

The use of mass spectrometry in lipidomics

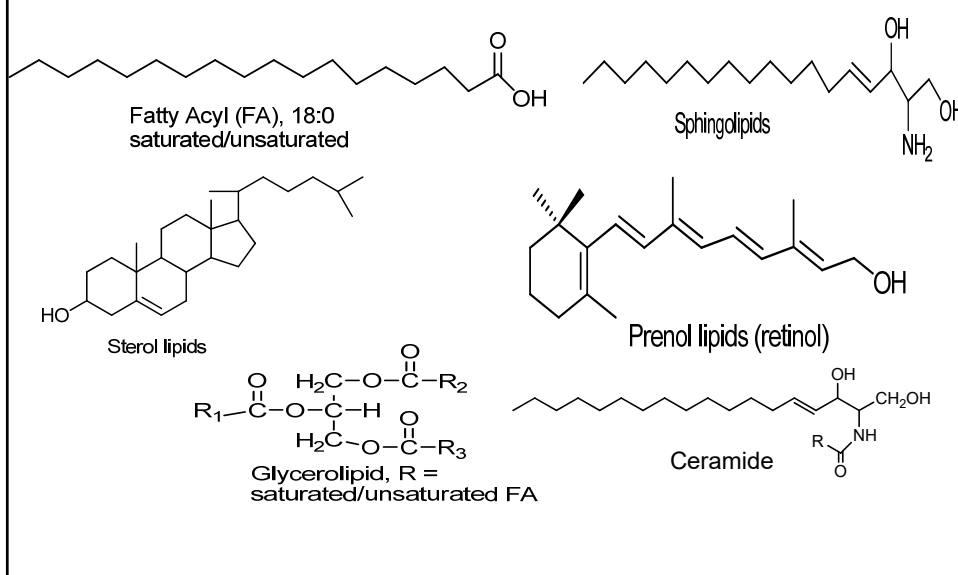
Jeevan Prasain
jprasain@uab.edu
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Outlines

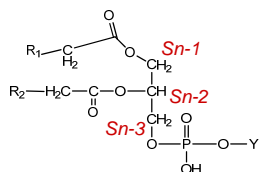
- **Brief introduction to lipidomics**
- **Analytical methodology: MS/MS structure elucidation of phospholipids**
- **Phospholipid analysis in lean and ob/ob mice by mass spectrometry**
- **LC-MS/MS quantification of ceramides**

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

Structures of different lipids classes



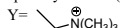
Structures of main phospholipids



Phosphatidylethanolamine (PE)



Phosphatidylcholine (PC)



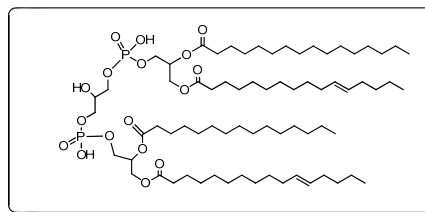
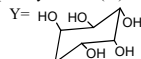
Phosphatidylglycerol (PG)



Phosphatidylserine (PS)



Phosphatidylinositol (PI)



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 °C until analysis.

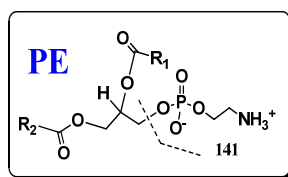
Shotgun lipidomics: intrasource separation of lipids for quantitative lipidomics

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.

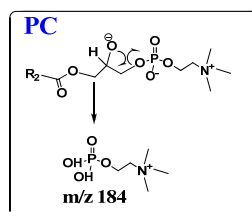
The ionization efficiency of an analyte greatly depends on the electrical propensity of an individual analyte in its own microenvironment to lose or gain a charge

Source: Gross and Han,, 2004

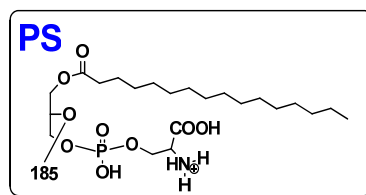
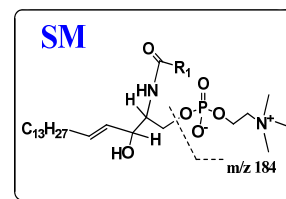
Profiling phospholipids and sphingosines in a complex mixture using MS/MS



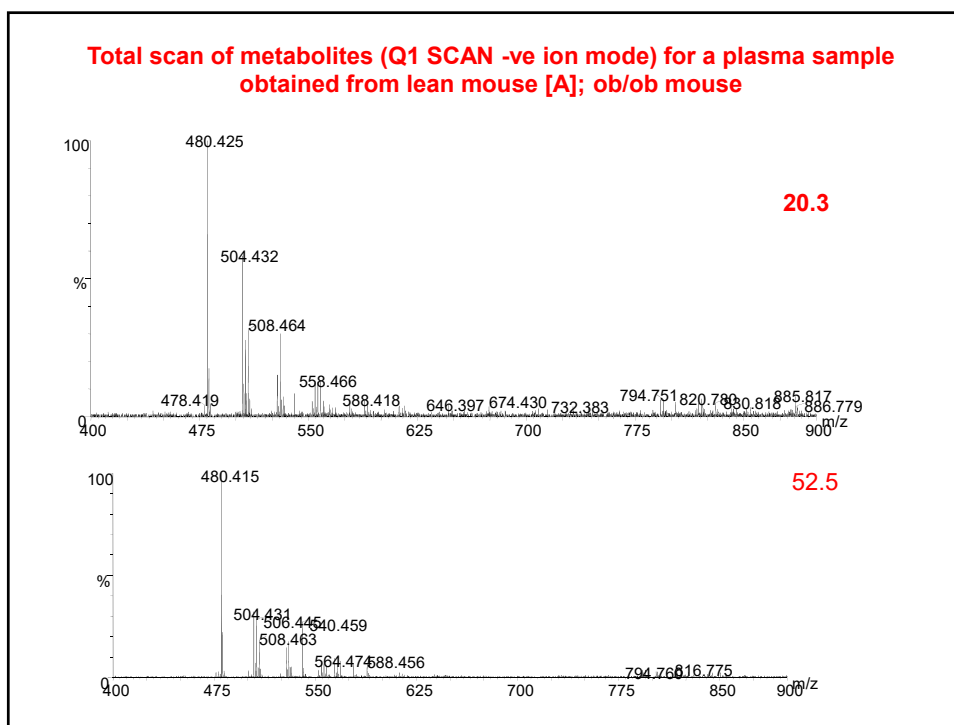
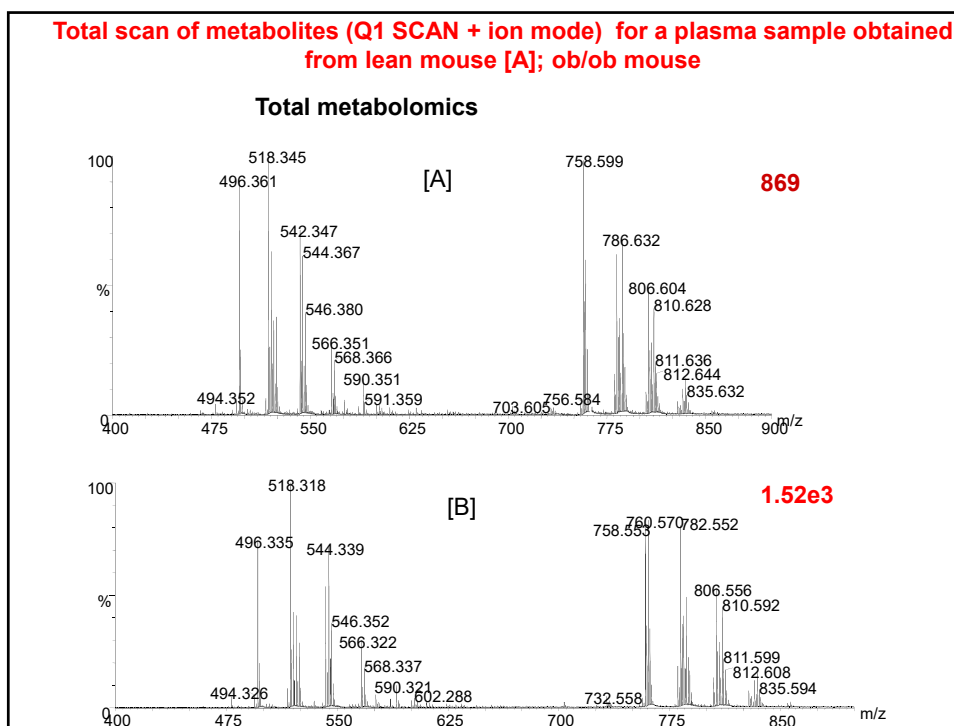
PE
Neutral Loss scan 141

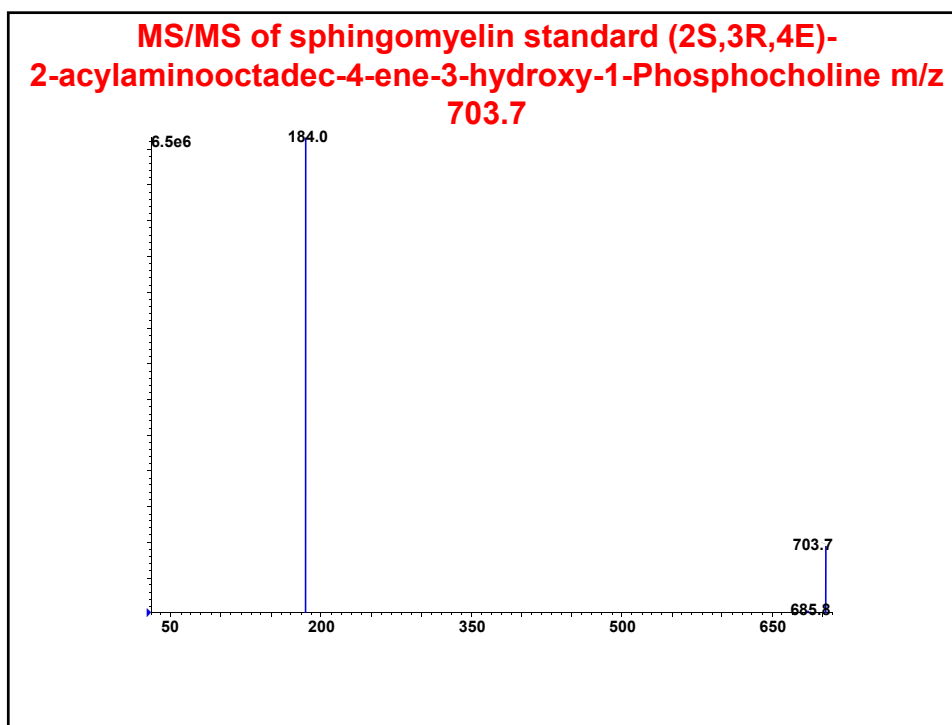
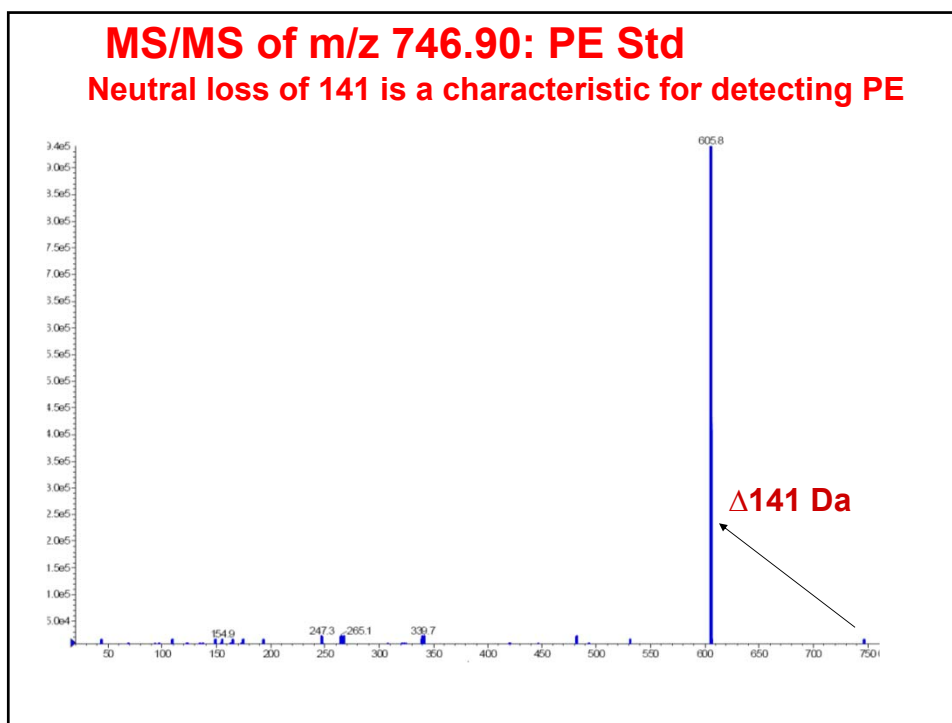


PC & SM
Precursor ion scan 184



PS
Neutral Loss scan 185

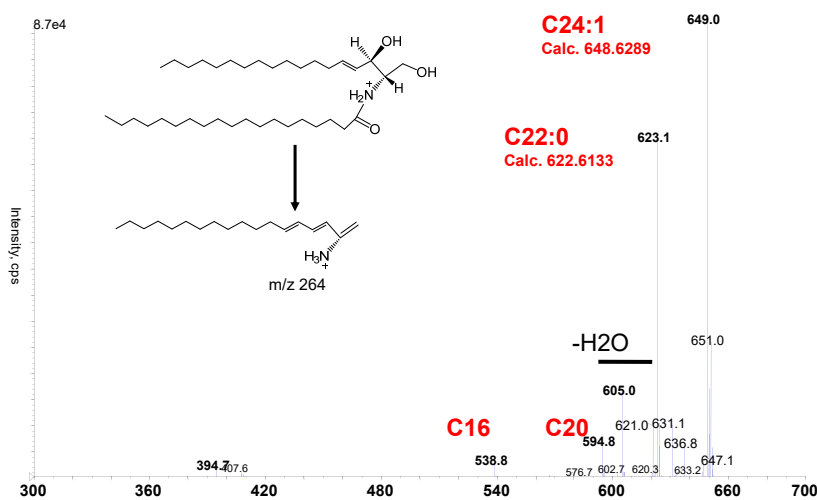




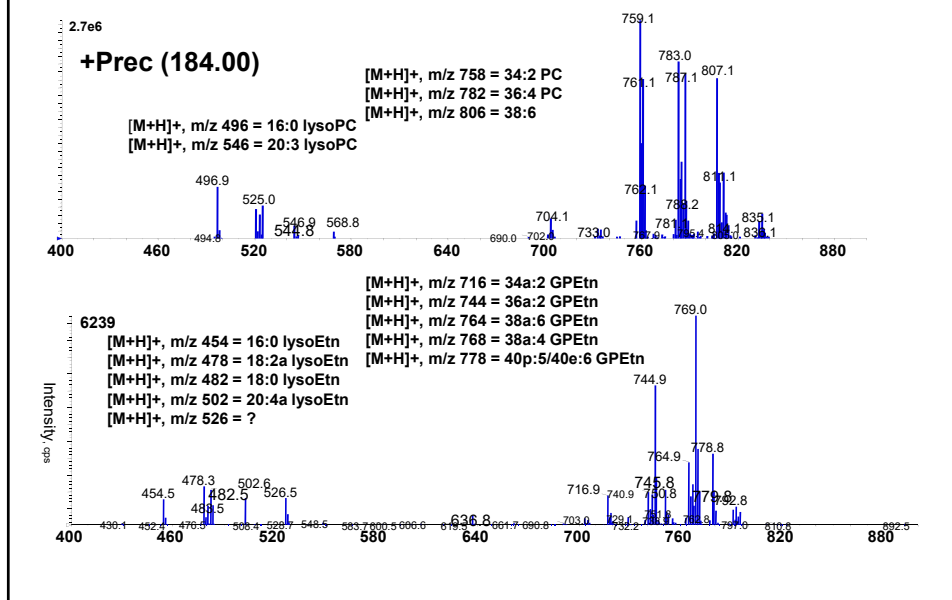
ESI-MS/MS analyses of various lipids

Lipid Class(s)	Precursor Ion	MS/MS Mode & Conditions	Fragment
cardiolipin	[M-2H] ²⁻	PI, <i>m/z</i> 153.0, 35 eV	glycerol phosphate derivative
PtdGro, PtdH	[M-H] ⁻	PI, <i>m/z</i> 153.0, 35 eV, *	glycerol phosphate derivative
PtdIns	[M-H] ⁻	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] ⁻	PI, <i>m/z</i> 321.1, 53 eV	phosphoinositol phosphate
PtdInsP ₂	[M-H] ⁻	PI, <i>m/z</i> 401.1, 62 eV	diphosphoinositol phosphate
PtdSer	[M-H] ⁻	NL, 87.0 amu, 25 eV, *	serine
		PI, <i>m/z</i> 153.0, 35 eV	glycerol phosphate derivative
sulfatide	[M-H] ⁻	PI, <i>m/z</i> 97.0, 65 eV	sulfate
acylCoA	[M-2H] ²⁻	PI, <i>m/z</i> 339.0, 30 eV, *	doubly-charged CoA derivative
PE, lysoPE	[M-H] ⁻	PI, <i>m/z</i> 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- <i>trans</i> -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, <i>m/z</i> 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, <i>m/z</i> 227(243), -45 eV	Li(Na)+galactose derivative
DGDG	[M+Li(Na)] ⁺	PI, <i>m/z</i> 227(243), -66 eV	Li(Na)+galactose derivative
acylcarnitine	[M+H] ⁺	PI, <i>m/z</i> 85.1, -20 eV, *	carnitine
chol. ester	[M+NH ₄] ⁺	PI, <i>m/z</i> 369.3, -50 eV, *	cholestane cation
TAG	[M+Li] ⁺	NL, X amu, -35 eV	a fatty acid

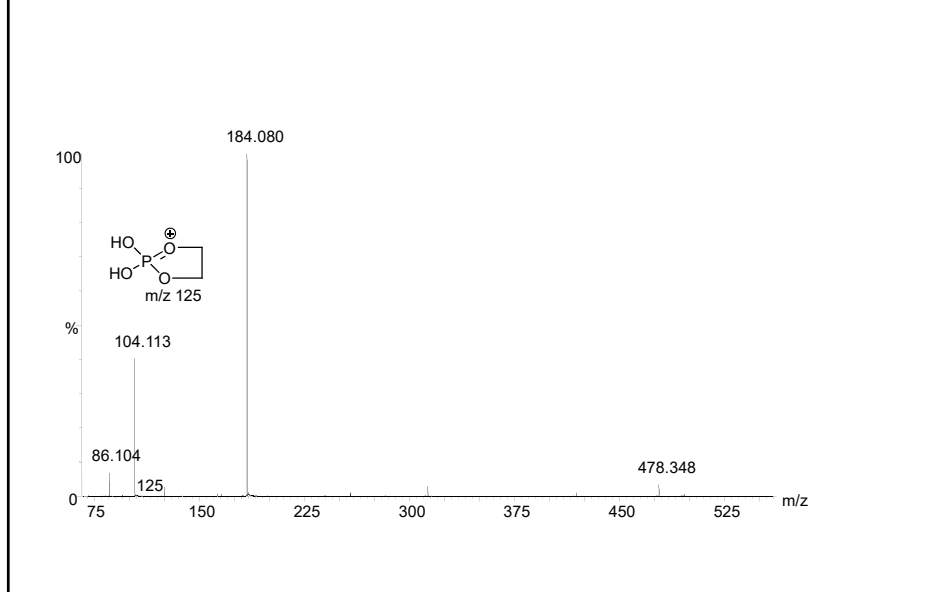
Source: Gross and Han,, 2004

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

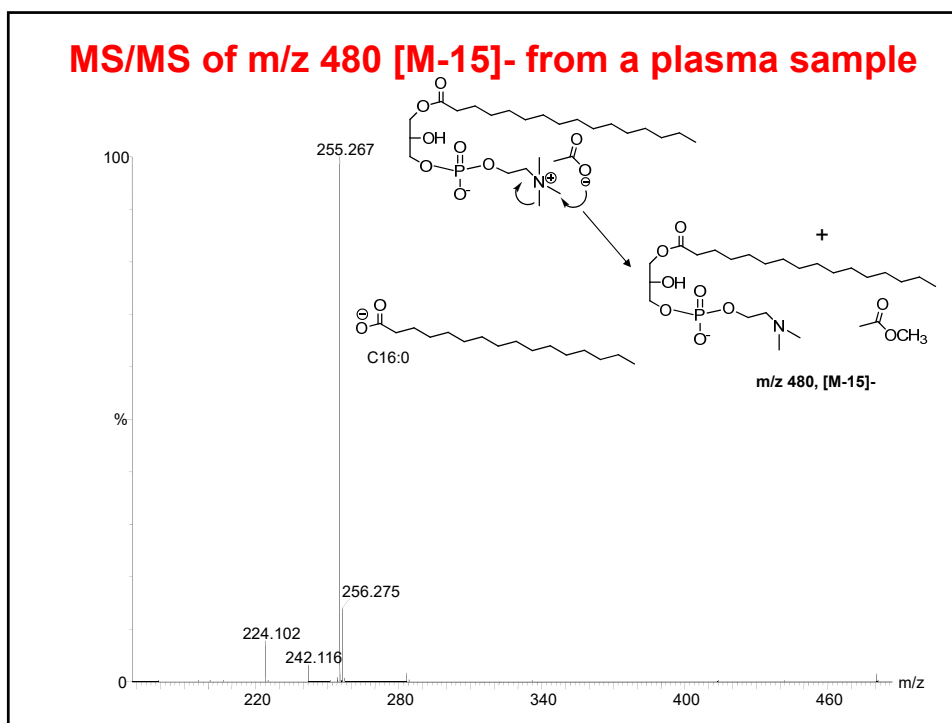
Profiling of phospholipids using precursor ion m/z 184 and neutral loss scan 141 for PC, SM and PE



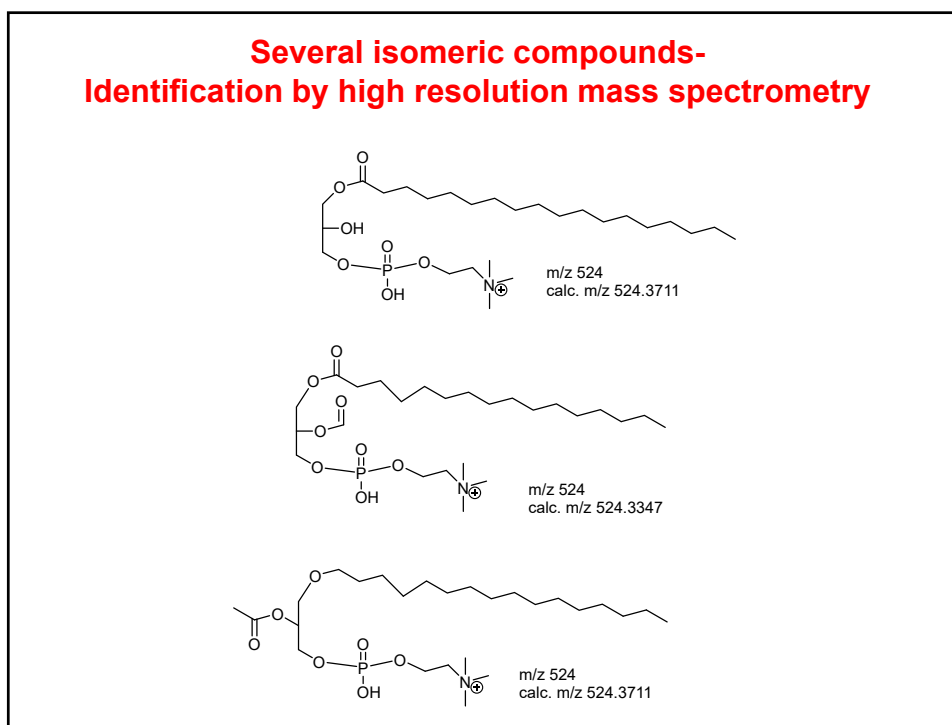
MSMS fragmentation of m/z 496 obtained from a plasma sample in positive ion mode



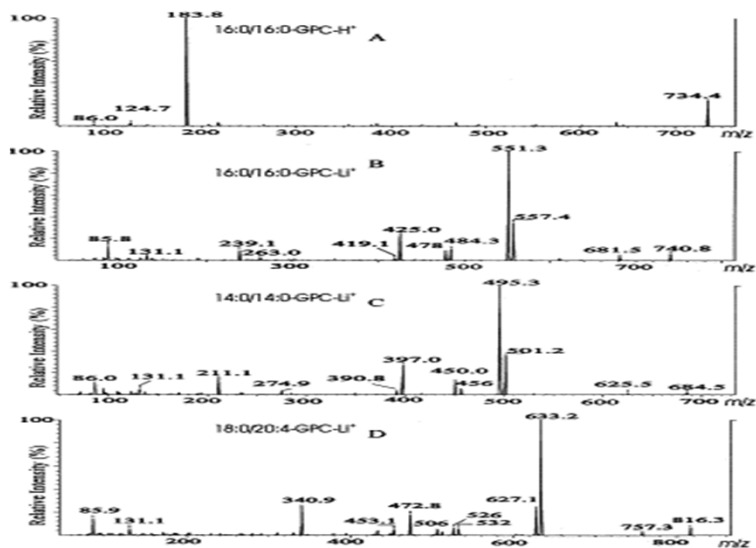
MS/MS of m/z 480 [M-15]- from a plasma sample



Several isomeric compounds- Identification by high resolution mass spectrometry

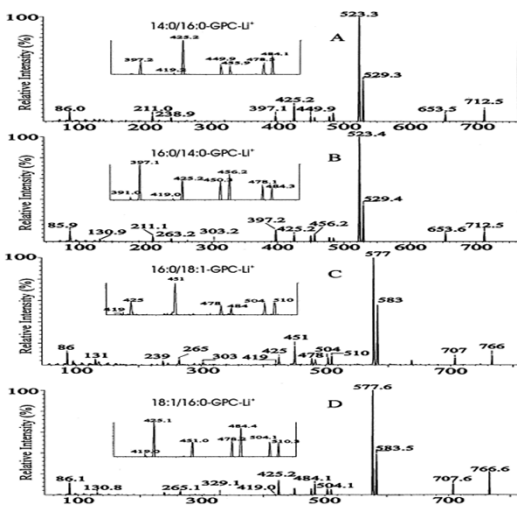


Lithiated adducts of phosphocholine provide more structural information in their MS/MS spectra



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

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

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LMSD: Lipid classification search results

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

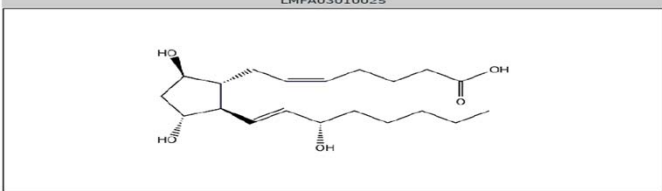
LM_ID	Common Name	Systematic Name	Formula	Mass
LMFA03000001	8(9)-EpETE	(+/-)-8(9)-epoxy-5Z,11Z,14Z,17Z-eicosatetraenoic acid	C ₂₀ H ₃₀ O ₃	318.22
LMFA03000002	11(12)-EpETE	(+/-)-11(12)-epoxy-5Z,8Z,14Z,17Z-eicosatetraenoic acid	C ₂₀ H ₃₀ O ₃	318.22
LMFA03000003	14(15)-EpETE	(+/-)-14(15)-epoxy-5Z,8Z,11Z,17Z-eicosatetraenoic 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 ₃₀ O ₃	324.27
LMFA03000006	17R,18S-EpETE	17R,18S-epoxy-5Z,6Z,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 ₃₀ O ₃	324.27
LMFA03000009	11S-HEDE	11S-hydroxy-12E,14Z-eicosadienoic acid	C ₂₀ H ₃₀ O ₃	324.27
LMFA03010000	Prostanoic acid skeleton	-	-	-
LMFA03010001	6-keto-PGF1a	6-oxo-9S,11R,15S-trihydroxy-13E-prostanoic acid	C ₂₀ H ₃₄ O ₆	370.24
LMFA03010002	PGF2a	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	PGF2a-d4	9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid (3,3,4,4-d4)	C ₂₀ H ₃₀ D ₄ O 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 ₄ O 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 ₄ O ₅	356.25
LMFA03010009	PGG2	9S,11R-epidioxy-15S-hydroperoxy-5Z,13E-prostadienoic acid	C ₂₀ H ₃₂ O ₆	368.22

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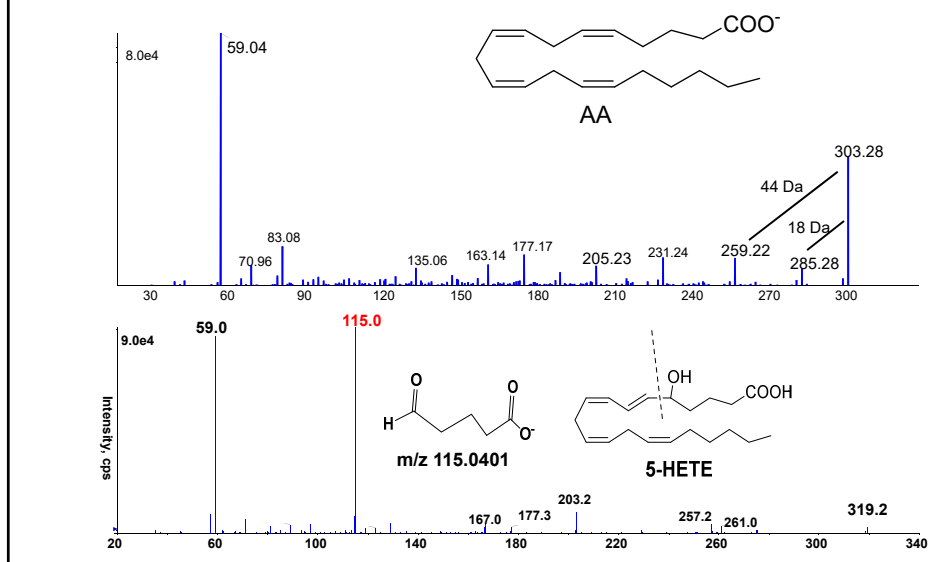
Structure database (LMSD)

LMFA03010025

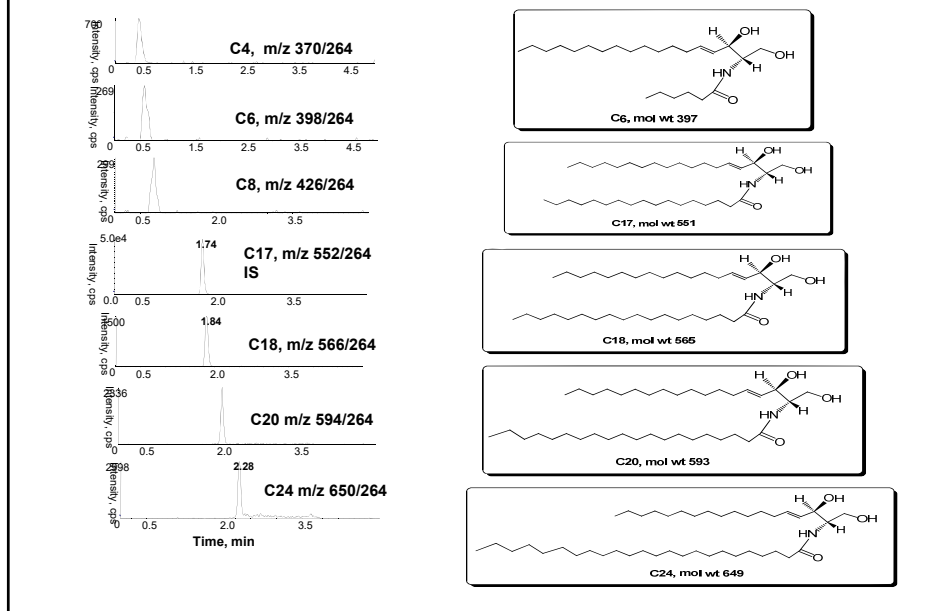


LM ID LMFA03010025
Common Name PGF2β
Systematic Name 9R,11R,15S-trihydroxy-5Z,13E-prostadienoic acid
Synonyms -
Exact Mass 354.24
Formula C₂₀H₃₄O₅
Category Fatty Acyls [FA]
Main Class Eicosanoids [FA03]
Sub Class Prostaglandins [FA0301]
LIPIDBANK ID [XPR1764](#)
PubChem Substance ID (SID) [4265968](#)
KEGG ID -

Product ion spectra of deprotonated arachidonic acid [AA] and its oxidation product 5-hydroxy-eicosatetraenoic acids [5-HETE]

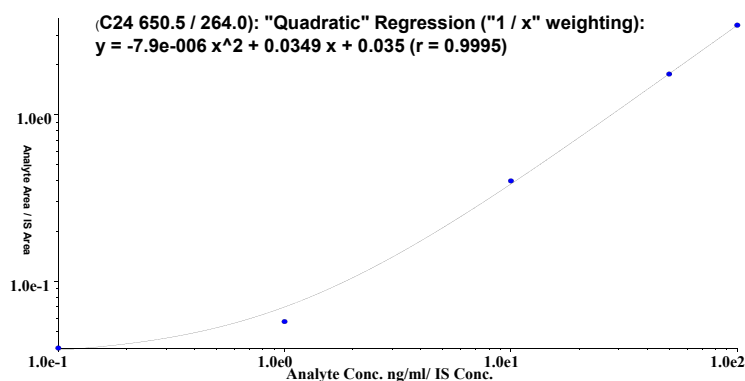


MRM chromatograms showing simultaneous determination of ceramides (C4-C24)

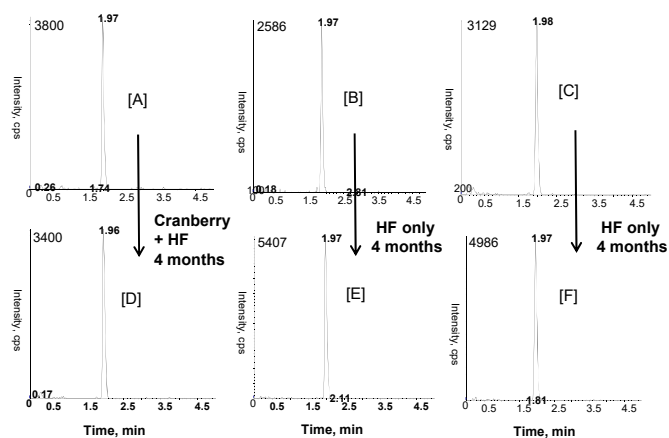


A linear response for Cer C24:0 was observed over a range of 0.1-100 ng/ml with correlation coefficient greater than 0.99

Sample Name	Analyte Peak Name	Calculated Concentration (ng/mL)	Accuracy (%)
Ceramide Standard 100 ng/ml	C24 650.5 / 264.0	100	100
Ceramide Standard 50 ng/ml	C24 650.5 / 264.0	49.8	99.6
Ceramide Standard 10 ng/ml	C24 650.5 / 264.0	10.5	105
Ceramide Standard 1 ng/ml	C24 650.5 / 264.0	0.634	63.4
Ceramide Standard 0.1 ng/ml	C24 650.5 / 264.0	0.132	132



Cranberry fruit powder treatment reduced the HF induced increased levels of Ceramide C20 in rats



[A]-[C] represent base line plasma ceramide C20 (594/264) from three animals
 [D] after 4 months treatment with cranberry (1 g/kg b. w. and high fat diet
 [E] & [F] after 4 months treatment with high fat diet only

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.**
- **A rapid five minute liquid chromatography tandem mass spectrometry (LC-MS/MS) method operating in multiple reaction ion monitoring mode (MRM) was developed for identification and simultaneous quantification of six ceramides.**