

# The use of mass spectrometry in lipidomics

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6-2612

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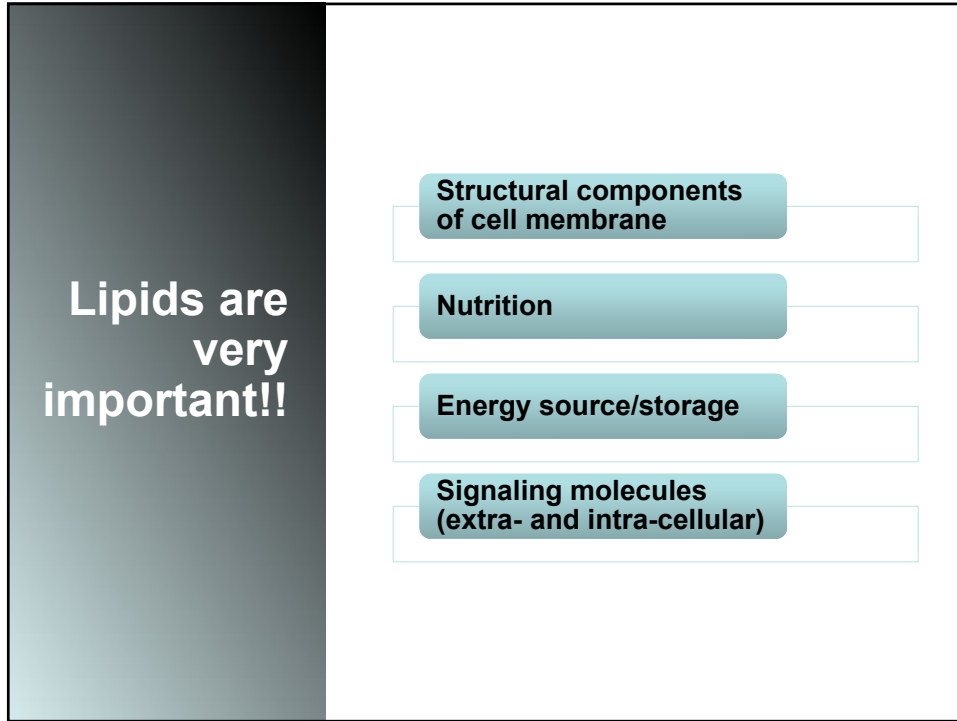
## Outlines

Brief introduction to lipidomics

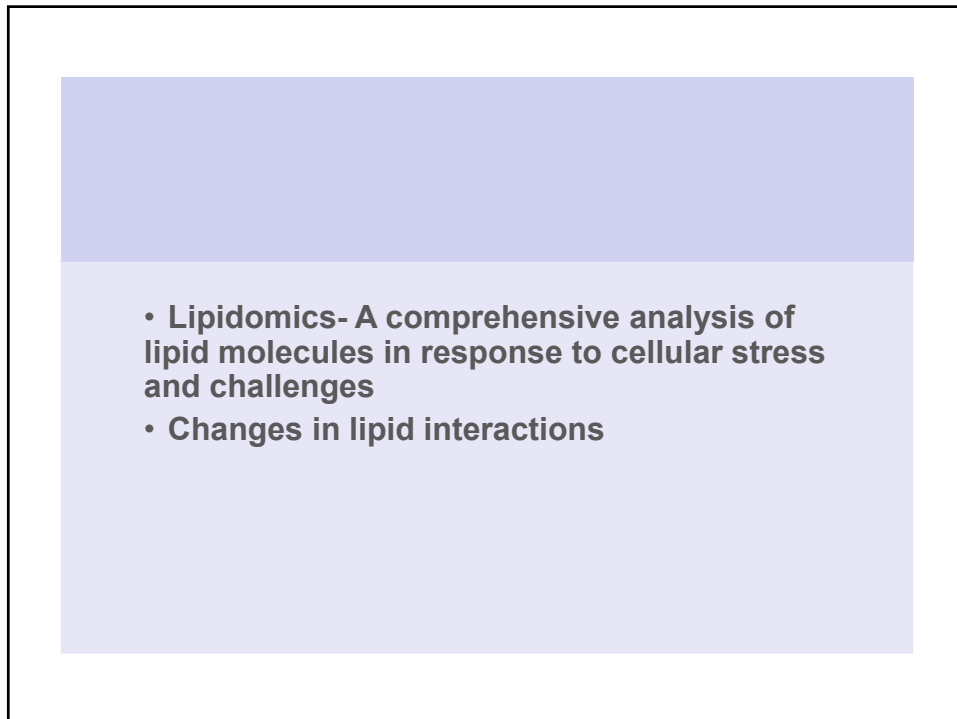
Analytical methodology:  
MS/MS structure elucidation  
of phospholipids

Phospholipid analysis in  
lean and ob/ob mice by  
mass spectrometry

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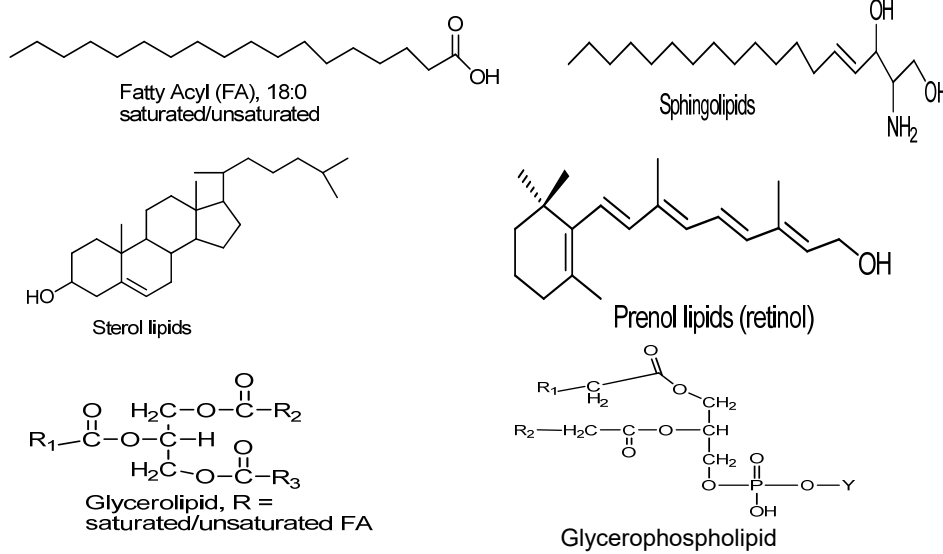


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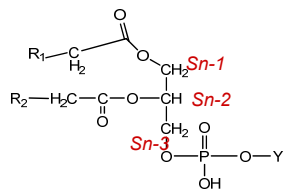
4

## Structures of different lipids classes

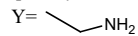


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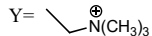
## Structures of main phospholipids



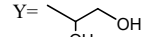
Phosphatidylethanolamine (PE)



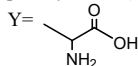
Phosphatidylcholine (PC)



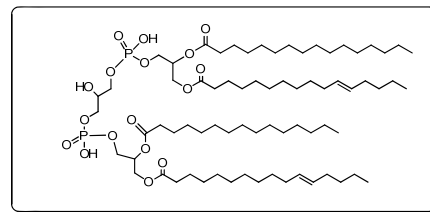
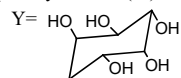
Phosphatidylglycerol (PG)



Phosphatidylserine (PS)



Phosphatidylinositol (PI)



Cardiolipin (diphosphatidylglycerol)

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## 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 °C until analysis.

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## Shotgun lipidomics: intrasource separation of lipids for quantitative lipidomics

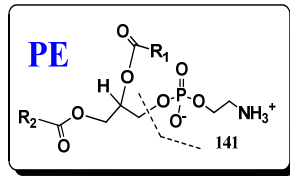
Group	Electrical Propensity	Lipid Classes
Anionic lipids	Carry net negative charge(s) at physiological pH	Cardiolipin, acylCoA, sulfatide, PtdIns (PtdInsP, PtdInsP <sub>2</sub> , PtdInsP <sub>3</sub> ), PtdGro, PtdSer, PtdH, etc.
Weak anionic lipids	Carry a net negative charge at alkaline pH	PE, lysoPE, 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

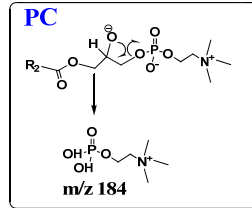
Source: Gross and Han, Mass Spec Rev. 2005

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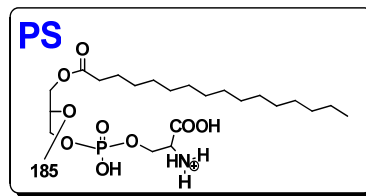
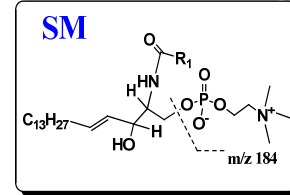
## Profiling glycerophospholipids in a complex mixture using MS/MS



**PE**  
Neutral Loss scan 141



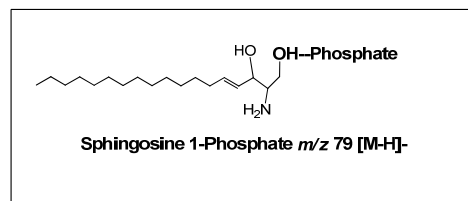
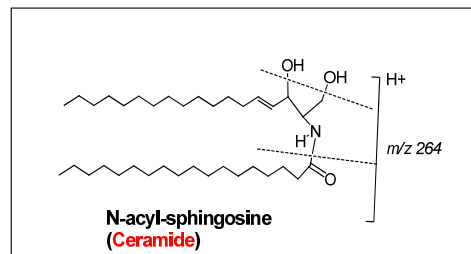
**PC & SM**  
Precursor ion scan 184



**PS**  
Neutral Loss scan 185

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

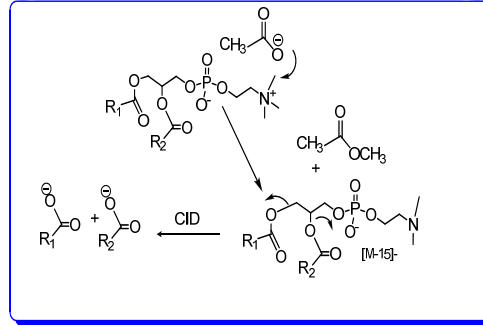


**m/z 264 is a characteristic ion for all compounds containing a sphingosine backbone in +ve ion mode**

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## 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.



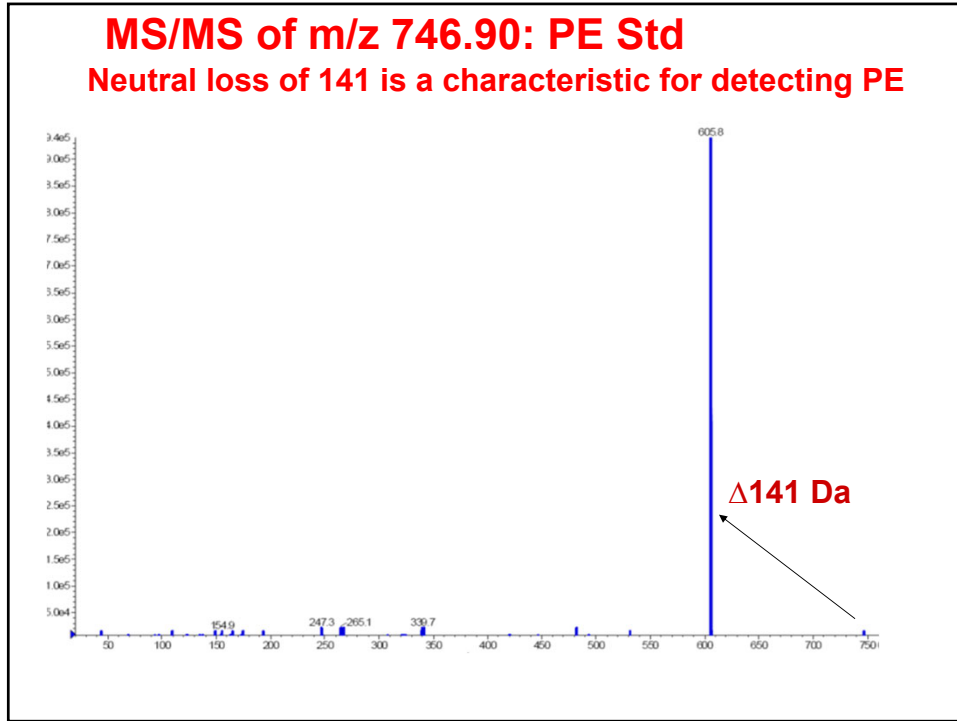
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## ESI-MS/MS analyses of various lipids

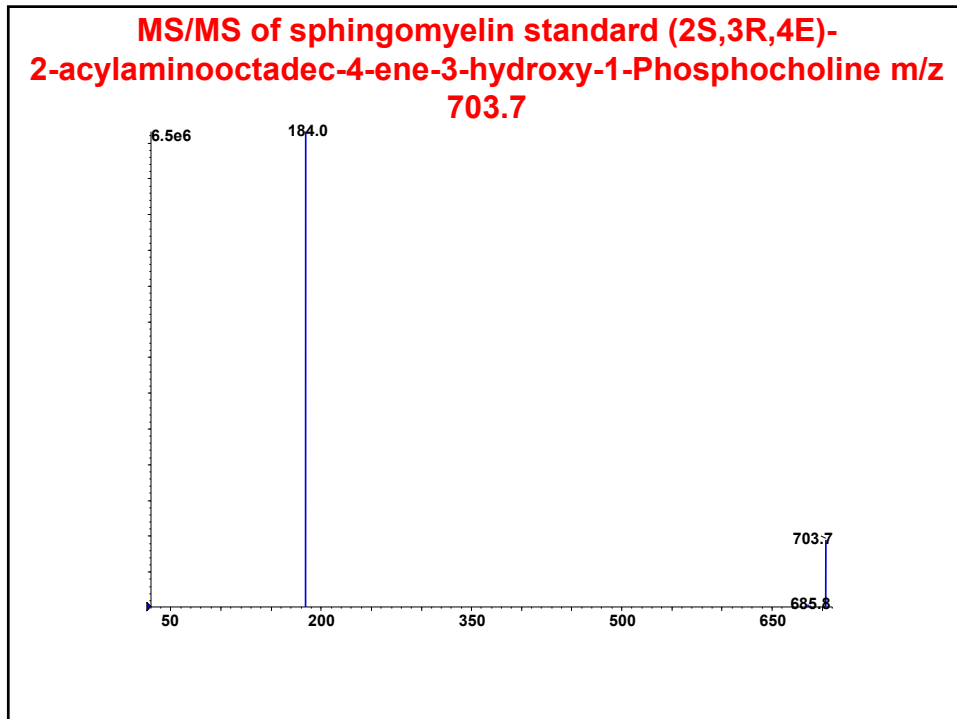
Lipid Class(s)	Precursor Ion	MS/MS Mode & Conditions	Fragment
cardiolipin	[M-2H] <sup>2-</sup>	PI, <i>m/z</i> 153.0, 35 eV	glycerol phosphate derivative
PtdGro, PtdH	[M-H] <sup>-</sup>	PI, <i>m/z</i> 153.0, 35 eV, *	glycerol phosphate derivative
PtdIns	[M-H] <sup>-</sup>	PI, <i>m/z</i> 241.1, 45 eV	cyclic Inositol phosphate
		PI, <i>m/z</i> 153.0, 35 eV	glycerol phosphate derivative
PtdInsP	[M-H] <sup>-</sup>	PI, <i>m/z</i> 321.1, 53 eV	phosphoinositol phosphate
PtdInsP <sub>2</sub>	[M-H] <sup>-</sup>	PI, <i>m/z</i> 401.1, 62 eV	diphosphoinositol phosphate
PtdSer	[M-H] <sup>-</sup>	NL, 87.0 amu, 25 eV, *	serine
		PI, <i>m/z</i> 153.0, 35 eV	glycerol phosphate derivative
sulfatide	[M-H] <sup>-</sup>	PI, <i>m/z</i> 97.0, 65 eV	sulfate
acylCoA	[M-2H] <sup>2-</sup>	PI, <i>m/z</i> 339.0, 30 eV, *	doubly-charged CoA derivative
PE, lysoPE	[M-H] <sup>-</sup>	PI, <i>m/z</i> 196.0, 50 eV	glycerol phosphoethanolamine derivative
ceramide	[M-H] <sup>-</sup>	NL, 256.2 amu, 32 eV *	
		NL, 327.3 amu, 32 eV	
		NL, 240.2 amu, 32 eV *	2- <i>trans</i> -palmitoyl alcohol
PC, lysoPC, SM	[M+Li(Na)] <sup>+</sup>	NL, 59.1 amu, -28 eV, *	trimethylamine
	[M+Li(Na)] <sup>+</sup>	NL, 183.1 amu, -32 eV	phosphocholine
	[M+Li] <sup>+</sup>	NL, 189.1 amu, -42 eV	lithium cholinephosphate
	[M+Na] <sup>+</sup>	NL, 205.1 amu, -35 eV	sodium cholinephosphate
	[M+H] <sup>+</sup>	PI, <i>m/z</i> 184.1, -30 eV, *	phosphocholine
	[M+Cl] <sup>+</sup>	NL, 50.0 amu, 24 eV, *	methylchloride
cerebroside	[M+Li] <sup>+</sup>	NL, 162.2, -50 eV, *	
	[M+Cl] <sup>+</sup>	NL, 36.0 amu, 30 eV	hydrogen chloride
MGDG	[M+Li(Na)] <sup>+</sup>	PI, <i>m/z</i> 227(243), -45 eV	Li(Na)+galactose derivative
DGDG	[M+Li(Na)] <sup>+</sup>	PI, <i>m/z</i> 227(243), -66 eV	Li(Na)+galactose derivative
acylcarnitine	[M+H] <sup>+</sup>	PI, <i>m/z</i> 85.1, -20 eV, *	carnitine
chol. ester	[M+NH <sub>4</sub> ] <sup>+</sup>	PI, <i>m/z</i> 369.3, -50 eV, *	cholestane cation
TAG	[M+Li] <sup>+</sup>	NL, X amu, -35 eV	a fatty acid

Source: Gross and Han, Mass Spec Rev. 2005

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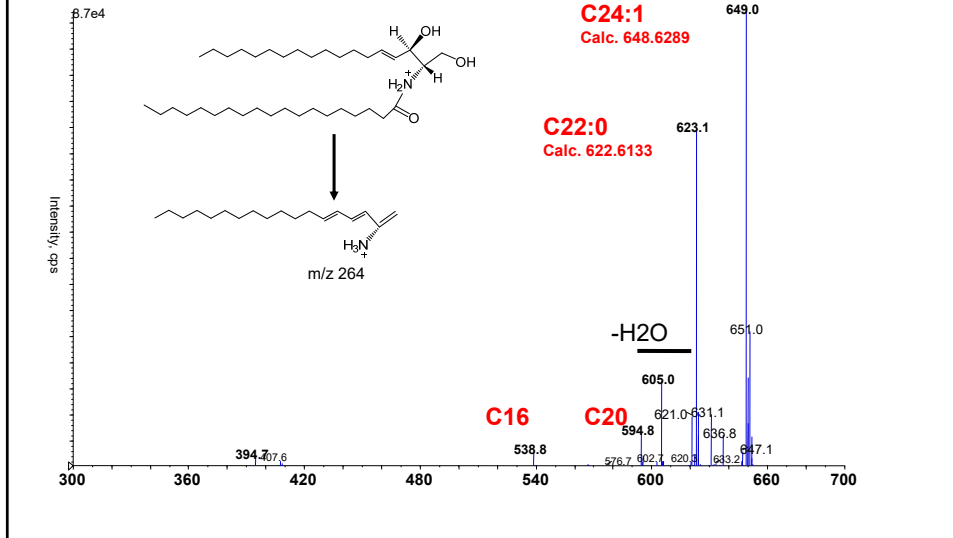


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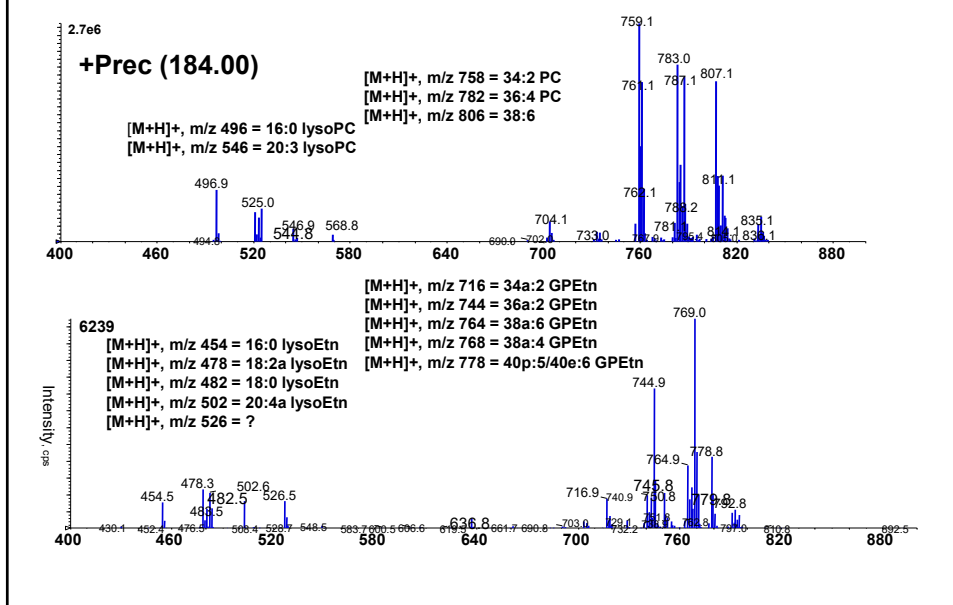
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**Precursor ion scan m/z 264 in +ve ion mode is specific to identify ceramides in a sample**



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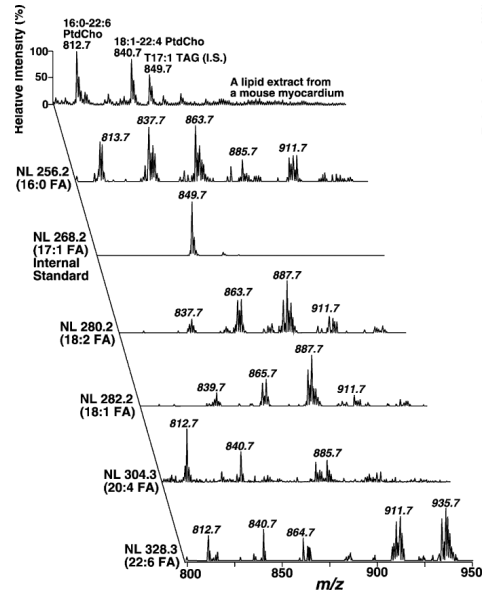
**Profiling of phospholipids using precursor ion m/z 184 and neutral loss scan 141 for PC, SM and PE**



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



Source: Gross and Han, Mass Spec Rev. 2005

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

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**LMSD: Lipid classification search results**

Fatty Acyls [FA] (W) -> Eicosanoids [FAO3]

LM_ID	Common Name	Systematic Name	Formula	Mass
LMFA03000001	0(9)-EpETE	(+/-)-0(9)-epoxy-5Z,11Z,14Z,17Z-eicosatetraenoic acid	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	318.22
LMFA03000002	11(12)-EpETE	(+/-)-11(12)-epoxy-5Z,8Z,14Z,17Z-eicosatetraenoic acid	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	318.22
LMFA03000003	14(15)-EpETE	(+/-)-14(15)-epoxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	318.22
LMFA03000004	17(18)-EpETE	(+/-)-17(18)-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	318.22
LMFA03000005	11(R)-HEDE	11R-hydroxy-12E,14Z-eicosadienoic acid	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	324.27
LMFA03000006	17R,18S-EpETE	17R,18S-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	318.22
LMFA03000008	15(R)-HEDE	15R-hydroxy-11Z,13E-eicosadienoic acid	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	324.27
LMFA03000009	11S-HEDE	11S-hydroxy-12E,14Z-eicosadienoic acid	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	324.27
LMFA03010000	Prostanic acid skeleton	-	-	-
LMFA03010001	6-keto-PGF <sub>2α</sub>	6-oxo-9S,11R,15S-9-hydroxy-13E-prostanic acid	C <sub>20</sub> H <sub>32</sub> O <sub>5</sub>	370.24
LMFA03010002	PGF <sub>2α</sub>	9S,11R,15S-9-hydroxy-5Z,13E-prostanic acid	C <sub>20</sub> H <sub>34</sub> O <sub>5</sub>	354.24
LMFA03010003	PGI <sub>2</sub> (D)	9-oxo-11R,15S-9-hydroxy-5Z,13E-prostanic acid	C <sub>20</sub> H <sub>32</sub> O <sub>5</sub>	352.22
LMFA03010004	PGI <sub>2</sub> (S)	9S,15S-9-hydroxy-11-oxo-5Z,13E-prostanic acid	C <sub>20</sub> H <sub>32</sub> O <sub>5</sub>	352.22
LMFA03010005	PGA <sub>1</sub>	9-oxo-12S-hydroxy-10Z,13E-prostanic acid	C <sub>20</sub> H <sub>32</sub> O <sub>5</sub>	336.23
LMFA03010006	PGF <sub>2α</sub> -44	9S,11R,15S-9-hydroxy-5Z,13E-prostanic acid (3,3,4,4-tetrahydro)	C <sub>20</sub> H <sub>34</sub> O <sub>5</sub>	350.27
LMFA03010007	PGI <sub>2</sub> -44	9S,15S-9-hydroxy-11-oxo-5Z,13E-prostanic acid (3,3,4,4-tetrahydro)	C <sub>20</sub> H <sub>32</sub> O <sub>5</sub>	356.23
LMFA03010008	PGE <sub>2</sub> -44	11R,15S-9-hydroxy-9-oxo-5Z,13E-prostanic acid (3,3,4,4-tetrahydro)	C <sub>20</sub> H <sub>34</sub> O <sub>5</sub>	356.23
LMFA03010009	PGG <sub>2</sub>	9S,11R-epidioxy-15S-hydroperoxy-5Z,13E-prostanic acid	C <sub>20</sub> H <sub>32</sub> O <sub>6</sub>	368.22

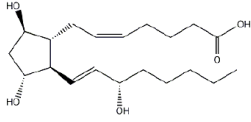
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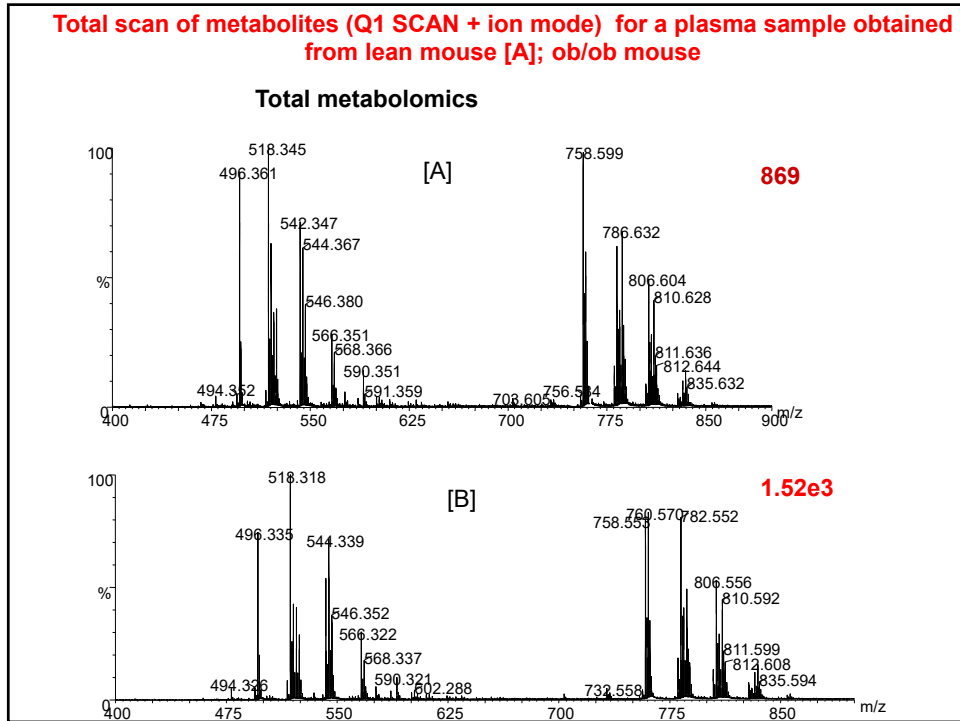
Structure database (LMSD)

LMFA03010025

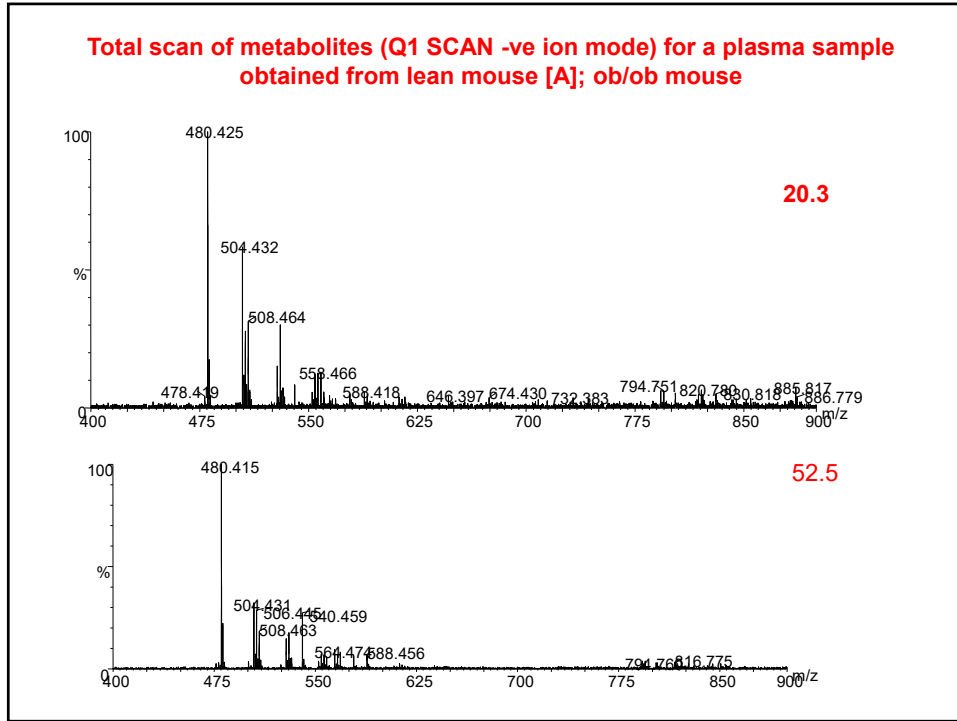


LM ID	LMFA03010025
Common Name	PGF2β
Systematic Name	9R,11R,15S-trihydroxy-5Z,13E-prostadienoic acid
Synonyms	-
Exact Mass	354.24
Formula	C <sub>20</sub> H <sub>34</sub> O <sub>5</sub>
Category	Fatty Acyls [FA]
Main Class	Eicosanoids [FA03]
Sub Class	Prostaglandins [FA0301]
LIPIDBANK ID	<a href="#">XPR1764</a>
PubChem Substance ID (SID)	<a href="#">4265968</a>
KEGG ID	-

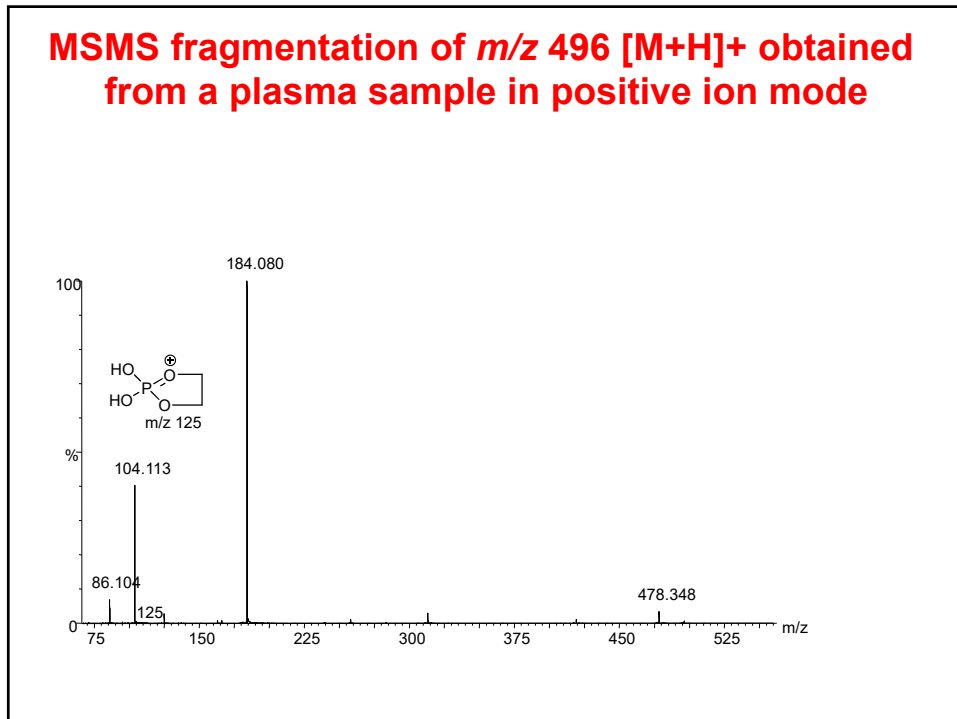
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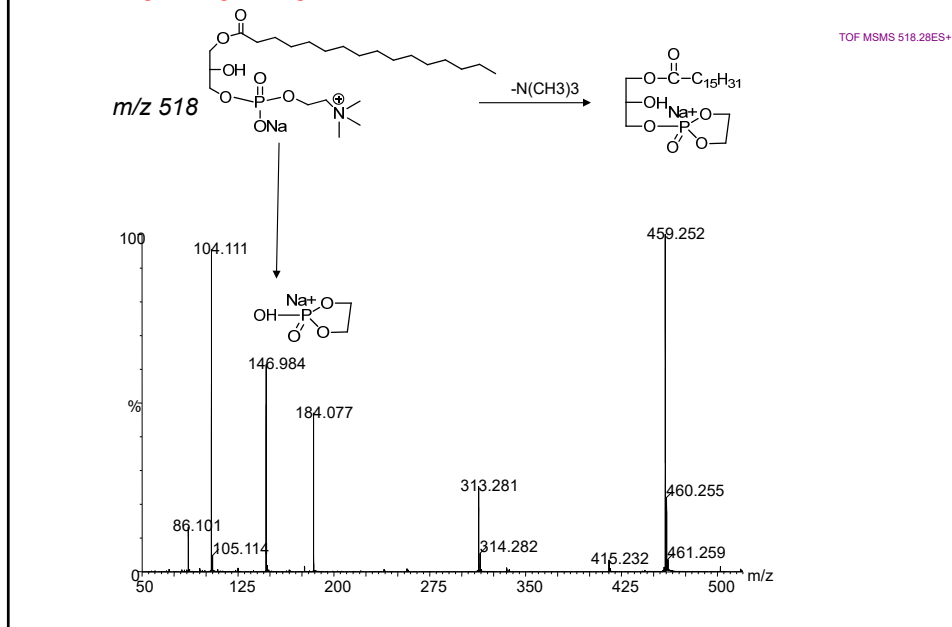


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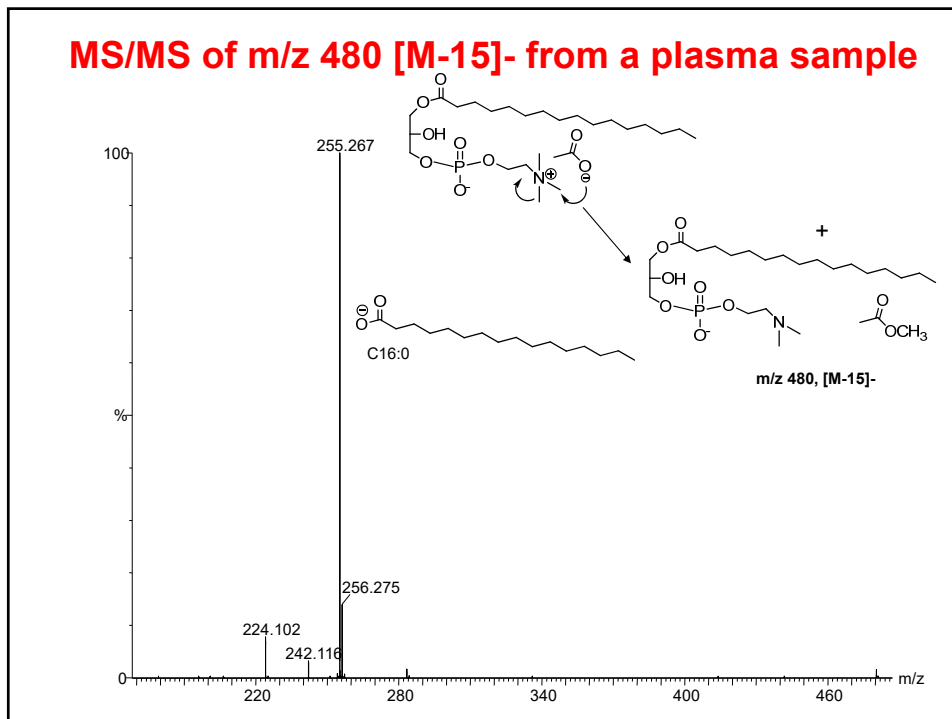
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**MS/MS of m/z 518 [M+Na]<sup>+</sup> indicates that it may be a sodiated ion of a phospholipid with mol. Wt. 495 Da**



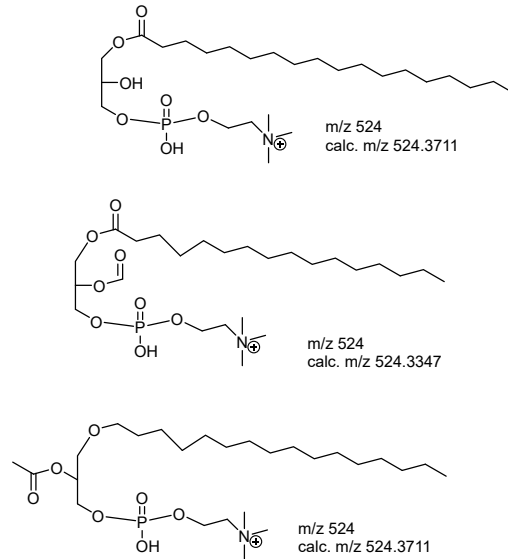
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**MS/MS of m/z 480 [M-15]<sup>-</sup> from a plasma sample**



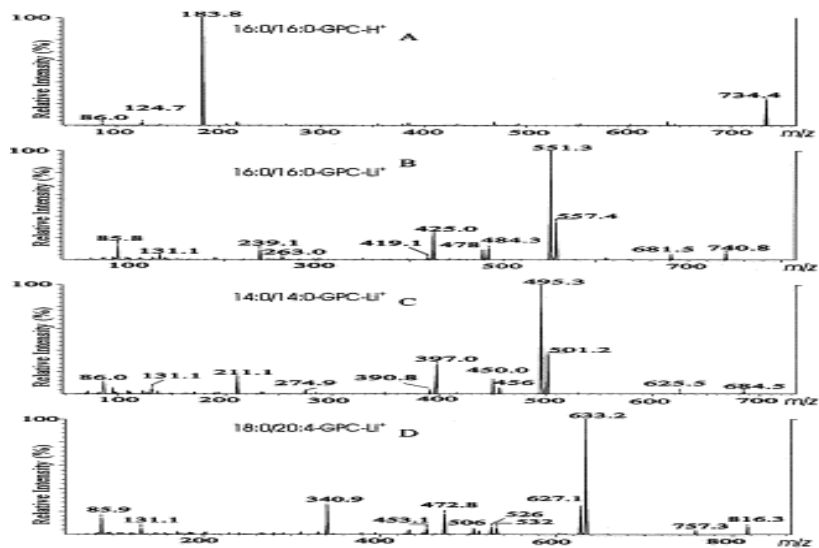
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## Several isobaric compounds- Identification by high resolution mass spectrometry



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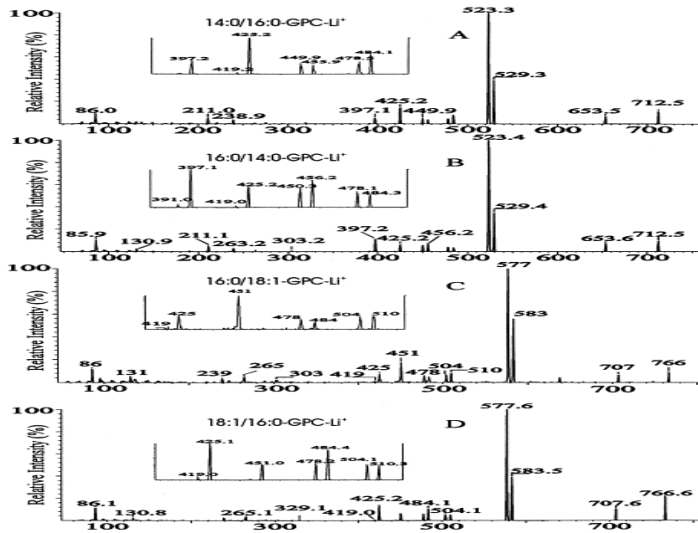
## Lithiated adducts of phosphocholine provide more structural information in their MS/MS spectra



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

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**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

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## 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.

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