"Insights into the use of tandem mass spectrometry in lipidomics"

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Insights into the use of tandem mass spectrometry in lipidomics

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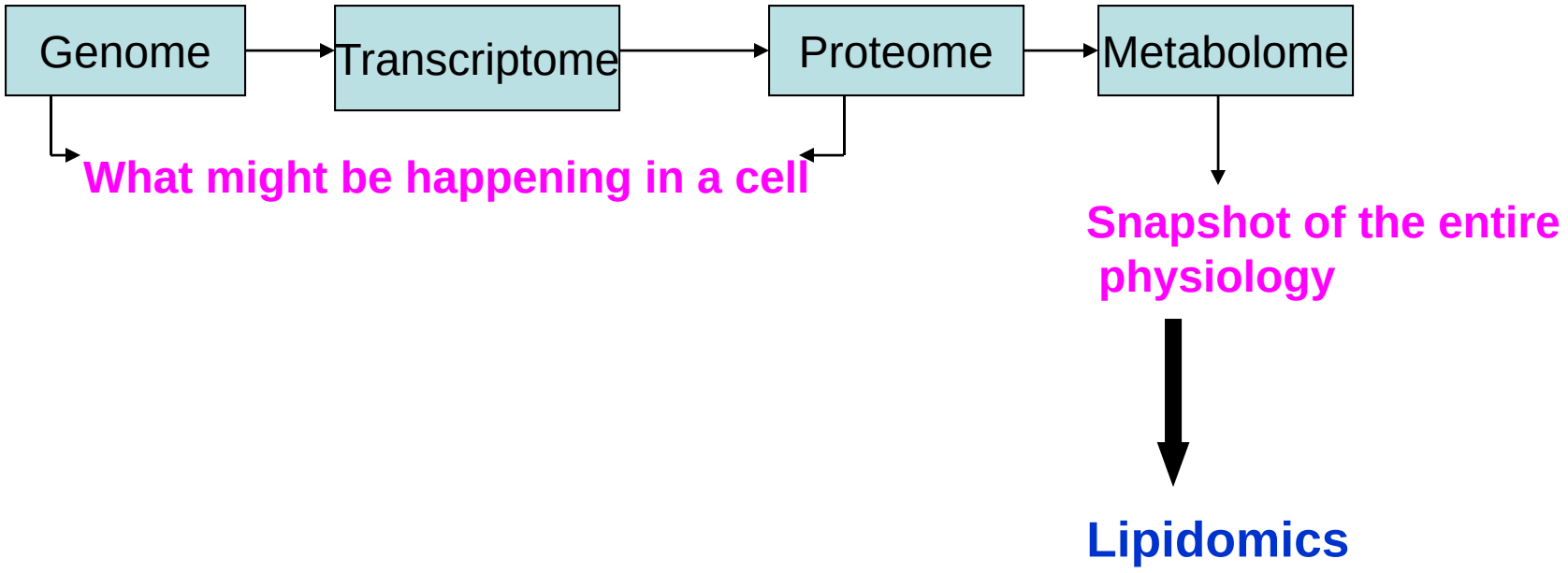
Outlines

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

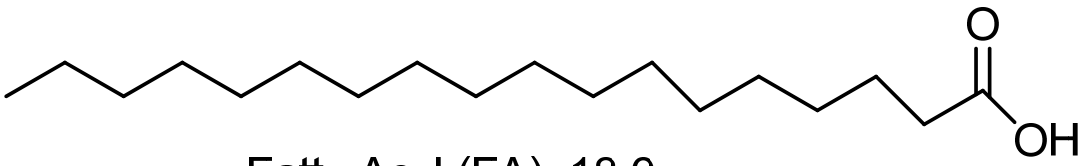
Lipidomics in the context of other omics



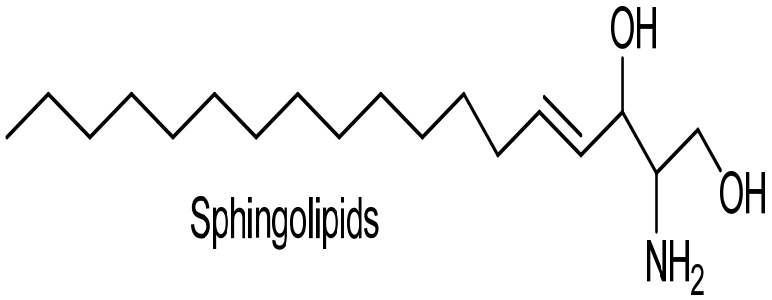
Lipids are important- as a membrane bilayer
- provides hydrophobic environment for protein function
- reservoir of energy
- signaling molecules

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

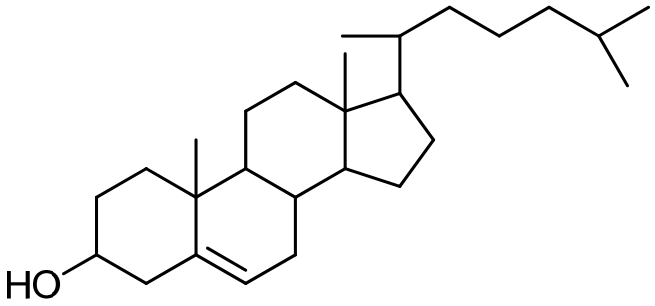
Structures of different lipids classes



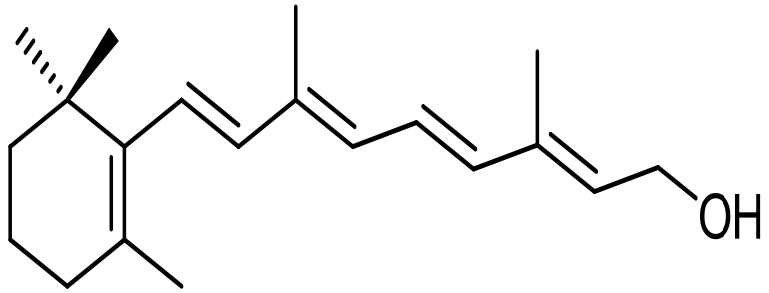
Fatty Acyl (FA), 18:0
saturated/unsaturated



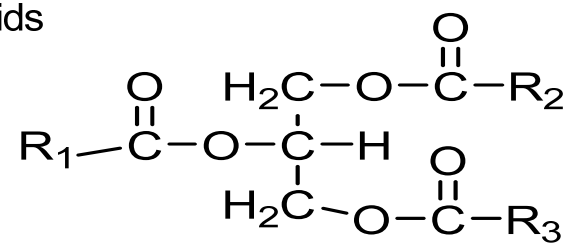
Sphingolipids



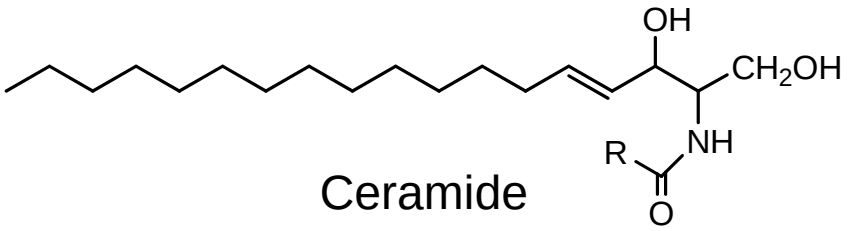
Sterol lipids



Prenol lipids (retinol)

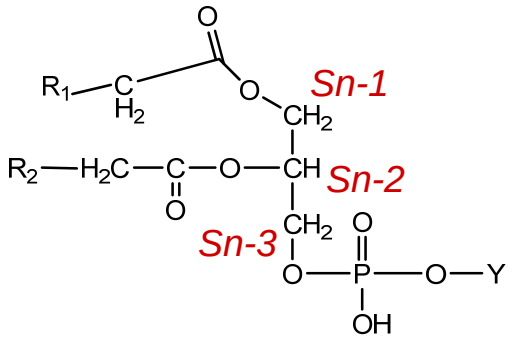


Glycerolipid, R =
saturated/unsaturated FA

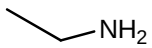


Ceramide

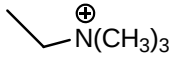
Structures of main phospholipids



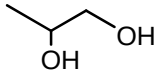
Phosphatidylethanolamine (PE)

Y= 

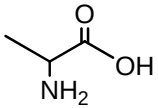
Phosphatidylcholine (PC)

Y= 

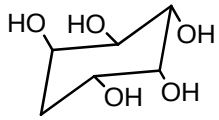
Phosphatidylglycerol (PG)

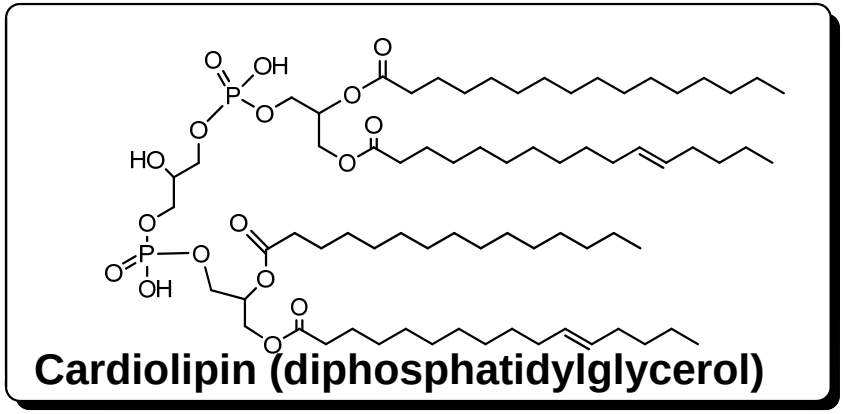
Y= 

Phosphatidylserine (PS)

Y= 

Phosphatidylinositol (PI)

Y= 



Cardiolipin (diphosphatidylglycerol)

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

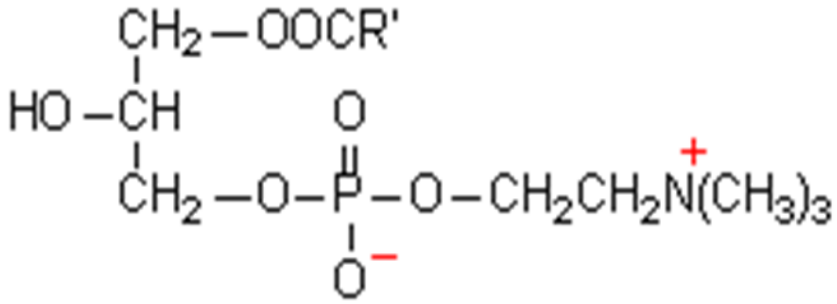
Which ionization mode for which phospholipids?

Positive ion mode

PC
LPC
PE
LPE
SM
PS

Negative ion mode

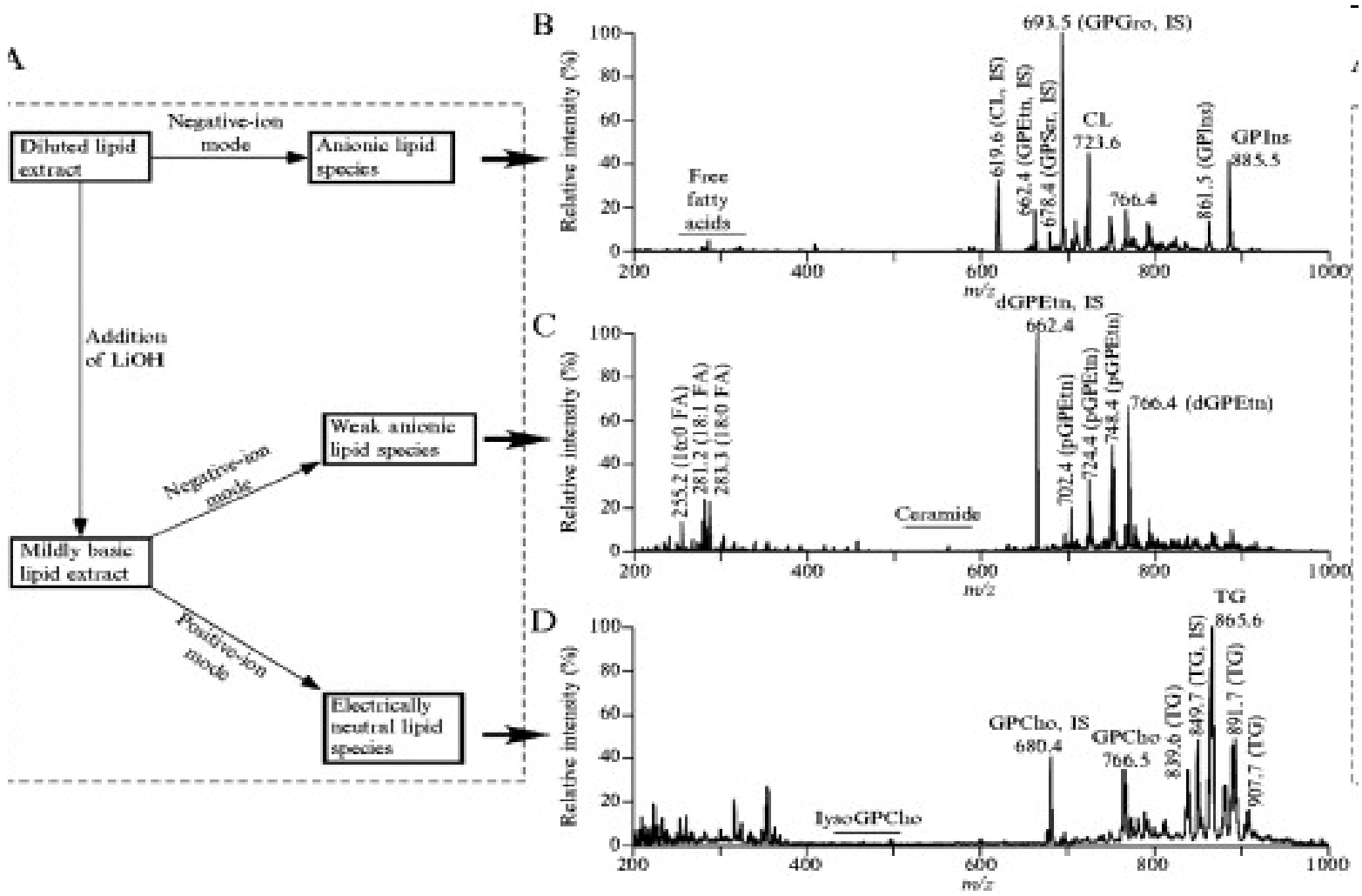
PE
PA
PI
PI
PG
PIPs



lysophosphatidylcholine

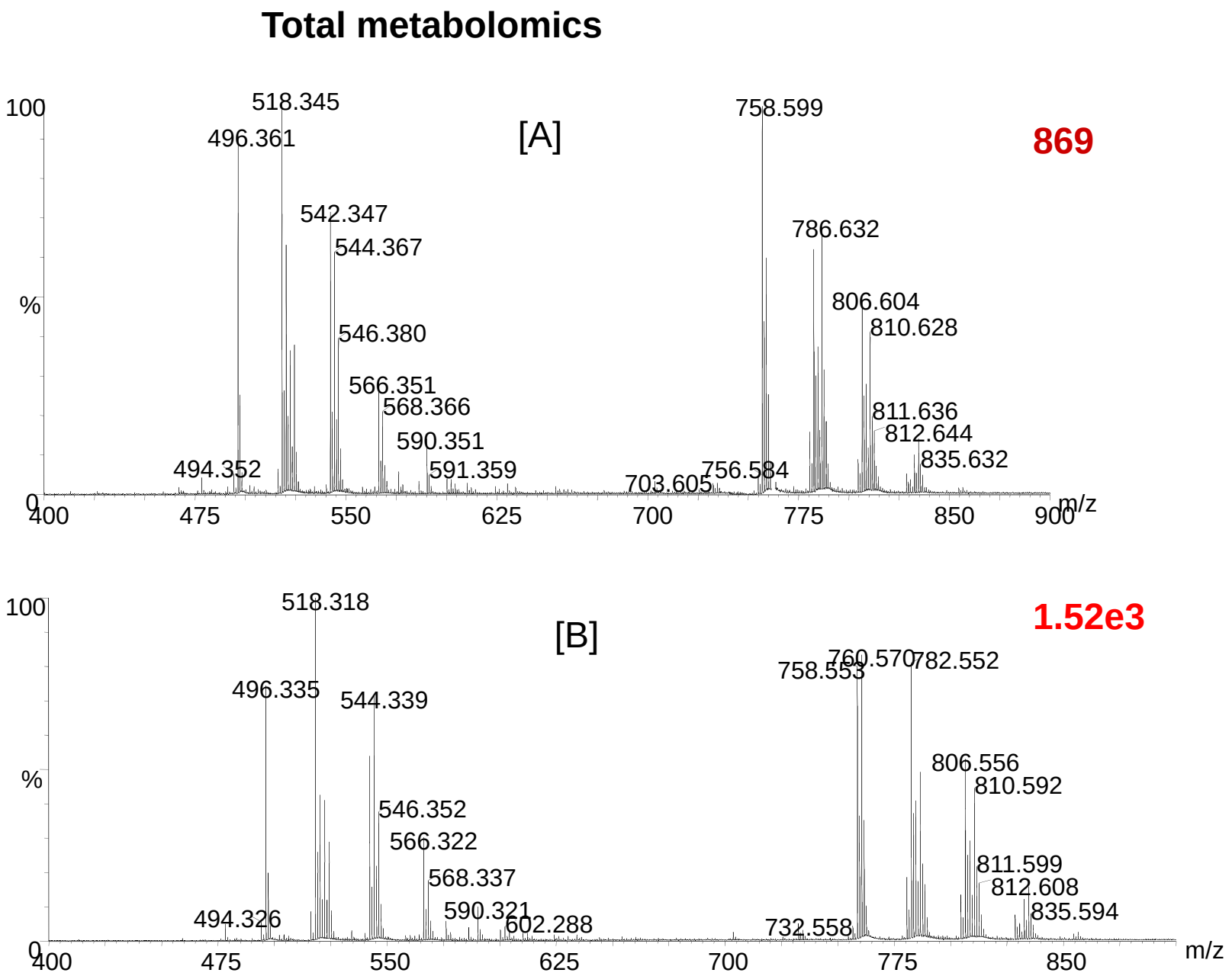
PC = phosphatidylcholine
PA = phosphatic acid
PE = phosphatidylethanolamine
PS = phosphatidylserine
PG = phosphatidylglycerol
PI = phosphatidylinositol
PIP = PI monophosphate
SM = sphingomyelin
LPE = lysoPE

Application of shotgun lipidomics: intra-source separation of lipids

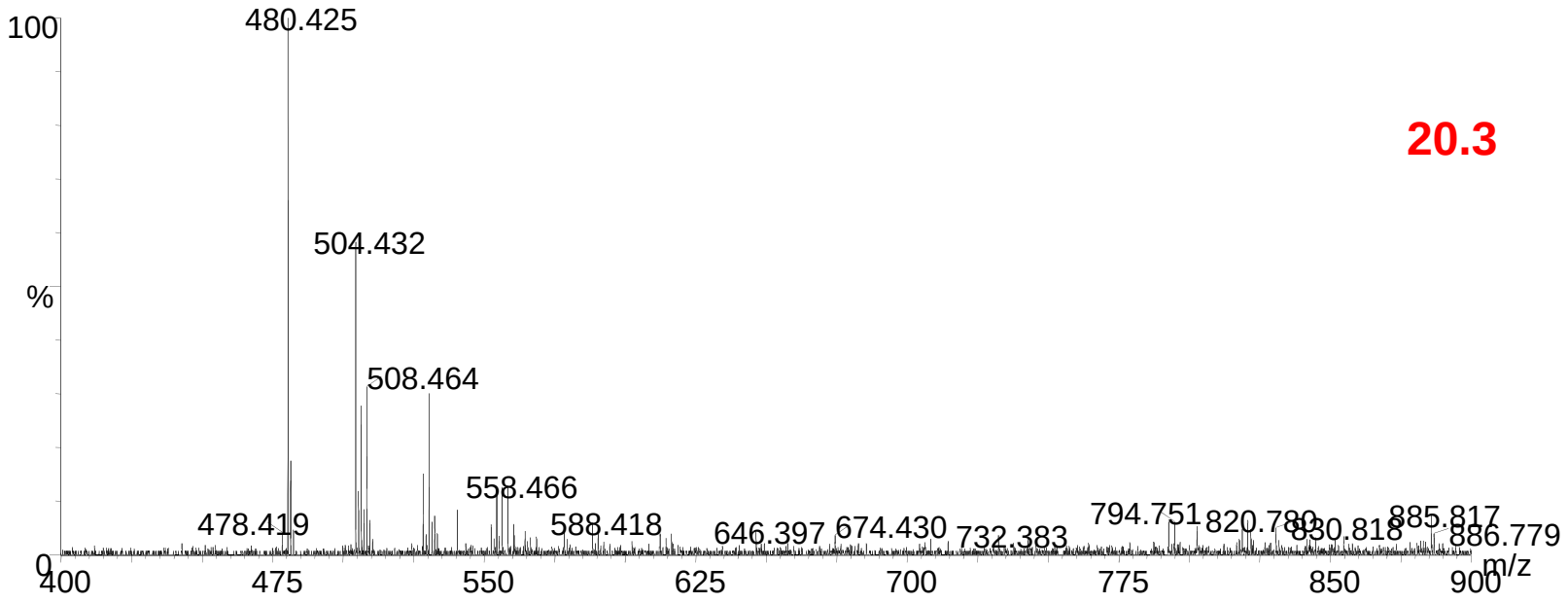


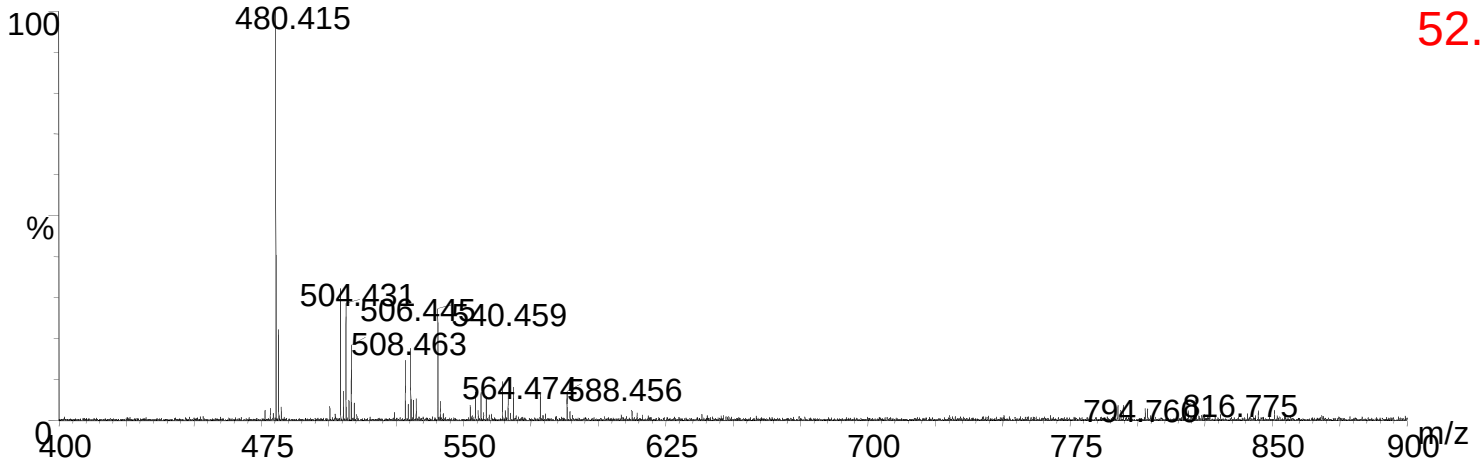
Source: Gross and Han, methods in Enzymology, 2007

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

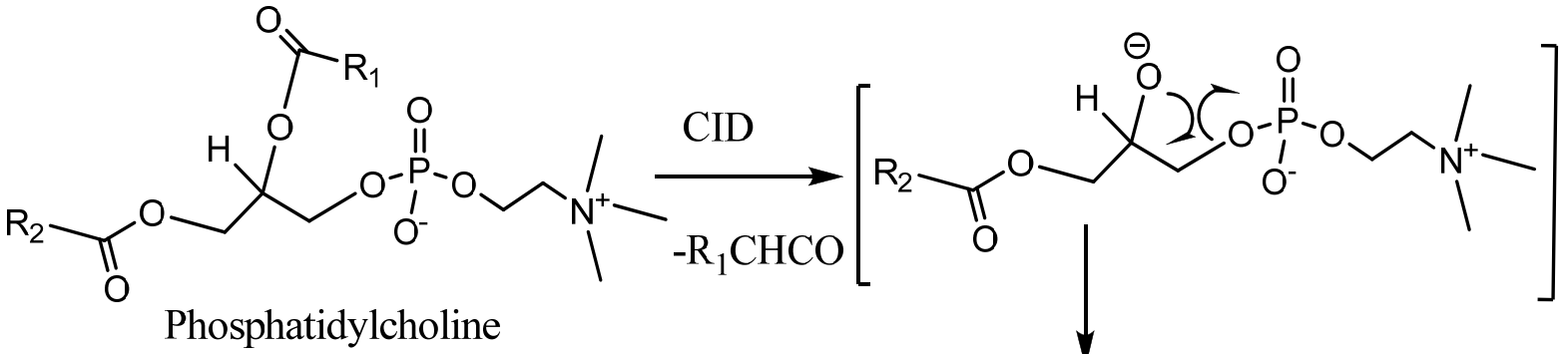


Total scan of metabolites (Q1 SCAN -ve ion mode) for a plasma sample obtained from lean mouse [A]; ob/ob mouse

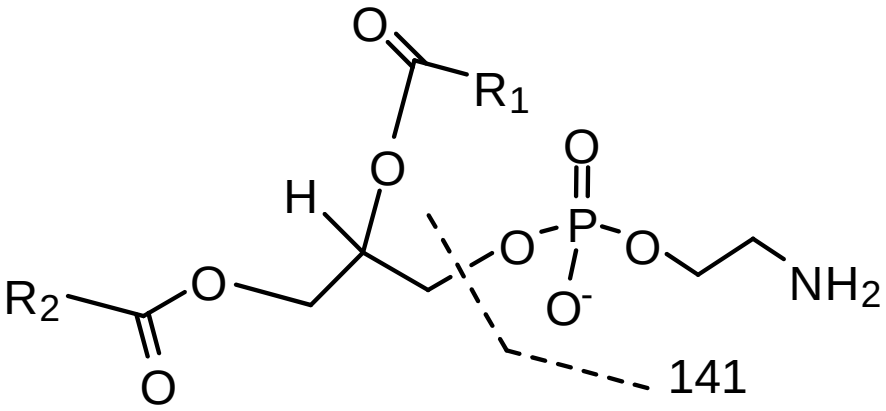




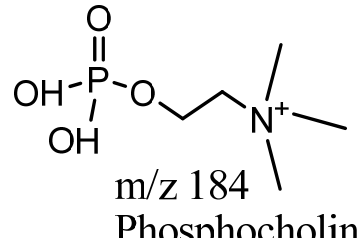
Tandem mass spectrometry (MS/MS) of phospholipids



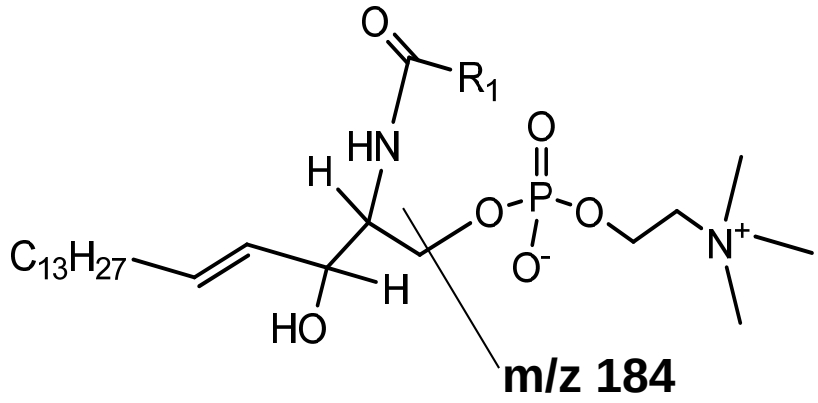
Phosphatidylcholine



Phosphatidylethanolamine

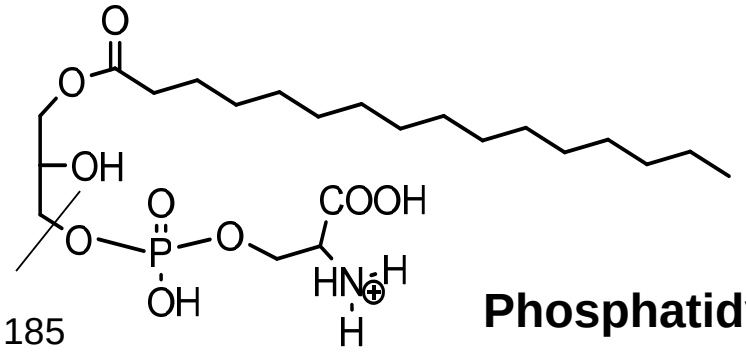


m/z 184
Phosphocholine



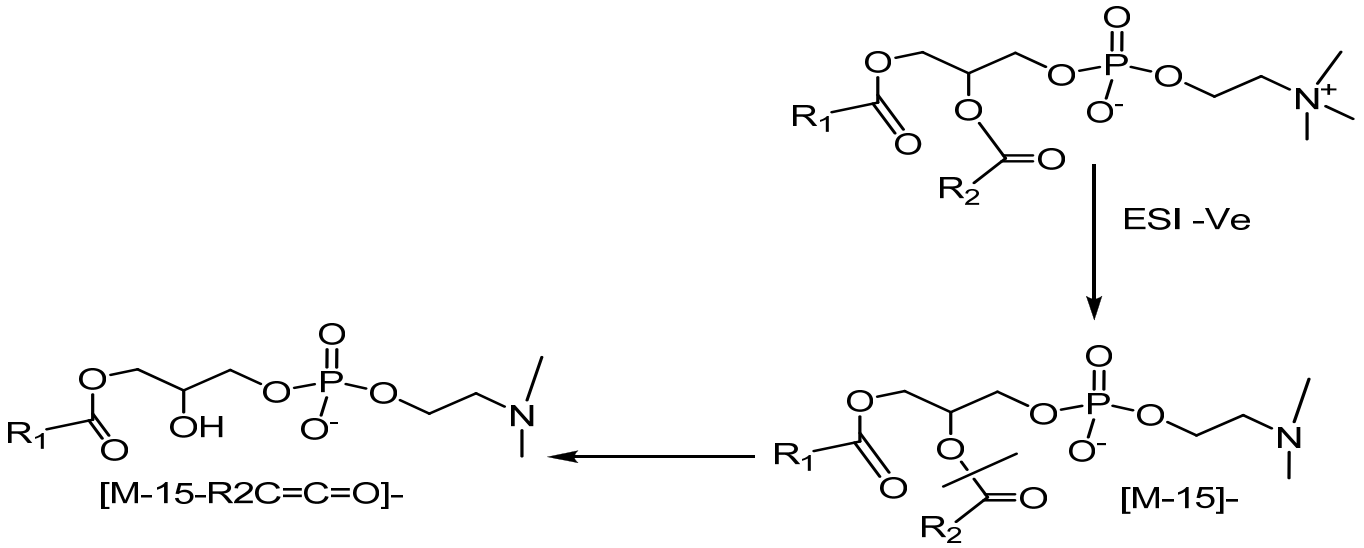
m/z 184

Sphingomyelin



Phosphatidylserine

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



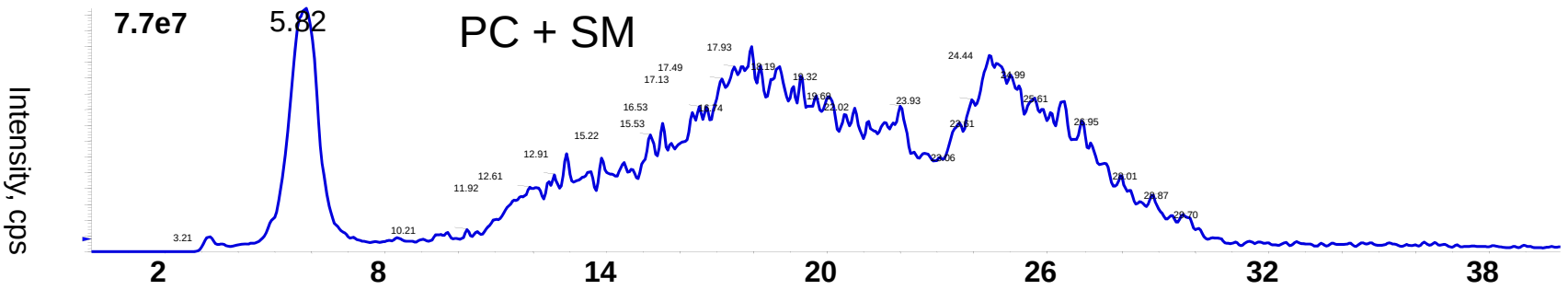
ESI-MS/MS analyses of various lipids

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

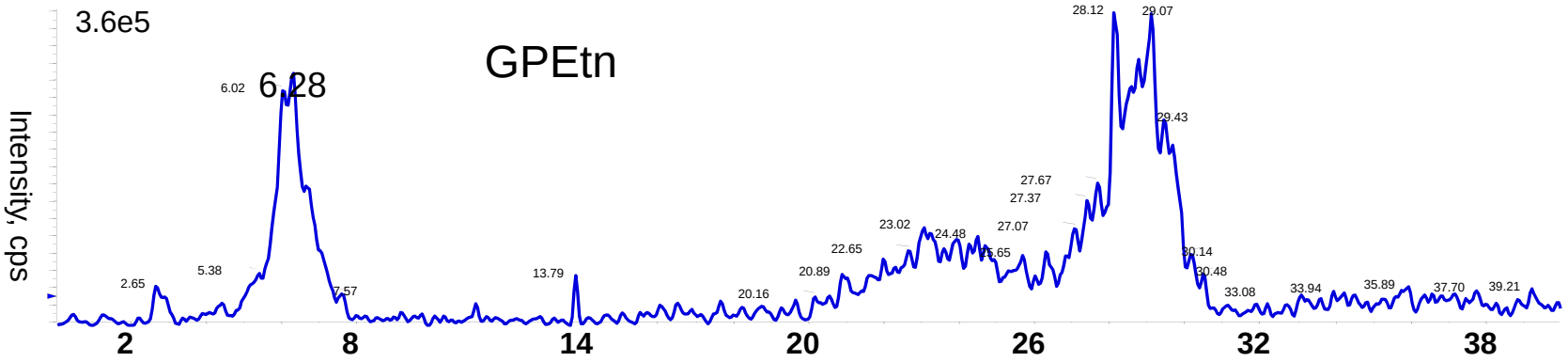
Source: Gross and Han,, 2004

LC-MS/MS analysis of phospholipids from a mouse plasma sample

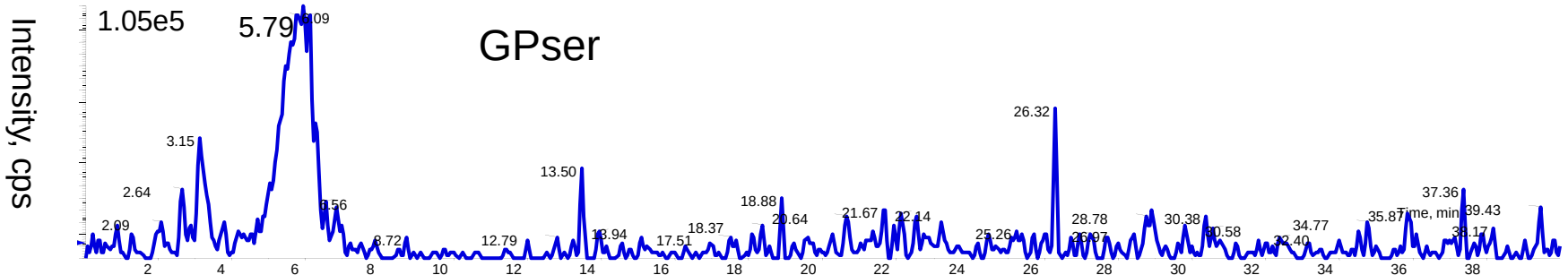
TIC of +Prec (184.00): Exp 1, from Sample 11 (Lean 53 NK) of ZhangSET1.wiff (Turbo Spray), Smoothed, Smoothed



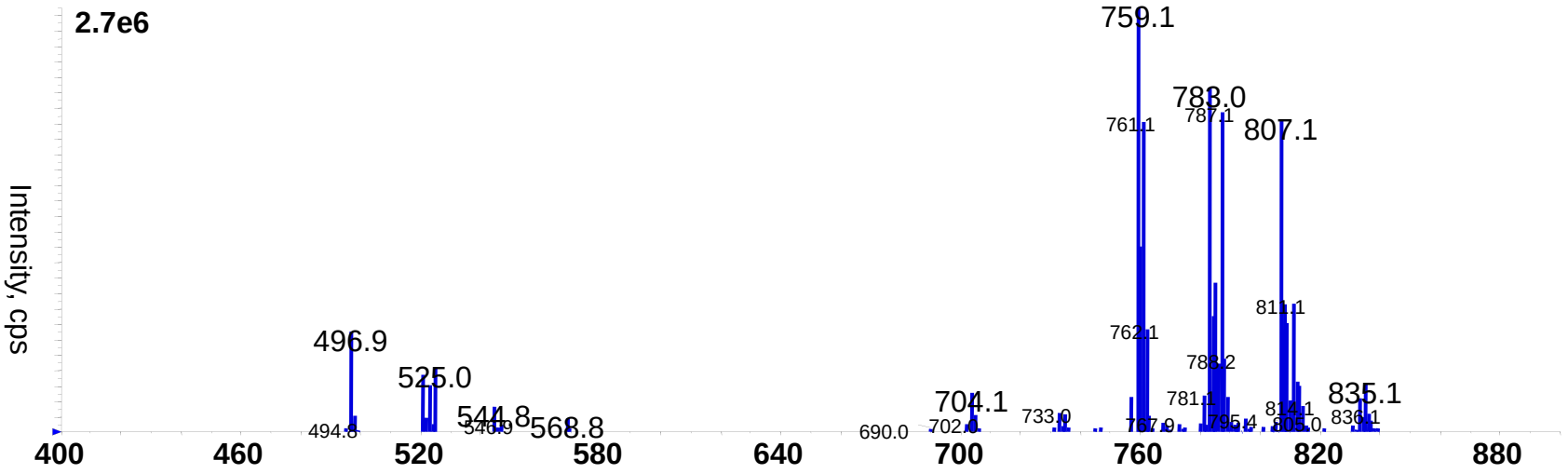
TIC of +NL (141.00): Exp 2, from Sample 11 (Lean 53 NK) of ZhangSET1.wiff (Turbo Spray), Smoothed, Smoothed



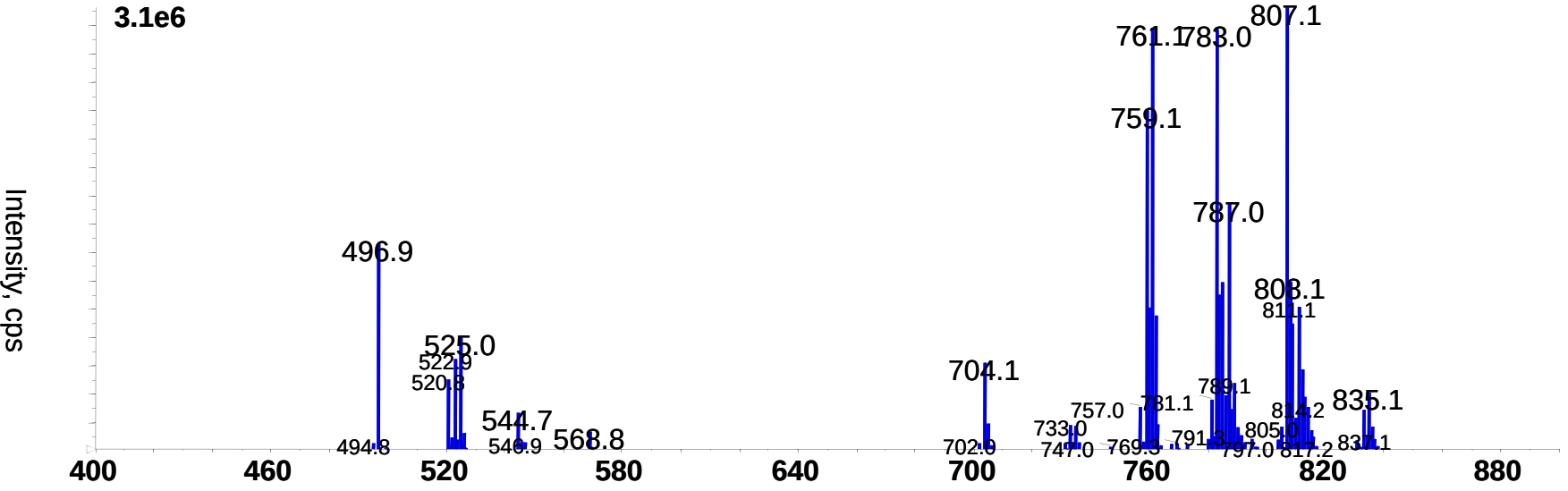
TIC of +NL (185.00): Exp 3, from Sample 11 (Lean 53 NK) of ZhangSET1.wiff (Turbo Spray), Smoothed



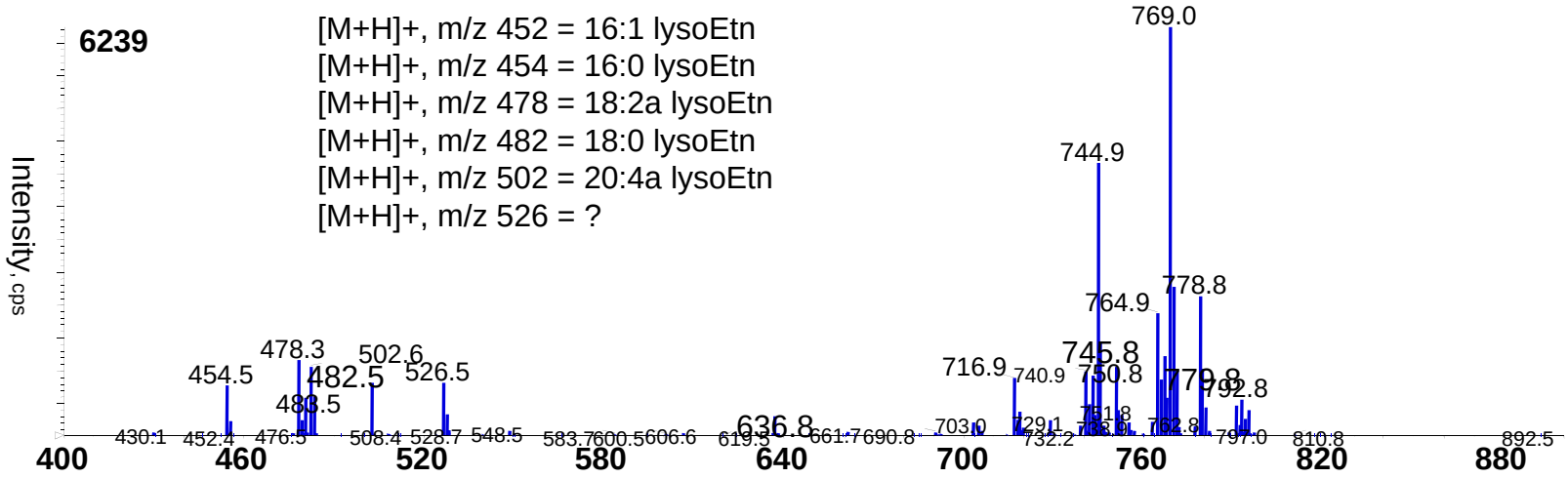
+Prec (184.00): Exp 1, 2.764 to 30.604 min from Sample 11 (Lean 53 NK) of ZhangSET1.wiff (Turbo Spray), Centroided



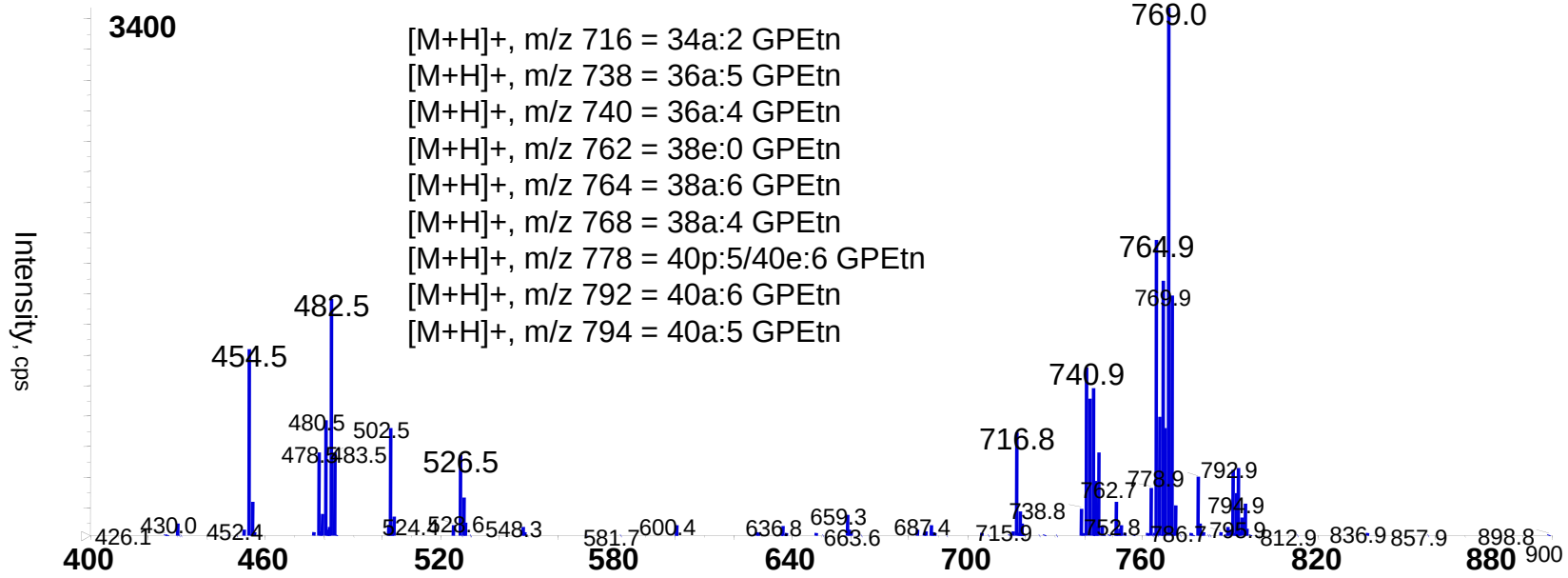
+Prec (184.00): Exp 1, 2.764 to 30.603 min from Sample 16 (Ob 27 NK) of ZhangSET1.wiff (Turbo Spray), Centroided



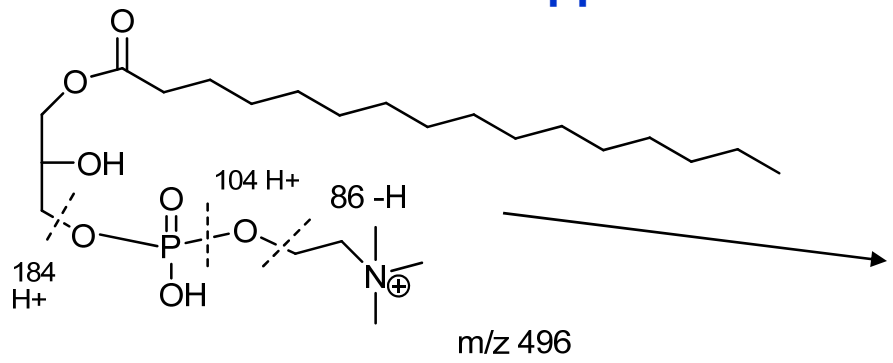
+NL (141.00): Exp 2, 2.278 to 33.485 min from Sample 11 (Lean 53 NK) of ZhangSET1.wiff (Turbo Spray), Centroided

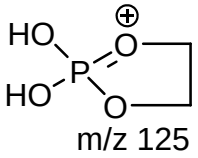


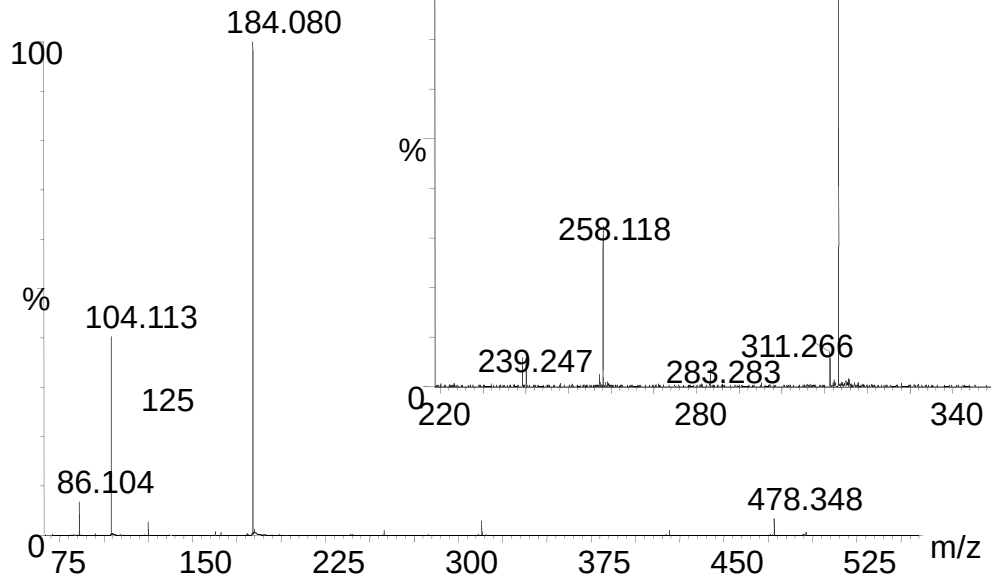
+NL (141.00): Exp 2, 2.278 to 33.485 min from Sample 15 (Ob 26 NK) of ZhangSET1.wiff (Turbo Spray), Centroided

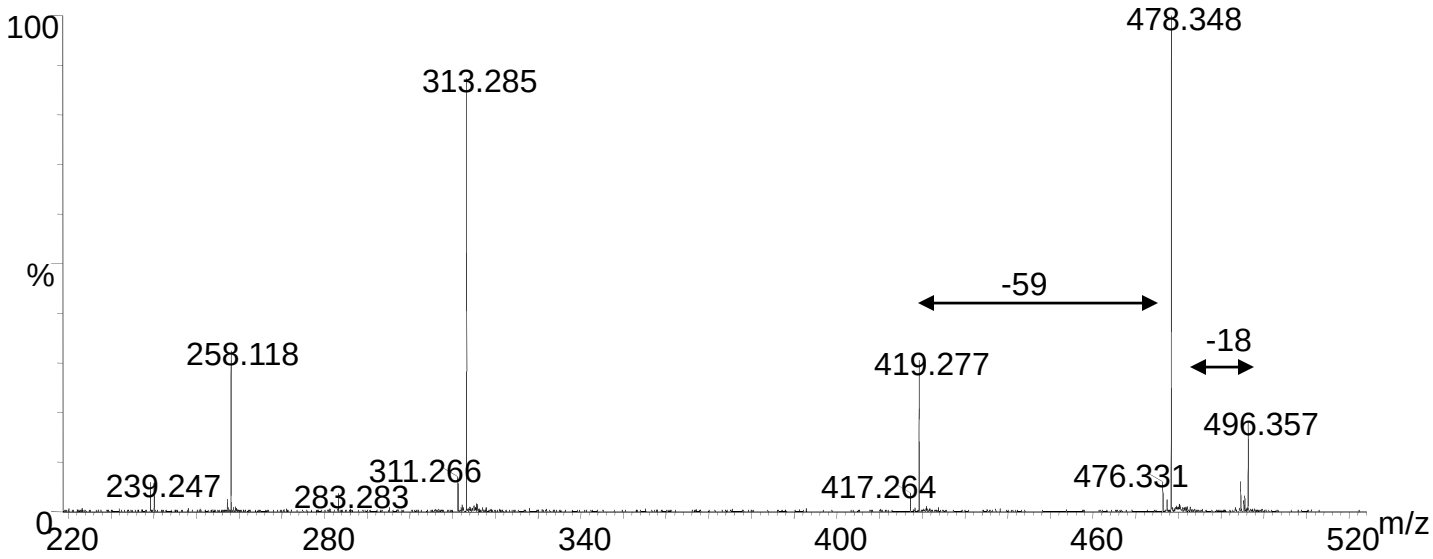


MSMS fragmentation of *m/z* 496 obtained from plasma of ob/ob mouse supplemented with kudzu



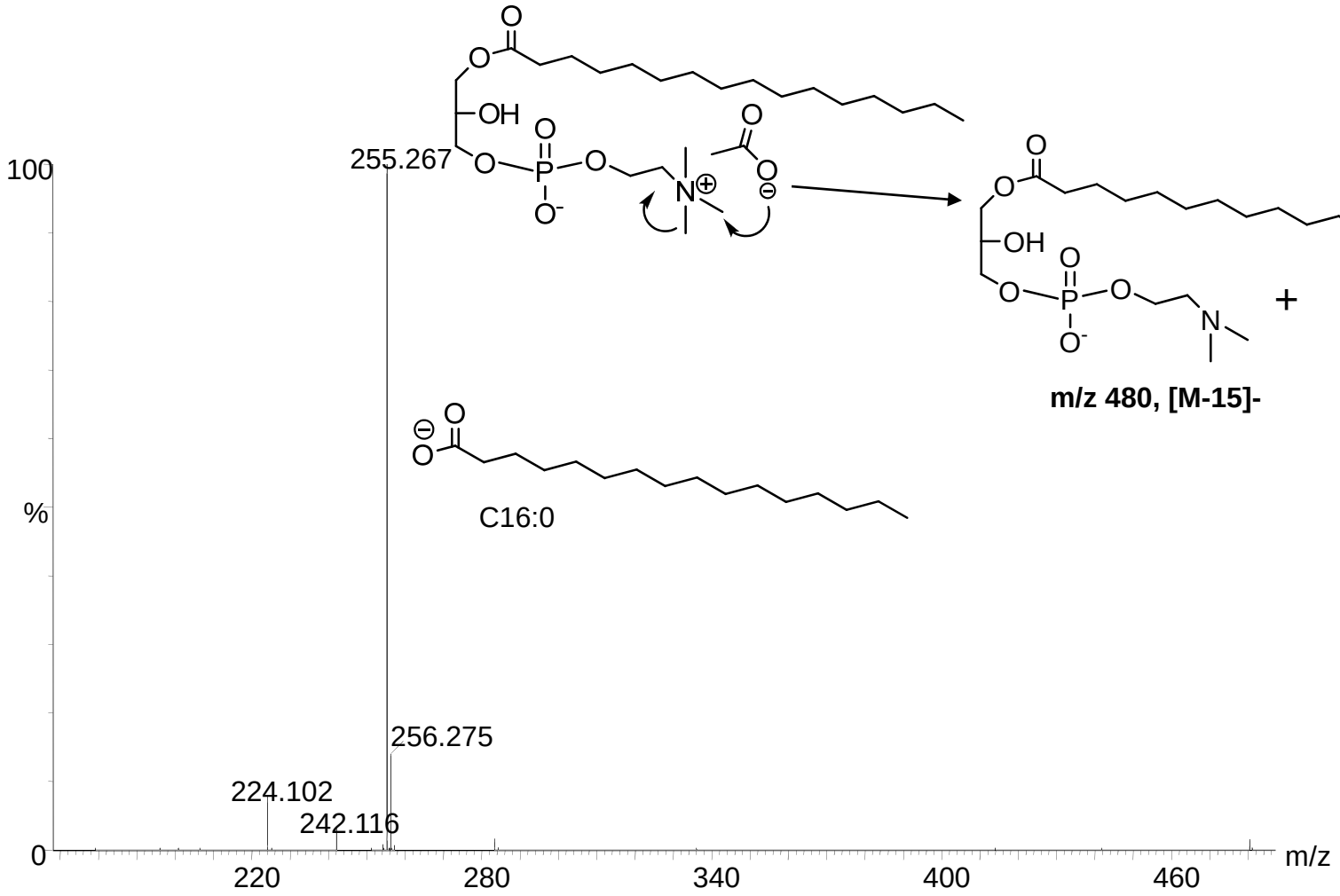




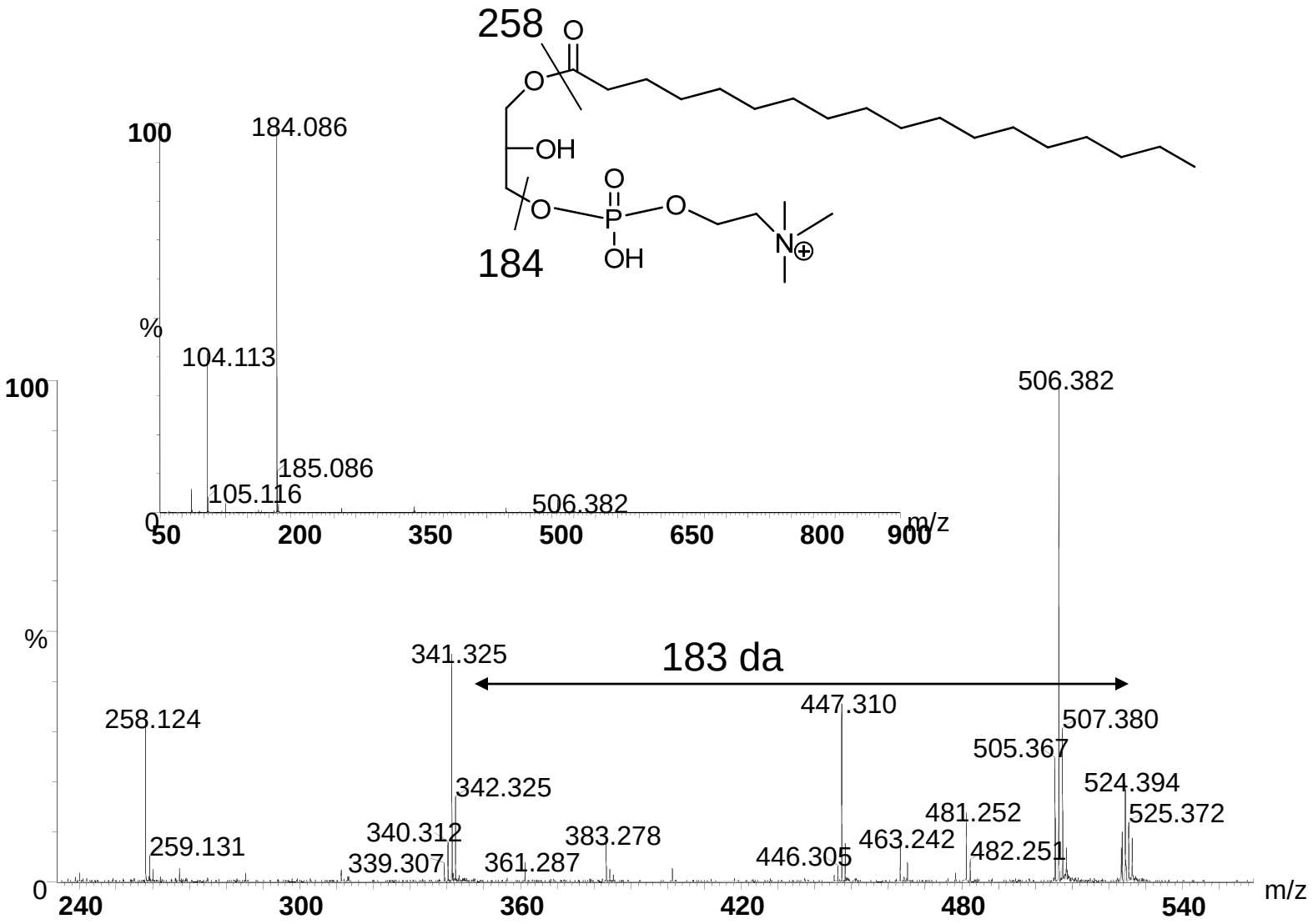


MS/MS of m/z 480 [M-15]- from a ob/ob no kudzu supplemented plasma sample

TOF MSMS 480.40ES-

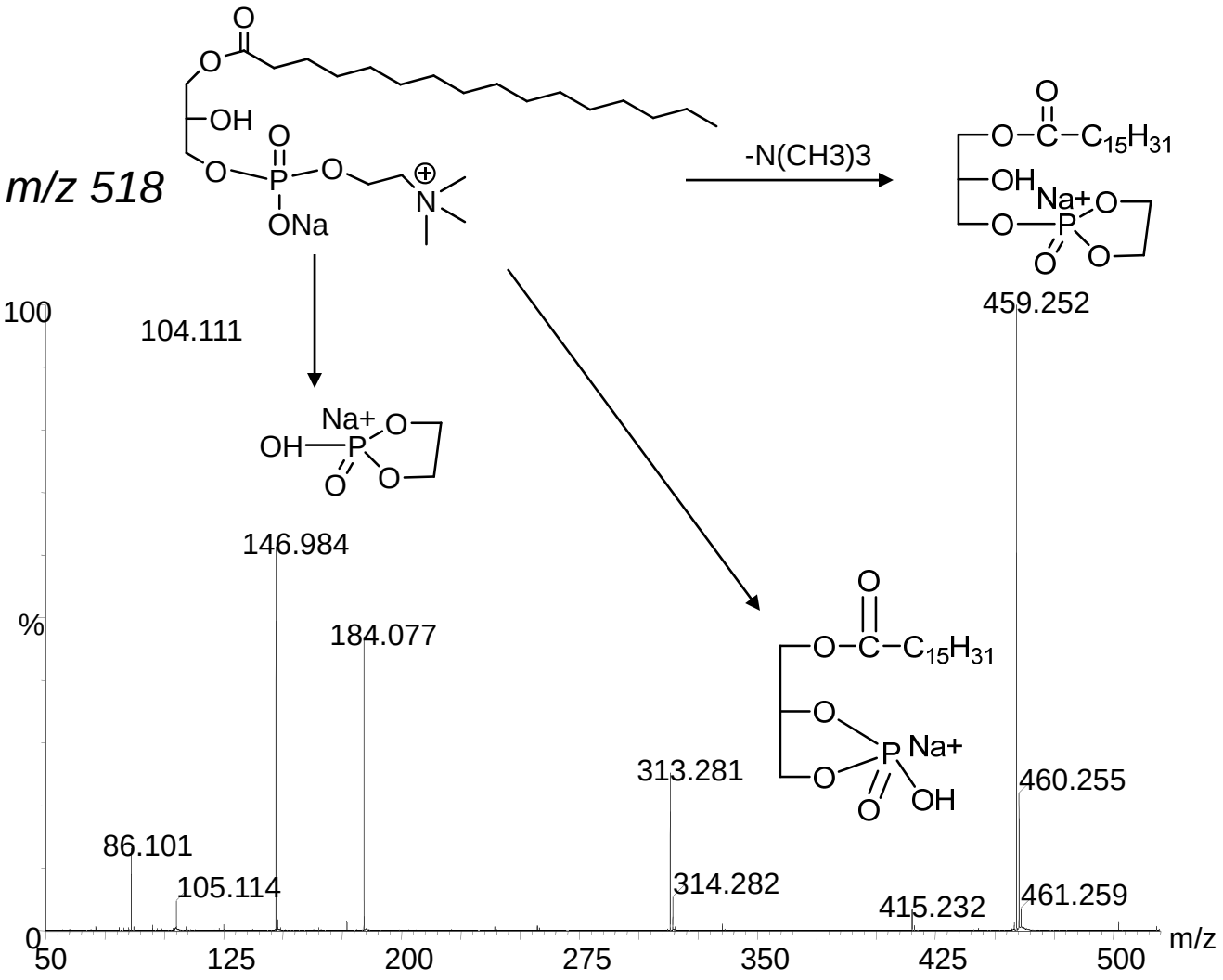


MSMS of ion m/z 524 in +ve ion mode



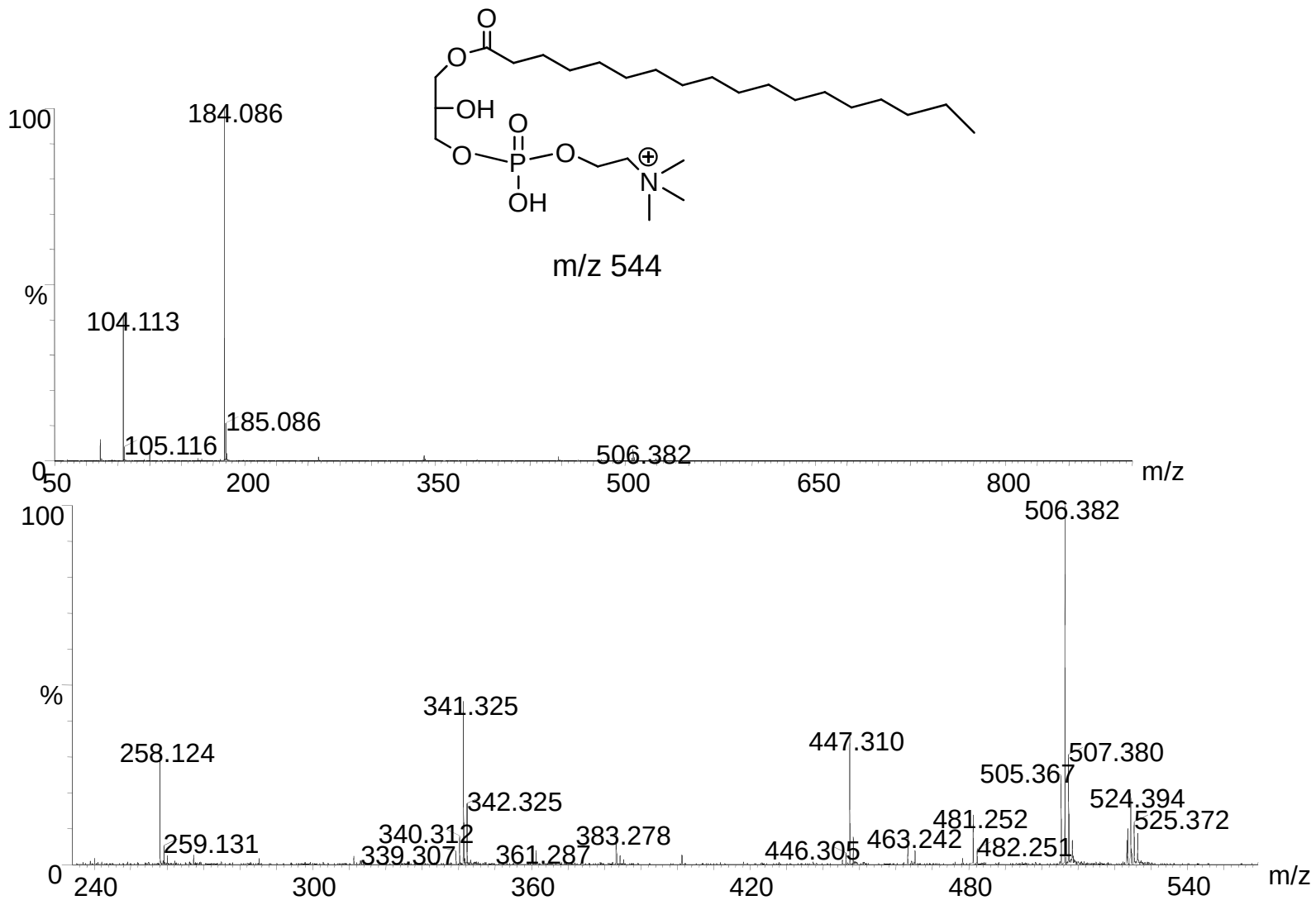
MSMS of m/z 518 [M+Na]+ indicates that it may be a sodiated ion of a phospholipid with mol. Wt. 495 Da

TOF MSMS 518.28ES+

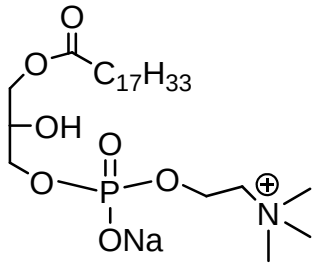


MSMS of m/z 524 indicates that this lysophosphatidylcholine is 18:0

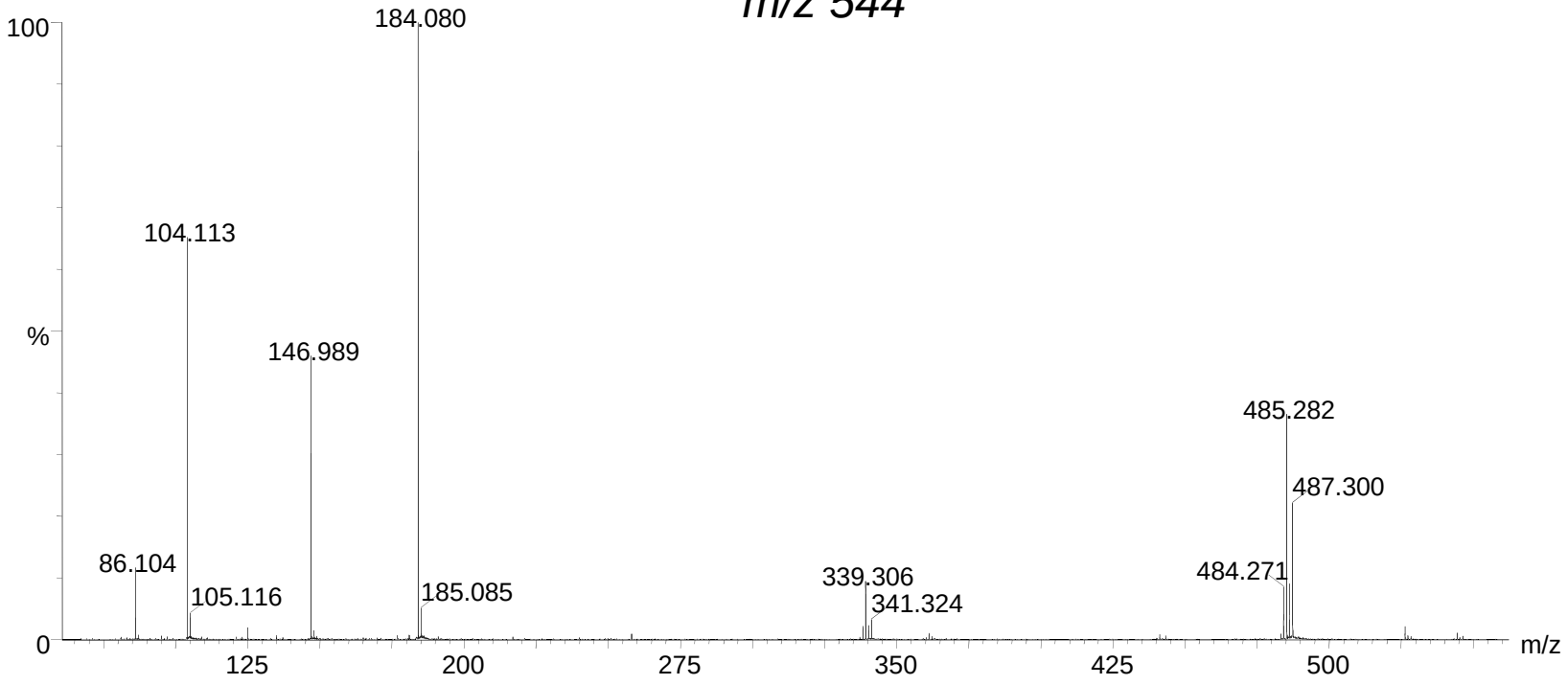
TOF MSMS 524.38ES+



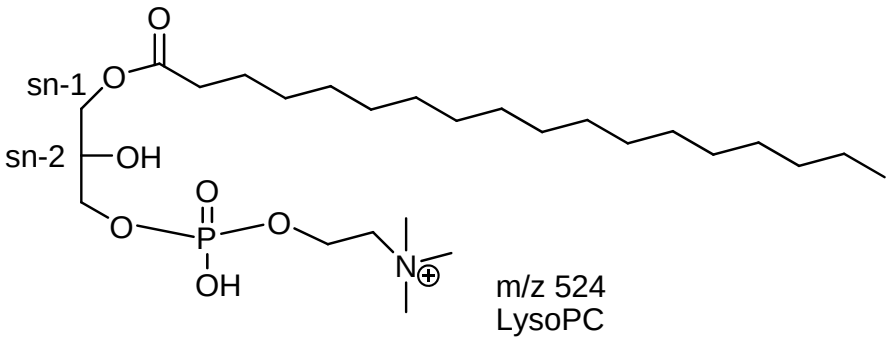
MSMS of m/z 544 indicated that it is a sodiated ion of mol. Wt. 521



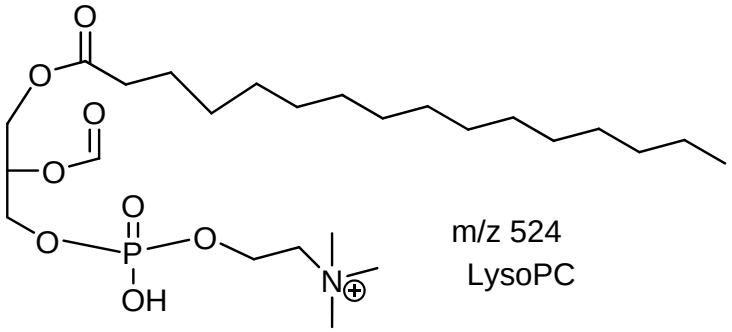
m/z 544



Several isomeric compounds exits and unambiguous identification is a challenge



m/z 524
LysoPC



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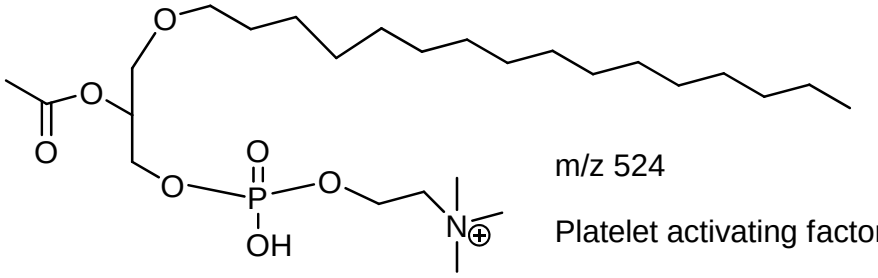
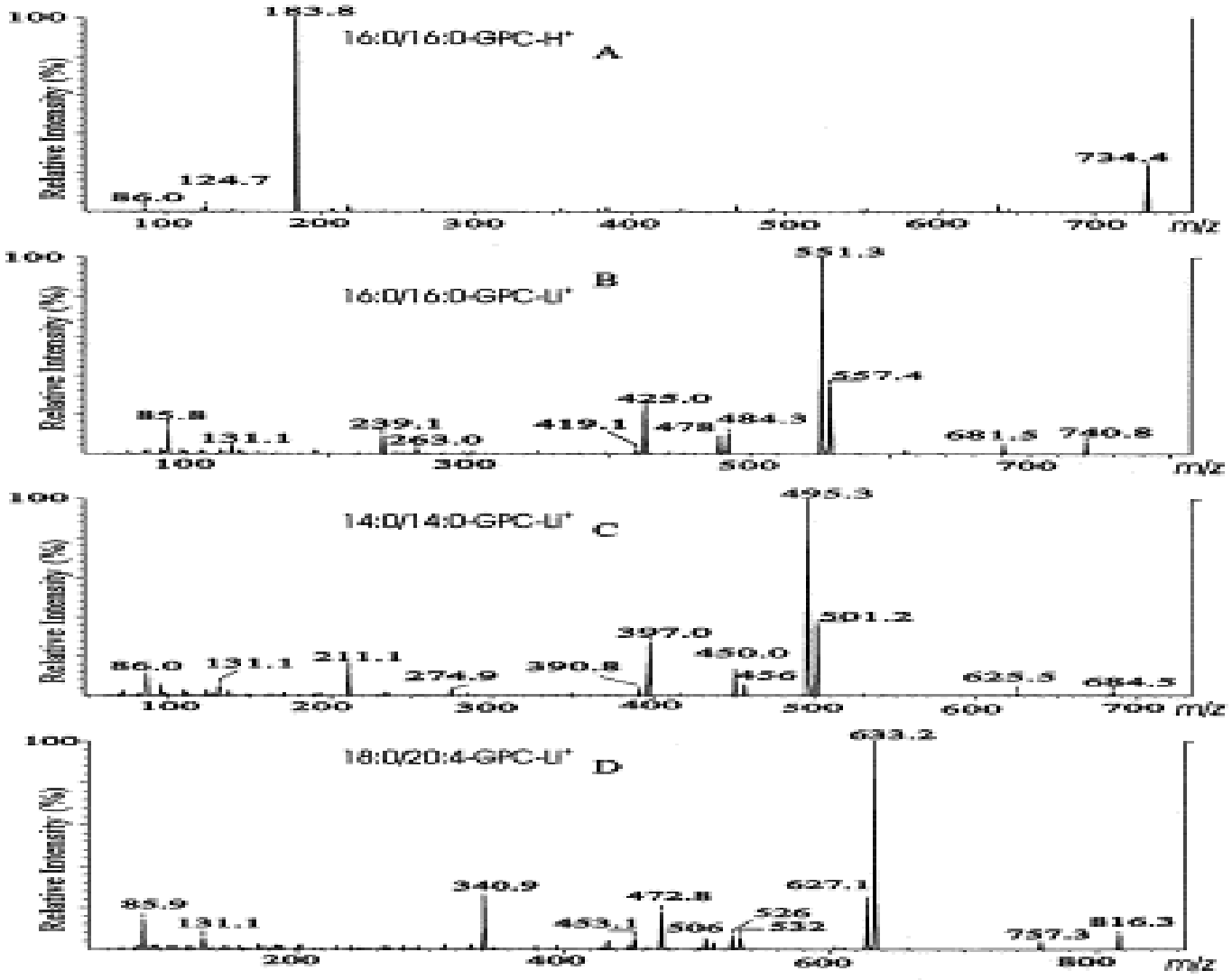


m/z 524

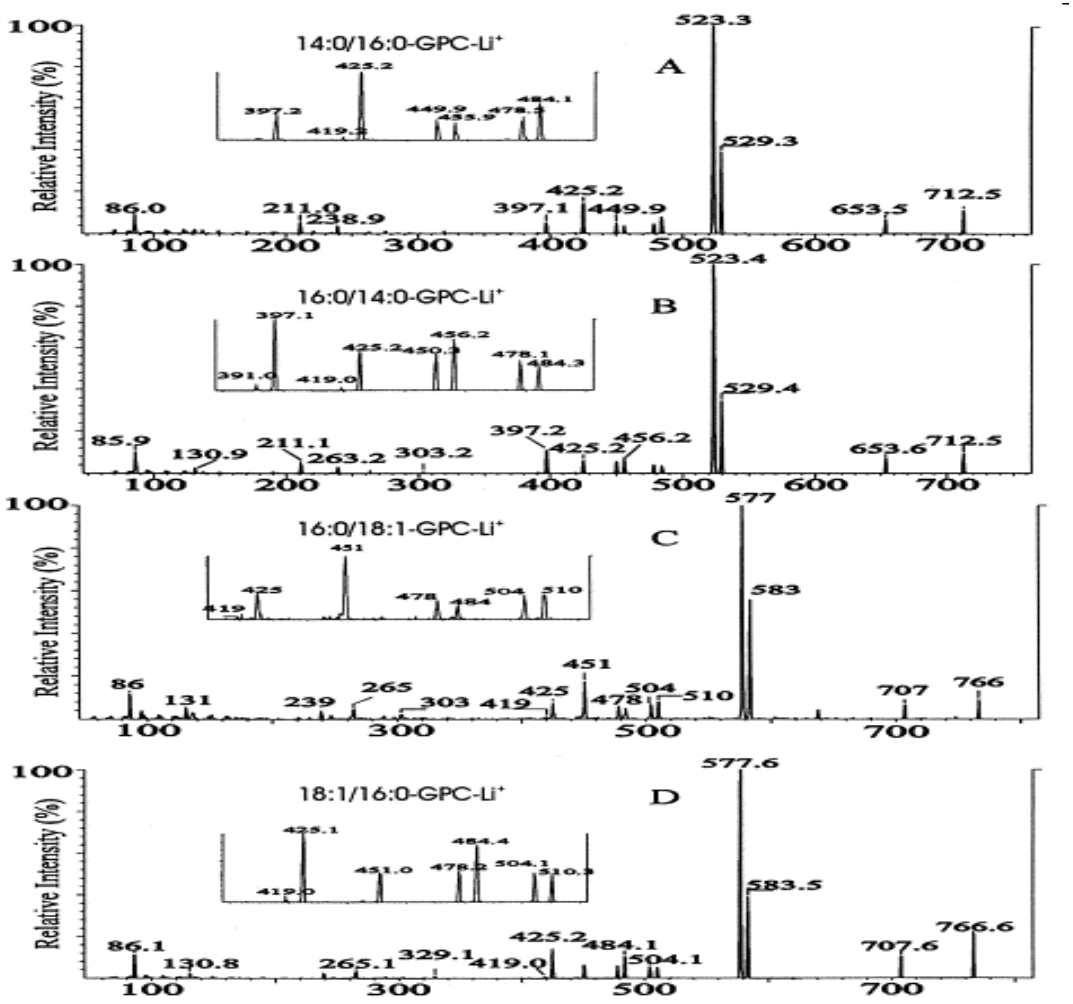
Platelet activating factor

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



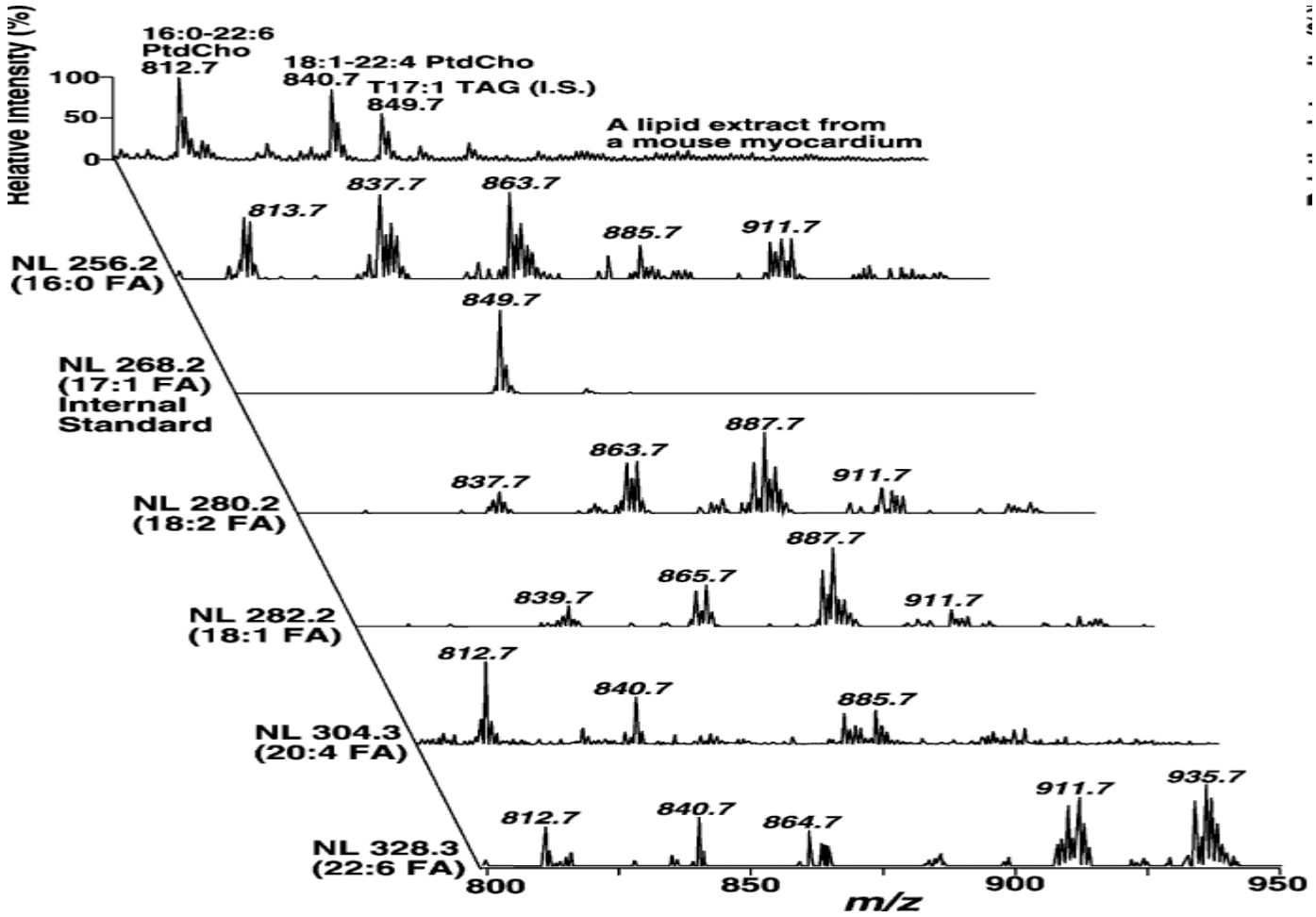
Source: Hsu et al. J. Am Soc. Mass Spectrom, 1998

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

Conclusions

- **Shotgun lipidomics approaches are high throughput and applicable to perform profiling as well as quantitative analysis of various lipids in biological samples.**
- **Reversed-phase LC-MS/MS can be used to separate phospholipids analysis of phospholipids indicated the separation based on lipophilocity (fatty acyl chain determine the elution sequence). Column carryover is a major problem in LC-MS analysis of phospholipids, especially lipids with di- or tri-acyl fatty chains.**
- **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.**