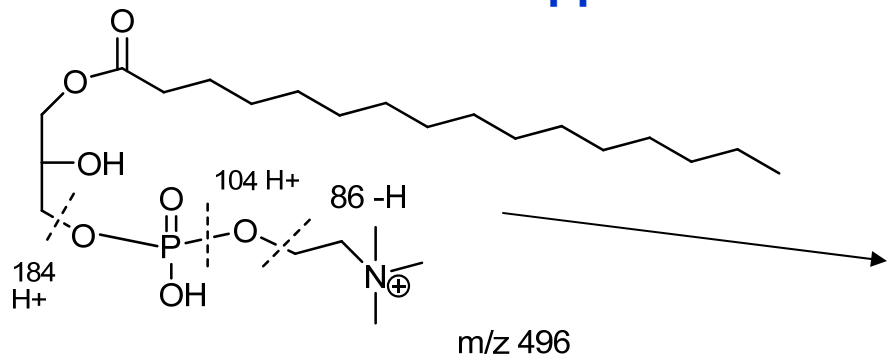
+NL (141.00): Exp 2, 2.278 to 33.485 min from Sample 11 (Lean 53 NK) of ZhangSET1.wiff (Turbo Spray), Centroided

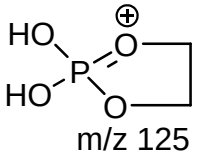


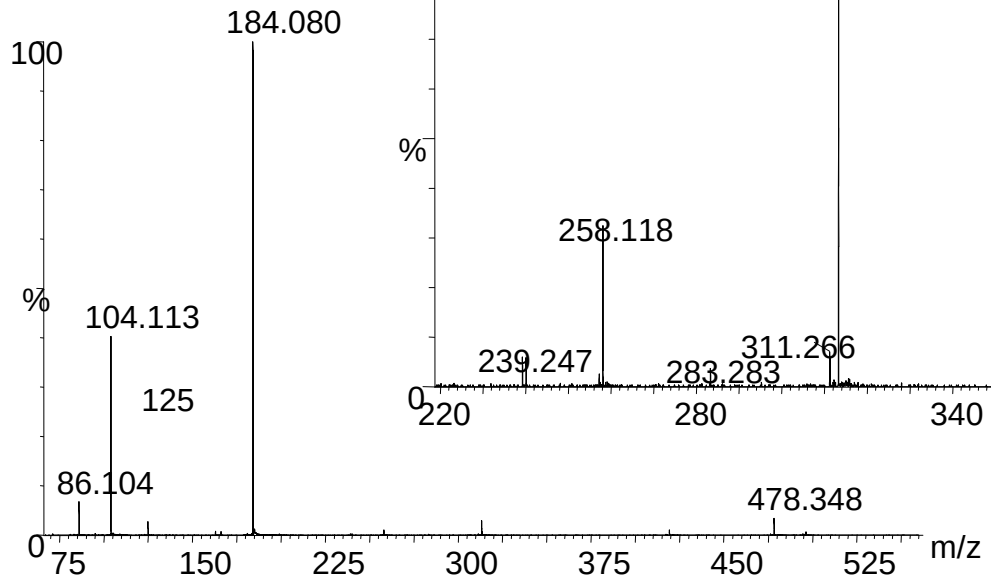
+NL (141.00): Exp 2, 2.278 to 33.485 min from Sample 15 (Ob 26 NK) of ZhangSET1.wiff (Turbo Spray), Centroided

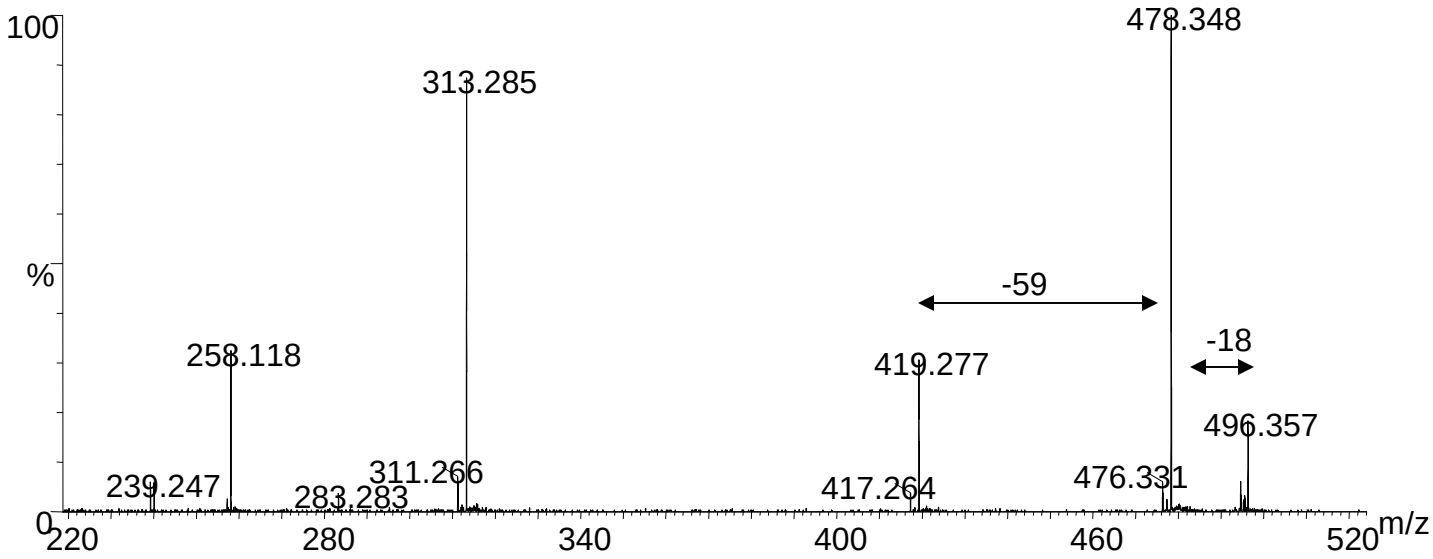


MSMS fragmentation of *m/z* 496 obtained from plasma of ob/ob mouse supplemented with kudzu



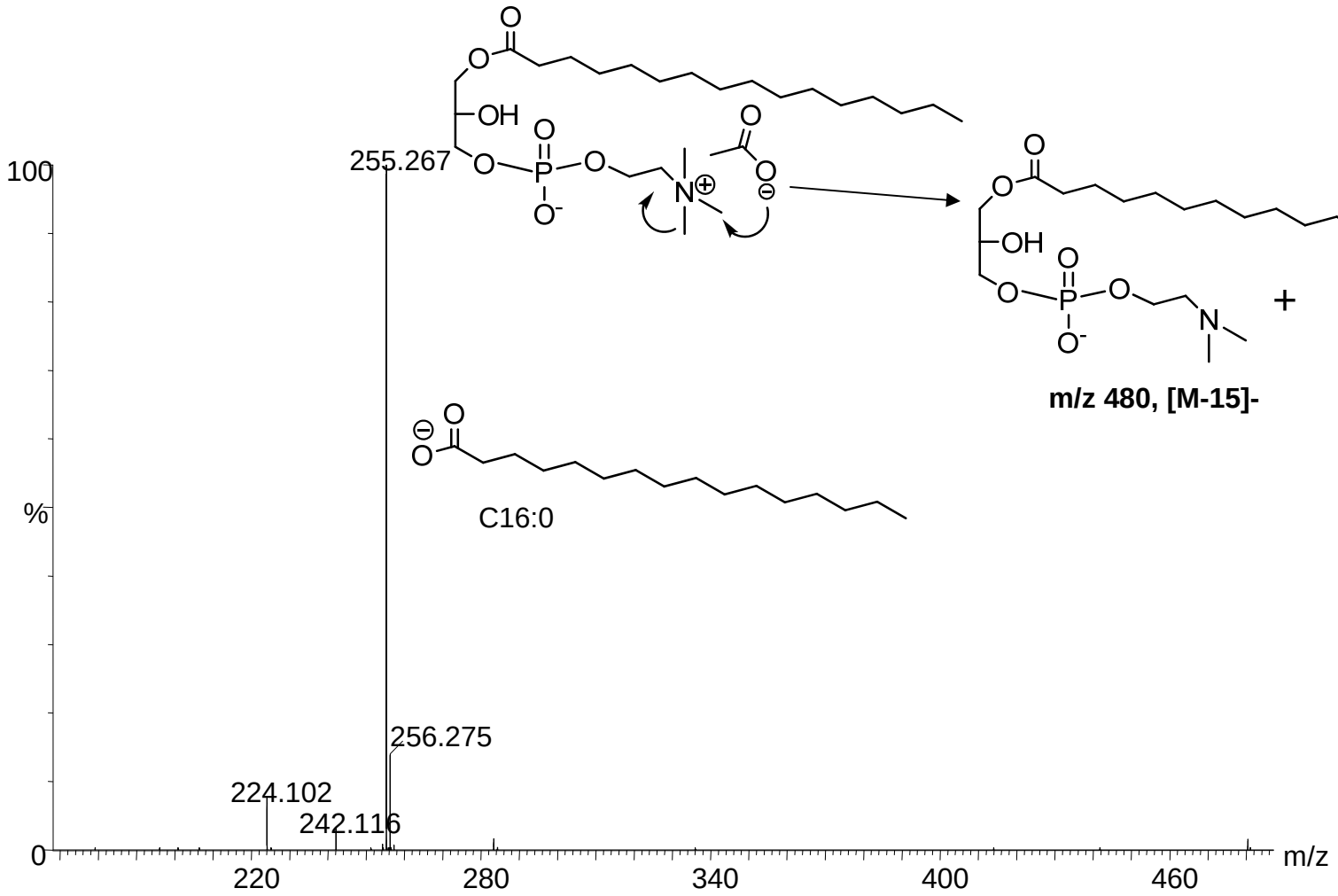




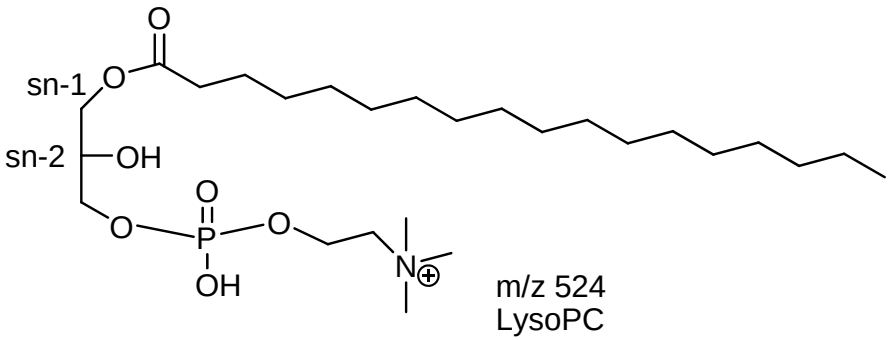


MS/MS of m/z 480 [M-15]- from a ob/ob no kudzu supplemented plasma sample

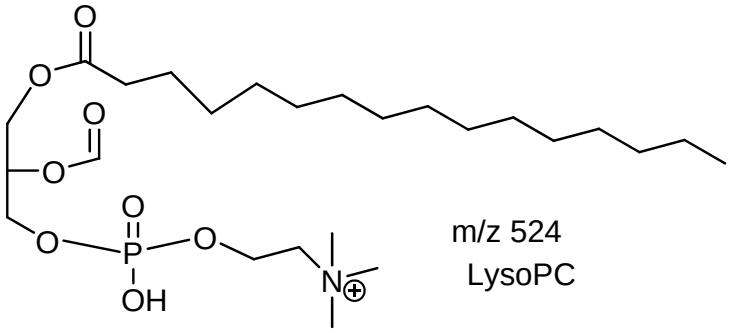
TOF MSMS 480.40ES-



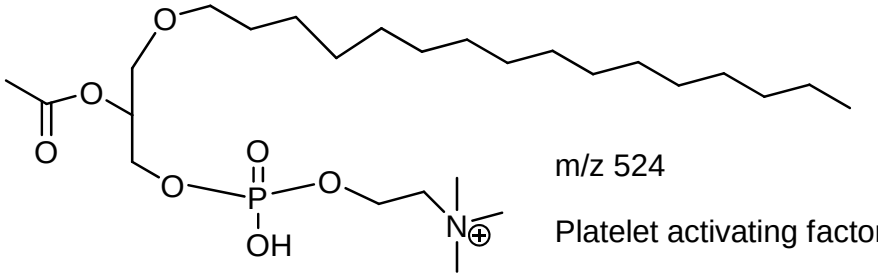
Several isomeric compounds exits and unambiguous identification is a challenge



m/z 524
LysoPC



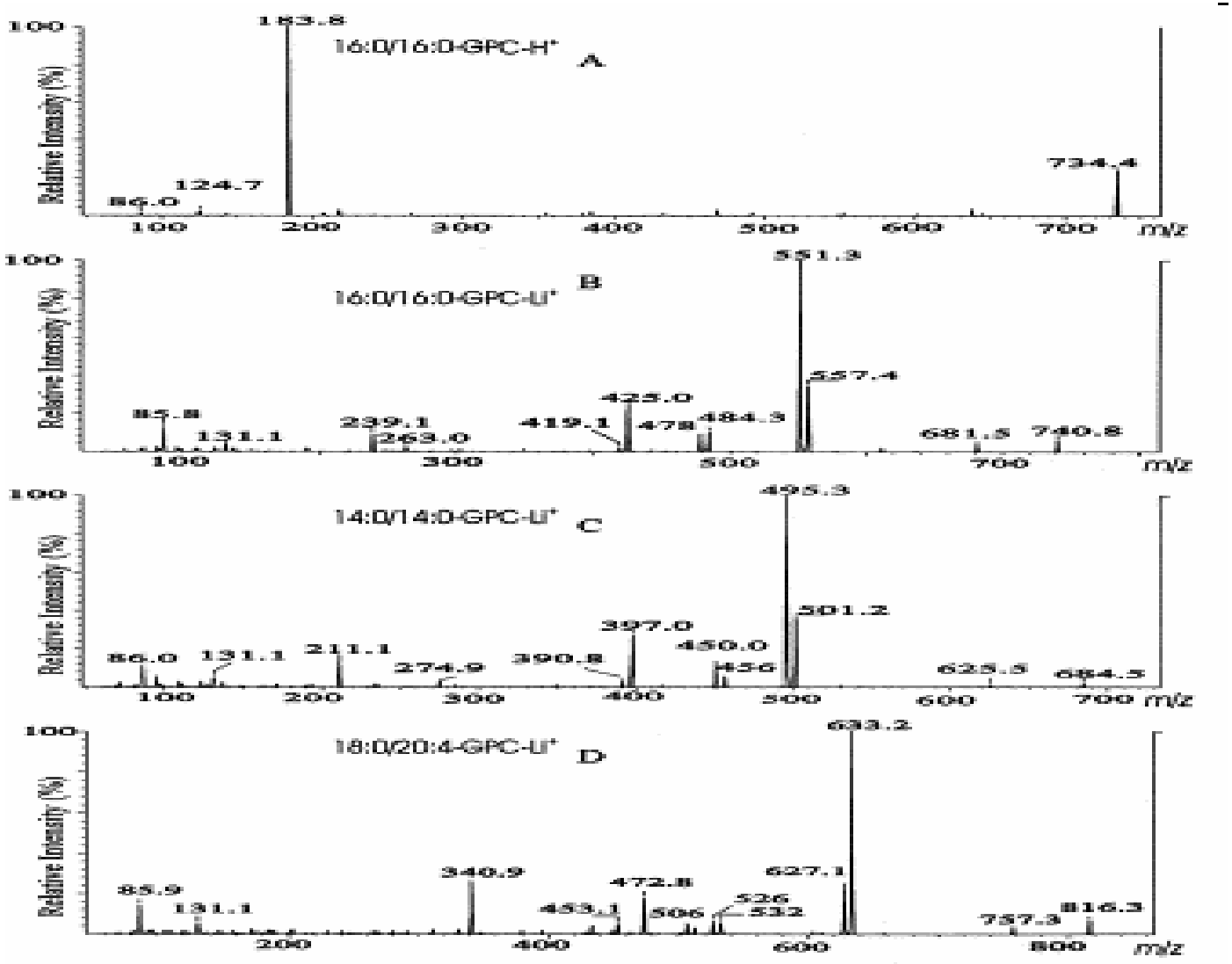
m/z 524
LysoPC



m/z 524

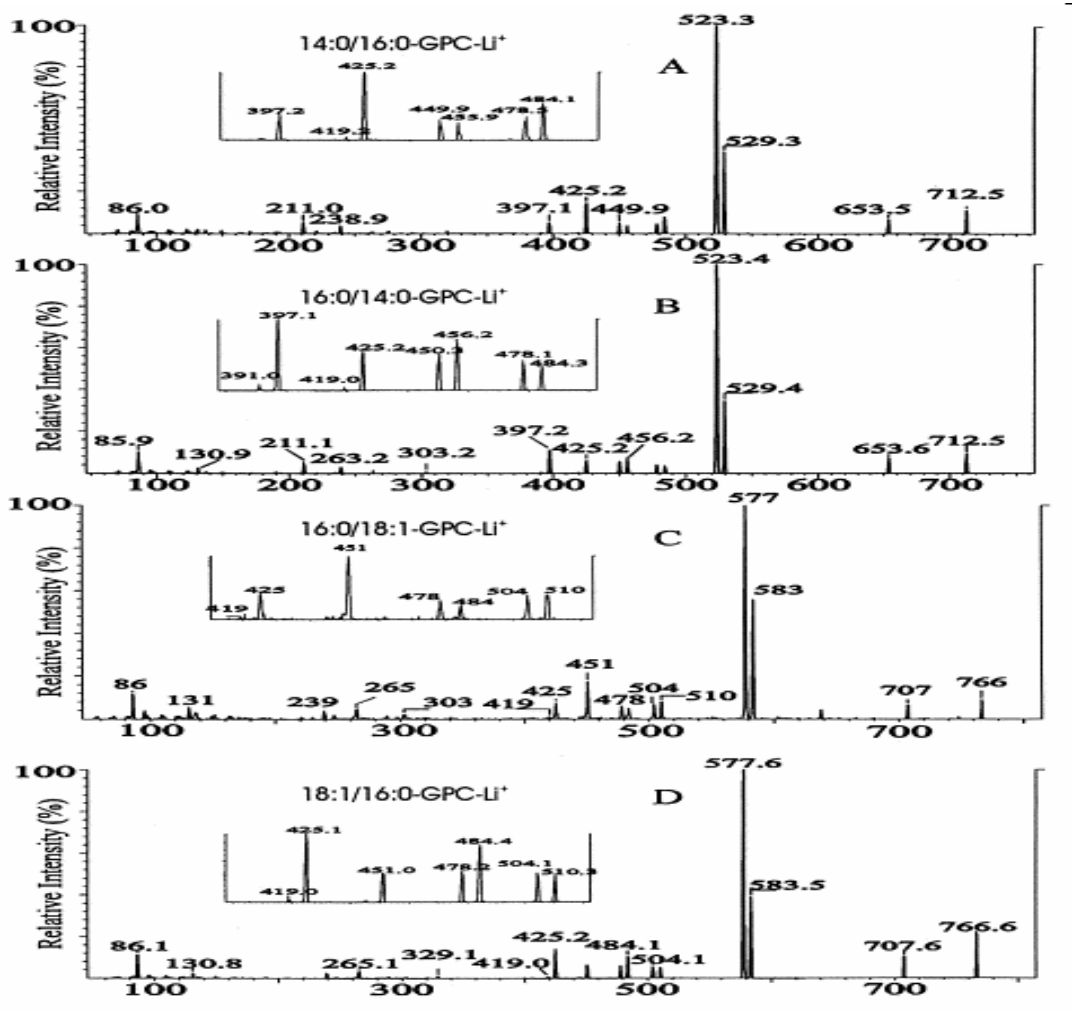
Platelet activating factor

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



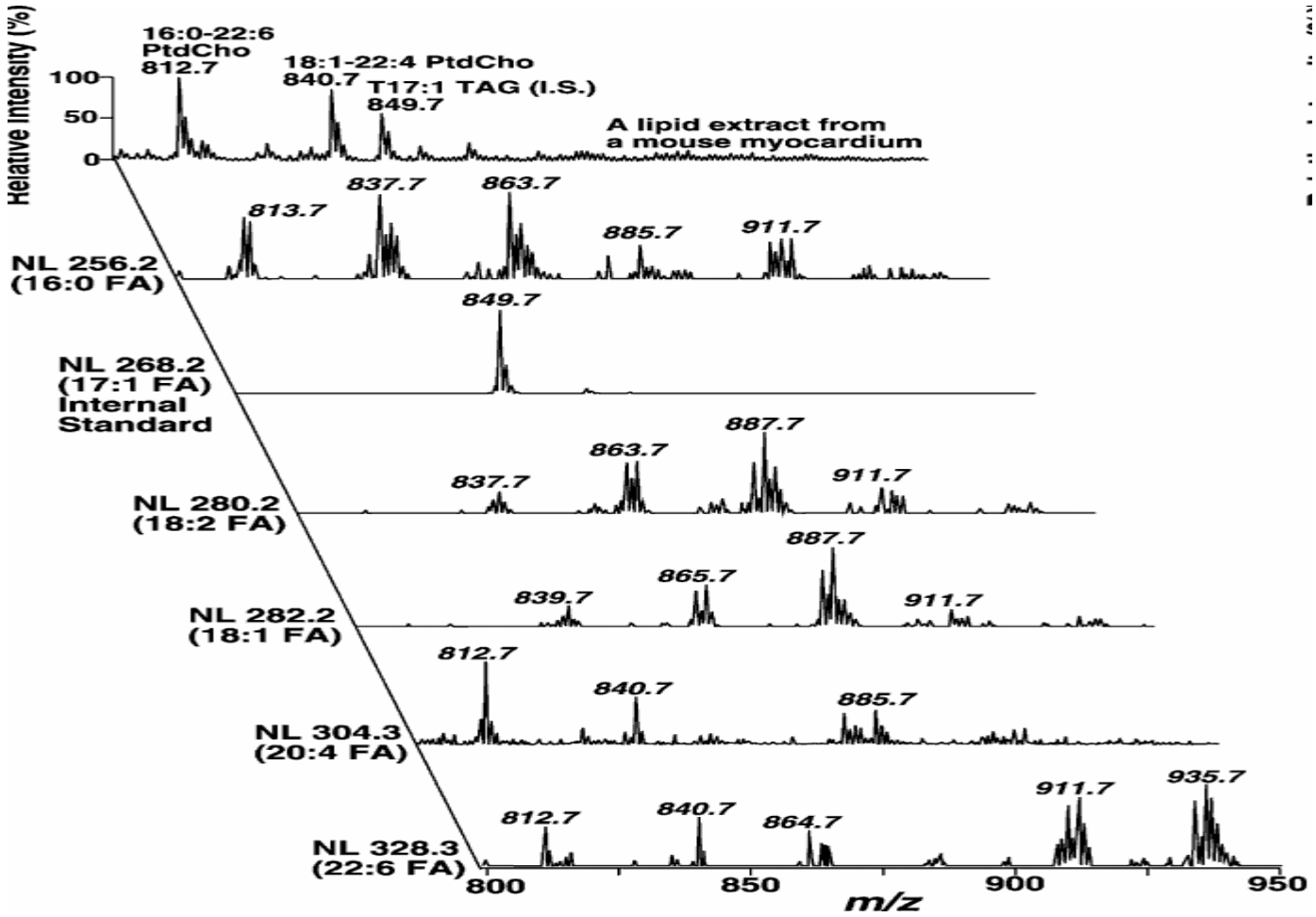
Source: Hsu et al. J. Am Soc. Mass Spectrom, 1998

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

A 2D ESI mass spectrometric finger print for TG molecules



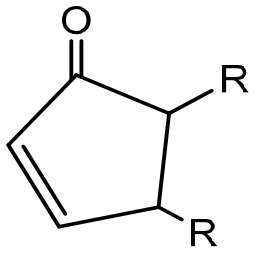
Source: Han and Gross, 2004

MS/MS analysis of eicosanoids

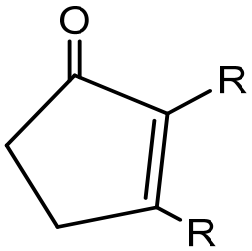
Eicosanoids, meaning 20
derived from a 20-carbon acid,
arachidonic acid

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

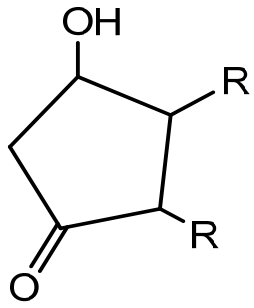
Structural representation PG based on ring features



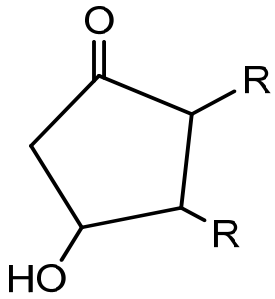
PGA



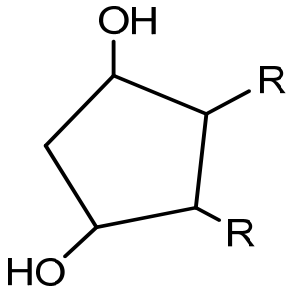
PGB



PGD



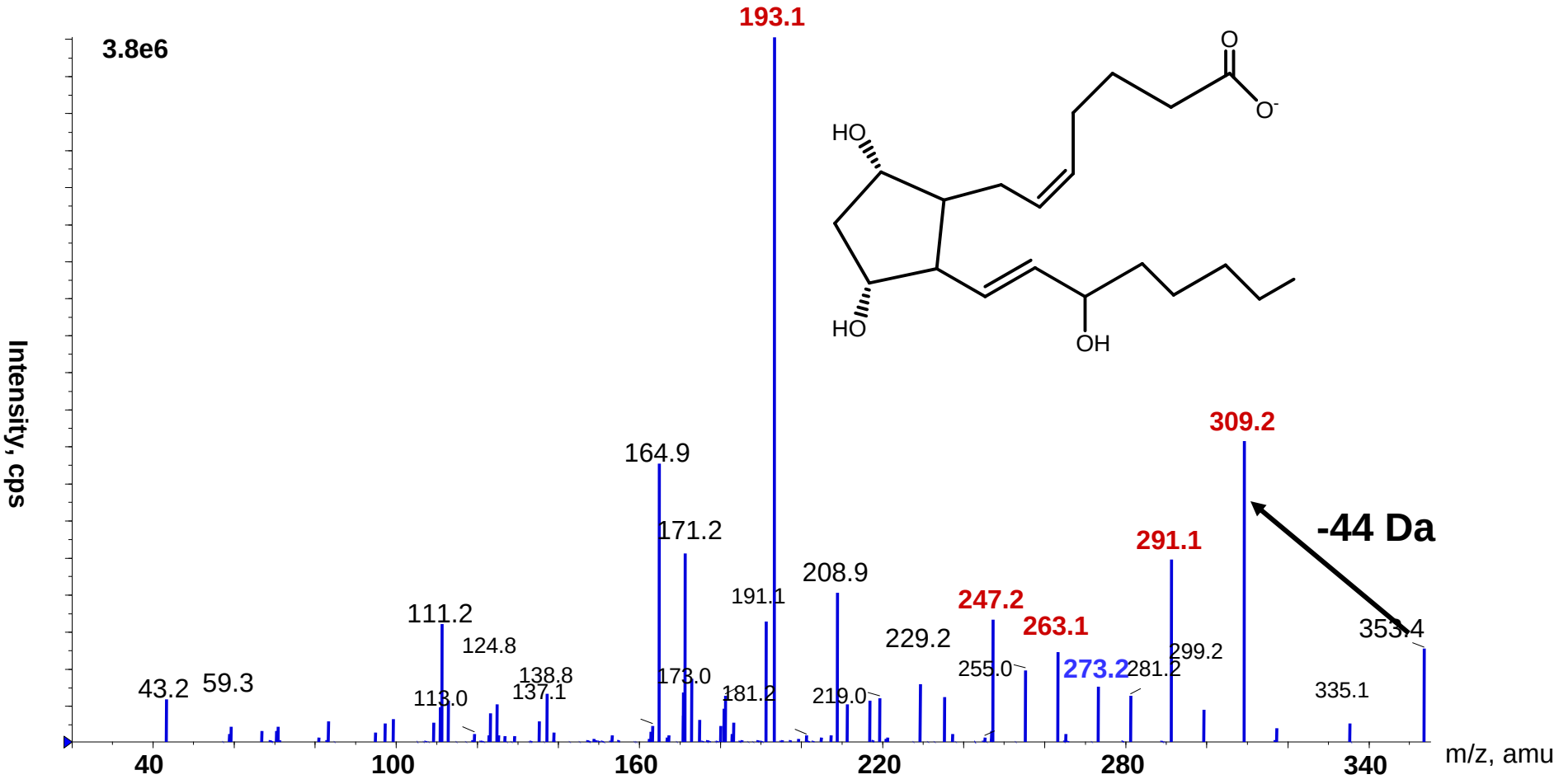
PGE



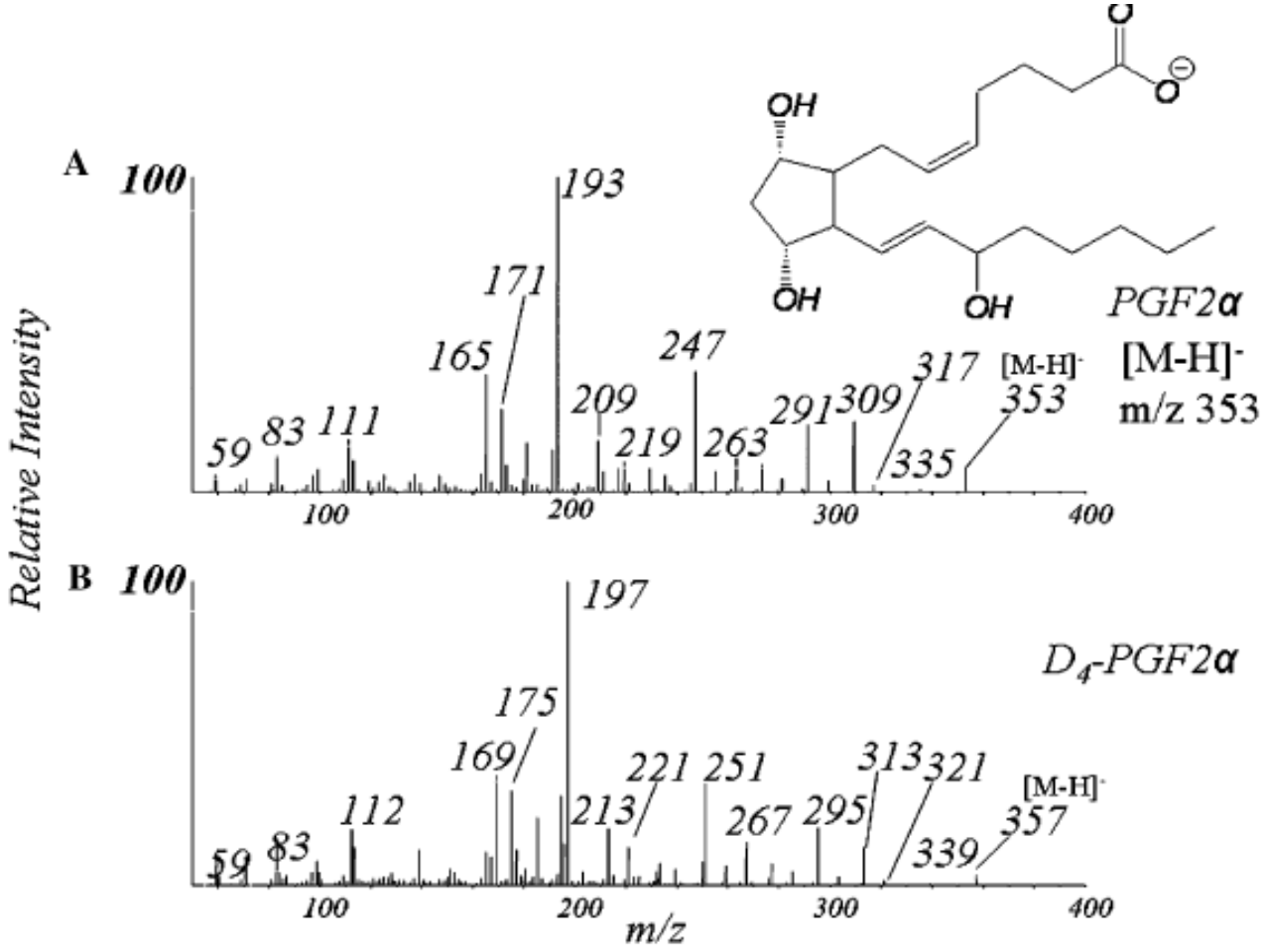
PGF

R = aliphatic chain

ESI-MS/MS of the [M-H]- from PGF2α m/z 353 using a quadrupole mass spectrometer

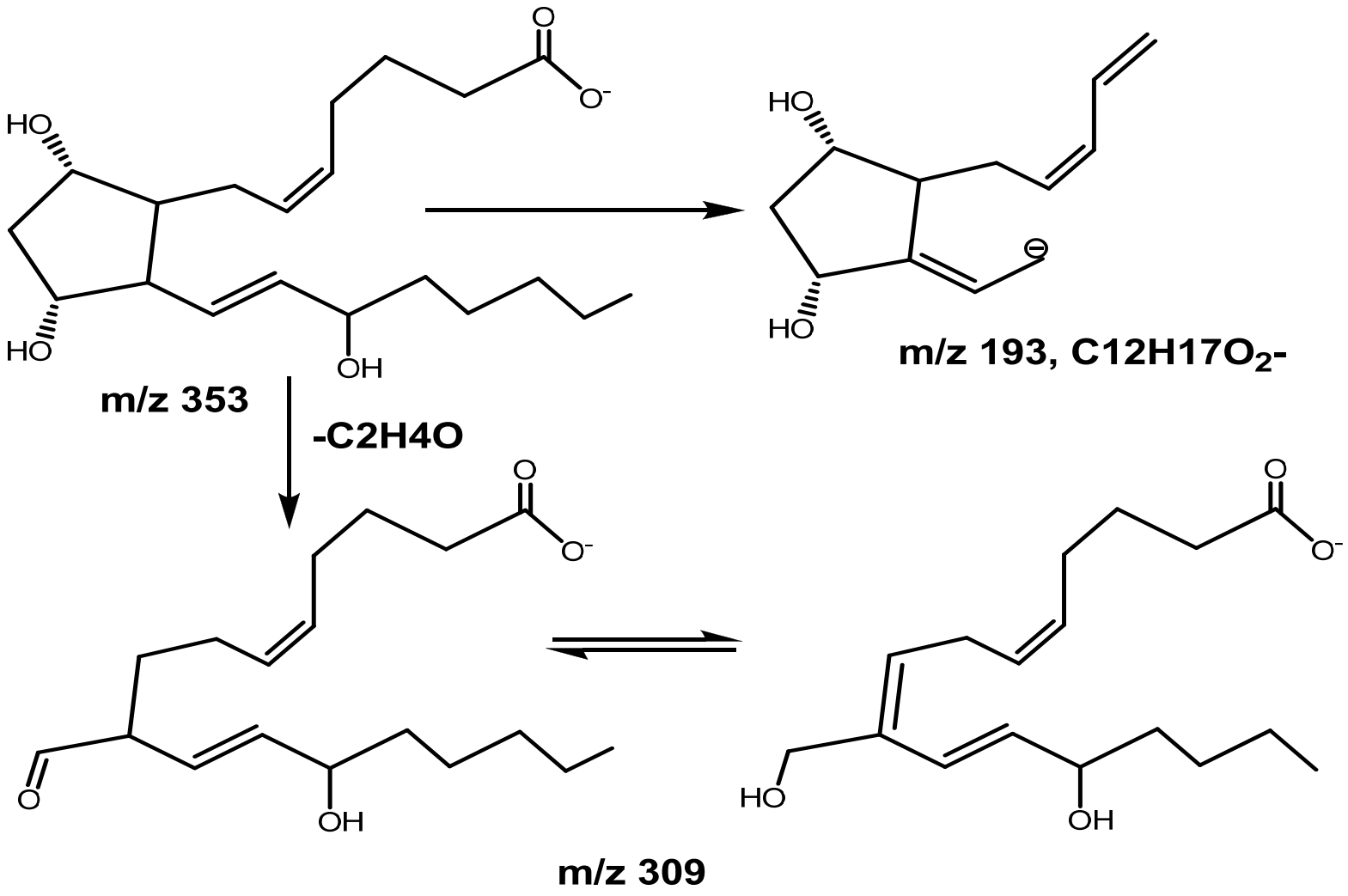


What information does deuterium labeling at C-2 and C-3 of PGF2 provide us for structure elucidation of PG?



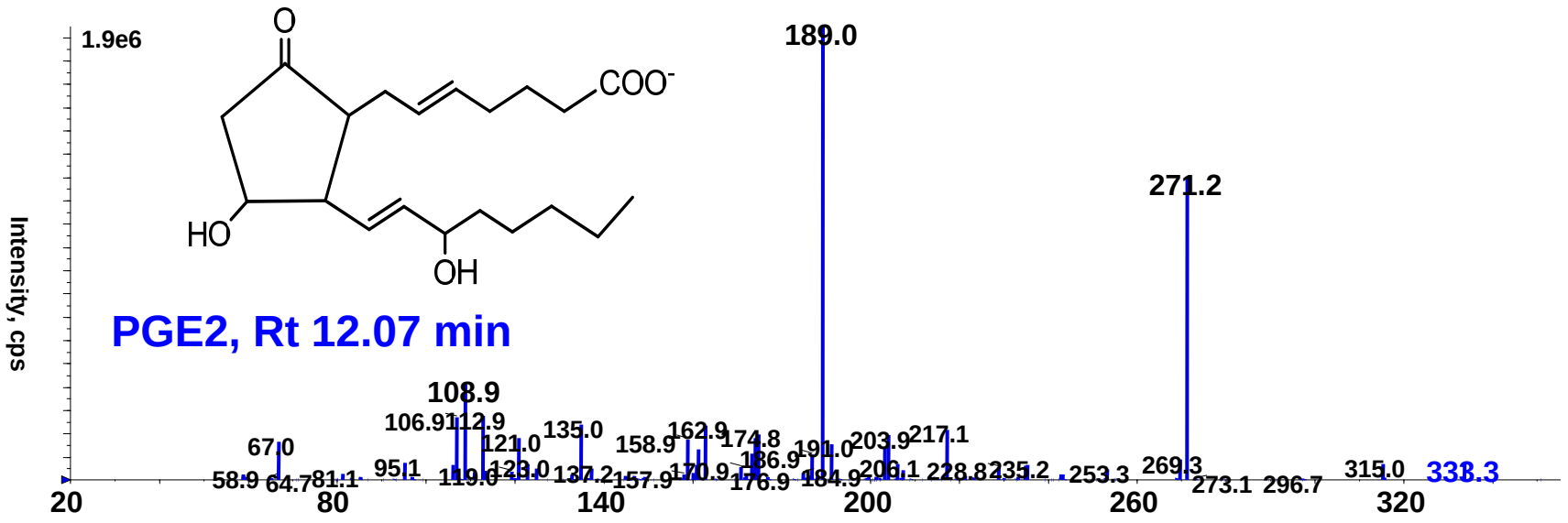
Source: Murphy et al. Analytical Biochemistry, 2005

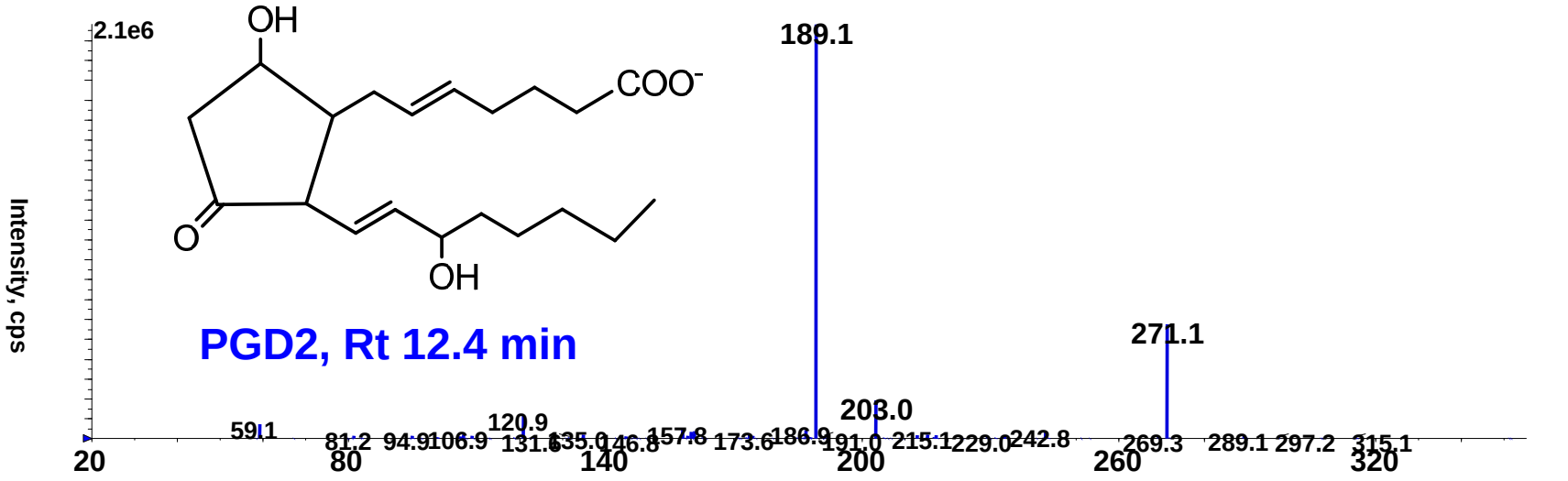
Fragmentation scheme of PGF2α [M-H]$^-$ m/z 353



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

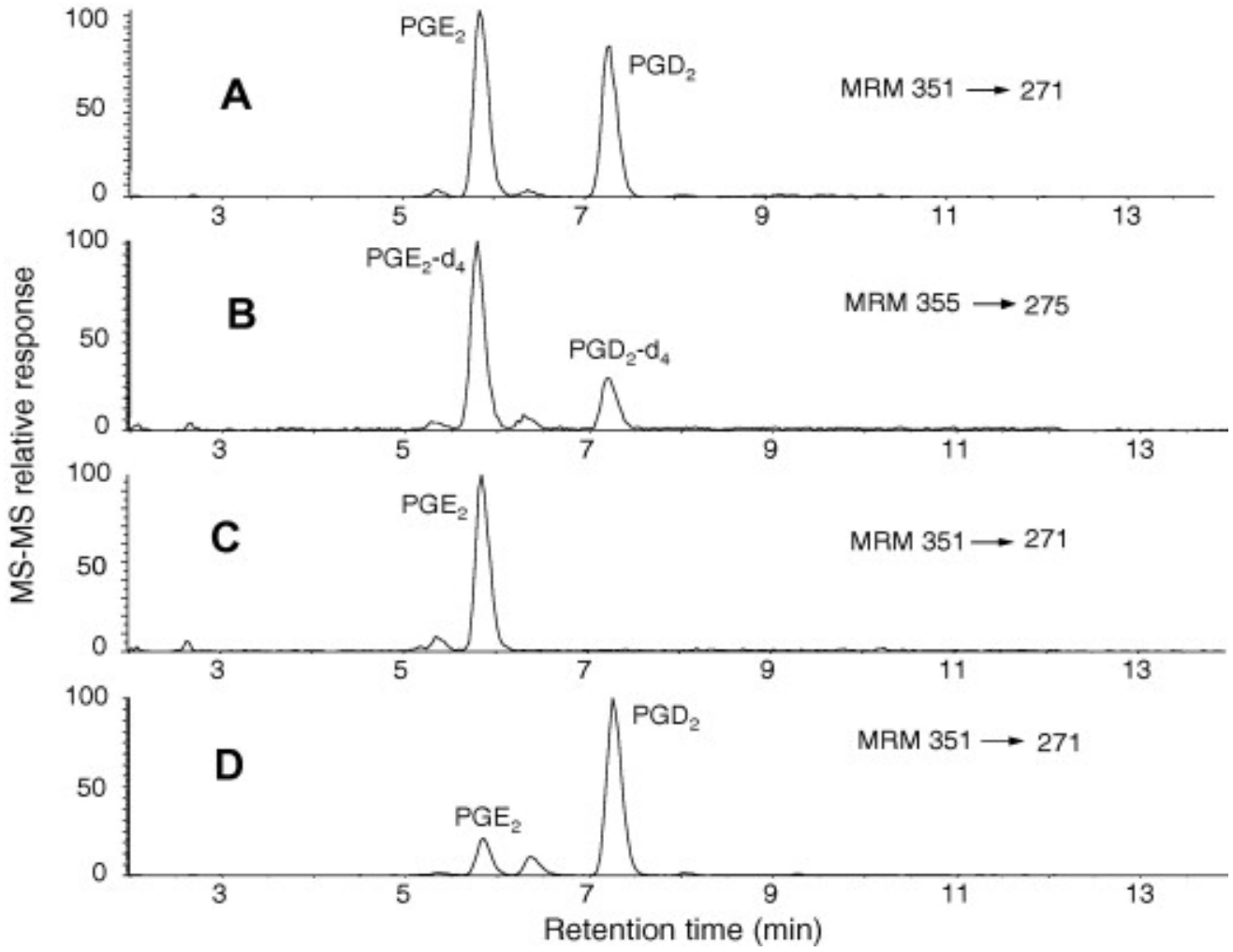
MS/MS fragmentation of PGE2 and PGD2 m/z 351.00





m/z, amu

Deuterated PG standards are used for quantitative analysis of PGs in a extract



Source: Cao et al. Analytical Biochemistry, 2008

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

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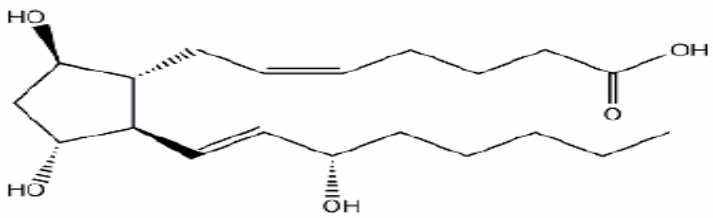
LMSD: Lipid classification search results

Fatty Acyls [FA] (W) --> Eicosanoids [FA03]

LM_ID	Common Name	Systematic Name	Formula	Mass
LMFA03000001	8(9)-EpETE	(+/-)-8(9)-epoxy-5Z,11Z,14Z,17Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000002	11(12)-EpETE	(+/-)-11(12)-epoxy-5Z,8Z,14Z,17Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000003	14(15)-EpETE	(+/-)-14(15)-epoxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000004	17(18)-EpETE	(+/-)-17(18)-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000005	11(R)-HEDE	11R-hydroxy-12E,14Z-eicosadienoic acid	$C_{20}H_{36}O_3$	324.27
LMFA03000006	17R,18S-EpETE	17R,18S-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	$C_{20}H_{30}O_3$	318.22
LMFA03000008	15(R)-HEDE	15R-hydroxy-11Z-13E-eicosadienoic acid	$C_{20}H_{36}O_3$	324.27
LMFA03000009	11S-HEDE	11S-hydroxy-12E,14Z-eicosadienoic acid	$C_{20}H_{36}O_3$	324.27
LMFA03010000	Prostanoic acid skeleton	-	-	-
LMFA03010001	6-keto-PGF1α	6-oxo-9S,11R,15S-trihydroxy-13E-prostenoic acid	$C_{20}H_{34}O_6$	370.24
LMFA03010002	PGF2α	9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid	$C_{20}H_{34}O_5$	354.24
LMFA03010003	PGE2 (W)	9-oxo-11R,15S-dihydroxy-5Z,13E-prostadienoic acid	$C_{20}H_{32}O_5$	352.22
LMFA03010004	PGD2 (W)	9S,15S-dihydroxy-11-oxo-5Z,13E-prostadienoic acid	$C_{20}H_{32}O_5$	352.22
LMFA03010005	PGA1	9-oxo-15S-hydroxy-10Z,13E-prostadienoic acid	$C_{20}H_{32}O_4$	336.23
LMFA03010006	PGF2α-d4	9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid (3,3,4,4-d4)	$C_{20}H_{30}D_4O_5$	358.27
LMFA03010007	PGD2-d4	9S,15S-dihydroxy-11-oxo-5Z,13E-prostadienoic acid (3,3,4,4-d4)	$C_{20}H_{28}D_4O_5$	356.25
LMFA03010008	PGE2-d4	11R,15S-dihydroxy-9-oxo-5Z,13E-prostadienoic acid (3,3,4,4-d4)	$C_{20}H_{28}D_4O_5$	356.25
LMFA03010009	PGG2	9S,11R-epidioxy-15S-hydroperoxy-5Z,13E-prostadienoic acid	$C_{20}H_{32}O_6$	368.22

Structure database (LMSD)

LMFA03010025



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	-

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.**